Early Exclusive Use of the Affected Forelimb After Moderate Transient Focal Ischemia in Rats
Functional and Anatomic Outcome
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Background and Purpose—Previous work by researchers in our laboratory has shown that in the rat, the exclusive use of the affected forelimb during an early critical period exaggerates lesion volume and retards functional recovery after electrolytic lesions of the forelimb sensorimotor cortex. In the present study, we examined the effects of exclusive use of the affected forelimb after middle cerebral artery occlusion (MCAO).

Methods—Ischemia of moderate severity was produced in male Long-Evans rats through 45 minutes of occlusion of the left middle cerebral and both common carotid arteries. Exclusive use of either the affected or unaffected forelimb was forced through immobilization of either the ipsilateral (MCAO ipsi) or contralateral (MCAO contra) forelimb, respectively, for 10 days in a plaster cast, or the animal was left uncasted (MCAO nocast). Sham surgeries were performed, and animals were also casted for 10 days or left uncasted. Sensorimotor testing was performed during days 17 to 38. At the end of sensorimotor testing, cognitive performance was tested with use of the Morris water maze. In a separate experiment, temperatures and corticosterone levels were measured during the 10-day period after 45-minute ischemia and casting.

Results—The MCAO ipsi group performed worse on sensorimotor tasks than the MCAO contra, MCAO nocast, and sham groups. Infarct volume was significantly larger in the MCAO ipsi group than in the sham and MCAO contra groups but not in the MCAO nocast group. No group differences were found with the Morris water maze, and no group differences were found in either temperature or plasma corticosterone level.

Conclusions—The exclusive use of the affected forelimb immediately after focal ischemia has detrimental effects on sensorimotor function that cannot be attributed to hyperthermia or stress. (Stroke. 2000;31:1144-1152.)

Key Words: middle cerebral artery occlusion ■ motor activity ■ stroke, experimental ■ rats

Early after some types of brain injury in rats, the exclusive use of the impaired forelimb may be detrimental to outcome. The forced exclusive use of the affected forelimb through immobilization of the unaffected forelimb in a 1-sleeve plaster cast was shown to retard functional recovery after electrolytic lesions of the forelimb sensorimotor cortex (FL-SMC). In addition, there was a gross exaggeration of the original lesion size when animals were killed 33 or 60 days after the injury. One week of exclusive use immediately after the injury, but not 1 week of exclusive use beginning 7 days after the injury, results in exaggeration of injury and worsened functional outcome. Early exclusive use is also deleterious after fluid percussion injury in rats.

Immobilization of the ipsilateral (unaffected) arm of patients with stroke, thus forcing exclusive use of the affected arm, has been shown to improve long-term functional outcome. Taub et al5,6 and others7,8 have shown that constraint of the unaffected arm in combination with physical therapy, when implemented several months after disruption of function due to unilateral stroke, leads to significant improvements in the function of the impaired arm.

Risedal et al9 recently reported that moderate motor “training” in rats exaggerated the infarct size if it occurred during the first week after middle cerebral artery occlusion (MCAO) in spontaneously hypertensive rats (SHR) but not if it occurred during the second week. The adverse training began 24 hours or 7 days after MCAO distal to the striatal branches. The training regimen consisted of an enriched environment plus 2 regimens of motor challenges that took place on alternate days for 5 days. Poststroke exposure to enriched environments without specific motor training improves functional outcome.10–12 Experiments such as these raise further concerns about what the most appropriate rehabilitation regimen might be after stroke.
To determine whether limb-specific early rehabilitation affects functional outcome after ischemic stroke, we examined the effects of casting after moderate transient MCAO with the use of a 3-vessel occlusion model in rats. Because the window of vulnerability to exclusive use is not known after ischemic stroke, animals remained casted for 10 days. The immobilization of 1 limb may be stressful to animals, so we monitored plasma corticosterone levels throughout the casting period. Metyrapone, a corticosterone synthesis inhibitor, can reduce infarct volume after MCAO,\textsuperscript{13} so it seemed possible that corticosterone, if increased by casting, might contribute to an exaggeration of injury. It also seemed possible that the casting regimen might result in hyperthermia, which has been shown to have detrimental effects on outcome after MCAO,\textsuperscript{14–16} even if the hyperthermia is delayed for 24 hours.\textsuperscript{17} Therefore, temperatures were monitored throughout the casting period via thermistors chronically implanted in the temporalis muscle.

