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Motor Skill Training, but not Voluntary Exercise, Improves Skilled Reaching After Unilateral Ischemic Lesions of the Sensorimotor Cortex in Rats

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Abstract

Background and Purpose—Exercise and rehabilitative training each have been implicated in the promotion of restorative neural plasticity after cerebral injury. Because motor skill training induces synaptic plasticity and exercise increases plasticity-related proteins, we asked if exercise could improve the efficacy of training on a skilled motor task after focal cortical lesions.

Methods—Female young and middle-aged rats were trained on the single-pellet retrieval task and received unilateral ischemic sensorimotor cortex lesions contralateral to the trained limb. Rats then received both, either, or neither voluntary running and/or rehabilitative training for 5 weeks beginning 5 days postlesion. Motor skill training consisted of daily practice of the impaired forelimb in a tray-reaching task. Exercised rats had free access to running wheels for 6 h/day. Reaching function was periodically probed using the single-pellet retrieval task.

Results—In young adults, motor skill training significantly enhanced skilled reaching recovery compared to controls. However, exercise did not significantly enhance performance when administered alone or in combination with skill training. There was also no major benefit of exercise in older rats. Additionally, there were no effects of exercise in a measure of coordinated forelimb placement (the foot-fault test) or in immunocytochemical measures of several plasticity-related proteins in the motor cortex.

Conclusions—In young and middle-aged animals, exercise did not improve motor skill training efficacy following ischemic lesions. Practicing motor skills more effectively improved recovery of these skills than did exercise. It remains possible that an alternative manner of administering exercise would be more effective.

Keywords

Exercise; Rehabilitation; Skilled reaching; Experience-dependent plasticity

Behavioral experience can enhance function after brain injury. For example, rats exposed to complex housing environments pre- and/or postinjury typically have improved functional outcome compared to animals in standard housing.^{1,2} Another source of activity-dependent plasticity is general exercise such as wheel running, which influences neuronal circuitry of brain and spinal cord when initiated pre- and postinjury (reviewed in³). In both young and aged intact animals, exercise increases synaptic plasticity-related proteins within the hippocampus and enhances cognitive function, as measured using hippocampal-dependent tasks, including the Morris water maze.^{4,5} In models of injury, exercise is beneficial for increasing axonal

regeneration in sensory neurons,⁶ restoring plasticity-related proteins after spinal cord injury,^{7,8} and protecting from damage caused by cerebral ischemia.⁹

Although exercise can be beneficial, learning motor skills results in neuronal structural and functional plasticity in the motor cortex and cerebellum that is not found with simple exercise or repetition of previously learned skills.¹⁰⁻¹² For example, intact rats trained on a skilled reaching task exhibited a significant increase in the representation area of the wrist and digits detected using intracortical microstimulation mapping, compared to animals trained on an unskilled, bar-pressing task.¹³ Similarly, in the cerebellum, acrobatic training, but not voluntary or forced exercise, induced synaptogenesis.^{11,14} Thus, learning may more effectively induce activity-dependent plasticity than mere use, at least in some of the brain regions that mediate motor movements. After brain damage, motor skill training has also been found to drive apparently adaptive neural plasticity. For example, training monkeys or rats on skilled reaching tasks after focal lesions of the sensorimotor cortex (SMC) prevents the further loss and promotes functional reorganization of movement representations,¹⁵⁻¹⁷ suggesting that mechanisms of plasticity involved in learning a new task in intact animals also play a role in maintenance and reorganization of remaining cortex postinjury.

This study focuses on the possibility that exercise could be used as a positive modulator of motor skill “re-learning” after brain damage. Training animals in skilled reaching after unilateral cerebral infarcts has been found to improve function in the injury-impaired forelimb,¹⁸⁻²⁰ and therefore we refer to such training as “rehabilitative.” However, skill training is often insufficient to return animals to preinjury levels of performance. This study examined whether general exercise could augment the efficacy of rehabilitative training on a skilled motor task after a unilateral focal cortical lesion. After acquisition of the single-pellet retrieval task, groups of young adult female rats received unilateral ischemic damage to the forelimb representation region of the SMC. The animals then received rehabilitative training on a tray-reaching task, voluntary exercise on a running wheel, or a combination of the two. We expanded this study to include middle-aged female rats undergoing rehabilitation to test whether exercise would be more beneficial in older, presumably less spontaneously active, animals. We also investigated the possible effects of exercise and motor training on immunohistochemical measures of the plasticity-related proteins, brain-derived neurotrophic factor (BDNF), spinophilin, microtubule associated protein-2 (MAP-2), and N-methyl-D-aspartate receptor subunit 1 (NMDA-1), in the perilesion tissue because of previous studies indicating that exercise or postoperative experience can increase these proteins.^{5,6,18,21,22}

