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Research report

# Consequences of forced disuse of the impaired forelimb after unilateral cortical injury

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## Abstract

Extreme over-reliance on the impaired forelimb following unilateral lesions of the forelimb representation area of the rat sensorimotor cortex (FL-SMC) leads to exaggeration of injury when overuse is begun during the first week, but not later periods, after injury. Behavioral impairment is partially worsened by the additional tissue loss. In the present study, we show that complete *disuse* of the impaired forelimb during the first post-operative week renders surviving tissue vulnerable to later overuse of the same limb, in effect extending the window of vulnerability in which use-dependent exaggeration of brain injury can occur. Behavioral recovery is disrupted by complete disuse, but the degree of impairment is variable depending on the nature of the behavioral test employed. Our results uphold the idea that *mild* rehabilitative training early after injury is beneficial, while either extreme overuse or complete disuse may disrupt functional recovery.
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*Keywords:* Disuse; Sensorimotor cortex; Recovery of function; Use-dependent; Forelimb use; Exaggeration of injury

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## 1. Introduction

The rat sensorimotor cortex (SMC) participates in the processing of afferent sensory information and the production of appropriate motor responses. Unilateral injury to this area of the brain results in both sensory and motor deficits on the opposite side of the body [8–11,32]. The forelimb representation area of the SMC (FL-SMC) regulates forelimb dexterity and fine movements [5,6,34]; thus, unilateral lesions of the FL-SMC cause motor and sensory deficits specific to the contralateral (opposite) forelimb [13,14,19,21,23,33]. Deficits in use of the contralateral forelimb result in preferential reliance on the ipsilateral, unaffected forelimb, creating a limb-use asymmetry that dwindles in severity with the passage of time [1,13,14,21].

Detrimental anatomical and functional effects of forced overuse of the impaired forelimb after injury to the FL-SMC have been documented [13,14,21]. Forced motor rehabilitation in the first week following focal cortical (but not striatal) infarction also has been shown to increase infarct size [26] and functional outcome [2]. The precise mechanism by which overuse causes exaggeration of neuronal injury remains unknown. The glutamatergic system appears to be involved in this process, since the NMDA receptor antagonist, MK-801, protects neurons from the damaging effects of forced overuse. Levels of extracellular glutamate, however, do not appear high enough after cortical injury and forced overuse to be considered excitotoxic [13]. Whatever the mechanism, it is apparent that in the FL-SMC ablation model its detrimental effects occur only during the first week after injury. If animals are forced to overuse the impaired forelimb during the second week following injury, minor behavioral deficits result, but use-dependent exaggeration of injury does not occur [14].

Although complete *overuse* of the impaired forelimb adversely affects both surviving tissue and functional recovery, the effects of complete *disuse* of the impaired forelimb have not been systematically investigated in this model of cortical injury. Results obtained by Kozlowski et al. [21] indicate that although disuse of the impaired forelimb does not detectably exaggerate the size of the original lesion, it does hinder behavioral recovery. Furthermore, 10 days of disuse following stroke impairs functional recovery [2]. The absence of obvious neural damage paired with exaggerated behavioral deficits is compelling, and suggests that disuse of the impaired forelimb produces subtle changes in tissue surrounding the lesion, compromising its ability to support

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the recovery of function observed in non-casted controls, or to withstand a later challenge of intense motor activity.

The present study investigates the effects of complete disuse of the impaired forelimb on later overuse of the same limb. After FL-SMC injury, rats were forced to rely only on the *unimpaired* forelimb for either 1 or 2 weeks following surgery, and then on the *impaired* forelimb for 1 week. Of particular interest was whether the vulnerable period for use-dependent exaggeration of injury could be pushed beyond the first week following injury by forcing behavioral quiescence of the impaired forelimb immediately after injury.

## 2. Materials and methods

### 2.1. Subjects

Eighty-nine male hooded Long-Evans rats (4–8 months of age at the time of surgery) were used for this study. Rats were housed in groups of 2–3 in transparent Plexiglas cages. Food and water were always available, and the animal room was maintained on a 12 h/12 h light/dark cycle. All behavioral testing was conducted during the light phase. Prior to inclusion in the study, all animals were tamed by gentle handling to get them accustomed to, and comfortable with, behavioral testing.

### 2.2. Surgical procedures

Equithesin, a cocktail of chloral hydrate (150 mg/kg) and sodium pentobarbital (25 mg/kg; 0.35 cc/100 g, i.p.), was used to anesthetize the rats. After the rat was placed in a stereotaxic apparatus, the skin was incised and retracted in order to expose the surface of the skull. The skull was removed between 3.0 and 4.5 mm lateral to the midline suture, and between 0.5 mm posterior and 1.5 mm anterior to bregma. A platinum electrode was lowered 1.7 mm below dura, and unilateral lesions of the FL-SMC were made by moving the electrode in slow, even traverses through the exposed cortex, while a 1 mA anodal current was delivered. Sham-operated animals received identical anesthesia and incision procedures, but the skull was not drilled.

### 2.3. Study design

This study comprised 12 groups of animals. Each group of lesioned animals ($n = 9$–10 per group) was complemented by a sham group ($n = 5$ per group) that received identical treatment except for the lesion. Table 1 depicts the design of this study.

