

Use-Dependent Exaggeration of Neuronal Injury after Unilateral Sensorimotor Cortex Lesions

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Unilateral injury to the forelimb representation area of the sensorimotor cortex (FL-SMC) in adult rats causes over-reliance on the unimpaired forelimb for postural-motor movements, as well as overgrowth of layer V pyramidal cell dendrites in the homotopic cortex of the noninjured hemisphere. The overgrowth appears to be use-dependent because it can be prevented by restricting movements of the unimpaired forelimb. Additionally, restricting the unimpaired forelimb in animals with FL-SMC damage results in significantly greater behavioral dysfunction when examined 2 d after cast removal (compared to that after impaired-limb immobilization, or no limb immobilization). In the present study, the long-term behavioral and anatomical effects of limb immobilization were examined. Animals with FL-SMC lesions were fitted with casts immediately after the lesion that immobilized the impaired forelimb, the unimpaired forelimb, or neither forelimb for 15 d. Immobilization of the nonimpaired forelimb resulted in chronic prevention of dendritic growth and

severe and chronic behavioral deficits. In addition, immobilization of the nonimpaired forelimb resulted in a dramatic exaggeration of the neuronal injury, presumably attributable to forced overuse of the impaired limb. Immobilization of the impaired forelimb resulted in no detectable neural changes and in only slightly increased and longer-lasting behavioral asymmetries (compared to nonimmobilized, lesioned animals), presumably attributable to mild disuse of the impaired limb. Immobilization of a single forelimb in nonlesioned rats resulted in no significant behavioral or anatomical changes. Together, these results suggest that although behavioral experience can enhance neural growth after brain injury, the region surrounding the injury may be vulnerable to behavioral pressure during the early postlesion period.

Key words: recovery of function; stereology; use-dependent; sensorimotor cortex; forelimb immobilization; neuronal degeneration

Unilateral injury to the forelimb-representation area of the sensorimotor cortex (FL-SMC) in adult rats leads to biphasic neuro-anatomical events in the intact, contralateral, homotopic cortex: use-dependent increase in the number of dendritic branches observed in layer V pyramidal cells (which may reflect dendritic growth), followed by glutamate-dependent reduction of the dendritic branches (which may reflect dendritic pruning) (Jones and Schallert, 1992, 1994; Kozlowski et al., 1994; Schallert and Jones, 1994). Dendritic enhancement is maximal in tissue examined 18 d after surgery, and dendritic reduction occurs thereafter. The enhancement of dendritic processes has been linked to a lesion-induced hyper-reliance on the unimpaired forelimb (i.e., the forelimb opposite the neuronal morphological changes; Jones and Schallert, 1994). When one-sleeved casts were used to prevent animals with an FL-SMC lesion from using their unimpaired forelimb during the period of expected overgrowth, lesion-induced dendritic enhancement was not present when examined 2 d after cast removal. This manipulation also interfered with performance on experience-dependent tests of postural-motor function also examined 2 d after cast removal (Jones and Schallert, 1994). Forcing nonlesioned (sham-operated) animals to use only one limb did not detectably alter dendritic morphology and

did not cause behavioral impairments. Thus, dendritic growth may be associated with an interaction between brain injury and learning-dependent use of the intact forelimb in compensating for lesion-induced deficits in the impaired forelimb.

The present study examined the long-term anatomical and behavioral consequences of immobilizing the unimpaired forelimb (i.e., forcing use of the impaired forelimb). The unimpaired forelimb in lesioned rats was immobilized with a one-sleeved cast during the period of dendritic enhancement (days 1–15 postlesion). After the casts were removed, behavioral events and neural morphology were examined at different time points. The initial aim of the study was to address whether there was an optimal period for lesion-induced changes in the contralateral homotopic cortex and for behavioral recovery. Specifically, would the enhancement of dendrites resume after removal of the cast, following the same time course and achieving the same level observed immediately after a unilateral lesion; and would animals that were prevented from using their unimpaired limb (forced to use only their impaired limb) during the first 2 weeks postlesion regain symmetrical forelimb use and recover from deficits in postural-motor and sensorimotor tasks? Finally, the long-term effects of limb immobilization on the lesioned area were examined using stereological analysis for the first time.

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MATERIALS AND METHODS

Animals

One-hundred and eighteen male, hooded Long-Evans rats weighing between 400 and 600 gm were used. Rats were individually housed in clear Plexiglas cages with wire bottoms and maintained on a 12 hr light/dark cycle. Food and water were available *ad libitum*. Animals were

tamed by gentle handling for at least 5 min/d for 3 weeks before surgery and behavioral testing.

Surgical procedures

Before being placed in a stereotaxic apparatus, the animals were anesthetized with Equithesin (25 mg/kg pentobarbital and 150 mg/kg chloral hydrate, 0.35 cc/100 gm, i.p.) followed by atropine sulfate (0.1 mg/kg, i.p.) to facilitate respiration. A unilateral lesion of the FL-SMC (Hall and Lindholm, 1974; Neafsey et al., 1986; Wise and Donoghue, 1986) was performed ($n = 67$) by removing a piece of the skull between 3.0 and 4.5 mm lateral to midline, -0.5 mm posterior, and $+1.5$ mm anterior to bregma to pass a 1 mA current through a platinum electrode lowered 1.7 mm below dura. The current was delivered during equally spaced traverses through the exposed cortex for a total of 2 min. Sham-operated animals ($n = 51$) received all standard stereotaxic surgical procedures up to, but not including, removal of the skull. After surgery, all animals were placed in incubators to minimize hypothermia.

Forelimb immobilization (casting) procedures and treatment groups

Animals were randomly assigned to one of four treatment groups: (1) Lesion + Ipsilateral (to the lesion) Cast (Lesion+Ipsi), (2) Lesion + No Cast (Lesion+No), (3) Sham + Cast (Sham+Cast), and (4) Sham + No Cast (Sham+No). They were also randomly assigned to be killed at one of three time points: on day 18 (Lesion+Ipsi, $n = 10$; Lesion+No, $n = 9$; Sham+Cast, $n = 9$; Sham+No, $n = 8$), day 33 (Lesion+Ipsi, $n = 11$; Lesion+No, $n = 9$; Sham+Cast, $n = 9$; Sham+No, $n = 8$), or day 60 (Lesion+Ipsi, $n = 10$; Lesion+No, $n = 8$; Sham+Cast, $n = 9$; Sham+No, $n = 8$) postlesion. These time points were chosen to correspond to the time course of dendritic enhancement seen immediately after a lesion: (1) day 18 was the time at which maximum dendritic enhancement was seen in noncasted lesioned animals previously demonstrated in Jones and Schallert (1992, 1994); (2) day 33 was 18 d after cast removal, which was the number of days sufficient, immediately after a lesion, for dendritic enhancement to occur; and (3) day 60 was 45 d after cast removal, the number of days necessary immediately after a lesion for dendritic pruning to occur). An additional group of rats was assigned to a Lesion + Contra (to the lesion) cast (Lesion+Contra, $n = 10$) and killed on day 60. This group was added to examine the long-term behavioral consequences of immobilizing the impaired limb. Dendritic morphology was not examined in these animals because in previous work it was shown that this procedure did not impair dendritic growth in the intact hemisphere (Jones and Schallert, 1994).

Immediately after surgery, animals in the "cast" groups were fitted with casts composed of plaster of paris designed to immobilize the designated limb. The designated forelimb was placed in a naturally retracted position against the sternum, and strips of casting material were formed around the limb and upper torso until they resembled one-sleeved jackets (Jones and Schallert, 1994). A small, loose stitch was placed connecting the back of the cast to the back skin of the rat to ensure placement and discourage removal of the cast. The casts were removed at day 15 postsurgery. During the 15 d cast period, the animals were groomed daily with cotton swabs and brushed to maintain a healthy coat.

