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STUDIES OF MECHANISMS OF PHARMACOLOGICAL ENHANCEMENT OF FUNCTIONAL RECOVERY AFTER CORTICAL CONTUSION

FINAL REPORT

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Electrophysiology, autoradiography, histochemistry, biochemistry, anatomical, and behavioral research techniques were used in studies to examine remote functional depressions, and the effects of drug treatments on some of these measures, in rats with unilateral ablation of the sensorimotor cortex (SMCx) or traumatic brain injury (contusion; TBI) centered over the SMCx. In addition to d-amphetamine (d-AMPH), drugs increasing norepinephrine (NE) release were found to accelerate beam-walking (BW) recovery in rats with SMCx ablation. The dose of d-AMPH required to induce BW recovery after TBI was dependent upon whether the right or left hemisphere was damaged and/or the extent of damage to midline cortex. Drugs decreasing NE levels or blocking alpha1-NE receptors were found to reinstate BW deficits in rats after SMCx ablation. Unilateral cerebellar lesions produced more enduring BW deficits than SMCx injury, these deficits were adversely affected by either early d-AMPH and/or haloperidol treatment,

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and drugs reinstating BW deficits in rats after SMCx injury did not affect BW in cerebellar-lesioned rats recovered on the BW task. Rats with unilateral lesion of the red nucleus (RN) recovered from BW deficits at a rate similar to those with SMCx ablation, but addition of SMCx injury to rats recovered from RN damage resulted in very prolonged BW deficits. Infusions of drugs into the RN revealed that neural activity in this structure is necessary for both spontaneous BW recovery and maintenance of that recovery. Gait analysis measures, shown to be less sensitive and more variable than BW for detecting motor dysfunctions in SMCx ablated rats, were found to be valuable in assessing changes in gait and limb control patterns in rats with RN injury.

Electrophysiological studies revealed that: 1) TBI or undercuts of SMCx, but not ablation, increased the thresholds within tissue anterior to these injuries required to elicit forelimb movements. All injuries resulted in loss of ability of normal threshold microstimulation to induce movements in contralateral limbs, indicating that cortical reorganization does not underly functional recovery that occurs after SMCx injury. 2) In the hippocampus ipsilateral to TBI, seizure-like activity occurs for up to 3 hours after injury. 3) From 18-30 days after TBI, LTP can be elicited in the the hippocampus contralateral, but not ipsilateral, to TBI. Anatomical studies revealed that secondary cell damage in the hippocampus ipsilateral to TBI occurred within 2 to 6 hours after injury, with cell loss peaking from 2-5 days after injury. During tests of ability to perform in a water maze, rats with TBI were also found to have severe learning deficits, but not retrograde amnesia. Metabolic studies revealed that: 1) Glycolytic metabolism in contused cortex and underlying hippocampal regions was increased for up to 30 minutes postTBI, decreased in these regions and many subcortical areas ipsilateral to TBI by 24 hours, and returned toward normal by 10 days after TBI. 2) Cerebral oxidative metabolism was decreased in ipsilateral cortex and many subcortical regions by 48 hours after TBI or ablation of the right SMCx. 3) Administration of d-AMPH 24 hours after injury attenuated effects of injury on cerebral oxidative metabolism; these drug effects were less robust after TBI than after SMCx ablation. 4) Differential BW experience did not alter measures of oxidative metabolism in normal rats. After TBI, cerebral blood flow (CBF) was reduced in ipsilateral cortex and hippocampus for up to 6 hours after injury and, at 24 hours after TBI, surrounding cortex and subcortical regions were either hyperemic or CBF was near normal at this time. d-AMPH treatment produced a global increase in CBF, but drug effects in any one specific region after TBI did not reach significance. Microdialysis combined with HPLC revealed that cerebellar NE levels were decreased by 24 hours after TBI; d-AMPH significantly elevated NE levels in both controls and TBI rats. The NE levels in frontal cortex, but not dopamine levels in the caudate, were found to decrease bilaterally by 50-70% of basal levels within 4 hours after TBI.

FOREWORD

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INTRODUCTION

There is a clear need for research aimed at delineating the pathophysiology which occurs after brain injury and for development of rational, empirically based pharmacotherapies and rehabilitation strategies that may promote functional recovery after central nervous system injury. Amongst the civilian population in the United States, there are over a half million new cases of brain damage from stroke and trauma every year, and traumatic brain injury (TBI) leads to significant and enduring impairments of physical, cognitive, and/or psychosocial functions in an estimated 50,000 individuals each year (1). Closed head injury and/or concussion are even more prevalent in combat soldiers than in the general public. In both military and civilian populations, medical treatment and rehabilitation of the brain-injured patient is a prolonged, costly, and labor intensive endeavor. The economic costs, estimated to average over $100,000 in the first year after injury (2), and emotional difficulties resulting from these injuries place an enormous burden and strain on patients, families, and the health care system. The traditional indirect approach to the treatment of brain injury after trauma or stroke, including attempts to limit the spread of cerebral damage by improving cerebral blood flow or reducing metabolic demands in ischemic tissue has not been successful in improving outcome (3). For patients with completed injuries only physical and/or cognitive therapy is generally used and, while beneficial, these rehabilitation efforts may not promote functional recovery. Thus, a significant reduction in the duration and cost of medical interventions and a hastening of the return of patients with TBI to a functional role in society may be accomplished through research aimed at better understanding: 1) the pathophysiological events occurring after TBI; 2) the mechanisms underlying spontaneous recovery after TBI; and, 3) development of pharmacological interventions that might reduce pathophysiological reactions and improve the rate of functional recovery.

In recent years laboratory research has demonstrated that the rate of spontaneous recovery in locomotor (beam-walking) ability of rats and cats with unilateral (right) sensorimotor cortex (SMCx) damage is significantly improved following treatment with d-amphetamine (d-AMPH) sulphate (4-11). This improved rate of motor recovery is obtained with delayed administration of the drug, administered 24 hours postinjury in the rat and as late as 10 days after injury in the cat. In rats, this d-AMPH-facilitated recovery was obtained after a single intraperitoneal (i.p.) administration of a 2 mg/kg dose of the drug following either right SMCx ablation (4,8,9) or right SMCx contusion (11). The d-AMPH-induced improvement in beam-walking ability following contusion produced via a weight-drop method (12) was limited by injury severity, since the drug improved the rate of recovery in rats with a 400 g-cm contusion to the right SMCx but did not alter the rate of spontaneous recovery following an 800 g-cm contusion to the right SMCx (11).

The mechanisms by which d-AMPH accelerates functional recovery after cortical injury are not known at this time. However, the

effects of d-AMPH on behavioral recovery after cortical ablation or stroke are blocked by co-administration of haloperidol (4,13) or phenoxybenzamine (7) suggesting that the catecholaminergic systems are mediating this drug's effects on recovery. Other studies have indicated a primary role for norepinephrine (NE) in recovery from hemiplegia induced by SMCx injury. Following either SMCx ablation or contusion, intraventricular infusion of NE, but not dopamine (DA), enhanced the rate of beam-walk recovery in rats (14,15). Infusion of NE into the cerebellar cortex contralateral, but not ipsilateral, to SMCx ablation results in a dramatic improvement in recovery of beam-walking ability in hemiplegic rats (16). Increasing NE release by administration of the alpha2-NE receptor antagonist idazoxan 24 hours after unilateral SMCx ablation also enhances beam-walking recovery in rats (8). However, administration of the alpha2-NE receptor antagonist yohimbine to rats with unilateral contusion injury of the SMCx did not significantly affect beam-walk recovery (17). The depression of local cerebral metabolic rates for glucose (LCMRglc) that occurs 48 hours postinjury in structures remote from a SMCx lesion (e.g., the red nucleus, cerebellum, locus coeruleus) is alleviated by administration of d-AMPH, and worsened by haloperidol treatment, 24 hours after this injury (18). The transient depression of staining for the oxidative enzyme alpha-glycerophosphate dehydrogenase in cortical regions following unilateral SMCx injury (19) is: 1) worsened by prior lesion of the locus coeruleus; 2) blocked by d-AMPH treatment; and, 3) unchanged by d-AMPH treatment if haloperidol was administered concomitantly with d-AMPH (18). In addition, the accelerated recovery of tactile placing exhibited by d-AMPH-treated cats with bilateral visual cortex ablation was associated with an increase in cytochrome oxidase (CO) activity in the superior colliculus of these animals (20). These studies have led to the proposal that d-AMPH improves behavioral outcome following cortical injury by preventing or reversing a "remote functional depression" or "diaschisis" of metabolic processes and/or noradrenergic neurotransmission in structures remote from the injury site (5-7).

In contrast to the above data, drugs that reduce NE release or block postsynaptic alpha-NE receptors may adversely affect functional recovery. For example, a single dose of haloperidol (4), prazosin (17), or clonidine (21) administered 24 hours after SMCx injury has been reported to retard recovery of beam-walking in rats. Administration of phenoxybenzamine to gerbils with unilateral carotid artery ligation increases the incidence of stroke, worsens neurological deficits, and increases mortality (22). Haloperidol, prazosin, phenoxybenzamine, and clonidine have all been reported to produce a re-emergence of deficits in rats or cats recovered from SMCx injuries (5-7,23,24). These data imply that the noradrenergic system may mediate the maintenance of recovery that occurs in animals with unilateral SMCx damage.

The two years of research supported by this grant used a variety of experimental approaches to further investigate the pathophysiology (i.e., alterations of cerebral blood flow (CBF), metabolism, electrophysiology, biochemistry, anatomy, and behavior) that follows SMCx injury and the effects of treatments with d-AMPH or alpha-NE

agonists and antagonists on some of these pathophysiological events. Multiple lesion paradigms and histochemistry techniques were utilized in attempts to delineate the contributions of neuronal pathways and/or nuclei to beam-walking ability in normal and/or SMCx injured rats. In addition, we examined the effects of SMCx injury on additional measures of sensorimotor recovery and on behavioral tasks designed to assess cognitive deficits. These studies have: 1) increased our understanding of the diverse physiological and behavioral changes that occur after cortical injury; 2) helped elucidate the potential role of various subcortical structures and pathways in spontaneous and/or d-AMPH-induced recovery; and, 3) strengthened the hypothesis that d-AMPH may improve recovery by alleviating injury-induced reductions in neurotransmitter and/or metabolic activity.

SUMMARY OF RESEARCH FINDINGS

Experimental Models of Injury and Research Methodology:

In the projects performed during this grant period, we employed two basic methods for inducing SMCx injury. In some projects SMCx injury was induced using suction ablation, as described in detail elsewhere (4,8,9). In other projects, SMCx injury was induced via contusion of the cortex through a craniotomy site. These contusions were induced by either dropping a weight down a guide tube to strike a footplate resting on dura (described in detail in ref. 12) or by rapid compression of the cortex by an impactor tip attached to a pneumatically-driven piston (2.25 m/s impact velocity; described in refs. 25-27). Both contusion models produce similar deficits in locomotor ability (11,25,26) and result in hippocampal and subcortical neuronal damage (15,26,28-30) that is not observed after SMCx ablation. In addition to SMCx injury, studies were designed to explore the role of other motor structures in beam-walking ability. In these studies cerebellar injury was induced using suction ablation, and red nucleus lesions were induced by injection of quisqualic acid into this structure.

In all studies described below, young adult male rats (Sprague-Dawley strain, purchased from either Harlan or Zivic-Miller) were used as experimental subjects. The animals were housed individually, in standard wire-mesh cages, on a 12/12 hour light/dark cycle. Food and water available ad libitum, with exception of a 6-8 hour fasting period prior to surgeries.

All surgical procedures were conducted using aseptic conditions, and animals were anesthetized throughout all "survival" surgical procedures with either halothane or a combination of ketamine and sodium pentobarbital. "Non-survival" surgeries were conducted using urethane of chloral hydrate anesthesia.

Numerous research techniques were utilized in the conduct of studies supported by this grant. Unless sufficient methodological details are given in prepared manuscripts that have resulted from this research, the particular techniques used in each project are elaborated upon in the following subsections of this report.

Diaschisis Within the Neocortex:

It has long been noted that spontaneous recovery from motor impairments contralateral to a unilateral cortical injury occurs

within a few weeks of injury. This recovery of behavioral function has often been attributed to either reorganization processes that occur within the remaining cortical tissue, or to the spontaneous dissipation of a transient diaschisis or functional depression that has occurred in tissue adjacent or connected to the injured area. In a prior study, it was reported the time-course of d-AMPH-induced beam-walking recovery in cats with bilateral SMCx ablations was similar to that of animals with unilateral SMCx injury (31). In addition, the best spontaneous recovery was obtained in an animal with a very large bilateral ablation, extending beyond the boundaries of the SMCx. These findings imply that spontaneous motor recovery after SMCx injury is not due to reorganization within spared remnants of SMCx and that the contralateral SMCx is not mediating the accelerated recovery induced by d-AMPH treatment. To further test whether reorganization or diaschisis mechanisms within the cortex might account for some of the recovery observed after unilateral SMCx injury, a study was conducted to examine cortical microstimulation thresholds in the rat (32).

Using cortical microstimulation techniques in chronically anesthetized adult rats, movements evoked in hindlimbs (HL) and forelimbs (FL) following stimulation of contralateral cortex and areas bordering either SMCx ablation, contusion (30 g weight dropped from a height of 20 cm), or undercut laceration (4 mm diameter undercut, 2.5 mm from cortical surface) were studied from 6 hours through 475 days after injury (32). After unilateral undercut laceration or suction ablation of the HL area, no HL movements/responses could be evoked by stimulation of the ipsilateral dorsal cortical surface in any preparations studied from 6 hours through 475 days after injury. Similar data on FL responses were found in animals with FL area undercuts or ablations. After contusion injury, HL responses could be elicited in some animals for up to 15 days, but were never observed in contused rats studied from 30-290 days postinjury. The presence of stimulus-evoked movements in some rats early after contusion illustrates the slow-growing nature of this type of injury (12,27) compared to either laceration or ablation injury models. Stimulation of the contralateral cortical surface was never observed to result in movement of the limbs ipsilateral to any type of SMCx injury. These data suggest that reorganization, either within the injured or the contralateral cortex, cannot account for the functional recovery that occurs within weeks of such injuries. Although thresholds to elicit movements remained unchanged (up to the edge of the cavity) following ablation injuries, a marked elevation of thresholds to evoke FL movements following stimulation of intact cortex 0.5 to 1.0 mm anterior to HL contusion or laceration injuries was observed at 1 and 4 days postinjury, returning to normal by 9-15 days after injury. These latter data support the hypothesis of remote, transient depressive effects after cortical trauma. However, the lack of changes in thresholds after ablation injury suggests that the threshold changes near the boundary of cortical injuries may be uncorrelated with both the onset of functional motor impairments and with functional recovery. Thus, while results of this study indicate that a "cortical diaschisis" does occur after unilateral SMCx damage induced

by either contusion of undercut laceration, the data also imply that the onset and/or the alleviation of these effects may not contribute to either the onset or the recovery from the behavioral dysfunctions that occur after SMCx injury.

Oxidative Metabolism After Injury, d-AMPH, or Beam-walk Training:

To further explore functional depressions that might occur after unilateral SMCx injury we studied changes in cerebral oxidative metabolism, using histochemical staining for cytochrome oxidase (CO), in rats with right SMCx ablation (33). Sham operates and injured animals were treated with either saline or d-AMPH (2 mg/kg) 24 hours postsurgery and then sacrificed 24 hours later. Optical densitometry methods were utilized to quantify the intensity of CO staining in cortex and subcortical regions.

Results of this study showed that right SMCx ablation significant reduced CO activity within the remaining ipsilateral cortical tissue and in the ipsilateral nucleus accumbens, caudate-putamen, globus pallidus, superior colliculus, and red nucleus of saline-treated rats. Contralateral to injury, CO activity in saline-treated animals was significantly decreased in the cerebral cortex, superior colliculus, and red nucleus. Treatment with d-AMPH blocked this injury-induced metabolic depression, and the CO activity in all structures examined in the SMCx injury plus d-AMPH treated rats did not differ significantly from the metabolic activity of corresponding structures of sham operates given either saline or d-AMPH treatment.