### Materials and Methods

All experimental procedures were performed in accordance with the University of Texas Medical School Animal Care and Use Committee protocols.

#### Animals

We used 52 male Long-Evans rats (Harlan Sprague-Dawley) that weighed between 300 and 350 g at the time of surgery. Thirty-four animals were used in the behavioral studies, and 18 animals were used in the corticosterone and temperature studies (9 of the animals in the corticosterone study were also used in the temperature study). Animals were kept on a 12-hour light/dark cycle and allowed rat chow and water ad libitum.

#### Animal Stroke Model

Animals were deprived of food but not water overnight before surgery. Animals were anesthetized with chloral hydrate (490 mg/kg IP). Surgical procedures were performed under an operating microscope. Both common carotid arteries (CCAs) were isolated via a midline neck incision and tagged with loose sutures. A skin incision was made horizontally between the left eye and ear. The skull was exposed by parietal bone dissection, with care taken not to damage the facial nerve. A 2-mm burr hole was made by drilling through the squamous temporal bone 2 mm anterior to its junction with the zygomatic arch to expose the MCA. The dura mater was cut above the MCA, and a 3-mm length of stainless steel wire (0.005-inch diameter) was inserted between the MCA and the parenchyma, lifted, and turned 90° counterclockwise. Both CCAs were then occluded with the use of atraumatic aneurysm clips. Interruption of blood flow was verified by the measurement of cerebral perfusion with a laser Doppler flowmeter (LDF) (model BPM2; Vasamedics, Inc). At the end of the 45-minute ischemic period, blood flow was reestablished by first turning the occluding wire clockwise and removing it and then removing the clips from the CCAs. Sham surgery included isolation of the CCAs, exposure of the MCA, and cutting of the dura mater. Animals were maintained at 37°C during all surgical procedures and during recovery. Because we were concerned about the effects of femoral artery cannulation on behavior, mean arterial blood pressure and blood gases were not measured.

#### Measurement of Cerebral Perfusion

To verify occlusion of the MCA, cerebral perfusion in the region previously shown to correspond to the core of the infarct\textsuperscript{18} was measured as previously described.\textsuperscript{18} Briefly, immediately after the burr hole exposed the MCA, a second burr hole 1 mm in diameter was made 4 mm dorsal to the first burr hole for the introduction of a 0.8-mm-diameter fiber-optic probe, which was coupled to the LDF. Care was taken to avoid damage to the dura. Under the operating microscope, the fiber optic probe was held in a vertical position in the burr hole touching but not compressing the dura, which was moistened with 0.9% saline. Cerebral perfusion (mL \( \cdot \) min\(^{-1} \cdot \) 100 g \(^{-1} \)) was measured before (baseline) and after occlusion of the MCA and both CCAs.

#### Casting Procedure

Before recovery from anesthesia after MCAO or sham surgery, animals were either fitted with 1-sleeve plaster casts or left uncasted. The upper torso 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.\textsuperscript{19} Animals remained in the casts for 10 days.

#### Temperature Analysis

Animals had remote temperature transponders (BMDS) implanted in the exposed temporalis muscle immediately after reperfusion and before closure of the incision. Temperature was measured with a digital reader (BMDS) 5 hours after reperfusion and on postsurgery days 1, 2, 3, 5, 7, and 10 between 12 noon and 3 PM.

#### Corticosterone Analysis

Tail blood was collected in capillary tubes that had been flushed with sodium heparin solution. Blood was collected 1 day before surgery and on postsurgery days 1, 4, and 10. The blood collection procedure was completed within 3 minutes of removal of the animal from the home cage and was performed between 12 noon and 3 PM. The plasma fraction was stored at \(-20^\circ\text{C}\) for later analysis of corticosterone with a \(H\) immunoassay kit (ICN).

#### Behavioral Testing Procedures

All sensorimotor testing was performed during the light cycle. Animals were pretrained the day before surgery and then tested once each week beginning 7 days after cast removal on postsurgery days 17, 24, 31, and 38. Water maze testing was performed between 6 and 11 PM and began on day 39 postsurgery.

#### Measurement of Forelimb Placing

Animals were held by their torso with the 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 1 was given each time the rat placed its forelimb on the edge of the tabletop in response to the vibrissae stimulation. Percent successful placing responses were determined (number correct \(\times 10\)) for contralateral and ipsilateral responses.

