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Experience-Dependent Structural Plasticity in Cortex Heterotopic to Focal Sensorimotor Cortical Damage

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Structural plasticity following focal neocortical damage in adult rats has recently been found to be sensitive to postinjury rehabilitative training. Experience on a complex motor skills task, the acrobatic task, after unilateral lesions of the forelimb representation region of the sensorimotor cortex (FLsmc) enhanced synaptic structural changes in the cortex contralateral and homotopic to the lesions. Using tissue from this previous study, the present study examined whether a heterotopic region of the sensorimotor cortex of either hemisphere, the hindlimb representation area (HLsmc), would undergo structural changes following unilateral FLsmc lesions and whether these changes would also be sensitive to postinjury training on the acrobatic task. Stereological methods for light and electron microscopy were used to assess structural changes in lesion or sham-operated rats following 28 days of postoperative acrobatic training or simple repetitive exercise (motor controls). In the HLsmc contralateral to the lesions of rats receiving acrobatic training, there was a subtle, but significant, increase in cortical volume and in layer II/III neuropil and dendritic volume per neuron in comparison to shams. In rats receiving simple exercise after the lesions, these changes were not significantly different from shams. Acrobatic training also prevented a loss of cortical volume in the HLsmc adjacent to the lesion in comparison to shams. These data suggest that behavioral training following cortical injury facilitates structural plasticity in behaviorally relevant areas of the neocortex other than the homotopic cortex contralateral to the lesion. This structural plasticity might be relevant to the development of behavioral compensation after cortical injury. © 2000 Academic Press

Key Words: **rehabilitative training; behavioral compensation; motor skills learning; forelimb representation area; hindlimb representation area; synaptogenesis; dendritic growth.**

INTRODUCTION

Previous research has identified the motor cortex as a site of significant neuronal structural and physiological change in response to motor skills learning. Increases in the dendritic arborization of neurons in layers II/III and V of the forelimb region of the sensorimotor cortex have been found in adult rats after forelimb reach training (11, 43). Training on a more complex motor skills task, the acrobatic task, has been found to result in synaptogenesis in layer II/III (27) and layer V (19) of the forelimb representation region of the sensorimotor cortex (FLsmc) of adult rats in comparison to animals receiving simple repetitive exercise. In addition, microstimulation studies suggest major changes in the motor cortical representations of movements involved in motor learning tasks in both monkeys (35) and rats (25). These and related lines of research (e.g., 2, 24, 38) suggest that plasticity in the motor cortex may underlie major components of motor skills learning. This has important implications for recovery from neocortical damage because this recovery may involve the compensatory development of new motor behaviors and be sensitive to rehabilitative motor skills training.

Cortical injury has been found to result in neuronal growth, synaptogenesis, and reorganization of cortical representations in remaining regions of the cortex (e.g., 5, 17, 20, 29). Recent research has supported that at least some of these cortical changes vary dependent upon postinjury behavioral experience. Unilateral FLsmc lesions result in impairments in forelimb use on the contralateral body side and a heightened dependence on the nonimpaired ("intact") forelimb for postural motor behaviors (22, see also 32). The compensatory reliance on the nonimpaired forelimb is likely to contribute to much of the measured recovery on tests of

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coordinated forelimb use (41). In layer V of the FLsmc contralateral and homotopic to the lesion, neuropil volume, dendritic arborization, and synaptic connections have been found to increase in a time-dependent manner after the lesions (18, 21, 22). The postlesion increases in dendritic arborization were linked to the compensatory reliance on the intact forelimb (23) and the synaptogenesis was enhanced by postoperative training on the acrobatic task (19). However, forced reliance on one forelimb in intact animals was not sufficient to reproduce the dendritic growth effects found after lesions (23) and acrobatic training in sham-operated animals results in a less robust synaptogenic response than training in rats with FLsmc lesions (19), suggesting that the structural changes are a result of a lesion–behavior interaction. Although the focus of these previous studies has been on changes in the cortical region contralateral and homotopic to the damage, it seems possible that postinjury behavioral training may also influence structural plasticity in other cortical regions.

The purpose of the present experiment was to determine whether motor skills training following unilateral FLsmc lesions influences structural plasticity in a heterotopic neocortical region, the hindlimb representation area (HLsmc), of either hemisphere. Electrophysiological and microstimulation mapping studies have indicated that reorganization of representation maps in the cortex adjacent to a focal injury is influenced by postinjury behavioral experiences and that the extent of reorganization is related to the extent of behavioral recovery from lesion-induced impairments (7, 17, 33, 34, 36, 45). In the present study, the HLsmc was chosen for analysis because it is located proximal to the FLsmc in both hemispheres, permitting analysis of the same subregion both contiguous to the lesion and in heterotopic (to the lesion) contralateral cortex. Furthermore, the hindlimbs may be involved, bilaterally, in the development of compensatory strategies as a result of lesion-induced impairments in one forelimb and the acrobatic task requires the animals to develop new skilled use of the hindlimbs in coordination with the forelimbs. The HLsmc is also readily identified using cytoarchitectonics (9, 44). Although acrobatic training has previously been found to result in structural changes in the forelimb representation area in intact animals (19, 27), the HLsmc, which borders the caudal and medial extent of the forelimb area, has not previously been investigated in intact rats after acrobatic training.

This study uses tissue generated in a previous study in which we found that acrobatic training following unilateral FLsmc injury enhances synaptogenesis in the contralateral homotopic cortex in comparison to animals that received either the training or the lesions alone (19). Adult rats with unilateral FLsmc lesions or sham surgeries were trained on the acrobatic task or received simple, repetitive exercise (motor controls). At the end of training, behavioral measures were used to test for an improvement in coordinated hindlimb placement in comparison with motor control animals. Structural changes in the hindlimb representation area of the sensorimotor cortex (HLsmc) of both hemispheres were assessed using stereological methods for quantitative light and electron microscopy. Layers II/III of the HLsmc were targeted for analysis in this study because the pyramidal neurons in these layers of motor cortex have been implicated as key mediators in cortical plastic events (13, 14, 37) and because, at the time of onset of this study, neocortical acrobatic training effects had as yet only been demonstrated in layer II/III of the FLsmc (27).