In a similar study (Bramlett and Feeney, unpublished observations), CO activity was assessed in various regions of the cerebrum of sham operates and rats with right SMCx contusion (20 g weight dropped 20 cm). The animals were treated with saline or d-AMPH (2 mg/kg, i.p.) 24 hours postsurgery and sacrificed at either 2 or 6 days after surgery. Compared to saline-treated sham operates, CO activity was significantly depressed in the caudate putamen, ventrobasal thalamic nuclei, and the cortical tissue medial and lateral to contusion in SMCx-contused rats treated with saline. By 6 days after injury these structures as well as the medial geniculate and substantia nigra of saline-treated rats exhibited a significant paling of CO staining intensity. Treatment with d-AMPH partially attenuated the injury-induced depression of oxidative metabolism in the caudate putamen, ventrobasal thalamus, and medial cortex at 2 days postinjury and in the caudate putamen and substantia nigra at 6 days postinjury. Although the metabolic activity within these subcortical structures in d-AMPH-treated rats with SMCx contusion was not significantly increased relative to CO activity in saline-treated counterparts, it was not significantly lower than that of saline-treated sham operates.

The results of these two studies are compatible with the hypothesis that d-AMPH may improve behavioral outcome after SMCx ablation or contusion by either blocking injury-induced depressions of metabolism in remote structures or by stimulating metabolic activity in these remote regions. We have previously reported that d-AMPH blocks the injury-induced depression of glycolytic metabolism in subcortical regions and the transient depression of staining for the oxidative oxidative enzyme alpha-glycerophosphate dehydrogenase

in cortical regions (18). Other investigators have reported that the pervasive depression of LCMRglc seen after cortical infarction is reversed or attenuated by postinjury administration of d-AMPH (34). The ability of d-AMPH to stimulate a resumption of activity in transiently depressed, but otherwise functional and apparently undamaged, nuclei remote from the site of injury may explain the ability of this drug to improve the rate of behavioral recovery in animals with SMCx damage.

An important theoretical issue in recovery of motor ability after cortical injury is whether or not the same neural systems controlling behavior in normal situations are involved in the mediation of spontaneuous or drug-induced recovery. For example, if alternate neural networks are utilized for the expression of a particular behavior after injury to the SMCx, it would be important to identify the neuronal populations and pathways in order to optimize rehabilitation or therapeutic strategies aimed at strengthening those alternate pathways. Since the CO histochemistry technique was sensitive to injury-induced changes of metabolism within various pyramidal and extrapyramidal structures, this technique might be useful in delineating structures that are more or less involved (i.e. metabolically active) in beam-walking activity. If this proved to be the case, then this technique might be utilized to differentiate between subcortical structures that were actively involved in mediating beam-walking ability after SMCx injury. To examine this possibility, normal rats were given differential experience on the beam-walking task and brain tissue was subsequently analyzed for CO activity.

For this study animals were first trained on the beam-walk task until they reached a criteria of three successive "7" ratings on a standardized beam-walk rating scale (see refs. 4-6,11,15,17). Two groups of rats (N=8/group) were subsequently required to traverse the beam either 5 or 40 trials per day for five consecutive days (for a total of 25 or 200 trials, respectively). During this 5 day period each animal in beam-walk groups was matched with a "stress" control animal that was handled and subjected to the bright light and white noise (used to "motivate" the animals to traverse the beam and terminated when animals enter a goal box at the end of the beam), but not placed on the beam. A "non-stressed", or "normal", control group (N=6) was not exposed to either beam-walking or to the light/noise conditions, but remained in similar housing conditions within the animal colony for the same duration as animals in the other experimental conditions. All animals were sacrificed 1-2 hours after their final beam trial, light/noise exposure, or equivalent days in the colony, and brain tissue was processed for CO histochemistry. The density of CO staining was evaluated bilaterally, using microdensitometry, in the SMCx, caudate-putamen, subthalamic nucleus, substantia nigra, red nucleus, locus coeruleus, and cerebellar cortex.

Analysis of data revealed that, although there were regional differences in CO staining intensity and slight changes of oxidative metabolism within various brain structures across groups, none of the groups differences in CO activity were significant (see Figure 1). Thus, while the CO technique appears to be sensitive to changes of

metabolic activity induced by SMCx damage and/or drug interventions, this procedure does not reveal changes in oxidative metabolism within brain structures after exposing rats to differential amounts of locomotor activity or exposure to potentially stressful conditions.

Hippocampal Damage After SMCx Contusion:

Prior work with the weight drop method of inducing SMCx contusion has revealed extensive pyramidal cell loss in the CA3 and hilar sectors of the ipsilateral hippocampus at 20 to 30 days postinjury (15,29,30). Pneumatically-controlled contusion of the SMCx leads to cell damage in the ipsilateral hippocampus by 6 to 24 hours after injury, with progressive cell loss and atrophy of the dorsal hippocampus occurring from 8 to 30 days after injury (28). Although similar hippocampal pathology has been reported following fluid-percussion injury to the rat parietal cortex (35) and after acceleration-induced head injury in primates (36), secondary neuronal death in hippocampus was not seen after ablation of the right SMCX (15,30) and therefore appears unique to trauma. The prior work describing hippocampal cell loss after weight drop-induced contusion of the SMCx did not allow for determination of when hippocampal pathology is initiated after injury, nor was any conclusive data on the mechanisms underlying this cell loss collected. Establishing both the time-course and potential mechanism of hippocampal damage after unilateral SMCX contusion is important, since knowledge of the onset and mechanisms of cell damage may allow for development of treatments to reduce or prevent secondary neuronal damage. To investigate these events, we conducted the following study (37).

To investigate the time-course of pathological changes in the hippocampus ipsilateral to cortical injury groups of rats with right SMCx contusion (400 g-cm impact; 20 g weight dropped from 20 cm height) were sacrificed at 2 or 6 hours and at 1, 2, 3, and 4-5 days following injury. Coronal sections of brain tissue were processed for either Fink-Heimer silver staining (38,39) to detect argyrophilic cells or stained with thionin (TH) followed with acid fuchsin (AF; 1%) staining to detect acidophilic cells and loss of Nissl staining (40-42). The distribution of: 1) argryrophilic neurons, 2) AF-positive neurons, and 3) regions showing loss of Nissl staining, were plotted on templates of the hippocampus at three anterior-posterior (AP) levels (-2.5, -3.8 and -5.6 mm from bregma). The amount of cell damage (Cell Damage Index) revealed by each of the three stains was quantified by sector (CA1, CA3, Hilus, Dentate) within each coronal plane, using a three point rating scale developed by Auer et al. (42). With this scale, a rating of 1 denotes less than 10% damaged neurons, 2 = 10-50% damaged neurons, and 3 = more than 50% damaged neurons.

Ratings of cell damage observed on silver-stained tissue sections are depicted in Figure 2. Argryrophilic neurons in the CA3 and hilar sectors of the hippocampus ipsilateral to SMCX contusion were observed at 2 hours postinjury and increased in numbers and distribution by 6 hours. At the light microscopic level, these neurons presented as intensely black pyramidal perikarya and neurites that stood out clearly against a golden-yellow background. By 24 hours postinjury argyrophilic neurons appeared as pyramidal cells

with shrunken somata and the neurites were less prominent compared to 6 hours. By 2-5 days after injury the numbers of argyrophilic neurons decreased, and those remaining appeared to be severely atrophic.

At 2 or 6 hours after right SMCX contusion pyramidal neurons in the ipsilateral CA3 and hilar sectors exhibited an affinity for both TH and AF stains. AF-positive cells were triangular in shape with brilliant red cytoplasm. A loss of Nissl-positive pyramidal neurons in the ipsilateral CA3 sector was not observed at 2 hours, but appeared as early as 6 hours and was more extensive by 24 hours after injury. By 24 hours postcontusion, numerous bright-red AF-positive neurons were seen in the ipsilateral CA3 and hilar region. These neurons did not stain for Nissl substance. By 2 to 3 days after contusion peak numbers of AF-positive neurons were observed in the hippocampal CA3 and hilar sectors, where reduced Nissl staining was observed. Interestingly, the loss of Nissl staining in the CA3 and hilar pyramidal cell layers reached its maximum extent by 2 days postinjury. The contralateral hippocampus of contused rats had no discernible cell loss at any of the time-points analyzed, and argyrophilic or acidophilic neurons were never observed in sham operates. Ratings of cell loss (Nissl material) is shown in Figure 3, and the ratings of cell damage as judged in AF-stained material is shown in Figure 4.

To assess electrophysiological activity within the hippocampus, chloral hydrate-anesthetized rats had stainless steel electrodes lowered into the left and right dorsal CA3 sector (-3.8 mm from bregma, 3.7 mm lateral of midline and -3.3 to -3.9 mm from dura). Single unit activity was recorded on tape for off-line analysis (Brainwave Systems Inc.). After approximately 30 minutes of preinjury recording the electrodes were retracted, animals received a 400 g-cm contusion to the right SMCx, and the electrodes were returned to the original coordinates as rapidly as possible. Postcontusion recording was conducted for up to 6 hours. In 4 animals these acute recording methods were begun 24 hours after contusion.

Examples of electrophysiological activity recorded from hippocampi in rats prior to contusion are shown in Figure 5. Following contusion of the right SMCX, an isoelectric period was detected in both the left and right hippocamal CA3 regions. In the CA3 sector ipsilateral to injury this was followed by the onset of aberrant, epileptiform activity that endured for up to 3 hours after injury (see Figure 6). This seizure-like electrical activity was never observed in the hippocampus contralateral to injury. Following the period of hyperactivity, from 4 to 24 hours after injury, normal unit activity was recorded in the contralateral hippocampus, but few spontaneously active units were encountered in the ipsilateral hippocampus (Figure 7).

The histological data from this study indicate that the onset of subcortical neuronal damage has begun by 2 hours after cortical contusion. The numbers and distribution of hippocampal cells damaged (detected with silver or acid fuchsin stains) appears to increase thereafter, peaking between 2-3 days after injury. Hippocampal

neuronal death (as indicated by peak reduction of Nissl staining), is complete by two days postcontusion. Although only suggestive, the electrophysiological data point to posttraumatic seizure activity as a possible contributer to the onset of hippocampal cell death following cortical contusion. These data suggest that early interventions may be needed to: 1) reduce seizure activity; and, 2) prevent the progressive damage and loss of hippocampal neurons that occurs after TBI.

Contusion-Induced Temperature Changes and Pathology:

Recent data from ischemia (43) and concussive (44) brain injury models indicate that temperature (T) changes produced by anesthesia/surgery may be either exacerbate (e.g. hyperthermia) or attenuate (e.g. hypothermia) primary and secondary neuronal damage. However, none of the previous studies utilizing weight drop-induced SMCx contusion have addressed the issue of T monitoring or control during or subsequent to injury. Thus, a study was conducted to examine the relationship between brain and body T changes and their relationships to behavioral and anatomical outcome in rats with SMCx contusion (45,46).

Results of this study indicate that surgery/anesthesia alone lowered brain (but not body) T and cortical contusion induced an additional transient (2-6 minutes) drop in brain T. Over the next hour, both brain and body T were elevated in contused rats compared to control animals. Behavioral outcome measures (beam-walking, weight loss) did not correlate with either preinjury or postinjury absolute levels of brain or body T. However, the level of brain, but not body, T at the time of SMCx contusion was positively correlated with the severity of both ipsilateral hippocampal (CA3 sector) cell loss and thalamic gliosis (lower brain T leading to less cell damage/gliosis). The change (increase) in brain, but not body, T from the time of contusion to either 5 or 10 minutes after termination of anesthesia was found to be negatively correlated with both behavioral and anatomic outcome measures. That is, a more rapid return of brain T toward normothermia lead to less post injury weight loss, a more rapid recovery of beam-walking ability, reduced cell loss in the ipsilateral CA3 sector of the hippocampus, reductions in gliosis in the ipsilateral thalamus and medial geniculate, and smaller volumes of cortical cavitation necrosis. These overall findings indicate that SMCx contusion induces a hyperthermic response in the injured brain and that the rate at which brain T returns towards normothermia may be a good indicator or "predictor" for the severity of TBI.

CBF Alterations After SMCx Contusion and/or d-AMPH:

In the rodent models of focal cortical contusion, no studies on the role of CBF changes after SMCx injury and/or d-AMPH treatment have been performed. Although the failure of atropine to block the effect of d-AMPH on functional recovery was interpreted as ruling out CBF as an important factor in drug-facilitated recovery (6), CBF was not directly evaluated in that study. Cortical necrosis after focal SMCx contusions in the rat appear to involve secondary injury, as these injuries progress from hemorrhages in the contused cortex,

underlying white matter, and subarachnoid space in the first hours posttrauma and slowly expand to a necrotic cavity over the subsequent 2 to 4 weeks (12,27). These hemorrhages may cause cerebral vasospasm and reduction of CBF. Microdialysis studies following TBI describe a massive release of glutamate and aspartate and disruption of metabolism in the contused cortex (47). Glutamate release induced by subarachnoid hemorrhage has been shown to correlate with reductions in cortical CBF (48). Since vasospasm and reduced CBF may directly contribute secondary injury after TBI (49) and d-AMPH has well known vasodilatory effects (50), a study was conducted to evaluate changes in CBF after cortical contusion and/or d-AMPH treatment (51,52).

Rats with SMCx contusion (400 g-cm impact) were prepared for measures of regional CBF 22 hours after injury. CBF was measured with the quantitative iodo-14C-antipyrine (IAP) autoradiographic technique (53). At 24 hours after TBI, animals (N=5) were administered a single dose of d-AMPH (2 mg/kg, i.p.) or saline (N=6). Control rats with no injury (N=5) were administered saline. The IAP injection and procedures for measurements of regional CBF were initiated 1 hour after injection of drug or saline. After developing the autoradiographs and standards, global CBF was evaluated by measuring the average flow in the entire left and right hemispheres (collapsed across readings from 10 evenly spaced coronal sections). When averaged over all sections, CBF (ml/g-1/min-1) in saline-treated rats was not different for the left (1.69 ± 0.09) and right (1.68 ± 0.17) hemispheres. d-AMPH produced a significant global increase in hemispheric CBF, particularly in the contralateral hemisphere, but the average global CBF in the left (2.42 ± 0.24) and right (2.23 ± 0.3) hemispheres of d-AMPH-treated rats were not significantly different.

The following regions were quantified for regional CBF using microdensitometry: 1) cerebral cortex at the epicenter (ischemic core), lateral, and medial borders of the contusion site; 2) homologous regions of cortex in the contralateral hemisphere; 3) the left and right dorsal hippocampus; 4) the left and right thalamus; and, 5) the left and right dorsal striatum. As shown in Table 1, TBI produced a profound reduction of CBF in the impacted cortical tissue, with hyperemia apparent in the ipsilateral hippocampus, striatum, and cortex lateral to TBI at 24 hours post injury. This pattern of injury-induced changes in regional CBF was unchanged by d-AMPH treatment.

To more thoroughly analyze changes in cortical tissue, CBF was measured bilaterally at 9 to 12 medial-to-lateral cortical sites from 10 evenly spaced coronal sections between the frontal and occipital poles. These data confirmed that a significant reduction in CBF occurred in the traumatized hemisphere at the center of the contusion and that d-AMPH-treatment did not affect this phenomenon. At coronal planes located at the anterior, but not posterior, borders of the contusion, d-AMPH-treated animals had significantly increased CBF in the cortex contralateral to SMCx contusion. However, d-AMPH did not significantly increase CBF at any location within the injured hemisphere.

Table 1. Values (mean±SEM) of regional CBF in brain regions, expressed as percentage of contralateral hemisphere, of saline- and d-AMPH-treated rats after unilateral cortical TBI.

