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PRINCIPAL INVESTIGATOR: Dennis M. Feeney, Ph.D.

CONTRACTING ORGANIZATION: University of New Mexico
Albuquerque, New Mexico 87131

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6. AUTHOR(S)
Dennis M. Feeney, Ph.D.
Michael R. Weintraub
Susan R. Queen

Authors added per Rebecca 6-27-91

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of New Mexico
Albuquerque, New Mexico 87131

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The seven experiments reviewed in this report utilize anatomical, metabolic or biochemical measurements to analyze the mechanism(s) of amphetamine accelerated recovery from the hemiplegia induced by ablation or contusion injury to rat sensorimotor cortex. After a unilateral contusion produced by focal impact trauma, but not following ablation injury of the same cortical area, secondary neuronal death occurs in the dorsolateral striatum, medial geniculate body and hippocampal CA3 pyramidal and hillar regions of the ipsilateral hemisphere. The severity of this remote histopathological reaction is influenced by the anesthesia employed and is not detectably different when the location of the contusion within the sensorimotor area is varied. The severity of the cell death in the hippocampus but not other areas is significantly reduced by the selective alpha$_1$ noradrenergic receptor agonist methoxamine administered 24 hours posttrauma. Amphetamine had no such protective effect even though it reduced the extent of cortical necrosis after trauma to posterior sensorimotor cortex, perhaps because of its weak

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alpha$_1$-noradrenergic receptor agonist effects compared to methoxamine. The secondary cell death in the hippocampus may be attributed to excitotoxicity a hypothesis supported by a study of posttraumatic oxidative metabolism. The pyramidal cells of the CA3 and hillar regions receive major excitatory input from the ipsilateral entorhinal cortex. At two days posttrauma (but not after ablation), this cortical area shows an increase in cytochrome oxidase activity compared to the contralateral hemisphere. A central role for norepinephrine in the amphetamine effects on functional recovery after cortical injury was supported by two studies. Using reserpinized SMCX injured rats, in which amphetamine increases striatal dopamine (DA) release but not norepinephrine (NE), amphetamine temporarily increased locomotor performance. But in contrast to nonreserpinized animals, hemiplegia remained at baseline disability levels. This reserpine blockade of recovery from hemiplegia occurs during the potentiation of amphetamine induced stereotypies, thought to be mediated by DA. Data from biochemical studies of SMCX injured rats indicated that 2 days after unilateral SMCX ablation there is a bilateral depression of NE, DA, and metabolite levels. In contrast, the metabolite of serotonin, 5-HIAA is markedly elevated in structures ipsilateral to the injury. This diffuse monoamine response to injury is likely a result of damage to the axons of the locus coeruleus (LC) by the SMCX injury. The elevation of 5-HIAA may result from a release of raphe serotonergic neurons from LC inhibition. Amphetamine administration after SMCX injury produces an enduring normalization of depressed NE levels, but not 5-HIAA, suggests the drug effect is at LC terminals. In summary, changes of monoamine metabolism within structures remote from a cortical injury, may play a diverse role in behavioral deficits, metabolic abnormalities and secondary cell death.

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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General Introduction

The experiments conducted in the last year of this contract (from April 14, 1986 to April 13, 1989) and the continued work (until the end of 1990) were very productive. We have made considerable progress toward achieving the goal of developing a pharmacological treatment regimen for promoting functional recovery after traumatic brain injury. This report describes the objectives, results and discusses the significance of seven completed but not yet published experiments. These research findings are currently being replicated or written for publication.

Research described in this report utilized three approaches to the analysis of pharmacological effects on functional recovery after unilateral injury to the sensorimotor cortex (SMCX) of the rat: First, anatomical description of secondary neuronal death following traumatic SMCX injury in the rat. This preliminary work and studies still in progress examined the effects of anesthetic conditions, location of the contusion within the SMCX and delayed pharmacological treatment with amphetamine or methoxamine on body temperature and some neuropathological sequalae of cerebral trauma. Second, histochemical studies of oxidative metabolism using a stain for the mitochondrial enzyme cytochrome oxidase, were conducted. This study tested the hypotheses that posttraumatic entorhinal cortex neuronal hyperactivity produced secondary cell death of pyramidal cells in the CA3 and hillar regions of the ipsilateral hippocampus. Third, the hypothesis that the well documented effects of amphetamine and other drugs effects on functional recovery after sensorimotor cortex injury (6, 7, 14, 15, 21, 26, 33, 34, 35, 40) was tested in two experiments. Pharmacological depletion of catecholamines by reserpine administration blocked the beneficial effects of a single low dose of amphetamine on recovery from hemiplegia without altering the drug induced stereotypies or increases in spontaneous activity. The other experiment examined the effects of sensorimotor cortex ablation and amphetamine treatment on levels of catecholamines and their metabolites and a serotonin metabolite (reportedly increased by freeze injury to cortex). The effects of focal trauma and amphetamine treatment on these biochemical measures were described in the 1988 annual report. Each of these experiments is presented and discussed separately below.

CONTUSION AND SECONDARY CELL DEATH

Traumatic brain injury in the rat, produced by contusion of the sensorimotor cortex (SMCx), produces gliosis and/or neuronal loss in areas remote from the primary cortical necrosis. This secondary damage is quite prominent in the ipsilateral hippocampal CA3 pyramidal cells and thalamic ventral basal complex (1). Similar effects have also been reported following fluid-percussion injury to the rat parietal cortex (2). Such remote pathological reactions may be due to many factors, including excitotoxicity or degeneration of fibers projecting to or from the primary injury site. This study compared the neuropathologic reactions remote from the cortical cavitation produced by either contusion or ablation. Then the protective effect of a high dose of the noncompetetive N-methyl-D-aspartate antagonist, ketamine, following contusion was evaluated on those areas exhibiting neuronal necrosis following contusion, but not ablation.

Eleven Sprague-Dawley albino rats weighing between 300 and 350 g on the day of surgery, individually housed in standard wire-mesh cages, maintained on a 12:12 h light/dark cycle, and given free access to food and water throughout the experiment were used.

Two methods were used to produce SMCx damage, suction ablation and a 400 g/cm contusion. For surgery, animals were anesthetized with a 4% halothane to oxygen mixture delivered at a 2.0 L/min flow rate. For ablation, a craniotomy was performed extending from 2mm anterior to 4mm posterior to bregma and to 5 mm lateral from the sagital sinus. The dura was then removed and the right SMCx ablated by suction. The resultant cavity was filled with sterile gelfoam and the scalp sutured closed For contusion, a small bone flap was removed and a focal injury produced with a contusion device. (Fig. 1)(adapted from 3), which produces a well-characterized focal cavitation necroses (4). The footplate of the device was centered at 1.5 mm posterior and 2.5 mm lateral to bregma. Following contusion, the bone flap was replaced over the surface of the brain, covered with bone wax, and the scalp sutured closed.

Eight animals were contused and three ablated. In addition, three of the contused animals received a single 150 mg/kg dose of ketamine (i.p.) five min post injury. Within two to four weeks after surgery, all animals were killed with a lethal dose of sodium pentobarbital, and perfused with saline followed by 10% formalin. After three days in a 10% formalin/20% sucrose solution, frozen, 40 micron, coronal sections were cut begining just anterior to the caudate nucleus and extending to the middle of the cerebellum. Selected sections were mounted on slices, stained with thionin, and coverslipped. Tissue sections were studied at three planes, bregma 0.48, -3.8, 15.8 mm, by experimenters (uninformed of treatment) and the following structures ipsilateral to the injury were rated for degree of neuronal loss and gliosis, relative to that in the contralateral homologous structures: medial septum, dorsolateral striatum (neuronal loss was not examined in the structure due to difficulaty in making definitive judgements), medial geniculate nucleus, substantia nigra, red nucleus, habenula, dentate gyrus, hillus of the dentate gyrus, CA1 and CA3pyramidal cells of the hippocampus, lateral posterior thalamic nucleus (laterocaudal and mediorostral parts), dorsolateral and ventrolateral geniculate nuclei, the posterior thalamic nuclear group, the ventral posterolateral and posteromedial thalamic nuclei, and arcuate hypothalamic nucleus. The following four-point rating scale was used:
1 = no. 2 = slight. 3 - moderate, and 4 = severe gliosis or neuronal

loss. The volume of primary cavitation necrosis was also determined for each of the groups.

Unilateral contusion of the sensorimotor cortex area in rats produces gliosis and or neuronal loss in the medial geniculate nucleus and CA3 region of the hippocampus, and gliosis in the dorsolateral striatum of the injured hemisphere (Fig. 2). These neuropathological reaction remote from the primary injury are unlikely due to antero- or retrograde degeneration of neuronal pathways since they are not produced by sensorimotor cortex ablations. Some of these secondary effects may be the result of NMDA receptor mediated excitotoxicity since ketamine administered 5 minutes after contusion reduced them significantly (Fig. 3). In addition, ketamine reduced the primary cavitation necrosis produced by contusion, suggesting that this necrosis is mediated by a similar mechanism (Fig. 4). However, these effects may be due to other factors such as altered cerebral blood flow or reduced body temperature produced by ketamine.

ANESTHETIC EFFECTS ON NEUROPATHOLOGY

Our previous work on focal contusion injrry to the rat sensorimotor cortex described the post traumatic evolution of primary cavitation necrosis, remote metabolic disturbances and recovery from a transient hemiplegia (4, 5, 6). Pharmacological studies using this model of severe traumatic brain injury indicate a central role for norepinephrine (NE) in recovery from hemiplegia. Delayed administration of drugs enhancing NE neurotransmission (e.g., a single low dose of d-amphetamine given 24 hours after injury) produces an enduring facilitation of functional recovery (6, 7). The many reports of the N-methyl-D-aspartate (NMDA) receptor complex-mediated excitotoxicity (8) and the attenuation of excitotoxicity by NE activation (9) prompted this investigation. Using our rat cortical contusion model, we evaluated the effects of anesthetic condition and delayed administration of ketamine (KET), a noncompetitive NMDA antagonist, on the magnitude of primary cortical necrosis, remote hippocampal and thalamic neuronal loss and gliosis, and recovery from hemiplegia. In brief, we observed that KET markedly lessens the hippocampal CA3 cell loss and reduces cortical necrosis but worsens neuronal loss and gliosis in the ventral basal complex thalamic nuclei and slows recovery from hemiplegia.

50 male Sprague-Dawley albino rats weighing between 300 and 350 grams on the day of surgery, individually housed in wire mesh cages, maintained on a 12:12 hour light dark cycle, and given free access to food and water throughout the experiment were used.

Sensorimotor cortex (SMCx) damage was produced by a 400 gram/square centimeter contusion. Animals were anesthetized with halothane, pentobarbital, or ketamine and pentobarbital. For contusion, a small bone flap was removed and the contusion device (Fig. 1) was placed over the exposed dura centered 2.5mm posterior to bregma and 3.5 mm lateral to the sagital suture. Following the contusion, the bone flap was replaced, covered with bone wax, and the scalp sutured closed.

10 animals were contused with halothane anesthesia, 10 with pentobarbital, and 13 with ketamine/pentobarbital anesthesia. In addition, 10 animals contused under halothane anesthesia were given a high dose of ketamine 5 minutes post-concussion and seven were given a comparable volume of saline. Three to four weeks post-injury, the rats were perfused with 10% formalin. After three days in 10% formalin/20%

sucrose solution, frozen 40 micron sections were mounted on slides, stained with thionin or thionin counterstained with acid fuchsin. Tissue sections at 3.8mm posterior to bregma were studied by trained observers uninformed of treatment condition. The thalamic ventral basal complex and hippocampal CA3 region ipsilateral to the SMCx contusion were rated for cell loss and gliosis as compared to the contralateral homologous structure. the following 4 point rating system was used: 0=no cell loss or gliosis, 1=mild cell loss or gliosis, 2=moderate cell loss or gliosis, 3=severe cell loss or gliosis. The volume of primary cortical necrosis was also calculated for the groups receiving ketamine or saline post contusion.

Unilateral SMCx contusion produces a dramatic loss of ipsilateral hippocampal CA3 pyramidal cells seen in thionin-stained sections at 20 days post injury when performed under halothane or pentobarbital anesthesia. Contusions under low dose ketamine combined with low dose pentobarbital significantly attenuate hippocampal CA3 pyramidal cell loss (Fig. 5). There was no significant effect of anesthesia on thalamic ventral basal complex cell neuronal loss and gliosis. Post-contusion administration of high dose ketamine produce significant protection of hippocampal CA3 pyramidal neurons and significantly decreased the volume of cortical necrosis (Figs. 6 and 7). The reduction of CA3 cell loss by post-contusion administration of high dose ketamine is not clearly seem at 2 days post-contusion, but is quite dramatic at 20 days after injury. Recent work has failed to replecate the protective effect initially observed with post-contusion ketamine administration. Continuing investigations are aimed to elucidate the reasons for this discrepancy.Unlike the ketamine attenuation of primary cavitation and secondary cell loss in the hippocampus there was no significant effect on thalamic ventral basal complex neuronal loss and gliosis (Fig. 8). No effects of anesthetic condition on thalamic ventral basal complex neuronal loss and gliosis were detected (Fig. 9). This thalamic gliosis was significantly correlated with the number of trials contused animals required to recover from hemiplegic conditions (Fig. 10). Animals given high dose ketamine post-contusion were significantly slower to recover to baseline locomotor ability than saline controls (Fig. 11). No effects of anesthetic conditions were detected on beam-walking recovery rate (Fig. 12).

METHOXAMINE AND NEUROPATHOLOGY

Because of the recent report (10) that methoxamine, an alpha-1 noradrenergic (NE) receptor agonist, reduces pyramidal cell loss in the CA1 sector of the hippocampus after transient ischemia in gerbils, the present study was conducted to examine the effects of this drug on both primary and secondary neuropathology produced by focal impact injury to the sensorimotor cortex in rats. Additionally, an examination of the effects of methoxamine on rectal temperature was conducted because of recent reports that hypothermia may mediate the neuroprotective effectiveness of some drugs after ischemia (11, but see 12).

Using a model of traumatic brain injury, it has been observed that focal impact to the sensorimotor cortex in rats produces a well-characterized evolution of cortical necrosis at the primary site of impact (4), as well as secondary neuropathology not seen after ablation of the same cortical region (1, 13). To date, this secondary neuropathology,

in terms of gliosis and/or cell loss, has been detected in the CA3 sector of the hippocampus, dorsolateral striatum, and medial geniculate. Moreover, both this secondary neuropathology and primary cortical necrosis are attenuated by ketamine pre- or posttreatment (1, 13).

Forty-seven Sprague-Dawley albino rats weighing between 300 and 350 g were individually housed in standard wire-mesh cages, maintained on a 12:12 h light/dark cycle, and given free access to food and water throughout the experiment.

Animals were anesthetized by administration of 60 mg/kg ketamine HCL and 0.08 ml atropine at 0.54 mg/ml im., followed 5 min later with 21 mg/kg sodium pentobarbital (Nembutal) ip. A craniotomy was performed over the right SMCx, and a focal impact injury produced with a contusion device (Fig. 1) adapted from Allen (3). Twenty-four hours postinjury, the animals were given a single injection (ip.) of either saline or methoxamine (1, 4, or 8 mg/kg).

At 30 days postinjury, animals were killed with a sodium pentobarbital (1 ml, ip.), and perfused with saline followed by 10% formalin. The brains were extracted and stored in 10% formalin until the time of histological sectioning. At this time, the brains were transferred to a 10% formalin/20% sucrose solution for three days. Frozen, 40 micron coronal sections were cut on a cryostat microtome, and every fifth section placed on slides, stained with thionin, and coverslipped. Using tissue sections at bregma -0.26 -3.8 and -6.04 mm, the degree of gliosis and/or neuronal loss in the CA3 region of the hippocampus, dorsolateral striatum, and medial geniculate was estimated by a trained observer blind to experimental treatment using the following 4-point rating scale: 0 = no, 1 = slight, 2 = moderate, and 3 = severe cell loss or gliosis. In addition, the volume of primary cortical necrosis was estimated by computing the sum of the cross-sectional areas (mm2) of primary cortical necrosis taken from the following seven sections: A9650, A8920, A7190, A5150, A2790, A2180, and A1610 microns, relative to the interaural line (14, 15).

Five rats weighing between 300 and 350 g were used to assess the effects of methoxamine on rectal temperature (Fig. 13). Animals were anesthetized with a 4% halothane to oxygen mixture delivered at a flow rate of 2.0 L/min and maintained with a 2% mixture at the same flow rate while the animals were secured in a restraint apparatus (approximately 10 minutes). Immediately thereafter, anesthesia was terminated, rectal temperature monitored for 45 minutes, the animals injected with either saline or methoxamine (4 or 8 mg/kg, ip.), and rectal temperature monitored for 2.5 hours.

Apriori pairwise comparisons using Tukey's HSD revealed that, compared to saline controls, the administration of 8, but not 1 or 4 mg/kg of methoxamine significantly reduced the amount of hippocampal CA3 pyramidal cell loss (Fig. 14) when given 24 hours following focal impact injury to the right sensorimotor cortex in rats [$T(43) = 2.08$, $p < .05$; $T(43) = 0.065$; and $T(43) = 1.064$, $p = .293$, respectively]. No effects of methoxamine (8mg/kg) on the estimated volume of primary cortical necrosis or on gliosis or cell loss in the medial geniculate, dorsolateral striatum were detected. Both 4 and 8 mg/kg of methoxamine lowered rectal temperature by approximately 2 degrees Celsius for approximately 2 hours (total observation period) postinjection.

A single administration of the alpha-1 adrenoceptor agonist, methoxamine (8 mg/kg, ip.) 24 hours following focal impact injury to the right sensorimotor cortex in rats significantly reduces pyramidal cell loss in the CA3 sector of the hippocampus. Unlike ketamine treatment, no

effects of methoxamine (8 mg/kg, ip.) on gliosis and/or cell loss in the cortex, medial geniculate, or dorsolateral striatum were observed. The mechanism mediating the neuroprotective effect of methoxamine observed in the present study remains undetermined. Possibilities include: effects of accompanying hypothermia, attenuation of excitotoxicity by inhibition of CA3 pyramidal cells or their afferents preclusion of CA++ influx by the release of intracellular stores of this cation produced by alpha-1 adrenoceptor stimulation.

This study investigated the effects of amphetamine on primary and secondary neuropathology in a model of severe traumatic brain injury in the rat. Contusion or ablation of the right sensorimotor cortex (SMCx) produces a transient left hemiplegia which can be alleviated by delayed administration of d-amphetamine (AMPH) and other drugs increasing noradrenergic neurotransmission (14). Additionally, in a model of embolic stroke, a single dose of AMPH administered 24 hours post injury markedly reduced cortical infarct volume, but was ineffective in the attenuation of subcortical pathology (16).

Sensorimotor cortex contusion and ablation produce different morphologic (13) and metabolic consequences (17). Secondary pathology is observed in the hippocampal CA3, medial geniculate nucleus, and dorsal lateral striatum after focal impact injury to the SMCx (1). In models of hypoxia and ischemia the hippocampal CA1 pyramidal cells exhibit selective vulnerability, while the CA3 is conspicuously resistant. Alternatively, in focal cortical impact injury, the CA3 pyramidal neurons are susceptible whereas the CA1 remains intact (2). Furthermore, ablation of the SMCx, induces none of the secondary pathology observed in the above models (13).

AMPHETAMINE AND CONTUSION LOCUS

In the present study, the effects of delayed AMPH administration were observed on cortical cavitation necrosis, ventricular dilation, and subcortical pathology. We now report enduring ventricular dilation ipsilateral to contusion injury. Additionally observed was a decrease in cavitation necrosis after contusion to the posterior (POST), but not anterior (ANT), SMCx if treated with AMPH.

Twenty-four male Sprague-Dawley rats weighing between 300 and 350 g at the time of surgery were used in this study. Originally, the POST and ANT groups were from two separate experiments but their surgical treatment differed only in the placement of their contusion.

Animals were anesthetized by administration of 60 mg/kg ketamine HCL followed 5 min later with 21 mg/kg sodium pentobarbital (Nembutal) ip. A craniotomy was performed over the right SMCx, centered over bregma (ANT) or 2.25 posterior to Bregma (POST), as illustrated in Figure 2. A focal injury of 400 g/cm was produced with a contusion device (Figure 1) adapted from Allen (3). Twenty-four hours postinjury, the animals were given a single injection (ip.) of either saline or AMPH (2 mg/kg).

Sixty (POST) or 85 (ANT) days postinjury, animals were killed with sodium pentobarbital (1 ml, ip.). Frozen, 40 micron coronal sections were cut on a cryostat microtome. Every fifth section was mounted on slides and stained with thionin. Using tissue sections at bregma -0.26 -3.8 and -6.04 mm, the degree of gliosis and/or neuronal loss in the CA3 region of the hippocampus, dorsolateral striatum, and medial geniculate was estimated by a trained observer blind to experimental treatment using the following 4-point rating scale: 0 = no, 1 = slight, 2 = moderate, and 3 =

severe cell loss and/or gliosis.