MATERIALS AND METHODS

Subjects and Experimental Conditions

This study used samples of the HLsmc collected from 40 adult, 4- to 5-month-old, male Long–Evans rats that were used in a previous study of acrobatic training effects on the FLsmc (18). Rats were housed in pairs, permitted food and water *ad libitum*, and were maintained on a 12:12-h light:dark cycle. Animals were handled regularly and made tame prior to surgery. Animals were randomly assigned to the following groups: (i) Sham Motor Controls (Sham-MC, $n = 10$); (ii) Sham Acrobats (Sham-AC), $n = 10$; (iii) Lesion Motor Controls (Lesion-MC), $n = 9$; and (iv) Lesion Acrobats (Lesion-AC), $n = 11$.

Surgical Procedures

Electrolytic lesions were aimed at the caudal forelimb representation area at coordinates corresponding to the overlapping somatic–sensory and motor representation of the forelimb (FLsmc, 12, 44, Fig.1). Following anesthetization with Equithesin (34 mg/kg pentobarbital and 150 mg/kg chloral hydrate), the skull and dura were removed between 3.0 and 4.5 mm lateral to midline and between 0.5 mm posterior and 1.5 mm anterior to bregma. An uninsulated 30-gauge platinum wire electrode was lowered 1.7 mm below dura and anodal current (1 mA) was delivered for 120 s as the electrode was moved continuously in eight equally spaced horizontal traverses through the exposed cortex. These lesions were found to produce complete to near complete damage to the overlapping somatic–sensory and motor representation area of the forelimb as well as considerable damage to nonoverlapping primary sensory and primary motor forelimb representational areas (19, Fig. 1). For sham-operated animals, all surgical procedures were performed up to, but not including, removal of the skull. Ten animals which developed postsurgical adynamic ileus linked to one

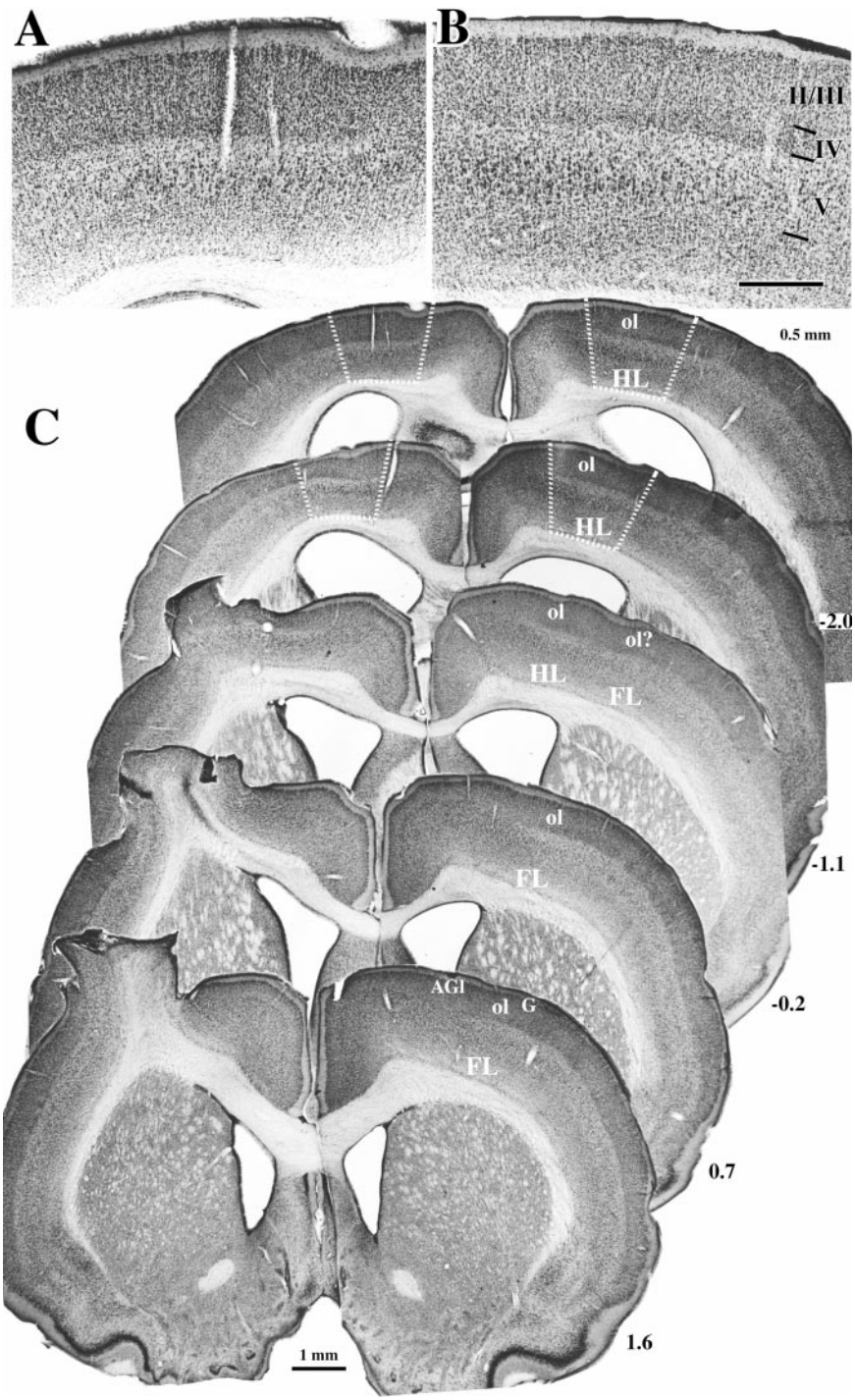

FIG. 1. Representative examples of regions chosen for analysis in the cortex ipsilateral, A, and contralateral, B, to unilateral lesions. Neuronal, synaptic, and dendritic measures were obtained from layer II/III. Photomicrographs are of coronal sections stained with methylene blue azure-II. Bar, 0.5 mm. C, lower magnification view of coronal hemisections of a representative lesion (left) and the region sampled (white dashed outlines) with corresponding sections of left and right hemispheres aligned. The caudal most pair of sections is the same as that used for the photomicrographs shown in A and B. The sample placement takes advantage of the very distinct cytoarchitectural characteristics of the caudal forelimb (FL) and the hindlimb (HL) representation regions of the sensorimotor cortex (8, 9, 44). The primary motor cortex as identified in microstimulation studies is found in the lateral agranular cortex (AGl) which is characterized, cytoarchitecturally, primarily by the presence of large pyramidal neurons in layer V and, in nonoverlapping zones, by the absence of a distinct layer IV. The primary sensory cortex as identified in electrophysiological studies is found in the granular cortex (G) which is characterized by the presence of a densely packed layer IV interrupted by dysgranular strips. The primary sensory and primary motor regions overlap (ol) partially in the forelimb region and overlap almost completely in the hindlimb region, but do not overlap in any other somatic–sensory and motor representation areas. Numbers to the right are approximate coordinates in mm relative to bregma. Bar, 1 mm.

batch of Equithesin were dropped from the study prior to the completion of behavioral training.

