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Maladaptive effects of learning with the less-affected forelimb after focal cortical infarcts in rats

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Abstract

It is common following stroke to focus early rehabilitation efforts on developing compensatory use of the less-affected body side. Here we used a rat model of focal cortical infarct to examine how motor skill acquisition with the less-affected (“intact”) forelimb influences sensorimotor function of the infarct-impaired forelimb and neural activity in peri-infarct cortex. Rats proficient in skilled reaching with one forelimb were given focal ischemic lesions in the contralateral sensorimotor cortex (SMC). Recovery in this forelimb was tested following a period of reach training focused on the intact forelimb or control procedures. Quantitative measures of the cumulatively expressed transcription factor, FosB/ΔFosB, were used to assay intact forelimb training effects on neuronal activity in remaining SMC of the infarcted hemisphere. Intact forelimb training worsened behavioral recovery in the impaired forelimb following unilateral focal ischemia. Furthermore, it decreased neuronal FosB/ΔFosB expression in layer II/III of peri-infarct SMC. These effects were not found in sham-operated rats trained sequentially with both forelimbs or in animals receiving bilateral forelimb training after unilateral infarcts. Thus, focused use of the intact forelimb has detrimental effects on recovery of impaired forelimb function following a focal ischemic injury and this is linked to reduced neuronal activation in remaining cortex. These results suggest that peri-infarct cortex becomes vulnerable to early post-stroke experience with the less-affected forelimb and that this experience may drive neural plasticity here in a direction that is maladaptive for functional outcome.

Keywords

motor rehabilitation; interhemispheric competition; learned nonuse; skilled reaching; ischemia; brain damage; motor cortex; FosB/ΔFosB; bimanual training

Introduction

Following unilateral cerebral damage, humans and other animals develop a compensatory hyper-reliance on the less-affected body side ipsilateral to the injury (Schallert, 2006). The development of compensatory behaviors is an advantageous strategy in that it permits performance of daily activities despite impairments. The cortex contralateral and homotopic to unilateral sensorimotor cortex (SMC) damage has increased neuroplasticity, e.g., dendritic

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growth (Jones, 1999; Jones, Kleim, and Greenough, 1996), which appears to facilitate the ability to acquire new skills with the less-affected body side (Bury and Jones, 2002; Allred and Jones, 2004; Hsu and Jones, 2006). Furthermore, it is typical in human stroke patients to focus early rehabilitation on compensatory use of the less-affected body side (Dobkin, 2005). However, such compensation may contribute to learned disuse of the *impaired* body side (Taub et al., 2006A). Furthermore, recent studies in humans and animal models suggest that the intact hemisphere develops an exaggerated disruptive influence over the infarcted hemisphere which is linked with worsened outcome (reviewed in Ward and Cohen, 2004). For example, after visual cortex lesions in cats, reversal of visual hemineglect was accomplished by cooling regions of the contralesional hemisphere (Rushmore et al., 2006). During movements of the paretic hand in humans with strokes, there is an abnormal interhemispheric inhibitory influence arising from the intact motor cortex (Murase et al., 2004; Duque et al., 2005). Anesthetization of the intact upper extremity in both humans (Voller et al., 2006) and rats (O'Bryant et al., 2007) transiently improves function in the stroke-impaired limb. These and related findings suggest that the intact hemisphere can negatively impact function of the impaired body side. It seems possible that behavioral experience with the intact body side is a major contributor to these effects.

We recently found that, following unilateral ischemic SMC lesions, training behaviorally naive rats on a reaching task for 10 days with their intact forelimb worsened their ability to learn the same reaching task with their impaired forelimb compared to those without the training (Allred et al., 2005). However, it was unknown whether this behavioral experience would also influence *recovery* of preexisting (before the infarct) motor skills in the impaired forelimb and whether this effect requires *focused* training of the intact limb. Furthermore, the neural basis of this effect has previously been unexplored.

The present study was designed to test the behavioral and neural consequences of early intact forelimb experience using a focal model of cortical stroke in rats. Cortical damage in humans and rats can result in major deficits in distal forelimb behaviors (e.g., Desrosiers et al., 2003; Gharbawie et al., 2005). Given that impairments in hand and arm sensorimotor function and compensation with the other hand are typical after stroke, a skilled forelimb reach-to-grasp task in rats seemed ideal for studying bilateral behavioral experience effects on recovery of function. Rats readily learn to reach for and grasp palatable food pellets, mimicking similar movements performed by humans (Iwaniuk and Whishaw, 2000; Whishaw, 2003). The single pellet retrieval test, a skilled reaching test, was therefore used as a sensitive measure of distal forelimb impairments (Whishaw and Gorny, 1994) and as an experiential manipulation of forelimb experience.

