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FAILURE OF OPIOIDS TO MODIFY NORADRENERGIC NEURONAL RECOVERY FROM A DORSAL BUNDLE LESION IN NEONATAL RATS

A Dissertation Presented to
the Faculty of the Department of Pharmacology
Quillen-Dishner College of Medicine
East Tennessee State University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

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by
Ellen Kunkel-Bagden
May, 1985

APPROVAL

This is to certify that the Graduate Committee of

ELLEN KUNKEL BAGDEN

met on the

27th day of March 1985.

The committee read and examined her dissertation, supervised her defense of it in an oral examination, and decided to recommend that her study be submitted to the Graduate Council and the Dean of the School of Graduate Studies in partial fulfillment of the requirements for the degree Doctor of Philosophy in Biomedical Sciences.

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the Graduate Council

ABSTRACT

FAILURE OF OPIOIDS TO MODIFY NORADRENERGIC NEURONAL RECOVERY FROM A DORSAL BUNDLE LESION IN NEONATAL RATS

by

Ellen Kunkel Bagden

Morphine and exogenous opioid peptides alter the development of central noradrenergic neurons damaged by neonatal treatment with the neurotoxin 6-hydroxydopa. Transection of the dorsal noradrenergic bundle (DNB) in neonatal rats produces nearly the same alteration in the developmental pattern. It was of interest to determine whether morphine or an exogenous opioid peptide (met-enkephalin) was able to modify the recovery of noradrenergic neurons after neonatal surgical transection of the DNB. Neonatal rats were divided into two groups. Both groups received saline (0.85%) or naloxone (2.0 mg/kg) by the intraperitoneal (i.p.) route. In the first group the animals received an additional i.p. injection of either saline or morphine sulfate (3.33 mg/kg). The second group received an intraventricular injection of either saline, morphine (10 µg/5 µl), or methionine-enkephalin (25 µg/5 µl) ten minutes after the i.p. injection. Half the animals in these groups then received a DNB lesion, made with a blade 3mm (depth) by 5mm (width) at the level of the colliculi. At 6 weeks brains were removed for assay of norepinephrine (NE) content by a fluorometric method, and for determination of the rate of ^{3}H-NE uptake, in order to assess noradrenergic fiber number. It was found that in the cerebellum both the ^{3}H-NE uptake rate and NE content were significantly elevated by approximately 75% in the animals that received the lesion. The recovery in the anterior cortex (39%) was significantly greater than in the posterior cortex (27%), while recovery in the hippocampus (21%) was the least. This indicates a regional difference in recovery in the more distal projections of the DNB. Within the lesion group, however, there was no alteration in ^{3}H-NE uptake rate nor NE content in any of the above regions with any of the drug treatments. Therefore, none of the drug treatments effectively altered the recovery from surgical transection of the DNB. It is suggested that opioids are capable of modifying neurotoxin damage per se, but not capable of modifying regeneration of noradrenergic neurons.

Dedication

This manuscript is dedicated to my father. It was his love for science that inspired and fostered my dedication to my work. Although he will be unable to read and review this manuscript, his criticisms are within it.

Acknowledgements

I wish to express my sincere appreciation to my major advisor, Dr. Richard Kostrzewa. It was through his inspiration, guidance, and efforts that I was able to commence and complete this project. I also wish to thank my husband Alan and my little boy Alex for all their love, patience, and understanding during this difficult time; their help was indispensable. They, also, worked very hard to make this project possible.

I want to give a special thanks to all the members of my committee: Dr. Daigneault, Dr. Hoover, Dr. Ernst-Fonberg, and Dr. Baisden for their encouragement and constructive criticisms. I wish to express my gratitude to Judy Hardin, Steve and Mark from animal care, and all the members of the department of Pharmacology for their assistance. Without the help of all the aforementioned people this project would not have been possible.

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Chapter I

Historical Review

A. Introduction

The noradrenergic (NA) neuronal pathways in the central nervous system (CNS) have been well mapped and are known to extensively innervate virtually all major structures of the brain and spinal cord. One of the most common means of studying the distribution of this fiber network is the destructive elimination of portions of the pathways, followed by subsequent histochemical/biochemical mapping in the experimental and control groups. Removal of NA fibers can be brought about by administration of relatively selective neurotoxins such as 6-hydroxydopamine (6-OHDA) or 6-hydroxydopa (6-OHDOPA), or by mechanical transection of the fiber bundles.

In order to gain insights into the ontogenic development of the above system, the indicated procedures are often carried out in immature animals. Because of the growing body of evidence that opioids interact with the NA system in a variety of ways, it is considered to be of value to determine how opioids might alter the developmental recovery of the NA system after a mechanical injury to one of the major fiber bundles.

B. Projections of the Locus Coeruleus

The locus coeruleus, a compact pontine nucleus located

in the central gray of the isthmus, is comprised of about 1500 cells which project axons throughout the CNS. The largest fiber projection of this nucleus is the dorsal noradrenergic bundle (DNB), which transmits axons to many forebrain regions. This compact bundle joins the medial forebrain bundle, and ascends rostrally in the brain to the genu of the corpus callosum, whereupon it diverges into a highly collateralized network (Moore and Bloom, 1979). The fibers of the DNB terminate in the cerebral cortex, hippocampus, thalamus, geniculate bodies, colliculi, habenula, some dorsal hypothalamic nuclei, and olfactory bulb (see Jacobowitz, 1978). The locus coeruleus also provides fibers which innervate virtually all segments of the spinal cord, distributing widely to both dorsal and ventral horns. Projections from the coeruleus have additionally been demonstrated in the brainstem, predominantly to the sensory nuclei. A third area to which the locus coeruleus projects is the cerebellum. In this brain region a sparse input to the granule cell layer is noted, along with an extensive innervation of the Purkinje cells and molecular layer (Moore and Bloom, 1979).

In the rat the cells of the locus coeruleus differentiate between fetal days 10 and 13. The fiber connections to all the target areas of the nucleus have been made by birth (Lauder and Bloom, 1974), although the target areas <u>per se</u> may develop later. Groups of neurons in the locus coeruleus are known to be divergent, i.e., a

single neuron projects axons to two or more regions in the CNS, such as to the cerebellum and spinal cord; or the frontal cortex and cerebellum; or the neocortex and hippocampus; etc. (Steindler, 1981; Room et al., 1981; and Ader et al., 1980). Steindler (1981) estimated that in mice 6% of the locus coeruleus cells that project to the neocortex also provide collateral fibers to the cerebellum. Conversely, 61% of the cells that project to the cerebellum were found to have collaterals to the neocortex. The fiber projections to the cerebral cortex from the nucleus are of two types. The most common fiber type innervates a restricted region, while the other innervation is widespread to various areas of the cortex (Nagai et al., 1981).

C. Destruction of the Noradrenergic Fiber Pathways

Several methods are available to alter the pattern of distribution of the fiber projections from the locus coeruleus. Administration of 6-OHDA or 6-OHDOPA to neonatal rats during the first two weeks after birth results in degeneration of NA fibers. This is followed by a permanent hyperinnervation by central NA neurons in the pons-medulla, mesencephalon, and cerebellum, when treatment is prior to the fourth postnatal day. In contrast the NA input to forebrain regions remains permanently reduced after such treatment (Breese and Traylor, 1970; Jonsson et al., 1974; Zieher and Jaim-Etchevery, 1975; Pappas and Sobrian, 1972; and Kostrzewa and Garey, 1976). The principal nucleus

involved in this alteration of NA inputs is the locus coeruleus, and it is this nucleus that is associated with the major portion of NA sprouting or hyperinnervation (Schmidt and Bhatnagar, 1979). The result of neonatal neurotoxin treatment is a reduction in NA input to the forebrain regions accompanied by an elevation in the NA input to the hindbrain regions.

Neonatal surgical transection of the DNB produces nearly the same alteration in developmental pattern as neurotoxin treatment. Kostrzewa et al. (1985) found that a brainstem lesion, encompassing both dorsal noradrenergic bundles, in neonatal rats between postnatal days 1 and 7, produces significant alterations in the adult pattern of NA fiber distribution. As a result of this neonatal lesion animals develop into adulthood with a significant decrease in norepinephrine (NE) content and fluorescent NA fiber density (with the glyoxylic acid technique) in the cerebral cortex and hippocampus, but with an increase in these parameters in the pons-medulla and cerebellum. Jonsson and Sachs (1982), using either a bilateral lesion as above or a unilateral transection of the DNB, noted similar developmental changes. The animals as adults have decreased levels of NE, ^{3}H-NE uptake, and fluorescent fiber number in the cerebral cortex, and a corresponding increase in these parameters in the mesencephalon, pons-medulla, and cerebellum.

These alterations in NA input, demonstrated with neuro-

toxin treatment and transection of the DNB, have been explained by Jonsson and Sachs (1982) and Jonsson and Hallman (1982b) as due to the so-called "pruning effect." In this theory neurons are said to be programmed to form a defined quantity of nerve terminals. If one branch is prevented from growing then a compensatory outgrowth will take place in the intact branches proximal to the lesion. As stated previously the pattern of recovery is almost identical for neurotoxin treated and surgically axotomized animals, but the changes are less pronounced in surgically altered animals. However, the method of denervation of each of these techniques is uniquely distinct. The surgical lesion affects only the DNB, while neurotoxin treatment results in at least a partial denervation of virtually all NA terminal areas. The surgical lesion, by damaging the major ascending axonal fibers, results in degeneration distal to the cut. The brainstem lesion also severs blood vessels, other nerve fibers, and damages the parenchymal tissue, while the neurotoxin destroys principally NA fibers.

D. Opioids

Recent evidence indicates that opioid peptide-containing neurons are an integral part of the CNS. These neurons contain as neuromodulators or neurotransmitters endogenous peptides, which are part of the opioid families of enkephalins, endorphins, and dynorphins. The neurons

have been mapped by immunohistochemistry and their respective nuclei and patterns of distribution are different from each other (Cuello, 1983 and Bloom, 1983).

The enkephalins are formed from enzymatic cleavage of a prohormone. Methionine-enkephalin (met-enkephalin), specifically, is a pentapeptide (Tyr-Gly-Gly-Phe-Met) formed by cleavage of proenkephalin-A (Morley, 1983). Enkephalins can be released by electrical or high potassium induced stimulation. These peptides are inactivated by the cerebral peptidases, aminopeptidase and enkephalinase (Hughes, 1983). The concentration of met-enkephalin is higher throughout the brain than that of the other enkephalin, leucine-enkephalin. The striatum has the highest levels of met-enkephalin. Of the areas under study high levels of met-enkephalin are found in the pons-medulla, lower levels are detected in the cortex and hippocampus, and the lowest levels are present in the cerebellum (Cuello, 1983 and Yang et al., 1977). Enkephalin-immunoreactive nerve fibers are known to innervate the source nuclei of all three major monoamine systems: the substantia nigra, the locus coeruleus, and the raphe nuclei (Bloom, 1983).

Tsang et al. (1982) found that the highest postnatal developing levels of enkephalin and its binding sites were obtained within the first week for the cerebellum, during the second week in the brainstem, and in the third week within the forebrain and cerebral cortex.

Recently the interaction of the enkephalinergic neurons with NA neurons has been studied. Bloom (1983) noted that enkephalin-immunoreactive fibers innervate the locus coeruleus and the presence of opioid receptors in the coeruleus has been known for several years. Enkephalin-containing fibers have also been identified in all brain regions to which the locus coeruleus projects.

E. Opioids in Combination with Neurotoxins

The locus coeruleus perikarya are known to receive several inputs. The enkephalinergic input described earlier is considered to be an inhibitory one (Aghajanian and Cedarbaum, 1979). A substance P neuronal input onto the locus coeruleus cells is considered to be excitatory.

Several authors have found that morphine and the endogenous opioids potentiate the effects of neonatal treatment with 6-OHDA and 6-OHDOPA. Harston et al. (1981) demonstrated that treatment with morphine or naloxone in combination with 6-OHDOPA at birth results in increased adult NE levels and numbers of NA fibers in the pons-medulla and cerebellum. Similar effects were seen with β-endorphin, leu- and met-enkephalin, and d-ala^2-enkephalinamide. It was later shown that the morphine and 6-OHDOPA combination at 3 days after birth resulted in a duplication of the findings from treatment at birth, with the exception that now the changes were blocked by naloxone, also given at 3 days after birth (Kostrzewa and Klisans-

Fuenmayor, 1984). Jonsson and Hallman (1982a) conducted similar studies, administering 6-OHDA and morphine to newborn pups. The ^{3}H-NE uptake velocity in these animals, as adults, was significantly reduced in the olfactory bulb and frontal cortex and significantly increased in the cerebellum. Naloxone administration reversed the effects of morphine. Clonidine, another potent inhibitor of locus coeruleus activity, administered as above in combination with 6-OHDA, produced results similar to that of morphine (Bardo et al., 1983). Thus, opioids and other compounds that inhibit locus coeruleus activity potentiate the decreased NA fiber density in the forebrain, and the increased fiber number in the hindbrain following neonatal neurotoxin treatment.

Substance P, the peptide with excitatory effects on locus coeruleus activity, counteracts the actions produced by 6-OHDA (Jonsson and Hallman, 1982c and 1982d). When substance P and 6-OHDA were administered at birth, the adult NE levels and ^{3}H-NE uptake rate were increased in the frontal and occipital cortex and decreased in the cerebellum and pons-medulla. Results similar to these have been found with nicotine, a drug also known to alter locus coeruleus activity (Jonsson and Hallman, 1980).