REGIONS	SALINE rCBF	p	d-AMPH rCBF	p
Cortex (Ischemic Core)	25±4%	<.001	14±2%	<.001
Cortex Lateral to TBI	148±35%	ns	82±28%	ns
Cortex Medial to TBI	94±3%	ns	66±21%	ns
Striatum	163±24%	<.05	175±24%	<.05
Hippocampus	138±9%	<.02	175±52%	ns
Thalamus	88±13%	ns	87±21%	ns

p = statistical significance of difference from the mean of 100%.
ns = nonsignificant

The results of this study imply that the mechanism by which d-AMPH enhances behavioral recovery after cortical trauma is not by it's effects on regional CBF in any specific structure, although the global increase in CBF produced by AMPH may be related to this effect. The hyperemia observed in some cortical and subcortical regions in the current study may reflect changes in response to enhanced neural activity (e.g. "excitotoxic" effects potentially generated by excess release of excitatory amino acids). However, in the absence of metabolic data from similarly treated animals, these hyperemic responses to cortical injury are difficult to interpret. Futhermore, if regional CBF changes after TBI are related to the patterns of secondary cell damage previously reported for cortex, hippocampus, and striatum (12,15,17,26-30,37), the failure of d-AMPH to affect the regional patterns of CBF may explain why administration of this drug 24 hours after injury has not been shown to reduce secondary cell damage in animals with TBI. Recent data indicate that TBI-induced changes in CBF are initiated quite early after injury.

In the 1970's a number of reports appeared indicating that at least 50% of postmortem cases of human TBI showed evidence of global or focal ischemic damage in addition to the primary damage resulting from the injury (54,55). Despite these findings, numerous clinical investigations conducted in the 1980's, most performed in the first few days after injury, failed to support the hypothesis that ischemic episodes occurred following TBI. This led some investigators to conclude that, if ischemic conditions occur at all after TBI, such conditions must occur within the first few hours of injury (56,57), which is consistent with reports that 30-50% of TBI patients are hypoxic on admission to the emergency room (58). Subsequently, in studies using dynamic CT scanning, it was reported that 17 of 25 fatally injured patients exhibited severe ischemia within the first 2 hours of TBI (59). More recently, it was reported that 33% of TBI patients seen within 6 hours of injury showed evidence of ischemia (60). Clinical outcome was shown to be correlated with CBF values in the first 8 hours after injury, but this correlation was lost after 12 hours (60).

Preliminary studies were conducted to address the question of whether or not early changes in CBF, and concomitant changes in metabolism, occur in regions showing secondary cell death after cortical TBI in the rat (61,62). For these studies, rats with unilateral SMCx contusion (2.25 m/s velocity) or sham injury underwent studies of CBF (using the IAP technique) or of LCMRglc (using the [14C]2-deoxy-D-glucose technique) immediately, 30 min, 6 hours, 1 day, and 10 days after injury. For quantification of the IAP and 2-DG autoradiography, optical density measurements were taken bilaterally in cortex and subcortical regions.

Although too few animals (2-3/group) have been processed to allow for statistical evaluation of the data, several interesting changes in CBF and LCMRglc induced by TBI have been observed. In animals sacrificed immediately or 30 minutes after TBI, LCMRglc in contused SMCx and surrounding tissue increases by 55% or greater above control levels and the underlying dorsal hippocampus shows a similar percent increase in LCMRglc. At these same time-points, CBF within the contused cortex and in the underlying hippocampus decreased to ischemic levels. These data suggest that excessive neuronal activity in these regions occurs in the absence of adequate CBF to support aerobic metabolism, which would lead to increased lactate production and subsequent acidosis (63). These acute increases in LCMRglc and reductions in CBF occur in hippocampal regions where seizure-like activity (37) and subsequent neuronal death (15,28) have been observed in previous studies of SMCx contusion. By 6 hours postinjury the ipsilateral cortex (including frontal, parietal, temporal and occipital cortex) and hippocampus exhibited a diffuse metabolic depression, with LCMRglc decreased by as much as 70% from normal levels. This depression of LCMRglc in cortex and hippocampus was also present at 24 hours postcontusion, and somewhat less severe reductions (15 to 45%) in LCMRglc were apparent at 6 and 24 hours after injury in the ipsilateral ventral and anterior thalamic nuclei, striatum, lateral geniculate, red nucleus, and substantia nigra. CBF had returned to normal levels in most regions by 24 hours, but remained decreased in regions exhibiting low rates of glucose metabolism. By 10 days after TBI, CBF appeared to be within normal range in all regions, including cortical tissue surrounding the area of trauma, and LCMRglc had also recovered toward normal levels in most structures at this time. In contrast to the study on CBF after TBI and d-AMPH treatment (51,52), no hyperemic regions were detected in this study. Cortical necrosis and cell loss in hippocampal regions showing hypermetabolism and reduced CBF acutely after injury had developed by 10 days after contusion.

This work has clearly shown that there is an uncoupling of the normally close relationship between CBF and LCMRglc in the first 30 minutes after TBI. These acute changes may reflect neurochemical changes in the cellular milieu induced by the injury, such as those reported for lactate and glutamate in cortical and hippocampal regions acutely after contusion or concussive injury to the cortex (47,64,65) or after subdural hemorrhage (48). Given the rapidity at which these changes in both glycolytic metabolism and CBF occur, early interventions with pharmacotherapeutic agents may be necessary if the goal is to normalize CBF and/or LCMRglc. Such interventions may not only improve behavioral outcome, but may also reduce secondary neuronal damage following cortical contusion.

Catecholamine Levels After SMCx Contusion and/or d-AMPH:

Previous studies, utilizing animal models of stroke or cortical ablation and contusion, have reported that tissue content of catecholamines are decreased or depleted days to weeks post injury (5,6,15,66-70). These findings have, in part, shaped the hypothesis that d-AMPH may improve behavioral outcome following cortical injury by preventing or reversing a "remote functional depression" of noradrenergic neurotransmission in structures remote from the injury site (5-7). Although these studies have provided valuable data on the effects of brain injury on catecholaminergic functioning, the use of homogenized tissue extracts (containing both extracellular and vesicular stores of transmitters) has prevented understanding of the effects of injury on true extracellular release of catechols versus effects of the injury on neurotransmitter stores. The sensitive microdialysis techniques developed within the past decade provide methods that allow for sequential sampling of extracellular levels of neurotransmitters in the same animal, reducing the need for multiple groups of animals for conduct of studies on the effects of injury and/or drug treatments at several times after intervention(s). To date, no published manuscripts have appeared describing the effects of TBI on catecholamine levels assessed via microdialysis. In the studies described below, we employed microdialysis sampling of extracellular levels of NE or DA combined with high performance liquid chromatography (HPLC), to assess the effects of unilateral SMCx contusion and/or d-AMPH treatment on extracellular levels of these catecholamines.

In all studies, concentric dialysis probes similar to those described in detail by Abercrombie and Finlay (71) were used for extraction of neurotransmitters from brain regions. Probes were continuously infused with artificial cerebrospinal fluid (aCSF; 147 mM NaCl, 2.5 mM KCl, and 1.3 mM $CaCl_2$), at a flow rate of 1.5 µl/min. To determine the relative recovery of each probe for the catechol of interest (either NE or DA), probes were calibrated using the in vitro recovery method (71,72), where the amount of NA or DA in the dialysate sample was expressed as a percent of that contained in an equal volume of aCSF obtained directly from a beaker containing 5 uM concentrations of NE or DA. Twenty minute sampling periods for collection of dialysate prior to immediate injection into the HPLC unit were used during all studies, and the NE or DA levels in each dialysate sample were corrected for probe recovery.

Since the cerebellum has been implicated in mediating recovery of beam-walking ability after SMCx injury (16), shows up to a 60% reduction of NE levels after right cerebral artery occlusion (69) and an increased rate of NE catabolism (indicated by the ratio of NE to its metabolite 3-methoxy-4-hydroxyphenylglycol) after SMCx ablation (70), and has been implicated in NE-dependent learning of locomotor tasks (73), we first examined bilateral changes in NE within this structure after right SMCx contusion (74,75). Rats with contusion (400 g-cm impact) or no cortical injury had dialysis probes implanted in the left and right cerebellar cortex, probes were cemented in place, and the animals were then placed in cylindrical holding cages with the probe assemblies attached to a fluid swivel that allowed the animal to move about freely in the cage (71).

From 22 to 24 hours after surgery, basal levels of NE within dialysate samples were found to be significantly depressed in both the right (TBI=4.96±0.43 pg; controls=8.21±1.17 pg) and left (TBI=4.84±1.09 pg; controls=10.08±0.97 pg) cerebellum of contused rats. These approximately 50% decreases in cerebellar NE levels support the hypothesis that SMCx contusion induces a remote depression of neurotransmitter release and/or synthesis. At 24 hours after surgery, all animals were given a 2 mg/kg (i.p.) dose of d-AMPH and dialysis samples were analyzed over the subsequent 6 hour period. As shown in Figure 8, d-AMPH induced a large bilateral increase in the extracellular levels of NE of both sham operates and contused rats. Although the absolute level of NE released in the first 3 hours after d-AMPH was significantly less in contused than in control animals, the relative percent change from basal values were quite similar in both groups. These data indicate that d-AMPH-releasable stores of NE are present in cerebellar terminals of contused rats, implying that the synthesis and packaging of NE within synaptic vesicles is intact in these animals, even though spontaneous release of NE may be impaired. By 22 to 24 hours after d-AMPH treatment, NE levels within the cerebellum of both normal and SMCx-contused rats had returned to the predrug, basal levels. Since prior data indicate that a single d-AMPH treatment is sufficient to improve beam-walking recovery in rats with right SMCx contusion (11), these data indicate that a prolonged elevation of NE may not be needed to initiate this recovery process. However, we cannot rule out the possibility that the addition of beam-walking behavior during the period of d-AMPH intoxication, shown to be important for achieving behavioral benefits from this treatment (4,9), might somehow prolong NE release or otherwise interact with cerebellar circuitry to improve behavioral outcome. A transient increase in cerebellar NE, which is known to be involved in ability of rats to learn new locomotor tasks (73), may be sufficient to induce accelerated beam-walking recovery in animals with unilateral SMCx injury.

The mechanisms by which SMCx injury produces remote depressions in neurotransmitter levels is speculative. However, it is hypothesized that damage to cortical efferents arising from the somata of NE-producing cells in the locus coeruleus (LC) may result in retrograde reactions within these cell bodies. A subpopulation of these NE neurons are known to bifurcate and innervate both the neocortex and the cerebellar hemispheres (76,77), and injury to cortical efferents arising from the LC reduces the activity of enzymes involved in the synthesis of NE (78). If injury to LC efferents result in retrograde changes in LC somata that either reduce the spontaneous firing rates of these cells or the synthesis of NE, it is plausible that NE would be reduced in all regions innervated by the LC. To test this hypothesis, and to determine the rate at which unilateral SMCx injury induces changes in extracellular levels of NE, we conducted a microdialysis study to assess NE levels in the frontal cortex of contused rats (79).

In this study rats were continuously anesthetized with halothane and dialysis probes were implanted in the right and left cortex (1-1.5 mm anterior to bregma, 1.5-2.0 mm from midline). After a 20

to 30 minute "flush" and a 2 hour baseline sampling period, probes were removed. In one group of animals, TBI (2.25 m/s impact velocity) was induced over the right SMCx. Probes were reinserted (within 90 seconds of TBI, 10 minutes after removal in shams) and additional dialysis samples were collected for 4 hours.

As shown in Table 2, basal levels of NE did not differ between sham injury controls and animals assigned to the TBI condition. Following removal and reinsertion of probes, NE levels in shams did not change significantly from baseline over a 4 hour sampling period. The percent changes in NE levels from basal values during the first and last 20 minute dialysate samples (during the 4 hour period after reinsertion of probes) from sham animals are shown in Table 2. TBI resulted in a slight (not significantly greater than shams) increase in NE levels in the right cortex in the first 20 minutes postinjury, with NE levels in the contused hemisphere dropping from 62-77 percent of baseline within the first hour and to 50 percent of baseline by 4 hours postinjury (Table 2). The NE levels in the contralateral (left) cortex were significantly increased in the first 20 minutes after right SMCx contusion, with levels slowly dropping to a low of 71 percent of basal values by the fourth hour after TBI (Table 2).

Table 2. Shows the mean±SEM pg levels of NE in frontal cortex of control (sham) and TBI rats for the final hour of baseline sampling and the first and last NE sample, expressed as percent change from baseline, collected from these groups over a 4 hour period after probe reinsertion and/or TBI to the right SMCx.

	Basal NE		Reinsertion/PostTBI 20 minutes		% Change in NE 240 minutes	
Group	Right	Left	Right	Left	Right	Left
Sham:	15.6±1.2	13.7±1.1	112±8.3	99±7.7	92±13.5	98±15.9
TBI:	13.4±2.4	12.5±1.2	134±30.9	159±25.7	50±5.0	71±7.4

To confirm that we were indeed measuring NE with our chromatograpic conditions, and to determine if NE receptor-mediated events were intact after TBI, animals were given a 0.3 mg/kg (i.p.) dose of the alpha-2 agonist clonidine (which should decrease LC firing and NE release) 4 hours after TBI/probe reinsertion. This treatment further decreased NE levels in TBI rats (to 29-35% of baseline) and produced a 31-49% reduction of NE from basal levels in the control rats.

The bilateral decrease in cortical NE following unilateral SMCx injury, and the rapidity of this response, implies that the LC may begin to "shut down" almost immediately after cortical trauma. It is also interesting to note that the 50% reduction in extracellular NE levels in the contused cortex by 4 hours after TBI is almost identical to the percent reduction of NE levels in cerebellar tissue at 22-24 hours after right SMCx contusion (74,75). The rapid drop in cortical NE levels after TBI found in this study occurs during the period when TBI-induced increases of glutamate and aspartate are

present (47,64). Since NE (which is an inhibitory transmitter in cortex) modulates both calcium influx (80) and glutamate activity (81), the rapid TBI-induced reductions of cortical NE acutely after injury may increase the potential for secondary cell damage mediated by excitatory amino acids. Based on these data, it is hypothesized that early (< 1-2 hours postinjury) interventions with drugs such as d-AMPH or alpha-2 NE antagonists (to increase NE release) may be useful in attenuating TBI-induced reductions of NE and may provide some neuroprotection afcer such injuries.

As mentioned earlier in this report, substantial data supports the hypothesis that NE is the neurotransmitter underlying the beneficial effects of d-AMPH and several other drug treatments on improvement in the rate of beam-walking recovery after SMCx injury. In addition, several lines of evidence in our recent research indicate that early intervention with drugs increasing catecholamines, or NE, may provide both more neuroprotection and behavioral benefits than is obtained by witholding treatment for up to 24 hours. However, as was the case for NE, little or no data has been collected on the acute changes in DA after SMCx contusion. If drugs such as d-AMPH are to be utilized for acute treatments after cortical injuries, studies to determine DA levels subsequent to TBI must also be performed. Since elevated levels of DA within the striatum after ischemic injury have been shown to work concertedly with glutamate to increase neuronal damage (82), it is important to assess TBI-induced changes in striatal DA after cortical injury since this is one subcortical region exhibiting slow, progressive atrophy after unilateral SMCx contusion (28). If SMCx contusion elevates striatal DA acutely after injury, drugs increasing DA release, such as d-AMPH, may be contraindicated during the acute phase after TBI.

Pilot data on DA levels within the striatum of sham controls (N=3) and rats with right SMCx contusion (N=4; 2.25 m/s impact velocity) has been collected. Animals were prepared, and dialysate samples collected, as in the acute microdialysis study of cortical NE, except that probes were placed in the right and left striatum (1.5-2.0 mm anterior to bregma, 2.0 mm lateral from midline). As shown in Table 3, the mean$\pm$SD basal levels of striatal DA were comparable between sham and TBI groups and between hemispheres in either group. Furthermore, DA levels of animals in the sham control group and the TBI group showed very little percent changes from basal levels, in both the right and left striatum, during the entire 4 hour sampling period after probe reinsertion. The percent change from baseline for DA in the right and left striatum of both groups in the first and last dialysate sample collected after probe reinsertion and/or TBI is also shown in Table 3. These data, and the very similar average maximum increases of DA above basal levels observed in sham operates (right = 108$\pm$10.4% in the 160 minute sample; left = 115$\pm$2.3% in the 120 minute sample) and contused rats (right = 117$\pm$2.2% in the 120 minute sample; left = 115$\pm$9.4% in the 120 minute sample), indicate that unilateral SMCx contusion does not substantially affect spontaneous DA release within the right or left striatum in the acute phase after injury.

