Unilateral ischemic sensorimotor cortical damage in female rats: forelimb behavioral effects and dendritic structural plasticity in the contralateral homotopic cortex

Rachel P. Allred\textsuperscript{a}, Theresa A. Jones\textsuperscript{a,b,*}

\textsuperscript{a}Psychology Department, The University of Texas, Austin, TX 78712, USA
\textsuperscript{b}Institute for Neuroscience, The University of Texas, Austin, TX 78712, USA

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Abstract

Previous studies in male rats with unilateral sensorimotor cortical (SMC) damage have demonstrated dendritic structural plasticity in the contralateral homotopic cortex and an enhancement of skilled reaching performance in the forelimb ipsilateral to the lesion compared to sham-operated rats. The purpose of this study was to determine if these findings could be replicated in an ischemic lesion model in female rats. Female rats were given sham operations or unilateral ischemic (endothelin-1 induced) damage in the forelimb representation area of the SMC opposite their preferred forelimb. Animals then received either 20 consecutive days of training on a skilled reaching task with the non-preferred/unimpaired forelimb or no-training control procedures. The surface density of dendrites immunoreactive (IR) for microtubule-associated protein 2 (MAP2) was then measured in the motor cortex opposite the trained limb and/or lesion. Female rats with sufficiently large, but not very small, lesions performed better with the unimpaired forelimb than sham-operated rats on the reaching task. The post-lesion reaching performance was not found to be significantly dependent upon estrous stage at the time of surgery, in agreement with previous studies that failed to find sex or sex-hormone effects after other types of SMC damage. Additionally, there were major laminar-dependent increases in the surface density of MAP2 IR dendrites in the cortex opposite lesions and trained limbs. These findings in female rats are consistent with the dendritic and behavioral changes previously found in male rats. They extend these previous findings by indicating that lesion size is an important variable in the enhancement of reaching performance.

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Introduction

Rats given unilateral sensorimotor cortical (SMC) damage in the forelimb representation region develop impairments in the forelimb contralateral to the damage and a compensatory increased reliance on the unimpaired forelimb (e.g., Jones and Schallert, 1992). Recently, focal unilateral electrolytic (Bury and Jones, 2002) or ischemic (Luke et al., in press) SMC lesions in male rats were also found to enhance the acquisition of a motor skill with the unimpaired forelimb. This was evident in studies employing a unilateral skilled reaching task, in which animals extend one forelimb through a window to grasp and retrieve palatable food pieces. The behavioral changes in the unimpaired forelimb have been linked to cellular and structural changes in the contralateral homotopic motor cortex, including increased dendritic arborization (Jones and Schallert, 1992; 1994; Jones et al., 1996) and synapse number per neuron (Bury et al., 2000; Jones, 1999; Jones et al., 1996, 1999; Luke et al., in press). For example, unilateral electrolytic SMC lesions result in an increase in dendrites immunoreactive (IR) for microtubule-associated
protein 2 (MAP2) in layer V of the motor cortex opposite the lesions compared with sham-operated rats (Adkins et al., in press; Bury and Jones, 2002). Skilled reach training after the lesions further increased MAP2 IR dendrites (Bury and Jones, 2002). Increases in neuronal growth- and synaptogenesis-associated proteins (Cheng et al., 1997; McNeill et al., 1999; Stroemer et al., 1995), in measures of excitability and excitatory neurotransmitter activity (Redecker et al., 2002; Witte et al., 2000) and in the supply of crossed corticostriatal connections (Carmichael and Chesselet, 2002; Uryu et al., 2001) have also been reported contralateral to unilateral neocortical lesions in rats.

Because these previous studies were performed in male rats, whether the forelimb behavioral effects and contralateral neuronal structural effects of SMC lesions extend to female rats was unknown. Previous studies have demonstrated neuroprotective effects of estrogen and progesterone following brain injury (for reviews see Roof and Hall, 2000; Stein, 2001). For example, normally cycling and pseudo-pregnant (high in progesterone) females had reduced cerebral edema following bilateral medial frontal cortex contusions compared with male rats, an effect attributed to circulating progesterone (Roof et al., 1993a; see also Roof et al., 1996). Progesterone treatment in male rats also significantly reduced infarct volume following middle cerebral artery occlusions (Jiang et al., 1996) and improved Morris water maze performance after medial frontal cortical contusions (Roof et al., 1994) compared to controls. Following electrolytic entorhinal cortex lesions, male rats had more impaired water maze performance than female rats that received the lesions during proestrus, when estrogen and progesterone hormone levels are both high (Roof et al., 1993b). However, female sex hormones are not always linked to improved behavioral performance after brain damage. Grossman and Stein (2000) found that, following contusion to the forelimb representation area of the SMC, normally cycling and pseudo-pregnant females did not differ from males in functional recovery of forelimb sensorimotor asymmetries. Goldstein and Bullman (1999) found that age, but not sex, was a determinant of motor functional recovery, as measured on the beam walking task, following SMC suction-ablation. Thus, there is not an established role of sex and female sex hormones in the effects of damage to the SMC.