To assay functional activation of remaining cortex, we took advantage of a transcription factor, Δ FosB, which is cumulatively and persistently expressed in response to repeated neuronal activation, (McClung et al., 2004) making it suitable for investigations of practice-dependent plasticity, such as that required for motor skill acquisition and motor “re-learning”. Immunocytochemistry for FosB/ Δ FosB was used. Experimental designs are summarized in Figure 1.

Materials and Methods

Animals

Well-handled rats were pair housed with standardized housing supplementation (a PVC pipe piece and small wooden objects) on a 12:12 light/dark cycle. Animals were maintained on scheduled feeding (15–17g/day) to motivate reaching performance. Animal use was in accordance with a protocol approved by the University of Texas at Austin Animal Care and Use Committee.

Focal Ischemic Stroke Model

Unilateral SMC lesions were made in the caudal forelimb representation area opposite the animals' preferred-for-reaching forelimb. Rats were anesthetized with ketamine (10mg/kg) and xylazine (120mg/kg), Exp. 1, or Equithesin (150 mg/kg choral hydrate; 37mg/kg sodium pentobarbital, Exp. 2). A craniectomy ($-1.0/+2.0$ A/P; $2.0/4.5$ M/L) was made, dura was removed and $2.5\text{ }\mu\text{L}$ (200 pmol) of endothelin-1 (ET-1), a vasoconstricting peptide, was applied to the pial surface, as previously described (Allred and Jones, 2004). Sham-operated rats received procedures up to the craniectomy. Adams et al. (1994) have previously shown that skull removal can result in forelimb behavioral and neurochemical asymmetries and, in this study, it was important for these rats to serve as intact controls.

Behavioral Procedures

Skilled Reach Training—The single pellet retrieval task (Miklaeva and Whishaw, 1996) was used as an assay and manipulation of behavioral function. A design that forces rats to use the experimenter-designated forelimb(s) for reaching was used. Rats reached through a narrow window for 45 mg banana flavored food pellets (Bioserve Inc.) from a shallow well in a shelf, as previously described (Hsu and Jones, 2006; Allred et al., 2005). Wall(s) were placed inside the reaching chamber (1.5 cm from window) ipsilaterally to the trained forelimb. In experiment 2, to permit training of one or both forelimbs, there were two reaching windows on opposite sides of the chamber which rats reached through in alternating trials and partial walls (in the form of corners) next to each window spanning $\sim 1/4$ of the length of the chamber. For each trial, rats were permitted up to 5 reach attempts to successfully retrieve the pellet. Each extension of the forepaw through the window was counted as an attempt. Successful retrievals/reach attempt $\times 100$ are reported.

Pre-operatively, all rats were trained to 60% success rate (approximately asymptotic performance) on the reaching task with the preferred-for-reaching forelimb (the to-be-impaired limb). Early post-lesion animals underwent control procedures or were trained with their intact (non-preferred) limb for 15 days (60 trials/day; Experiment 1) or 12 days (30 trials/day; Experiment 2) (Fig. 1). Bilaterally trained animals received 15 trials with each forelimb in an alternating fashion. Following this, impaired forelimb recovery was assessed. Experiment 2 rats then underwent an additional period of retesting the intact forelimb. Controls received pellets on the chamber floor at approximately the same rate as trained animals retrieved them.

Footfault test—The footfault test measures coordinated locomotor forelimb use (Barth, Grant, and Schallert, 1990). Animals traversed an elevated grid-platform for 2 min. From slow motion playback, instances of slipping through the grid with either forelimb were recorded. In experiment 2, at the last time-point, the intact forelimb was anesthetized with lidocaine (0.20 cc of 1.0% lidocaine into the bicep muscle) to test its contribution to performance. Performance was recorded as slips/step $\times 100$.