Table 3. Shows the mean±SD pg levels of DA in the striatum of control (sham) and TBI rats for the final hour of baseline sampling and the first and last DA sample, expressed as percent change from baseline, collected from these groups over a 4 hour period after probe reinsertion and/or TBI to the right SMCx.

	Basal DA (pg)		Reinsertion/PostTBI 20 minutes		% Change in DA 240 minutes	
Group	Right	Left	Right	Left	Right	Left
Sham:	1152±165	1231±118	89±22.9	96±12.7	105±11.7	93±17.3
TBI:	1178±259	1126±207	88±25.1	110±14.5	108±4.3	105±11.7

While these data must be considered preliminary due to the small numbers of animals studied, they do suggest that there is not a generalized "catecholamine diaschisis" following unilateral cortical contusion. These data also support the hypothesis that depression of NE, but not of DA, may underly some of the behavioral deficits observed after cortical trauma and, the influence of catecholaminergic drugs on facilitation of behavioral recovery may be primarily due to the effects of these drugs on NE neurotransmission. Additional research will be needed to confirm these preliminary results and determine if alterations in the DAergic system, produced by either injury and/or drug treatments, influences histopathology observed in the striatum after SMCx contusion.

Behavioral Pharmacology Experiments:

We have proposed (5-7) that NE plays a central role in both the promotion as well as the maintenance of functional recovery after SMCx injury. This hypothesis predicts that, early after injury, 1) treatments which increase central NE release will enhance recovery from hemiplegia, and 2) treatments which either reduce NE release or block postsynaptic NE actions will retard recovery. After recovery has occurred, 3) administration of drugs which decrease NE release or block postsynaptic actions of NE should lead to a transient re-emergence (reinstatement) of hemiplegic symptoms. Although d-AMPH stimulates NE release, and NE acts upon both alpha and beta receptors, clarification and/or characterization of the specific NE receptor subtypes involved in beam-walk recovery has yet to be established. Since previous studies indicate that the alpha-NE receptors might be mediating the effects of d-AMPH or intraventricular NE on beam-walking recovery, and these receptors are known to regulate LC neuronal activity (83-86), a study utilizing drugs affecting various NE receptor subtypes was conducted (87).

Our first goal was to further characterize involvement of the alpha-NE receptor system in the promotion of BW recovery after unilateral SMCX ablation in the rat. Therefore, the effects of drugs hypothesized to be beneficial for locomotor recovery (l-phenylephrine, an alpha1-NE agonist, and yohimbine, an alpha2-NE antagonist) and those hypothesized as potentially detrimental for motor recovery (prazosin, an alpha1-NE antagonist and clonidine, an alpha2-NE agonist) were compared with saline and d-AMPH. To

further test the hypothesized role of the alpha-NE receptors in the maintenance of beam-walking ability, prazosin, clonidine and yohimbine were administered to injured rats that had recovered beam-walking ability after SMCX ablation.

Of all drugs and doses tested for effects on acceleration or retardation of recovery (administered 24 hours postinjury), only d-AMPH (2 mg/kg) and yohimbine (at 10 mg/kg, but not 0.5 or 5 mg/kg) were found to exert significant effects on beam-walking recovery in rats with right SMCx ablation. Both of these drugs were found to produce very similar improvements (vs saline-treated controls) of recovery rates in hemiplegic rats. This finding implies that the common action of these drugs, which is an increase in NE release, was responsible for their ability to improve the rate of beam-walking recovery. Interestingly, neither prazosin (2 or 4 mg/kg), clonidine (0.1 or 0.4 mg/kg), or phenylephrine (2, 4, or 8 mg/kg) exerted any positive or detrimental effects on the rates of beam-walking recovery when administered 24 hours after surgery. In experiments to examine the effects of alpha-NE drugs on maintenance of beam-walking recovery after SMCx ablation (18 days after injury), it was found that both clonidine (0.1 and 0.4 mg/kg) and prazosin (2 and 4 mg/kg) reinstated beam-walking deficits in recovered rats (87). These data were interpreted as support for the hypothesis that adequate levels of NE, and/or ability of NE to act upon postsynaptic alpha1 receptors, may be necessary for the maintenance of locomotor recovery after SMCx damage.

Some aspects of the data in this experiment deserve special attention. First, the dose of yohimbine found to accelerate recovery after right SMCx ablation was reported to slightly, but not significantly, improve beam-walking recovery after right SMCx contusion (17). Second, a 4 mg/kg dose of prazosin, found to exert no significant effects on recovery after SMCx ablation, was reported to significantly retard beam-walking recovery after unilateral SMCx contusion (17). These data illustrate that substantial differences in drug effects can be obtained when different injury parameters are employed for pharmacotherapeutic studies. Although procedural differences related to behavioral assessments, experimenter variations, etc. may partially explain these differential findings of drug effects between cortical ablation and contusion models of injury, these effects may be related to the fact that more extensive secondary damage is produced by contusion injuries, particularly in subcortical regions, than after cortical ablation (15,30). This factor of more extensive subcortical damage influencing a drugs ability to affect behavioral recovery has previously been hypothesized to explain the ability of d-AMPH (2 mg/kg) to improve recovery after a 400 g-cm cortical impact but not after an 800 g-cm impact to the right SMCx (11).

To further examine the effect of injury location on the ability of drugs to influence behavioral outcome, a study on the ability of d-AMPH to affect recovery after either left or right SMCx contusion was conducted (88). In our previous studies on cortical contusion, only behavioral deficits and effects of drug interventions after TBI to the right SMCx have been examined (11,15,17,18,30). Since differential behavioral and biochemical effects are reported to occur

following right or left middle cerebral artery occlusion (67,68) or suction ablation (89), it was considered necessary to see if similar effects obtained after TBI to the right versus left SMCx. The presence of hemispheric differences in response to TBI or drug therapies may have profound implications for the proper choice of pharmacological interventions, drug dosage, and rehabilitation strategies needed to improve functional recovery.

In this study, rats were trained on the beam-walking task and open field activity was assessed (24 hours) prior to injury. Beam-walking was rated as previously described (4,87) and open field activity was conducted in a 60 x 60 x 30 (W x L x H) cm wooden field that had 15 cm square grids demarcated on the floor surface. Numbers of rearings and numbers of grids crossed during a 5 minute activity period were counted. After presurgical training, sham operates and rats with either right or left SMCx contusion (400 g-cm impact) were randomly assigned to receive either saline (SAL) or one of two doses of d-AMPH (2 or 3.5 mg/kg). Twenty-four hours postsurgery all animals received a single trial to assess beam-walking ability and a 5 minute open field test. Immediately following these tests d-AMPH or saline was administered. Beam-walking ability was tested at 1, 2, 3, 6, and 24 hours after injection and every other day for 15 days. Open field testing was conducted every day for 15 days post surgery. The open field activity data were expressed as difference scores, obtained by subtracting each animals daily post surgery score from their pre-surgery, baseline score. After completion of behavioral testing animals were euthanized, perfused, and thionin-stained histological sections were analyzed to calculate: 1) the average lesion volume per group; 2) the maximum extent of damage to medial cortex in sections located +2.2, +1.2, -0.26, -2.12, -4.16, -5.6, and -6.04 mm from bregma; and 3) the maximum lesion depth in the same 7 coronal sections. Data on the maximum damage to medial cortex and lesion depth were subdivided into categories of either anterior (+2.2, +1.2, -0.26) or posterior (-2.12, -4.16, -5.6, -6.04) damage for statistical analyses.

No significant effects of TBI or drug treatment were found for measures of open field activity (for rearings or grid crossing) in this study. Although TBI tended to produce some hyperactivity, as evalated by grid crossings, that was slightly greater after left than right SMCx contusion, groups showed large variability and data from the TBI groups always overlapped with that of sham operates given equivalent drug or saline treatments. TBI groups given either 2 or 3.5 mg/kg of d-AMPH showed some reductions in the numbers of grid crossings from 1 to 4 days after treatment as compared to saline-treated counterparts, but these effects were also not significant due to large within group variability. On the beam-walking task, a 2 mg/kg dose of d-AMPH was found to significantly facilitate recovery over the first 3 days after surgery for the left, but not the right, SMCx contused group as compared to the SAL controls (see Figure 9). In contrast, the 3.5 mg/kg dose of d-AMPH produced a significant improvement in the rate of beam-walking recovery over the first 3 days after surgery for the right, but not the left, SMCx contused group (see Figure 10).

The lack of d-AMPH-facilitated recovery in right SMCx contused rats after a 2 mg/kg dose was puzzling, as we have previously found this dose to facilitate beam-walking recovery after a 400 g-cm impact to the right SMCx (11). Since the location, volume, and medial extent of cortical damage may influence the ability of d-AMPH to affect recovery (11,90), presumably by differential damage to cortical efferents arising from the LC (90), extensive histological anaylses were performed on tissue of left and right SMCx contused rats. These analyses revealed that the overall volume of cortical necrosis between groups with left and right contusion did not differ significantly and there were no significant differences in lesion depth amongst the various groups. However, the volume of injury in the right SMCx contusion plus 2 mg/kg d-AMPH group was significantly larger than that of the group with right SMCx contusion that received a 3.5 mg/kg dose of AMPH. In addition, the right SMCx plus 2 mg/kg d-AMPH group had a larger overall injury volume, with significantly more damage to medial cortex in anterior tissue sections, than did the left SMCx contusion plus 2 mg/kg d-AMPH group. These findings indicate that the failure of the 2 mg/kg dose of d-AMPH to improve the rate of beam-walking ability after right, but not left, cortical injury in the current study may be due to the increased volume of injury and/or the increased damage to anterior medial cortex in these rats. Since efferents from the LC project caudally from the frontal cortex along the midline, increased damage to these fibers may have produced greater reductions in NE in this group, and thus attenuated d-AMPH's ability to promote behavioral recovery. Although the right SMCx injury group treated with 3.5 mg/kg of d-AMPH had more damage to posterior medial cortex than the right injury group receiving 2.0 mg/kg of d-AMPH, this higher dose of d-AMPH was capable of improving the rate of beam-walking recovery. This finding implies that the higher dose of d-AMPH was able to "overcome" the hypothesized greater reductions of NE that would result from damage to medial cortex. Another interpretation is that damage to LC efferents in posterior portions of the midline cortex (in the right, 3.5 mg/kg group) does not influence behavioral deficits as much as damage to these efferents in anterior midline cortical regions (which occurred in the right, 2.0 mg/kg group).

Although this study has not provided conclusive data with regards to potential asymmetrical responses after left or right hemisphere damage, the data can be taken to suggest that the optimal dosage of d-AMPH (or other drugs) for promoting functional recovery may be dependent upon the hemisphere injured. Results from this study also indicate that the extent of cortical necrosis and/or the amount of damage to midline cortical tissue is as important, if not more so with respect to drug effects after cortical injury, than is the particular hemisphere suffering injury. Further studies, providing information of biochemical, metabolic, and anatomical measures of left versus right hemisphere injury and/or medial cortex damage, and the response of each to drug interventions, must be conducted before firm conclusions on the influence of these factors can be made.

As mentioned several times in this manuscript, the cerebellum has been implicated as one potential structure mediating both spontaneous and d-AMPH facilitated recovery of beam-walking ability after

unilateral SMCx damage. If that is the case, then damage to the cerebellum should result in deficits that are move severe than those observed after SMCx injury and, drugs affecting catecholamine neurotransmission should be less effective in improving beam-walking ability after cerebellar injury. To test this hypothesis, rats with unilateral cerebellar ablation were tested for beam-walking ability and the effects of catecholaminergic drugs, administered early of late after injury, on recovery were assessed (91).

Rats were pretrained on the beam-walk task and then underwent surgery to remove the left anterior and neocortical cerebellar tissue. After surgery, all animals were tested every other day from 1 to 30 days, at 5 day intervals through day 40, and at 10 day intervals until 80 days post surgery. Beginning 24 hours after injury, d-AMPH (2 mg/kg), haloperidol (0.4 mg/kg), d-AMPH plus haloperidol (2 and 0.4 mg/kg, respectively) or saline was administered (i.p.) every 5th day for a total of 6 injections. On the six drug days, animals were given additional beam-walk trials at 1, 3, and 6 hours after drug administration.

Saline-treated rats with left cerebellar cortex ablation showed a very slow, gradual improvement in beam-walking ability, although recovery was still incomplete at 80 days after injury. Since rats with unilateral SMCx injury generally show complete recovery on the beam-walking task within a maximum of 15-30 days after surgery, it is obvious that cerebellar damage results in more severe and enduring impairments of motor ability than does SMCx damage. In contrast to the many studies showing enhanced rates of beam-walking recovery after SMCx injury and d-AMPH treatments, rats with cerebellar damage plus d-AMPH showed slower recovery rates in beam-walking ability than their saline-treated counterparts. This retardation of recovery was also observed in cerebellar-lesioned rats treated with either haloperidol or d-AMPH plus haloperidol treatments (91). Regardless of drug condition, all animals with evidence of damage to the deep cerebellar nuclei showed absolutely no recovery of beam-walking ability.

Those animals that showed adequate recovery by 110 days after cerebellar ablation were administered either phenoxybenzamine (an alpha-NE blocker; 10 mg/kg, i.p.) or propranolol (a beta-NE blocker; 20 mg/kg) and tested on the beam-walk task for 3 hours postdrug. Compared to sham injury controls administered the same doses, neither of these drugs were found to reinstate beam-walking deficits in cerebellar-lesioned rats.

These data are consistent with the hypothesis that the cerebellum, and/or motor circuits influenced by cerebellar outputs, is critically involved in the performance systems that control beam-walking ability. The deleterious effects of both d-AMPH and haloperidol on recovery after cerebellar damage was surprising, and may be due to actions on other neuronal circuits comprising the motor system, either in the cerebrum or in the spinal cord. These data illustrate that drugs shown to facilitate recovery after damage to one portion of the motor system (i.e., SMCx) cannot be assumed to facilitate recovery from damage to another portion of this system. The failure of phenoxybenzamine to reinstate deficits in cerebellar injured rats may seem surprising, given that alpha1 receptor blockers

have been shown to reinstate beam-walking deficits in rats recovered from unilateral SMCx injury (5-7,87). However, this finding is compatible with the hypothesis that the detrimental effects of these drugs in rats with SMCx injury is via actions on cerebellar circuitry; following cerebellar cortex ablation, receptors being acted on by these drugs in order to produce a worsening of beam-walking (in the case of SMCx damage) may simply not be available.

Role of the Red Nucleus in Locomotor Ability:

As detailed above in the section on metabolic studies, unilateral injury to the SMCx produces depressed metabolic activity within the red nucleus (RN) and this dysfunctional state is alleviated by administration of d-AMPH (18,33). Since the RN is known to be an important component of the motor system, and receives afferent inputs from both the SMCx and cerebellum, it seems plausible that this structure may be involved in both spontaneous recovery of locomotor ability after SMCx injury as well as play some role in drug-facilitated recovery processes. To examine the role of the RN in locomotor ability, studies were conducted to: 1) assess deficits in beam-walking ability in rats following unilateral RN lesion alone; 2) assess deficits in locomotor tasks in rats with combined unilateral RN lesion and ipsilateral SMCx ablation; and 3) determine if temporary, "reversible" lesions of the RN in rats with unilateral SMCx injury would either prevent or slow spontaneous recovery or reinstate beam-walking deficits in rats recovered from SMCx lesions.

Unilateral (right) SMCx ablations in rats were conducted as previously described (4,87). To produce unilateral lesion of the RN, anesthetized rats had 2 µl of quisqualic acid injected into the region of the right RN (-5.8 mm from bregma, 0.9 mm from midline, -7.8 mm from skull surface).

Data from these experiments is still being collected and analyzed. Thus, the results discussed hereafter are based on cursory examination of both behavioral and anatomical data collected to date.

