



Research report

Early overuse and disuse of the affected forelimb after moderately severe intraluminal suture occlusion of the middle cerebral artery in rats

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Received 26 June 2000; received in revised form 30 March 2001; accepted 30 March 2001

Abstract

We have previously shown that early forced overuse of the affected forelimb worsens outcome following moderately severe transient focal cortical ischemic stroke in rats using a distal middle cerebral artery occlusion (MCAo) model. This effect may be site-dependent, because we have also found that early forced use of the affected limb after unilateral 6-OHDA induced degeneration of ascending nigrostriatal dopamine neurons markedly enhanced functional outcome and is neuroprotective. The present study examines the effects of early overuse and disuse following a moderately severe proximal MCAo model, by means of intraluminal suture occlusion. Ischemia was produced in male Long-Evans rats with 60 min of occlusion, or sham surgery was performed. Early overuse or disuse of the affected forelimb was forced by immobilizing either the ipsilateral or contralateral forelimb, respectively, in a plaster cast or the animal was left uncasted. Casts were removed on day 10 and sensorimotor testing was performed weekly during days 17–38. Animals were sacrificed on day 45 and brains were fixed for later cresyl violet staining. The MCAo + contralateral cast group performed worse than all other groups on tests of forelimb sensorimotor function. All MCAo groups regardless of cast condition had significant atrophy of the ischemic striatum, but there was no significant atrophy of the ischemic cortex in any group. Forced disuse, but not overuse, of the affected forelimb immediately following proximal ischemia using the intraluminal suture model has detrimental effects on functional outcome, without exaggerating anatomical damage. The effects of disuse and overuse during the first 10 days after stroke differ depending on cortical or subcortical involvement. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Ischemia; Stroke; Functional recovery; Plasticity; Striatum; Sensorimotor

1. Introduction

Although stroke represents the most prevalent disabling neurological disorder requiring rehabilitation [44], the ideal program of physical rehabilitation following ischemic stroke has not yet been established. Ag-

gressive regimens of physical therapy have proven beneficial in stroke patients when implemented long after the brain damage has occurred. Constraint therapy, which involves temporarily restraining the patient's unaffected arm to force the exclusive use of the affected arm, leads to significant improvements in the function of the affected arm when implemented several months after disruption of function due to unilateral stroke [24,35,36,45]. However, it is currently unknown whether aggressive programs of physical rehabilitation

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should be employed early after ischemic injury, nor is it known how early this rehabilitation can safely begin. An optimal therapeutic window may exist in which lesion-induced plasticity [7,8,32,42,43] may be exploited to enhance recovery without negative consequences.

Animal models have proven to be useful for investigating the effects of behavioral manipulations on functional recovery after brain injury. We have shown in a rat model that forced overuse, but not disuse, of the affected forelimb via immobilization of the ipsilateral or contralateral limb, respectively, in a unilateral plaster cast [18] early after mild [2] and moderate [4] distal middle cerebral artery occlusion (MCAo) resulted in exacerbation of functional deficits. The distal MCAo model used in those studies resulted in exclusively cortical infarction. The finding of use-dependent exaggeration of injury after cortical infarction is consistent with the work of Kozlowski et al. [20] and Humm et al. [14,15] who have demonstrated that early forced overuse via ipsilateral forelimb immobilization for either 2 or 1 week, respectively, immediately after electrolytic lesions of the forelimb representation area of the rat sensorimotor cortex (FL-SMC) resulted in long-lasting exacerbation of functional deficits. In the present study, we used the 10 day casting period that was shown to worsen functional outcome when implemented immediately after a moderate distal MCAo [4].

Forced overuse of the affected forelimb by ipsilateral casting has recently been shown to ameliorate the affects of dopamine depletion caused by 6-hydroxydopamine (6-OHDA) lesions of the basal ganglia [3,37]. Spontaneous limb use asymmetries and apomorphine-induced contralateral rotation, which were exhibited in animals that had received 6-OHDA lesions, were not exhibited by the animals that had received 6-OHDA lesions and then ipsilaterally casted for 1 week after the surgery. Rather, lesioned animals that had been casted were behaviorally similar to animals that had undergone sham surgery. Furthermore, levels of dopamine as well as dopamine transporter expression in lesioned and casted animals were also similar to shams. Improvements in outcome also resulted from training in a lever-pressing task after 6-OHDA treatment [38]. These findings suggest that neurons in the basal ganglia may respond differently to early demands than neurons in the neocortex. However, it is not known if early use is beneficial only after dopaminergic dysfunction or after other types of striatal damage.