Schallert cylinder test—Lesion-induced forelimb asymmetry was measured using the Schallert cylinder test (Schallert et al., 1997) which encourages upright postural exploratory movements. Animals were filmed in a Plexiglas cylinder (19 cm diameter) for 2 min and the first 30 instances of sole (impaired or intact) or simultaneous forelimb use for support against the cylinder wall were recorded using slow-motion playbacks of each session. The percent impaired forelimb use was calculated as (impaired use/total forelimb use) $\times 100$. The post-lesion difference in use of the impaired limb (postoperative – preoperative % impaired limb use) was then calculated.

Immunohistochemistry and Quantitative Microscopy

At the conclusion of each experiment, animals were overdosed with sodium pentobarbital (150mg/kg) and transcardially perfused with 0.1M phosphate buffer followed by 4% paraformaldehyde in the same buffer. Brains were removed and 50µm thick coronal sections of cerebrum were produced using a vibratome. Archived tissue from a previous experiment (Allred et al., 2005) was also used to test the reproducibility of Experiment 1 results. Immunoreactivity for FosB/ΔFosB was visualized using 3-3' diaminobenzidine tetrahydrochloride with nickel ammonium sulfate using a free-floating section method, as previously described (Allred and Jones, 2004). Relatively thick 50µm sections were used because they are most suitable for the optical disector method used to quantify neuronal density (Harding, Halliday, and Cullen, 1994). The primary antibody was FosB/ΔFosB sc-48 (1:400, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The secondary antibody (Sigma) was antirabbit IgG made in goat (1:200). Within experiments, each batch of immunohistochemistry included tissue from all animals as well as no-primary control sections.

The remaining SMC (inclusive of agranular and granular cortex medial and lateral to the lesion) in the rostral extent of the lesion territory was chosen for the quantitative analysis because this region contains remaining forelimb representation zones. Layer II/III was chosen because it has previously been found to undergo neuroplastic changes as a result of motor skill training in intact animals (e.g., Bury and Jones, 2002; Kleim, Barbay, and Nudo, 1998). Layer V was chosen because it is the major source of corticofugal projections and it has also been found to undergo synaptogenesis as a result of motor skill training (Jones et al., 1999). Four sample sites per layer in each of four sections were analyzed using a 100X oil immersion objective with a final magnification of X1250. FosB/ΔFosB neuronal expression was also examined in layers II/III and V of the non-infarcted cortex; however, there were no consistent effects of differential training between and within experiments and therefore these data are not shown. Granular insular cortex, responsive to thermal stimulation of the tongue, served as a control region.

Immunostained sections were counterstained with methylene-blue azure II, a Nissl stain. The density of FosB/ΔFosB positive neurons (brown) and FosB/ΔFosB negative neurons (blue) were estimated using the optical disector method (Korbo et al., 1990). N_v was calculated using the formula: $N_v = \Sigma Q^- / \Sigma v(\text{frame})$, where ΣQ^- is the sum of all FosB/ΔFosB neurons counted per brain and $\Sigma v(\text{frame})$ is the total sample volume. Slides were coded prior to quantification such that the experimenter was blinded to behavioral conditions.

Volume of Remaining SMC and estimate of lesion size and placement

Cortical volume measurements in the SMC region of both hemispheres were used as an indirect measure of lesion size. At 17X magnification, seven 50µm thick Nissl stained coronal sections within the caudal forelimb area of the SMC (between 2.2 mm anterior to and 0.80 mm posterior to Bregma) were traced using Neurolucida perimeter tracing software (Microbrightfield Inc). The Cavalieri method (Gundersen et al., 1988), the product of the distance between section planes and the summed cortical areas, was used to calculate volume. Lesion extent and placement were assessed by reconstructing lesions onto coronal schematic templates relative to cytoarchitectural and macrostructural landmarks (as previously described in Allred and Jones, 2004).

Statistical Analyses

Repeated measures analysis of variance (ANOVA), using planned comparisons, were carried out for behavioral tests. One-way ANOVAs or t-tests were used for within group and between 2 group comparisons. Statistics were performed using SPSS (SPSS, Inc) and GraphPad Prism

(GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA). Descriptive results are shown as means $\pm$ SEM. Results were considered significant at $p < .05$.