The thionin-stained sections were projected and traced onto a grid calibrated for millimeters. The area of necrotic cavitation was calculated from all sections throughout the injury and summed (range was 23 to 49). The rostral and caudal extent of each lesion was measured for the A-P length. The equation below was used to estimate the volume of cavitation necrosis in each brain:

(A-P length) * [(Summed Area) / # of sections]

where A is the rostral border of the injury (mm)
P is the caudal border of the injury (mm)
Area is the sum of the areas (mm2) over all sections.

Ventricular areas were similarly computed from serial drawings of each coronal section through the rostral-caudal extent of the ventricular enlargement. At each coronal level the area of the contralateral ventricle was subtracted from the area of the ventricle ipsilateral to the injury.

Sensorimotor cortex contusion produced ventricular dilation in the ipsilateral lateral ventricle. In only three of the twenty-four animals was there no observed ventricular enlargement.

Ventricular enlargement was greater in saline treated animals that underwent posterior contusion to the sensorimotor cortex than in any other treatment group, but was significant only from the posterior group treated with amphetamine ($p<.040$). It is not clear whether AMPH reduced dilation in POST group.

A single injection of 2 mg/kg amphetamine administered 24 hours after the posterior contusion injury resulted in a reduction of cavitation necrosis volume. Since this reduction was previously observed in the embolic stroke paradigm, a one tail t-test was conducted to yield $p<.031$. Although the graphs show similar trends, the enlargement of the ventricle and cortical cavitation necrosis are not correlated ($r = .16$). The present study revealed no significant differences in secondary pathology.

A single AMPH treatment given 24 hours after a POST, but not ANT, contusion of the right SMCx significantly reduces the volume of cortical cavitation necrosis (Fig. 15). Ipsilateral ventricular enlargement is consistently found after either ANT or POST focal cortical contusions (Fig. 16). Preliminary observations indicate the presence of gliosis in tissue adjacent to the enlarged ventricles. A single AMPH treatment 24 hours post injury significantly reduced the volume of ventricular enlargement in the POST, but not ANT, contusion group. The ventricular enlargement seen after contusion is NOT correlated with the volume of necrotic cavitation. Upon preliminary observation, the same magnitude of dilation is not seen after ablation as in contusion. Further, the ependymal lining of the ventricles appears to be disrupted in contused animals, but intact in ablated animals. Also the gliotic reaction near the ventricle is not observed in the ablation injury, as it is after the contusion injury. Post traumatic hydrocephalus is commonly diagnosed after brain injury in human. The model used in our laboratory should prove useful in delineating the mechanisms of this reaction.

Even in areas considered relatively homogeneous (i.e. the sensorimotor cortex) we found differential effects of the AMPH treatment on both cortical cavitation and ventricular dilation. The results of this study

indicate that effects of drug treatment after brain injury can be dramatically altered by slight variations in injury and these results may have profound implications for development of pharmacotherapies for treating post traumatic sequelae.

HIPPOCAMPAL CELL DEATH AND EXCITOTOXICITY

Traumatic brain injury of the right sensorimotor cortex (SMCX) produces a transient left hemiplegia and widespread metabolic depression which is alleviated by AMPH and worsened by haloperidol in the rat (18). Investigating mitochondrial oxidative respiration using measurements of cytochrome oxidase (CYO) histochemistry with computer-assisted microdensitometry (CAM) at 2 days following SMCX injury, we have found that there is a widespread depression of this oxidative enzyme in morphologically intact structures remote from the injury. This depression in extrapyramidal structures is alleviated by AMPH. Similar unpublished findings have been obtained in studies of local cerebral glucose utilization. Complimenting the metabolic experiments and also supporting the NE hypothesis for recovery from hemiplegia are recent findings indicating a remote depression of NE, reversible by AMPH, in intact structures after SMCX ablation (19). The current study investigated CYO histochemistry on contusion and ablation quantified by computer-assisted microdensitometry (CAM). At 2, 6 and 16 days post-contusion and at 2 days post-ablation, rats recieved either 2mg/kg AMPH or saline. Several areas were investigated, and areas of interest for this particular study were the entorhinal and auditory pyriform cortices.

Male Sprague-Dawley rats weighing 300 to 350 grams at the time of surgery received either sensorimotor cortex contusion ablation or sham surgery. The procedure for producing a focal cortical contusion is described in detail by Feeney et. al., 1981(4). The apparatus consists of a stainless steel guide tube through which a weight is dropped to produce an impact force of 400mg/cm^2. The device is mounted on a stereotaxic carrier and the base of the device consists of a stainless steel circular foot plate which the falling weight strikes. A craniotomy is performed over and the foot plate of the contusion device was centered at 2.5mm posterior and 3.25mm lateral to sagital suture. The footplate is positioned so that it rests upon the surface of the intact dura. To prevent contused cortex from herniating through the cranial defect, craniotomies are made only slightly larger than the diameter of the footplate after impact, the boneflap is replaced, sealed with bonewax and the scalp suture closed. The sham surgery will involve only the opening the scalp and resuturing.

After testing animals within their respective time frames, all animals were sacrificed with sodium pentobarbital and transcardially perfused with phosphate buffered saline and then with a fixative solution of sucrose/paraformaldehyde/gluteraldehyde. The brains were extracted and immersed in 10% sucrose solution at 4 degrees centigrade overnight. After three days the brains were frozen with CO_2 and then 40 micron sections were mounted on slides. Slides were stained with cytochrome oxidase (CYO) histochemistry following the method of Wong-Riley (1989)(20). Sections were analyzed using computer-assisted microdensitometry (CAM).

Prior to surgery all animals were trained to traverse the beam. The first day consists of giving the animals three non-rated trials using successive approximation procedure. On trial 1 the rat is placed on a beam

just outside the goal box; On trial 2 at the midway point on the beam; and on trial 3 at the start position. On the next day, and every other day thereafter, each rat receives a single, rated trial on the beam from the start point. Locomotor performance and ability in transversal the beam is rated by two observers, one blind to all treatment conditions, using a 7-point rating scale (21). Criterion for the successful completion of beam-walking training is defined as achieving a presurgery score of "7" on three successful trials.

After reaching criterion, rats were to receive either contusion, ablation or sham surgery. At 24±1hour post surgery the animals are given a single trial to assess the severity of their disability. 'within five minutes following this test, the animals were to receive AMPH (2mg/kg i.p.), or saline (SAL). Ablation animals were tested at 2 days (given AMPH or SAL) postinjury and contused animals were tested (with AMPH or SAL) at 2, 6 and 16 days postinjury.

Mean optical density readings of the ipsilateral vs contralateral entorhinal, auditory and pyriform cortices for the 2 day contusion animals showed a significant increase as compared to the contralateral side ($p<.05$) (See figures 17, 18, and 19). Mean optical density readings of ipsilateral vs contralateral entorhinal auditory and pyriform cortices for the 2 day ablation animals revealed no significant difference between the ipsilateral and contralateral side in any of these three cortical structures (See figures 20, 21, and 22). At 2 days following unilateral contusion injury but not after SMCX ablation, cytochrome oxidase activity is elevated in the ipsilateral entorhinal, auditory and pyriform corticies compared to these areas contralateral to the injury. Amphetamine administered 24 hours post contusion normalizes the observed CYO ipsilateral hyperactivity in the entorhinal and auditory corticies (Figs. 17 and 18) but not in the pyriform cortex (Fig. 19) At 2 days following unilateral SMCX contusion injury, but not after SMCX ablation, cytochrome oxidase activity is elevated in the ipsilateral entorhinal, auditory and pyriform cortices compared to these areas contralateral to the injury. A single dose of AMPH (given at 24 hours after sensorimotor cortex contusion) alleviates the ipsilateral increase in cytochrome oxidase histochemical staining in the entorhinal and auditory cortices but not pyriform cortex. The effect of AMPH given after SMCX injury is to normalize perturbed oxidative metabolism in selected areas. For example, following SMCX ablation, AMPH increases cytochrome oxidase activity in hypometabolic extrapyramidal structures (18) but decreases cytochrome oxidase activity in hypermetabolic entorhinal and auditory cortices following SMCX contusion. Sensorimotor cortex contusion and ablation produce different metabolic sequelae that may be related to posttraumatic secondary cell death observed after contusion but not ablation.

AMPHETAMINE AND FUNCTIONAL RECOVERY AFTER RESERPINE

Unilateral ablation of the sensorimotor cortex (SMCX) produces a transient contralateral hemiplegia, and by rating locomotor agility on a beamwalking (BW) task, rate of functional recovery can be readily measured. Using this model in the rat has shown that a single d-amphetamine (AMPH) administration or its analogs, (e.g. phentermine) at 24 hrs after SMCX injury, when combined with BW experience, produces an enduring facilitation of recovery of BW ability (21,7). Studies comparing various drugs using this model in rat and cat suggests a central role for

norepinephrine (NE) in this effect of AMPH (6,26).

This study utilized a different approach to evaluate the contribution of striatal dopamine (DA) in this potentially therapeutic effect of AMPH. Reserpine administration depletes vesicular stores of DA and NE by inhibiting vesicular uptake and storage (27). Reserpine pretreatment enhances AMPH evoked striatal DA release and DA stereotypies (28,29), while a mobilisation of vesicular NE is required for high and sustained rates of outward transport of NE from intact adrenergic varicosities (30,27). Therefore, if the AMPH facilitation of recovery from hemiplegia involves striatal DA, reserpine pretreatment should enhance this effect, whereas, if NE is essential, this AMPH effect should be blocked.

Fifty-four male Sprague-Dawley albino rats weighing between 300 and 350 gm at the beginning of the experiment were used in the 3 experiments. The animals where individually housed in standard wire-mesh cages, maintained on a 12:12 hr light:dark cycle, and given ad lib access to food and water.

To assess recovery from hemiplegia, the animals were trained to traverse a narrow wooden beam to escape a bright light (60 watt) and loud noise (68 dB). If necessary prodding of the animals by gently tapping them on the tail with a standard pencil when they did not attempt movement for 5 or more seconds. The trial ended when the rat entered a dark goal box similar in appearance to a home cage.

Under asceptic conditions SMCX ablation or sham surgery was performed under halothane anesthesia using a sterotaxic apparatus. A craniotomy extending from 2mm anterior and 4 mm posterior to bregma and 5mm lateral from the sagittal sinus was performed, the dura removed and the right SMCX ablated by suction. The wound cavity was filled with sterile gelfoam and the scalp sutured closed. For sham surgeries the same procedure was followed but the skull was not open as this induces minor motor deficits and small cortical lesions.

BW training consisted of three consecutive trials. First, the rat was placed just outside the goal box, second, half the distance from the goal, and third at the starting point of the beam. Each animal received one daily trial from the start point. Then locomotor ratings were made by two observers, one blind to experimental conditions using a scale described in Table 2 (21). A score of 7 is considered normal and 1 a complete inability to maintain balance.

Training was completed when an animal scored three consecutive scores of 7 and the animals were then randomly assigned to one of four treatment conditions 1) abl-AMPH, 2) abl-saline, 3) sham-AMPH, 4) sham-saline. At 19.5 +/- 1 hr post-surgery, the animals were given one BW trial to assess the extent of motor deficit. Immediately after this baseline trial all animals were given 5 mg/kg reserpine i.p. solubilized in saline with 6 mg/ml citric acid and returned to their home cages. At 4.5 hrs after reserpine administration each animal received a single BW and either a 2 mg/kg i.p. injection of d-AMPH sulfate or saline and returned to their home cages. At 15 minute intervals for 1 hr after AMPH administration the animals were observed and scored for the intensity of sterotypies using a 5-point scale (31) described in Table 1.

Three experiments assessed the effects of the drug manipulation on BW recovery.

EXP-1) Animals received a BW score post-drug administration at 1,2,3,6,24 hrs and every other day thereafter for 16 days post-surgery.

EXP-2) Because the reserpine pre-treatment increased stereotypies only during the first hour, in this experiment rats were BW tested at

15,30,45 minutes, 1,2,3,6,24 hrs and every day for 16 days following AMPH or saline administration.

EXP-3) To prolong the period of dopamine activation, AMPH was administered twice and BW tests given 15,30,45,60 minutes following each injection of AMPH or saline. Single BW trials were then run at 3,6,24 hrs post AMPH or saline and then every other day thereafter relative to the first drug administration.
Animals were sacrificed by decapitation at the end of the 16 day testing period.

Following administration of reserpine all animals were catatonic and severely impaired on the BW task. As has been frequently reported rats given AMPH displayed a marked increase in sterotypies and motor activity compared to those given saline (Fig 4,6) and this endured for 1 hour. Reserpine catatonia is characterized by ptosis, and an arched back position. The ability of the animals to traverse the beam was dependent upon the state of activity and disability level. This is most clearly seen in the reserpinized sham operate animals that for 24 hrs post-reserpine were completely incapable of BW except for 1 hr after given AMPH when they easily traversed the beam and returned to their normal pre-reserpine BW agility (Figs 3,5).

In EXP-1 reserpine blocked the facilitation of BW recovery (Fig 2) normally seen after AMPH using this post drug BW test schedule (refer to Fig 1). No effects of drug treatment on BW recovery were observed. The saline and AMPH lesioned groups showed a similar course of BW recovery. The sham-AMPH animals returned to pre-reserpine BW scores 24 hours after reserpine administration. EXP-1 clearly demonstrates that reserpine blocks the usual AMPH facilitation of motor recovery (21,7). Reserpine also blocks BW ability of sham operated animals. Other alpha-1 noradrenergic antagonists or alpha-2 agonists, (e.g. haloperidol (21) and clonidine (6)) selectively block BW recovery only in SMCX injured rats without affecting BW ability of sham operates. Perhaps the non-selective effect of reserpine may only occur at high doses. AMPH induced sterotypies and increased activity endured for only 1 hour after injection (Fig 4), thus in EXP-1 BW was measured only at one time at the end of AMPH activation. To fairly assess BW ability during AMPH activation in reserpinized rats, BW tests in EXP-2 and EXP-3 were scheduled so animals were tested during AMPH activation. The data from EXP-2 are depicted in Fig 3 and BW trials were conducted during the first hour after AMPH or saline injections. Note that sham lesioned rats returned to their pre-reserpine BW scores, whereas lesioned animals receiving AMPH showed a significant improvement compared to saline during the first hour after AMPH (ANOVA, 15-30 min, $F = 13.67$, $df = (1,9)$, $p = .005$: 45-60 min, $F = 7.14$, $df = (1,9)$, $p = .026$). However, the transient recovery seen is of particular interest since lesioned-AMPH animals did not improve above post-lesion disability scores (pre-reserpine), but only achieved scores to baseline post-lesion levels (t-test, $df = 9$, $p = .289$). Therefore, in reserpinized rats, AMPH appeared to alleviate the catatonia allowing the rat to temporarily perform on the BW task only to his level of post-lesion disability without the recovery seen in non-reserpinized animals. No significant differences on BW recovery was obtained over the 16 days post injury between groups (MANOVA, Roys = .5529, $df = (1,2,1)$, $p = .604$).

In EXP-3 a second injection of AMPH or saline was given 1 hour after the first drug administration, to prolong the period of behavioral activation allowing the number of BW trials conducted to be similar to those conducted in the non-reserpinized model (see Fig 1). This would

permit a similar amount of BW experience during AMPH, which has been demonstrated to be critical for AMPH facilitation of motor recovery (21,7). The results of EXP-3 are shown in Fig 5. Sham operates returned to pre-reserpine BW levels for 1 hr after each AMPH administration, corresponding with AMPH activation and stereotypies (see Fig 6).

All saline treated reserpinized animals showed no improvement of BW ability during the first 24 hours post injury whether SMCX lesioned or sham operate. As in EXP-2, lesioned animals receiving AMPH showed a transient (2 hr) significant improvement of BW ability compared to lesioned-saline animals (ANOVA: 15-30 min, $F = 15,6$, $df = (1,11)$, $p = .002$: 1hr 15-30 min, $F = 6.91$, $df = (1,11)$, $p = .023$). Again as in EXP-2, the BW performance of the lesioned-AMPH group did not significantly improve above post-injury baseline disability (t-test, $df = 11$, $p = .633$).

Thus EXP-2 and EXP-3 show that AMPH is able to reinstate the ability of reserpinized rats to traverse the beam, but only to the ability shown prior to reserpine treatment (post-lesion baseline disability). The time course of this reinstatement of BW ability parallels the time course of AMPH induced stereotypies (Fig 6). No significant differences were obtained between recovery rates of lesioned animals given AMPH or saline over the 16 day BW test period (MANOVA: Roys = .371, $df = (1,.5,3.5)$, $p = .223$).

In reserpinized, sensorimotor cortex ablated, catatonic rats amphetamine activation temporarily allows locomotor performance only to baseline disability levels. No improvement is seen in contrast to non-reserpinized rats. This reserpine blockade of recovery from hemiplegia occurs during the potentiation of amphetamine induced stereotypies, a behavior mediated by dopaminergic systems. These data considered together with previous work indicate that amphetamine facilitated recovery of locomotor function assesed by beamwalk ability is not mediated by release of reserpine insensitve stores of dopamine. Since in reserpinized rats, amphetamine increases striatal dopamine release but does not substantially release norepinephrine, these data provide further evidence that the AMPH facilitation of recovery from hemiplegia may be mediated through noradrenergic systems.

MONOAMINE AND METABOLITE LEVELS

The purpose of this study was to assess changes of monoamines and their metabolite levels after right sensorimotor cortex (SMCx) ablation. We also investigated the effect of amphetamine (AMPH) administration upon monoamine and metabolite levels after SMCx ablation, since AMPH has been shown to ameliorate numerous deficits after cortical injury.

For example, a single dose of AMPH restores binocular depth perception in cats after bilateral visual cortex ablation (32). Following motor cortex injury, AMPH facilitates recovery from hemiplegia (21,33,34) and transiently reinstates tactile placing (35). Using the same paradigm in rats, AMPH has no effect on recovery of forepaw grasping deficits (26). In addition, AMPH administration reduces morbidity and mortality after cerebral infarction in the rat (16), and alleviates depression of cerebral metabolism in some brain structures (18).

Several studies implicate norepinephrine (NE) in the AMPH effect on recovery (26) and neuronal plasticity (36). Prior work on cortical injury and monoamines report persistent bilateral depletions of NE and dopamine

(DA) in widespread regions of cerebral cortex and brainstem of rats (37,38). Also, Finklestein et al. (39) have reported decreases in NE and serotonin within the ipsilateral cortex, while 5-hydroxyindoleacetic acid (5-HIAA) levels were increased throughout ipsilateral cortex one week after cortical injury. In addition, they reported decreased monoamine levels and increases of the metabolites within subcortical structures. These results suggest that perturbations of monoamine metabolism within remote brain regions may underlie some behavioral deficits, metabolic changes, and secondary neuronal pathology observed after cortical injury.

Sprague-Dawley rats (250-350 grams), under ketamine-nembutal anesthesia, the right SMCx was ablated or a sham surgery performed using procedures described elsewhere (21,40). One day post-surgery, rats received either AMPH (2mg/kg i.p.) or saline, and were sacrificed by decapitation at 2 days post-surgery. The brains were quickly removed and the frontal pole (FP), cerebellar (CB) cortex, cortex posterior to the injury site (PC), hippocampus (HP), and caudate/putamen (CP) dissected and flash frozen. Analysis of norepinephrine (NE), dopamine (DA), the monoamine metabolites 3-methoxy-4-hydroxyphenylglycol (MHPG), 3.4-dihydroxy-phenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5-HIAA) levels were performed using high perofrmance liquid chromatography. The experiment began with eight animals per group, however, several animals were lost due to surgical complications. In addition, numerous brain samples were not used in the statistical analysis due to contamination of the sample and or poor chromatographic results. Therefore, the number of brain samples in each group analyzed statistically varied between N = 3-9.

All neurotransmitters within each brain region were tested for the main effects of drug treatment and surgery as well as the interaction at an alpha = .05 using ANOVA. A-priori between group comparisons (simple main effects) were done using MANOVA and tested at Bonferroni adjusted p-values = .025.

Because of the volume of information contained in the HPLC data, please see table 2 for details of the results.

The general finding of a widespread depression of monoamine levels after focal cortical injury, and the alleviation of this depressed activity by a single dose of AMPH, may be the basis for AMPH facilitation of behavioral and metabolic recovery after cortical injury.

The data from this investigation indicate that 2 days after unilateral SMCX ablation there is a bilateral depression of NE, DA, and DOPAC levels. In contrast, the metabolite of serotonin, 5-HIAA is significantly elevated ipsilateral to the injury. This diffuse monoamine respose to injury is likely a result of damage to the axons of the locus coeruleus (LC) by the SMCX ablation. The resulting retrograde reaction of LC somata is to change from transmitter synthesis to a repair process (41).

The elevation of 5-HIAA after injury may result from the depression of LC activity, due to a reciprocal inhibitory interaction between the LC and raphe serotonergic neurons (42). Since AMPH produces an enduring normalization of depressed NE levels, but not 5-HIAA, suggests the AMPH effect is at LC terminals.