Bardo et al. (1983) and Harston et al. (1981) suggested that the drugs which alter the response of the locus coeruleus neurons, do so by modifying or enhancing the degenerative response to the neurotoxins; that is, morphine

increases the toxicity of 6-OHDOPA and thus potentiates the response of neurons. Another explanation of the opioid effects is the "pruning" hypothesis (Jonsson and Hallman, 1982a and 1982c and Harston et al., 1982). According to this hypothesis, synaptic inputs onto the locus coeruleus govern the state of the nuclei at the time of treatment, allowing an altered response to the neurotoxins. The interaction of opioids with NA fiber development is better studied in animals where the NA network has been disrupted by a mechanical (non-chemical) means. With this technique there are no drug interactions to complicate the study. This type of damage will produce true pruning; that is, the cutting of one branch of fibers produces an outgrowth of the other branches. In contrast the neurotoxin treatment damages fibers throughout the brain, and this results in an outgrowth of some of these fibers. The present study thus represents a novel approach to define the role of opioid alteration of NA fiber recovery.

Chapter II

Preliminary Studies

To ascertain the feasibility of the present study preliminary work was done. Rats received a bilateral lesion at the level of the colliculi at 3 days of age to transect the dorsal NA bundles. Immediately prior to the cut animals received an injection of saline, morphine, naloxone, or a combination of morphine and naloxone. At six weeks of age the NE content was determined in the cerebellum, pons-medulla, hippocampus, and posterior cortex. It was found that animals receiving the neonatal lesions had significantly lower NE levels in the hippocampus and posterior cortex, and higher levels in the pons-medulla and cerebellum. Morphine treatment appeared to attenuate this effect in the pons-medulla and cerebellum, while the effect of naloxone was unclear. In contrast the NE concentration in the posterior cortex and hippocampus was significantly increased with morphine treatment. Naloxone administered alone did not vary the NE levels in these areas, but it reversed the effects of morphine. In this preliminary study morphine treatment in combination with a brainstem lesion significantly enhanced recovery of NE in the forebrain.

CHAPTER III

Objectives and Proposal

This project is designed to determine whether opioids, specifically morphine and met-enkephalin, alter the pattern of distribution of noradrenergic fibers in specific brain regions, subsequent to a gross surgical lesion encompassing the DNB. The hypothesis is that the opioid system does play a role in regeneration of noradrenergic fibers after a surgical lesion in the brainstem of developing animals. To demonstrate the effects of morphine and enkephalin on noradrenergic regeneration, measurements indicative of noradrenergic fiber number will be made in different brain regions of adult animals that were treated as neonates. Comparison of the test subjects with sham controls will provide the test of the hypothesis.

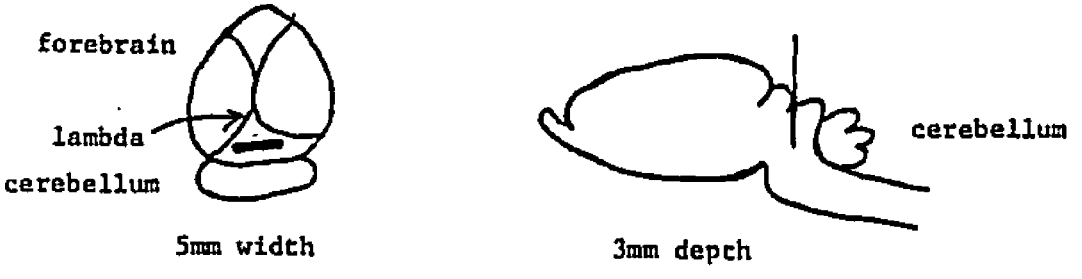
CHAPTER IV

Materials and Methods

A. Animals and Treatment

Animals from our colony of Camm-derived Sprague Dawley rats were used. Two groups of animals were treated at 3 days after birth. In the first group naloxone·HCl (2.0 mg/kg) was administered concurrently with saline (0.85%) or morphine sulfate (3.33 mg/kg) by the intraperitoneal (i.p.) route (table 1). A second group of rats received an i.p. injection of naloxone or saline, 10 minutes prior to an intraventricular (i.vtr.) injection of 5 µl of morphine sulfate (10 µg), or methionine-enkephalin (25 µg, Behring Diagnostics), or saline (0.85%) (table 1).

Half the animals treated with the above regimens received a surgical lesion, made with a blade 3 mm (depth) by 5 mm (width) under ice anesthesia (80 seconds), at the level of the colliculi, as illustrated below:



Group 1			Group 2		
i.p.		i.p.	i.p.		i.vtr.
Saline	+	Saline	Saline	+	Saline
Saline	+	Morphine	Saline	+	Morphine
Naloxone	+	Saline	Saline	+	Met-Enk
Naloxone	+	Morphine	Naloxone	+	Saline
			Naloxone	+	Morphine
			Naloxone	+	Met-Enk

Table 1

Animals listed in group 1 received a combination of two intraperitoneal (i.p.) injections of saline, morphine, or naloxone, as indicated. Animals in group 2 were injected with naloxone or saline (i.p.), 10 minutes prior to an intraventricular (i.vtr.) injection of saline, morphine, or met-enkephalin (met-enk), also in the combinations indicated.

The remaining half of the animals were anesthetized on ice, but did not receive the surgical lesion. The animals in group 1 received one of the injection combinations immediately following the surgical lesion. In the second group, animals received the i.p. injection 10 minutes prior to the i.vtr. injection, and were surgically lesioned immediately afterward.

Following treatment, litters were returned to their dams and kept in the animal care facilities on a 12 hour light/dark day cycle (on 0600) until sacrifice. The pups were weaned at 4 weeks, at which time the males and females were separated for group housing. All animals were sacrificed at 6 weeks of age unless otherwise indicated.

B. Histochemistry

Noradrenergic fibers were observed histochemically with the glyoxylic acid histofluorescence method of de la Torre and Surgeon (1976). In this procedure the whole brain was removed, mounted, and frozen onto a specimen plate. The tissue was then cut in cross sections at 30 μm in an IEC cryostat. Sectioned tissue from the cortex was then thawed onto glass slides and immersed in a glyoxylic acid solution (1%) containing sucrose (0.2 M) and potassium phosphate (0.236 M). After drying the tissue specimens were heated to 80°C for exactly 5 minutes, after which, the slides were coverslipped with mineral oil. Sections were viewed in an Olympus fluorescence microscope by two

experienced observers, who were unaware of the treatment grouping of each animal. After observation for green fluorescent fibers, photomicrographs were taken for a permanent record of the observation.

C. Determination of NE Concentration

Biochemical assessment of NA fiber input to the various brain regions was made with the trihydroxyindole fluorometric method of Hogans (Nagatsu, 1973). The cerebellum, pons-medulla, anterior cortex, posterior cortex, and hippocampus were dissected at the time of sacrifice, frozen on dry ice, and stored at -80°C until the time of assay. Using this procedure, tissues were homogenized in acidified butanol, and NE was extracted into a phosphate buffer (pH 6.5), for oxidation to the product, 3,5,6-trihydroxyindole, by iodine. The relative amount of fluorescent product was then determined in a spectrophotofluorometer at 385/485 (excitation (nm)/emission (nm)) wavelengths. The concentration of NE was expressed as nanograms NE per milligram tissue mass.

D. Measurement of ^{3}H-Norepinephrine Uptake Rate

The anterior cortex, posterior cortex, and cerebellum were dissected at the time of sacrifice and immediately homogenized on ice in 10 volumes (weight/volume) of 0.32 M sucrose. The homogenate was then centrifuged at 1,000 x g for 10 minutes to obtain the supernatent containing synaptosomes (Jonsson and Sachs, 1976). Analysis of protein

content was done on an aliquot of supernatent (Lowry et al., (1951). Aliquots of the supernatent (125 µl of posterior cortex or anterior cortex and 160 µl of cerebellum) were preincubated for exactly 5 minutes at 37°C with Krebs-Ringer bicarbonate in a total volume of 1.9 ml (pH 7.4 with 0.2 mg/ml ascorbic acid, 2.41 µg/ml nialamide and saturated with 95% O_2 and 5% CO_2). After addition of 0.1 ml of a ^{3}H-NE (10 µCi/ml of DL-[7-^{3}H(N)] NE·HCl) and NE·HCl (0.05 µm for posterior cortex or anterior cortex and 0.1 µm for cerebellum) solution, incubation was continued for another 5 minutes. The ^{3}H-NE uptake process was terminated by addition of 8 ml of cold Krebs-Ringer buffer. Samples were spun at 10,000 x g for 10 minutes and the supernatent was discarded. Absolute ethanol (1.2 ml) was added and the samples were allowed to stand overnight. The next day 1 ml of the ethanol containing the extracted radioactivity was counted in 10 ml scintillation fluid (Scintiverse, Fischer) using a Beckman liquid scintillation counter. The fmoles NE taken up was calculated by dividing the DPM's by the specific activity of the ^{3}H-NE solution.

E. Radioimmunoassay of Met-Enkephalin

A separate group of animals was surgically lesioned, as described earlier, at 3 days and sacrificed at 4 or 7 days after birth. During the dissection the pons-medulla, a portion of the pons-medulla containing the locus coeruleus, and the posterior cortex was removed. This tissue was then

stored at -80°C until time of assay.

Tissue was then weighed and heated in 1 M acetic acid (200 µl for locus coeruleus and 700 µl for pons-medulla and posterior cortex) at 95°C for 15 minutes. The samples were chilled, homogenized by ultrasound (Branson Model W-200 P) for 5 seconds, and after removal of an aliquot for protein, were centrifuged at 1,000 x g for 10 minutes. The supernatent was transfered to microfuge tubes and vacuum centrifuged until dry. The samples were resuspended in 500 µl of BSA-phosphate buffer with ultra-sound for 5 seconds, followed by shaking at 37° for 10 minutes. After centrifugation at 10,000 x g each aliquot was appropriately diluted with buffer. Met-enkephalin content was measured using a radioimmunoassay (RIA) kit from Immuno Nuclear Corporation.

F. Statistical Analysis

A 2 x 4 ANOVA was done using the NE content and ^{3}H-NE uptake values for the systemic administration of opioids. A 2 x 6 ANOVA was used for the data obtained after intraventricular administration of drugs. A single factor ANOVA and then a Newman-Keuls was used to analyze the per cent recovery in the anterior cortex, posterior cortex, and hippocampus. A single factor ANOVA was also used to compare met-enkephalin content in control and lesioned animals in the different areas studied.

Chapter V

Results

A. Verification of Transection of the Dorsal Bundle

Control and experimental animals receiving a lesion at 3 days postnatally were sacrificed at 7 days after birth. At this time there was no gross evidence of the lesion. However, using the histofluorescence method large numbers of brightly fluorescent, swollen and densely packed fibers were seen emanating in a rostral direction from the locus coeruleus of the animal receiving a lesion (fig. 1a). This contrasted sharply to the fine thin fibrils in the same position in control animals (fig. 1b). It is generally considered that the densely packed fluorescence indicates a retrograde accumulation of NE in portions of axonal fibers that are proximal to the transection.

B. Systemic Administration of Morphine

In figure 2 the NE concentration and rate of ^{3}H-NE taken up in the cerebellum is reported. The neonatal midbrain lesion resulted, at six weeks of age, in an increase in both parameters in each of the lesion groups. Both NE concentration and ^{3}H-NE uptake in the cerebellum at 6 weeks were not significantly different in any of the drug treatment groups. Similarly in the pons-medulla, the values for NE concentration and ^{3}H-NE uptake at six weeks were significantly increased in the lesion groups. However,

Figure 1

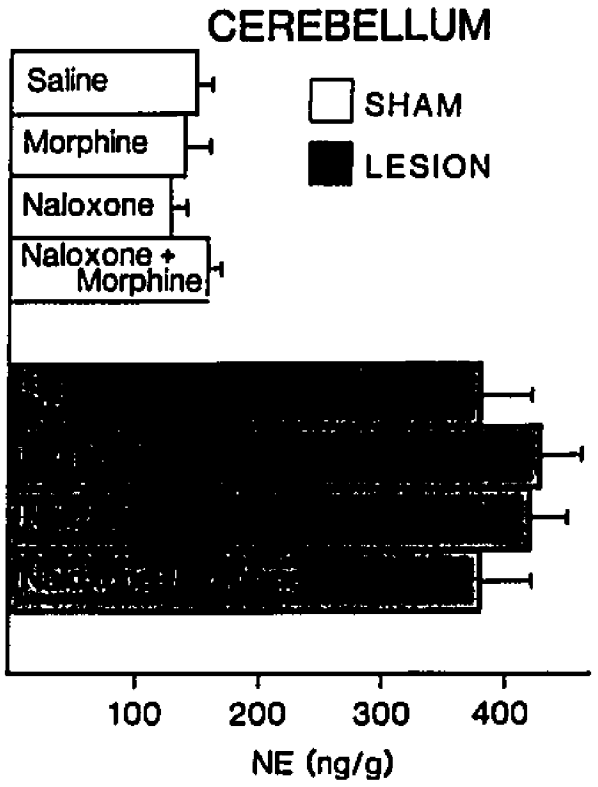
Photomicrographs of histofluorescent fibers originating in the locus coeruleus and traversing rostrally. Sections were taken from animals 7 days after birth, following either a brainstem lesion at 3 days after birth (a) or anesthesia only (b). The bar in (a) indicates 50 µm.