Results

Training the intact forelimb interferes with recovery of skilled reaching with the impaired forelimb

Intact forelimb training in the early post-infarct time period interfered with subsequent skilled reaching with the impaired forelimb (Figs. 2–3). Rats trained with their intact forelimb (IntactT) performed significantly worse during the impaired forelimb training period compared to Cont ($F(1,18) = 7.21$, $p < .05$, Fig. 2). In experiment 2 (in which rats received less intense intact forelimb training, 30 trials/day instead of 60), there was a significant group by day interaction ($F(9,144) = 3.03$, $p < .01$), but the main effect of group failed to reach significance ($F(1,16) = 3.58$, $p = .08$, Fig. 3). In post-hoc analyses, IntactT animals were significantly more impaired than Cont on Days 1, 2, 4 and 6 (p 's < 0.05). There were no group differences in the number of trials completed during each training session.

During the early training period, IntactT rats performed better with their *intact* forelimb compared to sham operated controls (Figure 2A; $F(1, 18) = 8.96$, $p < .01$). This was expected because these lesions facilitate acquisition of new skills in this forelimb, even in the presence of subtle impairments (e.g., Allred and Jones, 2004; Hsu and Jones, 2006). As expected, lesions also reduced reaching performance in the impaired forelimb compared to sham-operates (Fig. 2B).

Training the non-preferred forelimb in sham-operates does not impair the other forelimb

Sequentially training the forelimbs in the same order in sham-operates did not reproduce effects found after SMC lesions (Fig. 2B). Training the non-preferred-for-reaching forelimb after sham operations (Sham NPrefT) did not significantly change subsequent success rates with the preferred forelimb compared to Sham Cont ($F(1,18) = 2.86$, $p > .05$, Figure 2B). However there was a tendency for Sham NPrefT to achieve lower asymptotic performance compared with Sham Cont in later days of testing.

Training both forelimbs together does not worsen impaired forelimb performance

Rats trained bilaterally (BiT, Exp. 2) did not show a decrease in reaching performance with their impaired forelimb compared to Cont ($F(1,16) = 1.49$, $p > .05$) and this group performed significantly better than IntactT rats ($F(1,16) = 13.73$, $p < .01$, Fig 3).

Intact forelimb training results in abnormal reliance on this forelimb for coordinated forelimb behaviors

In the footfault task, rats traverse a grid floor by coordinated placement of their paws on the rungs of the grid. Recovery on this task after unilateral SMC lesions depends, in part, on compensatory ways of using the intact forelimb (Bury and Jones, 2002; Schallert et al., 2000). Rats compensating with their intact forelimb have increased errors with their impaired forelimb when the intact forelimb is anesthetized (the "lidocaine challenge"). In contrast, sham-operated animals show no impairment in the *unanesthetized* forelimb when the other limb is anesthetized (Bury and Jones, 2002). Rats trained with their intact forelimb early after SMC lesions, had increased misses (slipping through the grid) with their impaired forelimb during the lidocaine challenge compared to pre-lidocaine tests ($t(8) = -2.39$, $p < .05$) and compared to Cont rats ($F(1,16) = 5.25$, $p < .05$, Fig 4). BiT rats were not significantly different from Cont ($F(1,16) = .01$, $p > .05$).

As shown in Table 1, lesions reduced use of the impaired forelimb for postural support behavior as measured in the cylinder test during the early post-operative period. During the impaired limb training period, IntactT rats, but not Cont, remained significantly impaired in the cylinder test relative to the training-matched shams (Sham NPrefT, $p < 0.05$). However, the IntactT results failed to reach significance compared with lesion controls (Cont) in experiment 1 ($F(1,18) = 2.56$, $p = .13$) and 2 ($F(1,16) = 3.78$, $p = .07$).

Training the impaired forelimb does not worsen performance in the intact forelimb

After the end of the impaired forelimb training period, Experiment 2 rats received tests of intact forelimb reaching. Compared with their asymptotic performance levels during the earlier training period, there was no significant change in the performance of the intact forelimb in the IntactT rats (mean $\pm$ SEM retest % success = 62.25 ± 2.20 versus 58.35 ± 6.30 , $t(8) = -0.733$, $p > .05$) and BiT rats (52.73 ± 2.77 versus 48.82 ± 3.22 , $t(8) = -1.57$, $p > .05$). In Cont rats, which were being tested for the first time on this forelimb, performance was similar to performance of IntactT and BiT rats during their initial acquisition (e.g., Cont = 37.74 ± 3.61 versus 34.78 ± 2.99 in the first 5 days of early training in IntactT).