Behavioral Training and Testing

Acrobatic training. Beginning 2 days after surgery, lesion or sham-operated rats received 28 days of training on a complex motor skills task, the acrobatic task (adapted from 4), or received simple repetitive exercise (motor controls). The acrobatic condition animals were trained to complete an obstacle course that required the development of new coordinated motor skills. The animals were trained to traverse a wooden rod, a rope, and a link chain; climb and descend a ladder; scale barriers and parallel beams; traverse a grid floor; and leap a chasm, as described previously (19). The training sequence involved an initial exposure period (Day 2) in which the rats were acclimated to the testing room and placed on the first task on the obstacle course, heavily aided training (Days 3–4) in which the rats were assisted through the entire sequence of obstacles, full training (Days 5–13) in which the rats completed four trials of the obstacle course per day, and a final phase of light training (Days 14–28) in which the rats completed two trials of the obstacle course per day. The motor control animals were yoked to acrobatic rats and received simple exercise in a straight runway for the duration of each acrobatic trial.

Measures of hindlimb use on the footfault test. On Day 29, the animals were administered the footfault test to measure the coordinated use of their limbs (3). Rats were placed on an elevated grid platform (33L by 30W cm, 6.25-, 8.4-, and 12.25-cm^2 openings) and videotaped for 2 min. Errors in limb placement were evident when the paw(s) slipped through the grid openings. Although this task was initially designed to measure coordinated forelimb placing during locomotion, the inclusion of larger grid openings also permitted the assessment of hindlimb placing in the present study. Because hindlimb errors were rare on the small and medium grids, only the stepping behavior on the largest of these grid openings was included in this analysis. In video playbacks, the total number of steps and the number of errors were recorded for each hindlimb. A hindlimb placing error was identified when the animal missed the grid support such that the heel of the hindpaw slipped through the grid opening. Hindlimb placing errors were calculated as the percentage of hindlimb misses per hindlimb step. Forelimb footfault data had been collected and reported previously (19).

Tissue Preparation

On Day 30, the animals were anesthetized with sodium pentobarbital (120 mg/kg) and perfused intracardially with 0.1 M phosphate buffer followed by 2% paraformaldehyde and 2.5% glutaraldehyde in the same buffer. The brains were removed, halved at midline, and sliced coronally with a vibratome into 300- and 100-μm-thick sections. The 100-μm sections were collected at 900-μm intervals, stained with methylene blue-azure II, and used for cortical volume measurements. Samples within the cytoarchitecturally distinct HLsmc were collected from the 300-μm sections using a dissecting microscope and postfixed overnight. As shown in Fig. 1, the strategy for localizing these samples permitted consistency in the cytoarchitectural placement between hemispheres and animals but avoided the lesion border region, which typically included at least superficial damage to the rostral extension of the HLsmc. The sections were washed with 0.05 M cacodylate buffer, postfixed in 2% osmium tetroxide and 1.5% potassium ferrocyanide in cacodylate buffer for 2 h, stained *en bloc* with 2% uranyl acetate, dehydrated through a series of alcohol and acetone washes, and gradually infiltrated with Eponate 12 resin. These samples were sandwich embedded, polymerized at 60°C, and mounted onto blocks to enable coronal semi- and ultrathin sectioning using an Ultracut R microtome (Leica). Semithin (0.8-μm) sections were collected from these samples at 1.6-μm intervals. These semithin sections were stained with toluidene blue and used for neuronal density measurements. Three sets of four serial 70-nm-thick (ultrathin) sections were then collected from layer II/III of the samples and stained with lead citrate. A Jeol 1200EXII electron microscope was used to produce electron micrographs (20,060× final magnification) which were used for synaptic and dendritic measurements.

Stereological Methods

Of primary interest in the present study was the detection of net changes in synapse number and dendritic volume in the HLsmc. Because cortical volume can change, measures of synaptic density and dendritic volume fraction (the percentage of tissue occupied by dendritic processes) are not adequate for this determination. Increases in cortical thickness and volume have been found in many studies of experience effects on cortical structure (e.g., 40, 42, reviewed in 10) and unilateral FLsmc lesions have been found to result in an increase in the volume of the contralateral homotopic cortex (21, 22). Volume estimates of cortical subregions are problematic because they require the precise delineation of subregion boundaries. (Although positioning samples *within* cytoarchitecturally defined subregions can be done with accuracy, boundary delineation is error-prone.) However, when cortical volume increases, neurons become pushed apart by the addition of neuropil. Thus, changes in synapse number are accurately estimated by the number of synapses per neuron when neuron number is stable (1). In this situation, measurement of cortical volume *per neuron*

(the inverse of neuronal density) is the most sensitive means of detecting volume changes within a cortical subregion. Furthermore, neuronal and electron microscopic measures can be obtained from the same resin-embedded samples, avoiding differential shrinkage effects. However, in the presence of neuronal loss (i.e., evidenced by either neuronal density decreases in the absence of changes in cortical volume or, conversely, by cortical volume decreases in the absence of increased neuronal density) the ratio of synapses and dendritic volume per neuron may be of interest, but these ratios do not reflect net changes. In the present study, synapse number per neuron and dendritic volume per neuron ratios were used when neuronal density changes paralleled cortical volume changes (i.e., in the HLsmc opposite the lesion). In the HLsmc of the lesion hemisphere, based on evidence of neuronal loss, changes in synapses and dendrites were interpreted with reference to cortical volume changes.