#### Measurement of Footfault Asymmetry

Animals were placed on an elevated grid, with openings of 2.3 cm\(^2\), for 2 minutes. 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 were also counted. A footfault index was computed ([contra faults–ipsi faults]/(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.

#### Morris Water Maze

Acquisition of spatial learning was tested using procedures adapted from Morris.\textsuperscript{20} The apparatus consisted of a 1.3-m-diameter white steel tank filled with 30 cm of water that was made opaque with white tempera paint and maintained at 24°C. The walls of the room contained visual cues that remained constant across the experiment. A ceramic 8-cm-diameter circular platform was submerged under 2.5 cm of water in 1 quadrant of the tank during all training trials. On the first training day, a single habituation trial was performed by placing each animal on the hidden platform for 60 seconds. If the animal fell or jumped from the platform, it was removed from the water and
placed back on the platform. On each of the following 4 days, 1 block of 4 training trials was performed for each animal. Animals were placed in the maze at 1 of 4 locations in a different randomized order for each block. Animals were allowed 60 seconds to find the platform or they were placed on it, and they were left on the platform for 30 seconds. Latency to find the platform, distance swum per quadrant, and time spent in each quadrant were determined with San Diego Instruments software. A probe trial was run 48 hours after the final training block. Before the probe trial, the platform was removed from the maze, and each animal was placed in the water at a predetermined location and allowed to search for the platform for 60 seconds. Time spent in the goal quadrant was analyzed for the probe trial.

Histology

Animals were deeply anesthetized with chloral hydrate and intrardially perfused with 0.9% saline followed by 4% paraformaldehyde with 15% picric acid in PBS. The brains were removed and postfixed in the same fixative for 24 hours and then cryoprotected in 10%, 20%, and 30% sucrose in PBS for 24 hours at each concentration. The brains were then snap frozen and stored at -80°C for later cryostat sectioning. Eight 50-μm sections were taken 1 mm apart and mounted onto Superfrost Plus slides for hematoxylin and eosin staining. Each section was scanned into a Macintosh computer with the use of Brain software. Each cortical hemisphere was measured through drawing with a cursor by an experimenter who was blinded with respect to the treatment conditions. Volumes of remaining tissue were computed for infarcted (ipsilateral) and contralateral cortical and striatal hemispheres through summing the 8 areas and then multiplying by the distance apart. Infarct volumes were computed by subtracting the ipsilateral (infarcted) hemisphere volume from the contralateral hemisphere volume, to reflect atrophy of remaining tissue in the infarcted hemisphere.

Statistical Analysis

There were no significant differences between the sham (nonischemic) groups in the 3 casting conditions, so their data were pooled and labeled "sham." Physiological and behavioral data were analyzed with mixed ANOVA with group (MCAO+ipsi, MCAO+contra, MCAO+nocast, sham) as the between-group variable and time as the within-subject variable, with the exception of probe trial data, which were analyzed with 1-way between-group ANOVA. Several brains were damaged due to technical problems, leaving 7 MCAO+contra, 7 MCAO+ipsi, 8 MCAO+nocast, and 3 sham brains for the histological analysis. Infarct volumes and volumes of remaining cortex were analyzed with 1-way between-group ANOVA. Fisher’s LSD was used for post hoc analysis of main effects, and simple effects tests were used for analysis of interactions.

Results

Cerebral Perfusion

There were no group differences in cerebral perfusion and no interaction. There was a main effect of time on cerebral perfusion; intraschisma cerebral perfusion was reduced (3.65±0.36 mL min⁻¹ 100 g⁻¹) to <4% of preischemia values (100.30±6.74 mL min⁻¹ 100 g⁻¹; F₁,2₄=210.63, P<0.001).

Forelimb Placing

Exclusive use of the affected forelimb after MCAO resulted in an exaggeration of a forelimb placing deficit. As shown in Figure 1A, MCAO+ipsi rats were more impaired in the percentage of successful placing responses with the contralateral forelimb than all other groups (P<0.05). No impairments were observed in placement of the ipsilateral forelimb in any of the groups (data not shown).

Footfault

Exclusive use of the affected forelimb after MCAO resulted in impaired performance on the footfault task. As shown in Figure 1B, all MCAO groups were significantly more impaired with the contralateral forelimb on the footfault task than were the sham group, as indicated by higher footfault index scores (P<0.01). Furthermore, MCAO+ipsi rats had higher footfault index scores than the MCAO+contra and MCAO+nocast groups (P<0.05), but the MCAO+contra and MCAO+nocast groups did not differ.