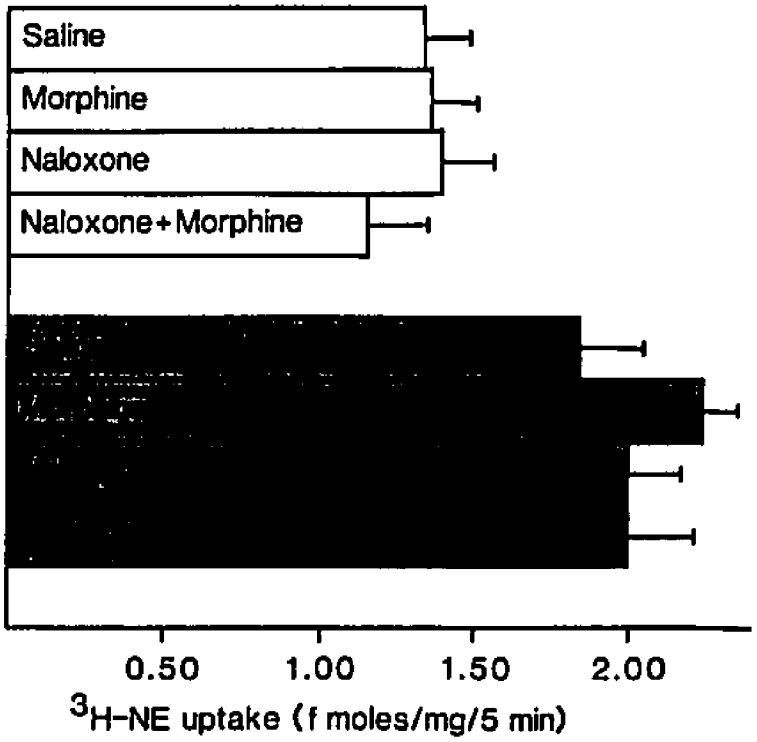
a

b

Figure 2

Mean (+SEM) NE concentration (ng/g wet weight) and rate of ^{3}H-NE uptake (fmoles/mg protein/5 min) in the cerebellum at six weeks after a neonatal midbrain lesion and intraperitoneal treatment with a combination of saline, morphine, and naloxone (group 1, N=6-11).


CEREBELLUM
Saline
Morphine
Naloxone
Naloxone + Morphine
SHAM
LESION
100
200
300
400
NE (ng/g)


Saline
Morphine
Naloxone
Naloxone+Morphine
0.50
1.00
1.50
2.00
3H-NE uptake (f moles/mg/5 min)

there was no significant difference in these parameters among the drug treatment groups (fig. 3).

In the anterior and posterior cortex (figs. 4 and 5) the lesion produced a dramatic decrease in the development of NE concentration and ^{3}H-NE uptake. The different drug treatments did not significantly alter either NE concentration or ^{3}H-NE uptake in any of the brain regions of animals at 6 weeks after birth. In Figure 6 the NE concentration in the hippocampus is illustrated. Within the lesioned groups of animals there was a decrease in NE concentration in the hippocampus similar to that seen in the cortical areas. Analysis of the Anova's on these measurements established a significant main effect of the lesion in all areas with all measurements, but there was no statistical variance with the drug treatments. Therefore, in all brain regions studied the measurements for ^{3}H-NE uptake and NE concentration were significantly different between sham and lesion animals, but within the sham and lesion groups the values were not significantly different between the drug treatment groups.

C. Intraventricular Administration of Opioids

The values obtained for NE concentrations and rates of ^{3}H-NE uptake of the animals as adults in group 2 are reported in figures 7 - 14. In these animals that received an injection of naloxone prior to an intraventricular injection of an opioid (i.e. morphine or met-enkephalin),

Figure 3

Mean (+SEM) NE concentration (ng/g wet weight) in the pons-medulla at six weeks after a neonatal midbrain lesion and intraperitoneal treatment with a combination of saline, morphine, and naloxone (group 1, N=6-12).

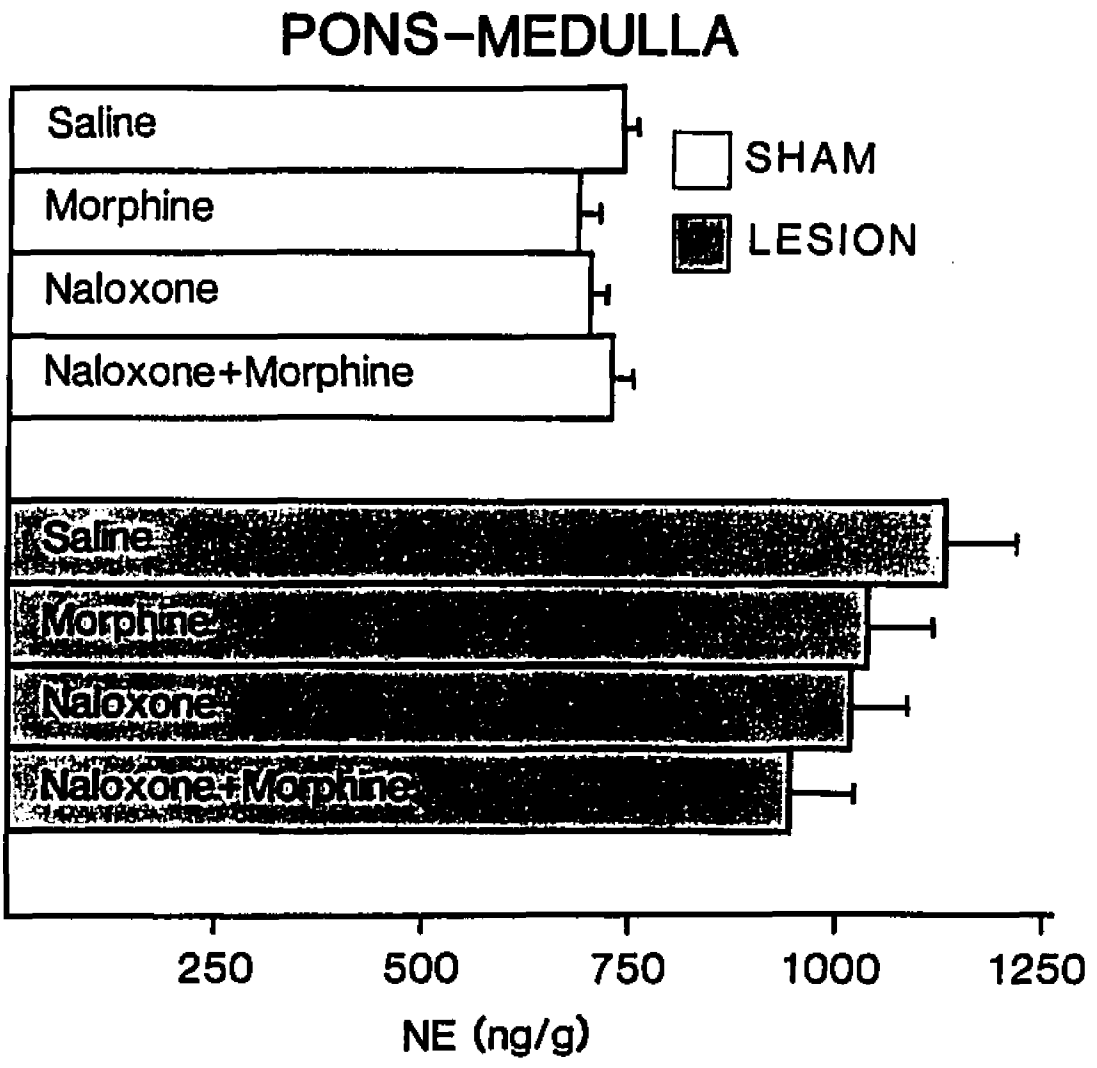
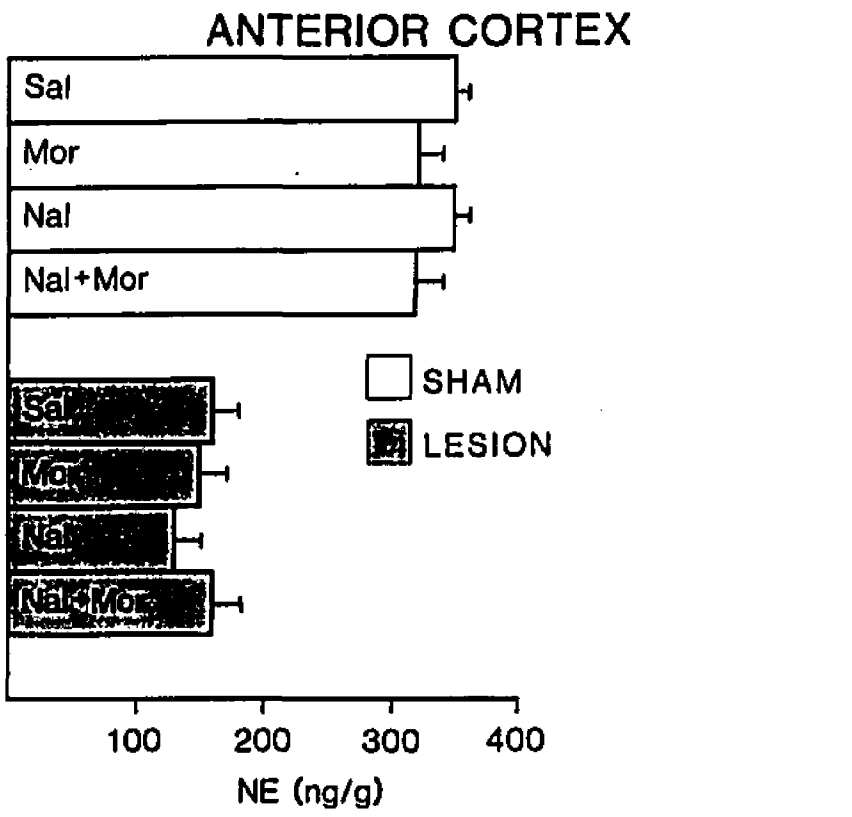

PONS-MEDULLA
Saline
Morphine
Naloxone
Naloxone+Morphine
SHAM
LESION
Saline
Morphine
Naloxone
Naloxone+Morphine
250
500
750
1000
1250
NE (ng/g)

Figure 4

Mean (+SEM) NE concentration (ng/g wet weight, N=6-12) and rate of ^{3}H-NE uptake (fmoles/mg protein/5 min, N=4-7) in the anterior cortex at six weeks after a neonatal midbrain lesion and intraperitoneal treatment with a combination of saline (sal), morphine (mor), and naloxone (nal) (group 1).


ANTERIOR CORTEX
Sal
Mor
Nal
Nal+Mor
SHAM
LESION
100
200
300
400
NE (ng/g)

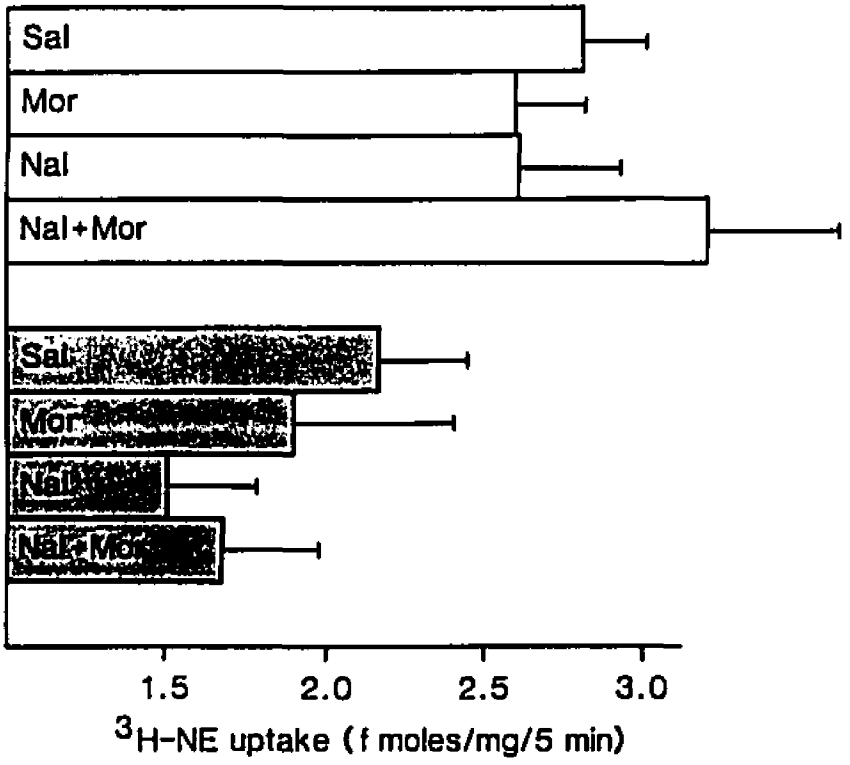
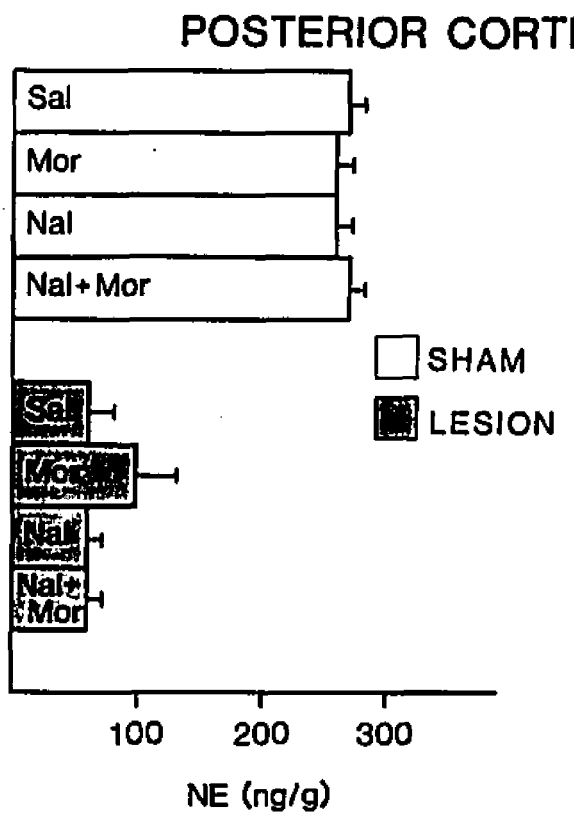

Sal
Mor
Nal
Nal+Mor
1.5
2.0
2.5
3.0
3H-NE uptake (f moles/mg/5 min)

Figure 5

Mean (+SEM) NE concentration (ng/g wet weight) and rate of ^{3}H-NE uptake (fmoles/mg protein/5 min) in posterior cortex at six weeks after a neonatal midbrain lesion and intraperitoneal treatment with a combination of saline (sal), morphine (mor), and naloxone (nal) (group 1, N=5-10).