Cortical volume measurements. Cortical volume measurements were obtained using the Cavalieri method and systematic point counting (31) with the aid of a light microscopic stereological workstation and NIH Image. Four 100-μm coronal sections 900 μm apart were analyzed using the rostral-most midline crossing of the anterior commissure as the landmark to identify the first section in the series. This sampling technique was used because it consistently included the HLsmc overlap zone but avoided the need to delineate the exact boundaries of the HLsmc. Because volume measurements were not limited to layer II/III of the HLsmc and relatively few sections were sampled per animal (because remaining sections were used for neuronal and synaptic density measures), volume measures were not intended to be sensitive to subtle changes but were intended to guide the interpretation of changes in neuronal density. Images of the sections were captured into NIH Image (×23) and the cortex was outlined. A grid of test points was randomly superimposed onto the image and the number of points that fell within the cortical boundaries was counted. HLsmc region cortical volume was then calculated using the formula

$$\text{Volume} = \Sigma P \times a(p) \times T,$$

where ΣP is the total number of points counted, $a(p)$ is the area represented by each point (0.30 mm^2), and T is the distance between section planes (900 μm). Some brains were not included in volume measurements (two whole brains from the Lesion-Acrobat group, one lesion hemisphere from the Lesion-Motor Control Group) because some sections did not adhere well to the slides, resulting in greater shrinkage during histological processing.

Neuronal density measurements. The physical disector method (31) was used for neuronal density measurements. In this method, neuronal nuclei that are present in one section, the "reference section" are counted if they are not also present in an adjacent "look-up" section. Reference samples from the semithin sections were captured in NIH Image (785× magnification) and the look-up samples from an adjacent coronal plane were viewed in a nearby monitor. Five samples per each of five sections were used. Samples were outlined by an unbiased sample frame positioned using systematic random sampling in upper layer II/III. Neuronal density was calculated using the equation

$$\text{Neuronal Density} = \Sigma Q^{-}/\Sigma[a(\text{frame}) \times T],$$

where ΣQ^{-} is the number of neuronal nuclei that were counted within a series, a(frame) is the area of the sample frame (37,250 μm^2, corrected for magnification), and T is the distance between look-up and reference section planes (1.6 μm).

Dendritic volume measures. Three electron micrographs (one from each set of the three series used for synaptic density measures) from each animal were analyzed to determine the average dendritic volume fraction (% of tissue occupied by dendritic processes). The processes of dendrites (Fig. 2) were identified primarily by the presence of microtubules and the absence of components characteristic of glial and axonal processes (e.g., glial filaments, small vesicles, myelin). A point-counting grid was randomly superimposed onto the images and the number of points that fell within the membranes of dendritic processes was counted. HLsmc dendritic volume fraction was then determined using the formula

$$\text{Dendritic Volume Fraction} = [\Sigma P \times a(p)]/\Sigma A_{\text{ref}},$$

where ΣP is the total number of points counted within the dendritic processes, $a(p)$ is the area per test point (0.26 μm^2), and A_{ref} is the sample area (99 μm^2). The average dendritic volume per neuron was then determined by dividing the dendritic volume fraction by neuronal density.

Synaptic measurements. The disector method was used to determine synaptic density. For each animal, three series of four serial, 70-nm-thick electron micrographs were examined. An unbiased sample frame was traced onto each micrograph and the synapses that fell within the inclusion boundaries were identified. A synapse was identified by the presence of at least three vesicles in the presynaptic bouton and a postsynaptic density (Fig. 2). Synapses were counted only if they were not present in the adjacent look-up section. This process was performed moving in both directions through the series and an average of the two synaptic