Table 1
The 12 groups comprising the present study and the number of animals in each group are shown below

| | Lesion | Sham |
|---|---|---|
| Contralateral limb immobilized for 14 days, then overused for 7 days | 9 | 5 |
| Contralateral limb immobilized for 7 days, then overused for 7 days | 10 | 5 |
| Contralateral limb immobilized for 7 days (disuse of impaired limb) | 10 | 5 |
| Ipsilateral limb immobilized for 7 days (early overuse of impaired limb) | 10 | 5 |
| Ipsilateral limb immobilized days 7–14 (late overuse of impaired limb) | 10 | 5 |
| Neither forelimb immobilized | 10 | 5 |

### 2.4. Casting procedures

Following surgery, while the animals were still under anesthesia, strips of felt were wrapped around the shoulders and sternum of animals in groups to be casted. The appropriate forelimb was then wrapped in felt and placed directly across the upper ribcage. More strips of felt were then wrapped around the limb, followed by strips of wet plaster. To prevent cagemates from chewing the cast off an individual rat, a spoonful of quinine (which tastes extremely bitter) was added to the water used to saturate the plaster. The result was a small cast that extended from the neck to just below the shoulders, leaving animals able to use only the uncasted limb. These casts were extremely light-weight and allowed considerable mobility. Upon recovery from anesthesia, and throughout the casting period, rats were observed to walk, rear and interact with cagemates, as well as drink freely from sipper tubes and feed and groom themselves.

### 2.5. Behavioral observations

All behavioral tests were performed prior to surgery, and then on post-surgical days 2, 4, 7, 10, 15, 18, 23, 29, 35, 40, 45, 52 and 59. If an animal was wearing a cast on a given testing day, the animal was not tested on that day. Animals were given at least 2 days following cast removal before behavioral testing commenced.

#### 2.5.1. Forelimb placing test

The vibrissae-stimulated forelimb placing test was performed as previously described [17,18]. Rats were held by the torso, with forelimbs hanging freely. The vibrissae on one side of the snout were brushed laterally against the edge of a countertop. Intact rats can quickly place the forelimb ipsilateral to the stimulated vibrissae onto the countertop. Typically, however, lesioned animals have difficulty placing the limb contralateral to the lesion. Ten trials were performed on either side, and behavioral asymmetry was calculated as the percent unsuccessful contralesional forelimb placing.

#### 2.5.2. Spontaneous limb use

Rats were videotaped in a transparent Plexiglas cylinder ($d$ 17.5 cm, $h$ 30.5 cm) for 3 min. The diameter of the cylinder was wide enough to allow rats to have all four limbs on the floor, without the snout touching the side of the cylinder. The following rating system (described in reference [28]) was used by a rater blind to condition: (a) the number of times the ipsilateral limb was used during landing from a rear; (b) the number of times the contralateral limb was used for landing from a rear; (c) the number of times both limbs were used simultaneously for landing from a rear; (d) the number of times the ipsilateral limb was used for weight support against the side of the cylinder (after rearing); (e) the number of times the contralateral limb was used for support against the side of the cylinder; and (f) the number of times both forelimbs were used simultaneously for support on the side of the cylinder. During a rear, the first forepaw to touch the wall of the cylinder was scored as an independent placement of that forelimb. If the other forepaw was subsequently placed on the wall while maintaining the initial placement, then a “both” wall movement was scored. In order for another independent wall movement to be scored, both forepaws had to be removed from the wall. If a rat explored the wall laterally using both forepaws simultaneously (wall stepping), the initial simultaneous placement was scored as a “both” and then every subsequent simultaneous movement also received a “both” score.

The following percentages were calculated: percent use of the ipsilateral − percent use of the contralateral forelimb (%ipsi − %contra) for (a) landing from a rear, and (b) weight support against the wall; and percent use of both forelimbs simultaneously for (c) landing from a rear, and (d) weight support against the wall. Percent ipsi − %contra use was scored because previous results indicated that independent use of the contralateral limb following injury is rare. Therefore, the higher this number, the greater the reliance on the ipsilateral forelimb (instead of the contralateral forelimb).

### 2.6. Histological procedures

All rats were overdosed with sodium pentobarbital (100 mg/kg, i.p.) on post-operative day 60, and perfused intracardially with saline, followed by a 10% formalin solution. The brains were removed, sliced into 100 μm sections, and stained with cresyl violet. Stereologic analysis was conducted using slices between 2.7 mm anterior and 1.0 mm posterior to bregma in order to determine the volume of intact tissue remaining in the lesioned hemisphere. A grid of points 20 mm apart was randomly placed over a 20× projection of each section between these coordinates. Volume of remaining tissue was computed using the Cavalieri formula, an unbiased method of volume analysis [27,37]. The formula is: $V = T(a/p)^2 \sum P$, where $T$ is the thickness of each measured slice (100 μm) + the distance between the measured slices (300 μm), $a/p$ is the size of the grid divided by the magnification (20 mm/20×) and $\sum P$ is the total number of grid points that touched intact tissue. The coefficient of error was 0.05 or less for each estimate of volume.

### 2.7. Statistical analysis

Anatomical data (volume of remaining tissue) were analyzed using a one-way ANOVA for Group, and post hoc analysis was carried out using Bonferonni-corrected planned comparisons.