Morris Water Maze

There was no effect of either casting or MCAO on performance in the Morris water maze. Latency to find the hidden platform for the 4 trials of each day was grouped into blocks for the within-subject variable of time (Figure 2A). All groups learned to find the hidden platform, as indicated by shorter latencies across days (P<0.01). There was no significant difference in latency to find the platform between...
groups and no interaction. Time spent in the goal quadrant during the probe trial not differ among groups (Figure 2B).

**Histology**

There was a significant effect of group on cortical infarct volume \( (P<0.01) \) as shown in Figure 3A. MCAO+ipsi rats had larger cortical infarct volumes than nonischemic sham rats \( (P<0.05) \), reflecting the atrophy of tissue in the infarcted hemisphere. No other MCAO groups were significantly different from sham. MCAO+ipsi rats had larger infarct volumes than MCAO+contra rats \( (P<0.01) \), and MCAO+nocast rats also had larger infarct volumes than MCAO+contra rats \( (P<0.01) \). There was a significant effect of group on cortex volume \( (F_{3,21}=5.98, P<0.01) \). All MCAO groups had smaller cortex volumes than sham rats \( (*P<0.05, **P<0.01, ***P<0.001) \). Values are mean±SEM of 7 or 8 rats in the MCAO groups and 3 in the sham group.

**Temperature and Corticosterone**

There was no effect of casting after MCAO on either temperature or plasma corticosterone level throughout the casting period. Temporalis muscle temperatures did not differ either between groups or across time, and there was no interaction, as shown in Figure 4A. There was a significant effect of time on plasma corticosterone level \( (P<0.01) \); basal corticosterone levels were higher than postsurgery levels. There were no differences between groups on plasma corticosterone level, as shown in Figure 4B.

**Discussion**

We examined the effects of early exclusive use and disuse of the affected forelimb, via immobilization of either the ipsilateral or contralateral limb with unilateral plaster casts, after transient MCAO in rats. A 45-minute MCAO resulted in sensorimotor deficits but not cognitive impairment compared with sham-operated rats. Early exclusive use, but not disuse, exaggerated sensorimotor deficits but did not affect cognitive function compared with uncasted rats.

These results extend the results of Kozlowski et al,1 Schallert and Kozlowski,2 and Humm et al,3 who reported that the exclusive use of an affected forelimb during the early

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**Figure 2.** Performance in the Morris water maze in rats that had been casted or left uncasted for 10 days immediately after MCAO or sham surgery. A, Neither MCAO nor early overuse had an effect on latency to find the hidden platform (main effect of group NS). All animals learned to find the platform (main effect of time \( F_{3,57}=69.06, P<0.001 \)). The 4 trials of each day are grouped into 1 block for each of the 4 training days (values are mean±SEM). B, Neither MCAO nor early casting had an effect on time spent in the goal quadrant during the probe trial (main effect of group NS). Values are mean±SEM of 6 rats.

**Figure 3.** Cortical infarct volume and volume of remaining cortex at postsurgery day 46 of rats that had been casted or left uncasted for 10 days immediately after MCAO or sham surgery. A, Infarct volume was computed by subtracting the infarcted (ipsilateral) from the contralateral cortex. There was a significant effect of group on infarct volume \( (F_{3,21}=4.99, P<0.01) \). MCAO+ipsi had larger infarct volumes compared with sham \( (P<0.05) \), whereas no other MCAO groups were significantly different from sham. MCAO+ipsi had larger infarct volumes than MCAO+contra \( (P<0.01) \), and MCAO+nocast also had larger infarct volumes than MCAO+contra \( (P<0.05) \). B, Volume of remaining cortex was computed by summing the infarcted (ipsilateral) and the contralateral hemispheres. There was a significant effect of group on cortex volume \( (F_{3,21}=5.98, P<0.01) \). All MCAO groups had smaller cortex volumes than sham rats \( (*P<0.05, **P<0.01, ***P<0.001) \). Values are mean±SEM of 7 or 8 rats in the MCAO groups and 3 in the sham group.
critical period after electrolytic lesions of the FL-SMC retards functional recovery and results in gross enhancement of the original damage. We found that there was no detectable exaggeration of cortical infarct volume resulting from early exclusive use after 45 minutes of MCAO. The exaggeration of anatomic damage after this model of MCAO appears to be less extreme than that after electrolytic lesions of the FL-SMC. This may due in part to the different location of the damage. An examination of triphenyltetrazolium chloride–stained slices 1 to 3 days after the surgery indicated that infarctions in this model of moderate distal MCAO include primarily parietal cortex, although the penumbra appears to involve FL-SMC in many animals. It is important to note that in contrast to focal electrolytic lesions, MCAO involves no mechanical damage to either cortical neurons or the corpus callosum, which may also explain the different effects of early exclusive use in these models. Early exclusive use did result in exaggeration of sensorimotor deficits in this model of transient MCAO. Sensorimotor function may be a more sensitive assay for the detection of neurological damage than a gross examination of tissue loss, especially when tested at multiple time points, as in the present study.