Supporting this hypothesis are data from studies of recovery from hemiplegia using the same paradigm as the present investigation. Infusion of NE into the contralateral, but not the ipsilateral, CB cortex facilitates recovery from hemiplegia. Additionally, NE, but not DA intraventricular infusion facilitates locomotor recovery (40). However, no significant effect of AMPH upon NE or MHPG levels was observed within

the contralateral CB cortex. Perhaps, microdialysis will provide more sensitive and reliable measures of monoaminergic activity.

In summary, changes of monoamine metabolism within structures remote from cortical injury, may play a diverse role in behavioral deficits, metabolic abnormalities, and secondary cell death.

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TABLES

Table 1

7 Animal that can traverse the narrow elevated beam normally with no more than 2 foot slips of the left hindlimb.

6 Animal that is able to locomote across the beam using the left hindlimb to aim in more than 50% of its steps on the beam.

5 Animal that is able to locomote across the beam using the left hindlimb to aid in less than 50% of its steps on the beam.

4 Animal that can traverse the beam, placing the foot on the left himdlimb on the horizontal surface of the beam without using the limb to aid in forward locomotion.

3 Animal that can traverse the beam while dragging the affected himdlimb or showing treading/stepping motions with the left hindlimb, but is not capable of placing the left hindlimb on the horizontal surface of the beam during traversal.

2 Animal that is unable to traverse the beam, but is able to place the left hind limb on the beam.

1 Animal that is unable to traverse the beam or place the left himdlimb on the beam.

TABLE 2

Effect of Cortical Injury
1. Right sensorimotor cortex (SMCX) ablation reduces DA and DOPAC levels bilaterally in the caudate/putamen (CP) compared to sham operates given saline (Figs. 24 and 25).
2. Right SMCX ablation reduces NE levels bilaterally in the frontal pole (FP) and posterior cortex (PC) compared to sham operates, whereas MHPG is elevated bilaterally in the cerebellar cortex (Figs. 27 and 28).
3. Right SMCX ablation elevates the serotonin metabolite 5-HIAA levels only within ipsilateral cortical and subcortical structures (26).

Effect of Amphetamine
4. Administration of AMPH to cortically ablated rats alleviates the depression of NE, DA, and DOPAC levels within some cortical and subcortical structures (Figs. 24, 25, and 27).
5. Administration of AMPH to cortically ablated rats had no effect upon the elevated 5-HIAA levels (Fig 26).
6. Administration of AMPH to sham operates reduced levels of NE bilaterally within the cerebral cortex measured 24 hours later (Fig 27).

Figure Captions

Figure 1.

Device for producing focal contusions of rat cerebral cortex. A 40 cm tube guided a falling weight onto a footplate that rested on exposed dura over the hindlimb area. A sleeve at the base of the tube was set to a height permitting a maximum of 2.5 mm penetration into the brain. This eliminated subcortical injury. A force of 400 gm/cm was produced by dropping a 20 g weight 20 cm.

Figure 2.

Mean(+SEM) rating of degree of gliosis in the medial geniculate nucleus (MGN), dorsolateral striatum (DLS), and CA3 region of the hippocampus (CA3) in the hemisphere ipsilateral to the SMCx injury, relative to the contralateral homologous structures, expressed as a function of ablation, contusion, and contusion/ketamine (CONT/KET/) conditions. Contusion injury produced significantly more gliosis than did ablation in the MGN, $t(8) = 3.0$ and DLS, $t(8) = 4.20$, all p's $< .05$. In addition, post injury ketamine treatment significantly reduced the amount of gliosis in both the DLS, $t(8) = 3.0$, and MGN, $t(8) = 2.27$, $p < .05$.

Figure 3.

Mean ($\pm$SEM) rating of degree of neuronal loss in the MGN and CA3 region in the hemisphere ipsilateral to the injury, relative to the contralateral homologous structures, expressed as a function of ablation, contusion, and contusion/KET, conditions. Contusion injury produced significantly more neuronal loss than ablation in the MGN, $T(7)=2.83$ and CA3 region, $t(8) = 2.0$, all p's $< .05$. Also ketamine treatment significantly reduced this loss in the MGN, $t(7) = 2.88$ and CA3 region, $t(8) = 3.45$, all p's $<.05$.

Figure 4.

Mean ($\pm$SEM) volume of primary cavitation necrosis (cubic mm) expressed as a function of ablation, contusion, and contusion/ketamine conditions. There was no significant difference between ablation and contusion procedures $t(8) = 2.213$, $p >.05$. Ketamine treatment reduced the amount of primary necrosis after the contusion injury $t(8) = 5.86$, $p < .001$.

Figure 5

Mean (+SEM) ratings (by blind observer; 0=none, 3=severe) of ipsilateral hippocampal CA3 pyramidal cell loss from frozen thionin-stained sections 3-4 weeks after SMCx contusion. There were no significant differences between the pentobarbital (60mg/kg) and halothane (4% halothane with 4 liters/minute oxygen for induction and 1.5% halothane with 1.5-2.0 liters/minute oxygen for maintenance) condition. However, there was a significant attenuation $F(2,30)=23.81$ $p<.001$, of cell loss was observed in animals contused with the combination of low dose ketamine (60mg/kg) and pentobarbital (21mg/kg). Only limited gliosis appeared in the CA3 region despite extensive neuronal loss. In other animals studied

at 6 days post contusion, a more severe gliosis was observed The selective loss of hypoxia and ischemia-resistant CA3 neurons while sparing CA1 pyramidal cells has also been described in rats following fluid percussion injury (2) or kainic acid administration (22).

Figure 6

Mean (±SEM) rating of hippocampal CA3 pyramidal cell loss. A high dose of Ketamine (150mg/kg) given 5 minutes after contusion dramatically blocks the CA3 neuronal loss, $F(1,18)=19.97$, $p<.001$.

Figure 7

Mean (±SEM) volume of cavitation necrosis. Post-contusion administration of a high dose of ketamine significantly reduced the volume of cortical necrosis, $t(8)=5.86$, $p<.001$.

Figure 8

Mean (±SEM) ratings of thalamic ventral basal complex gliosis. A slight, but nonsignificant, increase in VBC gliosis was observed in contused rats given a high dose of ketamine. The trend in similar to the slight increase in Ketamine/pentobarbital anesthesia animals in figure 2.

Figure 9

Mean (±SEM) ratings of thalamic ventral basal complex (including the somasthetic and cerebellar-cortical nuclei VPM, VPL, and VL) gliosis (VBC gliosis and neuronal loss were highly correlated, $r = .946$). Unlike hippocampal CA3 cell loss after contusion there is no significant effect of anesthetic condition.

Figure 10

The mean (±SEM) and regression lines for the number of days to recover baseline beam-walking performance. Hippocampal CA3 pyramidal cell loss was not significantly correlated to recovery. Thalamic ventral basal complex cell loss and gliosis was significantly correlated with beam-walking.

Figure 11

The mean (±SEM) number of days to recover baseline beam-walking ability. Animals given high dose of ketamine 5 minutes after contusion under halothane were significantly slower to recover to pre-contusion performance, $F(1,15)$ $p=.027$

Figure 12

The mean (±SEM) number of days to recover baseline beam-walking performance. There were no significant effects on the mean number of days to recover baseline performance across anesthetic conditions.

Figure 13

Compared to saline controls, the administration of 8, but not 1 or 4, mg/kg of methoxamine significantly reduced hippocampal CA3 pyramidal cell loss when given 24 hours following focal impact injury to the right sensorimotor cortex in rats. The data were analyzed with the following pairwise comparisons (ANOVA) using Tuckey's HSD: saline vs methoxamine-1 [$T(43) = .065$], saline vs methoxamine-4 [$T(43) = 1.064$, $p = .293$], and saline vs methoxamine-8 [$T(43) = 2.08$, $p < .05$].

Figure 14

Methoxamine at both 4 (n=2) and 8 (n=2) mg/kg lowered body core temperature, relative to saline (n=1), with the former producing a slightly larger effect than the latter in this small sample of normal rats. Future work will incorporate measures of brain as well as body temperature in both normal and injured animals.

Figure 15

Similar to pathology after embolic stroke (2), AMPH treatment significantly reduced the volume of cavitation necrosis in the POST group ($p<.05$ using a one tail Mann-Whitney test, and $p<.03$ using a one tail parametric t-test). The ANT SMCx had not previously been contused in our model, and interestingly, AMPH had no effect on the volume of cavitation in these animals.

Figure 16

Right SMCx contusion produced enduring enlargement of the lateral ventricle in 21 of the 24 animals. In the POST group, the enlargement was significantly reduced after AMPH treatment ($p<.04$ using a two tail t-test). Preliminary observation also revealed gliosis in most brain regions bordering the ventricle ipsilateral to contusions. No such reduction of ipsilateral ventricular enlargement was found for ANT contused animals receiving a single AMPH treatment. It is important to note that the volume of cavitation necrosis is NOT CORRELATED with the volume of ventricular dilation ($r=.16$). Thus, ventricular enlargement cannot be attributed to a single expansion of the ventricle to "fill in" for necrotic cortical tissue.

Figure 17

Represents mean proportional scores [1-(EC/CC)] and S.E.M. of CYO optical density readings for the ipsilateral entorhinal cortex (EC) contusion saline and amphetamine animals. Comparison are made to the sham saline and sham amphetamine animals. Proportional scores were done to minimize intra and inter-CYO staining variability (EC= Entorhinal Cortex, CC=Corpus Callosum). Their is a significant difference between the contusion saline animals optical density readings and the contusion amphetamine group ($p<.02$).

Figure 18

Mean proportional scores [1-(AC/CC)] and S.E.M. of CYO optical density readings for the ipsilateral auditory cortex (AC) contusion saline and amphetamine animals. Comparisons are made to the sham saline and sham

amphetamine groups. Note the marked difference of the contusion saline and contusion amphetamine animals to the sham groups. In comparison to Figure 1 (above), note that the contusion amphetamine group for the auditory cortex sh. .s a greater optical density reading than the contusion amphetamine animals for the entorhinal cortex.

Figure 19

Similar to the auditory and entorhinal cortex, the level of this mitochondrial enzyme is significantly higher ($p<.05$) in the pyriform cortex ipsilateral to a cortical traumatic injury compared to the contralateral pyriform cortex. This ipsilateral elevation of cytochrome oxidase activity is not observed following ablation (see figure 22). Unlike the hypermetabolism in entorhinal and auditory cortex, the pyriform ipsilateral-contralateral differences in cytochrome oxidase activity are NOT affected by AMPH treatment.

Figure 20

Mean proportional scores [1-(EC/CC)] and S.E.M. of CYO optical density readings for the entorhinal cortex (EC) ablation saline and ablation amphetamine animals. Comparisons are made to the sham saline and sham amphetamine groups. The ablation amphetamine animals show a marked increase in optical density readings as compared to the ablation saline and two sham groups. Note that this is a reversal of what was observed with the entorhinal cortex contusion animals in Figure 1.

Figure 21

Mean proportional scores [1-(AC/CC)] and S.E.M. of CYO optical density readings for the auditory cortex (AC) ablation saline and ablation amphetamine animals. Comparisons are made to the sham saline and sham amphetamine groups. Notice the almost identical optical density readings of these animal groups to the entorhinal cortex ablation animals in Figure 3.

Figure 22

After SMCX ablation, cytochrome oxidase activity is not significantly elevated in the ipsilateral pyriform cortex as compared to the contralateral pyriform cortex. Unlike entorhinal and auditory cortex there is no increase in the cytochrome oxidase activity of the ipsilateral pyriform cortex after AMPH administration.

Figure 23

Sequential (23) and feedforward (24) models of the anatomical connections of the trisynaptic circuit. The feedforward model differs from the traditional conception as it includes monosynaptic connections from the entorhinal cortex to hippocampal CA3 pyramidal neurons. This model is based on data from recent electrophysiological experiments (24). Anatomical experiments also support the feedforeward model of the trisynaptic circuit (25). Both models could account for excessive entorhinal cortex neuronal activity leading to excitotoxic CA3 pyramidal cell death but the trisynaptic circuit requires assumptions of granule

cell amplification of this input and/or resistance to glutaminergic toxicity. The monosynaptic feedforward model better accounts for the marked CA3 pyramidal cell death with little granule cell pathology following cortical trauma (1).

Figure 24

Effect of cortical injury

Following unilateral SMCX ablation, dopamine (DA) levels are decreased bilaterally in the caudate/putamen (CP) compared to sham operates given saline.

Effect of amphetamine

Administration of AMPH to cortically ablated rats increased DA levels bilaterally within the CP to levels of sham operate controls. (a: $p<.05$ abl/sal vs sham/sal; b: $p<.05$ abl/amph vs abl/sal; e: significant at post-hoc adjusted alpha for multiple comparisons.)

Figure 25

Effect of cortical injury

Following unilateral SMCX ablation, the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) levels are significantly decreased bilaterally in the caudate/putamen (CP) compared to sham operates given saline.

Effect of Amphetamine

Administration of AMPH to cortically ablated rats increased DOPAC levels bilaterally within the CP to levels of sham operate controls. (a: $p<.05$ abl/sal vs sham/sal; b: $p<.05$ abl/amph vs abl/sal; e: significant at post-hoc adjusted alpha for multiple comparisons.)

Figure 26

Effect of cortical injury

Following unilateral SMCX ablation, levels of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) are significantly elevated ipsilaterally in the frontal pole (FP), posterior cottex (PC), and the caudate putamen (CP) compated to sham operates given saline, as pervioulsy reported by Pappius (43) and Finkelstein (39).

Effect of Amphetamine

Administration of AMPH to cortically ablated rats had no effect upon the elevated 5-HIAA levels within the FP, PC, or CP. (a: $p<.05$ abl/sal vs sham/sal; c: $p<.05$ abl/amph vs sham/amph; e: significant at post-hoc adjusted alpha for multiple comparisons.)

Figure 27

Effect of cortical injury

Following unilateral SMCX ablation, norepinephrine (NE) levels are decreased bilaterally in the frontal pole (FP) and posterior cortex (PC) compared to sham operates given saline.

Effect of amphetamine

Administration of AMPH to cortically ablated rats slightly

increased NE levels only within the contralateral FP and PC compared to ablated rats given saline. The lack of a response to AMPH within the ipsilateral FP and PC is presumably due to transection of noradrenergic fibers.
Administration of AMPH to cortically ablated rats slightly increased levels bilaterally within the cerebellar cortex (CB) compared to ablated rats given saline.
The effect of AMPH is qualitatively different between injured and non-injured animals. For example, uninjured rats given AMPH had decreased NE levels within the FP, whereas AMPH elevated NE levels in injured rats. Similar results were pbserved in the PC and CB cortex. (a: $p<.05$ abl/sal vs sham/sal; c: $p<.05$ abl/amph vs sham/amph; d: $p<.05$ sham/sal vs sham/amph; e: significant at post-hoc adjusted alpha for multiple comparisons; $p<.04$ for the interaction in the left FP, right CB cortex, and left PC.)

Figure 28

Effect of cortical injury

Following unilateral SMCX ablation, 3-metohxy-4-hydroxypehiylglycol (MHPG) levels are elevated bilaterally in the cerebellar cortex and significantly reduced in the ipsilateral posterior cortex (PC) compared to sham operates given saline.

Effect of Amphetamine

Administration of AMPH to cortically ablated rats had no effect upon levels of MHPG within the CB cortex which remained elevated bilaterally compared to hsam operates.
Amphetamine administration to cortically ablated rats elevated MHPG levels to sham operate control levels within the ipsilateral PC. (main effect of injury: $p=.05$ for right CB cortex; $p=.028$ for left CB cortex; a: $p<.05$ abl/sal vs sham/sal; b: $p<.05$ abl/amph vs abl/sal; c: $p<.05$ abl/amph vs sham/amph.)

Figure 29

Effect of cortical injury

The NE/MHPG and DA/DOPAC ratio are considered indicators of neurotransmitter turnover. Following unilateral AMCX ablation, the NE/MHPG ratio in significantly decreased in the contralateral cerebellar (CB) cortex compared to sham controls given saline, even though NE and MHPG levels were not significantly different between groups.
Following SMCX ablation, the DA/DOPAC ratio is significantly increased in the contralateral caudate putamen (CP) compared to sham controls given saline, which paralells changes of DA and DOPAC levels.

Effect of Amphetamine

Administration of AMPH to cortically ablated rats had on effect upon the reduced NE/MHPG ratio within the CB cortex.
Administration of AMPH to cortically ablated rats returned the DA/DOPAC ratio to sham operate control valued within the CP. (a: $p<.05$ abl/sal vs sham/sal; b: $p<.05$ abl/amph vs abl/sal; d: $p<.05$ sham/sal vs sham/amph; e: significant at post-hoc adjusted alpha for multiple comparisons.)

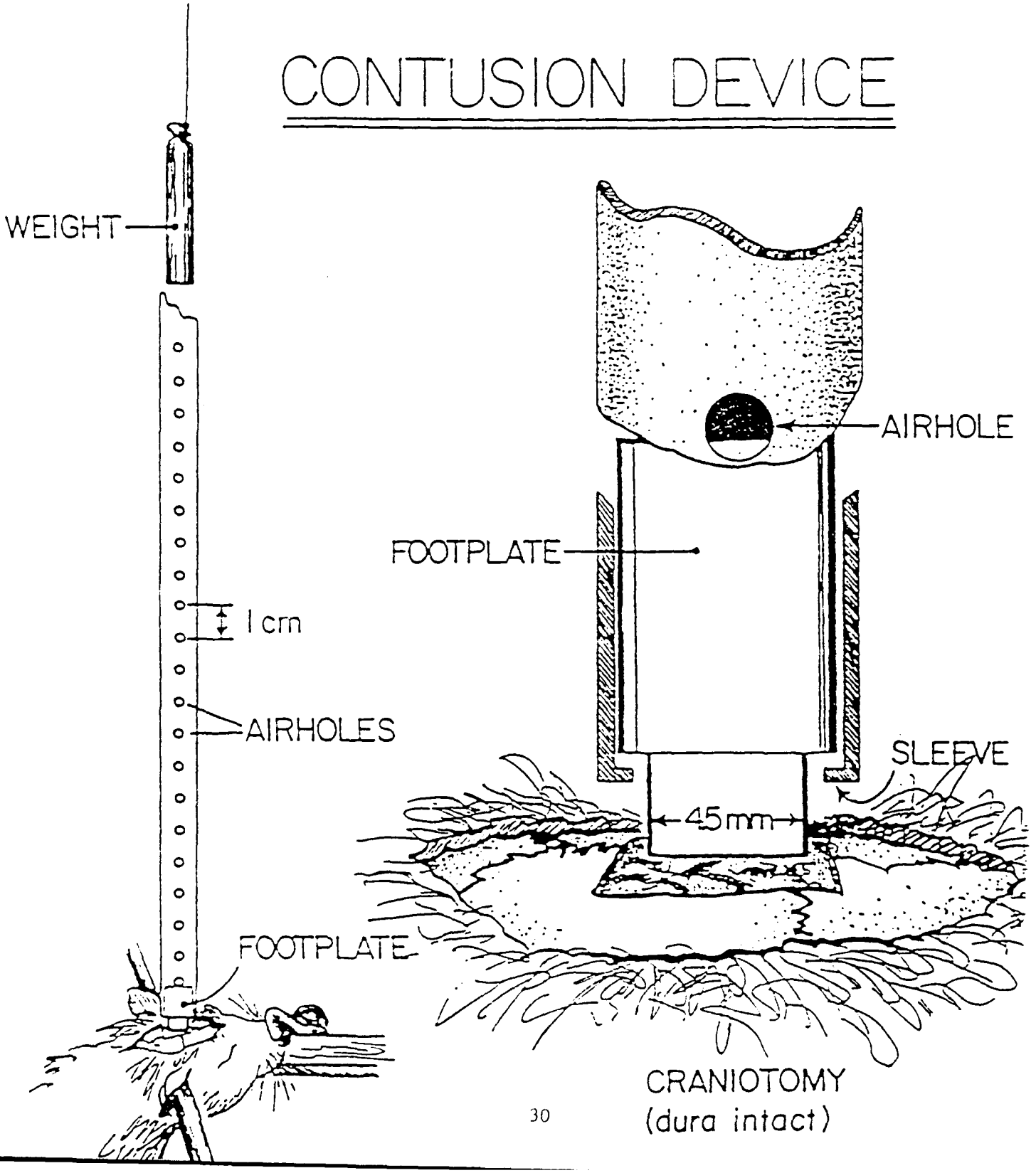

CONTUSION DEVICE
WEIGHT
AIRHOLE
FOOTPLATE
1 cm
AIRHOLES
SLEEVE
45mm
FOOTPLATE
CRANIOTOMY
(dura intact)