Because many animals in this study were wearing casts on behavioral testing days, and were therefore unable to undergo testing, there are large gaps in the behavioral data collected. While it would have been possible to begin testing on day 23 following injury, when all groups of animals had been removed from their casts, a stable baseline recovery curve for the control group (non-casted animals) was desired. Furthermore, since animals may show substantial recovery from deficits by day 23 on some of the behavioral measures used, recovery curves could not be obtained if all testing was begun on this day. Therefore, the statistical analysis of this study used hierarchical linear modeling (HLM), a model that can accommodate the gaps in the behavioral data. HLM calculates the slope of the recovery curve of every animal in every treatment condition. These slopes are then grouped into populations, according to treatment condition. HLM then compares the populations of slopes, regardless of when the behavioral data points begin. The first data point for each group on each test is called the intercept; HLM analyzed group differences in initial performance (intercept), as well as the overall slope of the recovery curves. For each behavioral measure, HLM was performed on the lesioned groups so that each one was compared to non-casted lesioned controls. Groups not different from each other were pooled together. This procedure was repeated for sham-operated groups, each sham group being compared to the non-casted, sham-operated animals. Another HLM was then performed to analyze differences between pooled lesion and pooled sham groups, as well as any groups that were different from the reference group in the initial HLM. Finally, an omnibus test of group differences was performed to ensure that group differences could be better predicted if group number was included in the analysis (rather than all animals being included as one group).

## 3. Results

### 3.1. Volume of remaining tissue

ANOVA indicated that the volume of intact tissue did not differ among the six sham-operated groups ($F(5, 24) = 0.855$, $P = 0.525$), so all sham animals were pooled into one group, called “Shams.”

One-way ANOVA revealed a significant main effect of Group ($F(6, 82) = 58.42$, $P = 0.0001$), and post hoc

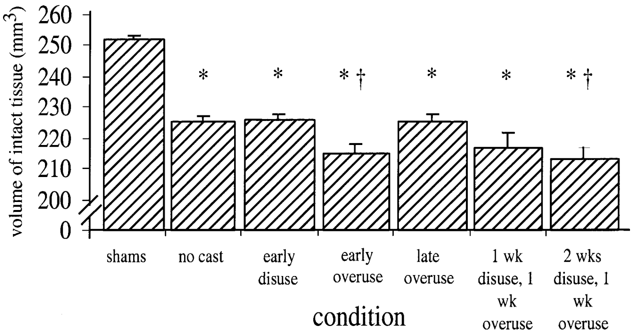

Fig. 1. Volume of brain tissue remaining in the lesioned groups, compared to sham-operated controls. $^{*}P < 0.001$, significantly different from sham, $\dagger P < 0.05$ significantly different from non-casted lesions.

analysis indicated that all lesioned groups had significantly less remaining tissue in comparison to sham-operated animals. Furthermore, post hoc tests indicated that overuse of the impaired forelimb during the first post-injury week led to a significantly smaller volume of remaining tissue in comparison to lesioned animals, although *disuse* during the same time period did not, replicating the findings of Kozlowski et al. [21] (see Fig. 1). Importantly, overuse of the impaired forelimb during the second post-injury week (Late Overuse group) did not result in less intact tissue, unless overuse in the second or third post-injury week was preceded by disuse immediately following injury (1 week disuse + 1 week overuse and 2 weeks disuse + 1 week overuse bars) (see Figs. 1 and 2). It should be noted that 1 week of disuse followed by 1 week of overuse was not sufficient to statistically affect volume of remaining tissue, although there is an obvious trend towards significance in this condition. Two weeks of disuse followed by 1 week of overuse produced a clear, statistically significant decrease in volume of remaining tissue.


no limb use restriction

impaired limb overused


impaired forelimb rested

impaired forelimb rested for 2 weeks, overused for one week

Fig. 2. Representative lesions from selected casted groups, shown at the approximate rostro-caudal midpoint of the damage.

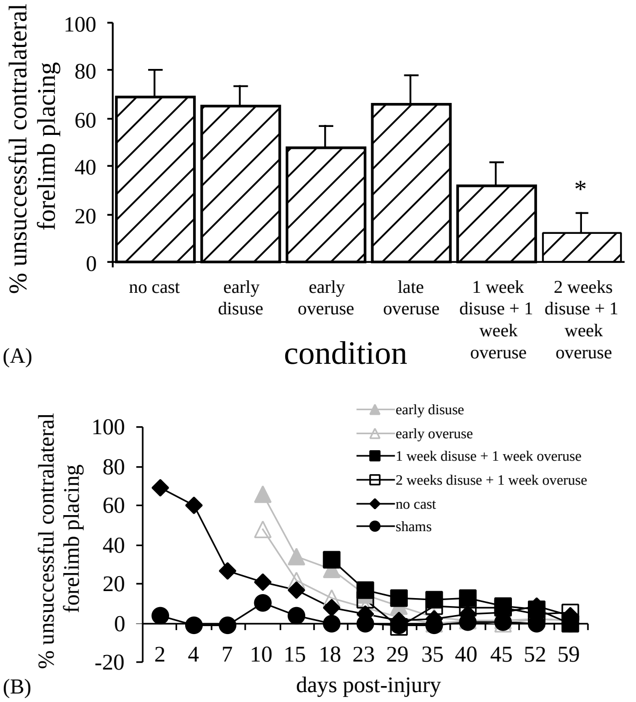

Fig. 3. Forelimb placing deficits of lesioned groups of interest. The *intercept* of each lesioned group is depicted in A. $^{*}P < 0.001$ significantly different from non-casted lesions. *Recovery curves* of lesioned groups are plotted vs. Shams and non-casted lesions in B.

