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Post-stroke protection from maladaptive effects of learning with the non-paretic forelimb by bimanual home cage experience in C57BL/6 mice

Abigail L. Kerr[a,1], **Malerie L. Wolke**[b], **Jared A. Bell**[c], and **Theresa A. Jones**[a,b]

[a]University of Texas at Austin, Psychology Department, 1 University Station, A8000 Austin TX, 78712, USA

[b]University of Texas at Austin, Institute for Neuroscience, 1 University Station, A7000 Austin TX, 78712, USA

[c]University of Texas at Austin, School Biological Sciences, 100 East 24th Street Stop, A6500, Austin TX 78712, USA

Abstract

Behavioral experience, in the form of skilled limb use, has been found to impact the structure and function of the central nervous system, affecting post-stroke behavioral outcome in both adaptive and maladaptive ways. Learning to rely on the less-affected, or non-paretic, body side is common following stroke in both humans and rodent models. In rats, it has been observed that skilled learning with the non-paretic forelimb following ischemic insult leads to impaired or delayed functional recovery of the paretic limb. Here we used a mouse model of focal motor cortical ischemic injury to examine the effects of non-paretic limb training following unilateral stroke. In addition, we exposed some mice to increased bimanual experience in the home cage following stroke to investigate the impact of coordinated dexterous limb use on the non-paretic limb training effect. Our results confirmed that skilled learning with the non-paretic limb impaired functional recovery following stroke in C56BL/6 mice, as it does in rats. Further, this effect was avoided when the skill learning of the non-paretic limb was coupled with increased dexterous use of both forelimbs in the home cage. These findings further establish the mouse as an appropriate model in which to study the neural mechanisms of recovery following stroke and extend previous findings to suggest that the dexterous coordinated use of the paretic and non-paretic limb can promote functional outcome following injury. Keywords: experience-dependent plasticity, learned nonuse, motor cortex, motor rehabilitation, stroke

1. Introduction

Stroke is among the leading causes of death and disability worldwide, with chronic upper limb impairment (usually unilateral) among the most common deficits reported in stroke survivors [1, 2]. Often this deficit presents as a loss of functional use of the hand or arm

All correspondence should be addressed to: Abigail L. Kerr, Illinois Wesleyan University, Department of Psychology, 1312 Park Street, PO Box 2900, Bloomington, IL 61702, Telephone: (309) 556-3803, Fax: (309) 556-3864, akerr@iwu.edu.
[1]Permanent address: Illinois Wesleyan University, Department of Psychology, 1312 Park Street, PO Box 2900, Bloomington IL 61702. Phone: (309) 556-3803. Fax: (309) akerr@iwu.edu

contralateral to the lesion locus (paretic body side; i.e, paretic limb). A natural response to loss of function is to develop alternative strategies to circumvent the problem. As a result, a common compensatory strategy among stroke survivors is to learn to rely on the less-affected, or non-paretic, limb for daily tasks involving skilled limb or hand use such as grasping and manipulating objects [3, 4]. However, the long-term neural and behavioral consequences of continued compensatory skill learning with the non-paretic limb use are not well understood.

Reliance on the non-paretic limb contributes to a learned non-use of the paretic limb [5, 6] and may limit long-term functional outcome following stroke. This is the basis of constraint-induced movement therapy (CIMT) [4, 7–10], whereby, in addition to intense rehabilitation, the non-paretic limb is bound for most waking hours, encouraging patients to use the paretic limb to complete daily tasks. While CIMT has been reported to significantly improve upper extremity deficits [11, 12], many stroke survivors continue to use their non-paretic limb for daily tasks when it is unbound [13], possibly affecting the rehabilitative potential of the paretic limb.

Unilateral motor cortex damage in the caudal forelimb representation area (CFA) of the rat results in contralateral-to-lesion forelimb impairments [14–24] and a reliance on the non-paretic body side [6, 17, 25–28], that resemble in many respects both the upper extremity impairments and learned non-use of the paretic limb observed in humans. Focused rehabilitative training of the paretic limb can improve motor function and structural and functional plasticity in remaining regions of cortex after stroke in rats [29– 36]. Following ischemic insult, contralesional cortex has been found to exhibit increased neuroplasticity [37, 38] that may facilitate the acquisition of new motor skills with the *non-paretic* limb [15, 17, 25]. Previously, our laboratory has reported that focused training of the non-paretic limb in a skilled reaching task impedes recovery of the paretic limb [39, 40] and disrupts functional reorganization in peri-infarct cortex that would otherwise contribute to improvements in functional outcome of the impaired limb [15, 40, 41], while promoting experience-driven plasticity in the contralateral-to-lesion cortex [17, 21, 22, 38, 42]. Rats that receive focused bilateral rehabilitative training on a skilled reaching task do not show the maladaptive effects and exhibit functional outcome that is similar to that of animals that receive focused rehabilitation of the paretic limb [40].