Materials and Methods

Subjects

Twenty-eight 4-month-old and twelve 1- to 1.6-year-old female Long-Evans hooded rats that were bred at the University of Texas at Austin were pair housed and kept on a 12/12 h light/dark cycle with water access *ad libitum*. Animals were frequently handled 2 to 3 weeks prior to the experiment, and all behavioral procedures were carried out in the same room in which the animals were housed. Prior to the start of behavioral methods, animals were placed on scheduled feeding of 15 g of rat chow given once per day (to ensure rats were not sated at the time of training).

Weights were monitored throughout the study, and, within ages, there were no group differences in weight change over the course of the experiment. In young females, the mean $\pm$ SEM starting and end weights (g) were 278.41 ± 4.7 and 294.8 ± 6.1 , respectively. In the middle-aged females, starting and end weights were 419.1 ± 17.4 and 339.2 ± 9.9 . Due to studies finding an influence of estrogen on dendritic outgrowth²³ and injury volume,²⁴ rats in this study were monitored for their estrous state throughout the experimental procedure using

cytological characterization of vaginal smears (as described in²²). The effects of estrous stage at the time of the twice-weekly probe trials could not be adequately assessed due to an insufficient sampling of estrous stages within subjects over days. Previous studies have found that moderate food deprivation has no effect on the estrous cycle.²⁵ Animals were randomly placed into different groups matched for preoperative reaching performance. Young rats (4 months) were divided into exercise and rehabilitation (Rehab+Run, n = 8), exercise alone (Cont+Run, n = 7), rehabilitation alone (Rehab+Cont, n = 7), and neither manipulation (Cont+Cont, n = 6). Aged rats (1-1.6 years) were divided into groups, matched for age, receiving either exercise and rehabilitation (Rehab+Run, n = 6) or rehabilitation alone (Rehab+Cont, n = 6).

Surgery

All animals were administered a focal unilateral lesion aimed at the forelimb representation area of the SMC contralateral to the preferred limb. Animals were anesthetized using intraperitoneal (i.p.) injections of Equithesin (140 mg/kg chloral hydrate and 35 mg/kg sodium pentobarbital) and placed in a stereotaxic apparatus. After a midsagittal incision, a craniectomy was made between 1.5 mm anterior, 0.5 mm posterior, and 3.0 to 4.5 mm lateral to Bregma. Pia mater was exposed by removal of dura in the area underlying the craniectomy. Endothelin-1 (ET-1, 80 μ M, 0.2 μ g/ μ l; 4 μ l total volume administered), a vasospasm-inducing peptide, was then topically administered with a Hamilton microsyringe and left undisturbed for 10 minutes before the skin was sutured, as described previously.^{26,27} Atropine (0.25 mg/kg) was administered i.p. to counter the respiratory depressive effects of Equithesin. Rats were allowed 4 days of recovery before postoperative behavioral manipulations began.

Training and Testing on the Single-Pellet Retrieval Task

The single-pellet retrieval task (Figure 1a) was used as a sensitive measure of deficits and recovery in skilled forelimb reaching behavior, and this required that rats be proficient in this task prior to the infarct. Training was conducted in a Plexiglas reaching chamber (length: 26 cm, height: 34 cm, width: 16 cm) consisting of a 1 cm by 23 cm window in the center of one 16 cm wide wall. Animals were first acclimatized to the chamber as previously described²² with 4.5 mg of banana-flavored food pellets (Bioserve, Frenchtown, NJ) placed on the floor. Rats have been found to have a limb preference when performing skilled tasks,^{28,29} and this preference was determined in all animals by shaping them to the task in 10-minute sessions administered for 2 to 10 days. Animals were first allowed to reach with either forelimb for centrally placed food pellets on a 3 cm high reaching shelf. When 20 consecutive reach attempts were made with one limb during a 10-minute session, this limb was identified as the preferred limb. For subsequent days of preoperative training, a wall was placed 0.5 cm from the window within the chamber and ipsilateral to the preferred limb. This enabled the animal to reach with only the preferred limb for pellets placed, one at a time, in a shallow well located contralateral to this limb at a distance of 1 cm from the reaching window.