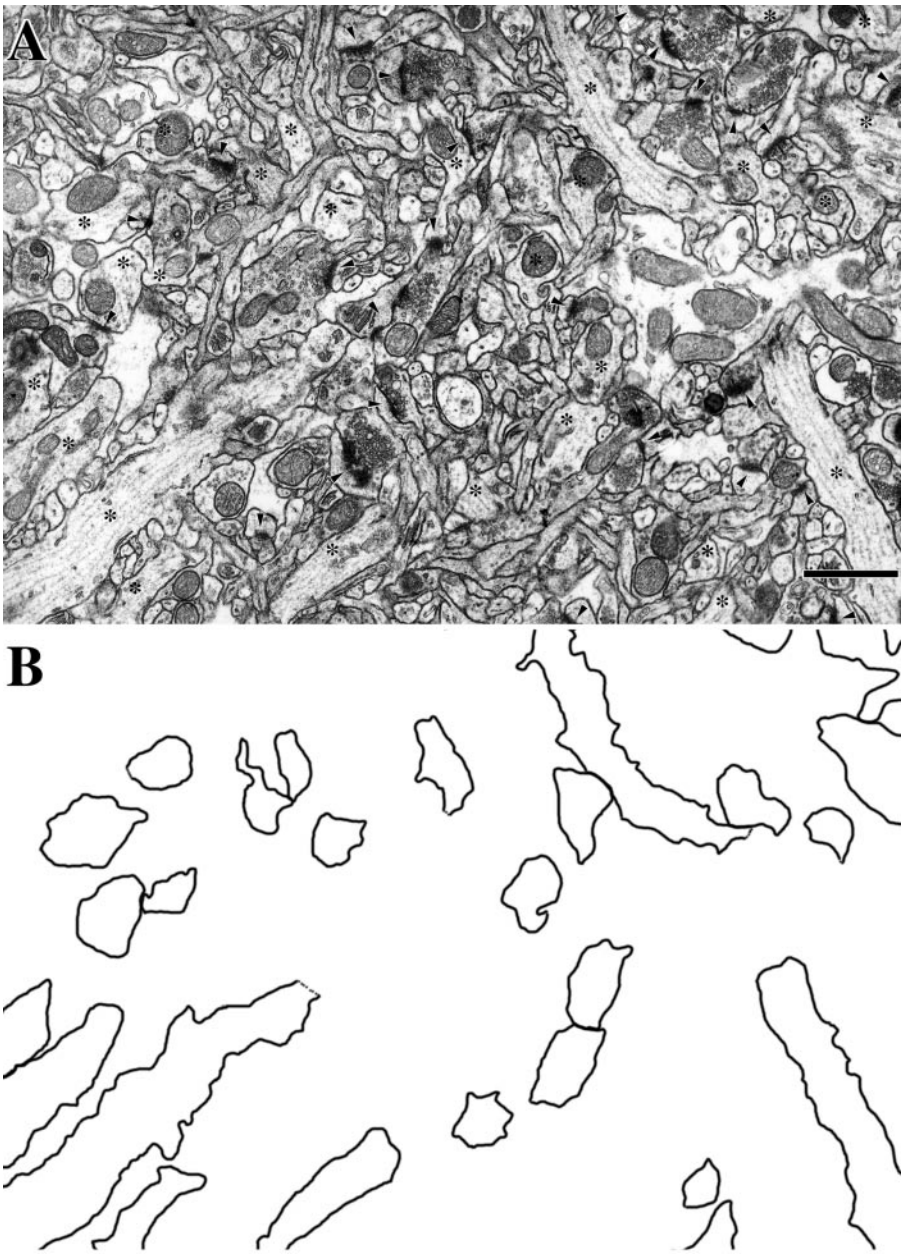

FIG. 2. A, electron micrograph of layer II/III of the HLsmc showing a relatively low magnification view of a field of dendritic processes (asterisks) and synapses (arrowheads). Bar, 1 μm. B, tracings of dendrites in A. Dendritic volume fraction and synaptic density were measured using stereological methods. The identification of dendrites was facilitated by the ability to follow processes through a series of sections.

counts was recorded. Synaptic density was then calculated using the equation

$$\text{Synaptic Density} = \Sigma Q^{-}/\Sigma[a(\text{frame}) \times T],$$

where ΣQ^{-} is the average number of synapses counted within a series, a(frame) is the area of the sample frame (99 μm^2, corrected for magnification), and T is distance between reference and look-up section planes (70 nm). The number of synapses per neuron was then determined by dividing synaptic density by neuronal density.

Statistical Analysis

For each behavioral and anatomical test, SAS general linear models procedure for contrasts was used to perform four planned comparisons. Comparisons of Sham-Acrobat vs Sham-Motor Control were performed to test for effects of motor skills learning. Sham-Motor Control vs. Lesion-Motor Control comparisons were

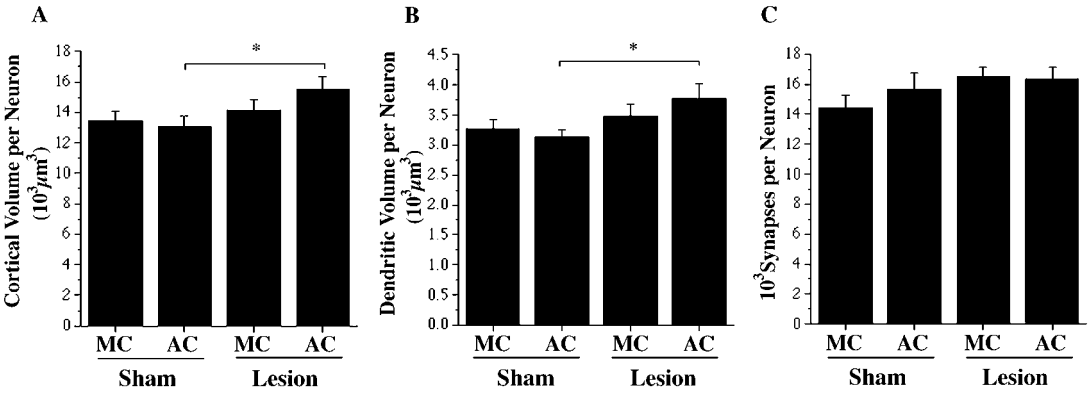

FIG. 3. Structural measures in layer II/III of the HLsmc opposite unilateral FLsmc lesions or sham operations in rats trained on the acrobatic task (AC) or receiving simple repetitive exercise as motor controls (MC). A, acrobatic training after the lesions resulted in a significant increase in layer II/III cortical volume per neuron (the inverse of neuronal density) in comparison to Sham-AC. The cortical volume per neuron in Lesion-MC was not significantly different in comparison to either Sham-MC or Lesion-AC. The overall cortical volume of the HLsmc region mirrored the increases in cortical volume per neuron (see Results). B, as with cortical volume increases, Lesion-AC rats showed a significant increase in the volume of dendritic processes per neuron in comparison to Sham-AC. Dendritic volume was not significantly increased in Lesion-MC. C, there were no significant group differences in the number of synapses per neuron in this region. Data are means ± SEM; *P $<$ 0.05.

performed to test for the effects of the lesion. Lesion-Acrobat vs Lesion-Motor Control comparisons were performed to test whether acrobatic training altered the lesion effects. Lesion-Acrobat vs Sham-Acrobat comparisons were performed to assess whether lesions altered acrobatic training effects. Only one hemisphere was analyzed for each sham-operated animal and these data were used for the comparisons with both ipsilateral and contralateral hemispheres of lesion animals.

RESULTS

Anatomical Changes in the Hindlimb Sensorimotor Cortex of the Intact Hemisphere

Changes in HLsmc region volume. In sham-operated animals, acrobatic training failed to result in a significant increase in the volume of the HLsmc in comparison to Sham-MC. The mean volume ± SEM was 60.32 ± 0.93 mm^3 in Sham-AC vs 62.39 ± 1.55 mm^3 in Sham-MC. In animals with unilateral FLsmc lesions, the volume of the HL region of the intact sensorimotor cortex tended to be elevated relative to shams. The mean volume was 62.88 ± 1.28 mm^3 in Lesion-MC and 63.76 ± 0.80 mm^3 in Lesion-AC. The volume in Lesion-AC was significantly increased relative to Sham-AC ($F(1,34) = 4.15$, $P < 0.05$). However, the volume was not significantly increased in Lesion-MC vs Sham-MC ($F(1,34) = 0.08$, $P > 0.05$) nor were the two lesion groups significantly different.

Changes in neuronal density. As shown in Fig. 3A, layer II/III volume per neuron (the inverse of neuronal density) in the HLsmc opposite the lesion mirrored the cortical volume measurements. The greatest cortical volume per neuron was found in Lesion-AC and this was significantly increased relative to Sham-AC ($F(1,36) = 10.06$, $P < 0.005$). Lesion-MC animals tended to show an increase in volume per neuron, but this was not significantly different from Sham-MC ($F(1,36) = 1.30$, $P > 0.05$). There was no significant difference between Lesion-AC and Lesion-MC, nor between Sham-AC and Sham-MC. Together with the cortical volume data, these data indicate that the reduction in neuronal density in the HLsmc opposite the lesion in Lesion-AC is a result of an expansion of neuropil volume and that this expansion is greatest in animals receiving acrobatic training after FLsmc lesions in comparison to other groups.