### 3.2. Forelimb placing

ANOVA found no differences among any of the groups before surgery ($F(11, 77) = 1.23$, $P = 0.2808$) on forelimb placing ability. HLM found no differences in forelimb placing ability among sham-operated groups (omnibus test of group differences: intercept, $P = 0.164$; slope, $P = 0.111$). Therefore, placing data for all sham groups were pooled into one group called “Shams.” When all lesioned groups were compared to the non-casted, lesioned controls, HLM revealed that the intercept for the 2 weeks disuse + 1 week overuse group was different. None of the other lesioned groups differed on intercept or slope, so all other lesioned groups were pooled and labeled “Lesions.” An overall HLM found no differences between the 2 weeks disuse + 1 week overuse group and Shams on either intercept ($P = 0.526$) or slope ($P = 0.982$). Lesions were significantly different from Shams on both intercept ($P < 0.0001$) and slope ($P < 0.0001$) (Fig. 3B).

### 3.3. Spontaneous limb use

#### 3.3.1. Use of the forelimbs for landing

Two different scores were calculated: use of both forelimbs simultaneously for landing from a rearing position, and percent ipsi − percent contra for landing from a rearing position. ANOVA found no differences among any of the groups before surgery ($F(11, 76) = 1.316$, $P = 0.2325$) for

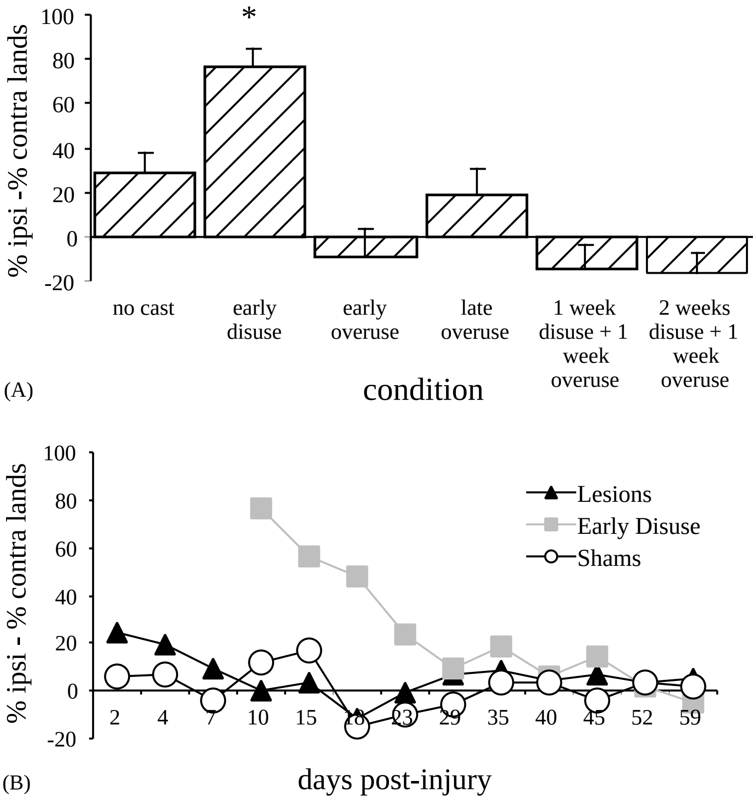

Fig. 4. Percent ipsilateral minus percent contralateral limb use for landing from a rear. The intercept for each lesioned group is shown in A, $^*P < 0.001$ significantly different from non-casted controls, %ipsi − %contra limb use for landing is plotted over time in B.

%ipsi − %contra limb use when landing from a rear. HLM indicated no differences among sham groups for %ipsi − %contra limb use for landing from a rear (omnibus test of group differences: intercept, $P = 0.0984$; slope, $P = 0.0526$), so data from all sham-operated groups were combined into one large group labeled "Shams." Fig. 4 shows that the only lesioned group that differed from the non-casted lesions (intercept, $P < 0.0001$; slope, $P < 0.0001$) was the Early Disuse group. Therefore, two lesioned groups were compared to Shams in the final HLM: Early Disuse, and all other lesioned groups pooled together (Lesions). The final HLM indicated that the Early Disuse group was different from both the Lesion and Sham groups which were not different from each other (see Fig. 4).

ANOVA revealed no group differences on pre-surgical data for percent use of both limbs simultaneously for landing from a rear ($F(11, 76) = 1.305$, $P = 0.2382$). Among sham-operated groups, when all casted shams were compared to the non-casted shams, HLM found the slopes of two groups, Early Disuse ($P = 0.0005$) and 1 week disuse + 1 week overuse ($P = 0.015$) to be different. All sham-operated groups except those two were combined into a larger group called "Shams." Among lesioned groups, HLM found the 2 weeks disuse + 1 week overuse (slope, $P = 0.03$) and the Early Disuse (intercept, $P = 0.005$; slope, $P = 0.005$) groups to be different from non-casted, lesioned controls. Therefore, all lesioned groups except those two were combined into a larger group called "Lesions." An overall HLM compared Lesions, Early Disuse (lesioned), 2 weeks disuse + 1 week overuse (lesioned), 1 week disuse + 1 week overuse (sham) and Early Disuse (sham) to the pooled sham group. The intercepts and slopes found to be significantly different from the pooled shams are shown in Fig. 5B.