Reaching success was chosen as the primary outcome measure because it is a highly sensitive measure of recovery of skilled motor function in rats.^{18,22,30,31} A successful reach occurred when the animal extended its forelimb through the window, grasped the pellet, moved the pellet to its mouth, and ate it. If the pellet was displaced from the well or if the animal dropped the pellet before consuming it, the trial was recorded as a “miss” or “drop,” respectively. In the presence of a pellet on the shelf, each extension of the trained limb through the reaching window was considered a reach attempt. Performance was measured as the total number of successes divided by the total number of reach attempts during a session. Young animals were trained preoperatively until they achieved a criterion of 30% successful retrievals per session. Older animals (which we had not previously investigated in our lab using this task) were preoperatively trained to a higher criterion (50% success) to ensure sensitivity in detecting

postoperative deficits and recovery patterns. To ensure equivalency in the recency of training experience, all rats also received a “refresher” session of training on the day prior to surgery.

Postoperative Training and Behavioral Measures

Postoperative training consisted of exercise, rehabilitation, or both that was initiated on postoperative day 5 and continued for 5 weeks. The delay in onset of the manipulations was to minimize possible influences of the behavioral manipulations on lesion size, which have been found in previous studies.^{5,32} Probe trials of reaching performance on the single-pellet retrieval task were performed after every fifth day of the rehabilitative training period for 2 successive days. Middle-aged animals received one additional probe trial 4 days after surgery. On probe trial days, animals did not receive rehabilitation, but the regular exercise regimen was provided every day of the 5 weeks. All reaching tasks were performed during the animal's light period. Reaching was then followed (within 3 hours) with voluntary access to a running wheel or novel cage. Access was initiated with 3 hours remaining in the light period and continuing for 3 hours of the dark cycle (total of 6 hours).

Rehabilitative Training: Tray-Reaching Task—The tray-reaching task (Figure 1b, adapted from^{20,33}) was used for motor rehabilitative training. This task differs from the single-pellet retrieval task in that a greater variety of reaching distances and trajectories is required and grasping more than one pellet at a time is permitted. Animals receiving rehabilitation (Rehab+Run and Rehab+Cont) were allowed to reach for 20 minutes for ~100 food pellets (4.5 g of 45 mg pellets) placed on an inclined tray. Young animals received an inclined tray made from 3 glass microscope slides (2.5 cm wide × 7.5 cm long) constructed into a trough with a 25-degree incline toward the reaching window. The tray was elevated so that the height of the lower point of the trough was 3 cm from the table top. Aged animals received a tray made from an aluminum block constructed to the same specifications of the glass tray. A plastic lip was attached to the base of the incline to prevent the pellets from rolling into the reaching chamber. A wall was placed ipsilateral to the impaired forelimb (the preoperatively preferred limb) to force the animals to reach with this limb. No-rehab control groups (Cont+Run and Cont+Cont) were placed in identical chambers for 20 minutes but received 4.5 g of pellets on the floor of the chamber. The experimenter recorded the number of pellets consumed by each animal.

Exercise: Running Wheel—Each day, exercised animals (Rehab+Run and Cont+Run) were individually placed for a 6-hour period (3 hours each in the light and dark cycles) in cages that were connected via wire-mesh tubes to metal running wheels (Figure 1c). The distance run in kilometers (calculated by a cycling computer, CatEye Enduro 8, attached to each running wheel) was recorded at the end of every 6-hour period. No-exercise controls (Rehab+Cont and Cont+Cont) were individually placed in cages for 6 hours (3:3 hours, light:dark) that were identical to those used for the exercised groups except that they had wire-mesh tubes connected to a novel metal box rather than the running wheels (Figure 1d). All animals had access to food (15 g) and water (ad libitum) during the 6 hours in the individual cages. After each session, rats were returned to their home cage with their cage-mate.

Coordinated Forelimb Use During Locomotion

Rats were administered the foot-fault test to measure coordinated forelimb placement during locomotor movements.³⁴ Animals were placed on an elevated grid platform (33 cm × 30 cm; grid openings: 8.4 and 6.25 cm²) and were videotaped exploring the surface for 2 minutes. As rats moved across the platform, placing their paws on the rungs of the grid openings, the experimenter counted the number of slips with either forelimb through the grid openings. Performance was evaluated as the number of errors (“foot faults”) that were executed in ratio to the total number of steps accomplished in the same time period during slow-motion video

playback. Following unilateral lesions, the forelimb opposite the lesion frequently has an increase in the number of foot faults through the grid openings.^{35,36} Animals were administered the foot-fault test preoperatively and 5 to 6 time points postoperatively using an apparatus and testing protocol that has previously revealed significant deficits in male rats with similar lesions.^{26,31}

Histology

Six weeks postlesion, all animals were given a lethal dose of sodium pentobarbital and transcardially perfused with 0.1 M sodium phosphate buffer (PB) followed by 4% paraformaldehyde in PB. Brains were isolated, placed in 4% paraformaldehyde solution, and stored at 4°C for 2 weeks. Six rostral to caudal sets of 50 µm thick coronal sections throughout the cerebrum were then made using a vibratome. One set was stained with toluidine blue to be used for reconstructions of the lesions, estimation of the volume of remaining cortex, and perilesion neuron counts. Adjacent sets were utilized for immunocytochemistry of plasticity-related proteins.