Behavioral manipulations did not significantly influence lesion size

Ischemic lesions produced damage to the caudal forelimb representation area of the SMC, as assessed using reconstructions of the lesion placement relative to cytoarchitectural and macrostructural landmarks. Lesions damaged most of the overlap zone of the caudal forelimb representation area of the SMC and considerable non-overlapping motor (lateral agranular) cortex, but spared most of the medial agranular cortex and much of the granular cortex lateral to the lesion. No striatal damage was evident, though approximately half of all lesion animals had at least subtle damage to the corpus callosum. Lesion placement and extent were similar between groups as evidenced in these reconstructions. Intact forelimb training did not increase infarct size as measured by remaining cortical volume (Table 2). Figure 5 shows coronal reconstructions of SMC lesions from Experiment 1. Rats in Experiment 2 tended to have smaller lesions than those in Experiment 1 (as evidenced by greater remaining cortical volume), but there was no differential training effect on this measure.

Training the intact forelimb decreases FosB/ Δ FosB expression in peri-infarct cortex

As shown in Figure 6, lesions increased FosB/ Δ FosB in remaining SMC, as assessed after the period of impaired forelimb training (Exp. 1). Intact forelimb training decreased expression of FosB/ Δ FosB in layer II/III compared to Cont ($F(1,19) = 5.92$, $p < .03$). In contrast, in layer V, where FosB/ Δ FosB neuronal expression was relatively low, both lesion groups had increased expression compared with sham-operates and there was no significant difference between lesion groups ($p > .05$). To assess the reproducibility of the intact training effect in layer II/III, FosB/ Δ FosB neuronal expression was measured in peri-infarct cortex of archived tissue from a previous study (Allred et al., 2005). The experimental design was similar to that of IntactT and Cont groups of Experiment 1 (Fig. 1A), with the exception that rats had no pre-operative reaching experience. Rats trained with their intact forelimb after unilateral SMC lesions also had decreased FosB/ Δ FosB neuronal density in peri-infarct cortex compared to Cont ($F(1,14) = 6.97$, $p < .02$).

In sham-operates, there was no significant decrease of FosB/ Δ FosB expressing neurons as a result of the prior forelimb training ($F(1,19) = 1.29$, $p > .05$) in layer II/III or in layer V ($F(1,19) = 1.58$, $p > .05$). FosB/ Δ FosB neuronal expression in layer V of granular insular cortex (GIC), a control region, was not significantly different between lesion groups ($F(1,14) = 2.79$, $p > .05$).

Rats in Experiment 2 had a training period focused on the intact forelimb before being sacrificed and this creates a potential confound to the interpretation of early intact training effects on peri-infarct cortex. Nevertheless, the FosB/ Δ FosB results showed a pattern of change consistent with that found in Experiment 1. The ratio between FosB/ Δ FosB positive and negative neurons was significantly reduced in IntactT compared to Cont ($F(1,16) = 27.18$, $p < .001$) in layer II/III peri-infarct cortex. BiT rats were not significantly different from either Cont ($p = .95$) or IntactT ($p = .13$).

DISCUSSION

Training the less-affected, “intact” forelimb in skilled reaching following unilateral SMC ischemic lesions increased deficits in reaching with the impaired forelimb. Additionally, this training reduced expression of a marker of persistent neuronal activation, FosB/ Δ FosB, in remaining motor cortex. It also increased reliance on the intact forelimb in tests of coordinated forelimb movements. In human stroke patients, it is common to focus post-stroke therapy on the execution of everyday tasks. This is frequently accomplished through compensatory use of the less-affected hand and arm. However, this may contribute to the phenomenon of learned nonuse, where disuse of the impaired arm is believed to further limit its recovery (Taub et al., 2006B). Our results suggest this lack of recovery may stem, not only from disuse, but also from disruptive influences of behavioral experience with the less-affected forelimb.

Our results indicate that peri-infarct cortex is vulnerable to early post-injury intact forelimb experience and suggest that neural activity in this region is disrupted by experience with the intact body side. Δ FosB, a splice variant of the FosB gene lacking 101 amino acids from the C-terminus (Nakabeppu and Nathans, 1991) is present for days after exposure to a stimulus and its activation is believed to initiate neuronal structural plasticity (McClung et al., 2004). In the present study, unilateral lesions increased FosB/ Δ FosB neuronal density in layer II/III of remaining SMC near the infarct after impaired forelimb training; however, early intact forelimb training reduced this expression. This is significant because the remaining cortex has been strongly implicated as a mediator of sensorimotor functional recovery in the impaired limb after unilateral cerebral damage (Nudo, 2003).