POSTERIOR CORTEX
Sal
Mor
Nal
Nal+Mor
SHAM
LESION
Sal
Mor
Nal
Nal+
Mor
100
200
300
NE (ng/g)

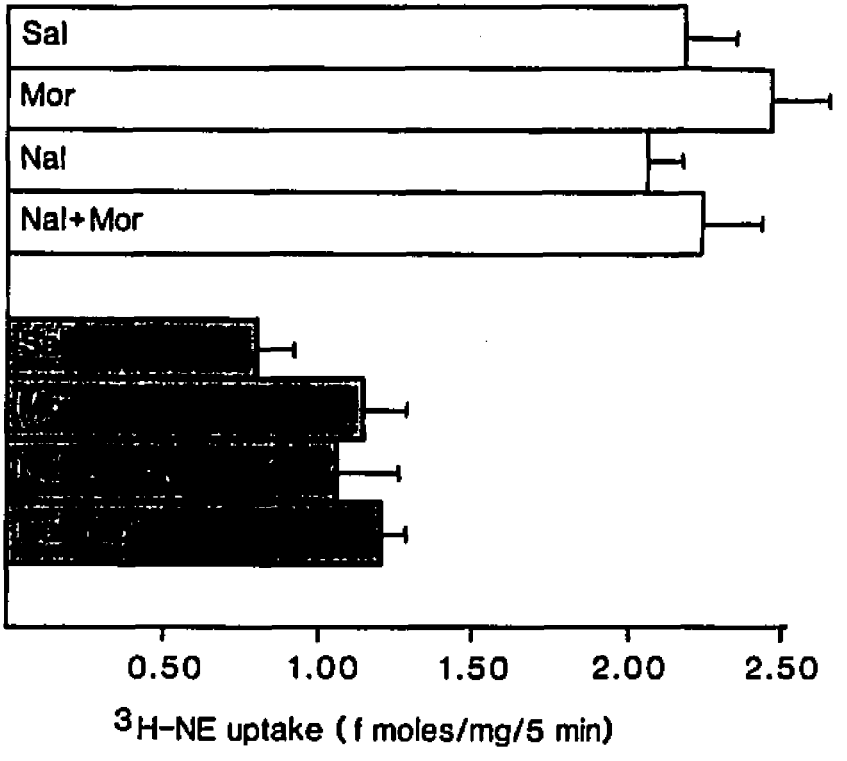
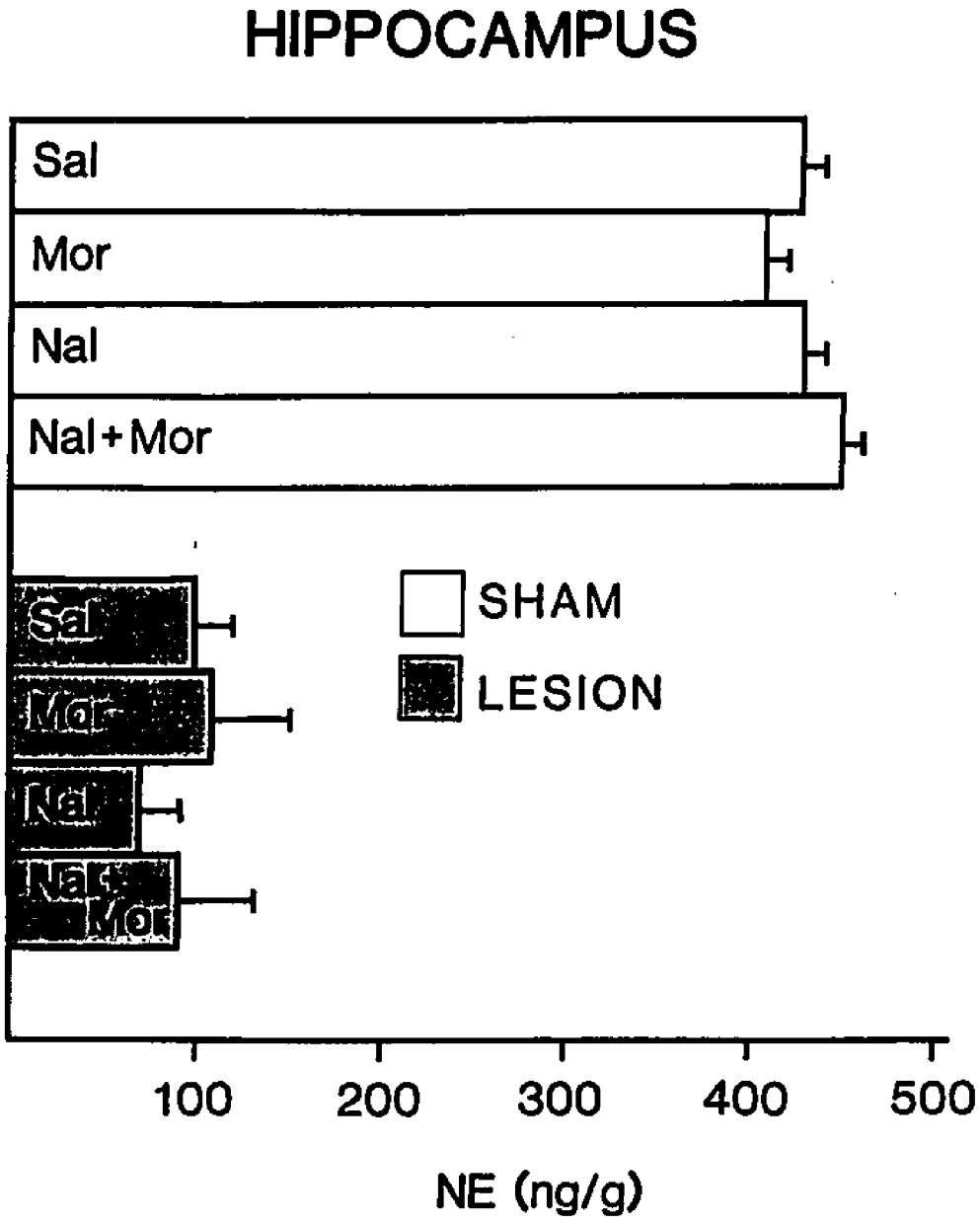

Sal
Mor
Nal
Nal+Mor
0.50
1.00
1.50
2.00
2.50
3H-NE uptake (f moles/mg/5 min)

Figure 6

Mean (+SEM) NE concentration (ng/g wet weight) in the hippocampus at six weeks after a neonatal midbrain lesion and treatment with a combination of saline (sal), morphine (mor), and naloxone (nal) (group 1, N=6-12).


HIPPOCAMPUS
Sal
Mor
Nal
Nal+Mor
SHAM
LESION
Sal
Mor
Nal
Nal+Mor
100
200
300
400
500
NE (ng/g)

the midbrain lesion produced an increase in the NE concentration of the cerebellum (fig. 7) and pons-medulla (fig. 8). This increase in NE concentration in the cerebellum and pons-medulla was similar in all the drug treatment groups. The drug treatment groups in these areas all had similar values for NE concentration in both the sham and lesion animals. In contrast the NE concentration in anterior (fig. 9) and posterior (fig. 10) cortex and hippocampus (fig. 11) was significantly lower in the groups that received a lesion.

Similar results were obtained when ^{3}H-NE uptake was studied in the different brain regions of lesioned animals. In the anterior cortex (fig. 12) there was a significant decrease in the rate of ^{3}H-NE uptake for those groups receiving the cut, but there were no significant changes with drug treatment. The variability seen in these measurements is probably not reflective of drug treatment, but due to smaller group size (N = 3-5). Similarly, in the posterior cortex (fig. 13) the ^{3}H-NE uptake was significantly lower in the lesion groups. However there again were no differences between the drug treatment groups. In the cerebellum (fig. 14), as in all areas previously reported, there was a significant effect of the lesion using ANOVA for the ^{3}H-NE uptake measurements, but the drug treatments resulted in no statistical alterations.

In figure 15 NE is expressed as percent of control for all the lesion animals in the different forebrain

Figure 7

Mean (+SEM) NE concentration (ng/g wet weight) in the cerebellum of 6 week old animals (group 2) after neonatal injections of combinations of naloxone or saline plus met-enkephalin (met-enk), or morphine, or saline in unoperated sham controls (N=7-9) or animals that received a neonatal brainstem lesion (N=10-12).

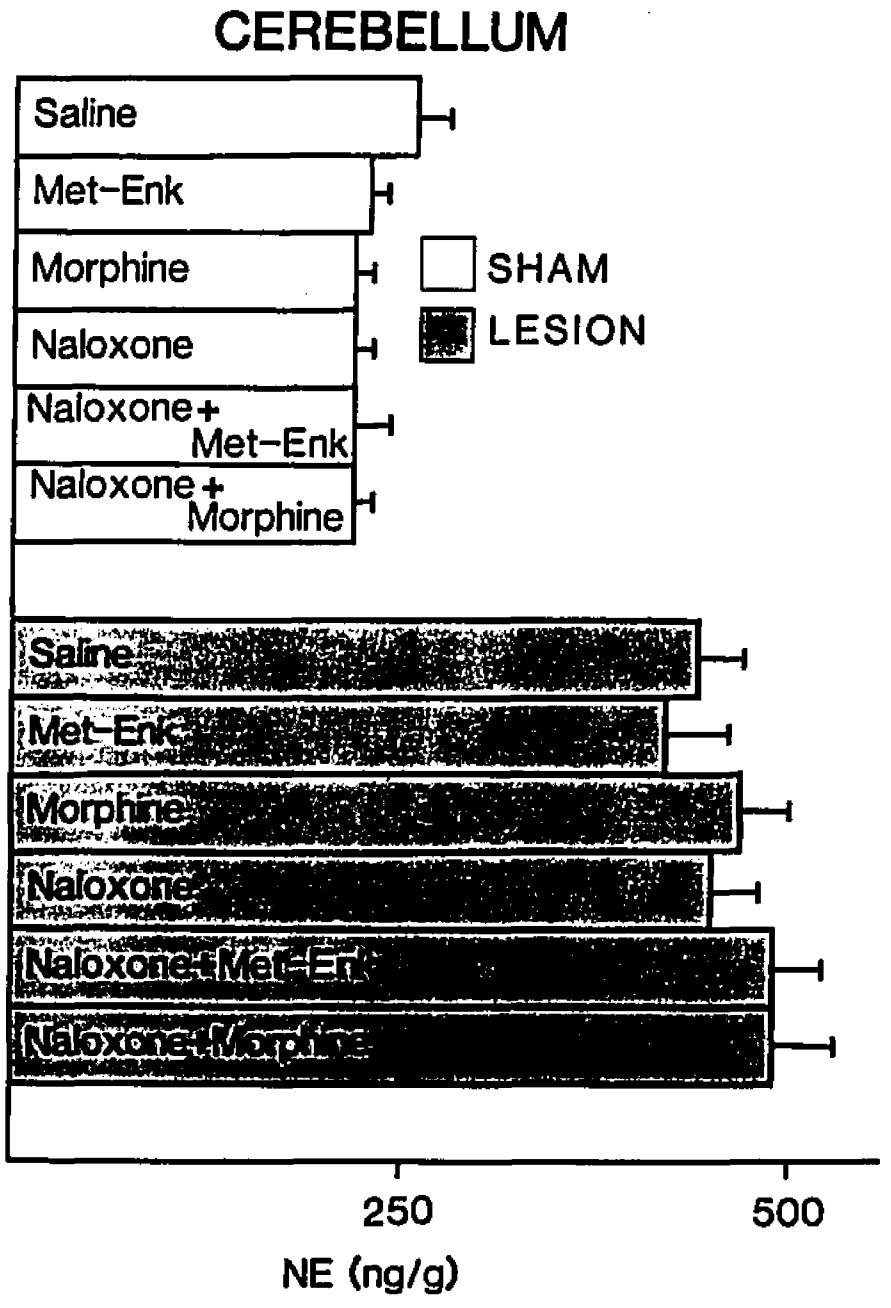

CEREBELLUM
Saline
Met-Enk
Morphine
Naloxone
Naloxone+ Met-Enk
Naloxone+ Morphine
SHAM
LESION
Saline
Met-Enk
Morphine
Naloxone
Naloxone+Met-Enk
Naloxone+Morphine
250
500
NE (ng/g)

Figure 8

Mean (+SEM) NE concentration (ng/g wet weight) in the pons-medulla of 6 week old animals (group 2) after neonatal injections of combinations of naloxone or saline plus met-enkephalin (met-enk), or morphine, or saline in unoperated sham controls (N=6-9) or animals that received a neonatal brainstem lesion (N=10-12).