The goals of the present study were to determine (1) if female rats given focal unilateral ischemic lesions of the forelimb representation area of the SMC would have enhanced reaching ability with the unimpaired forelimb on a skilled reaching task, the single pellet retrieval task, compared to sham-operated controls, (2) whether these lesions result in dendritic structural plasticity in the contralateral homotopic motor cortex, and if so, (3) whether motor skills training after the lesion would further enhance the dendritic changes. Ischemic lesions were induced using cortical surface application of the potent vasoconstrictor, endothelin-1 (Fuxe et al., 1997), and were made opposite the forelimb the animals preferred to use for the reaching task. Estrous stage at the time of surgery was monitored to assess its effects on lesion extent and behavior. Lesion- and sham-operated rats were then either trained for 20 consecutive days on the pellet-retrieval task with their non-preferred/unimpaired forelimb or received no-training control procedures. Changes in dendrites in the motor cortex contralateral and homotopic to the lesion and/or trained limb were assessed by quantification of the surface density of dendritic processes immunostained for MAP2, a cytoskeletal structural protein localized to neuronal soma and dendrites (Itoh et al., 1997; Sánchez et al., 2000).

Materials and methods

Animals

Sixty-three female Long-Evans hooded rats were obtained from Charles River Laboratories at approximately 1 month of age and used in the experiment between 3 and 4 months of age. Animals were housed 2–3 to a cage in standard laboratory conditions on a 12:12 h light/dark cycle. Animals were handled frequently beginning approximately 2–3 weeks before the experiment. Animals were moderately food restricted and body weights were reduced to 85–95% of initial weights before the onset of behavioral training. Mild food deprivation such as that employed in the present study has previously been demonstrated to not affect the estrous cycle of this strain of rat (Tropp and Markus, 2001). Animals were assigned to the following groups: animals receiving training on the single pellet retrieval task after unilateral SMC lesions (Les + Train, n = 21) or sham operations (Sham + Train, n = 20) and animals receiving no training control procedures after the lesions or sham operations (Les + Control and Sham + Control, n = 11). Surgical and behavioral procedures were performed in four rounds of approximately three to five rats per each of the four groups with the exception of Round 1, which excluded no-training control groups (n = 7, Les + Train, Sham + Train). For each round, animals were randomly assigned to groups after matching for pre-operative reaching task performance (described below). The protocol for this study was approved by the University of Texas at Austin Animal Care and Use Committee.

Surgical procedures

Unilateral ischemic lesions aimed at the forelimb representation area of the SMC were made using topical application of ET-1 (Adkins-Muir and Jones, 2003; Adkins et al., in press; Fuxe et al., 1997). Before surgical procedures, rats were anesthetized with Equithesin (approximately 140 mg/kg chloral hydrate and 35 mg/kg sodium pentobarbital). The skull was removed between 0.5 mm...
posterior and 1.5 mm anterior to bregma and between 3.0 and 4.5 mm lateral to bregma. The dura underlying the craniectomy was removed and ET-1 (80 μM) was applied to the cortical surface using a Hamilton microsyringe. The surgical site was then left undisturbed for 10 min before suturing. The quantity of ET-1 application was 2.0 μl (n = 18), 2.4 μl (n = 7), or 3.0 μl (n = 7). The ET-1 solutions used were from two different batches, which varied in age and storage conditions, and the quantities administered were not predictive of lesion size in this study. (The average for the small and large lesion groups described below was 2.53 and 2.05 μl, respectively.) Post-operative atropine sulfate (between 2.5 and 5 mg/kg) was administered to counterbalance the depressive effects of Equithesin on respiration. Rats receiving sham operations were treated the same as lesion-operated rats up to, but excluding removal of the skull. Previous findings have demonstrated that skull removal can result in behavioral asymmetries (Adams et al., 1994).

**Skilled reach training**

The single pellet-retrieval task was carried out in a Plexiglas reaching chamber (26 cm long by 34-cm high by 16-cm wide) with a tall narrow window (1-cm wide and 23-cm high) in the center of the 16-cm-wide wall. Animals were trained to reach with a forelimb through this window for 45 mg banana flavored food pellets (Bioserve Inc., Frenchtown, NJ) which were placed in one of two shallow wells on a block approximately 3 cm in height. The wells were centered with the left and right edges of the window at a distance of 1 cm from the window. A small metal rod approximately 2 mm in diameter adhered to the base of the reaching window created a barrier that prevented animals from scraping the pellets into the chamber and also reduced attempts to use the tongue to retrieve pellets. During training, a Plexiglas wall was inserted into the reaching chamber ipsilateral to the animal’s reaching limb and pellets were placed in the wells opposite the reaching limb (Fig. 1). This wall effectively forced the animals to use the forelimb chosen by the experimenter for the reaching task. In the initial design of the apparatus (used for Round 1), the inner chamber wall was placed at a distance of 1.5 cm from the reaching window. This was subsequently changed to a distance of 0.5 cm. (It was hypothesized that this would better fit the body size of the female rats, but this failed to significantly affect animals’ reaching success.)