The remaining cortex near an ischemic infarct undergoes a time-dependent cascade of degenerative and regenerative changes (Carmichael, 2006). Inhibition of peri-lesion cortex dramatically impairs recovery from cortical lesions in rats (Hernandez and Schallert, 1990). In humans, transient transcranial stimulation-induced “virtual lesions” of peri-infarct motor cortex worsens paretic hand function (Werhahn et al., 2003). Functional magnetic resonance imaging (fMRI) studies indicate that restoration of cortical activity in the infarcted hemisphere is positively correlated with functional recovery in rats (Dijkhuizen et al., 2003) and humans (Johansen-Berg, et al., 2002). Facilitative stimulation of peri-infarct motor cortex in rats (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2002) and squirrel monkeys (Plautz et al., 2003) further improves motor function and increases synaptic density in stimulated cortex (Hsu, Adkins, and Jones, 2006). Thus, there is a wealth of evidence to indicate that after unilateral cerebral damage, remaining cortical regions can mediate, or be driven to mediate, functional recovery. The present findings indicate that prior experience with the intact forelimb limits neuronal activation in remaining motor cortex, possibly suppressing or interfering with activity-dependent plasticity that could have mediated better recovery in the impaired forelimb.

Skilled reaching has been linked with lateralized neural plasticity in the motor cortex opposite the trained forelimb, including increased dendritic arborization, (Allred and Jones, 2004; Withers and Greenough, 1989), greater forelimb movement representations (Nudo et al., 1996) and behavioral LTP induction (Riout-Pedotti et al., 2000). Pyramidal neurons of layer

II/III of rat sensorimotor cortex are particularly responsive to motor learning (reviewed in Nudo, 1999) and changes here may be reflective of mechanisms related to long-term potentiation (e.g., Hess, Aizenman, and Donoghue, 1996). In contrast to FosB/ Δ FosB expression in layer II/III, neuronal labeling in layer V was not sensitive to the effects of intact forelimb training, though it was increased by the lesions alone. Both layers receive callosal projections (Donoghue and Parham, 1983), but dendritic spines in layer II/III are particularly responsive to manipulations of forelimb experience in intact rats (Adkins, Bury, and Jones, 2002). It may be that a greater duration of intact forelimb training is needed to influence FosB/ Δ FosB expression in layer V. In intact adult rats, 28 days of training on a complex motor skills task, the acrobatic task, increased layer V synapses per neuron (Jones et al., 1999). Given differences in baseline levels of FosB/ Δ FosB labeling between layers, these laminar differences should also be addressed using other measures of neuronal activation. Furthermore, the molecular mechanisms of these differential training effects and their relationship to synaptic structural plasticity require further investigation.

Constraint-induced movement therapy improves stroke-affected hand function (Mark and Taub, 2002; Wolf et al., 2006). However, extreme and early forced use of the impaired forelimb in rats increases lesion size and exacerbates behavioral deficits (Kozlowski, James, and Schallert, 1996; Schallert et al., 2000). Thus, there may be maladaptive consequences of overuse of *either* forelimb after unilateral brain damage. Our experiments used a challenging motor skills task, but this manipulation is subtle compared to behavioral manipulations found to exaggerate infarct size such as forced use (Kozlowski, James and Schallert, 1996). Furthermore, all rats with lesions increased reliance on the less-affected forelimb for postural support behavior, which presumably reflects asymmetrical forelimb use in the home cage. This raises the possibility that it is lateralized *skill acquisition* with the less-affected forelimb, rather than simply use of this limb, that alters behavioral outcome with the impaired forelimb. Following cerebral insult, animals have to “re-learn” new ways of using the impaired forelimb (Schallert, 2006; Shubring-Giese et al., 2007) and it is also possible that this is especially sensitive to experience with the less-affected forelimb. Previously, we found a large effect on impaired forelimb function following intact forelimb training in rats with little reaching experience prior to the infarct (Allred et al., 2005). Intact training effects may therefore be particularly maladaptive for learning *new* motor skills with the impaired forelimb.