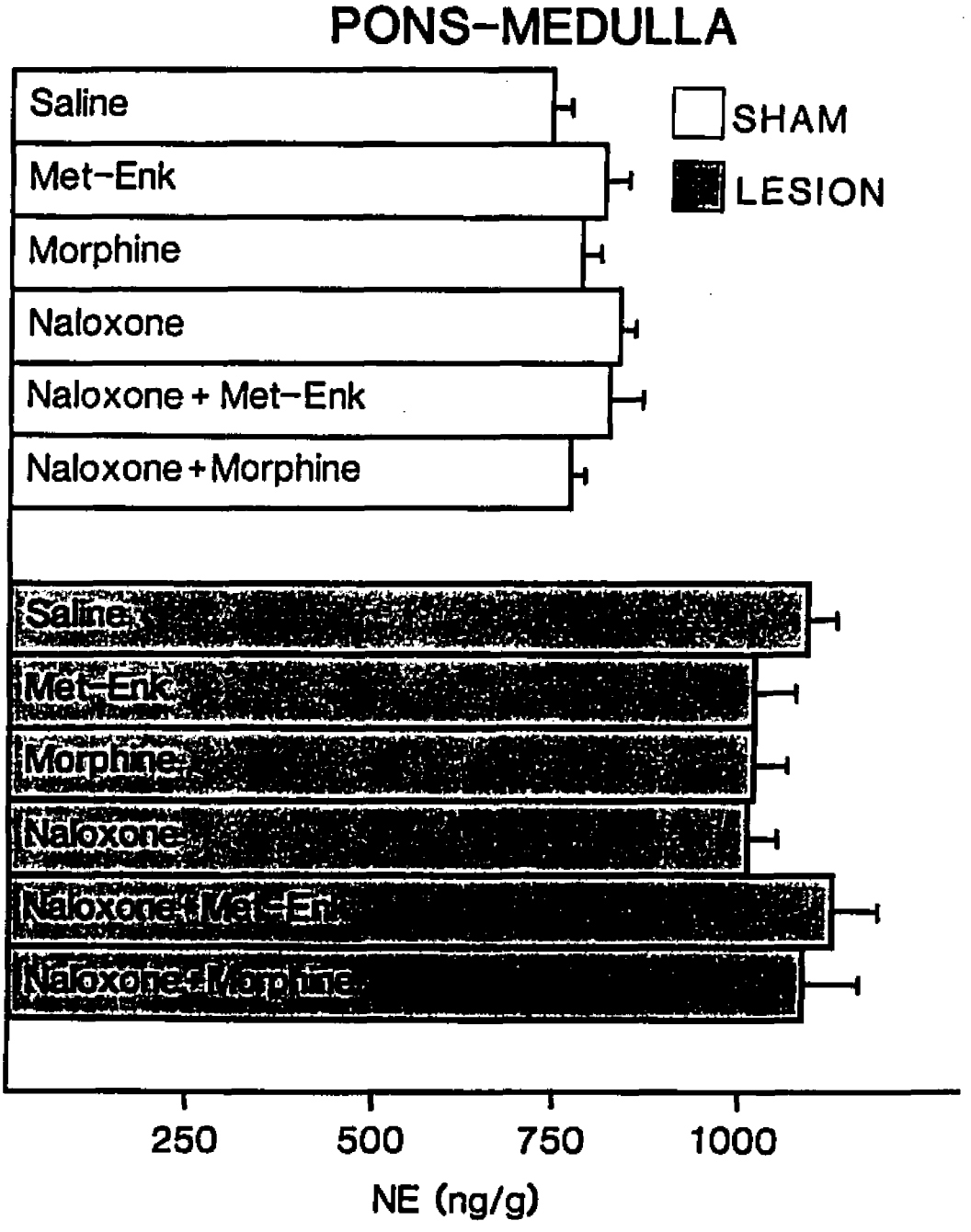

PONS-MEDULLA
SHAM
LESION
Saline
Met-Enk
Morphine
Naloxone
Naloxone + Met-Enk
Naloxone + Morphine
Saline
Met-Enk
Morphine
Naloxone
Naloxone + Met-Enk
Naloxone + Morphine
250
500
750
1000
NE (ng/g)

Figure 9

Mean (+SEM) NE concentration (ng/g wet weight) in the anterior cortex of 6 week old animals (group 2) after neonatal injections of combinations of naloxone or saline plus met-enkephalin (met-enk), or morphine, or saline in unoperated sham controls (N=7-9) or animals that received a neonatal brainstem lesion (N=8-12).

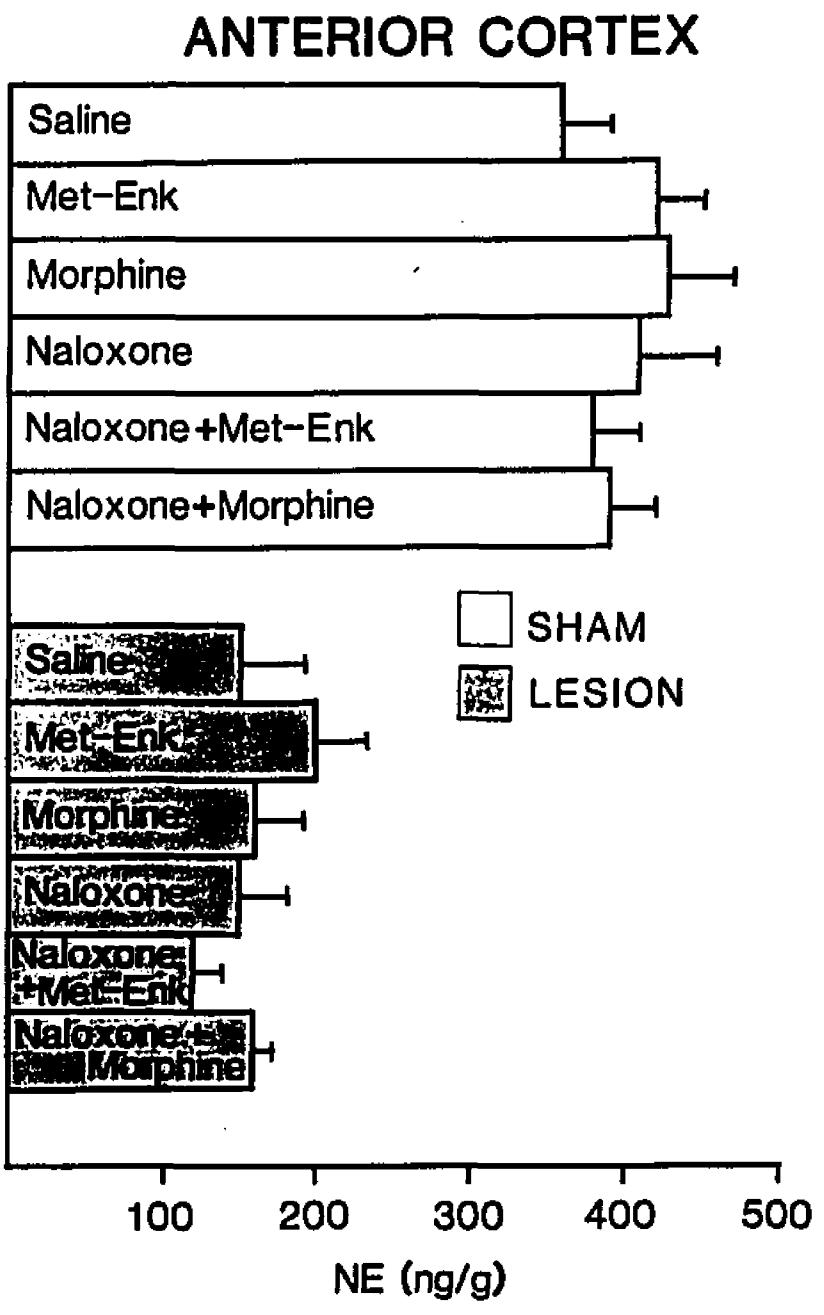

ANTERIOR CORTEX
Saline
Met-Enk
Morphine
Naloxone
Naloxone+Met-Enk
Naloxone+Morphine
SHAM
LESION
Saline
Met-Enk
Morphine
Naloxone
Naloxone +Met-Enk
Naloxone +Morphine
100
200
300
400
500
NE (ng/g)

Figure 10

Mean (+SEM) NE concentration (ng/g wet weight) in the posterior cortex of 6 week old animals (group 2) after neonatal injections of combinations of naloxone (nal) or saline (sal) plus met-enkephalin (met-enk), or morphine (mor), or saline in unoperated sham controls (N=7-9) or animals that received a neonatal brainstem lesion (N=10-12).

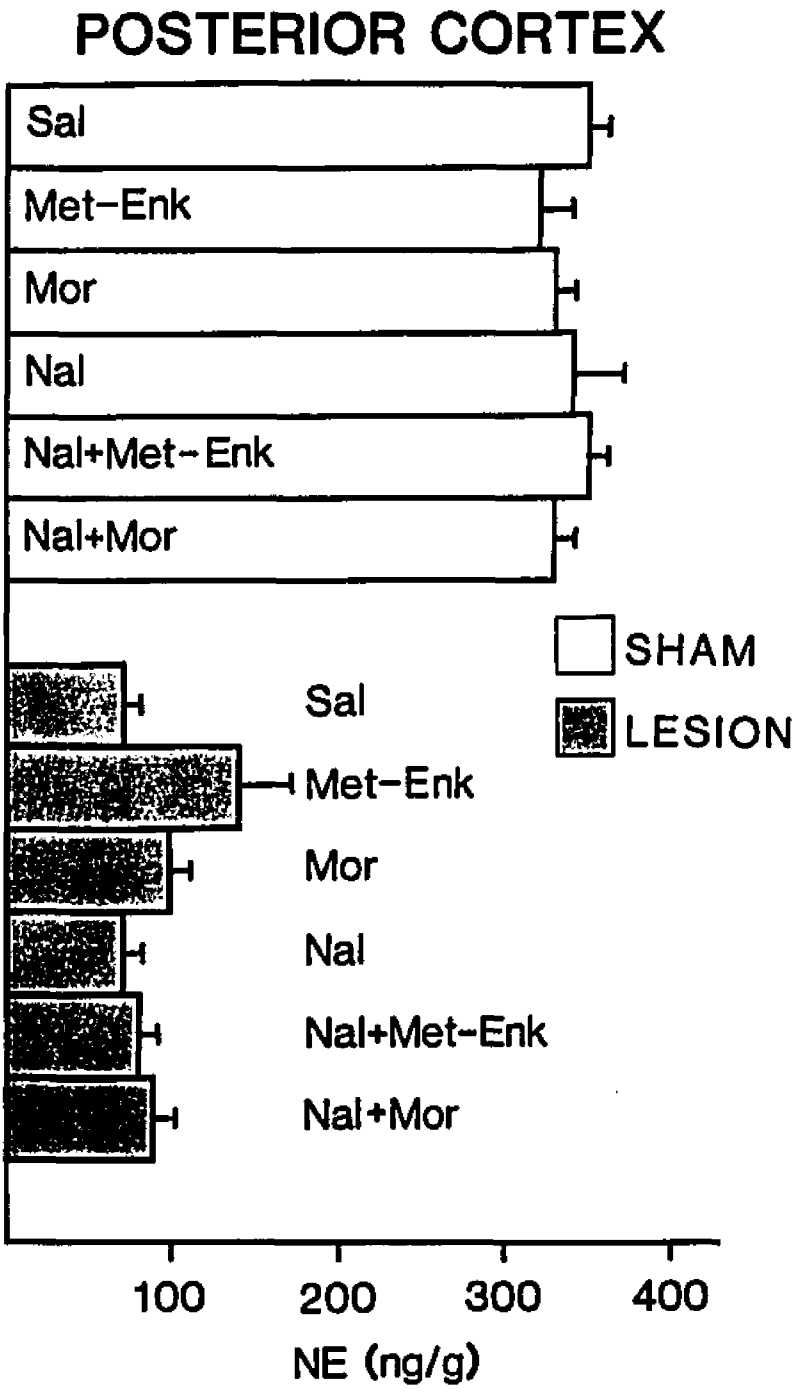

POSTERIOR CORTEX
Sal
Met-Enk
Mor
Nal
Nal+Met-Enk
Nal+Mor
SHAM
LESION
Sal
Met-Enk
Mor
Nal
Nal+Met-Enk
Nal+Mor
100
200
300
400
NE (ng/g)

Figure 11

Mean (+SEM) NE concentration (ng/g wet weight) in the hippocampus of 6 week old animals (group 2) after neonatal injections of combinations of naloxone (nal) or saline (sal) plus met-enkephalin (met-enk), or morphine (mor), or saline in unoperated sham controls (N=7-9) or animals that received a neonatal brainstem lesion (N=9-11).