Before surgery, animals were shaped on the pellet-retrieval task to determine a limb of preference. Previous studies have shown that rats have limb preferences which are task dependent (Greenough et al., 1985; Peterson, 1934; see also Whishaw, 1992). Shaping procedures typically lasted from 4 to 7 days. For each day of shaping, animals were placed in the reaching chamber without the Plexiglas wall for 10 min. When an animal made 20 consecutive reach
attempts with one limb on 1 day and 10 consecutive attempts with the same limb on the following day, this limb was defined as the preferred limb. Once animals reached these criteria, pre-operative shaping ceased. Five animals (not included in the n’s above) did not achieve shaping criteria and were excluded from the study. Four days after surgery, animals were trained on the pellet-retrieval task with their previously non-preferred limb for 20 consecutive days. In animals with lesions, the trained limb was also the unimpaired forelimb ipsilateral to the lesion. Animals were trained on 60 trials or a cutoff time, which ever came first. The cutoff time was reduced from 20 (round 1) to 10 min (rounds 2–4) because it was found that most animals finished the 60 trials within 11 min or less on most days of training. A reaching trial consisted of the animal either successfully grabbing the pellet and bringing it directly to its mouth (success), dropping the pellet before bringing it to its mouth, failing to grasp the pellet after five reaches or knocking the pellet out of its well. At the end of each reaching trial, a pellet was dropped into either the front or the back of the reaching chamber to “re-set” the animals and so that a new pellet could be placed into its appropriate well. Pellet-retrieval task performance was calculated by dividing the total number of successful reaches by the total number of reach attempts. No-training controls were yoked to the trained animals on each day of training and were placed in a reaching chamber with a Plexiglas wall ipsilateral to what would be their trained limb. The no-training control animals had pellets dropped into the reaching chamber at approximately the same rate as the trained animals received pellets. All training took place during the animals’ light cycle.

Measurement of forelimb asymmetry

The Schallert cylinder test (Schallert et al., 1997, 2000) was used as an assay of lesion-induced asymmetries in forelimb postural-motor behavior (Fig. 2). Placing rats in a cylinder (19 cm diameter) encourages upright exploratory movements during which asymmetrical forelimb use can be sensitively detected. Animals were filmed in the Plexiglas cylinder for 2 min at five different post-lesion time-points. Animals from Round 1 were filmed in the cylinder only on the day of perfusions and cylinder data from these animals were not included in the statistical analyses. From slow-motion playbacks of each session, the first 30 instances of sole use of either forelimb (ipsilateral or contralateral to the lesion) or simultaneous bilateral forelimb use for upright support against the cylinder wall were recorded. The forelimb asymmetry score was calculated using the formula: (total ipsilateral limb use + 1/2 bilateral) / total limb use × 100.

Estrous cycle monitoring

All animals’ estrous stage was measured at the time of surgery. In a subset of animals (n = 31), estrous cycle was also monitored at several time-points throughout training. Vaginal smears were collected by inserting a sterile cotton swab, soaked briefly in distilled water into the animal’s vagina, and rotating before withdrawing. The cotton swab was then smeared onto a slide and the smear was stained with Toluidine blue. Estrous stage was determined through examination of the vaginal cytology under a light microscope. Diestrus stages 1 and 2 were combined in this study and rats were therefore classified as being in estrus, diestrus, or proestrus at time of the vaginal smear.