Histological procedures

On day 18, 33, or 60, postsurgery animals were given a lethal dose of sodium pentobarbital anesthesia (1.0 ml) and perfused intracardially with saline followed by a 10% formalin solution. The brains were extracted, quartered, and placed in Golgi-Cox solution for 3 weeks. Following reaction and dehydration procedures adapted from Ramon-Moliner (1970), each cerebral hemisphere was embedded separately in epoxy resin and sliced on a microtome into 240 μ m sections separated by 150 μ m sections and mounted on slides. The slides were encoded by an independent researcher before quantification to minimize experimenter bias.

Analysis of the intact hemisphere

Dendritic quantification. Slices between 2.0 mm anterior to bregma and 1.0 mm posterior to bregma were chosen for quantification based on previous examination of the placement of the forelimb area in Nissl-stained sections (Jones and Schallert, 1989). This area encompasses approximately three to four slices. Pyramidal neurons from cortical layer V from the region between the dorsal peak of the corpus callosum and 4.0 mm lateral to midline were chosen from each of these slices based on absence from obstruction by astrocytes, capillaries, or neural processes of

neighboring pyramidal and drawn using a camera lucida at 475 \times magnification. The number of basilar dendritic branches was counted for 10 neurons per brain.

Analysis of the lesioned hemisphere

Volume analysis. To analyze differences in lesion size, the volume of the lesioned hemisphere and the hemisphere ipsilateral to the designated "sham" side was estimated using the Cavalieri principle. This has been found to be an unbiased and highly efficient method of analyzing the volume of regularly shaped objects (for review, see Uylings et al., 1986; Royet, 1991). Unbiased estimates of area were made using systematic point counting from coronal sections between approximately $+2.7$ and -1.3 mm (inclusively) relative to bregma. This area encompassed the forelimb motor representation area and surrounding cortex. A grid of points 2.0 cm apart was placed in a random position over 20 \times projections of these coronal sections in the hemisphere of interest. Volume estimates were computed using the formula $V = T \times (ap)^2 \times \Sigma P$, where V is the estimated volume, T is the distance between the face of section " n " and the face of section " $n + 1$ " (390 μ m), ap is the area per test point, adjusted for magnification (20 cm/20 \times magnification)², and ΣP is the total number of points found to touch brain tissue throughout all sections (Gundersen and Jensen, 1987; Korbo et al., 1990). The coefficient of error (SD/mean) (Gundersen and Jensen, 1987; West and Gundersen, 1990) was <0.06 for each estimate. Animals from the following groups were excluded from this analysis because of gouges or tears in the tissue or poor sections: Lesion+Contra ($n = 2$); Lesion+Ipsi ($n = 1$); Lesion+No ($n = 1$); Sham ($n = 5$).

Behavioral testing and limb use observations

A battery of behavioral tests was administered before surgery, 6–8 hr after removal of the cast at day 15, and then on postlesion days 17, 24, 31, 38, 45, 52, and 59. Additionally, limb-use observations were performed presurgery and on postlesion days 2, 4, 7, 10, 15 (6–8 hr after cast removal), 17, 24, 31, 38, 45, 52, and 59. The following tests have been shown to be sensitive to unilateral FL-SMC lesions and have been associated with experience-dependent anatomical changes such as dendritic growth (Jones and Schallert, 1992, 1994; Schallert and Jones, 1994; Jones et al., 1995, 1996).

Forelimb placing

Asymmetries in forelimb-placing behaviors were assessed using the Forelimb Placing Test. Animals were held by their torso with their forelimbs hanging freely. Each forelimb was tested independently by orienting one side of the animal toward a countertop and moving the animal slowly and laterally toward the edge of the countertop until the vibrissae of that side made contact with the edge. Intact rats typically place the forelimb (both ipsilateral and contralateral forelimbs) quickly onto the edge of the counter. Lesioned animals, on the other hand, typically place the limb ipsilateral to the lesion but show difficulties with the contralateral limb. Ten trials of each forelimb were performed in a balanced order. Placing asymmetries were recorded as the percent unsuccessful contralateral placements.

Limb use observations

Asymmetry in forelimb use was observed by videotaping the rat in a three-sided Plexiglas platform (53 \times 16 \times 26.5 cm³) for 4 min. Observations were made of the forelimb used for support during the following behaviors: during push off from the floor before rearing, during support behaviors against the walls of the platform both in the vertical and horizontal direction, and during landing after a rear. Jones and Schallert (1992, 1994) have found that these behaviors are easily quantified, frequently observed in both nonlesioned and lesioned rats, observed in both the home cage and on the platform with equal frequency, and appear to represent important and typical motor behaviors of rats. Limb use is presented as percent ipsilateral forelimb use.

Experience-independent test of somatosensory asymmetry

The bilateral tactile stimulation test and the somatosensory neutralization tests (Schallert et al., 1983; Schallert and Whishaw, 1984) were used to measure forelimb somatosensory asymmetries.

The animals were removed from their home cages, and small adhesive backed patches (113.1 mm²) were applied to the radial aspect of the wrist of both forelimbs. The rats were returned to their home cages, and the order and latency in which the animal contacted and removed the stimuli

were recorded. Four to five trials were conducted. Each trial was terminated after removal of both stimuli or after 2 min had passed. If the animal contacted and removed stimuli from only one side in 80–100% of the trials, a second phase of the test, somatosensory neutralization, was administered to assess the magnitude of the asymmetry. Preoperatively, if an animal showed a preference for stimuli placed on a given forelimb, the lesion was placed in the opposite hemisphere so that the postoperative effects were not masked by any endogenous asymmetry. Animals without a preoperative preference were randomly assigned to a lesion side.

In the somatosensory neutralization test, the stimulus on the limb ipsilateral to the response bias was decreased by 14.1 mm² while the stimuli contralateral to the response bias was increased by 14.1 mm². The ratio of the ipsilateral to the contralateral stimulus necessary to reverse or neutralize the response bias (e.g., the animals contact the contralateral and not the ipsilateral stimulus first) was recorded as the magnitude of asymmetry.

Unlike limb use and forelimb placing, the standard bilateral-tactile stimulation test is not influenced by experience. In a study by Schallert and Whishaw (1984), one group of lesioned animals was tested daily for 2 weeks, which provided an opportunity to practice the particular task; another lesioned group was not tested until the end of the 2 week period. Both groups received the same score (magnitude of asymmetry), suggesting that practice does not affect performance on this test. In another study, Rose et al. (1987) showed that this test, which they termed a test of “true recovery,” was not affected by postlesion enrichment. Additionally, studies have shown that this test does not correlate well with experience-induced alterations in neural morphology (Jones and Schallert, 1992, 1994).

Statistical analyses

Data analysis was accomplished by SAS procedures for general linear models with unequal cell sizes. Dendritic branches and volume data were analyzed by a two-factor ANOVA for Condition × Day of Killing treating neurons as nested variables within subjects for the dendritic analysis. Additionally, one-way ANOVAs for Group were performed at each time point. Post hoc analyses were conducted using SAS univariate contrasts for simple effects of Group and for simple effects of Group at different days postlesion.

Statistical analyses of behavioral data were conducted using SAS procedures for general linear models with unequal cell sizes. Behavioral data were analyzed at each separate time point with a repeated-measure, two-factor ANOVA for Group (i.e., treatment condition: Lesion+Ipsi, Lesion+No, Sham+Cast, Sham+No) × Day of behavioral observation (repeated measure). For each of the behavioral tests, performance on days 17 and 31 was analyzed individually for each group with a one-way ANOVA for Time Course (day 18, 33, or 60) to determine whether there were differences in behavior based on the time course group to which they were assigned. No significant differences of time course were found. Therefore, to best illustrate the behavioral recovery process, behavioral data from only the day 60 time course group are presented. Post hoc analyses were conducted using SAS univariate contrasts for simple effects of group at different test days.