As they are inexpensive to house and offer many transgenic lines that are suitable for *in vivo* imaging, mice are an important tool in understanding the impact of behavioral training on functional and structural recovery following stroke. We have determined that C57BL/6 mice have long lasting forelimb impairments following focal ischemic insult of the CFA [43] and exhibit improved functional outcome and structural plasticity following focused rehabilitative training of the paretic limb [44]. While it is clear that mice are an effective model of upper limb impairment and functional rehabilitation following stroke, the effects of non-paretic limb training, and thus their usefulness for investigating neural mechanisms of learned non-use, has not been established. The present study investigated the impact of skilled non-paretic limb use in our mouse model of focal ischemic insult. In addition, we explored the impact of coordinated, dexterous bimanual limb use in the home cage on non-paretic limb training effects. Our current findings lay the groundwork for further studies that will explore the neural consequences of non-paretic skill learning in both contra- and ipsilesional cortices.

2. Materials and Methods

Experimental designs are summarized in Fig. 1.

2.1. Subjects

A total of 94 well-handled, 3-month-old male C57BL/6 mice were housed in groups of four with standard housing supplementation (a small piece of PVC pipe, a cardboard roll, and small wooden objects for chewing) on a 12:12 light/dark cycle. Animals were maintained on a restricted feeding schedule (2.5–3g/mouse/day) to prevent satiation and promote reaching performance. Daily food restriction was monitored and adjusted such that no animal lost more than 10% of their free feeding weight, established prior to experimental procedures. In Experiment 1, 39 mice received intracortical infusions of the vascoconstricting peptide, endothelin-1 (ET-1), and 15 mice received a sham surgery consisting of intracortical infusion of 0.9% sterile saline. Six of the mice receiving ET-1 died during recovery from perioperative anesthesia and were consequently not included in the study. The surviving mice were separated into one of three groups on post-op day 5: paretic-trained (Par), non-paretic trained (NonPar), or Control (for all conditions, lesion: n = 11, sham: n = 5). Groups were matched on pre-operative performance levels.

In Experiment 2, 33 mice reached appropriate pre-operative reaching levels (defined in 2.3) and received intracortical infusion of ET-1. Five mice died during recovery from perioperative anesthesia. One additional mouse was excluded from the study because of severe motor deficits observed 48 hours after surgery. No sham operates were used in Experiment 2. Following surgery, mice were separated into one of four groups (matched on pre-operative performance): bimanual home cage enriched control (BE Control; n = 5), BE non-paretic limb trained (BE NonPar; n = 9), standard housed control (ST Control; n = 5), and ST NonPar (n = 8). Enrichment procedures are described below (see 2.4). Animal use was in accordance with a protocol approved by the University of Texas at Austin Animal Care and Use Committee.

2.2. Intracortical infusion of ET-1

Following pre-operative motor skill learning, mice received intracortical infusion of ET-1 as described previously [43]. Briefly, mice were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and placed into a stereotaxic frame (Stoelting, Wood Dale, IL). Lidocaine (2 mg/kg, s.c.) was injected into the scalp, and a midline incision was made. A small burr hole was drilled over the center of the forelimb region of SMC (0.3 mm anterior to Bregma; 1.5 mm from midline) contralateral to the preferred reaching paw. A total of 3 μl of ET-1 (American Peptide; 320 pmol, 0.2μg/μl in sterile saline) was injected into layer V of the motor cortex at a depth of 800μm below the surface of the cortex over the course of 10 minutes (1 μl at a time). The burr hole was then filled with gelfoam and covered with UV curing dental cement (Wave A2; Southern Dental Industries, Victoria, Australia). The wound was sutured and covered with antibiotic ointment. All mice were permitted to fully awaken in a heated chamber before receiving buprenorphine (3 mg/kg at 0.015 mg/mL in sterile saline, s.c.) and returning to the home cage. Mice in the sham group (Experiment 1) received an identical surgery with 3 μl of 0.9% sterile saline in the place of ET-1.

2.3. Behavioral methods: Pasta Matrix Reaching Test

Animals were trained on the Pasta Matrix Reaching Test (Figure 1A) as described previously [43]. Briefly, mice were trained to reach through a small slit found in the center wall of a Plexiglas chamber to break pieces (3.2 cm in height, 1mm in diameter) of vertically oriented, uncooked capellini pasta (De Cecco brand, Fratelli De Cecco di Filippo Fara San Martino S.p.A., Italy). Pasta pieces were located 2 mm apart in a heavy-duty plastic block outside of the reaching chamber. Half of each pasta piece extended above the block. This is a skilled reaching task that requires the animal to adjust its reach trajectory to obtain pasta pieces that are located increasingly further from the reaching aperture.

Prior to training, all animals underwent shaping procedures in which they were acclimated to the testing apparatus and limb preference was determined. Animals were placed individually in reaching chambers once daily for three to five days. During this time, the matrix stage (containing the pasta pieces) was completely filled, allowing animals to reach pasta pieces with both limbs. Each daily trial lasted for 10 minutes or until the mouse reached a minimum of 10 times. A reach was defined as extending the limb through the reaching aperture such that the entire forepaw was outside the chamber walls. Limb preference was determined when a minimum of 70% of an animal's reaches were made with either the right or left forelimb. Shaping trials continued once daily until each animal's limb preference was determined.