Histology and immunocytochemistry

On day 22 post-lesion, animals were given an overdose of sodium pentobarbital and transcardially perfused with 0.1M sodium phosphate buffer followed by a fixative solution (4% paraformaldehyde solution in the same buffer). Brains were removed, placed in fixative solution and sliced within 48 h. Six rostral–caudal sets of 50-μm thick coronal sections were taken throughout the cerebrum and then stored in cryoprotectant solution at −4°C before use. One set of sections was Nissl stained with Toluidine blue and used for lesion verification and measures of the volume of the remaining cortex. Another set of sections was processed using a free-floating immunocytochemistry method for microtubule-associated protein 2 (MAP2). Sections were first placed at room temperature in 0.3% hydrogen peroxide in 0.01M phosphate-buffered saline solution (PBS) for 30 min to inactivate endogenous peroxidase activity. Sections underwent several PBS washes and then were incubated in a block solution at room temperature for 2 h to prevent non-specific protein binding. The block solution included 0.2% Triton X-100,
0.1% bovine serum albumin and 2% horse serum in PBS. Sections were then rinsed in PBS and incubated in primary antibody solution at 4°C for 48 h. The primary antibody was anti-MAP2 (clone AP-20, 1:500, Sigma, St. Louis, MO, USA) which binds the higher molecular weight forms of MAP2 (2a + 2b). Following the 48-h primary antibody incubation, sections were rinsed several times in PBS and then placed in secondary antibody (1:200 biotinylated antirabbit IgG made in horse; Sigma) in 2% horse serum in PBS for 1 h at room temperature. After this incubation, sections were rinsed several times in PBS and incubated at room temperature for 2 h in a biotinylated horseradish peroxidase–avidin complex (ABC kit, Vector Laboratories, Burlingame, CA). MAP2 immunoreactivity was then visualized using 3-3′ diaminobenzidine tetrahydrochloride with nickel ammonium sulfate, a standard intensification process. Specificity of antibody binding was verified with tissue sections incubated without anti-MAP2 (no primary controls). These sections were present in each immunocytochemical run and did not contain evidence of distinct process staining. Each run of immunocytochemical processing included tissue from all groups to decrease the contribution of batch effects to the variability in immunocytochemistry staining (tissue from Round 1 was processed with tissue from Round 4).

**MAP2 quantification**

The surface density of MAP2 IR dendritic processes in layers II/III and V of the motor cortex (lateral agranular region) contralateral to lesions and trained limbs was measured using the cycloid-grid intersection method (Baddeley et al., 1986). Sections were coded so that the experimenter was blind to experimental groups. Systematic random sampling (Gundersen et al., 1988) was used to obtain five samples in each of three sections per brain. A cycloid grid reticle was placed in the left eyepiece of a Nikon Optiphot-2 light microscope and samples were viewed using a 100× oil immersion objective at a final magnification of ×1250. For each layer, the dorsal peak of the corpus callosum in each section was positioned just medially to the starting position. For each section, a random number table was then used to move the sample field laterally between 0 and 250 μm to take the first sample. Four more samples per section were then taken moving the sample field in steps of 250 μm laterally for each. One plane of focus was chosen per sample and intersections between the cycloid grid lines and MAP2 IR processes were counted. MAP2 IR somata were not included in the counts. All samples were aligned such that the apical dendrites were perpendicular to the minor axes of the cycloid arcs (i.e., apical shafts were used to define local vertical windows). MAP2 IR process surface density (Sv) was calculated using the formula $Sv = 2 \left( \frac{I}{L} \right)$ where $I = \text{total number of intersections summed across all samples per brain}$ and $L = \text{total cycloid test line length}$. In the Sham + Control group, the hemisphere measured was contralateral to what would have been the trained limb (and ipsilateral to the preoperatively preferred forelimb).

**Lesion analyses**

In brains with lesions, volume measures of remaining cortex were used as an indirect measure of lesion size. Area measures of remaining cortex in 50-μm-thick Nissl-stained coronal sections within the SMC region were obtained using Neurolucida perimeter tracing software (Microbrightfield Inc.) at a final magnification of ×17. The rostral most section traced was chosen based on the appearance of the corpus callosum (approximately 2.7 mm anterior to bregma). Moving caudally, six additional sections, each 600 μm apart, were then traced for a total of seven tracings. Volume was calculated using the Cavalieri method (Gundersen et al., 1988) as the product of the summed areas and the distance between section planes (600 μm). In addition to measurement of cortical volume, lesion extent and placement were reconstructed onto schematic templates of cortical coronal sections. Reconstructions within lesion groups were then overlaid onto one template and outer boundaries and shared regions of damage were outlined.

**Statistical analyses**

SPSS (SPSS, Inc.) repeated-measures analyses of variance (ANOVAs) were employed to examine reaching data across days, the effects of estrous stage on reaching success and cylinder test asymmetry score over post-operative days. Simple contrasts and one-way ANOVAs were used when needed to further analyze estrous stage data. Bivariate correlations were used to assess the relationship between average reaching success (pooled over reaching days 3–20) and average asymmetry score (pooled over 5 post-operative time-points).