RESULTS

Histological analysis of the intact hemisphere

Dendritic growth

The number of basilar dendritic branches in Golgi-stained neurons examined at day 18, 33, or 60 postlesion is presented in Figure 1. There have been concerns regarding the use of the Golgi stain to measure dendritic growth, specifically that it does not fully fill the dendritic tree, thus providing a biased measure. However, this method has consistently differentiated between lesioned and nonlesioned animals in numerous studies conducted in our laboratory and elsewhere (Kolb, 1995). In addition, dendritic growth and morphological changes in dendrites have been analyzed further in lesioned versus nonlesioned animals using electron microscopy (Jones et al., 1995, 1996). This study supported the idea that a unilateral lesion to the FL-SMC results in time-dependent dendritic growth in layer V of the contralateral homotopic cortex as predicted by the Golgi stain. By day 18 postinjury, a significant increase was seen in the volume of dendritic processes per neuron

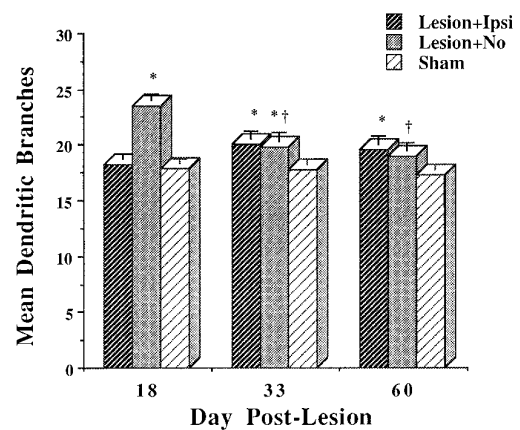


Figure 1. Mean dendritic branches in layer V pyramidal neurons in the intact, contralateral, homotopic cortex. At day 18, Lesion+No cast (*Lesion+No*) animals show an increase in dendritic growth compared with shams. This enhancement of dendritic morphology is blocked by immobilization of the ipsilateral forelimb. When the cast is removed and the animals are killed 18 and 45 d after cast removal (days 33 and 60 postlesion, respectively), there is a slight, but not significant, increase in dendritic growth. Additionally, the dendritic growth does not reach Lesion+No cast levels seen at day 18. Lesion+No cast animals show the previously demonstrated increase in dendritic growth followed by a decrease or pruning to near sham levels. * $p < 0.001$, significantly different from Sham; † $p < 0.05$, significantly different from Lesion+No group at day 18.

and in the surface area of dendritic membrane in lesioned animals in comparison to shams, thus suggesting dendritic growth. This was followed by an increase in synapse number per neuron.

In the current study, statistical analysis at all three time points indicated that there were no significant differences between Sham groups (i.e., forcing a nonlesioned animal to preferentially use one forelimb did not result in a change in dendritic growth). Therefore, both Sham+No and Sham+Cast groups were pooled and labeled “Sham” (day 18: $F_{(1,15)} = 3.18$, $p = 0.09$; day 33: $F_{(1,15)} = 0.28$, $p = 0.60$; day 60: $F_{(1,15)} = 0.00$, $p = 0.99$).

At day 18, 3 d after cast removal, one-way ANOVA revealed a significant effect of Group ($F_{(2,32)} = 39.60$, $p < 0.0001$). Significant enhancement of basilar dendritic branches was seen in Lesion+No animals (compared to sham, $p < 0.0001$) as in Jones and Schallert (1992). In addition, immobilization of the forelimb contralateral to the area of dendritic growth prevented the dendritic enhancement (i.e., dendritic levels were no different than those of sham animals; Jones and Schallert, 1994).

At day 33 (18 d after cast removal), of interest was whether the Lesion+Ipsi animals showed any increase in dendritic growth and, if so, whether it was similar to the levels seen in Lesion+No animals at day 18. One-way ANOVA revealed a significant effect of Group ($F_{(2,29)} = 3.58$, $p < 0.04$). Dendritic pruning began to occur in the Lesion+No group, but the number of branches was increased relative to that of sham animals ($p < 0.04$). The Lesion+Ipsi group showed a slight increase in dendritic arborization that was significantly greater than that of shams ($p < 0.02$), but not greater than Lesion+No; however, the increase did not reach the extent seen in Lesion+No animals at day 18 (even though the animals had 18 d after cast removal to permit growth, which was the number of days necessary for maximum dendritic enhancement in lesioned, noncasted animals).

By day 60, the dendrites in the Lesion+No group “pruned back” nearly to that of sham levels, but the dendrites in the

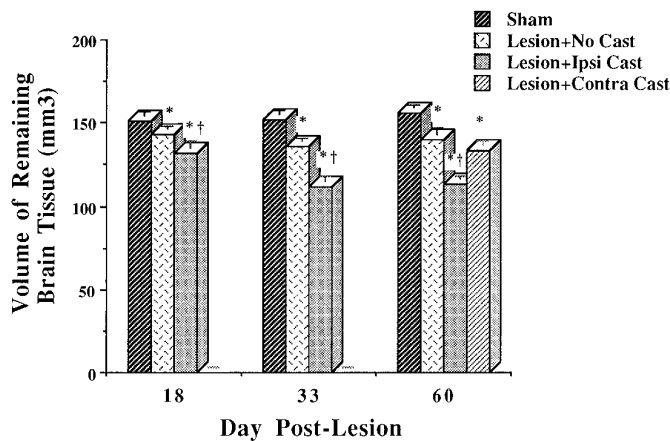


Figure 2. Volume of remaining brain tissue (in mm³). Immobilization of the ipsilateral forelimb (i.e., overuse of the impaired limb; *Lesion+Ipsi Cast*) resulted in a significantly larger lesion (less remaining brain tissue) compared with *Lesion+No Cast*, *Lesion+Contra Cast*, and *Sham* groups. Immobilization of the contralateral forelimb resulted in no significant differences in lesion volume compared with *Lesion+No* animals. * $p < 0.0001$, significantly different from Sham and *Lesion+No* groups; * $p < 0.0001$, significantly different from Sham.

Lesion+Ipsi group remained at the level seen at day 33, which was significantly different from sham ($p < 0.005$).

Group \times Day analysis revealed no significant effect of Day for the *Lesion+Ipsi* and the *Sham* groups ($p = 0.16$ and $p = 0.61$, respectively), suggesting that no significant changes in dendritic growth occurred over the three time periods. Therefore, immobilization of the nonimpaired forelimb prevented dendritic growth and dendritic arborization did not change over time. The failure to find a change in dendritic branching over time in the *Sham* group suggests that the changes in dendritic arborization over time in lesioned animals cannot be attributed to an age effect. In the *Lesion+No* group, there was a significant effect of Day ($F_{(2,22)} = 10.52$, $p < 0.006$). Noncasted, lesioned animals showed an enhancement of dendritic growth followed by a “pruning” of dendrites, as demonstrated previously in Jones and Schallert (1992).

Histological analysis of the lesioned hemisphere

Lesion volume

Brain volume measures did not differ between casted and noncasted sham-operated animals at day 18 ($F_{(1,14)} = 0.14$, $p = 0.71$), day 33 ($F_{(1,12)} = 0.73$, $p = 0.41$), and day 60 ($F_{(1,14)} = 0.19$, $p = 0.67$). Therefore, *Sham+Cast* and *Sham+No* groups were pooled and labeled “*Sham*.”