The present study investigated the behavioral and anatomical effects of early forelimb overuse and disuse after an ischemic insult designed to preferentially damage striatal neurons. Forelimb overuse and disuse were forced by immobilizing the ipsilateral or contralateral forelimb, respectively, in a unilateral plaster cast for the first 10 days after MCAo. We used the intraluminal suture MCA occlusion model, which was developed by

Longa et al. [23] and modified by Belayev et al. [1] to include coating with poly-L-lysine, a polycationic polymerized amino acid. Poly-L-lysine molecules adsorb strongly to solid surfaces, making the suture more adherent to the surrounding endothelium and thus reducing variability in infarct volume [1]. Belayev et al. [1] have demonstrated that 60 min of occlusion using this technique, which occludes the MCA proximal to the lenticulostriate branches, results in reliable and reproducible infarcts which are localized primarily in the lateral segment of the caudate putamen.

2. Materials and methods

2.1. Subjects

Forty-five male Long-Evans rats (Harlan Sprague-Dawley), weighing between 250–300 g at the time of surgery, were used. Animals were kept on a 12:12 h light:dark cycle and allowed rat chow and water ad libitum. Animals were tamed by gentle handling twice per week for 2 weeks before inclusion in the experiment.

2.2. Surgery

Animals were deprived of food but not water for 12–18 h prior to surgery. Occluding sutures were prepared using 30 mm lengths of 4-0 nylon monofilament suture (Ethicon) with a heat blunted end, which were coated with poly-L-lysine and dried for 1 h under a heating lamp. Animals were anesthetized with Equithesin (25 mg/kg pentobarbital and 150 mg/kg chloral hydrate, 0.35 cm³/100 g, IP) and placed in a supine position on a heating pad under an operating microscope. A skin incision was then made in the midline of the neck, and the left common carotid artery (CCA), internal common carotid artery (ICA), and external common carotid artery (ECA) were exposed using blunt dissection, and were dissected freed of surrounding nerves and fascia up to the basal cranium where the ICA enters the brain through the tympanic bulla. The ECA was ligated using two 6-0 silk sutures and transected, and a loose ligature was applied to the stump of the ECA. An atraumatic aneurysm clip was applied distal to the loosely tied ligature, at the bifurcation of the ECA and the ICA. A small incision was made in the ECA, and an occluding suture was introduced into the ECA lumen through this incision; the loose ligature was then tightened around it. The aneurysm clip was removed, and the occluder was advanced into the ICA 17–20 mm until a slight resistance was felt. This resistance indicated that the tip of the occluder had passed the origin of the MCA as it bifurcates from the ICA and has reached the anterior cerebral artery (ACA).

Thus, blood flow through the ICA was blocked, as was collateral blood flow through the circle of Willis. Fig. 1 depicts a dorsal view of the cerebral arteries and the location of the occluding suture. At the end of the 60 min ischemic period, the occluding suture was removed from the ECA and the loose ligature was permanently tied. Sham surgery included all procedures up to, but not including, insertion of the occluding suture. Core body temperatures were maintained at 37 °C (± 1.5) during surgery and recovery.

2.3. Casting procedure

Prior to recovery from anesthesia after MCAo or sham surgery, animals were either fitted with unilateral plaster casts or were left uncasted. The upper torso of casted animals was wrapped in soft felt, and either the ipsilateral (ipsi cast) or contralateral (contra cast) forelimb was wrapped in felt and positioned in a naturally retracted position against the animal's sternum. Plaster of paris strips were wrapped around the immobilized limb and upper torso. Casts were removed on day 10 post-surgery without anesthesia.

2.4. Behavioral measures

Animals were pretested 1–5 days before surgery, then tested weekly beginning on day 17 through day 38 post-surgery. All behavioral testing took place in the animal room by an experimenter blinded with respect to the treatment groups.