The surface density of MAP2 IR processes in each layer was analyzed using SPSS general linear model for univariate analysis of variance for a priori-planned comparisons for the effects of (1) the lesion: Les + Train and Les + Control animals were combined and compared to Sham + Train and Sham + Control animals, (2) the training: Les + Train and Sham + Train were compared to Les + Control and Sham + Control, (3 and 4) lesion and training combined: Les + Train animals were compared to Les + Control animals and to Sham + Train animals. The behavioral and anatomical data from the first round of animals (which were trained in a reaching chamber with different dimensions than the other rounds and without a complement of no-training controls) were included in all statistical analyses because there were no significant differences compared with the trained animals of the other rounds.
**Results**

**SMC lesion extent and placement**

Based on the reconstructions of lesion placement and extent, all lesions appeared to produce major damage to the forelimb representation area of the SMC (Fig. 3). Measurements of the volume of the remaining SMC revealed a similar lesion size between trained and untrained animals. The mean ± SEM volume of remaining cortex in mm$^3$ was 82.64 ± 1.16 in the Les + Train group and 80.04 ± 2.05 in the Les + Control group. However, within the group of trained animals, an effect approaching significance was found when volume measurements were correlated with reaching performance (pooled across training days 3–20; $r = -0.331$, $P = .072$, one-tailed) with animals with larger lesions having a tendency to outperform those with smaller lesions on the reaching task (Fig. 4). Because of this trend, lesion groups were subdivided based on remaining SMC volume. A cutoff value of 80 mm$^3$ was chosen as approximately half of each lesion group fell above this mark and approximately half fell below. Although all lesions in this study can be considered small cortical lesions, for convenience, the two lesion sizes are called “sm” for small and “lg” for large. The large lesion category included nine trained (lgLes + Train) and six untrained rats (lgLes + Control). The small lesion category included 12 trained (smLes + Train) and five untrained (smLes + Control) rats. Further statistical analyses for behavior and estrous stage data were based on these sub-categories. Two animals in the lgLes + Train group had penetration of callosal white matter and superficial dorsal striatal damage but the remaining lesions had no evidence of direct damage to striatum.

**Lesion effects on skilled reaching with the unimpaired forelimb**

As shown in Fig. 5, lesion-induced changes in reaching performance for female rats were dependent upon lesion size. Female rats with larger unilateral SMC lesions performed significantly better than sham controls using their unimpaired forelimb on the pellet-retrieval task. In repeated measures ANOVA, there was a significant effect for Group ($F(1,27) = 8.72$, $P < 0.01$) but no Group by Day interaction effect ($F(19,513) = .69$, $P = 0.83$). There was a significant Day effect, reflecting the improvements in performance in both groups over days of training.
There was no significant effect of Group (did not perform significantly better than sham controls). Larger lesions, female rats with small unilateral SMC lesions performed better in proestrus compared to estrus (Fig. 6). This effect appeared to primarily result from stage-dependent performance in sham-operated animals. Simple contrasts revealed that, within the large lesion group, there was no difference in reaching performance across estrous stage (P's > 0.05). However, sham-operated animals performed better in proestrus compared to diestrus (P < 0.05) and marginally better in proestrus compared to estrus or small (F(2,9) = 1.37, P > 0.05) lesion groups and there were no Stage by Day interaction effects (IgLes + Train: F(38,114) = 0.77, smLes + Train: F(38,171) = 0.34, P's > 0.05). Furthermore, there was no significant effect of estrous stage at time of surgery on the volume of the remaining cortex for all lesion animals analyzed using a 1-way ANOVA (F(2,20) = 0.66, P > 0.05).

Estrous stage in a subset of animals (n = 6 IgLes + Train, n = 10 smLes + Train, n = 15 Sham + Train) was monitored on several post-operative training days. Reaching performance (excluding days 1 and 2 of training) was pooled across stage for each animal and these data were analyzed using 2-way ANOVAs for the effects of Group by Stage. In this subset of rats, large (Group effect: F(1,19) = 9.49, P < 0.01), but not small (F(1,23) = 0.00, P > 0.05), lesions significantly improved reaching success compared with shams, consistent with the analyses including all animals. As shown in Fig. 6, the improvement in the large lesion group versus shams was dependent upon estrous stage. Although no significant main effect of Stage was found (F(2,38) = 0.07, P > 0.05), there was a significant Group by Stage interaction effect (F(2,38) = 3.34, P < 0.05). In post hoc analyses, there was significantly greater reaching success in IgLes + Train animals compared to Sham + Train animals in estrus and diestrus but not during proestrus (Fig. 6). This effect appeared to primarily result from stage-dependent performance in sham-operated animals. Simple contrasts revealed that, within the large lesion group, there was no difference in reaching performance across estrous stage (P's > 0.05).