Analyses of Cortical Damage

Lesion size was indirectly estimated by measures of remaining cortical volume in the SMC region. The perimeter of 50 µm thick Nissl-stained coronal sections, 600 µm apart, was traced using Neurolucida software (Microbrightfield Inc, Williston, VT) at a final magnification of $\times 17$. The appearance of the corpus callosum was used as a landmark for the most rostral section to be traced (approximately 2.7 mm anterior to bregma). The area of nonnecrotic/nongliotic cortex was measured in the next 5 sections, moving caudally (ie, the caudal-most section was approximately 0.3 mm posterior to bregma). Volume was then estimated using the Cavalieri method³⁷ as the product of summed area and the distance between section planes (600 µm). To visualize the extent of each lesion, they were reconstructed onto templates of cortical coronal sections (adapted from³⁸). For each group, reconstructions were overlaid to determine the representative and common regions of all lesions.

Given that neurons in peri-infarct tissue are vulnerable to use-dependent excitotoxicity,³⁹ we also assessed neuronal density in this region. For each brain, 3 sections each had 2 samples (1 medial, 1 lateral to the lesion) in each of layers II/III and layer V analyzed (total of 12 samples per brain per layer). Necrotic/gliotic tissue directly adjacent to the lesion was excluded. All samples were observed at 100 $\times$ oil objective with a final magnification of $\times 1680$. The optical dissector method and an unbiased sampling frame were utilized in obtaining the number of neurons per unit volume in an unbiased fashion⁴⁰ using Neurolucida software (Microbrightfield Inc). Neuron number per unit volume was calculated as the total neurons counted divided by the volume sampled (sample number $\times$ sample frame area $\times$ section thickness).

Immunocytochemistry for Plasticity-Related Proteins

We investigated expression of plasticity-related proteins BDNF and NMDAR-1, as well as the dendritic proteins spinophilin (a spine-enriched protein) and MAP-2 (a dendritic cytoskeletal protein), in young adults and BDNF in both young and older rats in layers II/III and layer V of remaining SMC near the lesion. BDNF optical density was also analyzed in hippocampal subregions. A free-floating immunocytochemistry method similar to that previously described^{22,26} was utilized for anti-NMDAR-1 (1:200; Chemicon, Temecula, CA), MAP-2 (1:500; clone AP-20, Sigma, St Louis, MO), spinophilin (1:500; Upstate Cell Signaling Solutions, Charlottesville, VA), and BDNF (1:1000; AB1779, Chemicon) using the avidin-biotinylated complex and 3-3' diaminobenzidine tetrahydrochloride visualization method. Immunoreactivity (IR) was analyzed using the cycloid grid intersection method (MAP-2;

described in²²) optical densitometry (NMDAR-1, BDNF, and spinophilin, described in³¹), and semiautomated puncta quantification using an Image J program (WCIF 1.34p; spinophilin).

Statistical Analyses

To analyze reaching performance across probe days, 3-way analysis of variance (ANOVA) for Exercise condition by Rehabilitative training condition by Day was performed using the SPSS program (SPSS Inc, Chicago, IL). Young and middle-aged rats, which were tested in separate experiments conducted at different times, were analyzed separately. To further analyze reaching performance between groups, simple mean comparisons or 1-way ANOVAs were utilized. One-way ANOVAs were also used to examine the effect of estrous stage at time of surgery on remaining cortical volume as well as the effects of treatment group on remaining cortical volume. To investigate the relationships between average distance run, reaching performance, and remaining cortical volume, bivariate correlations were employed.

Results

Lesion Extent

Young Adults—Lesion reconstructions revealed that the damage consistently involved the forelimb representational region of the SMC defined by cytoarchitecture (Figure 2). The remaining cortical volume in the SMC region was not significantly different between groups. The average volume (means $\pm$ SEM in mm³) was 71.8 ± 1.6 in the Rehab+Run, 71.7 ± 2.5 in the Cont+Run, 73.8 ± 2.2 in the Rehab+Cont, and 70.0 ± 2.8 in the Cont+Cont group ($P > .5$; $F[3, 27] = .43$).