Rats trained early after lesions with both forelimbs were not more impaired and tended to perform better than controls. Humans trained to use both arms to push and pull T-bar handles (following strokes) had increased activity in the affected hemisphere and better functional outcome with the paretic limb (Luft et al., 2004). Function in the paretic limb also improved following training of both limbs on reach-to-target tasks (Mudie and Matyas, 2000). It is important to note that BiT rats received half the intact training trials (15) compared to IntactT rats (30) and it is possible that the experience with the intact limb was simply too low in intensity to worsen function in the impaired forelimb in this group. Consistent with the possibility that training intensity is important, rats in Experiment 1 received 2.5 times as many intact training trials as rats in Experiment 2 and had more persistently worsened function in the impaired forelimb. However, this difference between experiments may also be related to infarct size, as rats in Experiment 2 tended to have smaller lesions. Differential stress can not be ruled out as a contributor to the present results, though this seems somewhat unlikely given that neither bilateral training, nor focused impaired limb training (e.g., Maldonado et al., in press), worsen function in the impaired limb on this reaching task.

In contrast to rats with infarcts, sequential forelimb training did not worsen behavioral performance with the preferred forelimb in intact animals. However, there was a non-significant tendency for training one limb to worsen function in the other limb in sham-operates. This is consistent with there being subtle, interhemispheric motor learning interference effects

that become exaggerated in rats with unilateral SMC lesions while re-learning to use the impaired forelimb. In both intact humans (Ward and Cohen, 2004) and rats (O'Bryant, Bernier, and Jones, 2007; Glazewski, Benedetti, and Barth, 2007), reduction in ipsilateral sensory activity has been found to improve function in the other body side. Sensory input from ipsilateral whiskers has been shown to result in a strong constraining influence on the receptive fields in the barrel field cortex (Glazewski, Benedetti, and Barth, 2007). Anesthetization of the less-affected hand improves performance of a finger tapping task with the paretic hand in stroke patients (Floel et al., 2004) and improves reaching function in the impaired forelimb in rats (O'Bryant, Bernier, and Jones, 2007). Moreover, the contralesional hemisphere can have an exaggerated inhibitory influence over the stroke-affected hemisphere during movements of the paretic hand compared with healthy subjects (Murase et al., 2004; Duque et al., 2005). Our findings suggest that these exaggerated interhemispheric effects are likely to be mediated, in part, by behavioral experiences with the intact body side.

Approximately 5% of the corticospinal tract descends ipsilaterally in rats (Brosamle and Schwab, 1997) and it is possible that this pathway contributes to the maladaptive effects of training the intact forelimb in this present study. The ipsilateral corticospinal tract has not been found to be a significant contributor to skilled forelimb function in rats (Whishaw and Metz, 2002), but this does not rule out a potential role in the exaggeration of forelimb impairments seen after training the intact forelimb.

It remains to be determined whether the post-injury time of onset of the training is an important variable in these effects. Furthermore, it is unknown whether the effects of learning with the intact forelimb extend to other types and modalities of function, such as less-skilled use of the forelimb, use of other body parts, and sensory functions. The behavioral and environmental experiences of the animals in this study were likely less intense and varied than the experiences of human stroke survivors. It is reasonable to hypothesize that training rats with their intact limb on motor skills of greater complexity (e.g., the pasta matrix task, Ballerman et al., 2001) or of greater variety (e.g., such as handling different kinds of pasta, Whishaw and Coles, 1996) would result in an even greater detriment in behavioral recovery of the impaired forelimb. It is also important to determine whether experiences with the less-affected body side influence behavior after infarcts in other brain regions and after other types of brain damage, including traumatic brain injury and Parkinson's disease, which frequently manifests asymmetrically.

The present results indicate that experiences with the less-affected forelimb can disrupt neuronal activity in the infarcted hemisphere in a manner linked to greater injury-induced impairments. Additional research is needed to address the timing and persistence of intact forelimb training effects, the causality of its relationship with peri-infarct neural activity, its molecular mechanisms, and the involvement of interhemispheric communication and corticospinal tract in these effects. There is also a need to assess the generalizability of reaching experience to other types of behavioral experience, including exercise. A better understanding of these effects could be of major relevance for rehabilitation approaches in human surviving stroke, and, possibly, other types of brain damage.

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Fig 1.