One-way ANOVA revealed significant effects of Group at day 18 ($F_{(2,32)} = 19.71$, $p < 0.0001$), day 33 ($F_{(2,29)} = 96.27$, $p < 0.0001$), and day 60 ($F_{(3,38)} = 83.43$, $p < 0.0001$) postlesion. As Figure 2 illustrates, there was a significant difference between all lesioned animals and sham animals at all three time points ($p < 0.01$). This indicated that there was significantly less brain tissue remaining in lesioned animals than in sham animals. In addition, there was a significant difference between *Lesion+Ipsi* and *Lesion+No* animals at day 18 ($F_{(1,32)} = 9.38$, $p < 0.004$), day 33 ($F_{(1,29)} = 52.39$, $p < 0.0001$), and day 60 ($F_{(1,38)} = 76.02$, $p < 0.0001$). Lesioned animals that had their nonimpaired limb immobilized (i.e., were forced to use their impaired limb) had significantly less brain volume than lesioned animals with no limb immobilization (i.e., the size of their lesion increased). However,

there was no difference in lesion size between lesioned animals that had their impaired forelimb immobilized (*Lesion+Contra*) and uncasted lesioned animals ($F_{(1,38)} = 3.16$, $p = 0.12$) at day 60. In summary, immobilization of the ipsilateral limb in lesioned animals, which may be associated with forced use of the impaired limb, resulted in a significantly larger lesion than in lesioned animals with immobilization of the contralateral limb, lesioned animals with no limb immobilization, and sham animals (with or without limb immobilization). The enhanced lesion size associated with casting the nonimpaired forelimb, which forces overuse of the impaired limb, may reflect a novel experimental finding: “use-dependent” exaggeration of neuronal injury.

The apparent size of the lesion in *Lesion+Ipsi* animals progressively increased over time ($p < 0.05$) (See Fig. 3). At day 18, the difference was not obvious on inspection, via qualitative comparisons using a microprojector (see also Jones and Schallert, 1994). Nonetheless, quantitative, stereological measurements resulted in a significantly larger lesion in *Lesion+Ipsi* animals than in *Lesion+No* animals at day 18. By day 33, quantitative (stereological) and qualitative (visual) inspection of the size of the lesion in *Lesion+Ipsi* animals showed a much larger lesion, expanding laterally, posteriorly, and anteriorly into the surrounding cortex ($p < 0.05$). Also, there occurred an expansion of the ventricle, loss of the corpus callosum, and loss of tissue in the dorso-medial striatum. On day 60, volume estimates indicate that the size of the lesion in *Lesion+Ipsi* animals was identical to the size at day 33. Nonetheless, there seem to be qualitative differences. At day 60, the borders of the enlarged lesioned area seemed to become more regular, looking as if tissue was “scooped out” of the brain.

Because this tissue was stained using Golgi procedures, a consecutive study was performed in which *Lesion+Ipsi* ($n = 5$), *Lesion+No* ($n = 5$), and *Sham* ($n = 4$) groups were killed at day 33, and their brains were sectioned using a vibratome (100 μm sections, separated by 300 μm) and stained with cresyl violet (a Nissl stain). Stereological analysis of this tissue showed that the effect was replicated (see Table 1). Additionally, this stain allowed for stereological analysis to be performed on the contralateral versus ipsilateral striatum, the contralateral versus ipsilateral total volume of the VPL, VPM, VLN, and VMN of the thalamus (which sends and receives projections to the SMC), and the contralateral versus ipsilateral sensorimotor cortex. At day 33, in lesioned animals, there was tissue loss in the lesioned cortex, the thalamus ipsilateral to the lesion, and the posterior portion of the striatum ipsilateral to the lesion (bregma -0.3 to -2.3). In the *Lesion+Ipsi* group, the use-dependent degeneration was restricted to the cortical areas at this time point. No differences were seen in the ipsilateral thalamic or striatal volumes between the *Lesion+No* and the *Lesion+Ipsi* group even though, on visual inspection, there seemed to be a decrease in size in the striatum. The lack of difference between the *Lesion+Ipsi* and the *Lesion+No* groups in the measures of the striatum may be attributable to the observation, in Golgi-stained tissue, of an extensive proliferation of astrocytes in the *Lesion+Ipsi* animals limited only to the dorsal regions of the striatum, an area that receives direct projections from the somatosensory cortex (Kolb and Tees, 1990). This proliferation was seen at days 18, 33, and 60. Proliferation of astrocytes is common around injured tissue; therefore, there may be neural degeneration occurring in the striatum that is not detectable by the stains used in this study. Further studies using stains specific to astrocyte proliferation (like GFAP) or neuronal degeneration (like cupric silver) would be beneficial to examine the proliferation of astrocytes in the striatum and the role that it

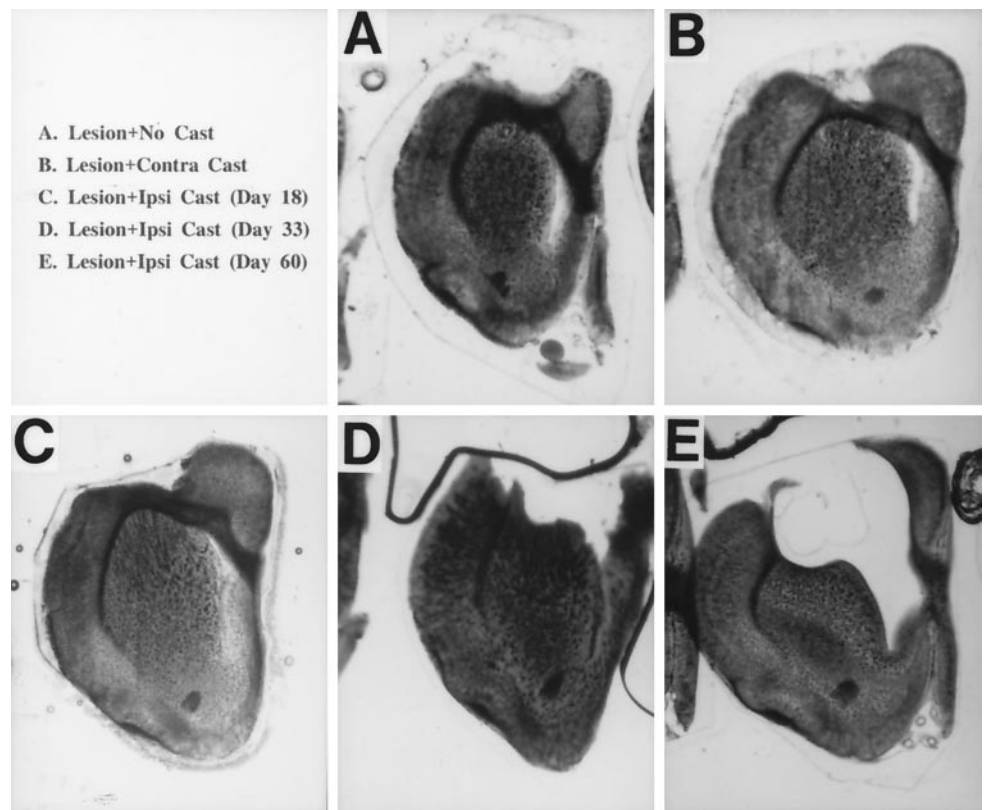


Figure 3. Representative photographs of the Golgi-stained lesioned hemisphere in the Lesion+Ipsi (*C–E*), Lesion+Contra (*B*), and Lesion+No (*A*) groups at different time points after injury.

plays. Nonetheless, preliminary microscopic examination of the Nissl-stained striatum showed less staining of neurons in the Lesion+Ipsi group.

Long-term behavioral effects of limb immobilization

Cast-weight analysis

Casts of lesioned and sham animals were weighed and analyzed after removal to eliminate the possibility that behavioral differences might be attributable to variability in the weight of casts. No significant differences were found in cast weight between groups at day 18, 33, or 60 postlesion ($F_{(2,26)} = 2.37, p = 0.11$).