When animals were separated by estrous stage at the time of surgery, a significant difference was found in remaining cortical volume ($P < .05$, $F[2, 27] = 3.4$). As shown in Figure 3a, animals in proestrus (Rehab+Run: $n = 4$, Cont+Run: $n = 3$, Rehab+Cont: $n = 3$, Cont+Cont: $n = 3$) were found to have significantly more cortical volume remaining than animals in either diestrus (Rehab+Run: $n = 2$, Cont+Run: $n = 3$, Rehab+Cont: $n = 4$, Cont+Cont: $n = 2$) or estrus stage (Rehab+Run: $n = 2$, Cont+Run: $n = 1$, Rehab+Cont: $n = 0$, Cont+Cont: $n = 1$). Neuronal densities assessed in the perilesion cortex were found to not be significantly different between groups in either layer II/III or layer V. In comparing remaining cortical volume and reaching performance (averaged over the 5 weeks of testing), no significant correlation was found, but it should also be noted that the correlation is limited by the restricted range of lesion sizes and the differential effects of training. With a median split of remaining cortical volume, reaching performance averaged $25.2\% \pm 2.4\%$ versus $28.6\% \pm 2.4\%$ in rats with smaller versus larger lesions, respectively. Power analysis indicates that 222 rats would be needed to detect a significant difference ($P < .05$) in these lesion size-dependent effects at 80% power.

Middle-Aged Animals—As with the younger rats, lesions were consistently placed in the forelimb representation region of the SMC (Figure 2). No significant differences in cortical volume remaining between groups or significant correlation between cortical volume remaining and reaching performance were found. Average remaining volumes were (means $\pm$ SEM in mm³) 77.9 ± 2.9 in the Rehab+Run and 79.2 ± 5.1 in the Rehab+Cont. Vaginal smears of these animals at the time of surgery revealed that most ($n = 10/12$) of the subjects were in anestrus, an indication of cessation of the rat's reproductive cycle. No significant difference in neuronal density in the perilesion cortex was found.

Exercise: Voluntary Wheel Running

Young Adults—Over days, both groups increased the distance run ($P < .001$, $F[34, 442] = 8.1$; Figure 4a). When pooled across the first 3 days of running, animals averaged 0.86 km, and by the last 3 days averaged 6.7 km distance run per day. No differences occurred between

groups in the distance run ($P > .05$, $F[1, 13] = 2.1$) or in group-by-day interaction ($P > .05$, $F[34, 442] = 0.84$). The cumulative distance run for groups Rehab+Run and Cont+Run was 177.8 ± 28.3 and 124.29 ± 22.7 mean $\pm$ SEM km. No relationship was found between the cumulative distance run across 5 weeks and performance on the reaching probe trials ($R = .115$, $P > .05$). However, a significant positive correlation was present between the cumulative distance run and the remaining cortical volume in the SMC region ($R = .6$, $P < .05$). Rats with larger SMC lesions tended to run less.

Middle-Aged Animals—Like the younger rats, middle-aged rats significantly increased in the distance run over days ($P < .05$, $F[1, 5] = 4.04$; Figure 4a), but they ran less than younger rats (Figure 4b). The average distance run in the first 3 days was 0.18 km, and by the last 3 days of the study, animals averaged 3.1 km. There was no correlation between cumulative distance run over 5 weeks and the reaching performance on the probe trials. In addition, no correlation was found for cumulative distance run and the remaining cortical volume in the SMC region.

Rehabilitation: Tray-Reaching Task

Young Animals—After the first day, all rats retrieved most or all pellets (~100 pellets; 4.5 g) with the impaired forelimb with no difference between groups (Rehab+Run and Rehab+Cont) in the average weight of pellets consumed during each day of the tray-reaching task. Averaged across groups, rats consumed 3.5 ± 1.1 g in the first 3 days of rehabilitation and 4.5 ± 0.1 g in the last 3 days.

Middle-Aged Animals—Although there was no difference between Rehab+Run and Rehab+Cont groups in the performance of the tray-reaching task, rats did show marked improvement of the task by having an increase in the amount of pellets consumed across the days of the study. Rats ate 1.5 ± 0.7 g in the first 3 days of training, and this gradually improved to 3.5 ± 1.0 g in the last 3 days of rehabilitation.