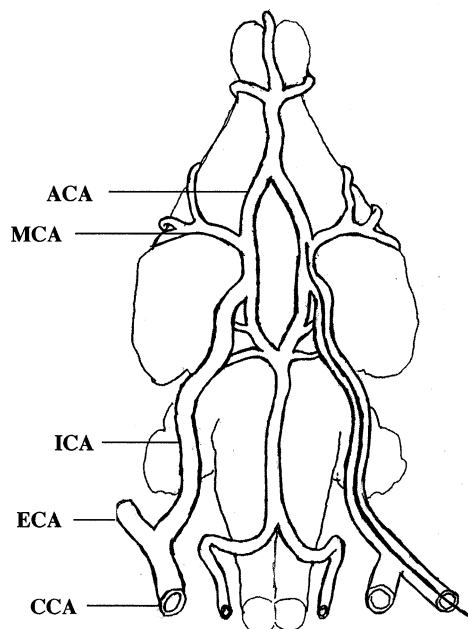


Fig. 1. A dorsal view of the rat cerebral arteries. The right side shows the placement of the occluding suture at the bifurcation of the MCA and the ICA by its insertion into the ICA lumen via the transected ECA. ACA, anterior cerebral artery; MCA, middle cerebral artery; ICA, interior carotid artery; ECA, external carotid artery.

2.4.1. Measurement of forelimb placing

Animals were held by their torsos with forelimbs hanging freely. Contralateral and ipsilateral forelimb placing responses were induced by gently brushing the respective vibrissae on the edge of a tabletop once per trial for 10 trials. A score of one was given each time the rat placed its forelimb on the edge of the tabletop in response to the vibrissae stimulation. Percent unsuccessful placing responses were determined (number incorrect $\times 10$) for contralateral and ipsilateral responses.

2.4.2. Measurement of footfaults

Animals were placed on an elevated grid, with openings of 2.3 cm², for 2 min. As the animals traversed the grid, a footfault was scored each time a paw slipped through an opening in the grid. The total number of steps was also counted. A footfault index was computed $[(\text{contra faults} - \text{ipsi faults}) / (\text{total steps})]$ such that a score of 0 represents no asymmetry, a positive score represents a contralateral deficit, and a negative score represents an ipsilateral deficit.

2.4.3. Measurement of spontaneous forelimb use

Use of the forelimbs during exploratory activity was assessed by videotaping each rat in a clear plastic cylinder for 3 min. The tapes were scored in slow motion by raters blinded with respect to the treatment groups. The following behaviors were observed for forelimb-use asymmetry during rearing, wall landing, and vertical movements along the wall of the cylinder: (a) independent use of the left or right forelimb on the wall; (b) independent use of the left or right forelimb to land after a rear; (c) simultaneous, or near-simultaneous, use of the left or right forelimb on the wall; (d) simultaneous, or near-simultaneous, use of the left or right forelimb for landing after a rear.

Each behavior was expressed in terms of: (a) use of the ipsilateral (non-affected) forelimb relative to the total number of ipsilateral, contralateral or simultaneous limb use observations; (b) use of the contralateral (affected) forelimb relative to the total number of ipsilateral, contralateral or simultaneous limb use observations; (c) simultaneous limb use relative to the total number of ipsilateral, contralateral or simultaneous limb use observations. Indices were computed to reflect ipsilateral biases for both wall-associated and landing-associated use with the following calculation: $(\text{ipsilateral} - \text{contralateral}) / (\text{ipsilateral} + \text{contralateral} + \text{simultaneous})$.

2.5. Histological procedures

Animals were sacrificed on day 45 post-surgery. Because infarcts have matured by this time post-stroke and dead cells have been phagocytized, ischemic damage is characterized by a loss of tissue in necrotic regions when late end points are used. Therefore, neu-

ronal death was measured by determining the volume of tissue atrophy using cresyl violet stained tissue. Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg) and intracardially perfused with 0.9% saline followed by 10% formalin. The brains were removed and post-fixed in formalin for at least 3 days prior to sectioning on a vibrating microtome (EMS). Eight 100 μm thick sections were taken 1 mm apart, mounted on Superfrost Plus slides (Fisher) and stained with cresyl violet. Each section was scanned into a Macintosh computer with Adobe Photoshop and then imported into NIH Image (a public domain program developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>) for analysis of tissue atrophy. Each cortical and striatal hemisphere and lateral ventricle was measured separately by tracing with a cursor and transformed to mm^2 with NIH Image. Percent atrophy was computed by dividing the ischemic (left) hemisphere from the intact (right) hemisphere, then multiplying by 100 for cortex and striatum. Ventricle volumes were computed by integrating by the distance between slices.