Following shaping procedures, mice were trained on the Pasta Matrix Reaching Test to establish the skill. During training, only half of the matrix stage was filled with pasta (contralateral to the preferred limb), forcing the mice to use their preferred to limb to reach for pasta pieces. Each daily trial consisted of 15 minutes or 100 reaches, whichever occurred first. Mice were trained for a total of 19 days, at which point performance was considered to be stable for all mice. To establish performance level, the average number of successful retrievals (i.e., number of pasta pieces broken) was calculated for the final three days of training. Previous results suggest that each mouse is only physically able to reach a maximum of 18 pieces of pasta in the matrix [43]. Eighty-seven mice (54 in Experiment 1; 33 in Experiment 2) reached criterion performance, defined as 9 successful retrievals, and received ET-1 or sham lesions (post-op day 0). On post-op day 4, performance of the affected limb was assessed on the Pasta Matrix Reaching Test. On post-op day 5, mice were separated into their respective groups (see 2.1) for post-operative training. For both experiments, Par and NonPar groups received daily training sessions (15 minutes or 100 reaches in length) of the paretic or non-paretic limb. Control mice were placed into reaching chambers with no matrix stage and ate a comparable amount of pasta broken into small nibblets. These training procedures took place for a total of 15 consecutive days.

Following post-operative training, all mice were assessed with their paretic limb on the Pasta Matrix Reaching Test with procedures identical to those during pre-operative training. Assessment took place over 7 consecutive days.

2.4. Bimanual home cage enrichment

Animals in the enriched groups (Experiment 2) received daily home cage enrichment that maximized novel dexterous bimanual forelimb use. Enrichment consisted of 10 pieces of 1.25 in long pasta and 6 sunflower seeds (in-shell) per mouse in addition to two square chewing blocks (5/8 mm^3) per cage per week. The dexterous manner in which rodents handle and consume long pasta pieces has been documented previously[43, 45–47]. It was noted in the current study that mice often shelled the sunflower seeds before consuming them as evidenced by empty shells found in the cages each morning, purportedly involving the dexterous use of both forelimbs. All other mice received a similar amount of pasta broken into small nibblets and shelled sunflower seeds. The square blocks were chosen for the enrichment condition as a novel shaped object that was distinct from those that the mice previously experienced in the baseline housing condition, which included round or smooth-edged wooden gnawing objects. Instead of square blocks, mice in the un-enriched condition received two round chew toys (5/8 mm in diameter) per cage per week, which were similar to those gnawing objects available during pre-operative baseline housing conditions. While mice in both conditions were assumed to use both limbs to manipulate the food and gnawing objects, the conditions of the bimanual enrichment were intended to promote experience with new bimanual handling patterns. Both enrichment and control cages consumed all of their allotted pasta and sunflower seeds (either shelled or unshelled) each day, and the wooden objects were observed to have been gnawed upon during weekly replacement. All

animals received either enrichment or control procedures concurrent with daily training sessions as described in 2.3. Following 15 days of post-operative training, paretic limb assessment commenced as described above. No enrichment (beyond the standard housing supplementation described in 2.1) was available to any mouse during paretic limb assessment.

2.5. Tissue processing and lesion analysis

Twenty-four hours after the final testing session (i.e., 27 days after ET-1 administration), mice were euthanized with an overdose of sodium pentobarbital (euthasol, 175 mg/kg, i.p.) and perfused intracardially with 0.1M phosphate buffer (PB) and 4% paraformaldehyde. Following perfusion, brain tissue was removed and stored in 4% paraformaldehyde at 4°C for a minimum of 72 hours before being sliced into 40 μm thick sections using a vibratome. Every sixth section was mounted onto gelatin-coated slides and Nissl stained with toluidine blue.

Neurolucida software was used to estimate lesion volume. Coronal sections were viewed at a magnification of 17x. The cortical areas of 10 coronal sections from approximately 2 mm anterior to 1.5 mm posterior to Bregma, each 240 μm apart, were measured by tracing cortical boundaries of both contralesional and ipsilesional cortex. The SMC fell within the area of tissue measured and no lesion extended beyond these boundaries. Cortical volume was estimated with the Cavalieri method, by multiplying the sum of section areas by the distance between sections[48, 49]. Lesion volume was calculated as the difference between contralesional and ipsilesional cortex.

2.6. Statistical analyses

SPSS software was used to conduct repeated-measures analyses of variance (ANOVAs) for Pasta Matrix Reaching Test performance, with Day as a within-subjects variable and Group as a between subjects variable. *Post hoc* analyses with a least significant difference (LSD) correction were conducted as necessary. A one-way ANOVA was conducted to compare the lesion extents of sham and ET-1 groups. In Experiment 1, there were no statistical differences between the performances of different sham groups ($p > 0.1$); all sham mice in Experiment 1were combined for statistical analyses. Likewise, there were no statistical differences between the performance levels of the two control groups in Experiment 2 ($p > 0.1$); mice in these groups were combined for statistical analyses. An α level of 0.05 was considered significant for all comparisons.