Forelimb placing

Immobilizing a forelimb in an unlesioned animal (i.e., sham) resulted in no significant behavioral differences between Sham+Cast and Sham+No groups ($F_{(1,15)} = 1.76, p = 0.20$). Therefore, they were pooled and labeled “Sham.” Two-way ANOVA revealed a main effect for treatment Group ($F_{(3,41)} = 119.81, p < 0.0001$), for Day ($F_{(8,327)} = 74.65, p < 0.0001$), and a significant Group \times Day interaction ($F_{(24,327)} = 27.95, p < 0.0001$) (See Fig. 4). Post hoc analyses revealed that on the day of, and 2 d after, cast removal, all lesion groups failed to place their contralateral (to the lesion) forelimb, in comparison to sham animals ($p < 0.005$), which placed both forelimbs successfully 100% of the time. The placing deficit in the animals whose ipsilateral forelimb was immobilized was significantly greater than in animals whose contralateral forelimb was immobilized or in animals that did not experience limb immobilization ($p < 0.005$). Deficits in contralateral forelimb placing also persisted much longer in the Lesion+Ipsi group than the other two lesion groups. Lesioned animals with no limb immobilization recovered forelimb placing behaviors by day 24 postlesion. At this time, both Lesion+Ipsi and Lesion+Contra groups were still showing con-

tralateral placing deficits that were significantly greater than that of both Lesion+No and Sham groups ($p < 0.005$). However, the placing deficit in the Lesion+Ipsi group was significantly greater than that of the Lesion+Contra group ($p < 0.0001$). Contralateral forelimb placing deficits in the Lesion+Contra group persisted slightly longer than those of the Lesion+No group (until day 31 postlesion). The animals in the Lesion+Ipsi group did finally recover forelimb placing behaviors at day 52 postlesion.

Forelimb somatosensory asymmetry

There were no significant differences between casted and non-casted sham groups in their responses to forelimb somatic sensation; therefore, they were pooled and labeled “Sham” for further analysis. Overall, two-way ANOVA revealed a main effect for treatment Group ($F_{(3,41)} = 5.88, p < 0.002$), for Day ($F_{(8,24)} = 5.95, p < 0.001$), and a significant Group \times Day interaction ($F_{(24,327)} = 1.76, p < 0.01$). Of primary interest was the chronic effect of the cast manipulations on forelimb somatosensory asymmetries. Post hoc analyses revealed that in the acute period after cast removal (days 15, 17, and 24), animals in both “casted” conditions showed a significantly larger “magnitude of asymmetry” (the ratio of the difference in stimulus size necessary to reverse response bias to bilateral simultaneous tactile stimulation), in comparison to sham animals ($p < 0.001$), but were not significantly different from Lesion+No animals (See Fig. 5). This may have been because immediately after cast removal there was great variability in the response to the previously casted limb in both casted groups (see Jones and Schallert, 1994). In the chronic time period after cast removal (days 31–60), animals that had their ipsilateral forelimb casted maintained a larger magnitude of asymmetry and, therefore, a greater forelimb somatosensory asymmetry compared with shams ($F_{(1,41)} = 15.65, p < 0.0003$),

Table 1. Volume of remaining brain tissue (in mm³) in Nissl-stained sections at day 33

Whole ipsilateral hemisphere		
Lesion+Ipsi	203.5 (5.21)*†	
Lesion+No	229.6 (1.92)*	
Sham	254.2 (3.29)	
Forelimb sensorimotor cortex		
Group	Ipsilateral hemisphere	Contralateral hemisphere
Lesion+Ipsi	69.6 (8.54)*†	101.1 (3.36)
Lesion+No	86.9 (7.4)*	105.5 (1.88)
Sham	96.8 (2.2)	95.8 (1.41)
Striatum (anterior and posterior)		
Group	Ipsilateral hemisphere	Contralateral hemisphere
Lesion+Ipsi	25.15 (3.16)	27.87 (1.25)
Lesion+No	26.62 (3.2)	29.37 (2.17)
Sham	26.53 (0.75)	26.76 (0.85)
Posterior striatum		
Group	Ipsilateral hemisphere	Contralateral hemisphere
Lesion+Ipsi	19.3 (3.15)*	26.3 (1.28)
Lesion+No	19.3 (1.84)*	25.1 (2.17)
Sham	25.0 (0.43)	25.1 (0.44)
Thalamus		
Group	Ipsilateral hemisphere	Contralateral hemisphere
Lesion+Ipsi	6.90 (0.34)*	7.90 (0.22)
Lesion+No	6.96 (0.11)*	7.84 (0.39)
Sham	8.27 (0.71)	8.13 (0.32)

Data are presented as mean ± SE. * $p < 0.05$, significantly different from Sham and/or from the contralateral hemisphere; † $p < 0.05$, significantly different from Lesion+No.

whereas both the Lesion+Contra and Lesion+No groups showed a forelimb somatosensory asymmetry similar to sham animals (i.e., recovery of a symmetrical response to somatic-sensory stimulation occurred). By the end of the study, 60 d postlesion, there was a significant difference between Lesion+Ipsi and Lesion+Contra ($F_{(1,41)} = 6.10, p < 0.01$) and between Lesion+Ipsi and Lesion+No groups ($F_{(1,41)} = 14.08, p < 0.0005$). Thus, the magnitude of asymmetrical responses to somatosensory stimulation was similar in all lesion groups immediately after cast removal (in the acute time period after cast removal); however, over time, lesioned animals with the contralateral limb or no limb immobilized recovered symmetrical responding to bilateral tactile forelimb stimulation, whereas the animals with the ipsilateral limb immobilized (those that were forced to use only the impaired limb during the first 15 d after the FL-SMC lesion) continued to show somatosensory asymmetries that were significantly larger than Lesion+Contra, Lesion+No, and Sham groups.

Limb use

Noncasted animals with an FL-SMC lesion showed a significant asymmetry in forelimb use immediately postlesion in comparison to shams. The animals with FL-SMC lesions preferred to use their ipsilateral (to the lesion) forelimb for postural-motor support behaviors (70–90% ipsilateral limb use), whereas sham animals used both forelimbs interchangeably (~50% ipsilateral limb use).

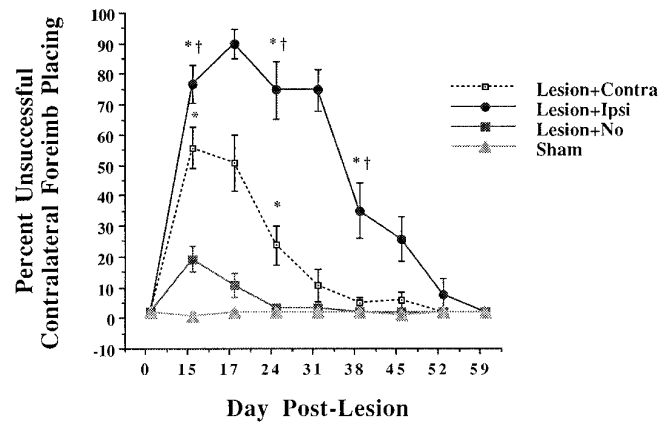


Figure 4. Percent unsuccessful contralateral forelimb placing. After cast removal, Lesion+Ipsi groups (filled circles) showed the largest and longest-lasting deficit. Lesion+Contra animals (open squares) showed a slightly larger deficit, lasting ~14 d longer, than Lesion+No animals (filled squares), which recovered by approximately day 24 postlesion. † $p < 0.001$, significantly different from Lesion+Contra; * $p < 0.001$, significantly different from Lesion+No and Sham.