Figure 2

GLIOSIS

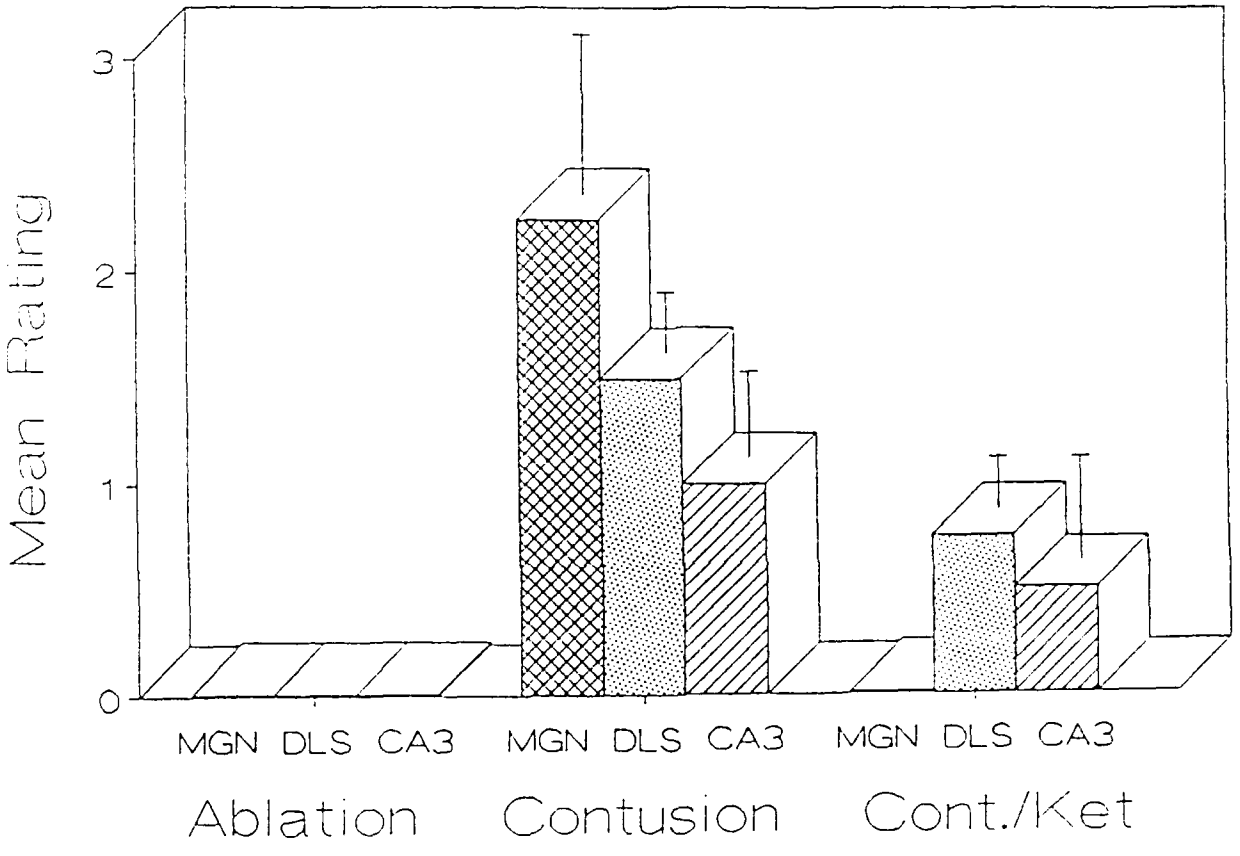
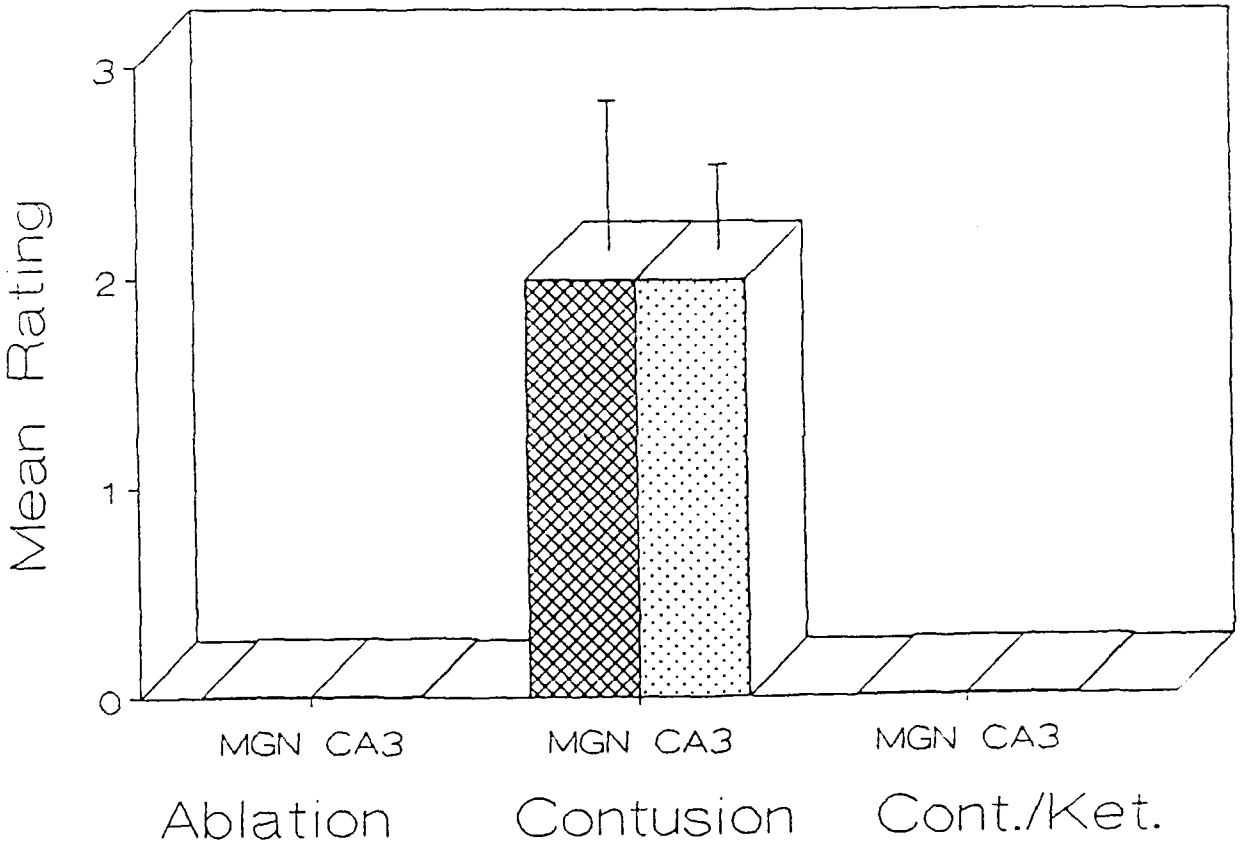


Figure 3

SECONDARY NEURONAL LOSS



VOLUME OF PRIMARY NECROSIS

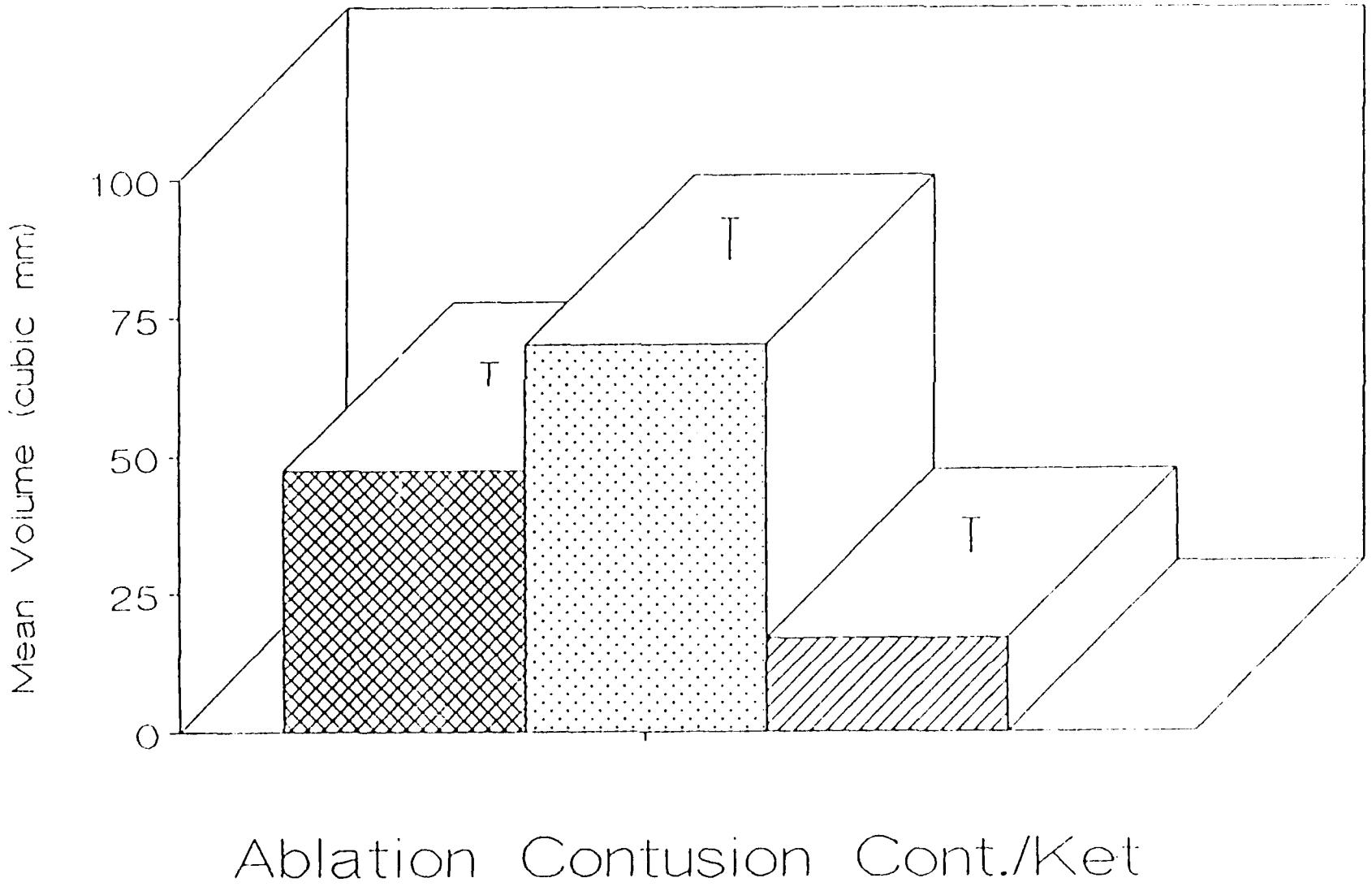


Figure 4

Figure 5

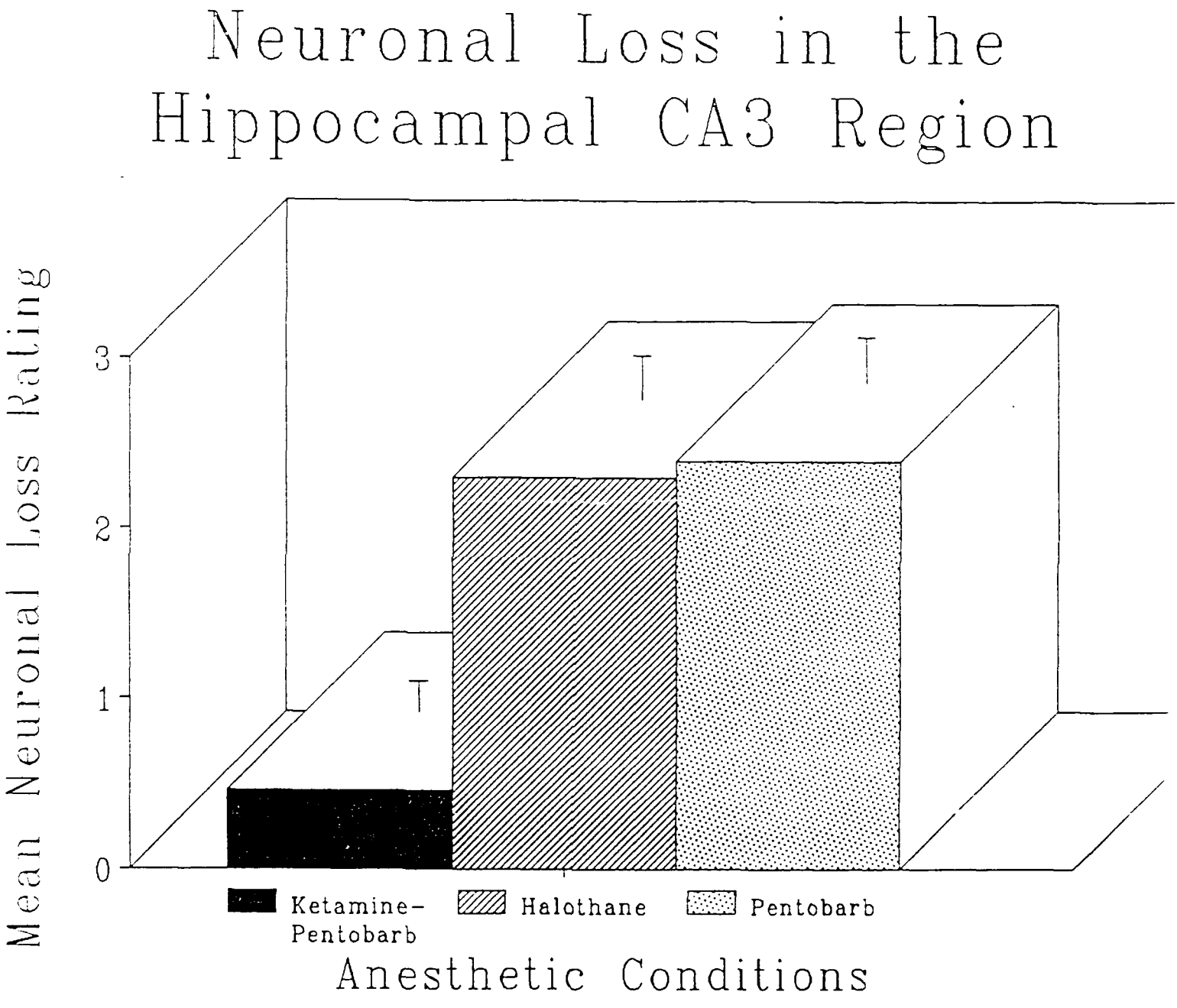
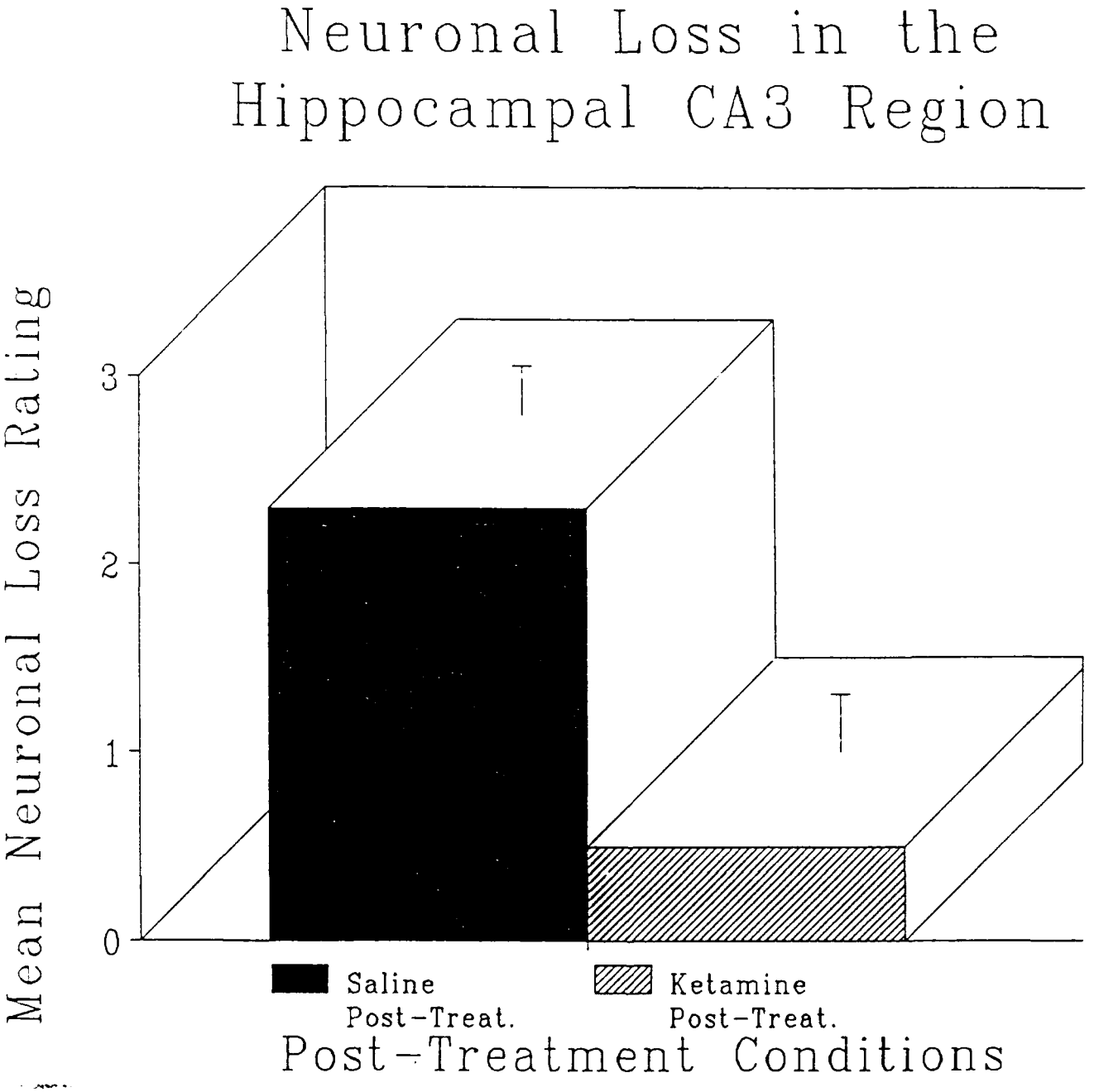

Neuronal Loss in the Hippocampal CA3 Region
Mean Neuronal Loss Rating
0
1
2
3
Ketamine-Pentobarb
Halothane
Pentobarb
Anesthetic Conditions

Figure 6


Neuronal Loss in the
Hippocampal CA3 Region
Mean Neuronal Loss Rating
0
1
2
3
Saline
Post-Treat.
Ketamine
Post-Treat.
Post-Treatment Conditions