Table 1

<table>
<thead>
<tr>
<th>Lesion size</th>
<th>Estrous stage at surgery</th>
<th>Mean reaching success (days 3–20) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>estrus</td>
<td>31.64 ± 12.95, n = 5</td>
</tr>
<tr>
<td></td>
<td>diestrus</td>
<td>28.90 ± 3.44, n = 4</td>
</tr>
<tr>
<td></td>
<td>proestrus</td>
<td>22.69 ± 8.60, n = 3</td>
</tr>
<tr>
<td>Large</td>
<td>estrus</td>
<td>23.03, n = 1</td>
</tr>
<tr>
<td></td>
<td>diestrus</td>
<td>49.40 ± 12.34, n = 5</td>
</tr>
<tr>
<td></td>
<td>proestrus</td>
<td>32.70 ± 13.95, n = 3</td>
</tr>
</tbody>
</table>

Fig. 5. The percentage of successful reaches on the pellet retrieval task after unilateral SMC lesions or sham operations. Training was with the preoperatively non-preferred forelimb and, in animals with lesions, the unimpaired forelimb. Rats with larger lesions achieved greater success on the task compared to rats with smaller lesions (P < 0.05) and compared to sham-operated rats (P < 0.01). These effects did not depend significantly upon the day of training. Data are means ± SEM.

(F(19,513) = 5.49, P < 0.001). In contrast to the effects of larger lesions, female rats with small unilateral SMC lesions did not perform significantly better than sham controls. There was no significant effect of Group (F(1,30) = 0.60, P > 0.05) nor a significant Group by Day interaction effect (F(19,570) = 1.09, P > 0.05), although there was a significant effect of Day (F(19,570) = 4.37, P < 0.001).

Finally, IgLes + Train animals performed significantly better than smLes + Train animals (Group effect: F(1,19) = 5.22, P < 0.05). There was also a significant effect of Day (F(19,361) = 3.42, P < 0.001) but not a Group by Day interaction effect (F(19,361) = 1.16, P > 0.05).
There was no difference in reaching performance between estrus and diestrus ($P > 0.05$) in the Sham + Train group.

**Asymmetries in postural-support behavior**

As shown in Fig. 7, large lesions resulted in a significant asymmetry on the Schallert cylinder test compared with shams in both reach-trained and untrained groups. LgLes + Train animals had a significantly higher asymmetry score compared to Sham + Train animals (Group effect: $F(1,18) = 4.85$, $P < 0.05$; Fig. 7A) and lgLes + Control animals had a higher asymmetry score compared to Sham + Control (Group effect: $F(1,15) = 6.33$, $P < 0.05$; Fig. 7B). No Day or Group by Day interaction effects were obtained. The small lesion groups of either training condition were not significantly different from either the large lesions or the shams of the same training condition. There were no significant Group or Group by Day interaction effects ($P's > 0.05$) in repeated measures ANOVAs of small lesion groups and the other groups. The average asymmetry score pooled over the five post-operative time-points of measurement was not significantly correlated with average post-lesion reaching ability pooled over training days 3–20 ($r = -0.30$, $P > 0.05$, 2-tailed for all trained animals).

**Lesion and training effects on motor cortical MAP2 immunoreactivity**

In contrast to the effects on reaching performance, there were no significant differences between large and small lesion groups of either training condition in the surface density of MAP2 IR processes for either layer II/III (large versus small lesion effects for training groups: $F(1,19) = 1.18$, no-training groups: $F(1,9) = .15$, $P's > 0.05$) or layer V (training groups: $F(1,19) = 0.52$, no training groups: $F(1,9) = 0.11$, $P's > 0.05$). For each layer, planned comparisons were thus performed to assess the effects of the lesion, of the training and the combination of the two (see Statistical Analyses). Representative micrographs of MAP2 immunoreactivity are shown in Fig. 8.

**MAP2 in layer II/III**

As shown in Fig. 9A, a lesion effect was found in layer II/III with lesion animals (Les + Train and Les + Control vs. Sham + Train and Sham + Control) having a significantly greater surface density of MAP2 IR processes than shams ($F(1,61) = 4.73$, $P < 0.05$). An effect of training was also found with trained animals (Les + Train and Sham + Train vs. Les + Control and Sham + Control) having significantly more MAP2 IR processes than untrained animals ($F(1,61) = 8.77$, $P < 0.01$). There was also an effect of combining lesion with training in comparison to the lesion alone. The Les + Train had a significantly greater surface density of MAP2 IR processes than untrained animals ($F(1,61) = 8.77$, $P < 0.01$). No significant correlation between reaching success and surface density of MAP2-positive dendrites in layer II/III was found (all trained animals: $r = 0.216$, $P > 0.05$, 2-tailed).

**MAP2 in layer V**

As shown in Fig. 9B, a significant increase in the surface density of MAP2 IR dendrites was found in layer V as a result of the lesions (Les + Train and Les + Control vs. Sham + Train and Sham + Control: $F(1,61) = 12.23$, $P < 0.001$). No effect of training was seen (Les + Train and Sham + Train vs. Les + Control and Sham + Control) ($F(1,61) = 0.35$, $P > 0.05$). There was also no further increase of MAP2 IR-positive processes in the Les + Train group compared to the Les + Control group ($F(1,30) = 0.42$, $P > 0.05$) though a significantly greater surface density of MAP2 IR processes was found in the Les + Train
group compared to the Sham + Train group \( F(1,39) = 8.02, P < 0.01 \). There was a significant correlation between MAP2 IR process surface density in layer V of trained animals and reaching success pooled over training days 3–20 (all trained animals: \( r = 0.412, P < 0.01, 2\text{-tailed} \)).