3. Results

3.1. Non-paretic limb training impedes functional outcome following ischemic stroke

Subjects were matched on pre-operative performance on the Pasta Matrix Reaching Test. All animals exhibited similar initial deficits in reaching performance (around a 40% decrease in successful reaching) after ischemic lesions (Fig. 2). Following 15 days of reach training procedures with either the paretic (Par) or non-paretic (NonPar) limb or control procedures, the reaching performance of the paretic limb was assessed in all animals. During initial post-training assessment, the performance of Sham and Par groups resembled pre-operative levels. Control and NonPar groups exhibited performance levels that were similar to that of the initial post-operative assessment. Over seven consecutive days of testing the paretic limb, Control mice gradually began to display behavioral outcome that resembled pre-operative performance levels. The performance of NonPar mice did not improve with seven days of paretic limb assessment and remained at initial post-operative levels. A repeated measures ANOVA confirmed a significant Group x Day interaction ($F_{(23,352)} = 9.04$, $p < 0.001$). *Post hoc* analyses with an LSD correction revealed significant differences between

NonPar animals and all other groups ($p < 0.001$ for all comparisons). In addition, there was a significant difference between Control and Sham groups ($p < 0.03$). No other *post hoc* comparisons reached statistical significance ($p > 0.1$ for all comparisons).

3.2.2. Bimanual home cage enrichment (BE) ameliorates non-paretic limb training effects following ischemic lesion—Experiment 2 assessed the value of dexterous coordinated use of both forelimbs in the home cage to functional outcome following non-paretic limb training. Following ET-1-induced ischemic infarcts, all groups exhibited similar deficits in reaching performance, with an approximate 50% decrease in successful reaching. Following 15 days of either standard housing or bimanual home cage enrichment and non-paretic or control training procedures, paretic limb performance was assessed for all mice over seven consecutive days (Fig. 3). Paretic limb assessment revealed that mice receiving bimanual home cage enrichment exhibited performance levels that were similar to those of Control mice. Both BE NonPar and Control groups improved their reaching performance over seven days of assessment, ultimately achieving performance levels that resembled pre-operative performance. ST NonPar mice did not improve their reaching performance with paretic limb assessment. Performance of ST NonPar mice remained similar to the initial deficit following stroke. A repeated measures ANOVA confirmed a significant Group x Day interaction ($F_{(16, 192)} = 9.361$, $p < 0.001$). *Post hoc* analyses with an LSD correction revealed that the performance of ST NonPar mice was significantly different from both BE NonPar and Control mice ($p < 0.001$ for both comparisons). No other comparisons reached statistical significance ($p > 0.1$).

3.1.1. Lesion volume and reconstruction—Representative images of Nissl stained tissue from both experiments are presented in Fig. 4A. Average lesion extent is outlined for all groups. ET-1 infusion resulted in damage in all mice, while sham procedures did not affect cortical volume. In Experiment 1, ET-1 infusion produced damage to the forelimb representation area of motor cortex extending in an approximately 1 mm radius from the infusion site (Figure 4A). Reconstruction of lesion placement and extent for Experiment 2 indicated that lesion placement was similar to that of Experiment 1, though the total lesion volume (contralateral-ipsilateral volume difference) tended to be larger (Figure 4A). No damage was observed in underlying white matter or striatum in either experiment.

As seen in Figure 4B (top), in Experiment 1 lesions (n = 33) resulted in a significant loss of ipsilesional cortical volume (as estimated by contralateral-ipsilateral volume difference) compared to sham operates ($F_{(1,46)} = 15.383$, $p < 0.001$). There was no significant difference in interhemispheric volume differences between the two lesion groups ($p > 0.1$; Figure 4B bottom). In Experiment 2, there was no statistical difference in lesion volume between Control (n = 10), ST NonPar (n = 8), and BE NonPar (n = 9) groups ($p > 0.1$; Figure 4C).

4. Discussion

We have previously established C57BL/6 mice as a reliable model for sensorimotor deficits following focal ischemic insult [43], with behavioral deficits resembling those produced by similar lesions in rats [14, 15, 17] and chronic impairments observed in humans (e.g., [50]). In this study, we have further developed our mouse model by exploring the impact of non-paretic limb training following focal insult. Our results indicate that the mouse, much like rats and humans, exhibits impaired functional recovery of the paretic limb following skilled use of the non-paretic limb after insult. We have further shown that the maladaptive effects of non-paretic limb training can be ameliorated with increased coordinated bimanual forelimb use in the home cage, suggesting that peri-lesion plasticity can be maintained and stimulated by minimal, unskilled, dexterous forelimb use. These results are in concert with previous findings that skilled bilateral training in rats [39, 40, 51] and humans [52, 53]

improves functional outcome and extend these findings by suggesting that bilateral limb use does not have to involve focused training, but simply coordinated, dexterous activity.

Mouse models of stroke are becoming increasingly important with the availability of transgenic lines, the affordability of housing, and the ease of *in vivo* imaging with the species. Because mice share homologies with rat and primate forelimb movements and motor system organization [32, 54–57], they are a strong candidate for modeling upper limb impairment following stroke. Previously, we have demonstrated that ET-1 ischemic lesions of SMC in mice produce behavioral deficits that are similar to those observed in the well-established rat model[25, 39]. Following ET-1 infusion, mice exhibit impaired skilled reaching abilities, asymmetrical responsiveness to tactile stimulation [43], and motor map reorganization [44], similar to that previously observed in rats and primates.