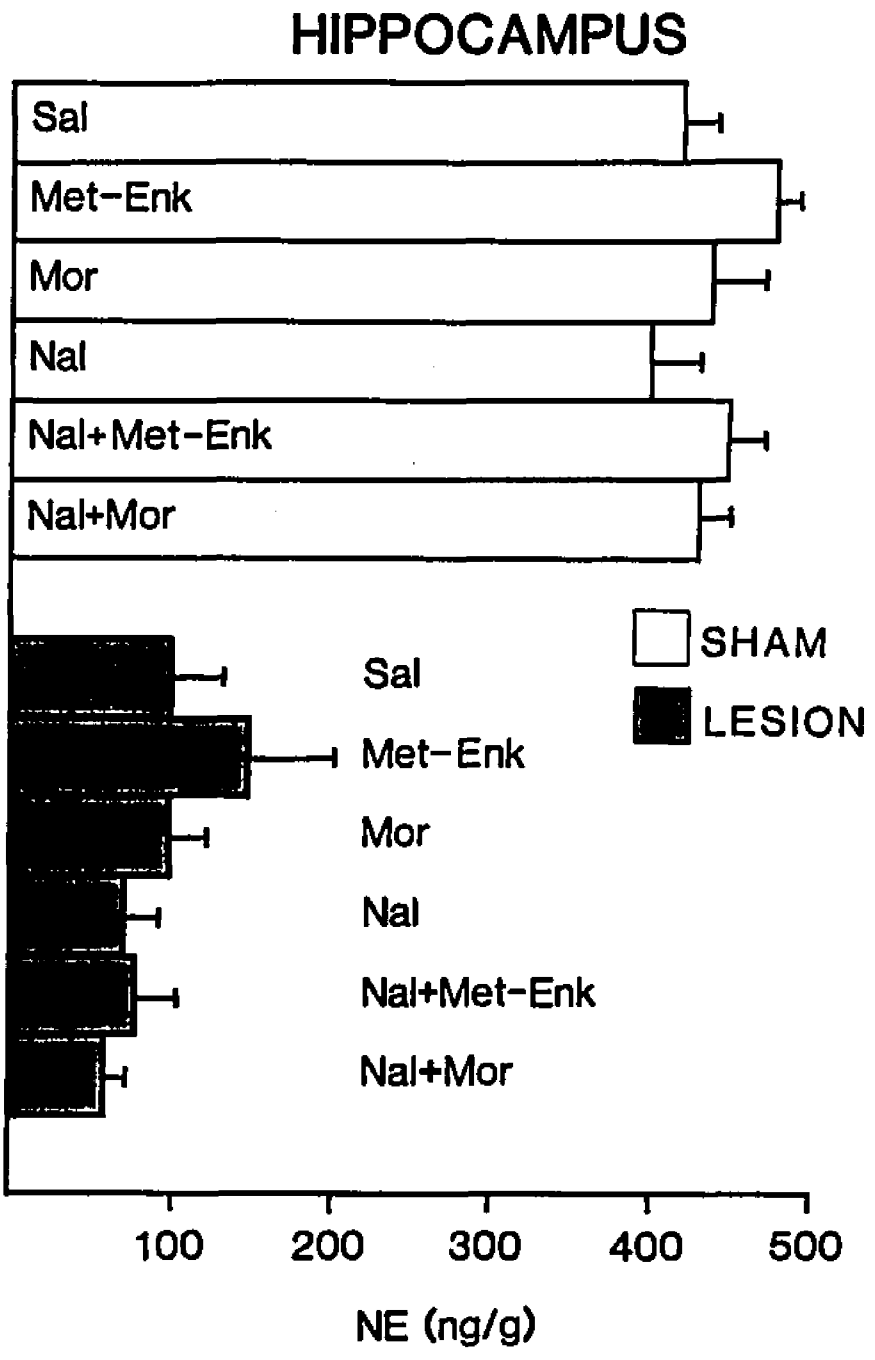

HIPPOCAMPUS
Sal
Met-Enk
Mor
Nal
Nal+Met-Enk
Nal+Mor
SHAM
LESION
Sal
Met-Enk
Mor
Nal
Nal+Met-Enk
Nal+Mor
100
200
300
400
500
NE (ng/g)

Figure 12

Mean (+SEM) rate of ^{3}H-NE uptake (fmoles/mg protein/ 5 min) in the anterior cortex of 6 week old animals (group 2) after neonatal injections of combinations of naloxone or saline plus met-enkephalin (met-enk), or morphine, or saline in unoperated sham controls (N=3-7) or animals that received a neonatal brainstem lesion (N=3-5).

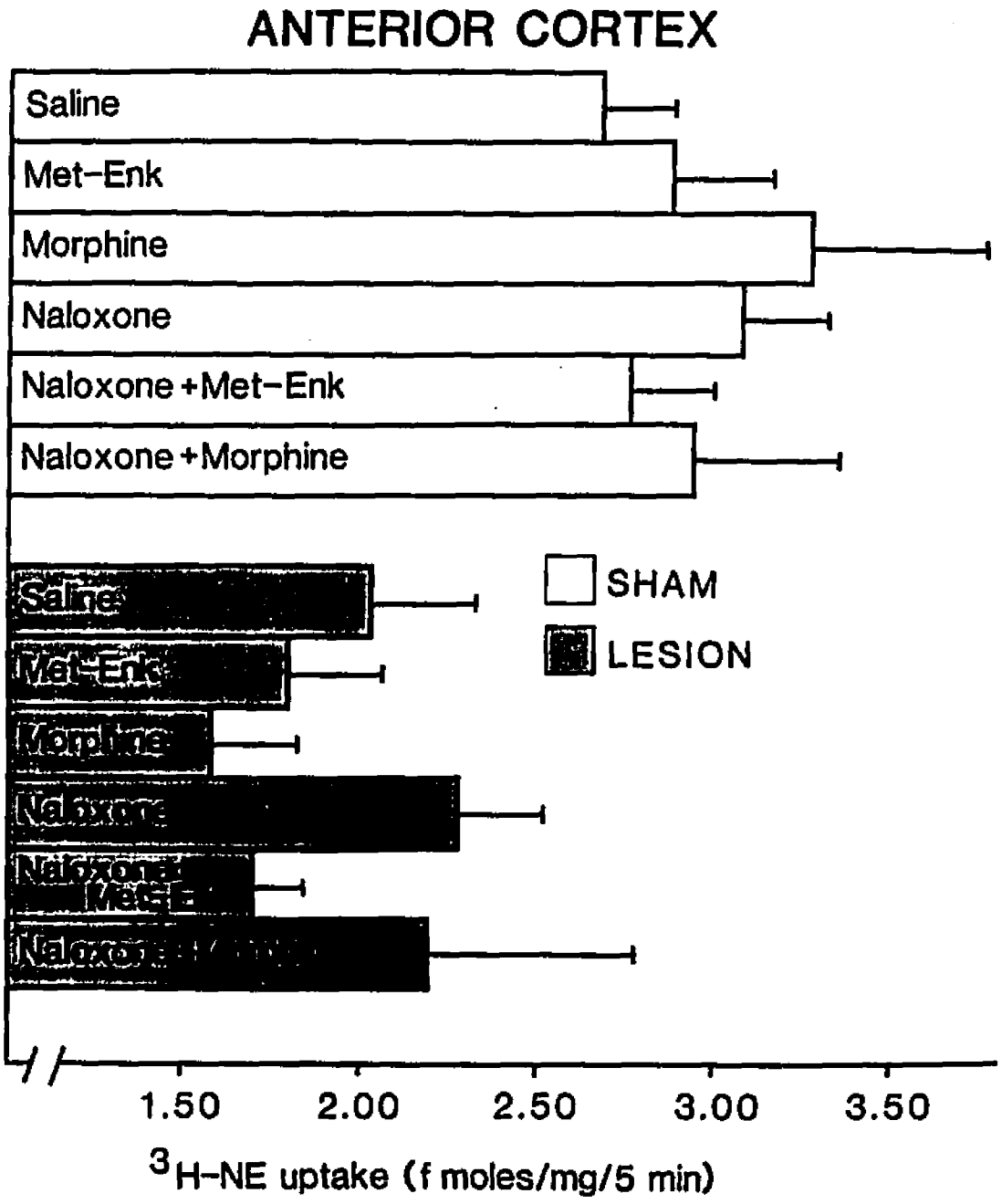

ANTERIOR CORTEX
Saline
Met-Enk
Morphine
Naloxone
Naloxone +Met-Enk
Naloxone +Morphine
SHAM
LESION
1.50
2.00
2.50
3.00
3.50
3H-NE uptake (f moles/mg/5 min)

Figure 13

Mean (+SEM) rate of ^{3}H-NE uptake (fmoles/mg protein/ 5 min) in the posterior cortex of 6 week old animals (group 2) after neonatal injections of combinations of naloxone or saline plus met-enkephalin (met-enk), or morphine, or saline in unoperated sham controls (N=4-8) or animals that received a neonatal brainstem lesion (N=6-10).

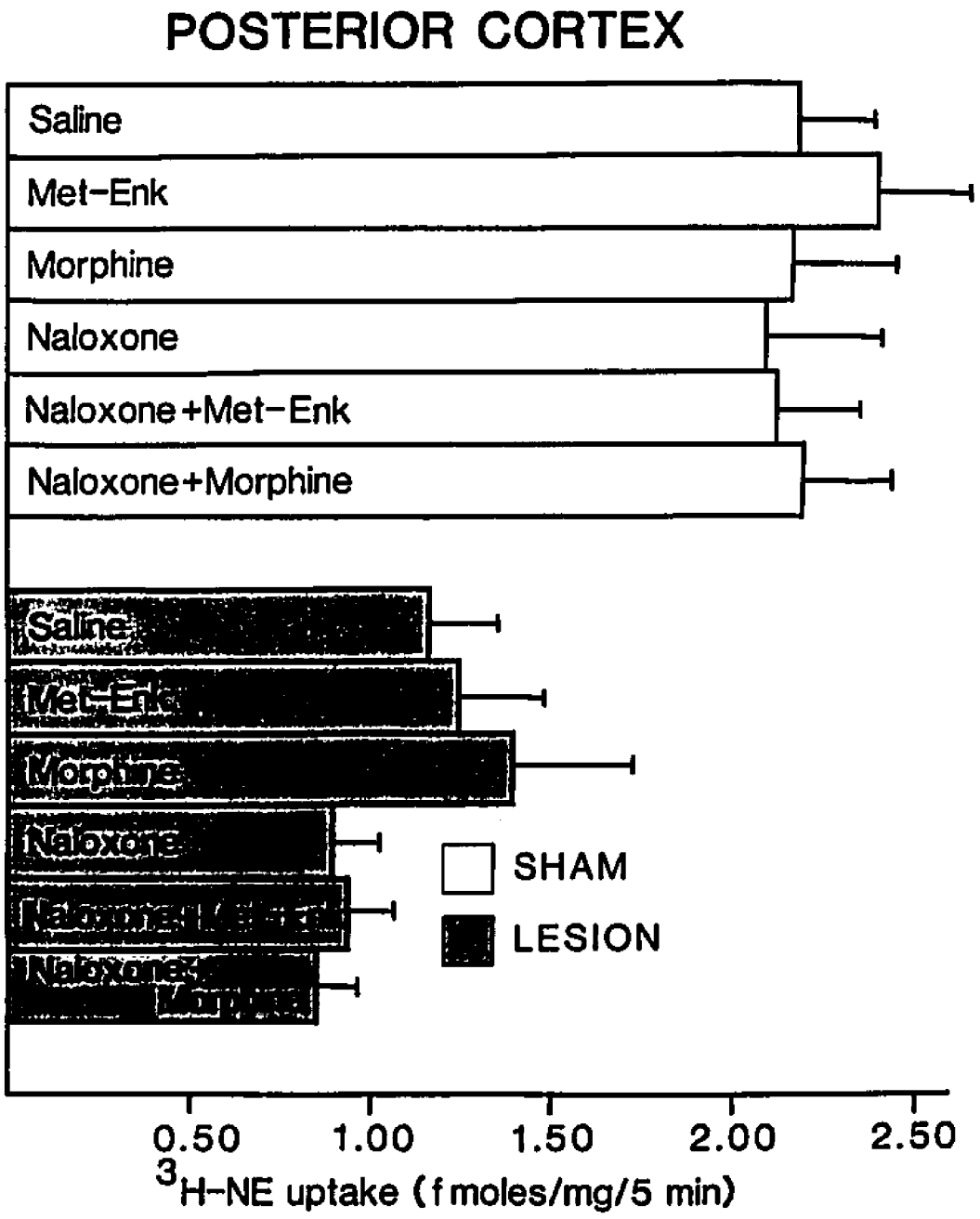

POSTERIOR CORTEX
Saline
Met-Enk
Morphine
Naloxone
Naloxone+Met-Enk
Naloxone+Morphine
Saline
Met-Enk
Morphine
Naloxone
SHAM
LESION
0.50
1.00
1.50
2.00
2.50
3H-NE uptake (f moles/mg/5 min)

Figure 14

Mean (+SEM) rate of ^{3}H-NE uptake (fmoles/mg protein/ 5 min) in the cerebellum of 6 week old animals (group 2) after neonatal injections of combinations of naloxone or saline plus met-enkephalin (met-enk), or morphine, or saline in unoperated sham controls (N=4-8) or animals that received a neonatal brainstem lesion (N=6-10).