It is interesting to note that MCAO+contra infarct volumes were similar to those of the sham rats, yet performance on sensorimotor tasks was similar to that of the MCAO+nocast group. An analysis of total cortical tissue (the sum of both hemispheres) reveals that all MCAO groups were different from the sham group. One possible explanation for the discrepancy between functional outcome and infarct volume in this group could be diffuse bilateral damage, which may result from ischemia in this rat model. This diffuse injury may be vulnerable to the effects of behavioral pressure that involve the limb ipsilateral to the infarcted hemisphere. Furthermore, casting of the impaired limb may prolong functional impairments, in the absence of gross anatomic damage, by prolonging metabolic depression; this depression was termed “diaschisis” by von Monakow. In rats, diffuse, long-lasting reductions in oxidative metabolism, as measured with cytochrome oxidase histochemistry, and in glucose metabolism, as measured with 2-deoxyglucose uptake, have been found after a mild concussive injury that caused no gross morphological damage. Cytochrome oxidase activity was reduced in subcortical structures for up to 30 days after visual cortex ablation in cats, and this was related to a tactile placing deficit. PET studies reveal metabolic depression of unaffected brain regions in humans after ischemic stroke and that this hypometabolism is related to impaired cognitive function. Recovery of language function in aphasic patients after stroke was shown to be associated with a regression of metabolic depression in unaffected regions, as measured with PET.

Although there were group differences in both the footfault task and the forelimb placing task, there was no effect of MCAO on spatial learning in the Morris water maze. Spatial learning may be dependent on an intact parietal cortex, but animals may be able to compensate after unilateral damage to the left parietal cortex, which was the side damaged in the present model of MCAO. Our results are in contrast to the findings of Markgraf et al, who report that cognitive deficits were found 8 weeks after permanent MCAO in Sprague-Dawley rats, even though recovery on sensorimotor tasks had occurred. Stroemer et al also found long-lasting learning deficits in the Morris water maze after permanent MCAO; however, performance on a footfault with large grid openings was also still impaired.

We have previously shown that after a more severe MCAO, exclusive use had no effect on functional or anatomic outcome. Rats were subjected to 90 minutes of 3-vessel occlusion, with a model similar to the present model, and were ipsilaterally casted or left uncasted for 14 days. At 30 days, there were no discernible differences in either sensorimotor function or infarct volume between animals that had been casted or left uncasted. Cortical damage may have been near maximal after this duration of ischemia and thus not vulnerable to the detrimental effects of exclusive use. However, Risedal et al reported an increase in infarct volume after moderate but “adverse” motor training in SHR during the first week after permanent distal MCAO, even though animals in this early training group had improved functional outcome relative to animals in the no-training and late-training groups. The motor training regimen used by Risedal et al may have allowed compensatory plasticity in both hemispheres to occur even while exacerbating damage to the infarcted hemisphere. Alternatively, it is possible that the
Contralateral cortex may have become thicker as a result of early training, giving the appearance of a smaller ipsilateral side.\textsuperscript{19}