In both the rat model and in human rehabilitation studies, skilled use of the non-paretic limb (forelimb in the rat) results in impaired functional outcome of the paretic limb. The current study establishes that this finding is also consistent in our mouse model. In fact, non-paretic training following ischemic stroke appears to not only impair, but to prevent functional recovery, as mice show no improvement in performance with seven days of focused paretic limb assessment (see Fig. 3 and 6). The current findings cannot be explained by lesion size as both paretic and non-paretic trained animals had similar lesion volumes assessed at the termination of paretic limb assessment. Therefore, non-paretic limb training interferes with functional recovery of the paretic limb, possibly by impeding on neural plasticity in peri-lesion cortex.

We have previously explored the impact of skilled bilateral limb training following similar insults in rats [40]. Our results indicated that focused training of both limbs prevents the maladaptive effects of non-paretic limb training after stroke. These results suggested that functional recovery is possible, even with a reliance on the non-paretic limb, as long as the paretic limb is also utilized in a focused rehabilitative fashion. However, humans often rely on their non-paretic limb to perform daily activities. This compensation both precedes and supersedes focused paretic limb rehabilitation, which is typically limited in frequency and duration. In the current study, we found that enhancing bimanual dexterous forelimb use for daily activities, such as eating, in mice was sufficient to avoid the deleterious effects of skill learning with the non-paretic limb [58, 59] and promote better functional outcome. Therefore, increasing coordinated bimanual limb use in daily activities may be an effective therapy to promote functional outcome without the inconvenience and discomfort of binding the non-paretic limb.

Following injury, the remaining cortex undergoes time-dependent degenerative and regenerative cascades that impact functional outcome [60]. Rehabilitative training interacts with this naturally occurring plasticity to impact both the structure and function of the central nervous system. Skilled unimanual training increases dendritic arborization [17, 25, 61, 62], the number of synapses per neuron [22, 63], and induces LTP-like mechanisms [64, 65] in contralateral-to-training motor cortex. In mice, skilled reaching training on the Pasta Matrix Reaching Test induces rapid synaptic remodeling observed *in vivo* [66]. When combined with the growth permissive and inhibitive post-stroke environment, skilled rehabilitative training induces motor map plasticity [31, 32, 44], dendritic plasticity [25, 38, 67], and improved motor function [30, 34]. These effects are time-dependent [68, 69] and often occur in areas of remaining ipsilesional cortex (i.e., peri-infarct cortex), which has been found to be especially important for behavioral outcome following injury. There is a correlation between behavioral outcome and movement related activation of the peri-infarct cortex in humans [70]. In mice, the area of peri-infarct cortex that is closest to the lesion is

particularly dynamic, with the greatest synaptic turnover found in areas within several μm of the lesion core [71].

Learned non-use may result, in part, because learning with the paretic limb somehow impedes or impairs the potential of residual cortex to mediate better function in the paretic limb. ET-1 induced lesions of the SMC facilitate learning the with non-paretic limb, possibly as a result of degeneration-triggered processes that facilitate synaptic changes that underlie learning [17, 22, 25, 72]. With this facilitated learning of the non-paretic limb comes additional, maladaptive plasticity including decreased neuronal activation that is associated with the initiation of plasticity in remaining ipsilesional cortex [40].

The neural basis of the present effects are poorly understood. We have previously demonstrated that the maladaptive effects of non-paretic training (our model for learned non-use) are mediated through interhemispheric connections of SMC, as contralesional cortex and transcallosal fibers are required for the appearance of non-paretic training effects. It has been suggested that learning to compensate with this limb exaggerates interhemisphere disruption that may occur following stroke [51]. This hypothesis is supported by human research whereby unilateral injury results in abnormalities in interhemispheric activity associated with decreased behavioral outcome [73–77]. It is possible that by increasing coordinated bimanual forelimb use in the home cage, we have increased interhemispheric communication and perhaps connectivity, preserving the neuroplastic capabilities of peri-infarct cortex. It is also feasible that increased bimanual limb use during non-paretic limb training periods preserves the function of peri-infarct cortex by stimulating neuronal activation associated with the initiation of neural plasticity and prevents maladaptive plasticity that results from non-paretic limb training from impeding on the structural rehabilitation of peri-infarct cortex. Coordinated bimanual limb use does not rescue damaged tissue in peri-infarct cortex as lesion sizes are similar between enriched and un-enriched animals.

As peri-infarct cortex undergoes remodeling following injury, and contralesional homotopic cortex is likely responsible for mediating changes in non-paretic limb use, these two locations offer the most fruitful exploration of neural mechanisms supporting the adaptive and maladaptive effects of rehabilitative training. Stroke induces both vascular and dendritic plasticity in a coordinated fashion in peri-infarct cortex [71, 78], and non-paretic limb training has been found to decrease neural activation associated with initiating neural plasticity in peri-infarct cortex [40]. Clearly, interhemispheric disruption impedes neural plasticity in peri-infarct cortex. One potential mechanism of non-paretic training effects is interference with dendritic and vascular plasticity in peri-infarct cortex as a result of increased plastic responses in contralesional cortex. Additional research is needed to assess the neural mechanisms that support both the maladaptive effects of non-paretic limb training and the amelioration of those effects by bimanual dexterous limb use in the home cage.