Skilled Reaching Performance

Young Adults—Twice-weekly probe trials of reaching performance on the single-pellet retrieval task were analyzed with a 3-way ANOVA for Rehab condition by Exercise condition by Day of testing. As shown in Figure 5a, rehabilitative training on the tray-reaching task had a major facilitatory effect on recovery of skilled reaching performance as measured using the single-pellet retrieval task. There was a significant main effect of Rehab condition ($P < .001$, $F[1, 24] = 49.2$) as well as a Rehab-by-Day effect ($P < .05$, $F[1, 24] = 2.8$). Post hoc comparisons revealed that the Rehab (Rehab+Run and Rehab+Cont) groups performed significantly better on the probe trials than the No-Rehab groups (Cont+Run and Cont+Cont) beginning in week 2 and thereafter. Exercise did not significantly affect performance in reaching probe trials or further enhance the effects of rehabilitative training on reaching performance. No main ($P > .05$, $F[1, 24] = 0.67$) or interaction effects of exercise condition (exercise by day: $P > .05$, $F[9, 16] = 0.34$, exercise by rehab: $P > .05$, $F[1, 24] = 2.51$, exercise by day by rehab: $P > .05$, $F[9, 16] = 0.54$) were found. When animals were separated by estrous stage at time of lesion application, there was no significant difference in reaching performance on the first probe days (postoperative days 9 and 10). Pooled across groups, on these days, the average percent success per reach attempt was 19.5 ± 3.4 , 20.8 ± 2.9 , and 17.1 ± 3.8 for rats in diestrus, proestrus, and estrus at the time of surgery.

Middle-Aged Animals—In repeated-measures ANOVA, no overall group effect was present, but a group-by-day effect was present ($P < .001$, $F[1, 10] = 3.5$). Rehab+Run performed significantly worse in the first week, significantly better in the second week, and was not significantly different from Rehab for the remainder of the study compared with Rehab+Cont

(Figure 5b). Thus, the pattern of recovery was significantly different between groups, but there was no consistent improvement in performance as a result of exercise.

Foot-Fault Test Performance

Young Animals—Compared to preoperative performance, no consistent mean decline in performance on the foot-fault task occurred, as first assessed 10 days after the ischemic lesions. (This is in contrast to effects in male rats with similarly sized lesions,^{26,31} and this task is potentially less challenging for the lighter weight female rats of this age.) When the animals were separated according to estrous stage at the time of surgery, however, a 1-way ANOVA revealed an overall significant difference in the number of errors per stage (Figure 3b). Animals in proestrus at the time of surgery made significantly fewer errors compared to those in diestrus ($P < .05$, $F[1, 23] = 5.13$) and those in estrus ($P < .05$, $F[1, 16] = 6.24$). Furthermore, the diestrus and estrus, but not proestrus, subgroups had a significant postoperative impairment in foot-fault test performance ($t = -2.5$, $P < .025$ prelesion versus day 10). No significant effects of exercise or training condition on this measure occurred when all animals were included in the analysis or when the proestrus-at-lesion subgroup of rats were excluded from the analysis. There were no significant differences between the diestrus and estrus subgroups.

Middle-Aged Animals—In middle-aged rats, lesions resulted in an increased error rate on the foot-fault task. We found no significant effect of exercise on the recovery pattern (Figure 6). A repeated-measures ANOVA revealed no significant difference between Rehab+Run and Rehab+Cont groups, but a significant effect of day was present, reflecting the improvement of performance during the 5 weeks of testing.

Immunocytochemistry for Plasticity-Related Proteins

With the exception of the spinophilin puncta quantification, no analyses revealed significant group differences, and these data are therefore not shown. Young adult rats receiving rehabilitation alone had significantly more spinophilin-IR puncta (eg, layer II/III mean $\pm$ SEM spinophilin-IR puncta per mm², $197,759 \pm 37,864$) compared to the combination of exercise and rehabilitation (Rehab+Run $108,449 \pm 10,239$, $P < .05$, $F[1, 13] = 5.8$) and Cont+Cont ($67,974 \pm 5,712$, $P < .05$, $F[1, 11] = 9.7$); this approached significance compared to exercised animals (Cont+Run $119,332 \pm 16,526$, $P = .082$, $F[1, 12] = 3.6$). However, when insular granular cortex of the same hemisphere was examined in Rehab+Cont and Cont+Cont, the Rehab+Cont group spinophilin-IR puncta was increased ($P < .05$, $F[1, 13] = 6.2$).

Discussion

In summary, young and middle-aged rats receiving 5 weeks of rehabilitative reach training had a significant enhancement of performance on a skilled reaching task after unilateral ischemic lesions compared to rats without training. Voluntary running had no major effect when administered alone or in combination with skill training. Furthermore, we found no correlation between distance run and recovery of skilled reaching performance. Exercise also failed to improve recovery of coordinated forelimb placement during locomotion, as assessed by the foot-fault test in middle-aged rats.