**Discussion**

In summary, female rats with sufficiently large SMC lesions performed significantly better with their unimpaired forelimb on the pellet-retrieval task compared with sham-operated controls, consistent with results using male rats (Bury and Jones, 2002; Luke et al., in press). Rats with very
small lesions, in contrast, did not perform significantly better than sham operates. It is important to note that even the lesions termed “large” in this study produced subtotal damage to the SMC and thus the enhanced reaching performance seems to require only a moderate amount of SMC damage. Also consistent with results from male rats, unilateral SMC lesions and reach training each resulted in major increases in the surface density of MAP2 IR dendritic processes in the motor cortex opposite the lesion compared with shams. These effects were laminar-dependent. Lesions increased the surface density of MAP2 IR processes in both layers II/III and V whereas reach training increased MAP2 only in layer II/III. In contrast to the behavioral effects of lesion size, no significant differences were found between small and large lesion groups in MAP2 IR dendrites in either layer. Finally, although estrous stage during training was a factor in reaching performance in intact animals, it was found not to influence post-lesion reaching performance or lesion size.

The finding in female rats that ischemic SMC lesions can improve reaching performance with the unimpaired forelimb compared to sham-operates replicates previous results using electrolytic (Bury and Jones, 2002) and ischemic (Luke et al., in press) SMC lesions in male rats. In potentially related findings in humans, anesthetization of one hand has been found to enhance tactile acuity in the other hand (Werhahn et al., 2002a,b). Unilateral nigrostriatal lesions and hemidecortication in rats have also been found to improve reaction time to tactile stimuli placed on the ipsi-lesional forelimb (Schallert and Whishaw, 1984; Schallert et al., 1982). The enhanced performance in the unimpaired forelimb may facilitate its involvement in behavioral compensation. However, as proposed previously by Bury and Jones (2002), it might also exacerbate the phenomenon of learned non-use of the impaired forelimb (Taub et al., 2002).

One issue is whether increased reliance on the ipsilesional forelimb improves its use in skilled reaching. In support of this possibility, an 8-day period of forced-use of one forelimb in intact rats improved its reaching task performance relative to controls (Bury and Jones, 2004). In the present study, the magnitude of forelimb asymmetry as measured in the Schallert cylinder test did not correlate with performance on the pellet-retrieval task. While it could be that this measure of asymmetry is not a sufficiently sensitive predictor of overall experience in asymmetrical forelimb use, Bury and Jones (2004) also found that rats with callosal transections (which did not have behavioral asymmetries) had dramatic improvements in the acquisition of the reaching task compared with sham-operated animals. Together, these findings support that either increased reliance on the non-impaired limb or denervation of transcallosal fibers can significantly enhance reaching performance. In animals with too small lesions, perhaps neither of these factors were sufficient to support improved reaching performance with the limb ipsilateral to the lesion. Furthermore, much larger cortical lesions than used in the present study have recently been found to produce ipsilesional forelimb deficits on the reaching task which impaired skilled reaching performance (Gonzales et al., in press). The presence of significant ipsilateral impairments could be expected to mask or inhibit lesion-induced facilitation of ipsilateral forelimb function.

The behavioral compensation with the unimpaired forelimb has previously been linked to dendritic growth in layer V of the homotopic-to-the-lesion cortex (Adkins et al., 2002; Bury et al., 2000; Jones and Schallert, 1994). Genes coding for activity-regulated cytoskeletal (Arc) protein and several proteins involved in neuronal signaling have also been found to be increased in the motor cortex opposite forced forelimb use (Keyvani et al., 2002). In the present study, lesions increased the surface density of MAP2 IR dendritic processes in both layers II/III and V in the contralateral motor cortex. In layer II/III, an effect of training and a combined effect of the lesion and training were found. The MAP2 effects are not revealing of the specific cytoskeletal changes occurring in dendrites, but changes in MAP2 IR processes should correlate with dendritic growth and restructuring (Audesirk et al., 1997; Philpot et al., 1997; Vaillant et al., 2002). Previously, lesion-induced increases in MAP2 immunoreactivity have been found at times corresponding to and preceding those times when dendritic growth was evident using Golgi stains or electron microscopy to visualize dendrites (Jones and Schallert, 1992; Jones et al., 1996; Monahan et al., 2000).