2.6. Statistical analysis

There were no significant differences between the sham (non-ischemic) groups in the three casting conditions so their data were pooled and labeled SHAM. Behavioral data were analyzed using mixed ANOVA with group (MCAo + ipsi, MCAo + contra, MCAo + nocast, SHAM) as the between groups variable and time as the within subjects variable. Three brains were damaged due to technical problems, leaving 10 MCAo + contra, 9 MCAo + ipsi, 10 MCAo + nocast, and 13 SHAM brains for the histological analysis. Cortical and striatal atrophy volumes were analyzed separately using one way between groups ANOVA. Fisher's LSD was used for post-hoc analysis of main effects. Interactions were analyzed by individual univariate ANOVA followed by Fisher's LSD. Alpha was set at 0.05 for post-hoc tests.

3. Results

3.1. Behavioral observations

3.1.1. Forelimb placing

ANOVA revealed a significant main effect of Group ($F(3, 41) = 5.22, P < 0.01$); post-hoc tests indicated that MCAo + contra were more impaired in the forelimb placing task than all other groups (Fig. 2). ANOVA also revealed a significant main effect of Time ($F(4, 12) = 3.25, P < 0.01$); post-hoc tests indicated that overall, animals were more impaired in the placing task post-surgery. The Group X Time interaction was sig-

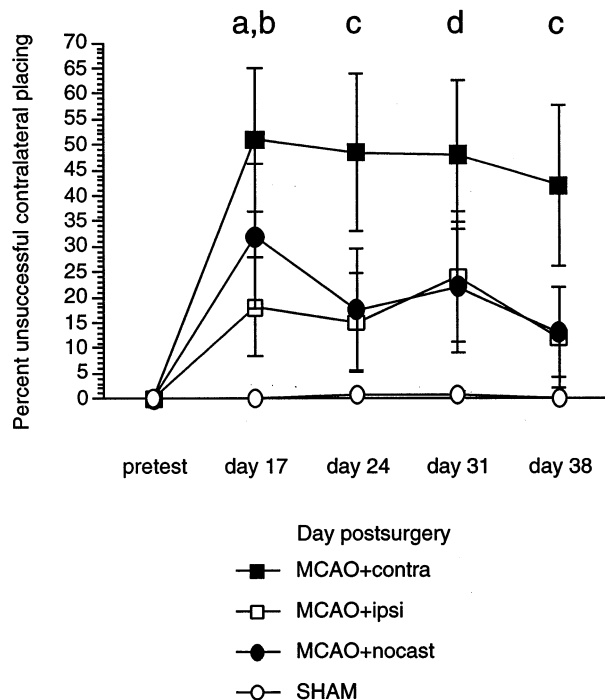


Fig. 2. Mean percentage of unsuccessful forelimb placing responses with the contralateral forelimb. Early disuse of the affected forelimb after MCAo (MCAo + contra) resulted in significantly more unsuccessful placing responses. SHAM represents the pooled data of ipsi-contra- and uncasted sham animals ($n = 5$ per condition). Error bars represent SEM. (a) MCAo + contra different from MCAo + ipsi and SHAM; (b) MCAo + nocast different from SHAM; (c) MCAo + contra different from MCAo + ipsi, MCAo + nocast, and SHAM; and (d) MCAo + contra different from SHAM.

nificant ($F(12, 164) = 3.25, P < 0.001$; Fig. 2). Post-hoc tests indicated that on day 17, MCAo + contra were significantly worse at placing the contralateral forelimb than MCAo + ipsi and SHAM, and MCAo + nocast were significantly worse than SHAM. On days 24 and 38, MCAo + contra were significantly worse at placing than all other groups. On day 31, MCAo + contra were significantly worse at placing than Sham. No groups were impaired at placing with the ipsilateral forelimb (data not shown).

3.1.2. Footfault

ANOVA revealed no significant main effects or interactions (Fig. 3), indicating that no groups were impaired in the footfault task. Because footfault deficits recover rapidly after primarily non-cortical forebrain injury, it seems likely that restorative mechanisms may have permitted recovery in this task prior to animals being tested on day 17.

3.1.3. Spontaneous limb use

Separate analyses of wall-associated and landing-associated limb use asymmetry were carried out. For landing-associated limb use asymmetry (Fig. 4A),

ANOVA revealed a significant main effect of Group ($F(3, 41) = 3.73, P < 0.05$); post-hoc tests indicated that MCAo + contra exhibited significantly greater ipsilateral biases than all other groups. For wall-associated limb use asymmetry (Fig. 4B), ANOVA revealed a significant main effect of Time ($F(4, 12) = 3.00, P < 0.05$); post-hoc tests indicated that overall, animals exhibited significantly greater ipsilateral forelimb biases post-surgery.