Two-way ANOVA during the casting period comparing Lesion+No and Sham groups showed a significant effect of Group ($F_{(1,14)} = 48.28, p < 0.0001$), Day ($F_{(3,42)} = 4.6, p < 0.007$), and a Group × Day interaction ($F_{(3,42)} = 2.79, p < 0.05$). By the end of the study, Lesion+No animals were using their ipsilateral forelimb at sham levels, i.e., 50% (see Fig. 6A). Immediately after cast removal (days 15 and 17), all casted animals showed preferential use of the noncasted limb (See Fig. 6B). By days 24–31 (acute time period after cast removal), both Lesion+Contra and Lesion+Ipsi groups showed preferential use of the ipsilateral forelimb compared to sham animals ($p < 0.001$), which was greater than the percent ipsilateral limb use seen in Lesion+No animals ($p < 0.05$). Between days 38 and 60 (chronic time period after cast removal), Lesion+Contra animals recovered symmetrical forelimb use to Lesion+No and Sham levels, i.e., ~50%. However, Lesion+Ipsi animals continued to show a preference for ipsilateral forelimb use during postural-motor behaviors, i.e.,

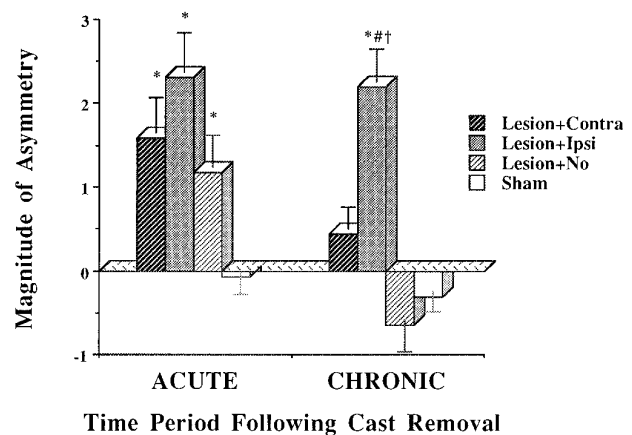


Figure 5. Magnitude of asymmetry during the acute (days 15–24) and chronic (days 31–60) time periods after cast removal. All lesion groups showed similar magnitudes of asymmetry immediately after cast removal. However, over time Lesion+Ipsi animals showed a persistently larger magnitude of asymmetry that did not recover to Sham levels by the end of the study. * $p < 0.05$, significantly different from Sham; †# $p < 0.05$, significantly different from Sham, Lesion+Contra, and Lesion+No.

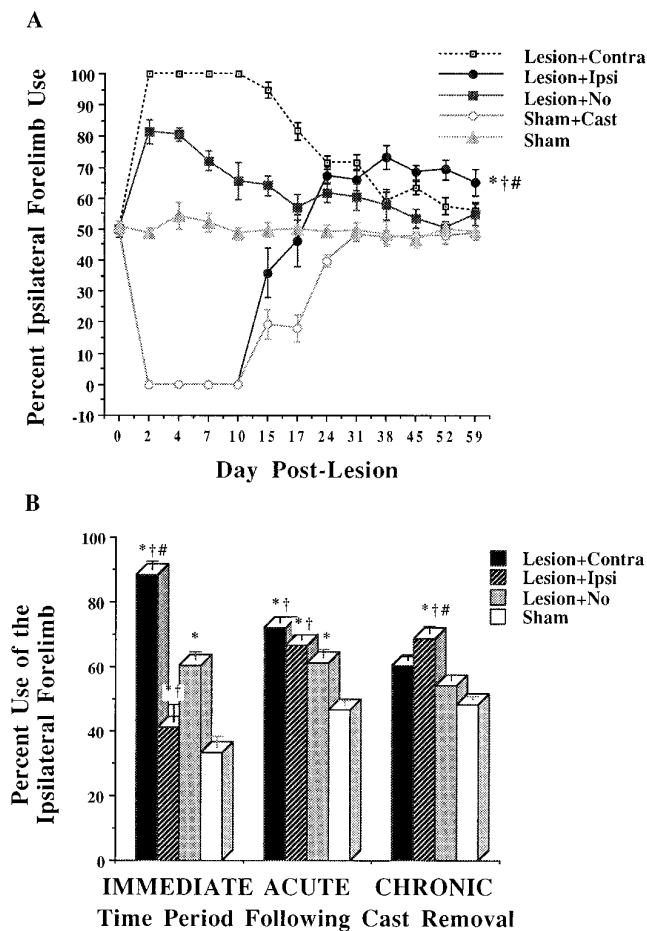


Figure 6. Asymmetry in limb use over days (*A*) and pooled into immediate, acute, and chronic time periods after cast removal (*B*). During casting, lesioned animals show a preference for the ipsilateral limb, which begins to become more symmetrical. Immediately after cast removal, all casted rats prefer to use the previously noncasted forelimb. Preferential use of the ipsilateral limb in both Lesion+Ipsi and Lesion+Contra casted animals is seen acutely after cast removal but is persistent in the Lesion+Ipsi group. * $p < 0.05$, significantly different from Sham; † $p < 0.05$, significantly different from Lesion+No; # $p < 0.05$, significantly different from either Lesion+Contra or Lesion+Ipsi.

~75% (significantly different from Lesion+No, Lesion+Contra, and Sham groups, $p < 0.03$).

Analysis of individual behaviors in the platform suggests that the overall return to more symmetrical limb use in Lesion+No animals may be deceiving. Figure 7*A* shows the results of percent ipsilateral limb use after landing, and Figure 7*B* shows the results of percent ipsilateral limb use for support along the wall. Observations of the limb used for landing followed the pattern of the analysis of overall limb use, placing and extinction placing tests, and the test of somatosensory asymmetry: (1) a significantly larger, slightly longer-lasting deficit in the Lesion+Contra group compared to Lesion+No group, and (2) a significantly larger, long-lasting, and perhaps permanent deficit in the Lesion+Ipsi group compared to Lesion+Contra and Lesion+No groups. Analysis of the landing behavior in Lesion+No animals also indicated that in the chronic period after cast removal (the period of time in which Lesion+No animals show a return to more symmetrical limb use), the Lesion+No animals preferred to use the ipsilateral limb significantly less than sham animals (i.e., they preferentially used the *impaired* forelimb for landing).

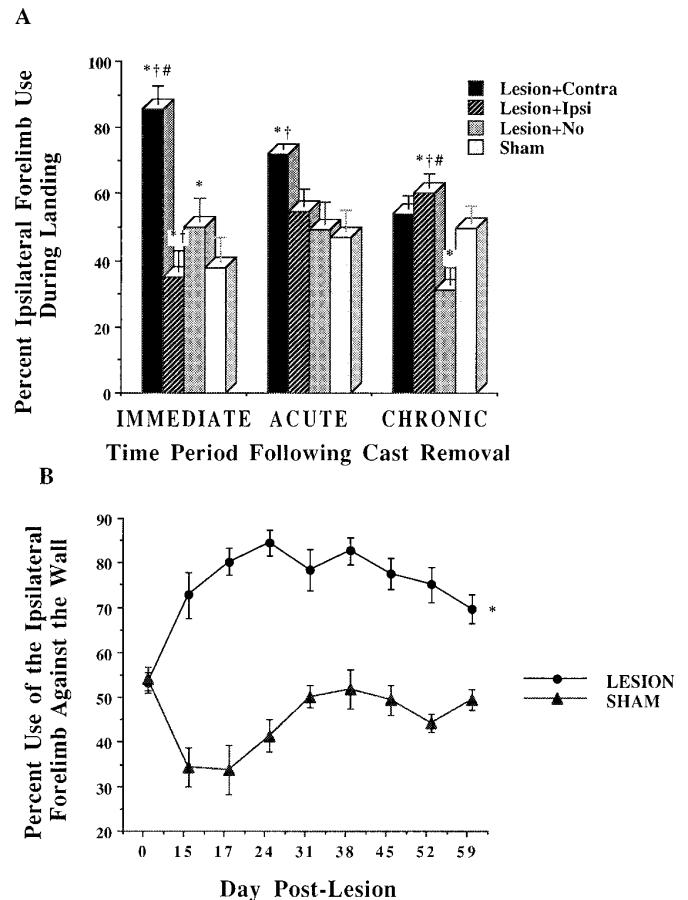


Figure 7. Asymmetries in landing on the platform (*A*) are transiently higher in the Lesion+Contra group and significantly higher and longer-lasting in the Lesion+Ipsi group. In the chronic time period, Lesion+No animals begin to preferentially use the impaired limb for landing. *B*, A significant preference for ipsilateral forelimb use along the wall is maintained throughout the experiment in all lesioned animals. *, Significantly different from Sham ($p < 0.05$); †, significantly different from Lesion+No ($p < 0.05$); #, significantly different from Lesion+Ipsi or Lesion+Contra ($p < 0.05$).