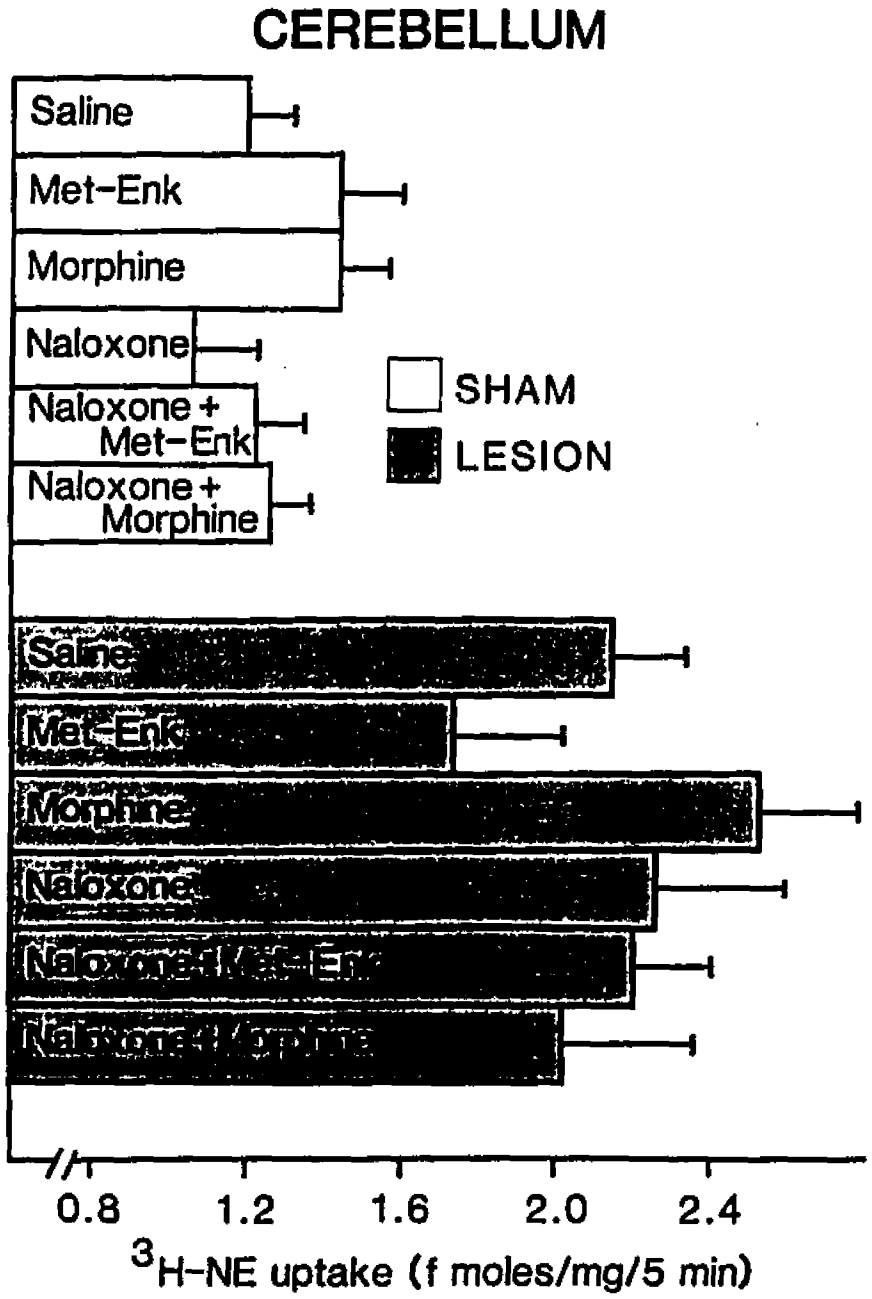
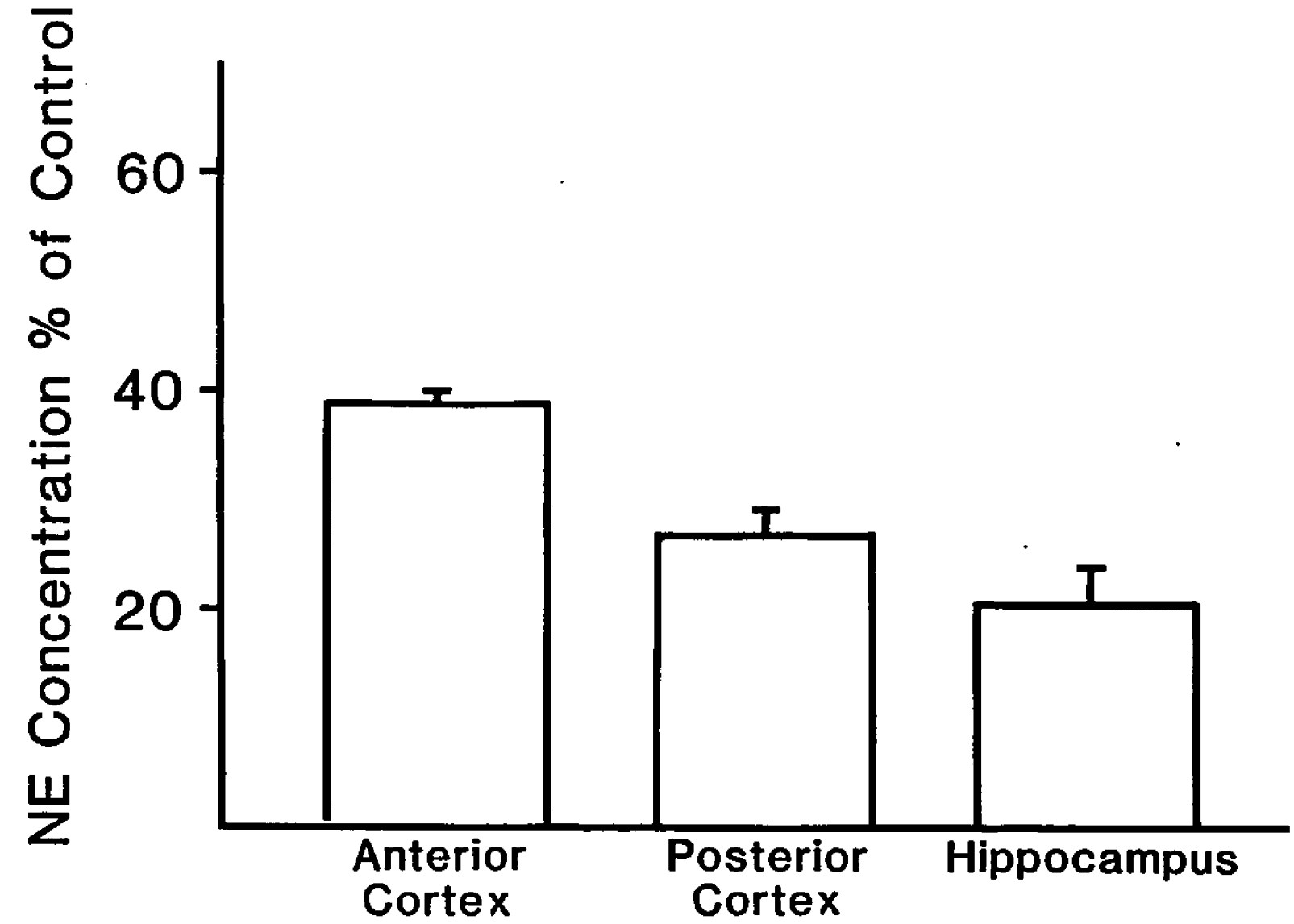

CEREBELLUM
Saline
Met-Enk
Morphine
Naloxone
Naloxone + Met-Enk
Naloxone + Morphine
SHAM
LESION
Saline
Met-Enk
Morphine
Naloxone
0.8
1.2
1.6
2.0
2.4
3H-NE uptake (f moles/mg/5 min)

Figure 15

Mean NE concentration (ng/g wet weight, percent of unoperated controls) in different forebrain areas of 6 week old animals that received a neonatal brainstem lesion (N=61-63). There is a significant difference ($p < 0.05$) between each region.


NE Concentration % of Control
60
40
20
Anterior Cortex
Posterior Cortex
Hippocampus

regions. The NE concentration of the lesioned animals in the anterior cortex was 39% of the control animals, while the posterior cortex was 27% of control, and significantly different from that in the anterior cortex. The lowest content of NE was found in the hippocampus, at 21% of control, and this was significantly different from that in either area of the cortex.

D. Met-Enkephalin Levels

There was no difference in met-enkephalin concentration, when expressed in terms of weight of the tissue, between the lesion and the control animals at 1 and 4 days after the lesion in any of the regions studied (table 2). Table 3 represents an expression of the met-enkephalin concentration per milligram of protein. On this basis there was a significant difference between the control and lesioned animals in the locus coeruleus region 1 day after the lesion. At this time point the lesion group had higher levels of met-enkephalin, but at 4 days after the lesion there was no longer a difference between the groups.

Area	Days Post-lesion			
	1 day		4 days	
	Control	Lesion	Control	Lesion
Locus Coeruleus Region	165.76 ± 26.4*	190.65 ± 27.1	220.93 ± 41.7	258.64 ± 52.8
Pons-Medulla	412.83 ± 44.5	425.88 ± 34.3	589.65 ± 29.8	652.48 ± 54.7
Posterior Cortex	72.61 ± 3.9	61.42 ± 12.1	83.67 ± 5.1	118.16 ± 16.1

*Mean ± SEM

Table 2

Met-enkephalin content (ng/mg tissue weight) in various brain regions of rats, 1 and 4 days after a dorsal bundle lesion made at 3 days after birth.

Area	Days Post-lesion			
	1 day		4 days	
	Control	Lesion	Control	Lesion
Locus Coeruleus Region	1.69 ± 0.34*	3.01 ± 0.34**	3.54 ± 0.48	3.09 ± 0.44
Pons-Medulla	8.06 ± 0.81	7.88 ± 0.68	11.36 ± 0.36	12.36 ± 1.13
Posterior Cortex	1.72 ± 0.12	1.35 ± 0.27	1.67 ± 0.15	2.34 ± 0.29

*Mean ± SEM
**$p < 0.05$

Table 3

Met-enkephalin concentration (ng/mg protein) in various brain regions of rats, 1 and 4 days after a dorsal bundle lesion made at 3 days after birth.

Chapter VI

Discussion

A. Opioid Effects on Noradrenergic Neurons

Neonatal treatment with morphine, met-enkephalin, or naloxone had no effect on NA fiber number in the brains of sham control rats at six weeks after birth. Within the sham groups there was no difference between the assorted drug treatments. In the animals that received a DNB lesion, there was also no difference among the drug treatment groups, in NE concentration and ^{3}H-NE uptake in the forebrain or hindbrain regions. The measurements of NE concentration and ^{3}H-NE uptake taken together are a good indication of NA nerve terminal density (Jonsson and Sachs, 1982). The surgical axotomy produced the expected increases in NA fiber number in the brainstem and cerebellum, and decreases in fiber number in the forebrain regions.

The previous findings, that morphine attenuated the NA effects associated with a DNB lesion, implied that in the present study the opioids would alter recovery of the NA fibers. Bardo et al. (1983) also found that morphine administration with a midcollicular hemisection reduced collateral sprouting in the ipsilateral cerebellar hemisphere, results similar to the preliminary study. Earlier studies with morphine and 6-OHDOPA, demonstrating that morphine potentiated the effects of neonatal neurotoxin treatment (Harston et al., 1981 and Jonsson and Hallman,

1982a), also suggested an opioid involvement in developmental recovery. Since recovery from neurotoxin and surgical axotomy is very similar, morphine was expected to alter the recovery from both manipulations. Further support for this hypothesis came from a report by Jonsson and Hallman (1983), in which substance P administration after unilateral transection of the DNB resulted in significant increases in NA fiber number in the cerebellum and pons-medulla. Substance P in that study did not counteract the damage in the cortex, but was effective in altering recovery of NA fibers in some areas.

The results of the preliminary study are considered to be unreliable, because of the large amount of variability in the lesion groups. The variability may have been the result of inconsistent transection of the DNB neonatally. If the lesion were not successful, the adult values obtained would be identical to that of the controls and thus inaccurately representing recovery. In the present study the surgical axotomy was considered to be successful. The ANOVAs on all the areas studied with all the different treatments demonstrated a significant main effect of the lesion. Also, the large number of animals surgically treated, has produced more consistent results. Thus in this study the large variability within the lesion groups was not present. The success of the lesion was verified in some animals by examining the fibers projecting from the locus coeruleus four days after the lesion. An

accumulation of NE was seen, indicating the fibers were cut. In these animals there was no visible clotting or scarring at the lesion site. Immunocytochemical localization of glial fibrillary acidic protein, a marker for glia, was used to study the glia after a neonatal brainstem lesion. An accumulation of the acidic protein indicates scarring has taken place. Examination of the distribution of the glial fibrillary acidic protein in the area of the lesion at six weeks revealed no accumulation of this protein and thus no scarring (unpublished results). Some of the values for NE content and ^{3}H-NE uptake, obtained for the lesioned animals, were closer to control, and thus suggested a degree of recovery of the NA fibers in these animals. Approximately one of eight surgically treated animals demonstrated this recovery, possibly indicating a percentage of unsuccessful cuts. However, in the preliminary study the frequency of these unusual animals may have been greater than in the present study.

Bardo et al. (1983) found that morphine administration with a unilateral lesion of the DNB reduced the sprouting in the ipsilateral cortex. These results have never been published completely. Thus the details of the study are not available. This group used a unilateral lesion in their study compared to a bilateral cut in the present study. The conflicting results in the two studies could be explained, if the lesion values obtained in the unilateral study were compared to values obtained from the

contralateral side rather than control animals. Bardo et al. (1983) also only mentioned effects in the cerebellum with no information about the changes in the cortex.

Jonsson and Hallman (1983) also used a unilateral lesion to show that substance P produced an alteration in noradrenergic fiber recovery only in the hindbrain regions and not in the cortical areas. In the previous work with neurotoxins, substance P could potentially modify recovery of all of the projections of the locus coeruleus. The authors suggest that the substance P alterations may be due to a growth-promoting effect of the compound in specific areas and thus not a general effect on the locus coeruleus to direct growth.