Volume of Cortical Necrosis produced by Sensorimotor Cortex Contusion

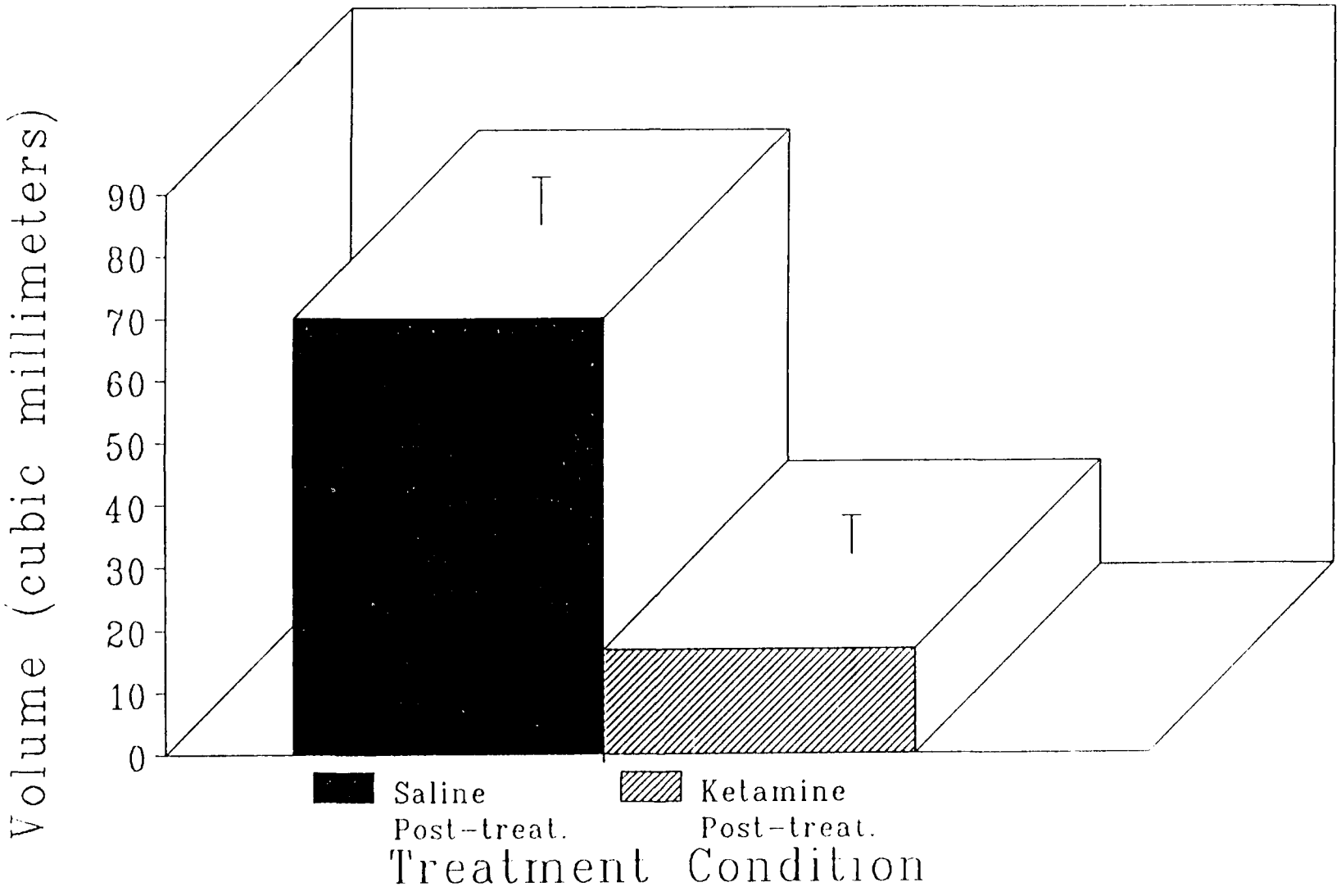
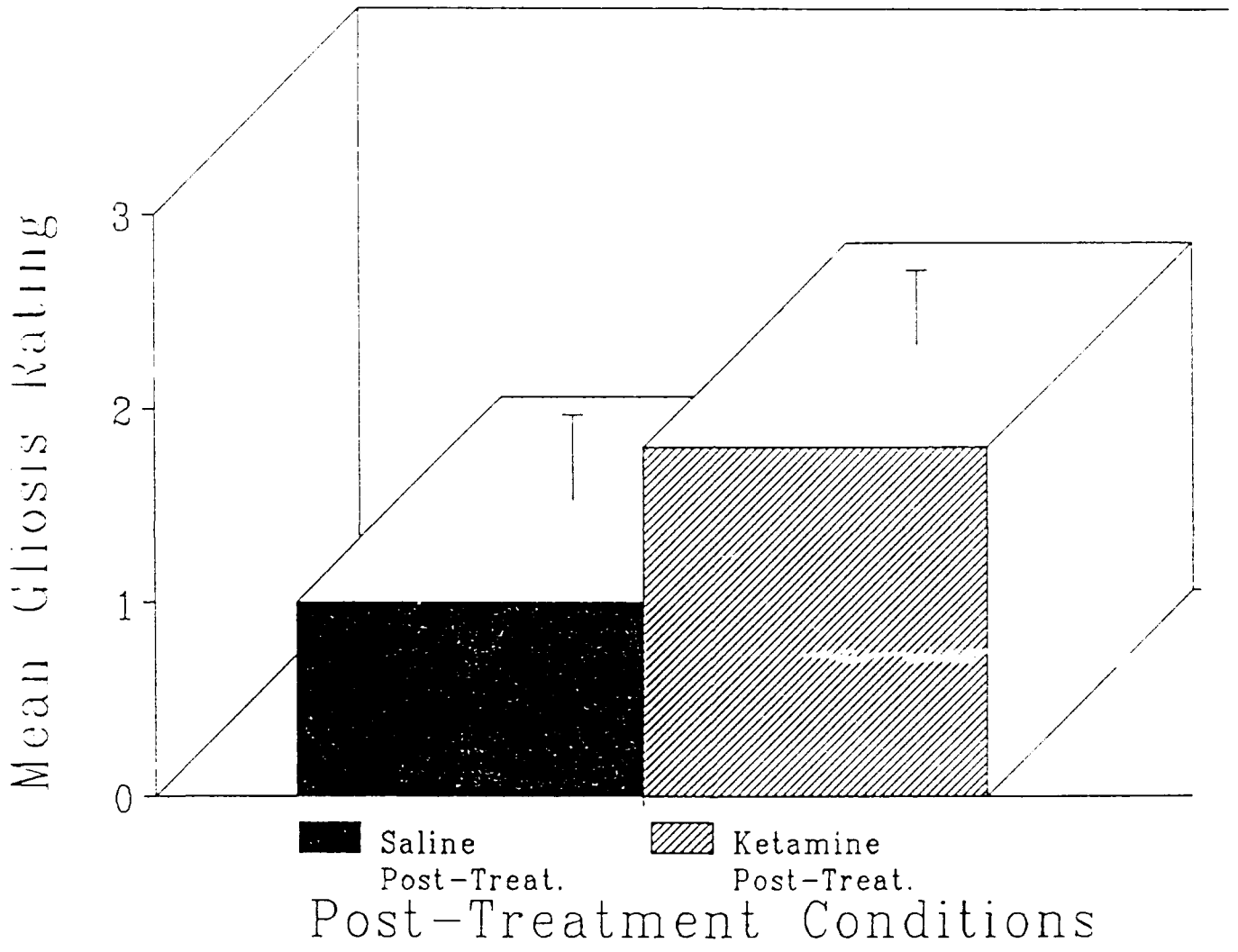


Figure 7

Gliosis in the Thalamic Ventral Basal Complex



Gliosis in the Thalamic Ventral Basal Complex

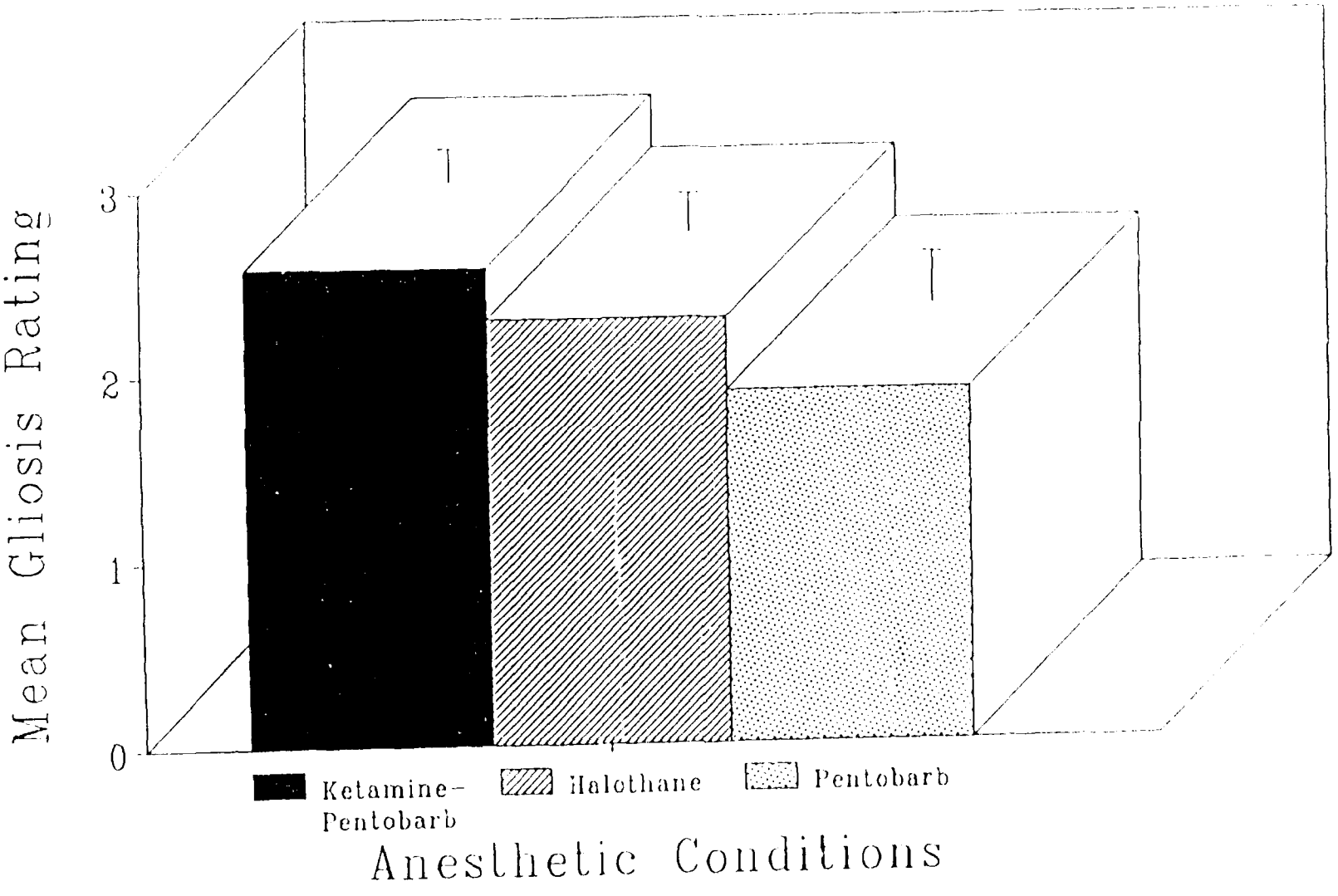


Figure 9

Figure 10

Relationship of Hippocampal and Thalamic Neuropathology to Recovery

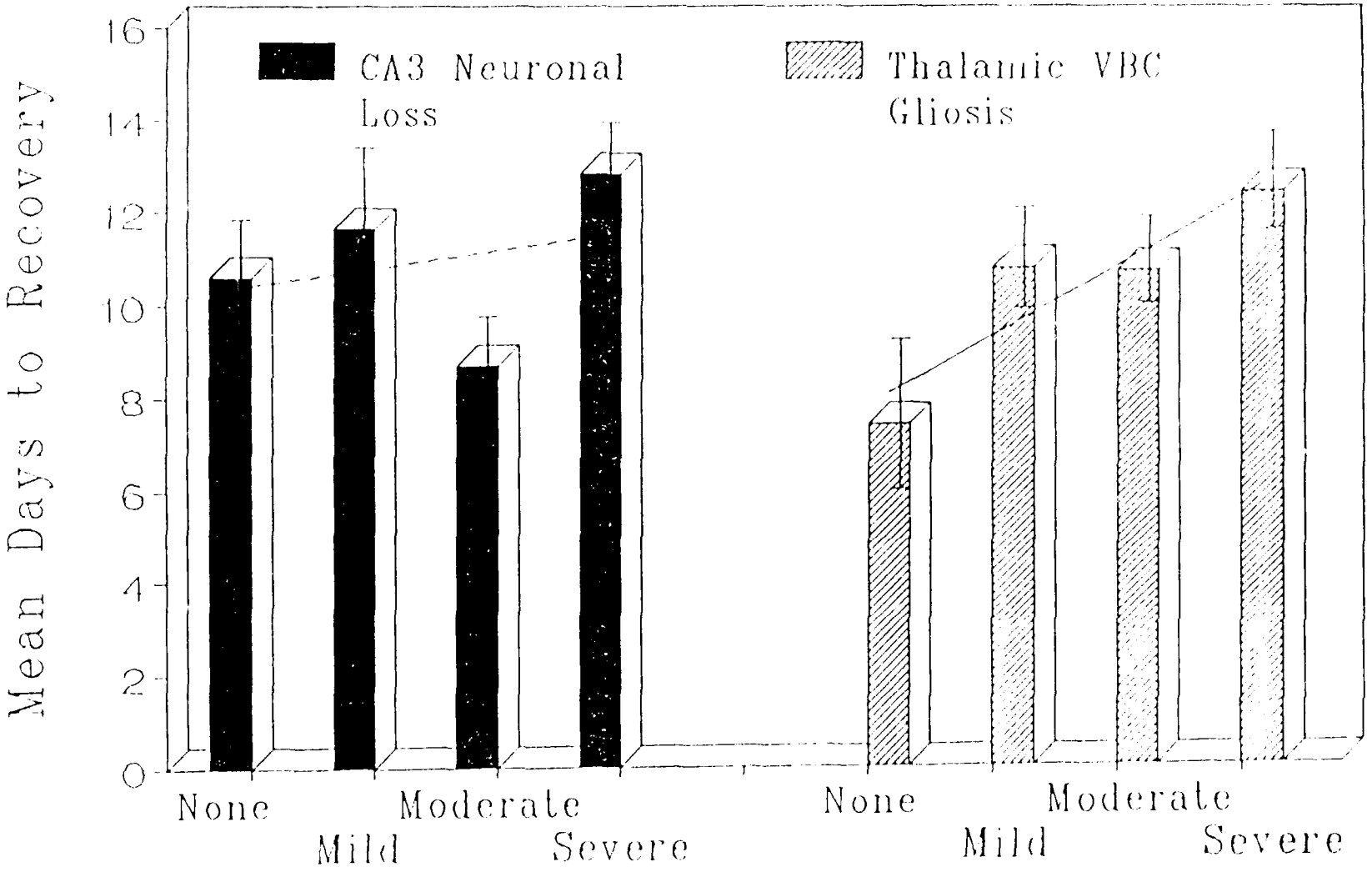


Figure 11

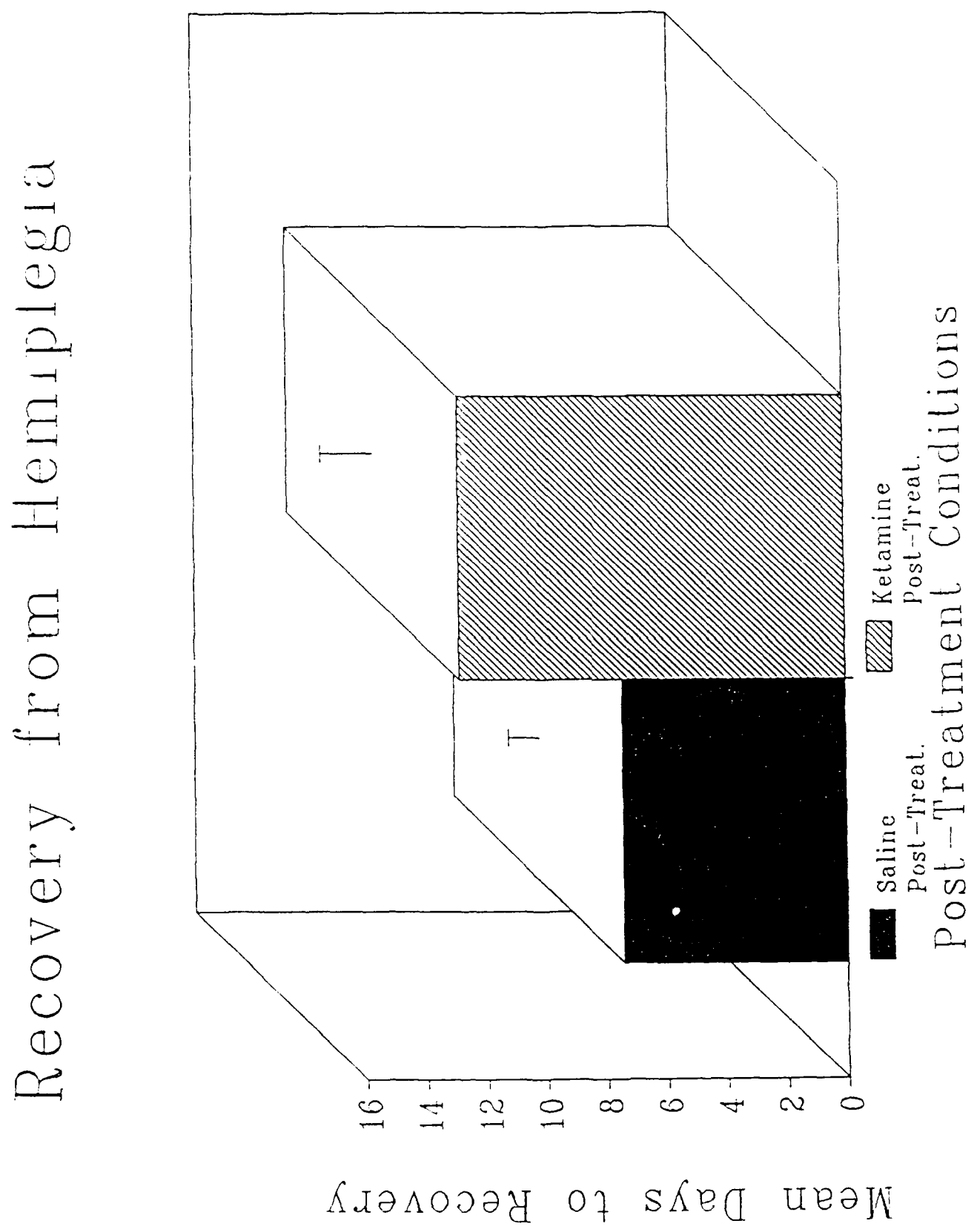

Recovery from Hemiplegia
Mean Days to Recovery
16
14
12
10
8
6
4
2
0
Saline
Post-Treat.
Ketamine
Post-Treat.
Post-Treatment Conditions

Figure 12

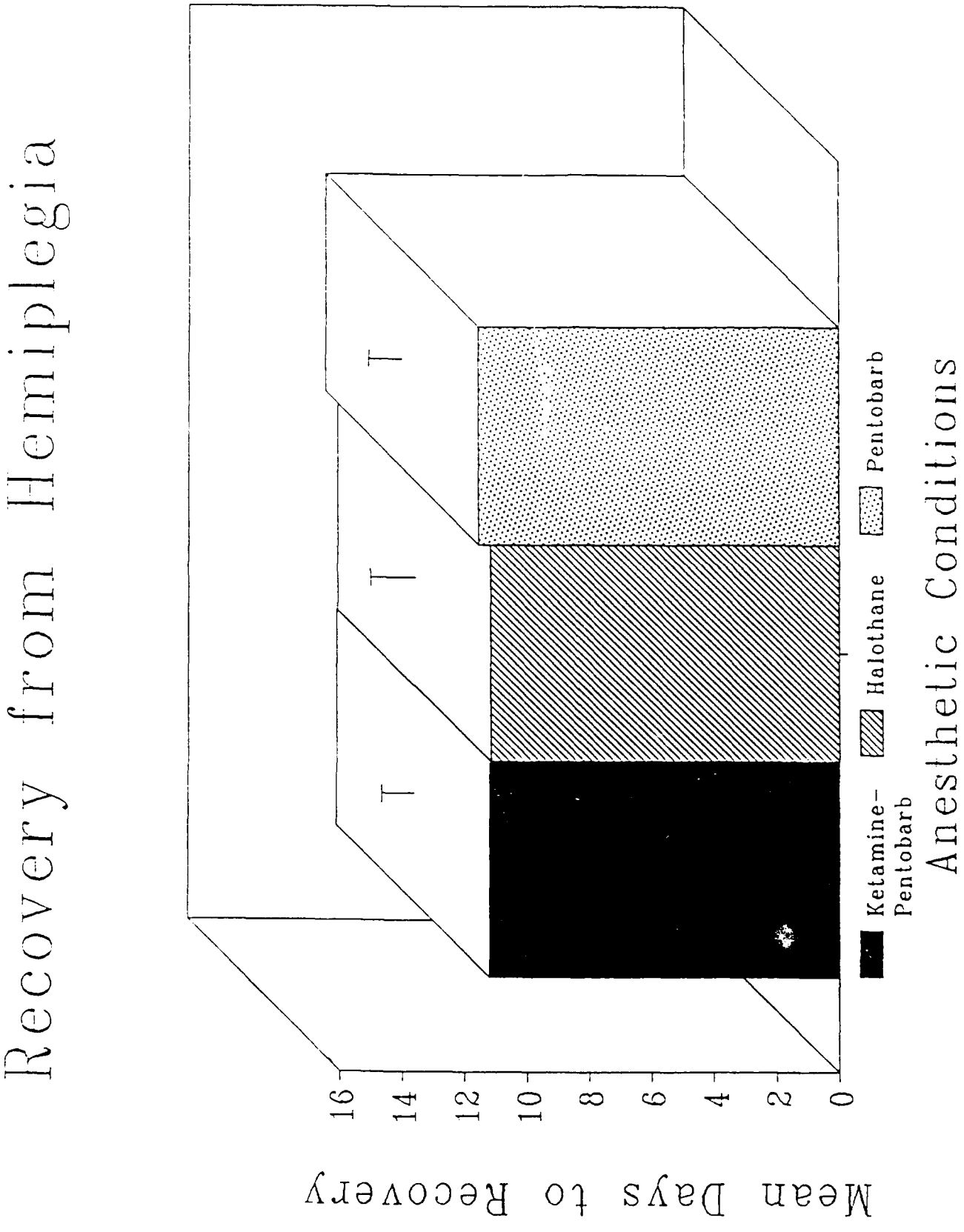
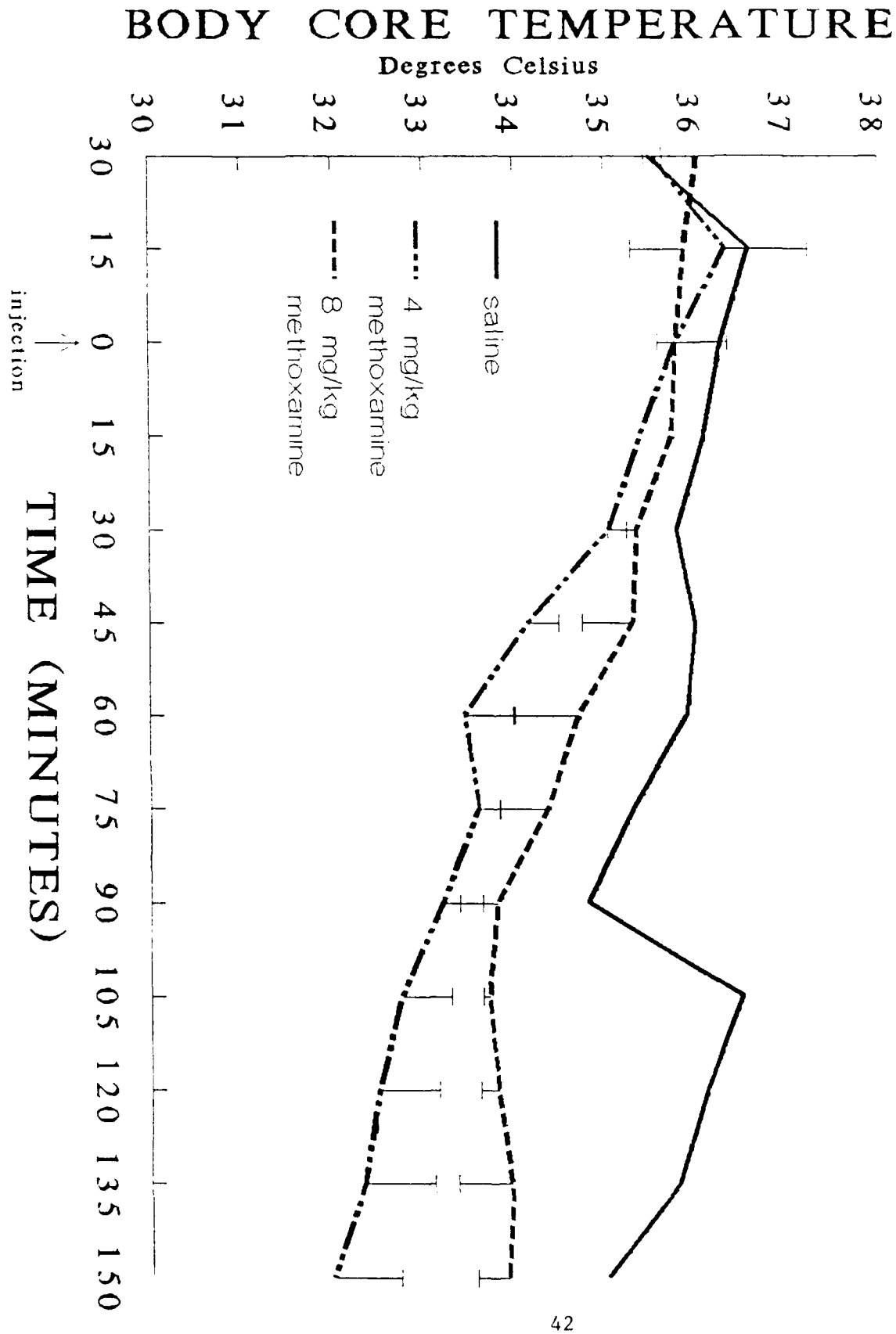