Analysis of the wall behavior indicated that all lesioned animals preferentially used their ipsilateral forelimb for support against the wall immediately after injury and continued to do so, as late as 60 d postlesion (significantly different from Sham, $p < 0.001$; see Fig. 7*B*). Furthermore, recent observations showed a deficit in this behavior in lesioned animals at 180 d postlesion (Kozlowski et al., 1995). Hence, symmetrical limb use in exploration of the wall may be a behavior that is permanently affected by FL-SMC lesions.

Taken together, analysis of the individual behaviors in the platform suggests that the return to symmetrical limb use previously reported in lesioned animals may reflect a redistribution of limb use tasks by the animal rather than general recovery of function. The symmetrical limb use in lesioned animals is *qualitatively* different from that seen in nonlesioned animals. Sham animals symmetrically use their forelimbs in all of the individual behaviors. In lesioned animals, a return to symmetrical use is seen in rearing behaviors. However, lesioned animals continue to use the *ipsilateral* forelimb preferentially for support along the wall and preferentially use the *contralateral* forelimb for landing (which, statistically, results in symmetrical limb use). Therefore, the return to symmetrical limb use in uncasted lesioned animals is

Table 2. Activity measures of the ipsilateral versus contralateral forelimb during and after casting

Group	During casting		After casting	
	Ipsi	Contra	Ipsi	Contra
Lesion+Contra	14.74 (3.1)*	0 (0)	20.7 (1.6) [†]	11.2 (1.3)
Lesion+Ipsi	0 (0)	13.2 (2.0)*	21.5 (2.4) [†]	11.1 (1.6)
Lesion+No	21.5 (2.1) [†]	7.9 (1.4)	18.6 (2.1) [†]	12.2 (1.4)
Sham	19.5 (1.0)	19.9 (1.5)	18.8 (3.7)	20.5 (0.8)

Data are presented as mean \pm SE. Lesion+Ipsi animals used their impaired forelimb more than Lesion+No animals. Lesion+Contra animals used their nonimpaired forelimb less than Lesion+No animals. * $p < 0.001$, significantly different from Lesion+No; [†] $p < 0.05$, significantly different from Contra forelimb.

qualitatively different from symmetry observed in sham animals. Thus, it is not a true measure of recovery but, rather, a measure of alternative, compensatory strategies. Lesion+No and Lesion+Contra groups successfully use these strategies, whereas Lesion+Ipsi animals do not. They continue to preferentially use the ipsilateral forelimb for all behaviors in the platform.

Activity measures

Measures of activity were obtained from observations of limb use on the platform both during the casting period and after cast removal (postcast). These measurements were taken for two main reasons. First, it was important to show that deficits in contralateral limb use were not attributable to increased activity in the ipsilateral forelimb. Second, it was important to determine that the ipsilateral cast caused overuse of the impaired forelimb relative to no-cast conditions. Because some groups were allowed to use only one limb during the casting period, separate activity measures of the ipsilateral (nonimpaired) and contralateral (impaired) limb were obtained. Differences between activity in the impaired and nonimpaired forelimb in the different treatment groups both during casting and postcast were analyzed. These measures are presented in Table 2. One-way ANOVA revealed a significant effect of Group for both use of the impaired limb and nonimpaired limb during casting ($F_{(3,31)} = 4.58, p < 0.009$; $F_{(3,30)} = 12.30, p < 0.0001$, respectively). Post hoc analyses revealed that during the casting period, casting the nonimpaired limb (Lesion+Ipsi) caused an increase in activity of the impaired limb compared to the activity of the impaired limb in Lesion+No animals ($p < 0.01$), whereas casting the impaired limb (Lesion+Contra) caused a decrease in activity of the nonimpaired limb compared to the activity of the nonimpaired limb Lesion+No animals ($p < 0.01$). However, Lesion+No animals showed the same amount of activity in the nonimpaired forelimb as did nonlesioned animals, whereas there was a lesion-induced decrease in activity of the impaired limb. Post-cast analysis revealed significantly less activity in the impaired limb in all lesion groups, compared to sham, but no significant differences in activity of the nonimpaired forelimb. Therefore, deficits in limb use cannot be attributed to an increase in use of the ipsilateral limb but, rather, to a decreased use of the contralateral, impaired limb (which altered the types of movements required by the nonimpaired limb; see Jones and Schallert, 1994). Also, behavioral and anatomical differences between groups after removal of the cast may be related to the use of their forelimbs during the casting period.

DISCUSSION

Long-term behavioral consequences of forelimb immobilization in animals with unilateral FL-SMC lesions

Immobilizing the ipsilateral forelimb in lesioned animals (forcing use of the impaired limb) resulted in severe and long-lasting behavioral deficits after cast removal in all of the behaviors measured: contralateral forelimb placing, responsiveness to forelimb somatic–sensory stimulation, and forelimb use for postural–motor support, which did not recover to noninjured (sham) levels. Immobilizing the contralateral forelimb in lesioned animals (preventing use of their impaired forelimb) resulted in slightly larger and longer-lasting behavioral deficits after cast removal (compared to noncasted, lesioned animals), which did recover to sham levels. Immobilization of a single forelimb in sham rats resulted in no significant behavioral changes.

Both behavioral and anatomical explanations for these data may be relevant. Studies have shown that functional recovery may be attributable to the development of alternate strategies involving compensatory behaviors (Honzick, 1936; Castro, 1977; Whishaw et al., 1986, 1991; Schallert, 1988). Lesion+No animals, which were allowed to use both forelimbs, may have developed the most efficient compensatory strategies and thus had the smallest, shortest-lasting deficits. The deficits seen in lesioned animals with contralateral forelimb immobilization may be a result of disuse of the impaired limb during the early postlesion period, i.e., missed therapeutic windows of opportunity. Moreover, in these animals the nonimpaired limb was not used to the same extent as in Lesion+No animals. The most surprising result was that immobilization of the ipsilateral (nonimpaired) forelimb, which would cause an increase in the use of the impaired forelimb, resulted in the largest behavioral deficits and the longest recovery period. This cannot be attributable to a lack of use of the impaired limb. In fact, Lesion+Ipsi animals used their impaired forelimb *more* than nonimmobilized, lesioned animals. It is possible that these deficits may be caused, in part, by *lack* of use of the ipsilateral forelimb as a “crutch” to compensate for deficits in the impaired forelimb during the early postlesion period. Indeed, recent work in this laboratory by R. Cocke, D. Kozłowski, J. Gotts, and T. Schallert (unpublished data) showed that in noncasted animals that appeared to have recovered from sensorimotor impairments, anesthetization of the ipsilateral, nonimpaired (crutch) forelimb reinstated severe impairments in the use of the impaired forelimb, whereas anesthetization of the impaired forelimb had little or no effect. However, this does not explain fully why the Lesion+Ipsi animals showed such a dramatic and long-lasting deficit compared to Lesion+Contra and Lesion+No groups. This may be explained neuroanatomically.

Long-term anatomical consequences of forelimb immobilization in animals with unilateral FL-SMC lesions

Prevention of dendritic plasticity

Preventing animals from using their nonimpaired limb during the early postlesion period resulted in a blocking of the use-dependent increase in dendritic growth of layer V pyramidal neurons in the contralateral, homotopic cortex (as in Jones and Schallert, 1994). After removal of the cast, there was a slight but not significant increase in dendritic growth, consistent with the possibility of an optimal period, or window of opportunity, for lesion-induced, use-dependent dendritic arborization. The reason for the lack of reinstatement in dendritic plasticity may be behav-

ioral. After removal of the cast, Lesion+Ipsi animals used their forelimbs asymmetrically, preferring to use their ipsilateral forelimb more than their contralateral forelimb, as do nonimmobilized lesioned animals immediately after surgery. However, in Lesion+Ipsi animals there was not a progression toward symmetrical limb use, as seen during the first 18 d postlesion in nonimmobilized animals. Thus, arborization may be attributable to the learning of compensatory strategies required for symmetrical limb use and not just to preferential use of the ipsilateral forelimb (Jones and Schallert, 1994). Greenough and colleagues have shown that learning, and not activity alone, is necessary for dendritic plasticity (Black et al., 1990, 1991; Greenough and Anderson, 1991). On the other hand, the reason for the prevention of dendritic plasticity may be more neuroanatomical. Immobilization of the nonimpaired limb may have prevented the astrocytic expansion seen in the FL-SMC of the intact hemisphere after injury, which has been postulated to play a role in dendritic plasticity by providing trophic support to prime for growth (Jones and Schallert, 1992). Additionally, it may be possible that the exaggeration of neural injury may have played a role in the prevention of dendritic growth.