Changes in dendrites. As shown in Fig. 3B, although there were no significant effects of either lesions or acrobatic training alone, Lesion-AC rats showed a significant increase in dendritic volume per neuron in comparison to Sham-AC ($F(1,36) = 5.74$, $P <$ 0.05). Lesion-MC rats showed dendritic volume per neuron means which were intermediate between Lesion-AC and Sham-MC, but which were not significantly different from either group. There were no significant changes in dendritic volume fraction (% of tissue volume occupied by dendrites) for any comparison. The volume fraction of dendritic processes was 24.32 ± 0.83% in Sham-MC, 23.98 ± 0.63% in Sham-AC, 24.52 ± 1.03% in Lesion-MC, and 24.20 ± 1.16% in Lesion-AC.

Changes in synapses. Although mean synapse number per neuron values tended to greater in the intact HLsmc in lesion groups and in Sham-AC relative to Sham-MC (Fig. 3C), this measure was not significantly different in any planned comparison. There were also no significant differences in synaptic density

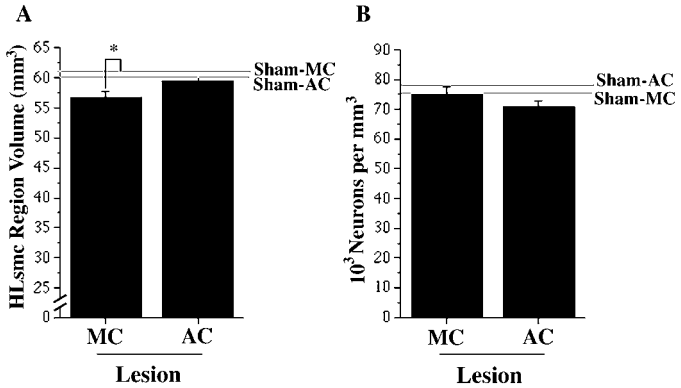

FIG. 4. Structural measures in the HLsmc ipsilateral to FLsmc lesions. A, unilateral FLsmc lesions resulted in a significant loss of volume in the HLsmc in Lesion-MC rats but not in Lesion-AC rats in comparison to sham groups of the same training conditions. B, neuronal density was not increased in Lesion-MC which suggests a loss of neurons given the reduction in overall cortical volume. In Lesion-AC, neuronal density was not significantly reduced in comparison to shams although mean values were lower. An approximation of total neuronal number is shown in Table 1. Data are means ± SEM; *$P < 0.05$.

for any planned comparison. Synaptic density in 10^8 synapses/mm^3 was 10.97 ± 0.84 in Sham-MC, 11.93 ± 0.73 in Sham-AC, 11.66 ± 0.41 in Lesion-MC, and 10.68 ± 0.68 in Lesion-AC.

Anatomical Changes in the Hindlimb Sensorimotor Cortex of the Damaged Hemisphere

Changes in volume. Lesion-MC animals had a significant decrease in cortical volume in the HLsmc contiguous to the lesion when compared to Sham-MC ($F(1,33) = 12.15$, $P < 0.002$; Fig. 4A). In contrast, the cortical volume of this region in Lesion-AC rats was not significantly different from Sham-AC ($F(1,33) = 0.25$, $P > 0.05$). The mean HLsmc volume in the Lesion-AC was elevated but not significantly increased when compared to Lesion-MC ($F(1,33) = 2.61$, $P > 0.05$).

Changes in neuronal density. There were no significant differences in layer II/III neuronal density in the HLsmc contiguous to the lesion for any planned comparison (Fig. 4B). There was, however, a nonsignificant tendency for Lesion-AC rats to have decreased layer II/III neuronal density in comparison to Sham-AC ($F(1,36) = 3.07$, $P = 0.09$). The neuronal density in the Lesion-MC group was similar to Sham-MC. The maintenance of normal neuronal density in Lesion-MC given the significant reduction in cortical volume is suggestive of a net loss of neurons. The tendency to reduce neuronal density in Lesion-AC in the absence of elevations in cortical volume is also suggestive of a loss of neurons in this group in comparison to shams. However, in Lesion-MC, in contrast to Lesion-AC, neurons appear to be lost together with neuropil volume.

An approximation of neuronal number was obtained by calculating the product of cortical volume and neuronal density. Although not an accurate estimate of the total number of neurons in the HLsmc region (because volume measures included all cortical layers), this measure is expected to be sensitive to the magnitude of neuronal loss in layer II/III. As shown in Table 1, this measure, reported in 10^5 neurons, reveals a moderate lesion-induced reduction in the number of neurons in this cortical region and this reduction was similar between the two lesion groups. There were no significant differences in this neuronal number estimate between the two lesion groups nor between the two sham groups. The Lesion-Acrobat group was significantly different from Sham-AC ($F(1,36) = 4.31$, $P < 0.05$) and Lesion-Motor Controls approached significance in comparison to Sham-MC ($F(1,36) = 4.04$, $P = 0.052$).

Changes in dendrites. As shown in Table 1, there were no significant differences in the average dendritic volume per neuron in layer II/III in the HLsmc contiguous to the lesion for any planned comparison. Approximation of the total dendritic volume (the product of layer II/III dendritic volume fraction and HLsmc region cortical volume) tended to be lower in lesion groups than in sham groups, but there were also no significant differences in these values. Dendritic volume fraction was similar between groups (% of tissue occupied by dendrites: 24.65 ± 0.75% in Lesion-MC

TABLE 1

Structural Changes in the HLsmc of the Lesion Hemisphere

	Sham-MC	Sham-AC	Lesion-MC	Lesion-AC
10^3-μm^3 dendritic volume per neuron	(Figure 3B)		3.35 (0.19)	3.16 (0.23)
10^3 synapses per neuron	(Figure 3C)		14.64 (1.10)	15.80 (0.98)
10^5 neurons[a]	46.86 (1.54)	46.73 (2.11)	42.14 (1.15)	42.09* (1.15)
Dendritic volume (mm^3)[a]	15.19 (0.70)	14.46 (0.44)	14.02 (0.63)	13.10 (0.74)
10^9 synapses[a]	67.61 (4.25)	72.27 (5.22)	61.57 (4.80)	66.14 (5.06)

Note. Data are means (±SEM). MC, motor control; AC, acrobat condition.