### *3.3.2. Use of the forelimbs for weight support during wall exploration*

Two different scores were calculated: use of both forelimbs simultaneously for weight support against the wall of the cylinder, and %ipsi − %contra forelimb use for independent weight support against the wall. For pre-surgery data, ANOVA revealed no differences among any of the groups on %ipsi − %contra use of the forelimbs for weight support against the wall ($F(11, 75) = 1.737$, $P = 0.0814$). HLM found no differences among sham-operated animals on this measure following sham surgery (omnibus test for group differences, intercept, $P = 0.102$; slope, $P = 0.0473$; for Bonferonni-corrected individual comparisons all $P$s $> 0.05$) and no differences among lesioned groups following injury (omnibus test for group differences, intercept, $P = 0.0449$; for Bonferonni-corrected individual comparisons all $P$s $> 0.05$; slope, $P = 0.0605$). All sham groups were therefore

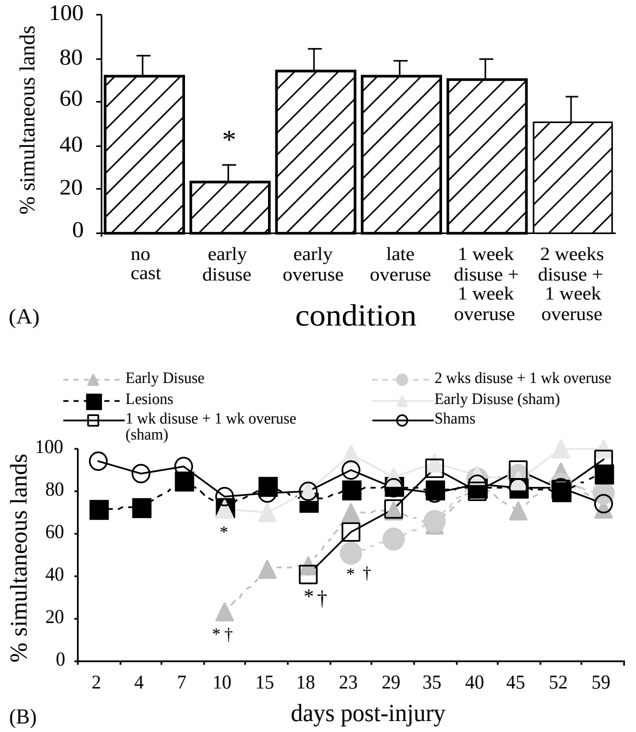

Fig. 5. Simultaneous use of the forelimbs for landing from a rear. The intercept of each lesioned group is depicted in A, where $^*P < 0.05$ significantly different from non-casted lesions. The performance of groups of interest is plotted over time in B. $^*P < 0.05$ *intercept* significantly different from Shams, $^\dagger P < 0.05$ *slope* significantly different from Shams.

combined into one group called "Shams" and all lesioned groups pooled into one large group called "Lesions." HLM revealed a significant difference between Lesions and Shams on the intercept ($P = 0.0001$) but no difference in the slope of the recovery curves.

ANOVA found no differences among any of the groups before surgery on simultaneous forelimb use against the wall ($F(11, 75) = 0.772$, $P = 0.6667$). HLM found no differences among sham groups post-operatively on simultaneous forelimb use against the wall (omnibus comparison of group differences, intercept, $P = 0.0628$; slope, $P = 0.0900$), so all sham groups were pooled into one large group labeled "Shams." Similarly, HLM found no differences among lesioned groups on simultaneous forelimb use against the wall (omnibus test of group differences, intercept, $P = 0.335$; slope, $P = 0.0718$), so lesioned groups were combined into one large group called "Lesions." A final HLM analyzing differences between Lesions and Shams found no significant differences in the recovery curves of these two groups (intercept, $P = 0.504$; slope, $P = 0.515$).

## 4. Discussion

The primary finding of this study was that disuse of the impaired forelimb immediately following injury had profound effects on the neuroanatomical consequences of subsequent overuse of the same limb. Disuse of the impaired forelimb extended the window of vulnerability during which injured tissue is susceptible to the effects of forced overuse. Normally, in this model, tissue remaining in the peri-lesion area can be destroyed by overuse of the impaired forelimb only if that overuse occurs during the first post-injury week. The results of this study indicate that if there is total lack of use of the impaired limb for either 1 or 2 weeks following injury, peri-lesion tissue remains vulnerable to the detrimental effects of overuse. In the past, we have found that both overuse [12–14,21] and disuse [2,21,35] of the impaired forelimb slow recovery of symmetrical limb use after unilateral brain injury.

### 4.1. Explanation of statistical findings

The statistical analysis performed was chosen because the first day of behavioral testing differed for each experimental group, depending on when the cast was removed. Hierarchical linear modeling can account for these differences in the onset of data collection. HLM showed that forelimb placing was profoundly affected by both early disuse and early overuse of the impaired forelimb, and, to a lesser extent, by 1 week of disuse followed by 1 week of overuse. This is illustrated by Fig. 3, which shows the forelimb placing performance of each lesioned group on the first day of testing. The non-casted control group shows a steady recovery curve (Fig. 3B), markedly improving by day 10 after injury, and reaching asymptotic performance by day 23. If placing ability was unaffected by the casting manipulations, then the performance of each group on its first testing day (the intercept) should be different from the performance of the non-casted control group on its first testing day. More specifically, the performance of each group on the first testing day should be *better* than that of the control group. Instead, as depicted in Fig. 3B, the intercept of every casted, lesioned group (with the exception of the 2 weeks of disuse + 1 week of overuse group) is not significantly different from the non-casted group's intercept. This indicates that the performance of these groups on the first day that each was tested was essentially the same as the performance of the control group on its first testing day. Therefore, it appears that both overuse and disuse of the impaired forelimb may delay recovery of forelimb placing ability, which is illustrated by Fig. 3B, where the recovery curves of lesioned groups of interest are plotted against the recovery curve of the control group.