In contrast to those findings with neurotoxins, it is now reported that opioids do not have an effect on recovery of a NA system damaged by surgical axotomy. Because opioids do modify recovery of NA fibers damaged by the neurotoxin, 6-OHDOPA (Harston et al., 1981), the present study indicates that this action probably results from a modification of the neurotoxic actions of the 6-OHDOPA. Failure of the opioids to modify recovery of NA fibers from a mechanical lesion suggests that the opioids are not capable of acting on the NA fibers to actually stimulate regeneration. Procedurally, neurotoxins damage all major projections of the locus coeruleus, by being taken up into the nerve terminal areas and destroying these regions. The surgical axotomy severs the connections of the locus coeruleus to the forebrain

projections only. The DNB lesion severs the neurons in only one major bundle. Opioids may be effective in altering nerve terminal input, but not in promoting major nerve fiber connections.

Previous work in our laboratory has established that neonatal 6-OHDOPA treatment (the same protocol as in the neurotoxin studies) does not alter the number of cells in the locus coeruleus (unpublished results). In a similar study with animals in which the DNB had been transected neonatally, the number of cells in the locus coeruleus was found to be decreased (Kostrzewa et al., 1985). The damage produced by transection of the DNB is thus very different from the neurotoxin damage.

The evidence suggests that opioid alterations are specific for neurotoxin induced damage. This may be a consequence of the opioids interacting with or combining with 6-OHDOPA, although there is no evidence at present for this. The drugs are toxic to the neurons and the opioids may also be toxic or just capable of enhancing the toxicity of the neurotoxins.

The "pruning effect," proposed by Jonsson and Hallman (1982a and b), suggests that nerve terminals are programmed to develop a certain number of arborizations to each of the target areas of the locus coeruleus. Following damage to one projection the neurons would develop an enhanced nerve terminal arborization in an intact collateral region. The synaptic inputs onto the locus coeruleus were thought to

influence this response to damage of the nuclei. Exogenous administration of opioids did not alter the response of the nuclei to surgical axotomy; opioids, therefore, are not working at the level of the perikarya, when altering the recovery from neurotoxin damage. The present study demonstrates that opioids do not alter true pruning, but rather alter recovery from nerve terminal damage as in the neurotoxin treated animals.

B. Met-Enkephalin Concentration in Neonatal Animals with a Severed Dorsal Noradrenergic Bundle

The animals that received the DNB lesion exhibited an increase in met-enkephalin levels in the locus coeruleus region, one day after the lesion. This result could indicate an increased met-enkephalin fiber activity, as a consequence to NA fiber injury. The effect was immediate, but not prolonged, since met-enkephalin content was not elevated four days after the lesion. The validity of this result is questionable, because an effect was not present when the met-enkephalin concentration was expressed in terms of tissue weight.

Additional studies would need to be done to clarify these results. Moreover, the locus coeruleus would need to be more discretely dissected in order to obtain a greater reliability of such findings. Either biochemical measurements of met-enkephalin with micropunch samples would be suitable, or immunocytochemical quantitative histochemistry

of met-enkephalin fibers in the locus coeruleus. More frequent sampling would show the time course of any changes in the met-enkephalin fibers resulting from NA fiber transection. Although met-enkephalin levels may increase with damage to the neurons, as indicated in this portion of the study, exogenous administration of met-enkephalin did not produce any apparent alteration in the recovery of locus coeruleus fibers.

C. Noradrenergic Recovery from a Dorsal Bundle Lesion

The DNB lesion was found to reduce NE content and ^{3}H-NE uptake in brain regions distal to the fiber transection (i.e. neocortex and hippocampus). At the same time a retrograde degeneration occurs in at least a portion of those fibers that were transected. Upon studying the recovery of the forebrain projections, it has been noted that NA fiber recovery in the anterior cortex recovered to a greater degree than was found in the posterior cortex. Recovery was least in the hippocampus.

The pattern of recovery suggests that NA fiber recovery is greater when axonal fiber length to a target area is short. A long axonal fiber length may result in fewer axons being able to regenerate across the longer fiber pathway. Because the degree of recovery of NA fibers was identical in each of the target regions at both 4 and 6 weeks, the recovery pattern appears to be permanent once an animal reaches adulthood.

D. Summary

Neither morphine nor met-enkephalin administration alters the recovery of NA fibers in various rat brain regions after a mechanical lesion of the DNB. The opioid system does not seem to play a role in regeneration of NA fibers after a surgical lesion in the brainstem of developing animals. Previous studies, using neurotoxins to produce damage, suggested that the opioid system was capable of altering the NA fiber development after damage to the system neonatally. The present studies indicate that the opioid system does not have a growth promoting effect on NA recovery from degeneration produced by a DNB cut, but that the specific opioid compounds are only effective in neurotoxin-induced degeneration.

A regional difference in recovery of the NA fibers after neonatal axonal transection has been noted. The present findings demonstrate that the degree of recovery of central NA fibers is related to the length of the axonal pathways. The distance the terminal areas are from the locus coeruleus seems to determine the amount of recovery.

REFERENCES

References

Ader, J.P., P. Room, F. Postema, and J. Korf 1980 Bilaterally diverging axon collaterals and contralateral projections from rat locus coeruleus neurons, demonstrated by fluorescent retrograde double labeling and norepinephrine metabolism. J. Neural. Transm., 49:207-208.

Aghajanian, G.K. and J.M. Cedarbaum 1979 Central noradrenergic neurons: interaction of autoregulatory mechanisms with extrinsic influences. In: Catecholamines Basic and Clinical Frontiers. E. Usdin, I.J. Kopin, and J. Barchas, eds. Pergamon Press, New York, Vol 1, pp. 619-621.

Bardo, M.T., R.H. Schmidt, and R.K. Bhatnagar 1983 Effects of morphine on 6-hydroxydopamine-or hemisection-induced sprouting of locus coeruleus fibers in the neonatal rat. Fed. Proc., 42:2719.

Bloom, F.E. 1983 Endogenous opioids: histochemistry, neurophysiology, and pharmacology. Psychiatr. Clin. AM., 6:365-75.

Breese, G.R. and T.D. Traylor 1970 Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholamine neurons. J. Pharmacol. Exp. Ther., 174:413-420.

Cuello, A.C. 1983 Central distribution of opioid peptides. Br. Med. Bull., 39:11-16.

de la Torre, J.C. and J.W. Surgeon 1976 A methodological approach to rapid and sensitive monoamine histofluorescence using a modified glyoxylic acid technique: the SPG method. Histochemistry, 49:81-93.

Harston, C.T., M.B. Clark, J.C. Hardin, and R.M. Kostrzewa 1981 Opiate-enhanced toxicity and noradrenergic sprouting in rats treated with 6-hydroxydopa. Eur. J. Pharmacol., 71:365-373.

Harston, C.T., M.B. Clark, J.C. Hardin, and R.M. Kostrzewa 1982 Developmental localization of noradrenergic innervation to the rat cerebellum following neonatal 6-hydroxydopa and morphine treatment. Dev. Neurosci., 5:252-262.

Hughes, J. 1983 Biogenesis, release, and inactivation of enkephalins and dynorphins. Br. Med. Bull., 39:17-24.

Jacobowitz, D.M. 1978 Monoaminergic pathways in the central nervous system. In: Psychopharmacology: A Generation of Progress. M.A. Lipton, A. DiMascio, and K.F. Killam, eds. Raven Press, N.Y., pp. 119-129.

Jonsson, G. and H. Hallman 1980 Effects of neonatal nicotine administration on the postnatal development of central noradrenaline neurons. Acta Physiol. Scand. [Suppl.], 479:25-26.

Jonsson, G. and H. Hallman 1982a Modulation of 6-hydroxydopamine-induced alteration of the postnatal development of central noradrenaline neurons. Brain Res. Bull., 9:635-40.

Jonsson, G. and H. Hallman 1982b Response of central monoamine neurons following an early neurotoxic lesion. Bibl. Anat., 23:76-92.

Jonsson, G. and H. Hallman 1982c Substance P counteracts neurotoxin damage on norepinephrine neurons in rat brain during ontogeny. Science, 215:75-77.

Jonsson, G. and H. Hallman 1982d Substance P modifies the 6-hydroxydopamine induced alteration of postnatal development of central noradrenaline neurons. Neuroscience, 7:2909-2918.

Jonsson, G. and H. Hallman 1983 Effects of Substance P on neonatally axotomized noradrenaline neurons in rat brain. Med. Biol., 61:179-185.

Jonsson, G., C. Pycock, K. Fuxe, and C. Sachs 1974 Changes in the development of central noradrenaline neurons following neonatal administration of 6-hydroxydopamine. J. Neurochem., 22:419-426.

Jonsson, G. and C. Sachs 1982 Changes in the development of central noradrenaline neurons after neonatal axon lesions. Brain Res. Bull., 9:641-650.

Jonsson, G. and C. Sachs 1976 Regional changes in ^{3}H-noradrenaline uptake, catecholamines, catecholamine synthetic and catabolic enzymes in rat brain following neonatal 6-hydroxydopamine treatment. Med. Biol., 54:286-297.

Kostrzewa, R.M. and R.E. Garey 1976 Effects of 6-hydroxydopa on noradrenergic neurons in developing rat brain. J. Pharmacol. Exp. Ther., 197:105-118.

Kostrzewa, R.M. and D. Klisans-Fuenmayor 1984 Development of an opioid-specific action of morphine in modifying recovery of neonatally-damaged noradrenergic fibers in rat brain. Res. Commun. Chem. Pathol. Pharmacol., 46:3-11.

Kostrzewa, R.M., D. Klisans-Fuenmayor and J.C. Hardin 1984 Naloxone-reversible morphine-induced destruction of coeruleus cells accompanies enhanced recovery of noradrenergic fibers in brains of rats treated as neonates with 6-hydroxydopa. Abstract to 9th Int'l Congress of Pharmacology, London.

Kostrzewa, R.M., D. Klisans-Fuenmayor and C.T. Harston 1985 Alterations in noradrenergic innervation of the brain following dorsal bundle lesions in neonatal rats. (Submitted to Brain Res. Bull.).

Lauder, J.M. and F.E. Bloom 1974 Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei, and substantia nigra of the rat I. Cell differentiation. J. Comp. Neurol., 155:469-481.

Lowry, O.H., A.L. Rosebrough, A.L. Farr, and R.J. Randall 1951 Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193:265-275.

Moore, R.Y. and F.E. Bloom 1979 Central catecholamine neuron systems: Anatomy and physiology of the norepinephrine and epineprine systems. Annu. Rev. Neurosci., 2:113-168.

Morley, J.S. 1983 Chemistry of opioid peptides. Br. Med. Bull., 39:5-10.

Nagai, T., K. Satoh, K. Imamoto, and T. Maeda 1981 Divergent projections of catecholamine neurons of the locus coeruleus as revealed by fluorescent retrograde double labeling technique. Neurosci. Lett., 23:117-123.

Nagatsu, T. 1973 Biochemistry of Catecholamines the Biochemical Method. University Park Press, Baltimore, p. 209.

Pappas. B.A. and S.K. Sobrian 1972 Neonatal sympathectomy by 6-hydroxydopamine in the rat: no effects on behavior but changes in endogenous brain norepinephrine. Life Sci., 11:653-659.

Patey, G., S. de la Baume, C. Gros, and J.C. Swartz 1980 Ontogenesis of enkephalinergic systems in rat brain: post-natal changes in enkephalin levels, receptors, and degrading enzyme activities. Life Sci., 27:245-252.

Pert, C.B. and S.H. Snyder 1973 Opiate receptor: demonstration in nervous tissue. Science, 179:1011-1014.

Room, P., F. Postema, and J. Korf 1981 Divergent axon collaterals of rat locus coeruleus neurons: demonstration by a fluorescent double labeling technique. Brain Res., 221:219-230.

Sachs, C. and G. Jonsson 1975 Effects of 6-hydroxydopamine on central noradrenaline neurons during ontogeny. Brain Res., 99:277-291.

Schmidt, R.H. and R.K. Bhatnagar 1979 Distribution of hypertrophied locus coeruleus projection to adult cerebellum after neonatal 6-hydroxydopamine. Brain Res., 172:23-33.

Steindler, D.A. 1981 Locus coeruleus neurons have axons that branch to the forebrain and cerebellum. Brain Res., 223:367-373.

Tsang, D., S.C. Ng, K.P. Ho, and W.K. Ho 1982 Ontogenesis of opiate binding sites and radioimmunoassayable B-endorphin and enkephalin in regions of rat brain. Brain Res., 281:257-261.

Yang, H.Y., J.S. Hong, and E. Costa 1977 Regional distribution of leu and met enkephalin in rat brain. Neuropharmacology, 16:303-307.

Zieher, L.M. and G. Jaim-Etcheverry 1975 6-Hydroxydopa during development of central adrenergic neurons produces different long-term changes in rat brain noradrenaline. Brain Res., 86:271-281.

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Kostrzewa, R.M., C.T. Harston, J.C. Hardin, and E. Bagden 1980 Enhanced regeneration of noradrenergic fibers in rat cerebellum by nerve growth factor. J. Histochem. Cytochem., 28: 28 (abstract).

Kunkel-Bagden, E., and R.M. Kostrzewa 1982 Influence of nerve growth factor (NGF) on growth of noradrenergic fibers into rat cerebellum. Trans. S.E. Pharmacol. Soc., 3 (abstract).

Kunkel-Bagden, E., and R.M. Kostrzewa 1984 Morphine-induced alterations of the development of noradrenergic fiber projections in rat brain following a dorsal bundle lesion. Fed. Proc., 43: 3463 (abstract).

Kunkel-Bagden, E., and R.M. Kostrzewa
1985 Opioids fail to alter recovery from a dorsal bundle lesion. Trans. Americ. Soc. Neurochem., 16: 364.

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