The data collected to date indicate that unilateral injury to the RN results in substantial deficits in beam-walking ability (deficient control of movements in limbs contralateral to the lesion), with spontaneous recovery in RN-lesioned animals occurring over a range of 1 to 6 weeks after injury. Ongoing histological analysis of lesion extents may show this recovery range to be related to extent of damage to either the RN or surrounding regions. However, the average time-course of spontaneous recovery following unilateral RN lesion is similar to that observed in rats with unilateral SMCx ablation alone. Some rats that recovered beam-walking ability after right RN lesion subsequently sustained a right SMCx ablation. Interestingly, these latter animals had beam-walking deficits that endured for over 90 days after the second injury.

To determine if temporary blockade of neuronal activity (both efferent and afferent) within the RN would affect recovery of beam-walking ability in rats with unilateral SMCx ablation, one group of rats were implanted with chronic guide cannulas into the region of the ipsilateral RN at the time of right SMCx injury. Every other day after injury these animals received a single infusion (0.75 µl) of

either 2% lidocaine (to silence red nucleus neural activity) or saline. Immediately after infusion the animals were given a single beam-walk trial. On days 7, 13, and 21 after surgery these animals were given a single beam-walk trial that was not preceded by lidocaine or saline infusion (probe trial). Lidocaine-treated rats consistently scored lower on these probe trials than did saline controls. This finding suggests that preventing the transmission of information from the RN to other structures involved in motor control, or blocking sensory feedback into this structure, during beam-walk performance after SMCx ablation will retard recovery.

The symptoms of hemiparesis that become evident after SMCx damage in rats made to traverse a narrow, elevated beam (4,92) are generally transient in nature, enduring for less than 2-3 weeks before spontaneous recovery is noted. Other investigators have reported that analysis of paw-print patterns ("gait analysis"), exhibited by rats or cats during locomotion, is a sensitive method for detecting deficits or altered limb use patterns following sciatic nerve injury (93), cortical ablation (94,95), or drug treatments (96). In a preliminary study, employing both beam-walking and gait analysis in animals with SMCx ablation, it was found that beam-walking assessment was both a more sensitive measure of locomotor disability and revealed more prolonged deficits than did the gait analysis procedure (97). Deficits in beam-walking in this study were apparent through 11 days after right SMCx ablation. A significant decrease in stride length occurred during the first postinjury paw-print test, (Figure 11), but stride length returned to baseline levels by the second postinjury test (3 days after SMCx ablation). No significant effect of injury on hindlimb angle measurements was found (Figure 12), and both measures of gait analysis were more variable than the ratings of beam-walking ability.

This gait analysis procedure was also employed for assessment of motor deficits in some rats with unilateral RN lesion. Unlike the data resulting from the unilateral SMCx study (97), data collected to date for the RN lesioned rats indicate that this injury leads to a more enduring (at least 2 weeks) deficit or change in gait. These animals show reductions in the width of the hindlimb stance (reduced base of support) and a decrease in the hindlimb angle measures. These changes seem to be related to effects of RN lesions on the timing of hindlimb movements, with lesioned rats showing a tendency to move both hindlimbs simultaneously during locomotion (loping or hopping pattern), rather than alternate limb movements as occurs in a normal step cycle.

To determine whether the RN is involved in the maintenance of beam-walk ability after recovery has occurred, SMCx-injured rats that recovered to preinjury beam-walk levels were given infusions of either lidocaine (2%, .5 µl), gamma-amino-n-butyric acid (GABA; 1 M, .4 µl), or kynurenine (75nM, 2 µl, the metabolically active precursor of kynurenic acid, a glutamate antagonist) immediately prior to beam-walk testing. Infusion of any of these drugs which reduce RN neural activity produced transient (5-10 minutes), but severe, beam-walking deficits in SMCx-injured animals. Uninjured control rats receiving these treatments were either unaffected or showed minor beam-walk deficits after infusions. These results suggest that RN is also involved in the maintenance of beam-walking ability in SMCx-ablated animals.

Cognitive Deficits After SMCx Contusion:

The demonstration of cognitive impairments in rodent models of TBI are crucial for modeling the human condition, as enduring and frequently severe cognitive impairments occur after human TBI (98,99). Although experimental models of TBI have traditionally focused on analyses of sensory and motor deficits, several other labs working with TBI models have also recently begun to utilize tasks to assess postinjury learning and memory deficits. Following bilateral TBI, long-term deficits in ability to learn an 8-arm radial maze task (100) and long-term deficits in acquisition of a Morris water-maze (MWM) task have been reported (101). Following unilateral TBI induced using the fluid percussion injury model, apparently mild and transient (48 hours) deficits in a previously learned MWM task were exhibited by injured rats (102). Since the MWM task is known to be sensitive to hippocampal damage such as that which occurs ipsilateral to unilateral SMCx contusions (15,28-30,37), studies have been conducted to ascertain whether or not rats with right SMCx contusion would exhibit learning and/or memory impairments in a MWM task.

Rats trained in a simple version (stationary platform) of the MWM for 4 days prior to injury and re-tested on this task from 22 to 28 days after right SMCx contusion (2.25 and 3.22 m/s injury velocities) exhibit mild, but significant, deficits in this spatial learning task (Sutton, unpublished observations). Although data from this study implied that TBI induced learning or acquisition impairments rather than deficits in memory or retention of previously acquired information, firm conclusions on this matter could not be made due to the experimental design employed. To further explore the nature of cognitive deficits in rats with unilateral SMCx contusion, we conducted experiments in sham operates and rats with a 400 g-cm impact to the right SMCx (103,104).

It was reasoned that naive rats (no prior training), tested shortly after cortical trauma, might exhibit more severe deficits in learning to navigate (swim) to a platform hidden in a MWM than would rats with MWM training prior to TBI. To test this hypothesis, our first study examined MWM performance in rats with no prior training in the MWM task. In this investigation we used a difficult version of the MWM (which tests "learning set" ability), where the location of the escape platform was moved daily. Testing was conducted for 12 days, beginning 4 days after surgery, with animals given 8 trials a day (2 blocks of 4 trials separated by a 5 to 10 minute rest period). Within each block of trials, the rat was placed at a different compass point (N, S, E, or W) at the start of each trial. Each swim trial lasted for a maximum of 60 seconds, and the rat was allowed to remain on the escape platform for 10 seconds at the end of each trial. The intertrial interval was 10 minutes.

As is illustrated in Figure 13, sham operated rats learned a search strategy in the MWM task that allowed them to rapidly find the escape platform from trials 2 through 8 on each testing day. Beginning on the first day of testing, rats with SMCx contusion had significantly longer latencies to find the escape platform during the second block of swim trial (trials 5-8) than did sham operates. These animals with TBI showed some improvement in MWM performance over the 12 days of testing (compare Figure 13A and 13D), but they

showed little or no improvement within the 8 trials conducted each day. These results indicate that unilateral cortical injury results in a severe anterograde amnesia, or inability to learn a spatial learning set. On days 13 and 14 after surgery these "naive" rats were tested on their ability to navigate to a visible, black platform within the water maze. As shown in Figure 14, all rats were equally proficient in learning to swim to the visible escape platform. In addition, calculations of the average swim speed (cm/second) for days 1, 6, and 12 of testing showed that there were no significant differences between groups on this measure (Figure 15). These findings suggest that potential sensory and motor deficits induced by the SMCx contusion cannot account for the reduced ability of these animals to find the hidden escape platform.

The 10 sham operates in the "naive" group, and 4 similarly trained rats, were randomly assigned to sham or TBI groups and then underwent surgery on the day following completion of visible platform testing in the experiment described above. Four days later, these "pre-trained" rats were tested using the MWM protocol detailed above. In contrast to the findings in "naive" subjects, SMCx contusion in rats who were pre-trained in the MWM produced only mild and transient (1-2 days) deficits in place learning ability (Figure 16). This finding implies that unilateral TBI in rats does not result in retrograde amnesia (i.e., disrupt memory of previously learned tasks). As for the "naive" rats, latencies to locate a visible platform and swim speed on days 1, 6, and 12 were not significantly different for sham operates and TBI rats in these "pre-trained" groups.

After completion of behavioral testing, sham operates and rats with unilateral TBI were anesthetized with urethane, and perforant path-dentate gyrus evoked potentials were examined in the hippocampus ipsilateral and contralateral to SMCx contusion. Single pulse stimulation was used to examine input-output (I-O) functions [field excitatory postsynaptic potentials (EPSPs) and population spike amplitudes] and high-frequency stimulation was utilized to examine induction of long-term potentiation (LTP). Comparisons of data derived from sham operates and TBI rats revealed that TBI did not significantly alter I-O curves within the hilar region of the hippocampus ipsilateral or contralateral to neocortical contusion. In the hippocampus contralateral to contusion, LTP was induced and maintained within normal ranges observed in sham operates. In contrast, only very short-lasting enhancement was obtained in the hippocampus ipsilateral to SMCx contusion and LTP could not be induced. This inability to induce LTP in the hippocampus ipsilateral to TBI is consistent with the findings that TBI induces severe learning deficits, since LTP is thought to be one physiological mechanisms underlying learning processes.

Histological analyses of brain tissue from rats in the MWM studies revealed that both the extent of cortical cavitation necrosis and the extent of cell loss in the hippocampus ipsilateral to contusion did not differ significantly between the "naive" and "pre-trained" TBI groups. Thus, differential damage to cortical or subcortical regions cannot account for the reduced impairments that were observed in the TBI rats that were trained in the MWM prior to induction of injury.

The results of these MWM studies extend the relevance of our injury model for comparison to human closed head injury, since these injuries often results in significant cognitive as well as sensorimotor impairments. Additional research will be required to determine if these learning deficits are amenable to pharmacotherapy, or if the same drugs capable of improving recovery from sensorimotor dysfunctions will also reduce or ameliorate cognitive dysfunctions resulting from TBI.

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APPENDIX 1

FIGURES AND FIGURE LEGENDS

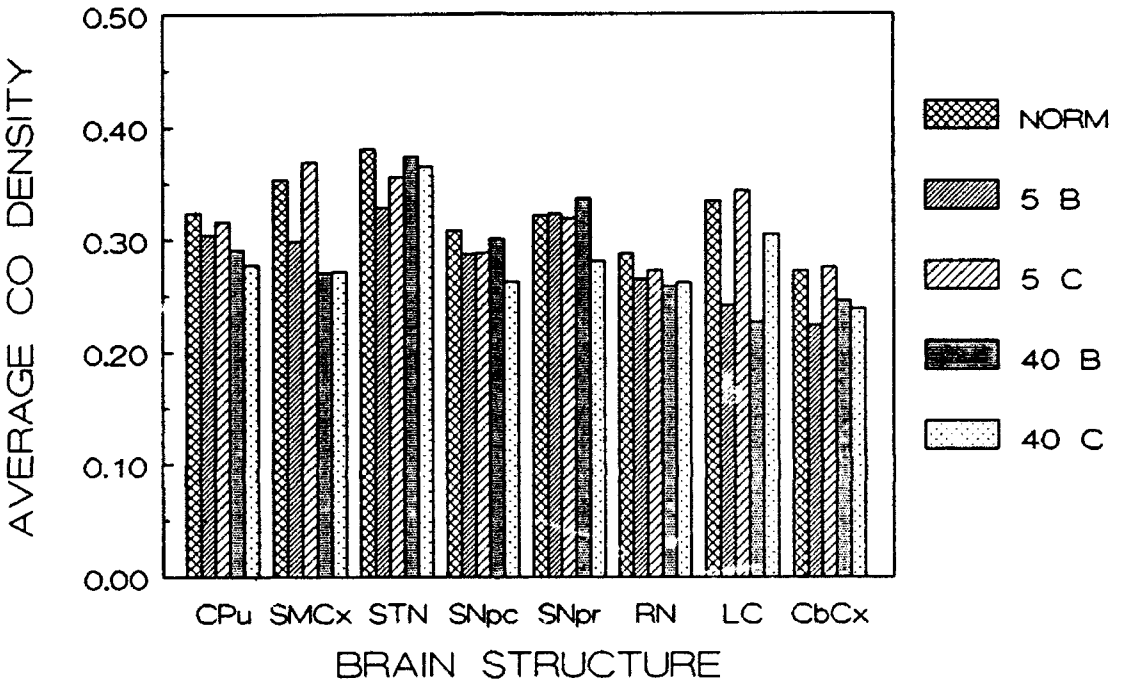

Figure 1. Illustrates the average density of cytochrome oxidase (CO) staining within various structures in left and right hemispheres (data collapsed across hemispheres) of rats in the "motor activation" study. No significant differences were found for CO activity within these structures between normal (NORM), "non-stressed" controls, rats with either 5 (5 B) or 40 (40 B) beam-walk trials on 5 consecutive days, and "stress" control rats (5 C and 40 C) exposed to the same amount of bright light and white noise as the 5 B and 40 B groups but not required to traverse the beam (see text for details). CPu = caudate-putamen; SMCx = sensorimotor cortex; STN = subthalamic nucleus; SN = substantia nigra [pars compacta (pc) and pars reticulata (pr)]; RN = red nucleus; LC = locus coeruleus; CbCx = cerebellar cortex.

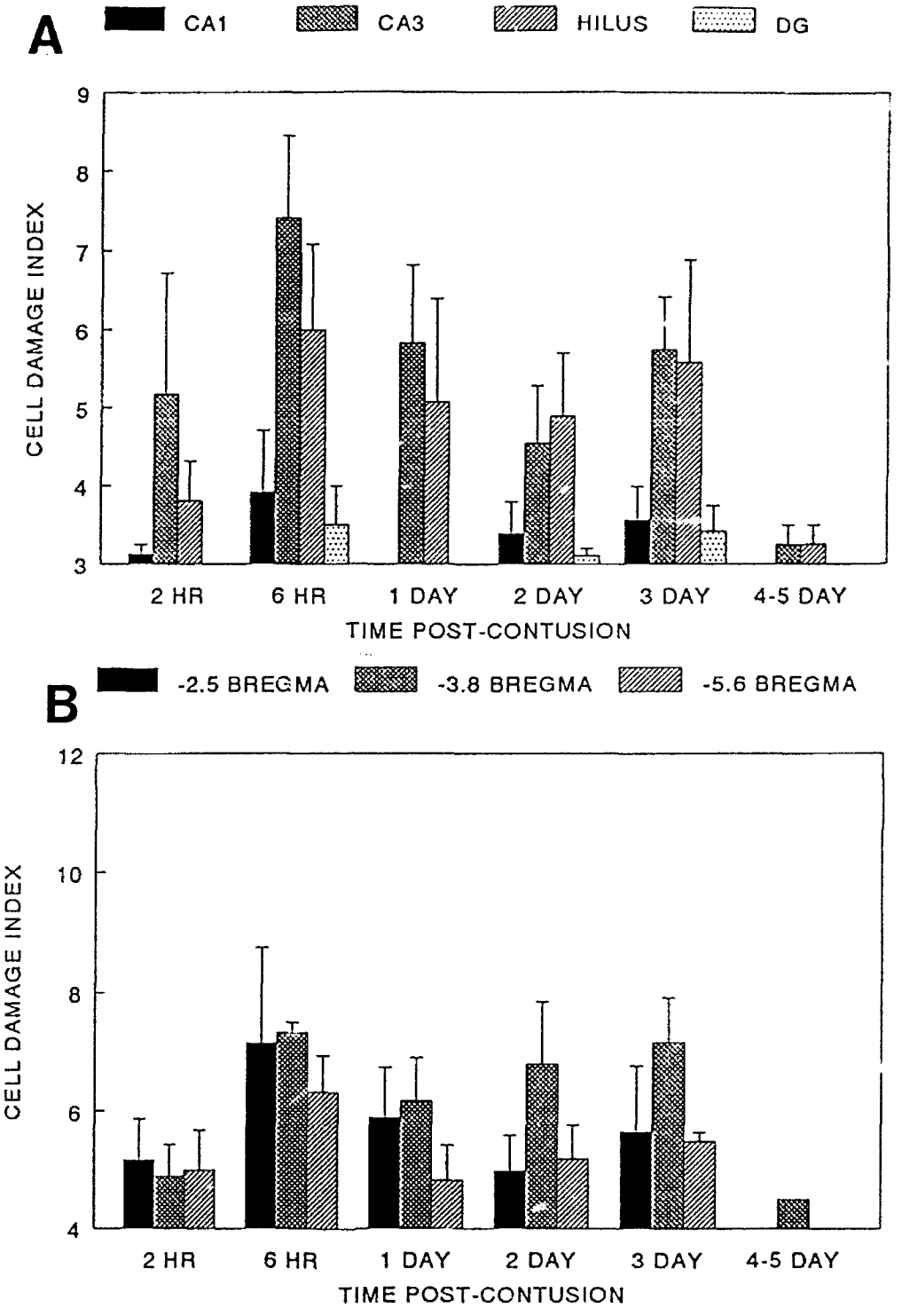

Figure 2. Ratings of ipsilateral hippocampal cell damage (argyrophilic neurons) at various time-points after right SMCx contusion in the rat, as assessed in silver-stained tissue sections. A) Cell damage in 4 hippocampal sectors summed over 3 coronal planes. B) Cell damage at each of 3 coronal planes, summed over sectors.