The beneficial effects of motor skills training are consistent with previous findings.² For example, Biernaskie and Corbett¹⁸ found that task-specific rehabilitation in combination with enriched environment housing improved performance on the Montoya staircase task after unilateral middle cerebral artery occlusion compared to untrained rats. Behavioral improvements resulting from motor skill training after unilateral cerebral injury have been linked with reorganization of cortical movement representations.^{17,41-43} Nudo and colleagues⁴⁴ trained animals on a skilled reaching task that increased intracortical

microstimulation-evoked movement representations within the motor cortex corresponding to fine digit movements used to perform the task. When the same animals were later trained on a wrist-dependent task, the representation of the digits decreased and wrist representational areas expanded. These findings support the specificity of behavioral effects on neural connections in the motor cortex. These previous findings also suggest that the tray-reaching task of the present study may have improved function because it engaged remaining cortical tissue sufficiently to reorganize it in a functionally beneficial manner.

In contrast to skill training alone, exercise failed to have major effects on skilled reaching recovery. Previously, Komitova et al⁴⁵ found that rats given voluntary running after cortical ischemic damage performed at the same level of deficit as control animals in traversing a rotating rod. Similarly, voluntary exercised animals performed worse in a battery of locomotor behavioral tasks (eg, traversing a beam or rotating pole) after ischemic damage to the cortex compared to animals given social interaction or an enriched environment.⁴⁶ Apparent benefits of physical activity, however, have been repeatedly found in other injury models (reviewed in³), including an increase in cognitive function after traumatic brain injury.⁵ Neuroplastic effects of exercise have predominately been examined in the hippocampus and cerebellum, where cellular proliferation and angiogenic effects are found.^{3,11,47,48} Exercise also increases expression of neurotrophins, especially BDNF, which mediate its effects on hippocampal-dependent tasks.⁴⁹ Exercise induces angiogenesis in the motor cortex,⁵⁰ but it is slow to show these effects. Swain and colleagues⁵¹ found that exercise induced angiogenesis in the primary motor cortex only after chronic (30 days) exposure to voluntary running and not at any time point earlier. Although the 5 weeks of exercise used in the present study was substantial, an even longer exercise regime may be necessary to facilitate motor recovery after ischemic damage. In contrast to our failure to find that skills training can be augmented by exercise, previous studies have reported that the efficacy of rehabilitative motor skill training can be improved by its combination with other treatments, including pharmacotherapies and facilitative stimulation.^{10,52,53}

With the exception of spinophilin-immunoreactive puncta, we saw no effect of either rehabilitation or exercise on immunoreactivity for plasticity-related proteins (BDNF, MAP-2, and NMDAR1), as assayed in layers II/III and V of the remaining SMC of the infarcted hemisphere of young rats. Furthermore, no differential immunoreactivity for BDNF in hippocampal subregions was found. There are several possible reasons for this. Brains were assayed at the end of the training/exercise period (6 weeks postlesion), and it is likely that earlier time points of analyses are needed to sensitively detect changes in at least some of these plasticity-related proteins. In intact animals, increased hippocampal BDNF mRNA²¹ and protein⁵⁴ have been found after 1 and 4 weeks of exercise, respectively. Elevated levels of BDNF protein in the motor cortex have been found after 14 days of exercise, but not later.⁵⁵ On the other hand, hippocampal BDNF protein was not significantly increased by exercise provided during the first 7 days after a fluid percussion injury⁵⁶ nor cortical BDNF protein with exercise given 5 to 12 days after an ischemic cerebral lesion.⁵⁷ It is also possible that our scheduled feeding procedures created a ceiling effect in the production of plasticity-related proteins, given the major influences of dietary manipulations on levels of neuroplasticity-related molecules.^{58,59} Finally, the nonexercised controls in this study were given access to a novel environment during the running period, and it is possible that this experience itself was sufficient to elevate these proteins.

We did find a significant effect of rehabilitative training alone for spinophilin, a dendritic spine protein^{60,61} in the perilesion motor cortex, which may indicate that tray reaching produced synaptogenesis in this region. However, the elevated levels also seen in the insular granular cortex, which was intended to serve as a control region, must be taken into consideration. This area is responsive to thermal stimulation of the tongue⁶² and has not previously been found

to undergo dendritic structural change in response to reach training in the contralesional cortex.³⁰ It is also possible that these data indicate a nonlocalized increase in spine density in the remaining cortex of the damaged hemisphere. If so, the failure to find this effect in the Rehab +Run group suggests that running reduces or alters the time course of this effect.