Enhanced brain damage does not appear to be inevitable after early overuse. The location and type of damage, as well as the timing and the intensity of the behavioral pressure, all appear to be important factors that contribute to use-dependent exaggeration of injury. Colbourne et al\textsuperscript{46} found that forced use of the hippocampus did not increase the extent of damage to hippocampal neurons after global ischemia with hypothermia in gerbils. Multiple behavioral testing involving hippocampus-dependent tasks failed to increase the number of damaged neurons in the CA1 subfield. It is possible that vulnerable hippocampal neurons may not be susceptible to overuse effects. However, it should be noted that the training regimen was relatively mild and was not implemented until 4 days after the insult in this experiment. Mild physical training after brain injury has not shown to be deleterious. Early training in food pellet retrieval tasks does not exacerbate cortical damage after focal stroke in primates and appears to promote reorganization of relevant cortical representation areas.\textsuperscript{37,38} Stoltz et al\textsuperscript{19} have shown that 5 minutes of swimming each day beginning 5 days after electrolytic lesions of the FL-SMC has no adverse effect on either lesion size or functional outcome in rats. Forced exercise on a running wheel during the early postlesion period led to improvements in functional outcome after focal cortical lesions and no change in lesion volume.\textsuperscript{40} These behavioral results of Hart et al\textsuperscript{40} are consistent with those of Risedal et al,\textsuperscript{9} who found improvements in functional outcome in animals that had received early “adverse” moderate motor therapy after permanent MCAO in SHR. Mild physical training may be beneficial when administered in combination with pharmacotherapy. For example, after suction ablation of the motor cortex in rats, beam walking performance was significantly improved after the administration of amphetamine only when animals were allowed practice trials on the beam and not when animals were given amphetamine without practice.\textsuperscript{41}

After some types of brain injury, early exclusive use with casting can reduce damage. Tillerson et al\textsuperscript{42} found that the exclusive use of the affected forelimb by ipsilateral limb immobilization for 7 days improved function in a rat model of Parkinson’s disease when rats were tested up to 60 days after the surgery. Rats that had been casted were similar to sham rats in spontaneous limb use, as well as in apomorphine-induced rotation. In addition, dopamine levels were closer to sham levels in rats that had been casted. It is possible that vulnerable striatal neurons respond differently to the demands of early exclusive use than do vulnerable cortical neurons. We are currently investigating the effects of early exclusive use on outcome after ischemic damage to the striatum with the poly-L-lysine–coated intraluminal suture model of MCAO,\textsuperscript{43} in which relatively short durations of occlusion preferentially cause subcortical damage.

The mechanism of use-dependent exaggeration of injury remains unclear. We have reported that neither contralateral nor ipsilateral casting causes hyperthermia. Hyperthermia has been well established as a moderator of neuronal injury after focal ischemia.\textsuperscript{14–17} However, we cannot rule out the possibility that local increases in brain temperature occur as a result of exclusive use. We have also demonstrated that the casting paradigm does not cause increases in the stress-related hormone corticosterone, which has been shown to be deleterious after focal ischemia and excitotoxic cortical injury\textsuperscript{13} and has long been known to be deleterious to hippocampal neurons both in intact animals\textsuperscript{44} and after global ischemia.\textsuperscript{45} Moreover, the inhibition of corticosterone synthesis during the casting period with metyrapone did not block use-dependent exaggeration of injury after FL-SMC lesions (S.T.B., J.L. Humm, and T.S., unpublished data). We have previously shown that blockade of the N-methyl-D-aspartate (NMDA) receptor by the noncompetitive antagonist MK-801 attenuates use-dependent exaggeration of injury after electrolytic lesions.\textsuperscript{46} A mechanism other than excessive release of glutamate into the extracellular space may be responsible, because forced exclusive use via casting did not result in increases in glutamate levels when measured with in vivo microdialysis.\textsuperscript{47} Enhanced NMDA-mediated excitatory postsynaptic activity occurs during the early period in the area surrounding cortical heat lesions,\textsuperscript{48} and inhibitory GABAergic activity is reduced.\textsuperscript{49} Glutamate receptors, including NMDA receptors, are upregulated in the striatum after lesions of the frontal cortex.\textsuperscript{50} It is possible that overactivation of these receptors by neuronal activity, even in the absence of high levels of glutamate in the extracellular space, is detrimental to outcome during this period of hyperexcitability. It is important to note that Qu et al\textsuperscript{51} demonstrated that NMDA receptor excitability is increased after stroke in mice for up to 28 days, yet behavioral pressure has not been shown to exacerbate damage when it is begun later than the first week after damage.\textsuperscript{3}

The present results are in contrast to several studies in humans that report the forced use of an impaired arm improves functional outcome after ischemic stroke.\textsuperscript{5–8} Taub et al\textsuperscript{5} has proposed that “constraint therapy” (ie, immobilization of the unaffected arm in combination with physical therapy) may allow the patient to overcome “learned nonuse” of the arm after brain damage. In these studies,\textsuperscript{5–8} forced limb use was begun several months after the damage had occurred. The effects of extreme regimens of physical therapy implemented during the acute phase after brain injury in human patients depend on many factors and are largely unknown.