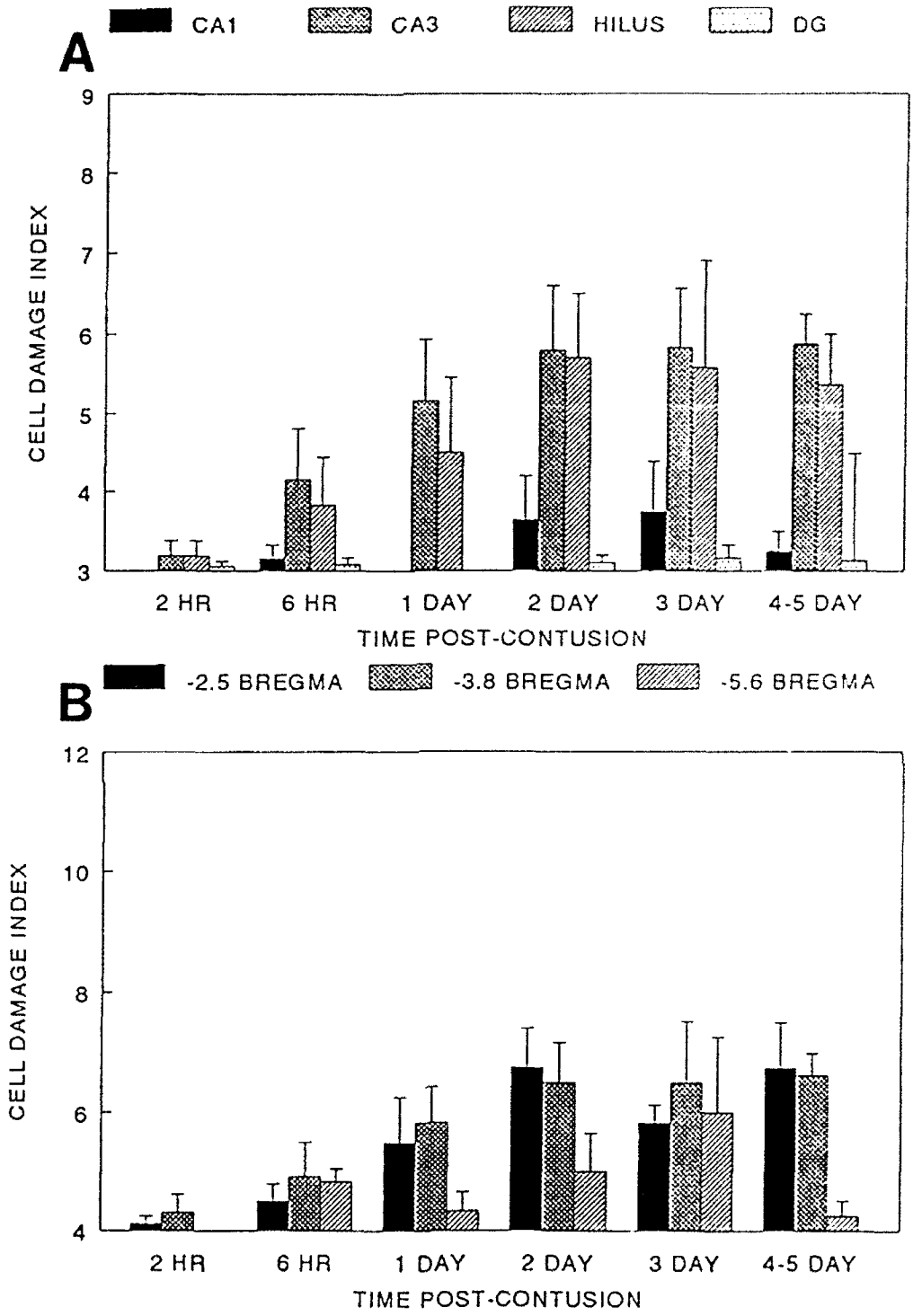

Figure 3. Ratings of ipsilateral hippocampal cell damage (loss of Nissl-positive neurons) at various time-points after right SMCx contusion in the rat, as assessed in thionin-stained tissue sections. A) Cell damage in 4 hippocampal sectors summed over 3 coronal planes. B) Cell damage at each of 3 coronal planes, summed over sectors.

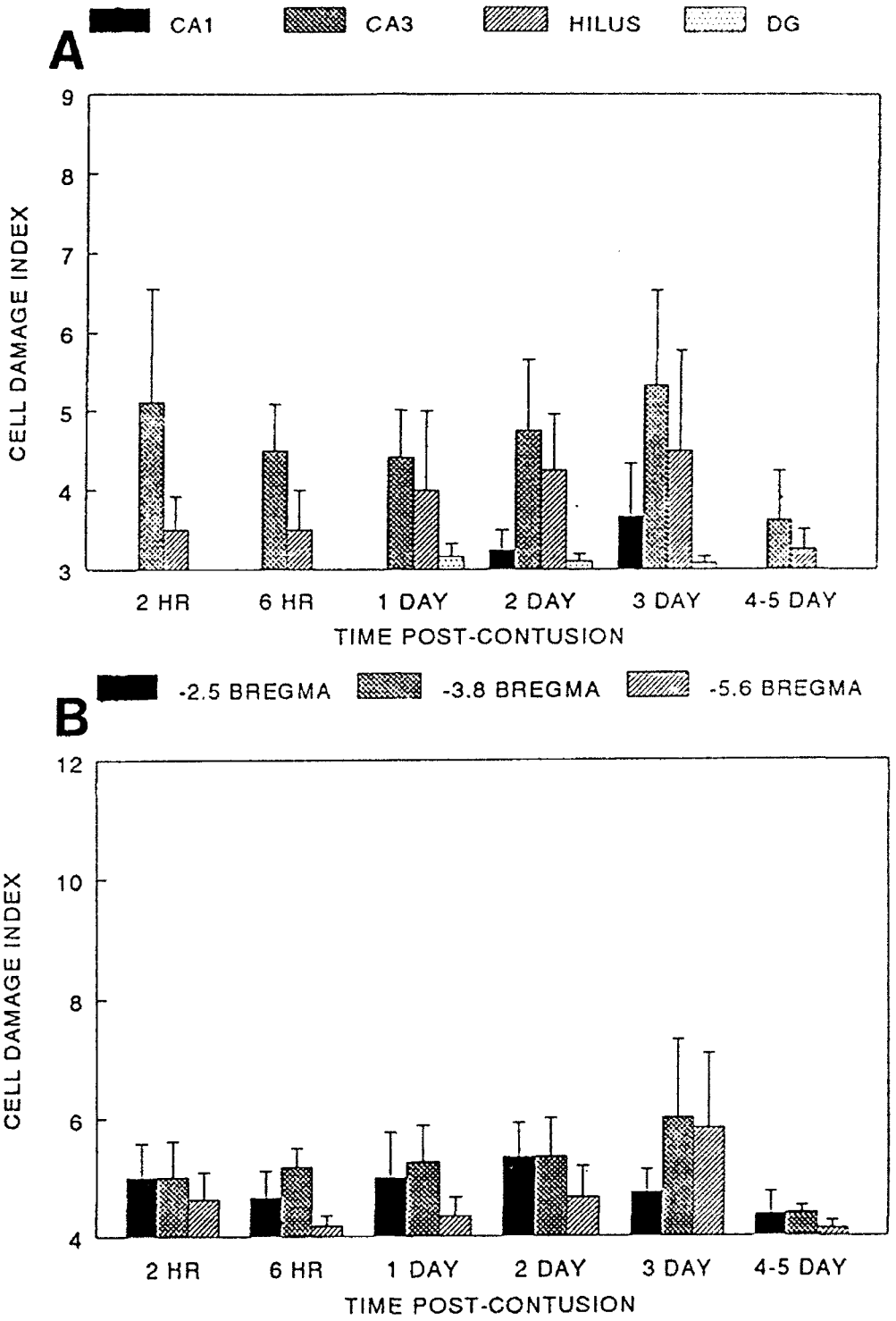

Figure 4. Ratings of ipsilateral hippocampal cell damage (presence of AF-positive neurons) at various time-points after right SMCx contusion in the rat, as assessed in tissue sections stained with acid fuchsin (AF). A) Cell damage in 4 hippocampal sectors summed over 3 coronal planes. B) Cell damage at each of 3 coronal planes, summed over sectors.

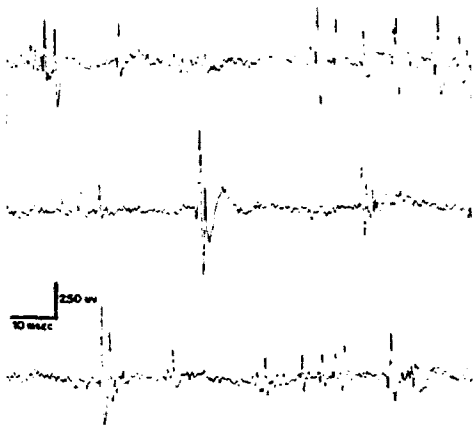

Figure 5. Three traces showing the typical patterns of spontaneous action potentials recorded from the CA3 sector of the hippocampus in rats prior to injury.

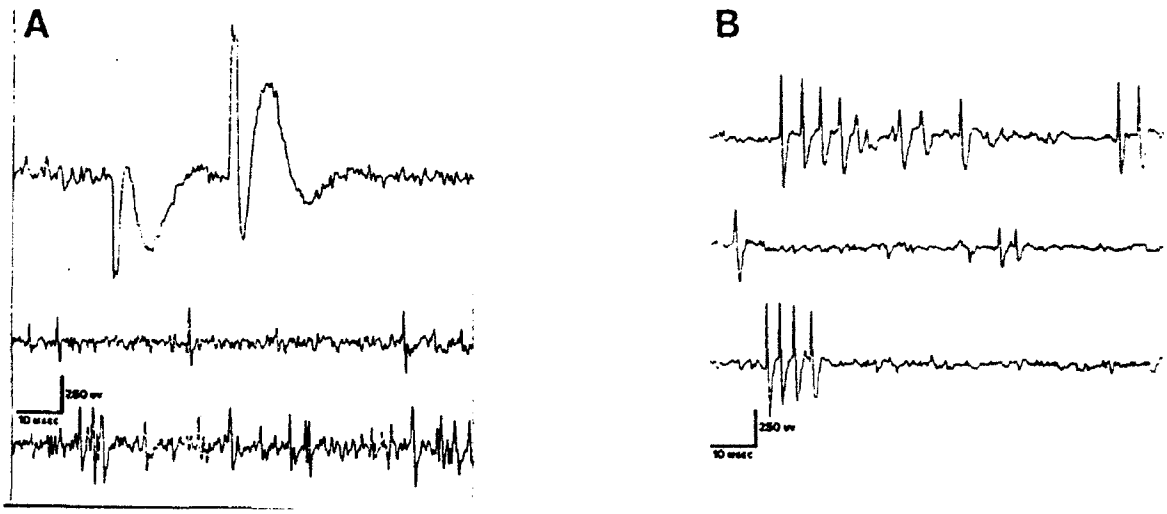

Figure 7. Following cessation of seizure and "epileptiform" activity in the CA3 sector of the hippocampus ipsilateral to cortical contusion, a reduction of unit activity occurred. This consisted of sporadic unit discharges and occassional large amplitude slow waves (A). Following a postcontusion isoelectric period, single unit activity in the CA3 sector contralateral to SMCx contusion appeared relatively normal (B).

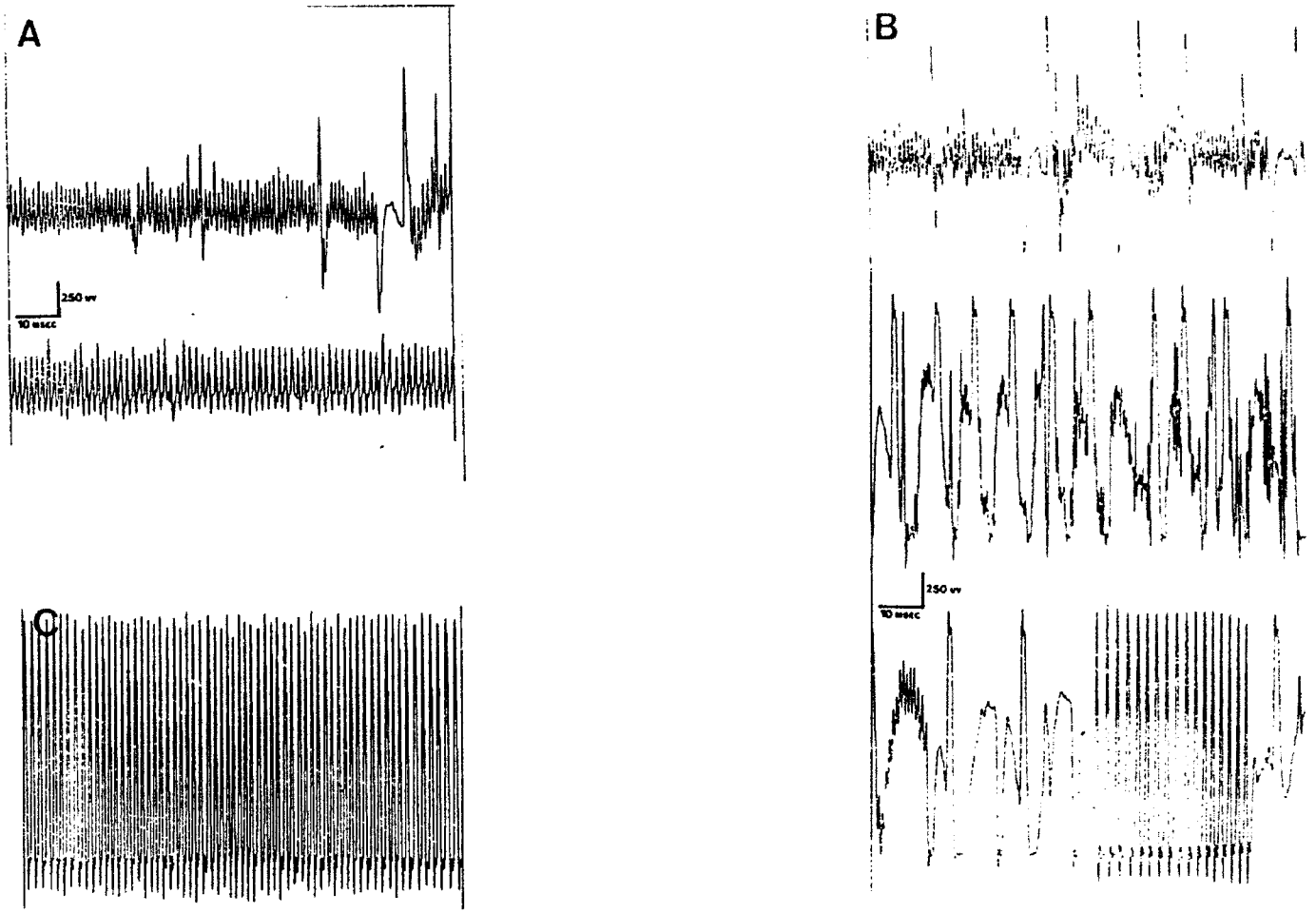

Figure 6. Following a transient (up to 20 minutes) isoelectric period following SMCx contusion prolonged single unit "epileptiform" activity was recorded in the CA3 sector of the ipsilateral hippocampus (A). This epileptiform type of discharge was interspersed with periods of large amplitude, slow wave activity of unknown origin (B). Bursts of seizure-like activity were recorded from the ipsilateral hippocampus for up to 3 hours after TBI (C), but was never observed in the contralateral hemisphere.