It should be noted that in the current study, our ischemic insult produced different sized lesions between Experiment 1 and Experiment 2, with the lesions in Experiment 2 being somewhat larger on average that those in Experiment 1. It is important to realize that the size of lesions produced by ET-1 infusion are often small and the lesion sizes in both experiments fall within the normal range of what we have previously observed with this technique[43]. While the lesion-induced decrements in behavior were somewhat different between experiments, with animals in Experiment 1 exhibiting a 40% decrease in reaching performance and animals in Experiment 2 exhibiting a 50% decrease, the response of all animals to non-paretic limb training was similar. That is, in both experiments training the non-paretic limb reduced functional improvements in the paretic limb assessment. Further, the amelioration of the non-paretic limb training effect was detected in Experiment 2,

following slightly larger lesions. As the larger lesion is associated with a larger impact on performance, it could be argued that effective rehabilitative strategies in Experiment 2 would also be effective in Experiment 1 and therefore do not limit our conclusions in the present study.

The current study is not without limitations. We assessed paretic limb performance for seven days following non-paretic training conditions. Additional research is also needed to determine the persistence of non-paretic training effects. In addition, the behavioral experience of our mice was limited in complexity, intensity, and variety and therefore does not accurately mimic the range of human bimanual experience. It is important to further explore the impact of intensity and variety of both behavioral experience and training on adaptive and maladaptive effects of post-stroke behavioral training. Finally, it would be useful to assess the generalizability of our findings to other disorders that present unilaterally or asymmetrically such as traumatic brain injury and Parkinson's disease. Our present results suggest that therapeutic strategies focused on increasing coordinated bimanual limb use, and minimizing reliance on the non-paretic limb, will result in the most effective functional outcome following injury. The current findings also raise the possibility that the effects of paretic limb training might be further improved by its combination with bimanual enrichment training, although this effect was not explicitly tested as we did not have a paretic limb trained enrichment group. A better understanding of both the neural mechanisms that support functional rehabilitation and maladaptive behavioral outcomes, and the generalizability of our findings, may lead to optimal applications of rehabilitative strategies, including CIMT, cortical stimulation [79– 81], behavioral experience [82, 83], and focused behavioral training, to promote more effective behavioral recovery following injury.

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Highlights

- We studied the effect of non-paretic limb training on paretic limb outcome in mice.
- Non-paretic limb training was found to impede functional outcome of the paretic limb.
- Home-cage bimanual limb use attenuated maladaptive effects of non-paretic training.
- Potential neural mechanisms that support functional outcome are considered.