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Over the past 2 decades there has been a remarkable increase in the number of laboratory investigations of brain injury. The earlier pessimism regarding treatment of brain injury changed to optimism based on laboratory observations of pharmacological reduction of delayed neuronal death. The brief therapeutic window of those agents coupled with their neurotoxic effects greatly limited their potential clinical utility. Other laboratory studies, measuring effects of pharmacological treatment on functional outcome rather than neuronal death, led to the strategy of combining routine physical therapy with pharmacotherapy which used drugs that increased noradrenaline release. Conversely, early administration of drugs blocking α1-noradrenergic receptors or reducing noradrenaline release slows spontaneous recovery. Data from a few controlled clinical studies2,3 appear promising in their support of this approach. Evaluation of the assumptions and hypotheses guiding rehabilitation medicine by controlled experiments, development of laboratory models, and interaction with other disciplines is essential, or the field will stagnate.

One of the earliest reports on the rehabilitation of hemiplegic stroke patients was published in 19154 and described improved outcome after passive and assisted movements of paretic limbs. This remains the routine method for rehabilitation of hemiplegic patients. An important change in this rehabilitation was the forcing of movements from the patient. In constraint-induced (CI) movement therapy,5 patients are forced to use the affected limb through restraint of the unaffected limb in a sling for 2 weeks and use of reward training to shape additional movements of the affected limb. Reportedly, CI is effective when initiated late after infarct, and improvement is attributed to changing the “learned disuse” of the paretic limb. However, there are no data to support a learning hypothesis, and even when alternate movement strategies are used, this does not exclude the involvement of other mechanisms.

The accompanying article by Bland et al extends the approach used by constraint but describes an unexpected deleterious effects on outcome when intense, forced movement of the affected limb is conducted early after stroke. Limiting use of the unaffected limb by a plaster casting of the unaffected forelimb forces exclusive use of the affected limb. When done early after ischemia involving sensorimotor cortex, it produces a worsening of symptoms. Using the same forced use procedure after other types of injury, they and others reported both an increased injury volume and slower functional recovery. However, in this study of ischemia there was a worsening of symptoms without any increase in the extent of ischemic damage. To account for the dissociation of forced use producing slower recovery without increasing lesion volume, the authors note that the area of ischemia was too large to detect increased cell death. While such an account is feasible, it is possible to infer that there is no causal relation between the exaggerated cell death and slower functional outcome. It seems unlikely that neurons, so close to the threshold for death that they die from behavioral pressure, make any contribution to recovery. It seems likely that the early behavioral demands are killing marginally alive neurons by the increased behavioral pressure pushing the cell into energy failure when it is unable to maintain ionic homeostasis. If measures of cerebral metabolism were taken during this behavior, it is likely to evoke a much greater amount of glucose utilization, and a more severe and widespread pos injury hypometabolism would be observed. Such a demand could expand the area or increase the severity of hypometabolism early after stroke and correlate with the slower behavioral recovery.

By measuring brain temperature and the stress hormone cortisol and finding no increases during forced use of the affected limb early after ischemia, these nonspecific factors were excluded from any role in the period of neuronal vulnerability early after brain injury. It may be useful to compare the period of posts ischemic vulnerability revealed by forced use to another method, ischemic tolerance,7 which also shows a transient vulnerability after ischemia. Repetition of ischemic events given at short time intervals of 1 hour or less displays a cumulative response. Two sublethal ischemic challenges, when spaced at a short interval, can add up to lethal levels. However, at intervals of at least 24 hours, ischemic tolerance is observed. If a sublethal ischemic challenge is administered and followed in 25 hours by a lethal insult, the tissue will show a diminished reaction to the second event. Thus, the factors producing early posts ischemic vulnerability to subsequent challenges may represent the beginning of a vulnerable period terminated when subsequent challenges are no longer cumulative but attenuated. Sublethal ischemia stops most protein synthesis in neurons but induces synthesis of “heat-shock proteins,” which are thought to play a role in maintaining the health of the neurons. The period of suppressed protein synthesis may be related to the vulnerability to behavioral pressure discussed by Bland et al. Some consideration of the parallels between these responses may be worthwhile, as both are altered by MK-801 and alter neurons in the penumbra of focal ischemia.

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