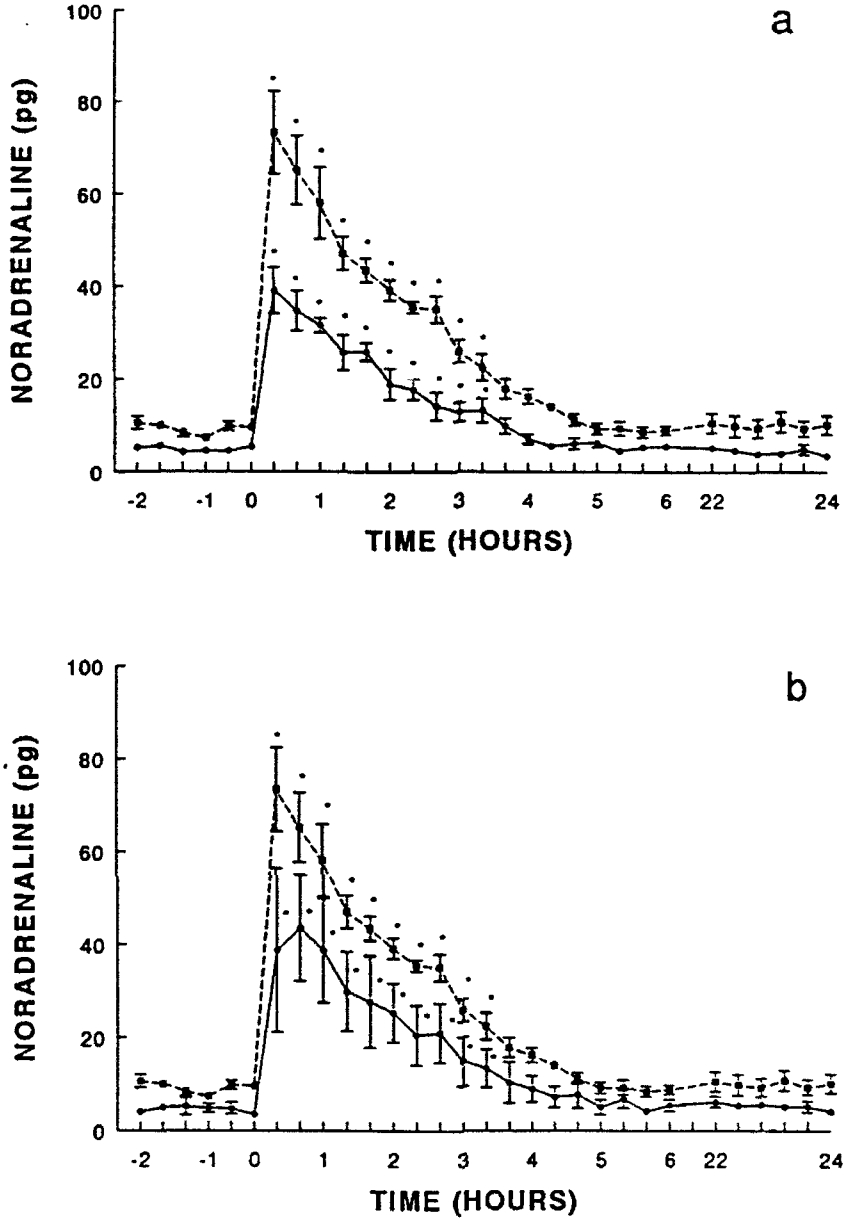

Figure 8. Illustrates the average extracellular levels of NE within cerebellar cortex of freely moving control (filled squares) and contused (filled circles) rats. Basal levels (-2 to 0 h) were collected from 22 to 24 hours after surgery and d-AMPH (2 mg/kg, i.p.) was administered 24 hours postsurgery (time 0 in the graph). Levels of NE in the left and right hemispheres of control animals were not significantly different, and these data were combined for the current illustration. The NE levels within the right (a) and left (b) cerebellar cortex of contused and control rats were significantly elevated above their respective basal levels for over 3 h and 20 min after AMPH administration (*p's<.0042, paired t-tests; Bonferroni procedure). Bars represent the SEM.

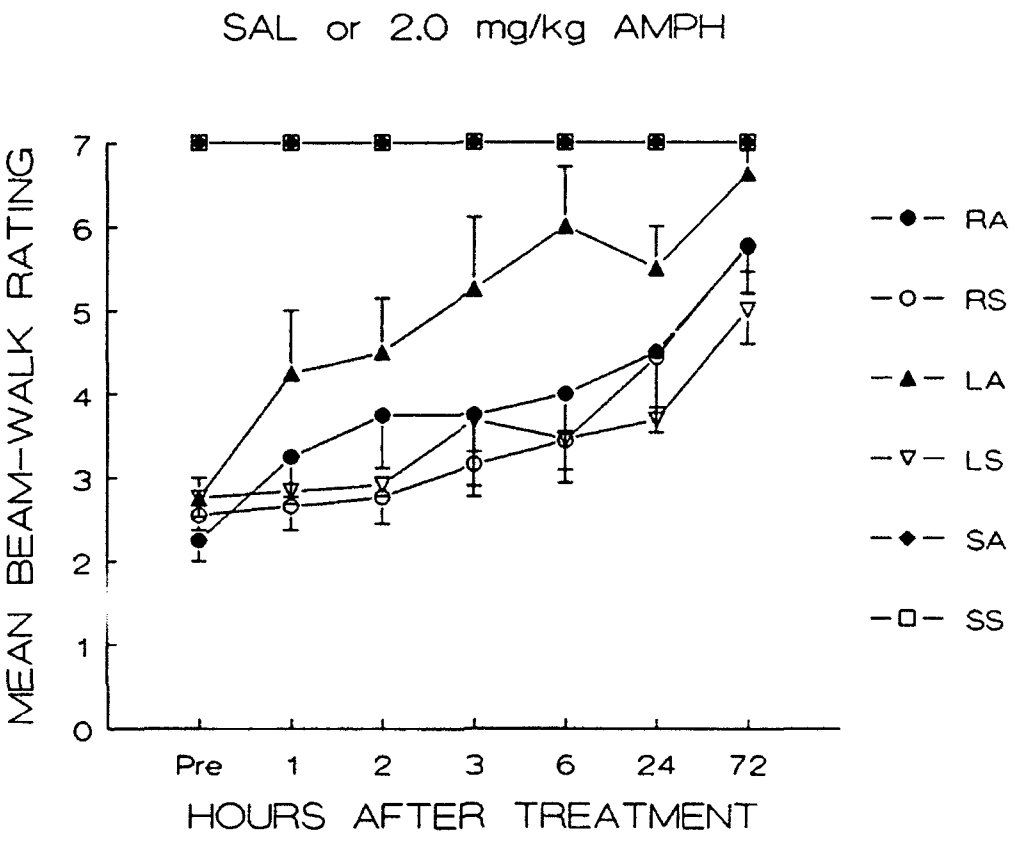

Figure 9. Illustrates the average beam-walking scores for sham operates (S) and rats with either right (R) or left (L) SMCx contusion after surgery. All animals had similar deficits in the pretreatment (Pre) trial conducted 24 hours after surgery. Animals with a left SMCx contusion that were given a 2 mg/kg dose of d-AMPH (LA) immediately after the Pre trial showed a significantly accelerated rate of beam-walking recovery over the first 3 days after treatmment compared to the contused groups administered saline (RS, LS). This dose of d-AMPH failed to accelerate recovery in the rats with right SMCx contusion (RA).

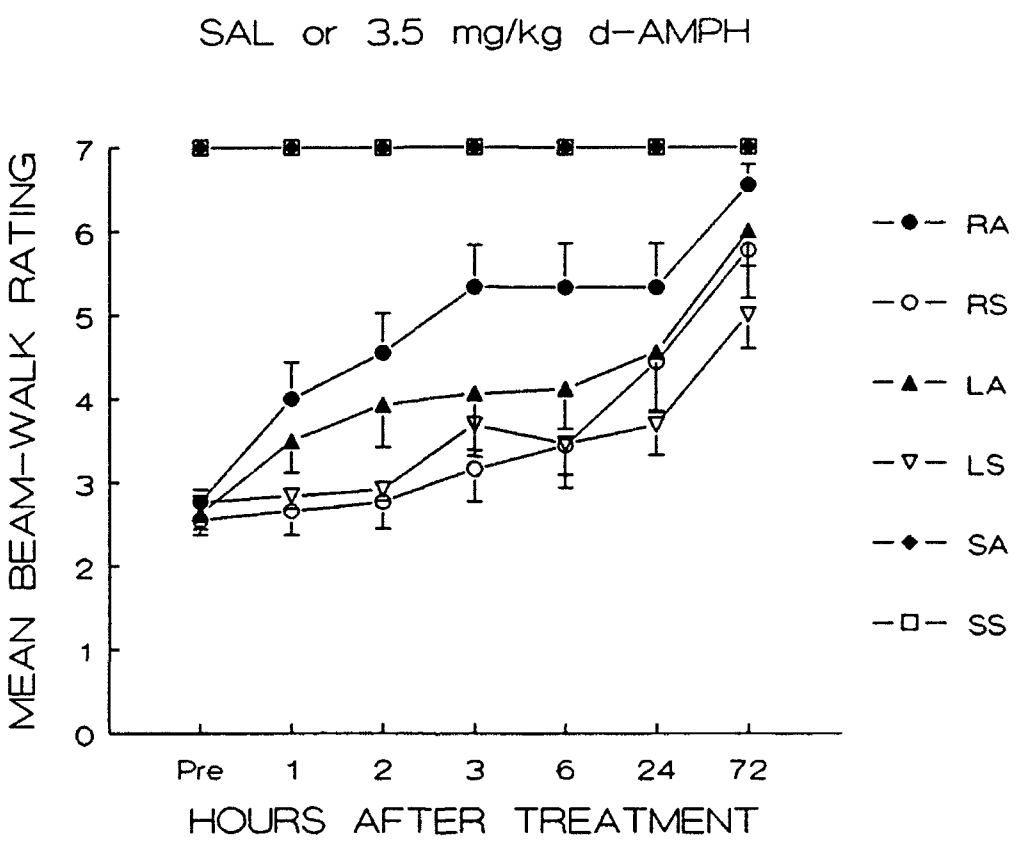

Figure 10. Illustrates the average beam-walking scores for sham operates (S) and rats with either right (R) or left (L) SMCx contusion after surgery. All animals had similar deficits in the pretreatment (Pre) trial conducted 24 hours after surgery. Animals with a right SMCx contusion that were given a 3.5 mg/kg dose of d-AMPH (RA) immediately after the Pre trial showed a significantly accelerated rate of beam-walking recovery over the first 3 days after treatmment compared to the contused groups administered saline (RS, LS). This dose of d-AMPH failed to accelerate recovery in the rats with left SMCx contusion (LA).

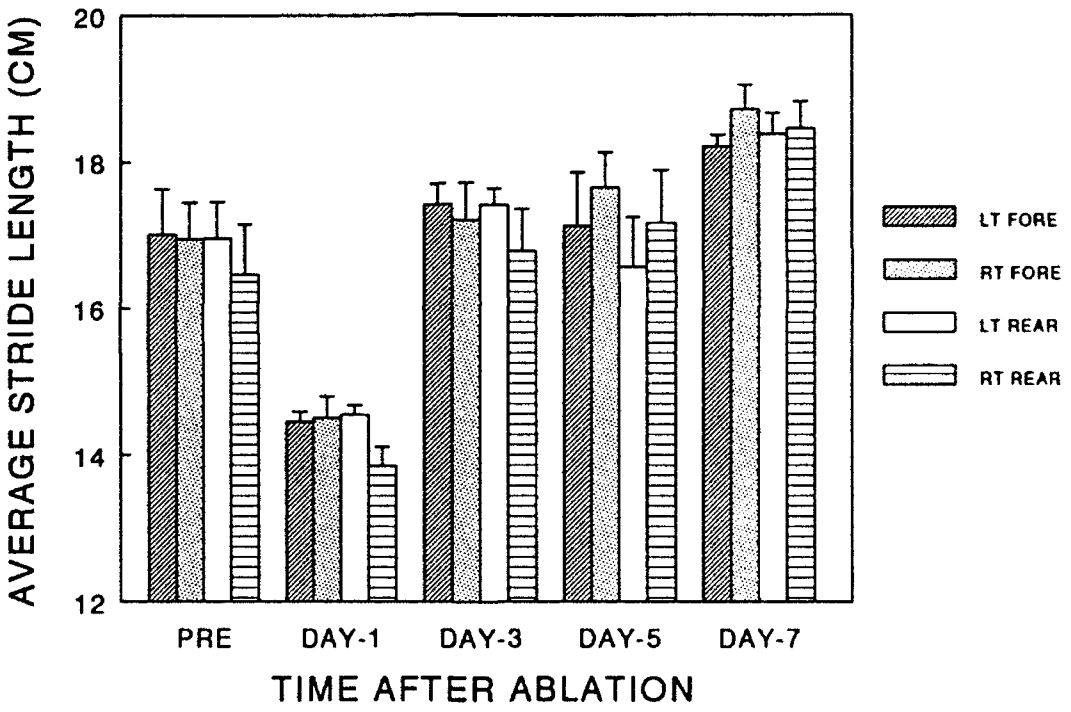

Figure 11. Shows the mild effect of unilateral SMCx ablation on stride length of right (RT) and left (LT) forelimbs (FORE) and hindlimbs (REAR) in rats. Compared to presurgical (PRE) measures, stride length was only significantly reduced (in all limbs) during the first postinjury testing session.

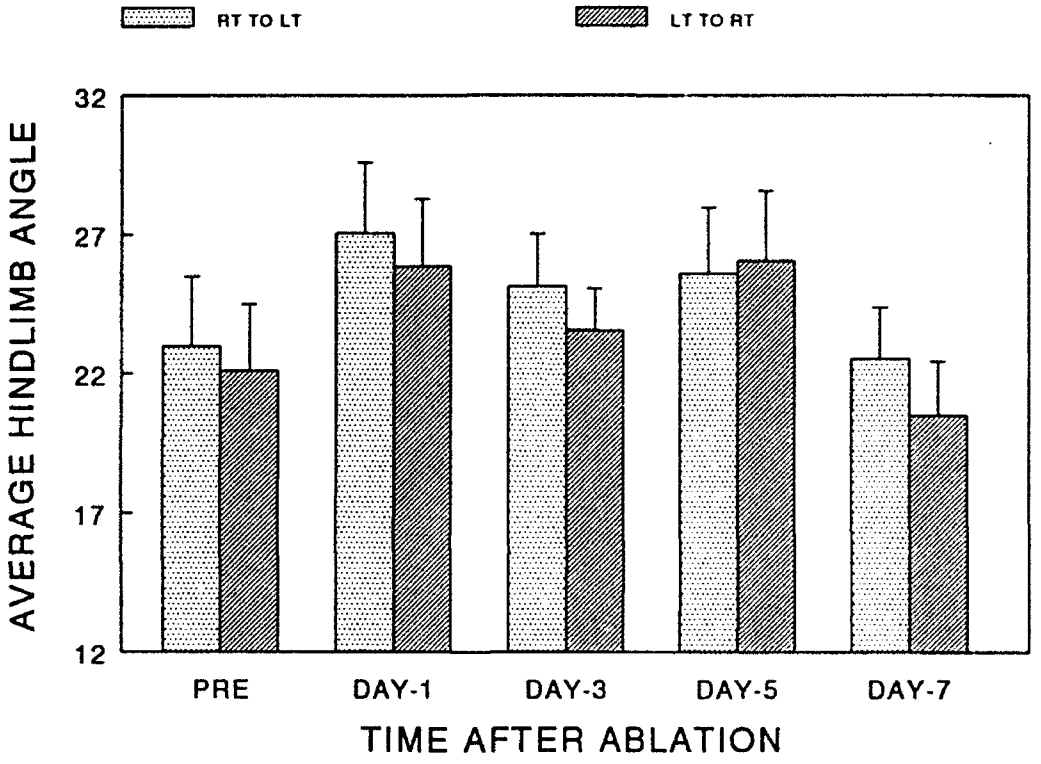

Figure 12. Illustrates the lack of effect of right SMCx ablation on measures of hindlimb angle (gait analysis) during the first week of postsurgical testing. Paw prints were made for each animal as they walked across a flat surface. The measures shown are obtained by calculating the angle formed by a line connecting two sequential paw prints of one hindlimb and another line connecting the first of these paw prints with the next paw print of the opposite hindlimb formed as the animal steps forward. The lack of effect of SMCx ablation on both left (LT) to right (RT) and RT to LT hindlimb angles, compared to presurgical (PRE) measures, suggests that the animals did not alter either the base of support or alternation of limbs during locomotion after injury.