3.2. Histological observations

MCAo resulted in significant tissue loss in the striatum but not in the neocortex (Fig. 5 and Fig. 6). For striatal atrophy, ANOVA revealed a significant effect of Group ($F(3,39) = 5.15, P < .01$); post-hoc tests indicated that all MCAo groups had greater striatal atrophy than SHAM. There was no significant difference between groups for cortical atrophy or for ventricle volume (data not shown).

4. Discussion

The present study investigated the effects of early overuse and disuse of the affected forelimb, by immobilization of either the ipsilateral or contralateral limb, respectively, using unilateral plaster casts after transient proximal MCAo in rats. We observed that 10 days of forced disuse of the affected forelimb beginning immediately after MCAo worsened outcome on two mea-

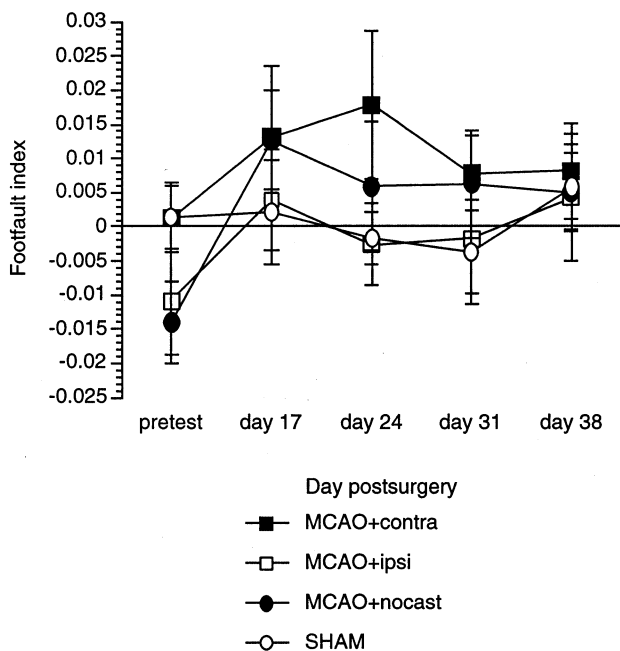


Fig. 3. Mean footfault index scores. No group differences were found in performance on the footfault task. Error bars represent SEM.