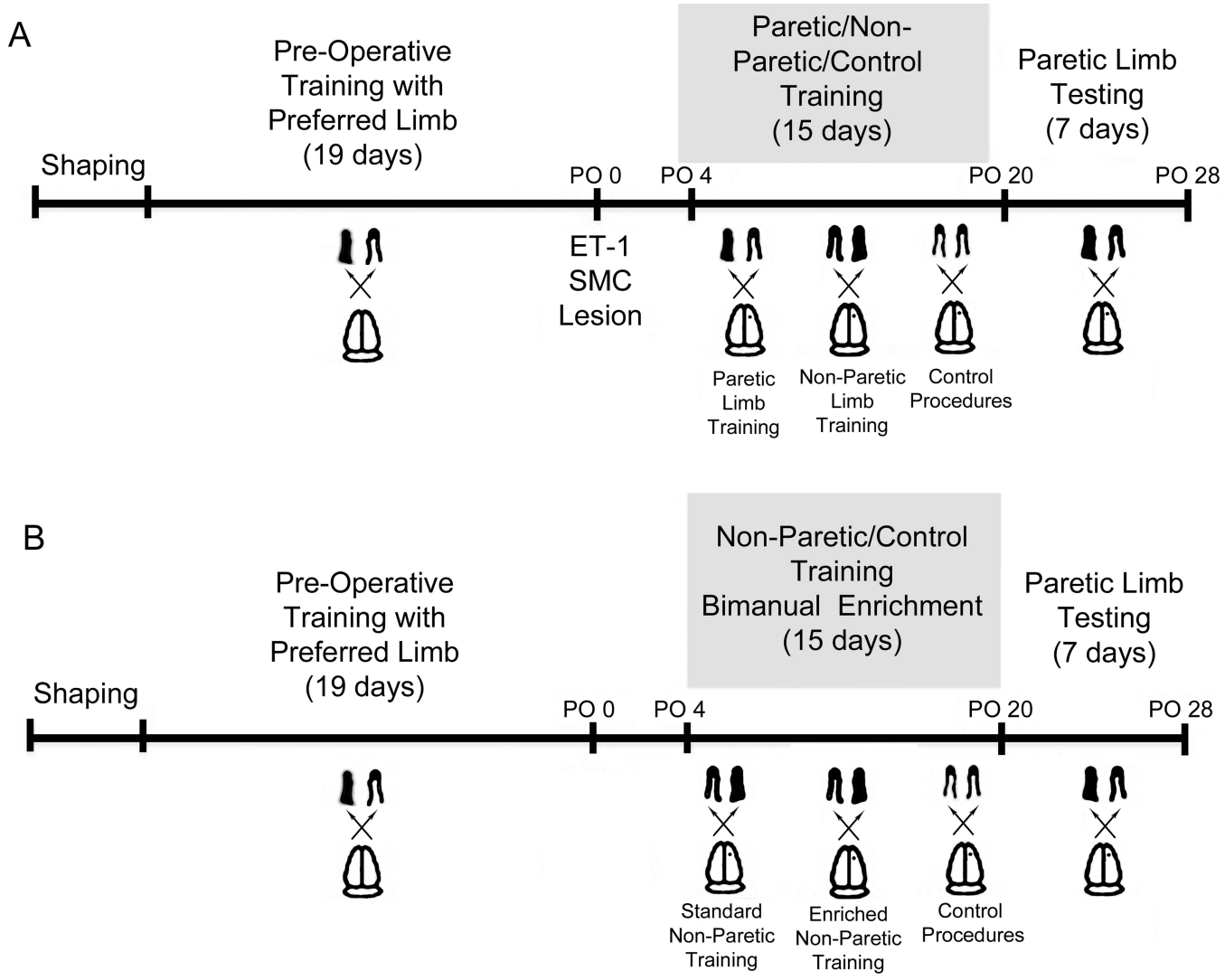

Fig. 1.

Experimental Design. In Experiment 1 (A), mice were given ET-1 induced ischemic lesions of the contralateral SMC following task acquisition. During postoperative training, mice trained with either the paretic (Par) or non-paretic (NonPar) limb on a reaching task or received control procedures. Following training, all mice were assessed on the Pasta Matrix Reaching Test using their paretic limb. Experiment 2 (B) was similar to Experiment 1. However, during post-operative training all mice were forced to use their non-paretic limb for reaching or received control procedures. In addition, half of the mice were given home cage enrichment that encouraged bimanual dexterous forelimb use (BE) versus standard housing conditioning (ST).

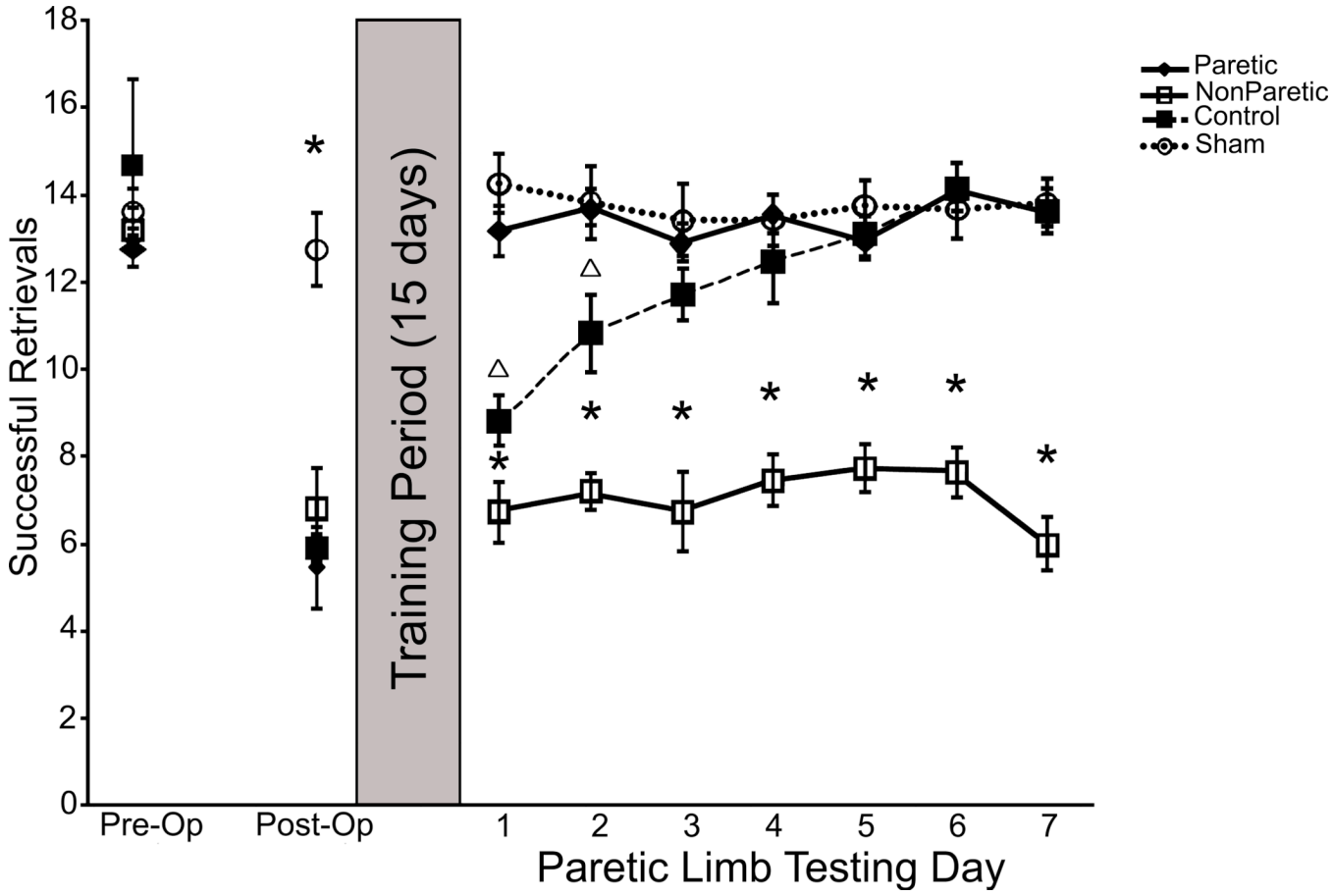

Fig. 2.