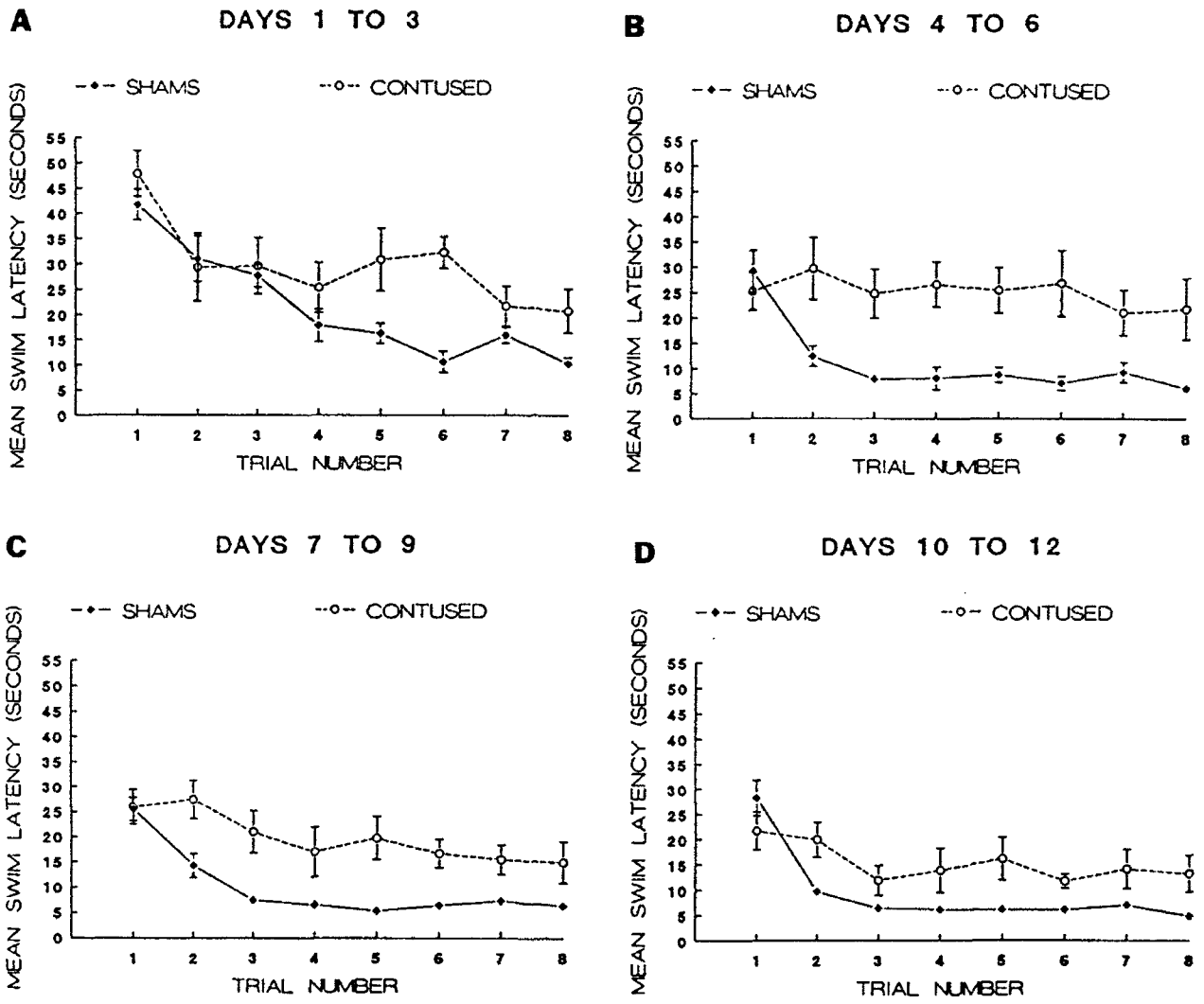

Figure 13. These graphs illustrate the average time required by "naive" sham operates and rats with right SMCx contusion to locate the escape platform during 8 daily trials in a MWM task. Data from the 12 days of testing are averaged over 3-day intervals. Note that while contused rats showed some improvements in locating the platform over the 12 days of testing, they consistently performed worse than the shams. SMCx contused rats showed little or no decrease in swim latencies from trial 1 to trial 8 within each daily testing session.

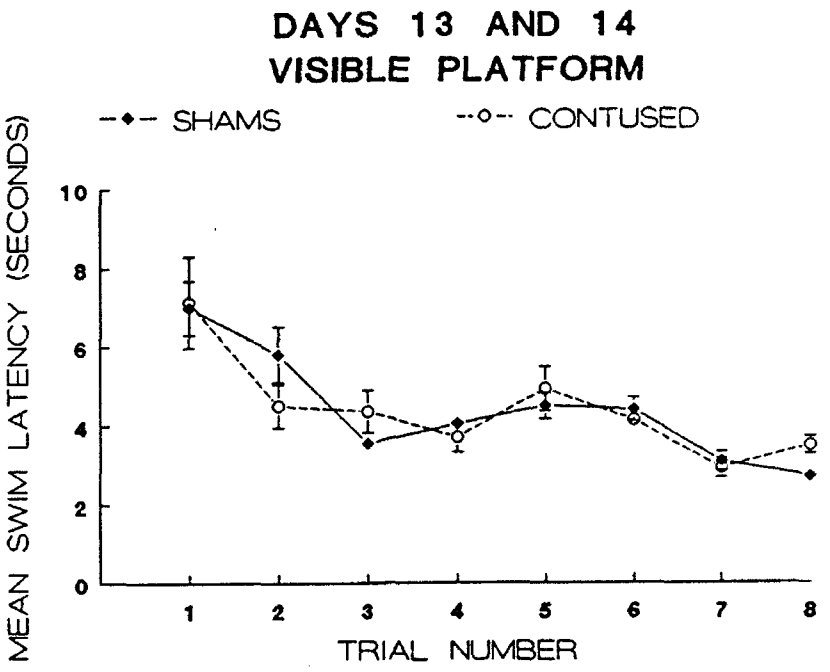

Figure 14. Average latencies to locate a visible platform in the MWM on Days 13 and 14 of testing for "naive" sham operates and rats with right SMCx contusion. Groups did not differ on this task, suggesting that sensory and/or motor deficits did not account for the poorer performance of contused rats during tests using a submerged escape platform (Figure 13).

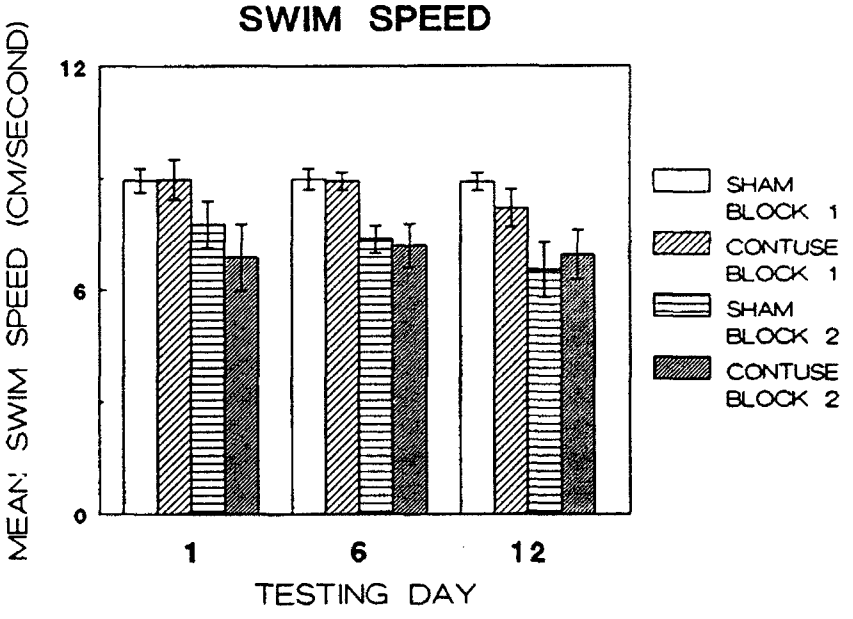

Figure 15. Shows the average swim speed of "naive" sham operates and rats with right SMCx contusion (CONTUSE) during Days 1, 6, and 12 of testing in the MWM. Although both groups showed some evidence of "fatigue" (slower swim speed) during trials 5-8 (BLOCK 2) on these days, groups did not differ significantly during BLOCK 2 or BLOCK 1 (trials 1-4/day).

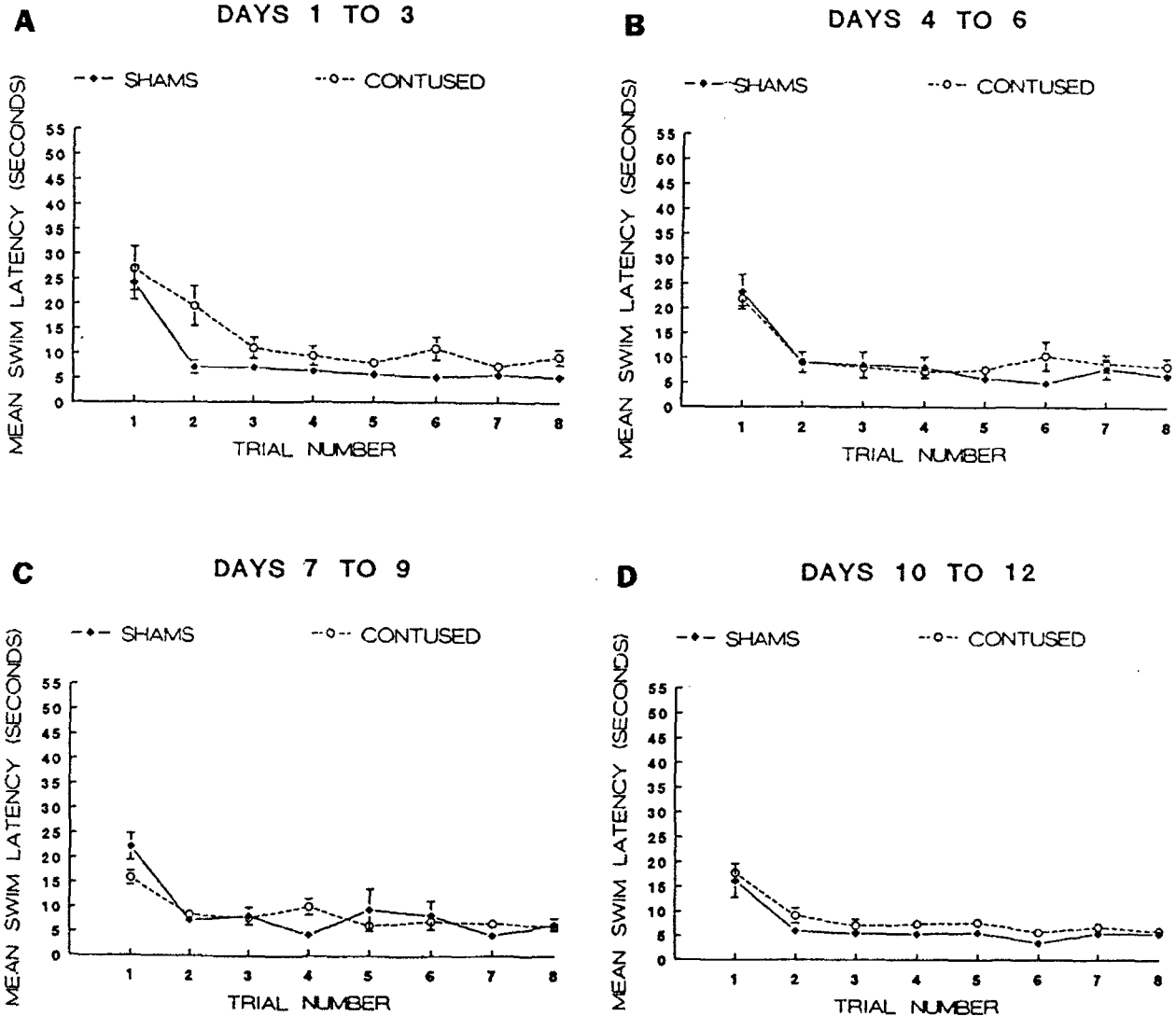

Figure 16. These graphs illustrate the average time required by "pretrained" sham operates and rats with right SMCx contusion to locate the escape platform during 8 daily trials in a MWM task. Data from the 12 days of testing are averaged over 3-day intervals. The rats pretrained in the MWM and then contused over the SMCx showed only mild and transient (Days 1 and 2 only) deficits in the MWM task.

APPENDIX 2

MANUSCRIPTS CITING U.S. ARMY GRANT DAMD17-91-Z-1006

Boyeson M.G., Feeney D.M., and Dail, W.G. (1991) Cortical microstimulation thresholds adjacent to sensorimotor cortex injury. J. Neurotrauma 8:205-217.

Boyeson, M.G. and Feeney, D.M. (1991) Adverse effects of catecholaminergic drugs following unilateral cerebellar ablations. Rest. Neurol. Neurosci. 3:227-233.

Sutton, R.L. and Feeney, D.M. (1992) -noradrenergic agonists and antagonists affect recovery and maintenance of beam-walking ability after sensorimotor cortex ablation in the rat. Rest. Neurol. Neurosci. 4:1-11.

Sutton, R.L. and Feeney, D.M. (In press) Noradrenergic pharmacotherapy and functional recovery after cortical injury. In: Neurological Rehabilitation: Rehabilitation of the Neurological Patient. (L.S. Illis, ed.). Oxford: Blackwell Scientific Publications Ltd.

Sutton, R.L., Chen, M.J., Hovda, D.A. and Feeney, D.M. (Submitted) Amphetamine attenuates the cerebral metabolic depression produced by unilateral sensorimotor cortex ablation: A quantitative cytochrome oxidase histochemical study in the rat. J. Cereb. Blood Flow Metab.

Krobert, K.A., Sutton, R.L. and Feeney, D.M. (Submitted) Spontaneous and amphetamine-evoked release of cerebellar norepinephrine after cortical contusion: An in vivo microdialysis study in the awake rat. J. Neurochem.

Weisend, M.P. and Feeney, D.M. (Submitted) Brain temperature before and after traumatic brain injury is related to outcome. J. Neurosurg.

Scrimen, O.U., Feeney, D.M. and Li, M.G. (Submitted) Cerebral blood flow after cortical contusion: Effects of amphetamine. Brain Res. Bull.

ABSTRACTS/POSTER PRESENTATIONS
CITING U.S. ARMY GRANT DAMD17-91-Z-1006

Scrimen, O.U. and Feeney, D.M. (1991) Effects of amphetamine on the regional cerebral blood flow changes produced by cortical trauma. (Abstract). Presented at the 9th Annual Neurotrauma Symposium, November 9-10, New Orleans, LA.

Sanchez, R.J., Bustos, E.A., Krobert, K.A. and Feeney, D.M. (1992) Comparison of recovery of beam walking and gait on a flat surface after sensorimotor cortex ablation in rats. (Abstract). Presented at the MBRS Conference, November 3-5, Washington, D.C.

Krobert, K.A., Salazar, R.A., Sutton, R.L. and Feeney, D.M. (1992) Temporal evolution of histopathology and unit activity in rat hippocampal CA3 region after focal cortical contusion. J. Neurotrauma (Abstract) 9:64.

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