Exaggeration of lesion size

The chronic, long-lasting behavioral deficits are also likely to be attributable to the dramatic exaggeration of the lesion seen in ipsilaterally immobilized animals. Immobilization of the forelimb ipsilateral to the lesion resulted in a dramatic increase in the extent of the volume of the lesion. It may be that forced overuse of the impaired forelimb caused chronic excitation of surviving tissue surrounding the lesioned area, which in turn resulted in a dramatic deterioration of the lesioned hemisphere. To our knowledge, this is the first report that behavioral events can exaggerate brain damage. It has been demonstrated, however, that in noninjured neural systems chronic depolarization produces retraction of and degeneration of phasic motor axons in the developing crayfish (Egid and Lnenicka, 1994) and that unilateral electrical stimulation of the sensorimotor cortex induces excitotoxic loss of GABAergic neurons in the striatum of rats (To et al., 1994).

Possible mechanisms for use-dependent exaggeration of neuronal injury

A number of possibilities exist (some of which are being currently examined) that may explain the mechanism behind use-dependent exaggeration of neuronal injury. The increase in lesion size might be attributable to behaviorally related enhancement of excitotoxic processes. Use-dependent increases in depolarization of glutamatergic neurons may result in an excitotoxic cascade, causing an increased flux of Ca^{2+} via glutamatergic action at NMDA receptors, which may eventually lead to neuronal death (Olney, 1983; Schwarcz et al., 1984; Choi et al., 1987; Choi, 1988; Cotman et al., 1988; Mattson, 1989; Mattson et al., 1989, 1993). If this is the case, then administering an NMDA-receptor antagonist (such as MK-801) during the time in which the animal's limb is immobilized may prevent the deterioration of the lesion and the chronic and long-lasting behavioral deficits. This possibility is currently being explored by J. Humm, R. Cocke, and T. Schallert (unpublished data), and preliminary data indicate that MK-801 prevents use-dependent exaggeration of neuronal injury.

Forced use of the impaired limb may mimic peri-infarct events seen in the penumbra of ischemia (Nedergaard and Astrup, 1986; Gill et al., 1992; Iijima et al., 1992). Ischemic insults produce a

focal lesion as well as an area of undamaged, yet vulnerable, neural tissue called the penumbra, which is characterized by the presence of spreading depression. In ischemia, there are two types of spreading depression-like depolarizations: one mediated by glutamate released from the core of the infarct (classical glutamate excitotoxicity), and one mediated by fluctuations of blood flow that result in transient episodes of hypoxia (Nedergaard and Hansen, 1993; Back et al., 1994). The hypoxia compromises protein synthesis and can result in progressive tissue injury and the expansion of the ischemic infarct core into the penumbral zone (Mies et al., 1991). It may be that forcing the use of the impaired limb results in spreading depression-like depolarizations that may lead to transient episodes of hypoxia in otherwise surviving, but vulnerable, tissue surrounding the lesioned area. The hypoxia may induce metabolic deficiency such as an inhibition of protein synthesis which, in turn, may produce the progressive neural damage seen in ipsilaterally casted animals.

On the other hand, it may be that a hypermetabolism, instead of a hypometabolism, is the cause. Studies have shown that animals that are placed in enriched environments (Black et al., 1991) or that engage in sustained motor activity, such as exercise (Black et al., 1990), have a significantly higher vascular density than non-enriched, nonactive rats so as to deal with the metabolic demand placed on them. In a compromised brain, however, initiation of these events may be deleterious. Increased or fluctuations in blood flow, hemorrhage, or increased intracranial pressure may act synergistically to cause permanent histological damage (i.e., expansion of the size of the lesion). Studies examining cerebral blood flow, protein synthesis, and oxidative and glucose metabolism in the lesioned area of ipsilaterally casted animals are currently being implemented to address these questions.

Finally, it was also hypothesized that the stress of immobilization may play a role. Animals that are being forced to use their impaired limb may be experiencing more stress than animals that are being forced to use their nonimpaired limb. It is well documented that stress causes an increase in glucocorticoids in the brain (for review, see Sapolsky, 1992) that has been linked to cell death in the hippocampus (Sapolsky and Pulsinelli, 1985) and the neocortex (Koide et al., 1986), presumably caused by the induced increase in excitatory amino acids (Moghaddam, 1993; Moghaddam et al., 1994). The role of stress is currently being explored, and preliminary results indicate that the level of corticosterone in animals with ipsilateral limb immobilization is not different from that of animals with contralateral limb immobilization or no limb immobilization (Kozlowski et al., 1995). Therefore, increased corticosterone levels per se do not seem to play a direct role in the exaggeration of neuronal injury.

Relationship between behavioral and anatomical consequences of forelimb immobilization

Regardless of the mechanism, the present study indicates that immobilization of the nonimpaired limb (i.e., forcing the use of the impaired limb) results in chronic and long-lasting behavioral asymmetries, disruption of neural plasticity in the intact hemisphere, and a dramatic exaggeration of neural injury in the lesioned hemisphere. At this time, it is not possible to determine whether the devastating behavioral effects of casting the nonimpaired limb are linked to the prevention of dendritic overgrowth, caused by the progressive expansion of the lesion size, or both.

To delineate the contributions of the anatomical consequences of limb immobilization to the behavioral deficits, studies must be

performed to isolate further the optimal periods for dendritic growth and use-dependent exaggeration of injury.

Analysis of forelimb use as a measure of behavioral compensation

Finally, this study resulted in an interesting observation regarding the measurement of asymmetrical limb use on a three-sided platform. Analysis of individual behaviors in the platform suggested that the return to symmetrical limb use seen in lesioned animals was qualitatively different from symmetrical limb use in nonlesioned animals. The symmetrical limb use seen in nonlesioned animals was attributable to equal use of both forelimbs in all of the behavioral measures. In contrast, the symmetrical limb use seen in “recovered” lesioned animals consisted of a preference for the ipsilateral forelimb on one behavior (for support against the wall) and an opposing preference for the contralateral forelimb on another behavior (landing on the platform after a rear or after exploration of the wall). Thus, the symmetrical limb use seen in lesioned animals represents an alternative, compensatory strategy used by the lesioned animals that is not analogous to the symmetrical forelimb use of nonlesioned animals. These findings have resulted in the development of new hemisphere-specific behavioral tests and manipulations to examine the compensatory use of the ipsilateral forelimb in lesioned animals (Schallert and Kozlowski, 1996).

Implications

The potential implications for rehabilitation suggested by this study may be worth noting, albeit with the usual caution about generalizing from one lesion model to others and about comparing animal research with human neurological treatment. This study, in conjunction with previous studies (Jones and Schallert, 1992, 1994), suggests that after brain injury there may exist (1) a window of opportunity for compensatory, use-dependent structural changes in the intact cortex, and (2) a region of sublethally injured, but use-vulnerable, tissue in the injured hemisphere. Although behavioral experience can enhance neuronal growth after brain injury in remote, intact brain areas, excessive behavioral pressure during the early postlesion period may cause irreversible damage to surviving, but compromised, tissue surrounding the injury. Therefore, a “use-it-early-or-lose-it” strategy of rehabilitation that was implied in Jones and Schallert (1992, 1994) may apply to the intact hemisphere but not to the injured hemisphere. Instead, a less aggressive “use-it-but-don’t-overuse-it” strategy perhaps should be adopted for optimal restoration of function of the forelimb corresponding to the injured hemisphere.

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