[a] These data are the product of cortical volume measured in layers I–VI and measures of neuronal density, dendritic volume fraction, and synaptic density, respectively, obtained from layer II/III.

* $P < 0.05$ significantly different from Sham-AC.

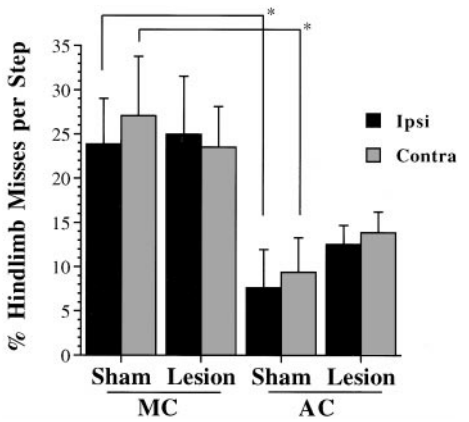

FIG. 5. Footfault test performance. This test was administered after the last day of training (on Day 29 postoperative) to measure coordinate limb placement during locomotion. Acrobatic training (AC) in sham animals significantly decreased hindlimb placing errors on this task in comparison sham motor controls (MC). Lesion-AC tended to make fewer errors than Lesion-MC (see Results). Data are means ± SEM; $*P < 0.05$.

and 22.06 ± 1.27% in Lesion-AC). (Sham values for dendritic volume fraction are reported above.)

Changes in synapses. There were no significant differences in the average number of synapses per neuron in Layer II/III of the HLsmc (Table 1) for any planned comparison. The product of layer II/III synaptic density and HLsmc region cortical volume (an approximation of synapse number) tended to be lower in lesion groups than in sham groups, but there were no significant differences in these values. Synaptic *density* was also similar between groups. Synaptic density (10^8 synapses per mm^3) was 10.87 ± 0.84 in Lesion-Motor Controls and 11.08 ± 0.79 in Lesion-Acrobats. (Sham values for synaptic density are reported above.)

Footfault Test Performance Using the Hindlimbs

As shown in Fig. 5, Acrobatic training significantly improved sham-operated rats' performance on the footfault test using the hindlimbs in comparison to Sham-Motor Controls ($F(1,39) = 9.33$, $P < 0.005$, left and right hindlimb data pooled). There was a nonsignificant tendency for acrobatic training to improve performance of the hindlimb ipsilateral to the lesion (the nonimpaired side; $F(1,39) = 3.56$, $P < 0.07$) and a less notable tendency for the contralateral-to-the-lesion hindlimb ($F(1,39) = 2.20$, $P = 0.12$). When the ipsilateral and contralateral footfault data were pooled, the tendency for Lesion-AC rats to have fewer total hindlimb errors than Lesion-MC approached significance ($F(1,39) = 3.93$, $P = 0.055$). There were no significant differences between Lesion-MC and Sham-MC in performance with either hindlimb.

DISCUSSION

Acrobatic training after unilateral FLsmc lesions affected the structure of the HLsmc of both hemispheres. In the HLsmc opposite the lesions, acrobatic training resulted in increases in cortical and dendritic volume that were not found as a result of the lesion or the training alone. In Lesion-AC there was an increase in total HLsmc region cortical volume, in layer II/III neuropil volume per neuron, and in dendritic volume per neuron in comparison to Sham-AC. In rats receiving simple repetitive exercise after the lesions (Lesion-MC), these variables showed only nonsignificant elevations in comparison to Sham-MC. No significant differences in synapse number per neuron were detected between these groups. Thus, in contrast to previous findings of major structural changes in the contralateral homotopic motor cortex (e.g., 21), FLsmc lesions may normally produce, at most, very subtle structural changes in the contralateral (heterotopic) HLsmc. Acrobatic training after the lesions may cause a modest enhancement of these structural changes, resulting in significant changes relative to intact animals. In the HLsmc ipsilateral to the lesion, acrobatic training appeared to reduce a lesion-associated loss of cortical volume. Lesion-MC animals, and not Lesion-AC, had a significant decrease in HLsmc region cortical volume in comparison to shams. Thus, motor skills training after FLsmc lesions may either spare the loss or promote the recovery of tissue volume in perilesion cortex. However, this greater volume did not include a greater number of neurons nor were there compensatory increases in synapse number or dendritic volume per neuron in Lesion-AC rats in comparison to shams.

The greater loss of HLsmc volume in Lesion-MC rats is unlikely to have resulted from a greater initial lesion size in this group relative to Lesion-AC. Reconstructions of the lesion based on cytoarchitectural delineation of cortical subregions indicated that there were no systematic differences in the placement or extent of the forelimb area lesions in acrobatic versus motor control rats (19). Most lesions did produce direct damage to the rostral extension of the HLsmc which borders the FLsmc but this lesion border region was avoided by the neuronal sampling strategy in the present study. A thinner appearance of the perilesion HLsmc relative to the intact hemisphere was apparent in many of the Lesion-MC brains (e.g., Fig. 1). It seems likely that the loss of volume and (in both lesion groups) of neurons in the present study largely reflects degenerative effects secondary to the lesion.

The motor skills training effects in the HLsmc of the lesion hemisphere are important in light of recent findings suggesting that it is possible to exaggerate perilesion tissue damage and worsen functional outcome using manipulations of postinjury behavioral experience. Fitting rats with vests which forced reliance on the

impaired forelimb during the first week after unilateral FLsmc lesions worsened the function of the impaired limb and increased the size of the lesion (16, 30). Administration of an NMDA receptor antagonist blocked these effects suggesting that they may result from a use-related exaggeration of excitotoxicity (15). Risedal *et al.* (39) have also recently found that postinjury behavioral manipulations can worsen the damage produced by middle cerebral artery ligation in spontaneously hypertensive rats. Rats that were housed in a complex environment beginning 24 h after the ligation and received, in addition, daily motor skills training had a larger infarct volume in comparison to animals with either no, or a later onset of, behavioral manipulation. In the present study, acrobatic training following damage to the FLsmc did not result in a greater lesion extent or in a further loss of neurons, synapses, or dendrites in the HLsmc contiguous to the injury, it clearly did not worsen function as measured by the footfault task, and it reduced a lesion-associated loss of volume in the HLsmc region. Performance using the forelimbs on the footfault test in these animals has also previously been found to be improved by acrobatic training (19). Thus, it is possible to undertake rehabilitative training which enhances functional outcome and which clearly does not sacrifice perilesion cortex. The behavioral manipulations in the present study were introduced gradually over the first several days after the injury and they never required that the rats spend more than 25 min training per day. It may be that this training procedure avoids a vulnerable time period and/or that the net use of impaired extremities is simply much reduced in comparison to the procedures which have been found to exaggerate injury extent.