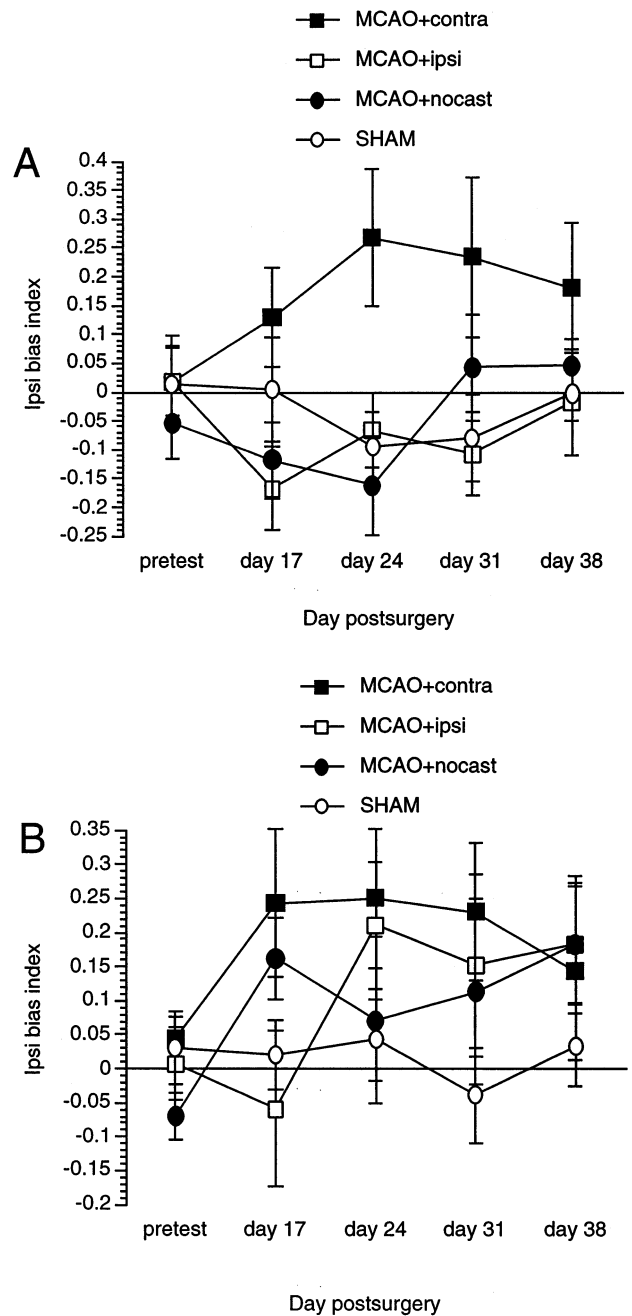


Fig. 4. Mean ipsilateral bias index scores for landing-associated (A) and wall-associated (B) limb use during spontaneous exploratory behavior in a clear cylinder. Positive scores indicate ipsilateral limb use bias, negative scores indicate contralateral limb use bias, and 0 represents no asymmetry. SHAM represents the pooled data of ipsi-contra- and uncasted sham animals ($n = 5$ per condition). Error bars represent SEM. Early disuse of the affected forelimb after MCAo (MCAo + contra) resulted in an ipsilateral bias in landing-associated limb use (A). There were no detectable group differences in wall-associated limb use (B).

asures of forelimb sensorimotor function, the forelimb placing task and landing-associated spontaneous limb use, but that forced overuse of the affected forelimb had no effect on any measure. Although we did not

perform behavioral tests during the early post-surgery period due to the presence of the casts on some rats, it is likely that this model of proximal MCAo resulted in acute behavioral dysfunction which spontaneously recovered, as reported by Belayev et al. [1], and that the disuse of the affected forelimb by immobilization blocked this recovery. This worsening of functional outcome was not accompanied by exaggeration of injury either to the striatum, which was reliably damaged in this model, or to the cortex, which was affected in only a small number of animals. These results are in contrast to previous work in our labs demonstrating that early overuse, but not disuse, of the affected forelimb after transient distal MCAo worsened functional outcome after both 30 min [2,31] and 45 min [4] of occlusion. It is likely that this discrepancy is due to the difference in the location of the damage in these two models. Our finding of reliable and almost exclusively striatal damage from 60 min of intraluminal MCA occlusion with a poly-L-lysine coated suture are

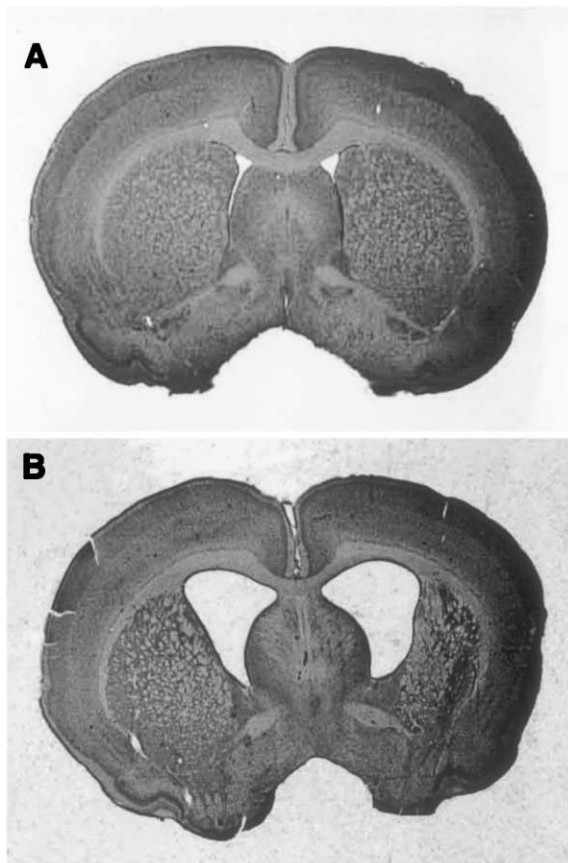


Fig. 5. Representative cresyl violet stained SHAM (A) and MCAo (B) brain slices at the level of the striatum. Right and left are reversed such that the right side represents the ischemic, left hemisphere. There is significant striatal atrophy in B (the ischemic striatum is 50% of the ischemic striatum) but there is no cortical atrophy in this animal. Lateral ventricle enlargement is seen bilaterally in this animal, however, there were no significant group differences in ventricle volume.