Recovery from Hemiplegia
Mean Days to Recovery
16
14
12
10
8
6
4
2
0
Ketamine-Pentobarb
Halothane
Pentobarb
Anesthetic Conditions

Figure 13



BODY CORE TEMPERATURE
RESPONSE TO METHOXAMINE

Figure 14

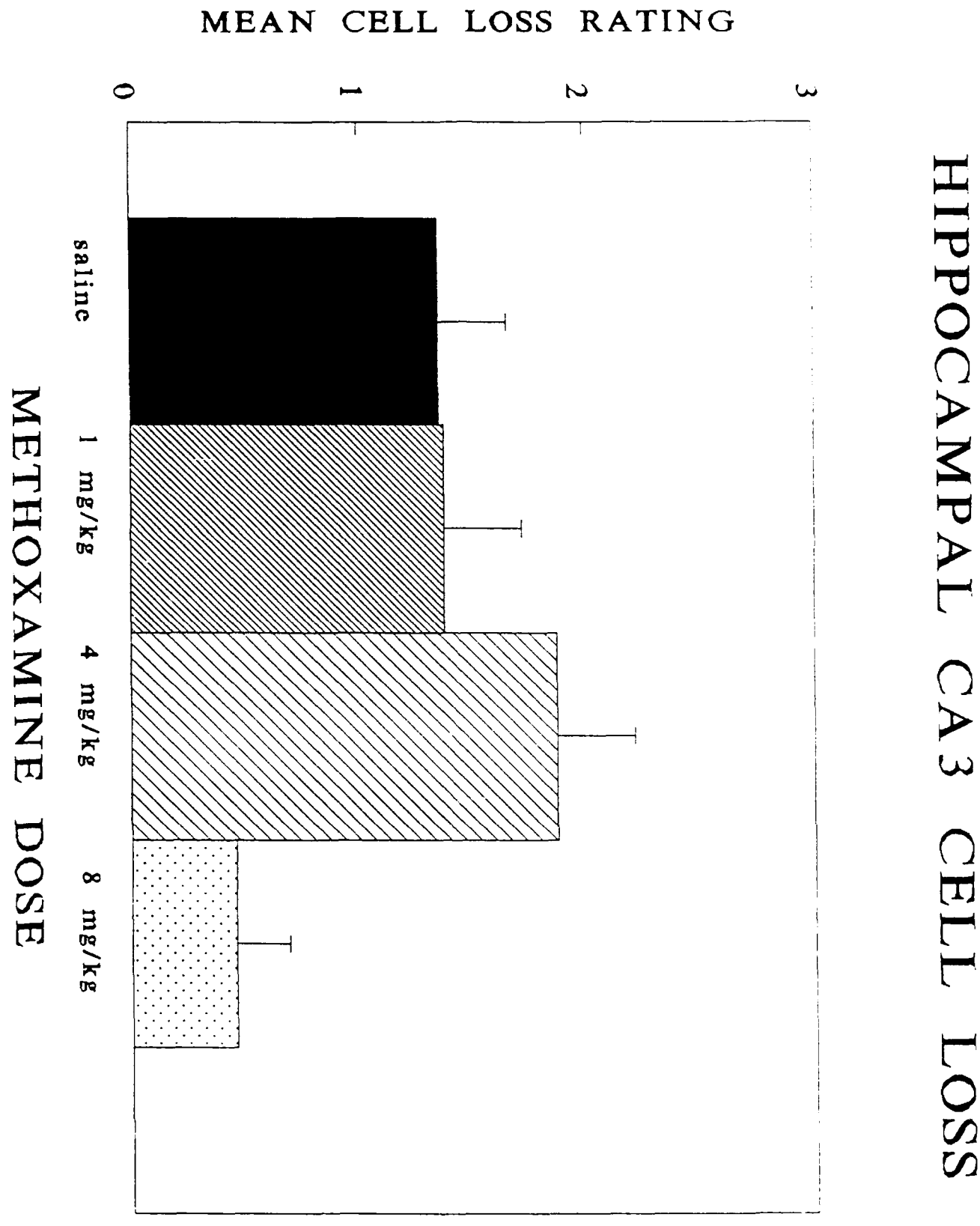

HIPPOCAMPAL CA3 CELL LOSS
MEAN CELL LOSS RATING
0
1
2
3
saline
1 mg/kg
4 mg/kg
8 mg/kg
METHOXAMINE DOSE

Figure 15

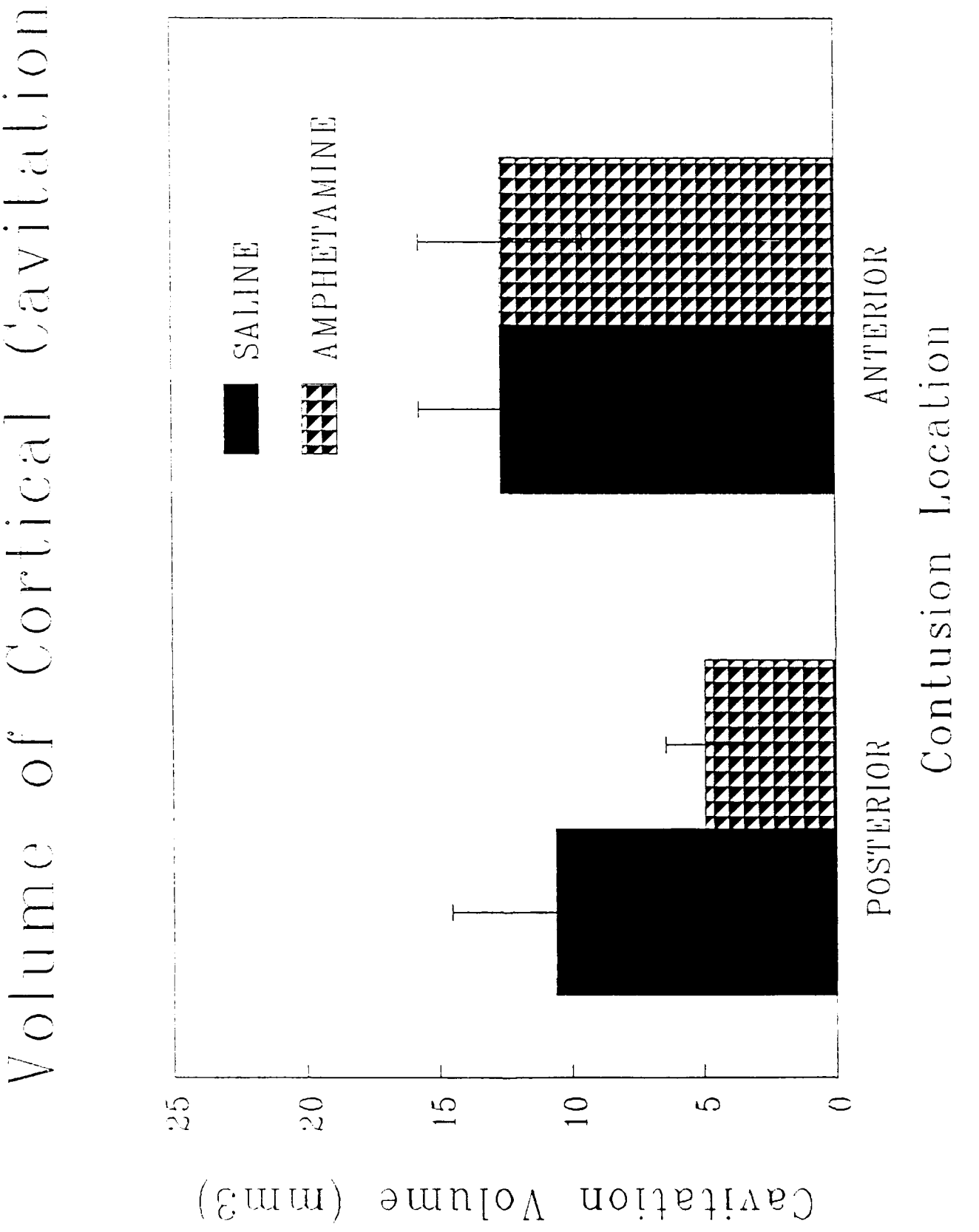

Volume of Cortical Cavitation
SALINE
AMPHETAMINE
Cavitation Volume (mm3)
0
5
10
15
20
25
POSTERIOR
ANTERIOR
Contusion Location

Figure 16

Ipsilateral Ventricular Dilation

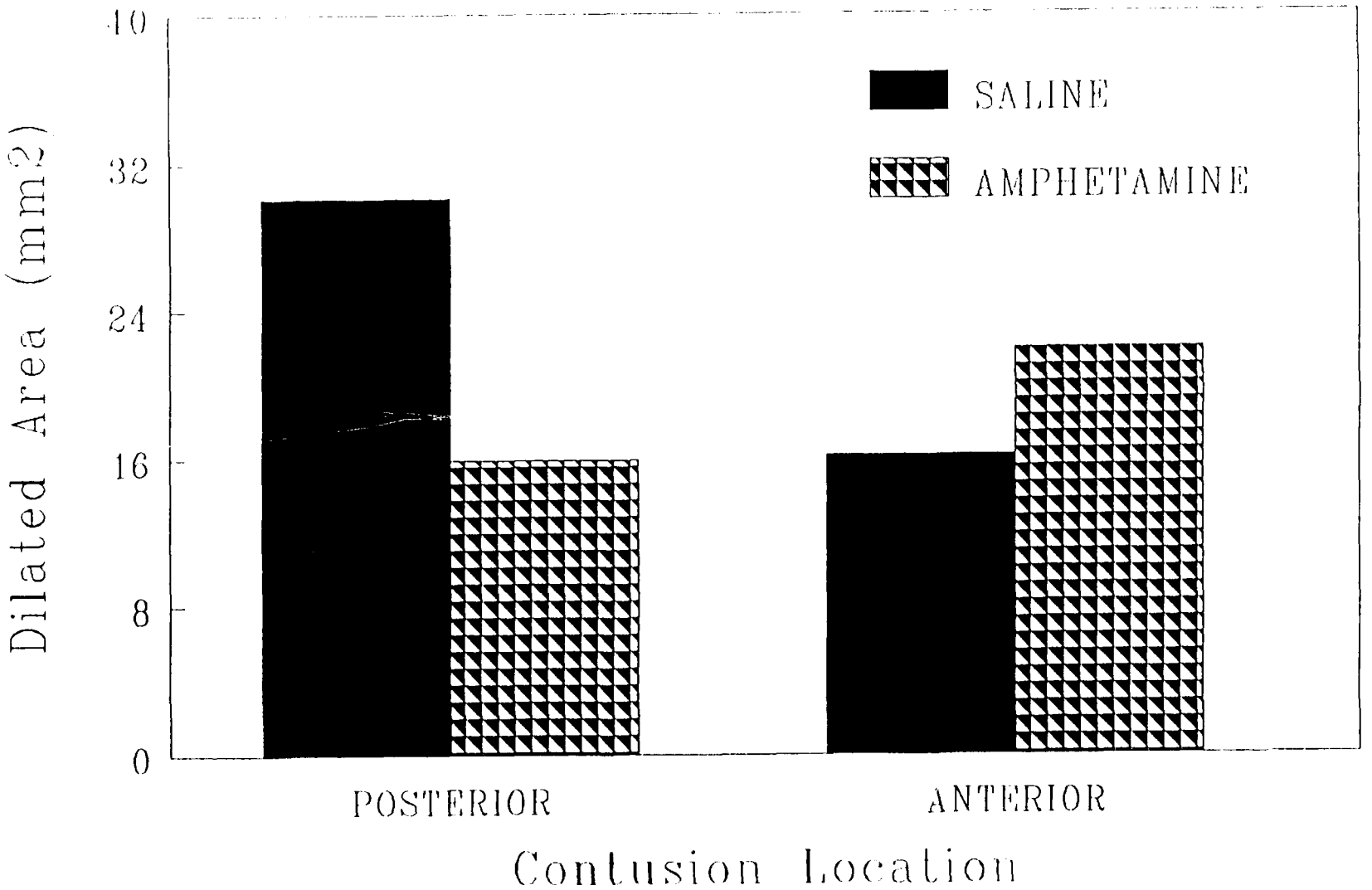


Figure 17

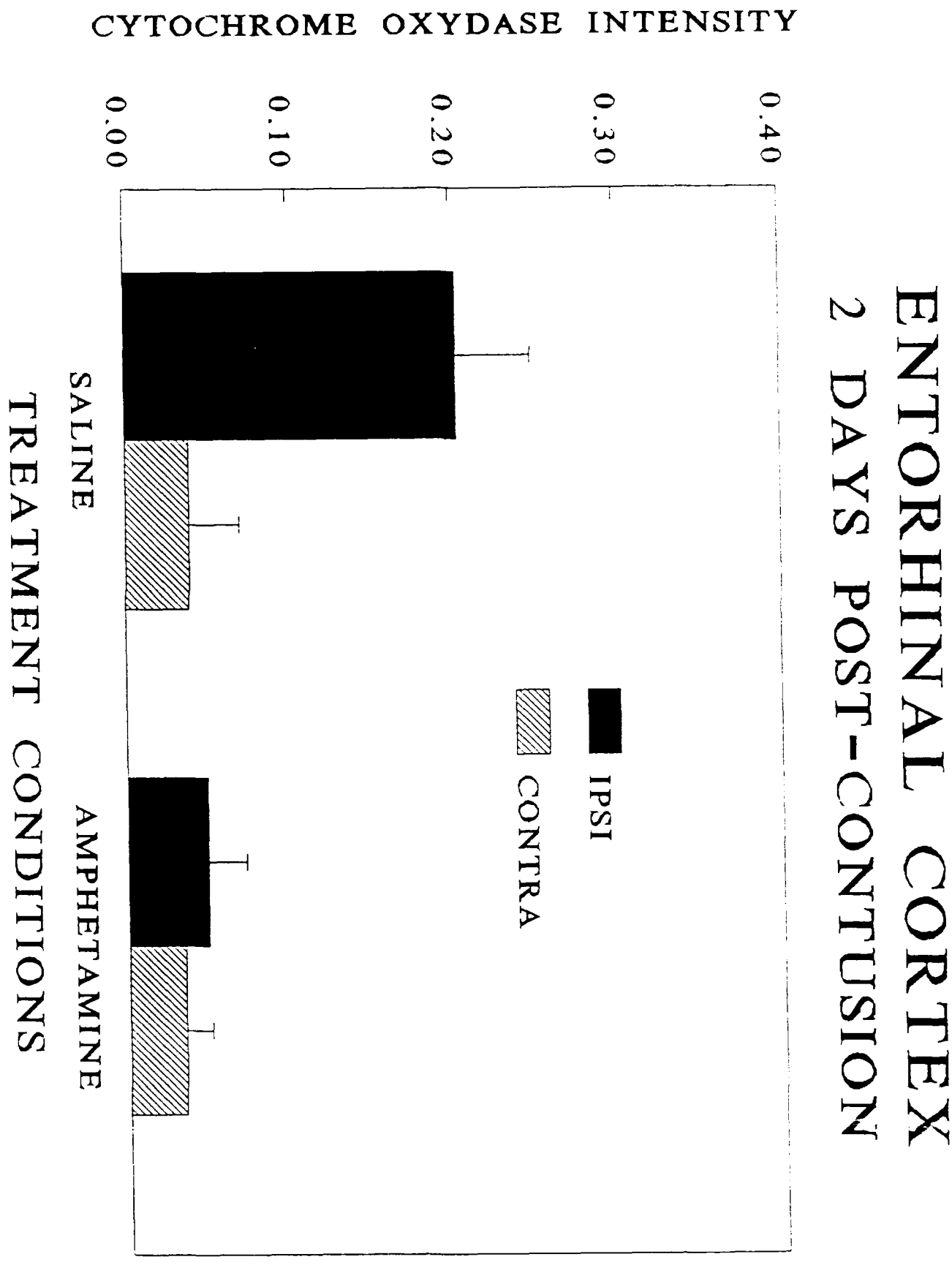

ENTORHINAL CORTEX
2 DAYS POST-CONTUSION
CYTOCHROME OXYDASE INTENSITY
0.00
0.10
0.20
0.30
0.40
IPSI
CONTRA
SALINE
AMPHETAMINE
TREATMENT CONDITIONS