Intact rats typically land on both limbs simultaneously, only occasionally using one limb or the other exclusively. This behavior changes following FL-SMC injury, as rats transiently rely on the non-impaired forelimb for landing, before readopting simultaneous use. Thus, for lesioned animals, use of the ipsilateral forelimb exceeds that of the contralateral forelimb for approximately 1 week after injury. Therefore, when percent use of the contralateral limb is subtracted from percent use of the ipsilateral limb (%ipsi − %contra), a positive score is obtained for the first several testing days after surgery. The higher the score, the greater the discrepancy between ipsilateral and contralateral limb use. Fig. 4A shows that the intercept of the Early Disuse group was significantly higher than that of the non-casted control group. Indeed, this was the only group in which this was the case, indicating that early disuse of the impaired forelimb greatly increased the tendency to use the ipsilateral limb when landing from a rear. Interestingly, early *overuse* of the impaired limb, and early disuse followed by overuse, did not significantly affect this score. This may be because the Early Disuse group was the only group in which disuse was the final manipulation. In the Early Overuse group, and the two groups in which disuse preceded overuse, the impaired forelimb was heavily used; in fact, it was the only limb possible to use for landing from a rear. Although all three of these groups showed overuse-dependent exaggeration of injury, it seems that practicing use of the impaired forelimb lessened the discrepancy between use of the ipsilateral and contralateral forelimbs.

Lesioned animals in the Early Disuse group were the only animals that had slowed recovery of simultaneous limb use when landing. As shown in Fig. 5A, this group was the only lesioned group with an intercept significantly different from that of the non-casted control lesions. The 2 weeks disuse + 1 week overuse group had a slope that was significantly different from controls as well, but the intercept was not different. The slope was probably different because the intercept for that group was lower (although not significantly) than that of the control group.

### 4.2. Implications of behavioral findings

One interesting aspect of the behavioral findings is the lack of major limb use deficits in the Early Overuse group. Despite the fact that the volume of remaining tissue of the brains in this group was significantly lower than that of the non-casted, lesioned animals, the behavioral deficits displayed were not as severe as those observed in similar groups of animals in past studies [13,14,21]. There may be several reasons for this. First, the casts placed on the animals in this study were markedly smaller and lighter than those used in prior studies, allowing a fuller range of movement. It seemed possible that allowing animals to use the impaired forelimb for an even wider variety of movements than animals in prior studies would only exaggerate the lesion damage (and thus the limb use deficit) further, but this was not found to be the case. A second possibility is a difference in housing of the animals in the present study, compared to the housing of animals in past studies. The animals in this study were housed with littermates in groups of two or three, while those of prior studies were housed individually. These individual cages were much smaller than those of the group-housed animals (24 cm × 21.5 cm × 20 cm versus 45.5 cm × 24 cm × 18 cm). The rats in the group-housed cages thus had the opportunity to move around more, and it is possible that this increased opportunity for movement constituted a form of self-rehabilitation by allowing the animals to extensively practice use of the impaired forelimb. Throughout this study, group-housed animals were observed to interact with cagemates by play fighting, social grooming and sleeping in close proximity. Interaction with cagemates thus encouraged animals to move, much more so than an animal housed alone would be encouraged to move. Activity may also have been encouraged by the sawdust bedding used in the group-housed cages, which provided the opportunity for digging and burrowing, and indeed, casted animals were occasionally observed engaging in these activities. In contrast, the individual cages had wire mesh bottoms.

There are other implications of the housing difference. In essence, the housing condition used in this study is the same as the Social Control (SC) condition used by many environmental enrichment (EE) studies. SC accounts for the social component of the enriched environment condition, without providing animals with the opportunity to use tunnels, running wheels, climbing ropes and other aspects of an EE. While SC has not been found to cause the extensive neuroanatomical changes induced by EE, it has been found to have a facilitory effect on recovery of function from brain damage. For example, social housing produced an enhancement of recovery from dorsal hippocampal lesions that was intermediate between that produced by EE and that observed in individually housed rats [7]. Furthermore, following medial preoptic area lesions, male rats that were socially housed after injury were able to copulate successfully, while rats that were housed alone showed a much lower copulatory success rate [22]. These findings suggest that social housing can enhance recovery of cognitive and sexual function. It may be that social housing can also enhance recovery of motor function. Indeed, Johansson and Ohlsson [15] found that socially housed rats had a level of recovery intermediate between that of EE and that of rats allowed access to a running wheel. Given the many reasons that SC housing might encourage movement, it is not difficult to imagine that this sort of environment might rehabilitate limb-use deficits or enhance mechanisms of plasticity that might attenuate adverse degenerative events linked to intense rehabilitative pressure. This explanation remains speculative, however, until this experiment has been run using individually housed animals.