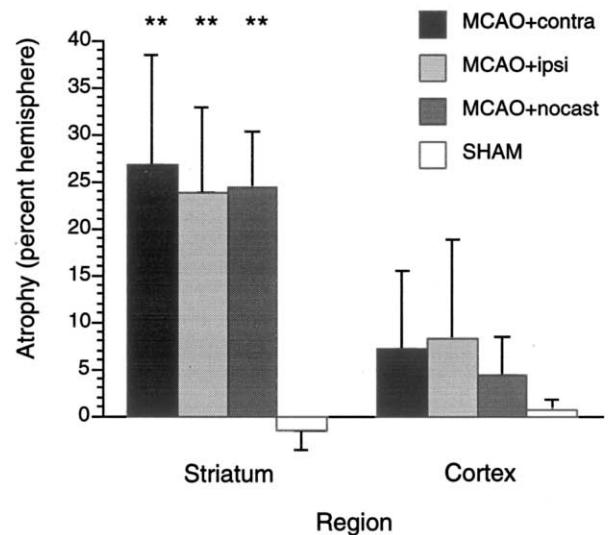


Fig. 6. Mean tissue atrophy in the ischemic striatum and cortex expressed as a percentage of the contralateral, intact hemisphere. All MCAo groups had significant striatal atrophy with respect to shams. There was no cortical atrophy in any group. SHAM represents the pooled data of ipsi- contra- and uncasted sham animals ($n = 5$ per condition). Error bars represent SEM. ** $P < 0.01$, different from SHAM.

in agreement with the results of Belayev et al. [1]. Conversely, we have reported significant cortical, but not striatal, damage after 30 [2] and 45 [4] min of distal MCAo using a three vessel occlusion model. Taken together, these studies suggest that there is a difference in the response to early post-ischemia demands on neurons in the cortex and in the striatum. It is important to note that in preliminary experiments using the present model, TTC staining 72 h post-stroke revealed that the ischemic damage was localized primarily in the lateral segment of the caudate putamen, similar to the results found by Belayev et al. [1]. We observed faint TTC staining in the caudate putamen in damaged areas, indicating the presence of some viable mitochondria. This suggests that there may be selective cell death and/or sparing of fibers of passage in this stroke model.

Early disuse of the affected forelimb resulted in worsening of function in the forelimb placing task and in landing-associated spontaneous forelimb use during exploration in a clear cylinder, but not in the footfault task nor in wall-associated spontaneous forelimb use in the cylinder. These tasks may differentially require an intact cortex, striatum, or both. Furthermore, they may be differentially sensitive to relative degrees of dysfunction in these structures, especially in cases where both cortical and subcortical damage has occurred. For example, we have recently reported differences in the initial deficit as well as differences in the rate and extent of recovery on these and other tasks in four models of CNS damage [30]. In that study, after focal electrolytic lesions of the FL-SMC moderate forelimb placing

deficits were found acutely and recovery to presurgery levels was found chronically, while after permanent distal MCAo (which results in cortical as well as subcortical infarction) severe forelimb placing deficits were found acutely with incomplete recovery chronically [30]. Forelimb placing deficits after nigrostriatal 6-OHDA lesions were also severe acutely, and no recovery was observed chronically [30]. In the cylinder test of spontaneous limb use, animals with FL-SMC lesions as well as with permanent MCAo showed moderate wall-associated limb use asymmetries acutely with some recovery chronically, and mild landing-associated limb use asymmetries both acutely and chronically. Animals with 6-OHDA lesions showed severe asymmetries in wall-associated, and moderate asymmetries in landing-associated limb use acutely, with no recovery in either measure [30]. It should be noted that 6-OHDA lesions, while primarily (though not exclusively) localized to the basal ganglia, preferentially destroy dopaminergic neurons, making comparisons with ischemic lesions of the basal ganglia difficult. Using a striatal hemorrhagic stroke model in the rat, which causes pan-necrotic neuronal death, we have observed greater deficits in forelimb placing than in the footfault task [unpublished data]. While comparisons of various behavioral tests between different injury models are somewhat difficult to interpret, the diversity in outcomes depending on the location and extent of the damage brings to light the value of performing a battery of behavioral tests, especially when evaluating interventions. We have recently summarized differences in recovery of sensorimotor function using several of our behavioral tests in rat models of stroke, parkinsonism, focal cortical injury, and spinal cord injury [30].