MAP2 increases were uncoupled with reaching success in that small and large lesions did not significantly differ in MAP2 effects, in contrast to the lesion size effects on reaching performance. This finding may indicate that the threshold of lesion size needed to induce dendritic cytoskeletal changes in the contralateral homotopic cortex is less than that needed to improve reaching performance. This does not indicate a lack of relationship between reaching and motor cortical MAP2 given that training in intact animals increased layer II/III MAP2 IR surface density. There was also a significant correlation between layer V MAP2 IR surface density and reaching success.

The laminar pattern of training-induced MAP2 changes (found in layer II/III but not V) corresponds well with recent findings of laminar-dependent increases in synapse number per neuron after reach training in male rats (Luke et al., in press). However, unilateral reach training can also result in dendritic growth and synapse addition in layer V of male rats (Greenough et al., 1985; Kleim et al., 2002, 2004; Withers and Greenough, 1989) and these structural changes are likely to be linked to long-term potentiation-like changes in neuronal activity (Monfils and Teskey, 2004; Monfils et al., 2004; Rioult-Pedotti et al., 1998, 2000). A major difference in the present versus these previous studies is the intensity of the reach training. Animals in the studies by Greenough et al. (1985) and Kleim et al. (2002, 2004) had more intensive training regimens than that used in the present study, which was intended to be sensitive to group differences in motor skills rather than being optimal for...
driving neuronal structural changes. Thus, more intensively administered reach training may have resulted in greater MAP2 changes in layer V of the sham-operates in the present study.

Female rats did not reach as well as male rats of previous studies (see Bury and Jones, 2002; Luke et al., in press). Furthermore, reaching success rates in the present study were also lower than those reported previously in intact females of other studies using similar tasks (Metz and Whishaw, 2000; Vergara-Aragon et al., 2003; see also Whishaw, 1992). It is important to note however that in both the study by Metz and Whishaw (2000) and Vergara-Aragon et al. (2003), intact animals were trained with their preferred limb. Training with the non-preferred forelimb, as done in the present study, is likely to make the task inherently more difficult. An unaddressed issue is whether there are sex differences in the ease in which rats learn to reach with the non-preferred limb.

Estrous stage at time of surgery did not have an effect on overall reaching success nor on lesion size in the present study. Several previous studies have found neuroprotective and behavioral sparing effects of female sex hormones (see Roof and Hall, 2000; Stein, 2001), but there has been a lack of evidence for these effects after injury to some regions of the frontoparietal cortex. Hormonal levels at the time of parietal cortical contusions were not found to affect performance on beam motor tasks (Wagner et al., 2004). Furthermore, as previously mentioned, Grossman and Stein (2000) did not find a difference in sensorimotor asymmetries after unilateral forelimb area SMC contusions in pseudo-pregnant female, normally cycling female and male rats. These authors hypothesized that edema may not be a major contributor to this type of brain damage and, therefore, progesterone’s role in reducing cerebral edema (see Roof et al., 1993a; Roof et al., 1996) failed to have a major impact on the effects of the lesion. Furthermore, Goldstein and Bullman (1999) failed to find sex differences in the motor effects of SMC aspiration lesions. However, it remains quite possible that there are sex differences in the extent of ischemic SMC damage and in the resulting behavioral effects that would be revealed in a direct comparison of male and female rats. Furthermore, estrous stage is far from a definitive assay of endogenous circulating hormones and a role for these hormones cannot be ruled out as a contributor to lesion size in the present study. Earlier, post-lesion time points are also needed to sensitively assess hormonal effects on the evolution of the lesions, such as effects on edema (Grossman et al., 2004) and apoptotic cell death (Djebaili et al., 2004). In support of a role for estrogen in ischemic damage, Li et al. (2004) have recently found that male and ovariectomized female mice given an estrogen implant before unilateral middle cerebral artery occlusion, had greater functional recovery of the cylinder test compared to oil-treated mice.

Although there was no effect of estrous stage at the time of surgery, estrous stage during training did influence performance. Rats with larger lesions were found to reach better than sham animals during estrus and diestrus, but not during proestrus. This effect was due, in part, to the sham-operates’ improved performance during proestrus, when estrogen and progesterone are typically high. This finding is suggestive of an influence of circulating hormones on motor performance in intact animals which calls for further inquiry using direct measures and manipulation of circulating estrogen and progesterone levels.

In conclusion, unilateral ischemic SMC lesions in female rats result in dendritic structural plasticity in the contralateral homotopic motor cortex and, if the lesions are of sufficient size, in enhanced reaching performance with the unimpaired forelimb. These findings replicate the major findings of similar studies using male rats (e.g., Bury and Jones, 2002; Luke et al., in press). Although estrous cycle was not found to be a significant contributor to either lesion size or acquisition of a skilled reaching task, possible roles of estrogen and progesterone in ischemic lesion size and skilled reaching performance have yet to be determined.

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