It may be surprising that the extensive lesion damage caused by overuse was not complemented by more impressive limb use deficits. However, lesion extent does not always correlate well with functional outcome, in part because the functional tests may be sensitive to damage to specific brain regions while increases in extent of injury may involve brain regions not necessarily tapped by the tests selected. Also, potential adverse effects of rehabilitative procedures may be obscured by beneficial effects. For example, EE has been found to facilitate recovery from ischemic injury, yet it did not reduce infarct size [24]. This suggests that EE is enabling animals to make better use of remaining circuitry, or enabling them to recruit other circuits to support recovery. By encouraging movement, the social housing environment in the present study may be enabling animals to develop new circuitry in remaining tissue, thereby preventing their behavioral performance from slipping below that of non-casted, lesioned animals, despite the more extensive tissue loss. Risedal and colleagues [26] found that EE housed animals forced to engage in extensive motor rehabilitation during the first week after stroke were not significantly more functionally impaired despite a doubling of infarct size, but were improved in motor tests if the motor rehabilitation was delayed a week. It is possible that the expanded infarct size did not extend into task-targeted brain areas sufficiently to allow detection of exaggerated deficits, that the tests were not sensitive enough to increases in injury severity, and/or that use-dependent events in undamaged regions essentially canceled the degenerative events. Indeed, although in general less severe brain injury should be detectable, outcome studies have struggled to disentangle delayed degenerative events associated with brain injury from plasticity related events [3,28,30]. The goal of rehabilitation, of course, is to maximize chronic outcome. If, in a clinical setting, the beneficial effects of early intense rehabilitation after cortical motor area injury can sometimes mask detrimental effects on brain tissue, it may be that brain imaging methods must be used as an adjunct to evaluating outcome [4,25].

With some brain injuries it may be that an early rehabilitation regimen is optimal. Intense overuse of the forelimb contralateral to the lesioned hemisphere can be beneficial following 6-OHDA a lesion [36]. With focal cortical injury it is possible that early rehabilitation is beneficial when graded in intensity in order to maximize chronic outcome in a wide array of functions. Mild acrobatic training instituted in a graded fashion in cortically injured rats, beginning 2 days after injury, can enhance functional recovery [16]. The current study, in combination with others, however, indicates that complete disuse of the affected limb following central nervous system injury is not advisable. Forced disuse of the impaired limb has been found to disrupt behavioral recovery following cerebral ischemia [2] and unilateral 6-OHDA injection [35]. Disuse following ischemia does not increase anatomical damage, consistent with the results of this study, although it does increase the loss of striatal dopamine after a mild 6-OHDA lesion. In summary, it seems that careful behavioral training early after injury may be conducive to functional recovery, while complete rest of the affected limb may lead to long-term behavioral deficits.

The results of this study indicate that early disuse of the impaired forelimb has considerable consequences when the same limb is subsequently overused. These data may have implications for motor rehabilitative studies in human patients. That is, if there is a window of time soon after brain injury during which intense motor experience may be detrimental to functional recovery and tissue integrity, it is possible that avoiding motor rehabilitation altogether during this period may extend the window of vulnerability to later periods that are otherwise safe from extreme behavioral demand (see references [20,28,31] for a review of post-operative treatments that affect recovery depending on the timing of their administration). Whatever effect resting the impaired forelimb is having on intact tissue, it has marked consequences for the integrity of that tissue [29]. The results of the present experiment suggest that restricting use of the impaired forelimb may disrupt functional recovery of that forelimb (without exacerbating neural injury), and may render surviving brain tissue vulnerable to subsequent intense rehabilitative measures.

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## References

[1] Barth TM, Grant ML, Schallert T. Effects of MK-801 on recovery from sensorimotor cortex lesions. Stroke 1990;21:III153–7.
[2] Bland ST, Pillai RN, Aronowski J, Grotta JC, Schallert T. Early overuse and disuse of the affected forelimb after moderately severe intraluminal suture occlusion of the middle cerebral artery in rats. Behav Brain Res 2001;126:33–41.
[3] Corbett D, Nurse S. The problem of assessing effective neuroprotection in experimental cerebral ischemia. Prog Neurobiol 1998;54:531–48.
[4] Crafton KR, Mark AN, Cramer SC. Improved understanding of cortical injury by incorporating measures of functional anatomy. Brain 2003;126:1650–9.
[5] Dolbakyan E, Hernandez-Mesa N, Bures J. Skilled forelimb movements and unit activity in motor cortex and caudate nucleus in rats. Neuroscience 1977;2:73–80.
[6] Donoghue JP, Wise SP. The motor cortex of the rat: cytoarchitecture and microstimulation mapping. J Comp Neurol 1982;212:76–88.
[7] Einon DF, Morgan MJ, Will BE. Effects of post-operative environment on recovery from dorsal hippocampal lesions in young rats: tests of spatial memory and motor transfer. Quart J Exp Psychol 1980;137–48.
[8] Finger S, Hart T, Jones E. Recovery time and sensorimotor cortex lesion effects. Physiol Behav 1982;29:73–8.
[9] Goldstein LB. Rapid, reliable measurement of lesion parameters for studies of motor recovery after sensorimotor cortex injury in the rat. J Neurosci Methods 1993;48:35–42.
[10] Goldstein LB. Right versus left sensorimotor cortex suction-ablation in the rat: no differences in beam-walking recovery. Brain Res 1995;674:167–70.
[11] Goldstein LB, Coviello A. Post-lesion administration of the NMDA receptor antagonist MK-801 does not impair motor recovery after unilateral sensorimotor cortex injury in the rat. Brain Res 1992;580:129–36.

[12] Gotts JE, Press C, Leasure JL, Schallert T. Focal brain injury FGF-2 and the adverse effects of excessive motor demand on cortical and nigral degeneration: marked protection by delayed intermittent exposure to halothane. J Neurotrauma 2000;17:1067–77.