To our knowledge, the present results are the first to describe the effects of complete rest of an affected forelimb after striatal ischemic damage in the rat. We observed a dissociation between functional deficits and anatomical damage. Rats that had disused the affected forelimb post-surgery by immobilization of the limb in a cast were more impaired functionally than animals that had been either forced to overuse the affected forelimb or simply allowed to use it spontaneously, yet there were no differences between these treatment groups on the extent of tissue atrophy. Post-injury disuse of the affected forelimb may block recovery associated with 'reactive plasticity' [8,32,42,43]. This type of plasticity may create a milieu in the injured brain similar to that of the immature brain, and may thus allow experience-dependent plasticity similar to that occurring during development [see [7] for review]. The expression of trophic factors, including IGF-1 [13], basic fibroblast growth factor (bFGF) [29], and transforming growth factor β (TGFB) [19], is transiently increased after brain injury. The post-injury expression of these trophic factors may allow reactive plasticity to

occur during the early post-injury period. Some injury-induced trophic factors, such as brain-derived neurotrophic factor (BDNF), are also known to be activity dependent, with increases in their expression resulting from neural activity [25]. Not all injury-induced trophic factors require neural activity for their increased expression, but it is possible that they require neural activity to occur coincident with their increased expression in order for optimal synaptic remodeling and functional recovery to occur. Until further experiments are done, however, it remains speculative that mechanisms such as these are occurring in the present model.

Another possible explanation for the worsened functional outcome resulting from forced disuse of the impaired limb is a prolongation of remote functional depression of brain regions distant to the injury site. This 'diaschisis' [40] has since been shown to occur in rats, cats, and human patients after brain injury. For example, after a mild concussive injury in rats, diffuse and long lasting reductions in cytochrome oxidase (CO) activity [11] and in glucose metabolism [22,47] have been reported. Visual cortex ablation in cats resulted in reductions in CO activity in subcortical structures for up to 30 days, and this CO reduction was related to a tactile placing deficit [12]. Evidence for diaschisis has also been found in human stroke studies. Positron emission tomography (PET) studies have revealed metabolic depression of unaffected brain regions in patients following ischemic stroke [21]. PET has also shown that recovery of language function in aphasic patients following stroke was associated with a regression of metabolic depression in unaffected regions [5]. It is likely that allowing some use of the affected forelimb after moderate MCAo can promote the resolution of diaschisis and that complete disuse, as in the present model, can prevent the resolution of diaschisis.

Regardless of the mechanism, it is apparent that moderate use of an affected limb is beneficial after many types of brain injury in animal models. Other groups have shown that encouraging the use of an affected limb, either by specific motor training [18,26,28,46], forced exercise [9], or exposure to spatially [17,16,27] or socially [17] complex environments can enhance functional recovery. Enhancing motor recovery after brain injury with pharmacotherapies such as amphetamine requires task-specific practice with the impaired limb in addition to the drug regimen in rats [10] and in humans [41]. It is important to note that functional recovery or its blockade need not be accompanied by changes in the extent of gross anatomical damage. Investigations of molecular and cellular events after brain damage reveal that more subtle changes, reflecting reorganization or optimization of existing neurons, are associated with changes in functional recovery [26,34,39,46].

These changes may explain why early training regimens are not always detrimental [6,33] and can have beneficial effects on functional recovery, even when these regimens are forced or seemingly adverse. One and one-half hour of daily forced exercise on a running wheel for the first 10 days after FL-SMC lesions in rats led to improvements in forelimb placing with no change in lesion volume [9]. Risedal et al. [28] found improvements in motor performance in rats that had received early 'adverse' moderate motor training after permanent MCAo in spontaneously hypertensive rats (SHR). The adverse training, when implemented in the 1st week, but not in the 2nd week after ischemia resulted in significantly larger infarct volumes in spite of the enhanced functional recovery in both conditions. In primates, retraining of skilled hand use within 5 days after focal ischemic infarcts of the hand territory of primary motor cortex resulted in behavioral recovery and cortical reorganization [26]. Similarly to specific motor training, post-stroke exposure to enriched environments, which allows free physical activity and social interactions, has been shown to be beneficial after focal brain ischemia in rats [16,17,27].

In conclusion, the results of the present study demonstrate that early chronic disuse of an affected forelimb is detrimental to behavioral, but not to anatomical, outcome after moderate proximal MCAo in the rat. These results suggest that in preclinical studies of striatal damage the moderate use of an affected forelimb should be encouraged. In addition, the present model could provide a valuable tool in studies of activity-dependent reactive plasticity and diaschisis.

Acknowledgements

Supported by NIH RO1-NS39367-01. We wish to thank Dr Nicholas van Bruggen and Harold Thibodeaux for help with establishing the MCAo model.

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