Another consideration across models of brain injury is the time after injury at which a physical intervention starts.^{63,64} In a traumatic brain injury model, animals receiving voluntary exercise early postinjury (days 0-6) were found to have reduced expression of plasticity-related proteins in the hippocampus and worsened water maze performance compared to animals exercised between 14 and 20 days after injury.⁵ Although it is unknown whether such sensitive periods exist for exercise after SMC lesions, a high level of overuse of the impaired forelimb too early after unilateral electrolytic lesions can exaggerate lesion size and limb use deficits.^{65,66} Other time-dependent effects for skilled rehabilitative tasks after unilateral cerebral infarcts have been shown. Greater behavioral improvements⁶⁷ and greater influences on cortical reorganization⁶⁸ were found with earlier onset of training. In the present study, each session of exercise *followed* each day's training on the reaching task. Many studies finding beneficial effects of exercise after injury used a regimen in which the animal experienced the exercise before the application of the injury (eg, 6^{47,69}). Exercise might also have a priming effect if administered *before* each session of rehabilitative training, although the possibility of fatigue during the performance of the reaching task would also need to be considered. It should be noted that, although there was no overall benefit of exercise in the middle-aged animals, they were capable of performing at preinfarct levels a week earlier than the rehabilitated-alone animals, supporting the possibility of subtle benefits of this manipulation that might be enhanced by an alternative administration regime.

Although lesion size, as assessed by remaining cortical volume, was similar between experimental conditions, rats with larger lesions tended to run less, which could be a result of either motor and motivation deficits resulting from larger lesions or a tendency for running to reduce lesion size. The latter possibility seems less likely given the 5-day delay in onset and the low initial rates of running. Consistent with the well-established neuroprotective roles of progesterone and estrogen,⁷⁰ we also found smaller lesions and better postischemic foot-fault task performance in those animals that were in proestrus, a period when estrogen and progesterone are at their peak, at the time of infarct induction. Also, animals in proestrus at the time of surgery had a significantly better postischemic performance on the foot fault test.

This study further supports the efficacy of motor skill training in improving functional outcome after a unilateral cerebral infarct. In the context of abundant evidence for beneficial effects of exercise in other behavioral measures and injury models,⁷¹ the present findings also provide important information about exercise parameters that are *unlikely* to be particularly beneficial for improving motor skills after cortical ischemia. Further investigations of the time of onset, task specificity, and duration of postischemic physical activity are needed to determine whether exercise can augment task-specific motor skills training.

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Figure 1.

Rats were trained to proficiency on the single-pellet retrieval task preoperatively, and this test was used to probe reaching function after the ischemic lesions (a). The tray-reaching task was used for rehabilitative training (b). Exercised rats (c) had access to a running wheels (via a chicken wire tube) for 6 h/day, whereas no-exercise controls were given access to a novel metal box (d).

Figure 2. Ischemic sensorimotor cortex lesions. Schematic representations of the damage common to all lesions, the range, and a representative lesion within each group. Numbers to the right indicate the distance from bregma of the coronal sections.

Figure 3.

Estrous stage effects at the time of lesion induction in young adults. Rats in proestrus (when estrogen and progesterone are at their peak) had (a) significantly more remaining cortical volume in the sensorimotor cortex region and (b) significantly fewer errors on the foot-fault test (a measure of coordinated forelimb use during locomotion) 10 days postinfarct, compared to animals in diestrus and estrus. Estrous stage distributions were approximately equal among experimental groups (see text for details). Most of the older rats were in anestrus at the time of lesion induction. Data are means $\pm$ SEM. * $P < .05$ compared with proestrus.

Figure 4.

Voluntary exercise. By the fifth week, both young adults and middle-aged adults increased the distance run compared to the first week (a). Although the average distance run in the middle-aged animals was less than the young adult rats, the performance in the skilled reach task averaged across all 5 weeks was similar (b). There were no significant correlations between distance run and reaching task performance (see text for details).

Figure 5.

Single-pellet retrieval task. Young adult rats (a) receiving rehabilitation on the tray-reaching task had a significant improvement in reaching performance in probe trials after lesions, whereas there were no significant main or interaction effects of exercise. In middle-aged animals (b), exercise influenced the pattern of recovery, but not the overall outcome (note that middle-aged animals were trained to a higher preoperative criterion than younger rats and received an additional probe trial prior to rehabilitation training onset). Data are means $\pm$ SEM.

* $P < .05$ and ** $P < .005$ rehab significantly different versus no-rehab.

Figure 6.

Foot-fault task. Middle-aged animals were found to have a significant increase of errors in the task 4 days after ischemic injury (post-op). However, after 5 weeks, animals were capable of completing the task with fewer errors. There were no significant effects of the exercise condition on this test.