[13] Humm JL, Kozlowski DA, Bland ST, James DC, Schallert T. Use-dependent exaggeration of brain injury: is glutamate involved? Exp Neurol 1999;157:349–58.

[14] Humm JL, Kozlowski DA, James DC, Gotts JE, Schallert T. Use-dependent exacerbation of brain damage occurs during an early post-lesion vulnerable period. Brain Res 1998;783:286–92.

[15] Johansson BB, Ohlsson AL. Environment, social interaction, and physical activity as determinants of functional outcome after cerebral infarction in the rat. Exp Neurol 1996;139:322–7.

[16] Jones TA, Chu CJ, Grande LA, Gregory AD. Motor skills training enhances lesion-induced structural plasticity in the motor cortex of adult rats. J Neurosci 1999;19:10153–63.

[17] Jones TA, Schallert T. Overgrowth and pruning of dendrites in adult rats recovering from neocortical damage. Brain Res 1992;581:156–60.

[18] Jones TA, Schallert T. Use-dependent growth of pyramidal neurons after neocortical damage. J Neurosci 1994;14:2140–52.

[19] Kawamata T, Dietrich WD, Schallert T, Gotts JE, Cocke RR, Benowitz LI, et al. Intracisternal basic fibroblast growth factor enhances functional recovery and up-regulates the expression of a molecular marker of neuronal sprouting following focal cerebral infarction. Proc Natl Acad Sci USA 1997;94:8179–84.

[20] Kleim JA, Jones TA, Schallert T. Motor enrichment and the induction of plasticity after brain injury. Neurochem Res 2003;28(11):1757–69.

[21] Kozlowski DA, James DC, Schallert T. Use-dependent exaggeration of neuronal injury after unilateral sensorimotor cortex lesions. J Neurosci 1996;16:4776–86.

[22] Meisel RL. Effects of postweaning rearing condition on recovery of copulatory behavior from lesions of the medial preoptic area in rats. Dev Psychobiol 1982;15:331–8.

[23] Napieralski JA, Banks RJ, Chesselet MF. Motor and somatosensory deficits following uni- and bilateral lesions of the cortex induced by aspiration or thermocoagulation in the adult rat. Exp Neurol 1998;154:80–8.

[24] Ohlsson AL, Johansson BB. Environment influences functional outcome of cerebral infarction in rats. Stroke 1995;26:644–9.

[25] Pineiro R, Pendlebury ST, Smith S, Flitney D, Blamire AM, Styles P, et al. Relating MRI changes to motor deficit after ischemic stroke by segmentation of functional motor pathways. Stroke 2000;31:672–9.

[26] Risedal A, Zeng J, Johansson BB. Early training may exacerbate brain damage after focal brain ischemia in the rat. J Cereb Blood Flow Metab 1999;19:997–1003.

[27] Royet JP. Stereology: a method for analyzing images. Prog Neurobiol 1991;37:433–74.

[28] Schallert T, Fleming SM, Leasure JL, Tillerson JL, Bland ST. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology 2000;39:777–87.

[29] Schallert T, Humm, JL, Bland ST, Jones TA, Kolb B, Aronowski J, Grotta JC. Activity-associated growth factor expression and related neuronal events in recovery of function after brain injury. In: Choi D, Dacey RG, Hsu CY, Powers WJ, editors. Cerebrovascular disease: momentum at the end of the second millennium. Armonk, NY: Futura Publishing Co. Inc.; 2001 [chapter 29].

[30] Schallert T, Woodlee MT, Fleming SM. Disentangling multiple types of recovery from brain injury. In: Krieglstein J, Klumpp S, editors. Pharmacology of cerebral ischemia 2002. Stuttgart: Medpharm Scientific Publishers. p. 201–16.

[31] Schallert T, Fleming SM, Woodlee MT. Should the injured and intact hemispheres be treated differently during the early phases of physical restorative therapy in experimental stroke or parkinsonism? Phys Med Rehab Clin 2003;14(1):1–20.

[32] Schmanke T, Barth TM. Amphetamine and task-specific practice augment recovery of vibrissae-evoked forelimb placing after unilateral sensorimotor cortical injury in the rat. J Neurotrauma 1997;14:459–68.

[33] Soblosky JS, Matthews MA, Davidson JF, Tabor SL, Carey ME. Traumatic brain injury of the forelimb and hindlimb sensorimotor areas in the rat: physiological, histological and behavioral correlates. Behav Brain Res 1996;79:79–92.

[34] Stashkevich ISB J. Correlation analysis of neuronal interaction in the motor cortex of rats during performance of a discrete instrumental reaction. Int J Neurosci 1981;12:1–6.

[35] Tillerson JL, Cohen AD, Caudle WM, Zigmond MJ, Schallert T, Miller GW. Forced nonuse in unilateral Parkinsonian rats exacerbates injury. J Neurosci 2002;22:6790–9.

[36] Tillerson JL, Cohen AD, Philhower J, Miller GW, Zigmond MJ, Schallert T. Forced limb-use effects on the behavioral and neurochemical effects of 6-hydroxydopamine. J Neurosci 2001;21:4427–35.

[37] Uylings HBM, VanEden CG, Hoffman MA. Morphometry of size/volume variables and comparison of their bivariate relations in the nervous system under different conditions. J Neurosci Methods 1986;18:19–37.