Figure 18

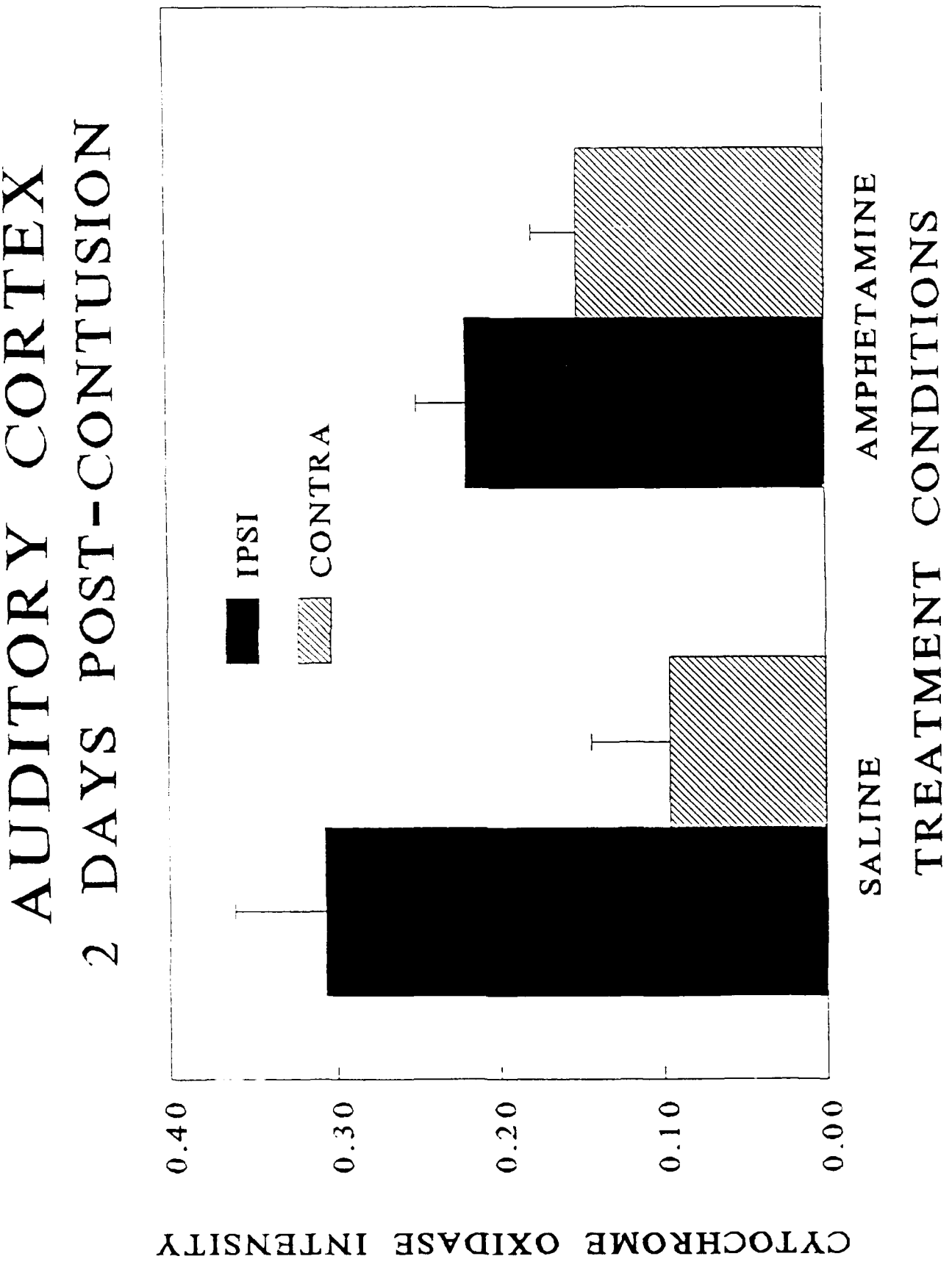
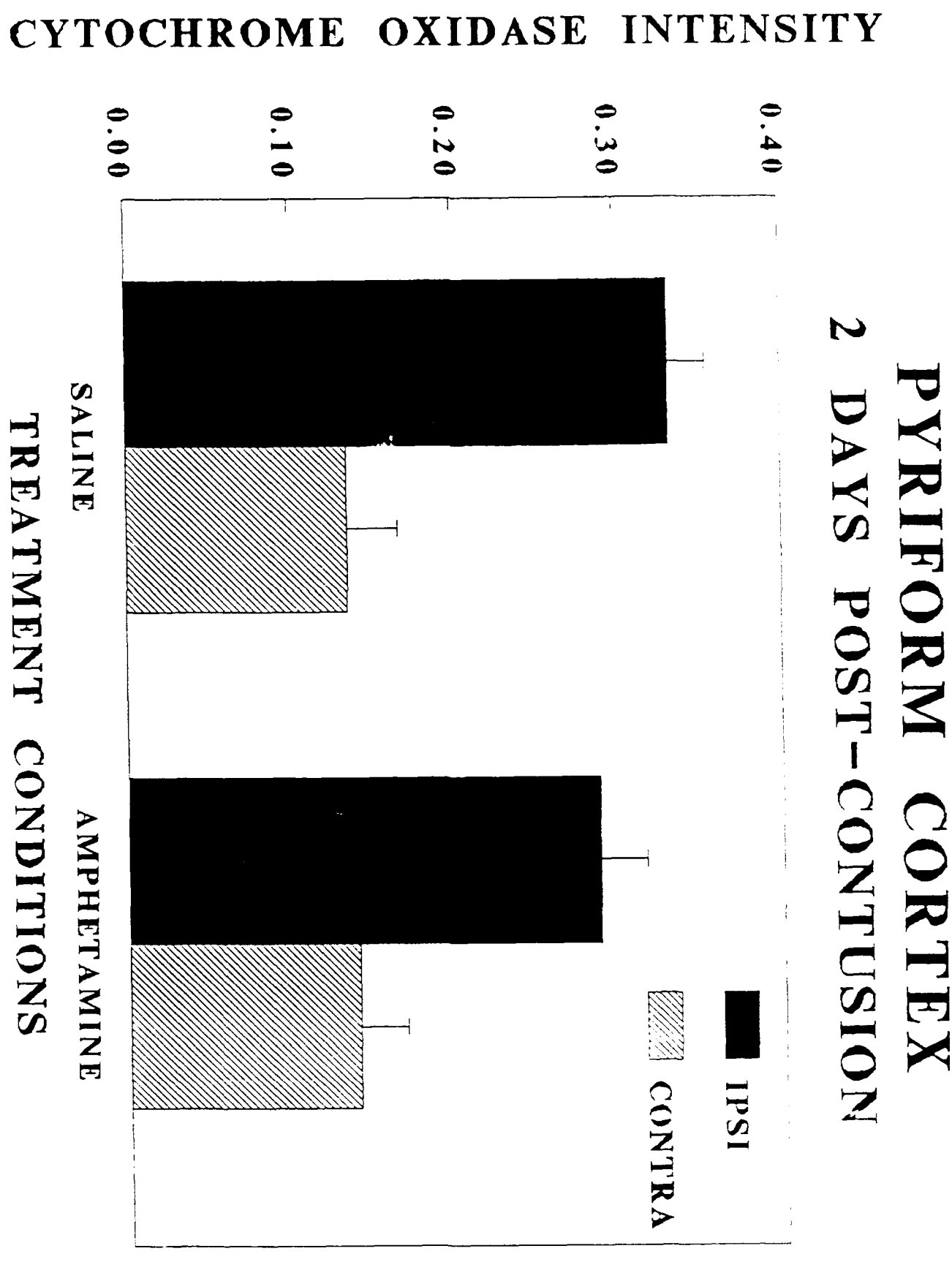

AUDITORY CORTEX
2 DAYS POST-CONTUSION
IPSI
CONTRA
0.40
0.30
0.20
0.10
0.00
CYTOCHROME OXIDASE INTENSITY
SALINE
AMPHETAMINE
TREATMENT CONDITIONS

Figure 19


CYTOCHROME OXIDASE INTENSITY
PYRIFORM CORTEX
2 DAYS POST-CONTUSION
0.00
0.10
0.20
0.30
0.40
IPSI
CONTRA
SALINE
AMPHETAMINE
TREATMENT CONDITIONS

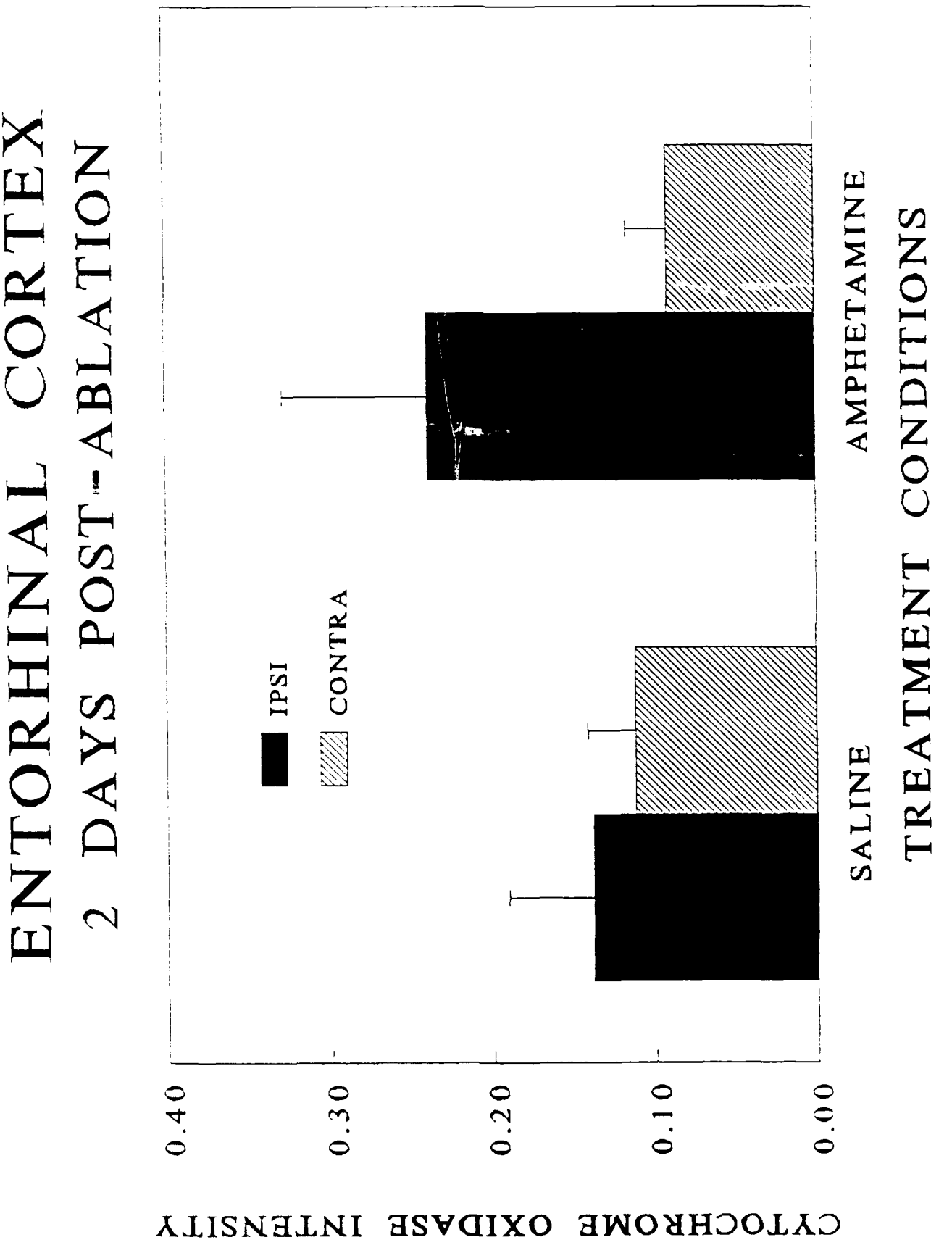

ENTORHINAL CORTEX
2 DAYS POST-ABLATION
IPSI
CONTRA
0.40
0.30
0.20
0.10
0.00
CYTOCHROME OXIDASE INTENSITY
SALINE
AMPHETAMINE
TREATMENT CONDITIONS

Figure 21

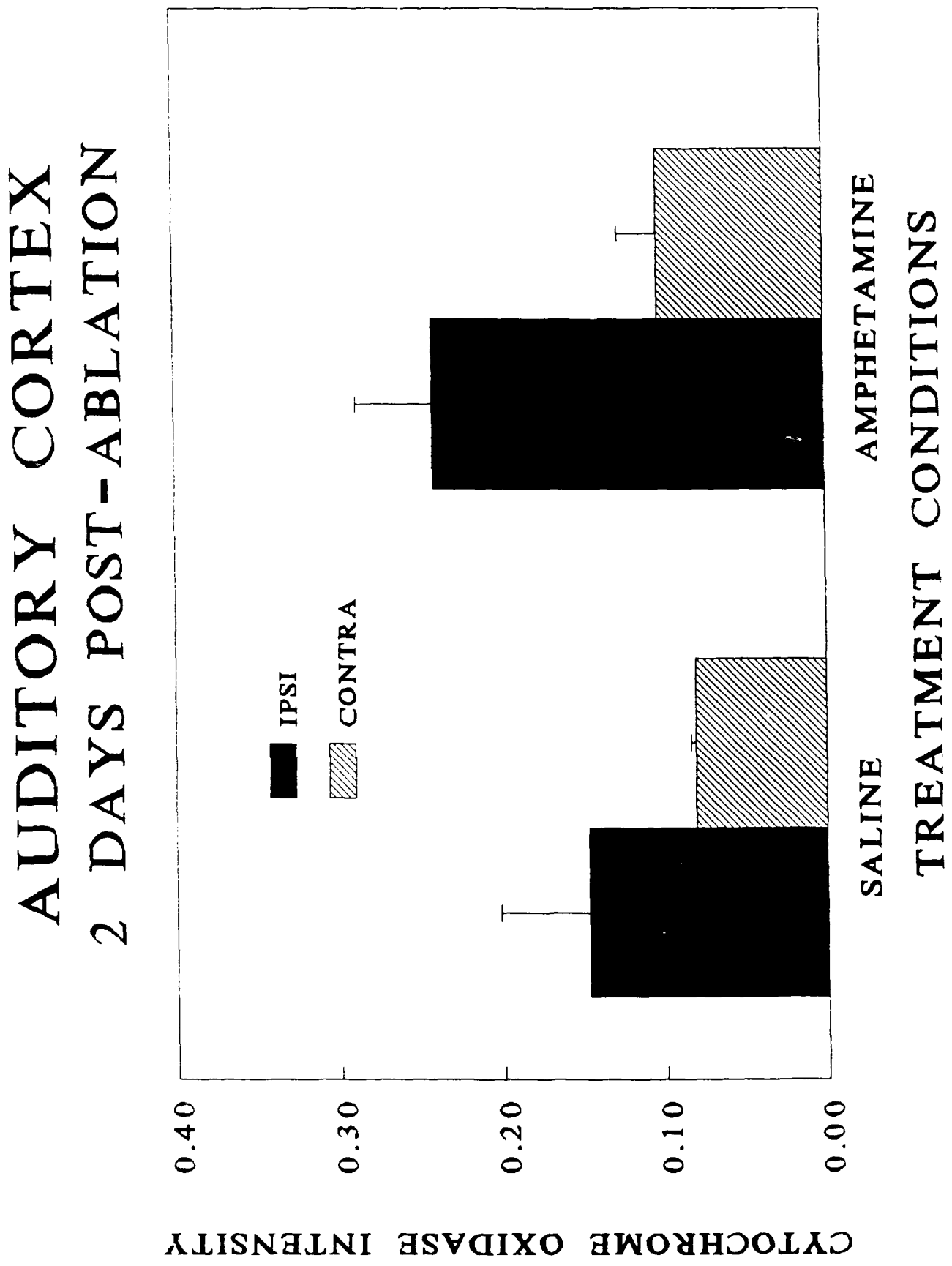
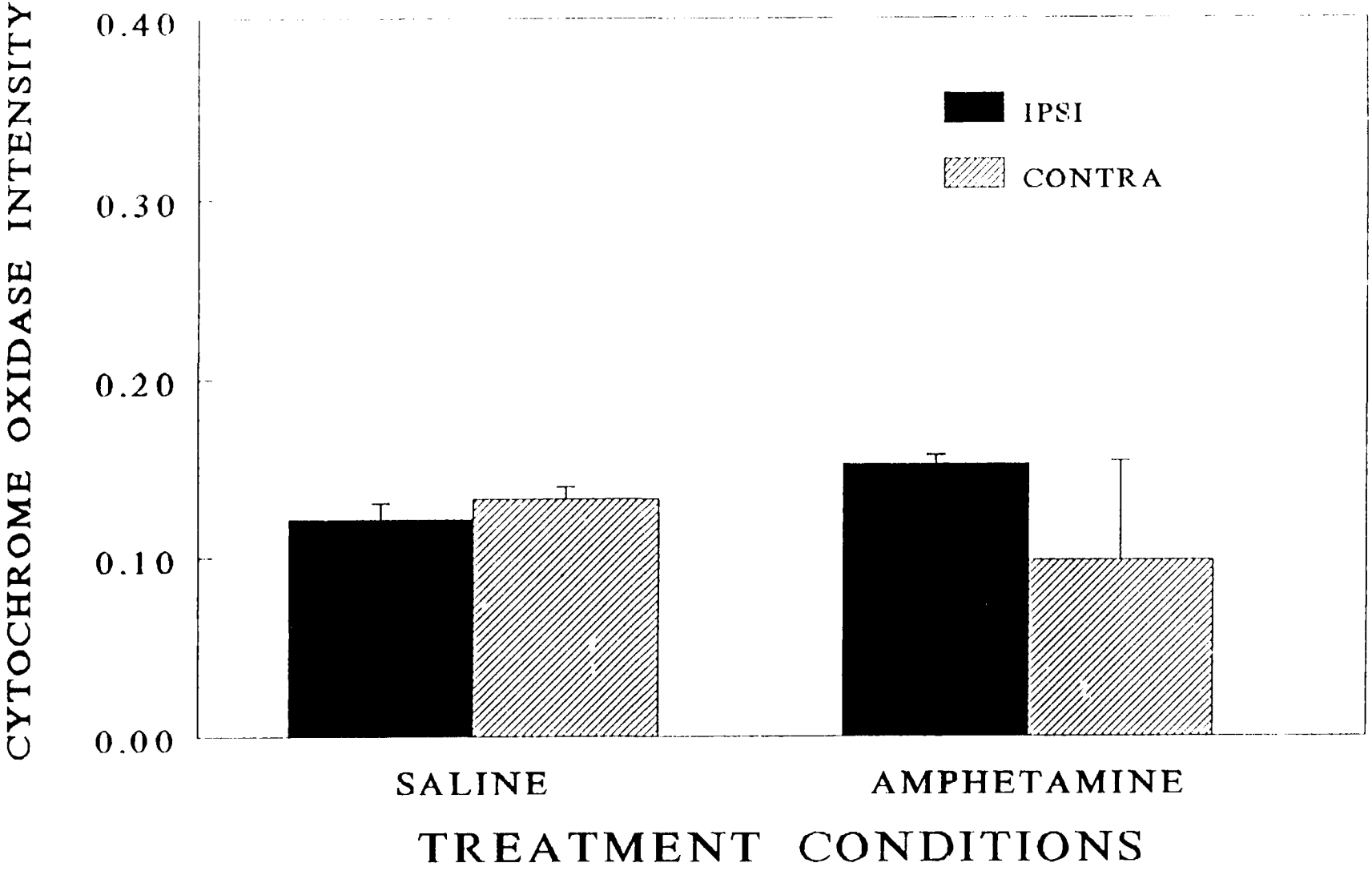
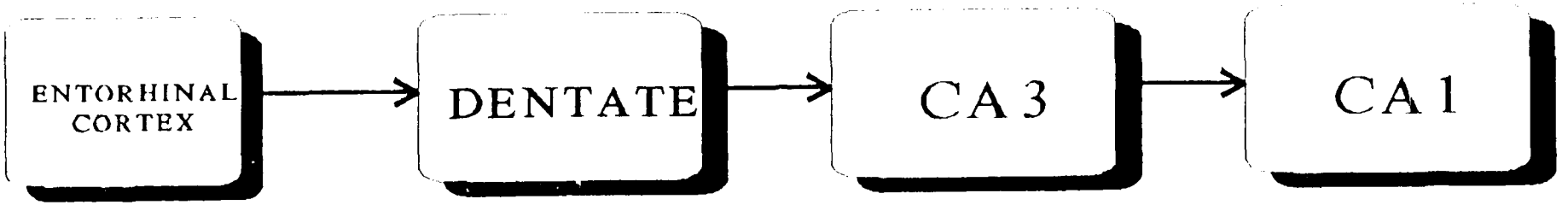

AUDITORY CORTEX
2 DAYS POST-ABLATION
IPSI
CONTRA
0.40
0.30
0.20
0.10
0.00
CYTOCHROME OXIDASE INTENSITY
SALINE
AMPHETAMINE
TREATMENT CONDITIONS