A question raised by the present results is the functional efficacy of the cortical volume which is spared (or restored) by acrobatic training after lesions. The greater volume of the ipsilesional HLsmc in acrobatic rats in the present study did not obviously reflect a greater number of neurons, synapses, nor volume of dendritic material and may instead be composed of, e.g., a greater volume of astrocytes and/or other glial cells. Of potential importance is whether this experience-dependent structural effect has any relationship to plasticity of perilesion representations as mapped by microstimulation and electrophysiological studies. Using microstimulation mapping, Nudo and colleagues have shown that very focal damage to digit representations of the motor cortex of monkeys causes surrounding undamaged representation regions to reorganize (33). Motor training involving heavy use of the digits after injury prevented a secondary loss of hand representation territory in perilesion cortex and allowed some of the directly damaged hand representation to be recovered (34, 36). This reorganization was coupled with improved skilled use of the impaired hand. In rats with bilateral FLsmc lesions, training on a forelimb lever pressing task using ventral tegmental area stimulation as a reinforcer resulted in the appearance of a forelimb representation in the rostral–lateral portion of the HLsmc bordering the FLsmc (7). This region is more rostral and lateral than the HLsmc sample placement used in the present study (outlined in Fig. 1), but it is nevertheless possible that subregions of the hindlimb representation area examined in the present study had undergone reorganization. One potential issue is whether greater regional volume and greater dilution of surviving neurons can contribute to the representational shifts revealed by microstimulation or electrophysiological mapping. These issues highlight the need to assess the relationship between training-dependent structural differences and training-dependent plasticity of maps in perilesion cortex. Kleim *et al.* have recently found that motor cortical map plasticity and synaptogenesis coincide following reach training in intact rats (26). Whether there is a similar relationship between perilesional plasticity of maps and synapses remains to be determined.

The failure to find synaptogenesis or dendritic growth in the HLsmc of intact rats (Sham-AC) indicates that this region does not display the type of training-induced structural plasticity that has been found in the FLsmc. Acrobatic training results in an increase in synapse number per neuron, in increases in perforated synapses, and in multiple synaptic boutons in the FLsmc (19, 27). This training also results in an increase in parallel fiber synapses per Purkinje neuron in the paramedian lobule of the cerebellum (28). In the present study, there were no significant effects of acrobatic training alone on HLsmc volume, neuronal density, the number of synapses per neuron, nor the dendritic volume per neuron. It is unlikely that the time point examined (28 days) was too early in the course of training to detect synaptic changes because increases in synapse number per neuron have been found as early as 10 days after the onset of acrobatic training in layer II/III of the FLsmc of female rats (27). Furthermore, rats clearly demonstrated learning in the use of the hindlimbs. Over days of training, an increase in the speed of task completion was coupled with a decrease in the number of both forelimb and hindlimb errors (footslips) per trial (19). Acrobatic training also resulted in improved coordinated placing using the hindlimbs as measured on the footfault test (Fig. 5). However, in observations of acrobatic task performance it seemed obvious that the learned movements required for fore- vs hindlimbs were quite different in quality. Rats appeared to use coordinated forelimb movements to guide forward locomotion (e.g., limb placement) on the task whereas hindlimbs appeared to be primarily involved in maintaining balance and whole-body postural stability. The relative contribution of motor cortical versus, e.g., cerebellar, plasticity to this type of motor skills learning is currently far from clear.

Even in Lesion-AC rats, the significant increases in neuropil volume and dendritic volume per neuron in the HLsmc opposite the lesion were subtle. Furthermore, changes in the number of synapses per neuron were not detected. Thus, the structural changes in the HLsmc are clearly less robust than the structural changes which have been found to occur in the neighboring FLsmc (e.g., 18, 19, 21). In the FLsmc, lesions of the opposite cortex result in a major (approximately 23%) increase in synapse number per neuron (19, 21) and acrobatic training after the lesions results in a further increase (19). The possibility that changes in HLsmc resulting from the lesion are delayed in comparison to changes in the FLsmc cannot be ruled out by the present results. Conceivably, the increases in dendritic volume per neuron found in the HLsmc of Lesion-AC rats might correspond to a transient stage of dendritic overgrowth which has been found to normally precede synaptogenesis in the cortex opposite FLsmc lesions. At 18 days after FLsmc lesions, increases in dendritic volume per neuron but not in synapse number per neuron were found in the contralateral homotopic FLsmc (21). Increases in FLsmc synapse number per neuron have been found at 30 days after the lesion (19, 21), the time point examined in the present study. An alternative possibility is that the degree to which a cortical region is denervated by the lesions and driven by behavioral change plays a critical role in the magnitude of lesion-induced neural restructuring. Unilateral FLsmc lesions result in a depletion of transcallosal afferents to the contralateral homotopic motor cortex (6). They also result in deficits in the use of the contralateral forelimb which leads the animals to develop compensatory reliance on the nonimpaired forelimb (23). Recently, denervation of transcallosal afferents via corpus callosum transections in rats that were forced to rely on a single forelimb was found to reproduce the type of dendritic growth which has been found in the forelimb region of the motor cortex opposite unilateral FLsmc lesions (6). Neither denervation alone nor forced forelimb use alone were sufficient to produce dendritic growth. It may be that the contralateral HLsmc is not sufficiently denervated by FLsmc lesions and/or not sufficiently driven by lesion-induced or training-induced behavioral change to show the same magnitude of structural plasticity as found in the forelimb region opposite the lesion. Nevertheless, the present results suggest that motor skills training after FLsmc lesions heightens structural plasticity in this nonhomotopic region of the contralateral cortex. Conceivably, this enhanced plasticity might represent a greater recruitment of this cortical region for involvement in the development of compensatory motor skills.

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