Summary of Experimental Designs. Experiment 1 was designed to test the hypothesis that training the intact forelimb worsens functional recovery in the infarct-impaired forelimb. After training to proficiency with the preferred forelimb on a skilled reaching task, the single pellet retrieval task, rats underwent either an ischemic sensorimotor cortex (SMC) lesion or a sham operation in the hemisphere contralateral to this limb. Beginning on postoperative day 5 (D5), rats received either 15 days of training (60 trials/day) with their intact/non-preferred forelimb (IntactT and Sham NPrefT) or control procedures (Cont, Sham Cont). All animals were then tested for 13 days (30 trials/day) with their impaired and/or preferred forelimb and then sacrificed to assay neuronal activation in peri-infarct cortex (n 's = 10/group). Experiment 2 was designed to test whether *focused* intact forelimb training is necessary to influence recovery of impaired forelimb function. Animals were trained pre-operatively as in Experiment 1. All animals then received an SMC lesion followed by 12 days of training (30 trials/day) with the intact forelimb (IntactT) both forelimbs (BiT) or control procedures (Cont). All rats were then tested with their impaired forelimb as in Experiment 1 followed by 5 days of intact forelimb retesting (n 's = 9/group).

Fig 2.

Training the intact forelimb in the skilled reaching task early after unilateral SMC lesions worsened subsequent performance in the impaired forelimb. A: Intact training period of Experiment 1. Lesion rats trained with their intact forelimb (IntactT) performed significantly better than sham (Sham NPrefT) rats, $p < .01$. B: Impaired testing period. Sham animals performed significantly better than lesion animals ($p < .01$). IntactT rats performed significantly worse with their impaired forelimb compared to Cont rats ($p < .05$). The shaded forelimb indicates the trained/tested forelimb. Data are means $\pm$ SEM.

Fig 3.

Training both forelimbs in skilled reaching early after the lesions did not reproduce the effects of *focused* intact limb training. IntactT rats performed significantly worse with their impaired forelimb compared to BiT rats ($p < .01$) and performed worse compared to Cont dependent on day ($p < .01$). Training with both forelimbs did not significantly change impaired forelimb performance compared to control animals ($p > .05$). These results are from Experiment 2. Data are means $\pm$ SEM. Note the difference in scale between Figure 2 and Figure 3.

Fig 4.

Reach training focused on the intact forelimb increased reliance on this limb in a measure of coordinated forelimb placement during locomotion (the footfault test). Unilateral lesions resulted in impairments in the contralateral forelimb on the footfault test which recovered by Day 31. IntactT rats had increased slips with their impaired forelimb during the lidocaine challenge test compared to Cont (* $p < .05$). Data are means $\pm$ SEM.

Fig. 5.

Schematic coronal sections throughout the sensorimotor cortex showing representative lesions in Cont and IntactT animals (from Experiment 1).

Fig 6. FosB/ΔFosB representative photomicrographs from layer II/III peri-infarct cortex (A,B, scale bars = 10 μm) and quantitative results (C) from Experiment 1. Unilateral lesions increased FosB/ΔFosB neuronal density in remaining SMC compared to sham-operates. Intact forelimb training significantly decreased FosB/ΔFosB neuronal density in layer II/III compared to Cont rats. A representative lesion is shown in panel A. Scale bar = 500μm. Data are means ± SEM. *p < .05.

Table 1
Intact training effects on use of the impaired forelimb in postural support behaviors.

Group	Early Post-operative	Impaired Forelimb Training
Experiment 1		
Cont	$-9.18 \pm 5.55\%$ *	$-4.46 \pm 4.03\%$
IntactT	$-12.16 \pm 6.66\%$ (p = .06)	$-17.69 \pm 6.24\%$ *
Sham Cont	$7.00 \pm 2.56\%$	$7.00 \pm 6.24\%$
Sham NPrefT	$5.33 \pm 5.49\%$	$9.29 \pm 8.93\%$
Experiment 2		
Cont	$-16.40 \pm 6.73\%$	$-14.48 \pm 4.71\%$
IntactT	$-26.23 \pm 7.35\%$	$-30.94 \pm 7.03\%$
BiT	$-18.57 \pm 3.76\%$	$-21.85 \pm 5.96\%$

Data are means $\pm$ SEM post-operative – pre-operative % impaired limb use.

* p < .05 compared to Sham of the same training condition.

Table 2
Volume of remaining infarcted sensorimotor cortex.

Group	Remaining SMC volume (mm ³)
Experiment 1	
Cont	82.12 ± 2.24
IntactT	83.20 ± 1.96
Experiment 2	
Cont	91.41 ± 2.33
IntactT	90.08 ± 2.37
BiT	95.70 ± 2.83

Data are means ± SEM.