Figure 22

PYRIFORM CORTEX
2 DAYS POST-ABLATION



SEQUENTIAL MODEL



FEEDFORWARD MODEL

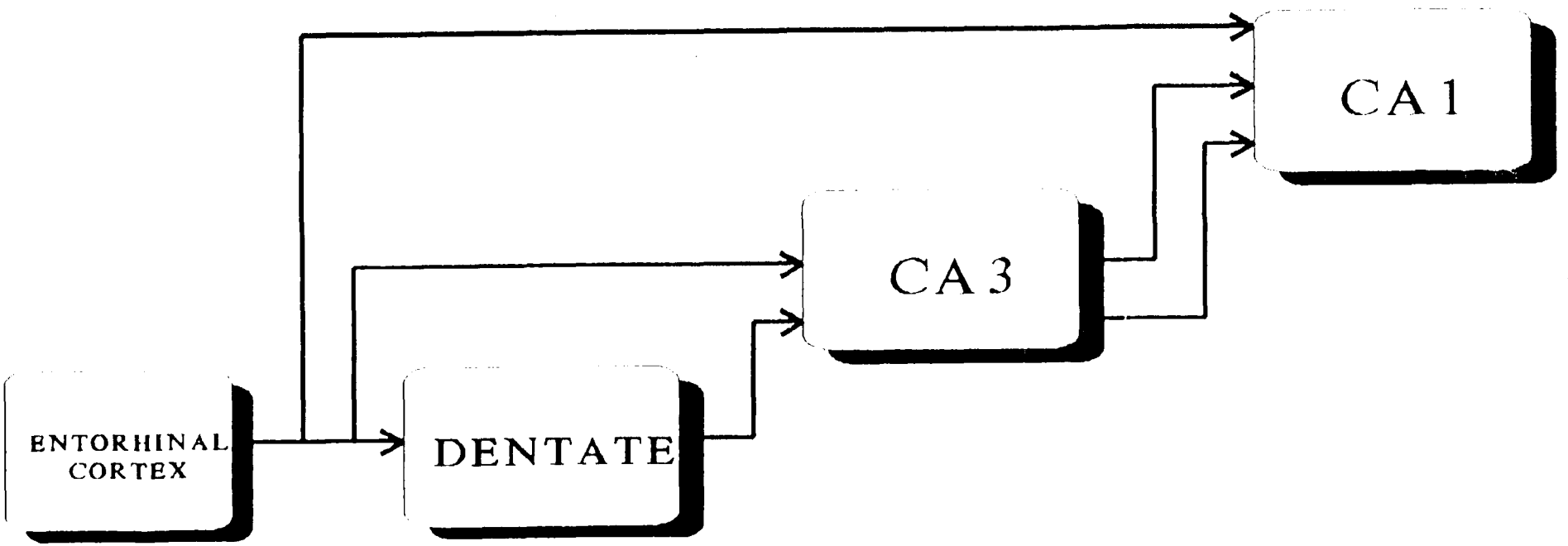


Figure 23

DOPAMINE LEVELS

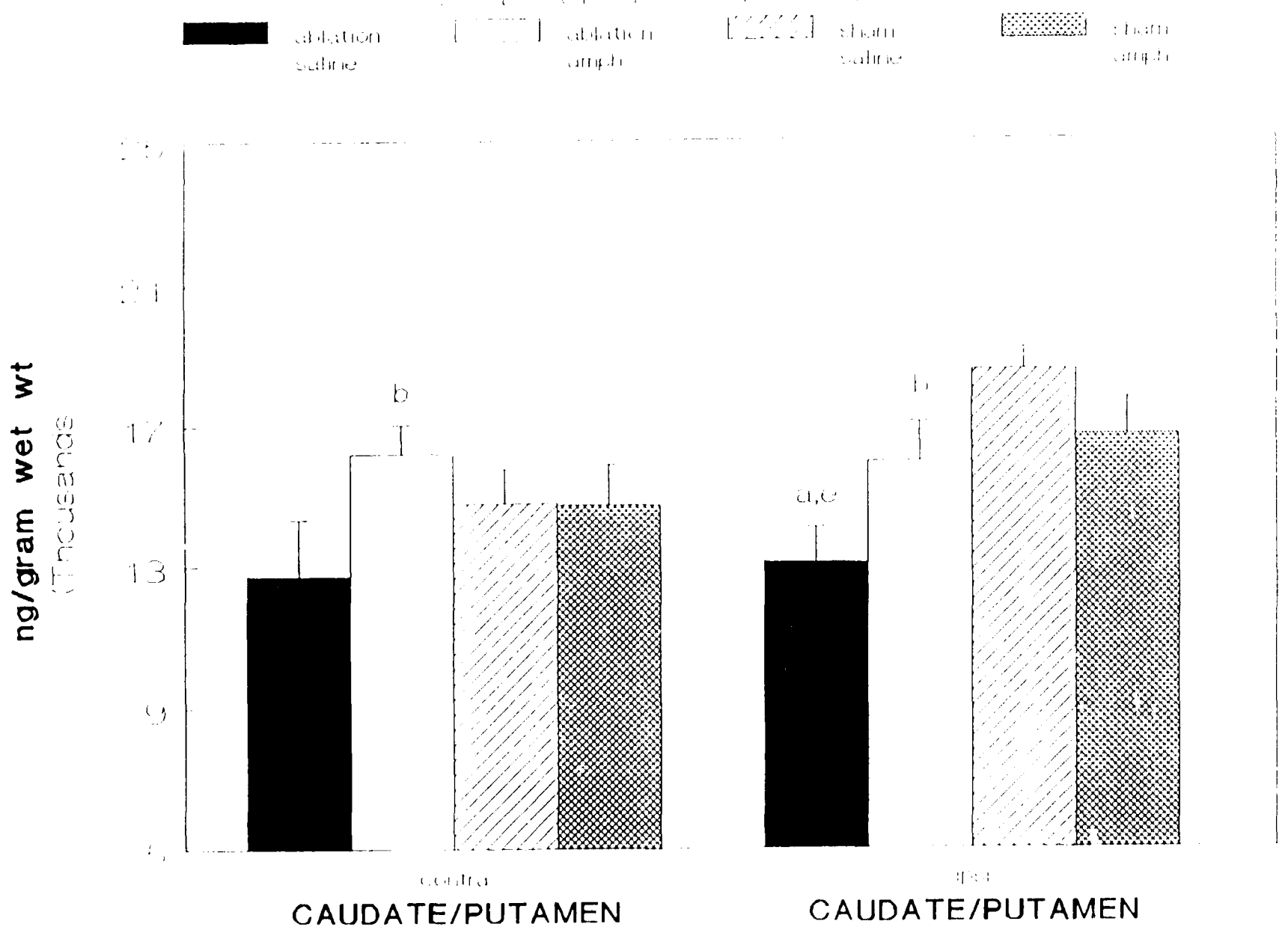


Figure 24

Figure 25

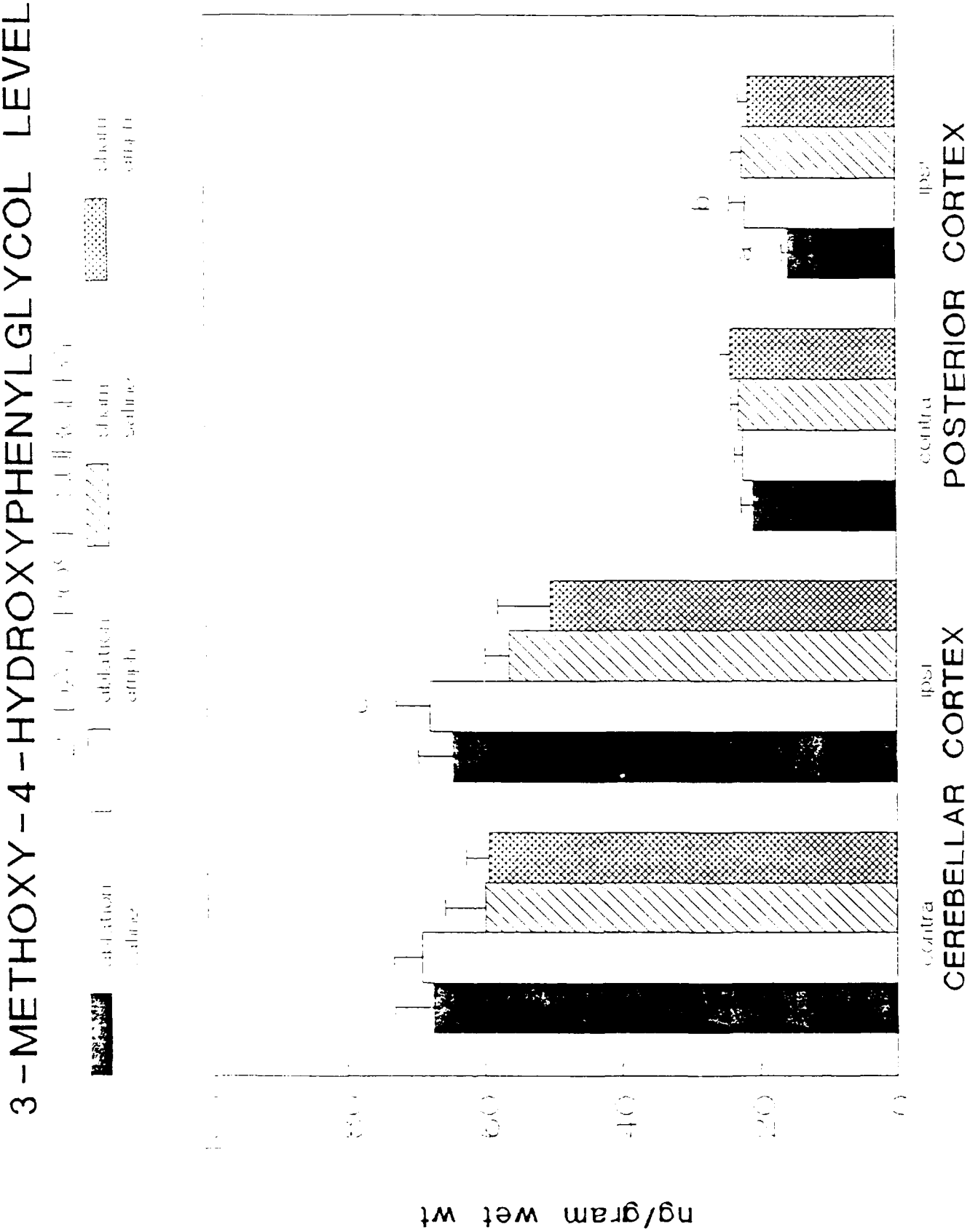

3-METHOXY-4-HYDROXYPHENYLGLYCOL LEVELS
ng/gram wet wt
CEREBELLAR CORTEX
POSTERIOR CORTEX
contra
ipsi

Figure 26

5-HYDROXYINDOLEACETIC ACID LEVELS
2 DAY POST-SURGERY
ablation saline
ablation amph
sham saline
sham amph
ng/gram wet wt
1000
800
600
400
200
0
a,e
c,e
contra
ipsi
FRONTAL POLE
POSTERIOR CORTEX
CAUDATE/PUTAMEN

Figure 27

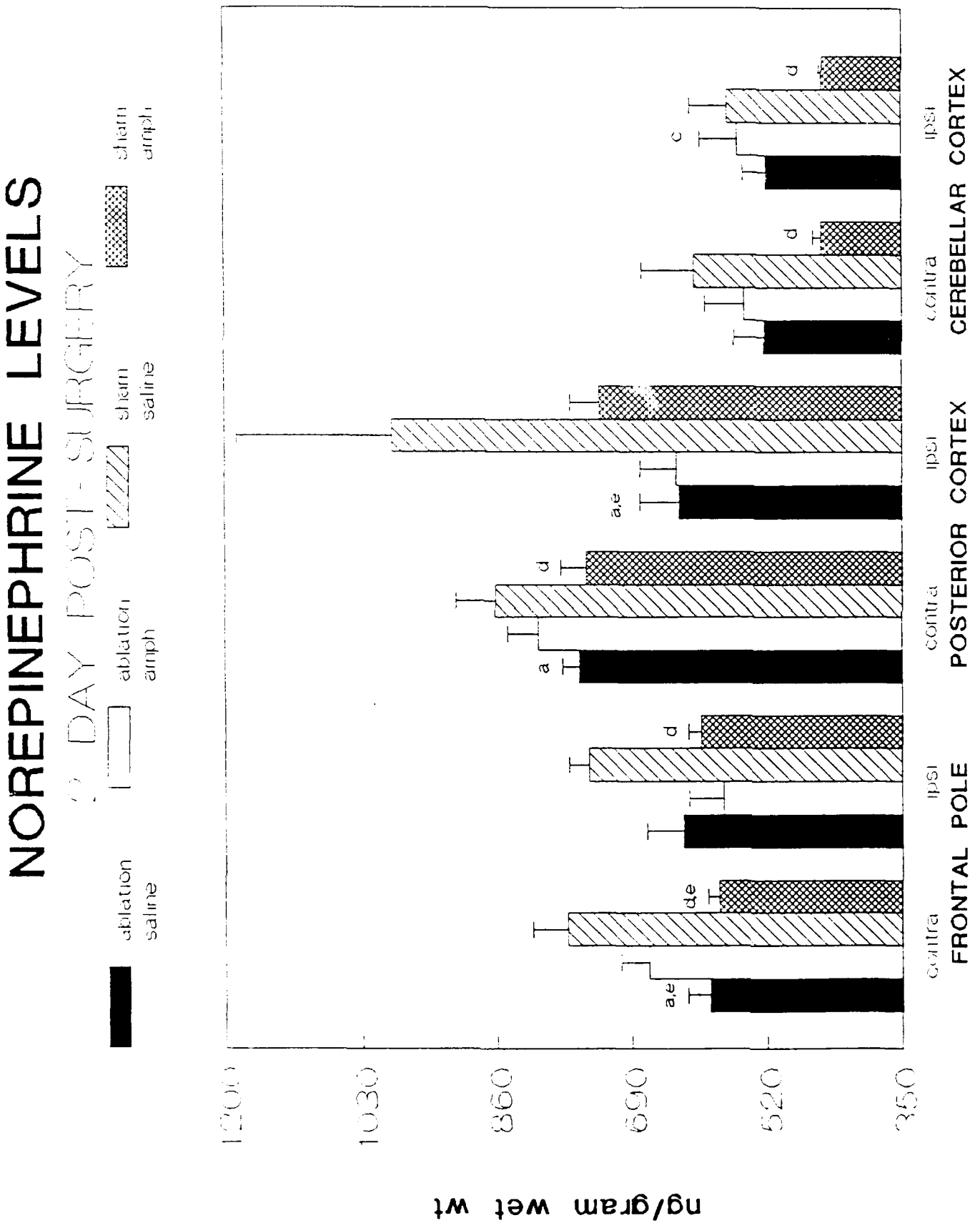
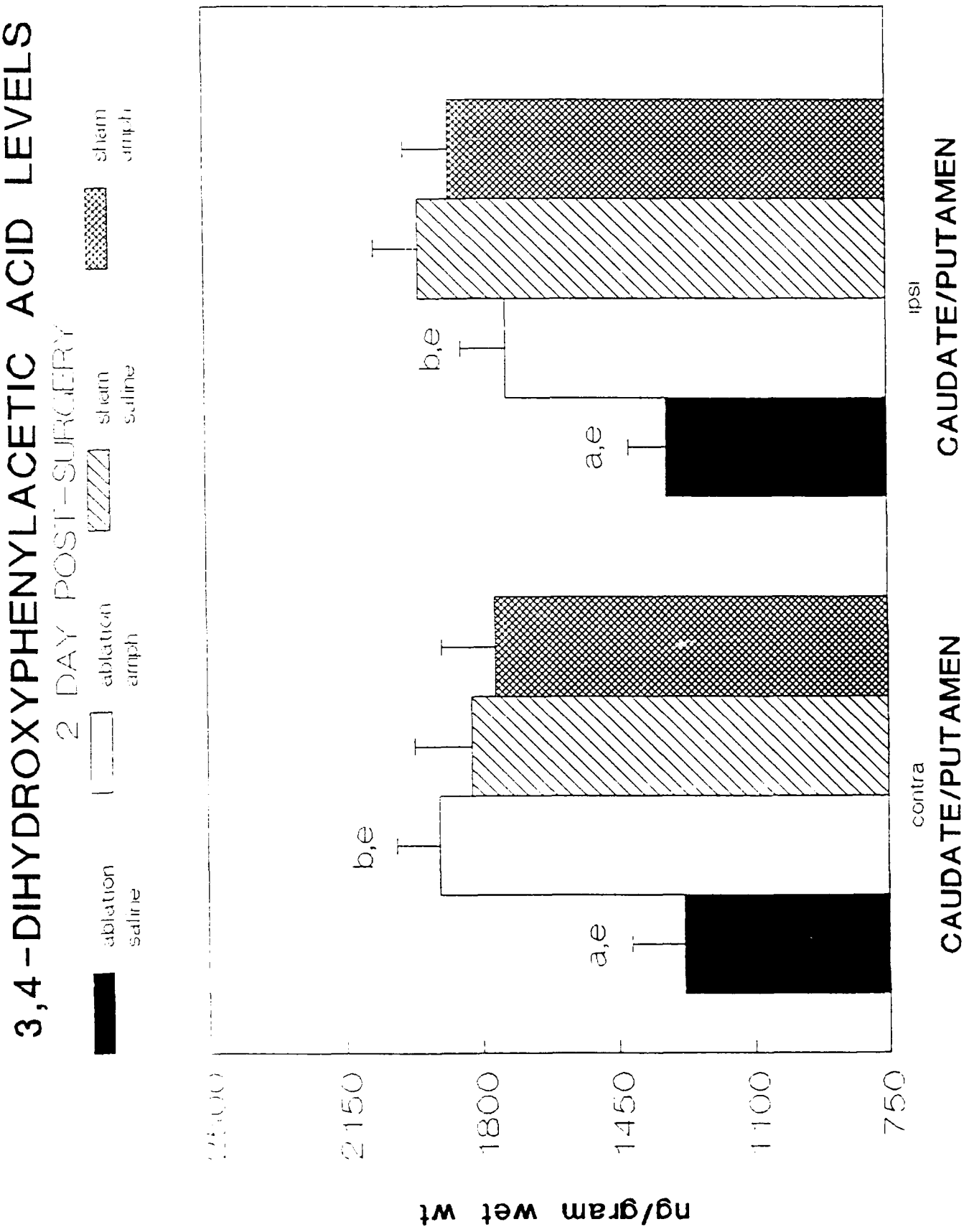

NOREPINEPHRINE LEVELS
2 DAY POST-SURGERY
ablation saline
ablation amph
sham saline
sham amph
ng/gram wet wt
1200
1030
860
690
520
350
a,e
de
d
a
d
a,e
d
c
d
contra
ipsi
contra
ipsi
contra
ipsi
FRONTAL POLE
POSTERIOR CORTEX
CEREBELLAR CORTEX

Figure 28


3,4-DIHYDROXYPHENYLACETIC ACID LEVELS
2 DAY POST-SURGERY
ablation saline
ablation amph
sham saline
sham amph
ng/gram wet wt
2150
1800
1450
1100
750
a,e
b,e
a,e
b,e
contra
ipsi
CAUDATE/PUTAMEN
CAUDATE/PUTAMEN

NE/MHPG and DA/DOPAC RATIOS

2 DAY POST-SURGERY

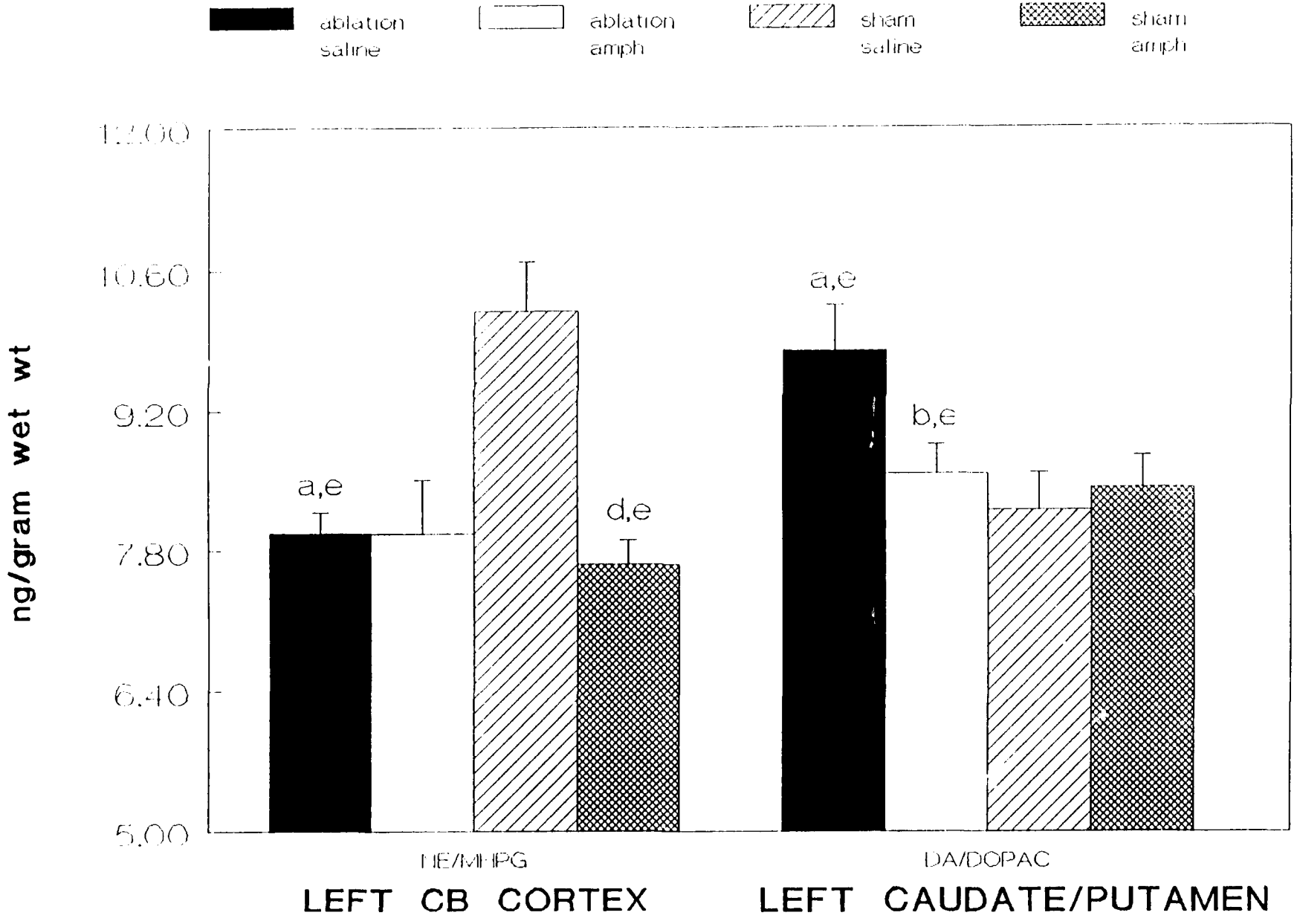


Figure 29