Experiment 1: Pasta Matrix Reaching Test. Mice receiving rehabilitative training of the paretic limb (Par) during the training period exhibited functional recovery of the paretic limb during testing. Control mice received no skilled training during the training period. With paretic limb assessment, control mice improved their performance to reach pre-operative levels. Mice that received focused training of the non-paretic limb (NonPar) during the training period did not exhibit recovery during paretic limb assessment. NonPar mice did not experience any improvement of the paretic limb during the experiment. Significant differences ($p < 0.05$) from all other groups is denoted by *. Significant differences ($p < 0.05$) from only Par and Sham groups is denoted by Δ.

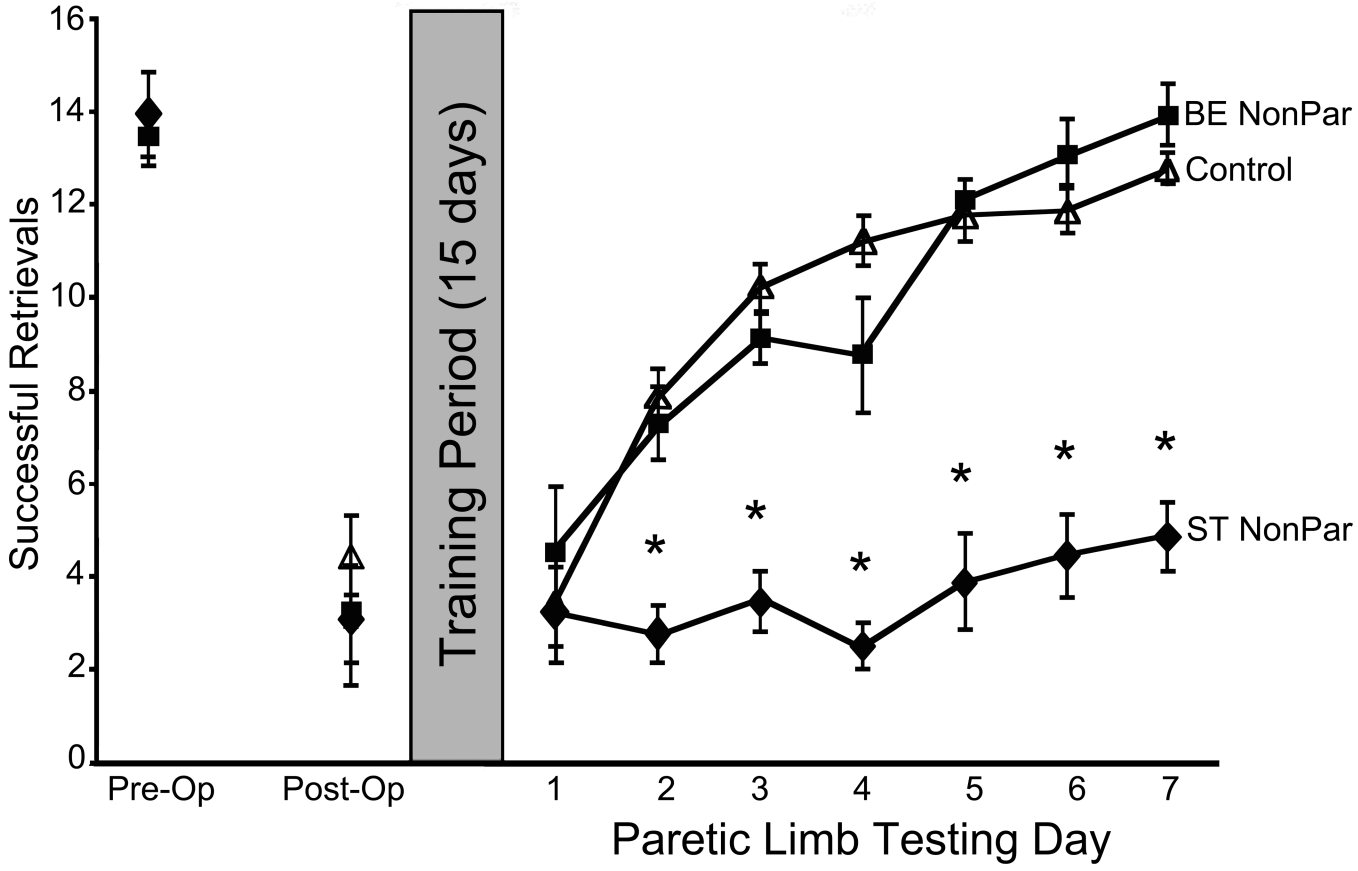

Fig. 3.

Experiment 2 Pasta Matrix Reaching Test. Home cage bimanual enrichment (BE NonPar) ameliorated the effects of non-paretic limb training (ST NonPar). BE NonPar mice performed similarly to Control mice. As in Experiment 1, ST NonPar mice did not show evidence of improved paretic limb performance during assessment. Significant differences ($p < 0.05$) from all other groups is denoted by *.

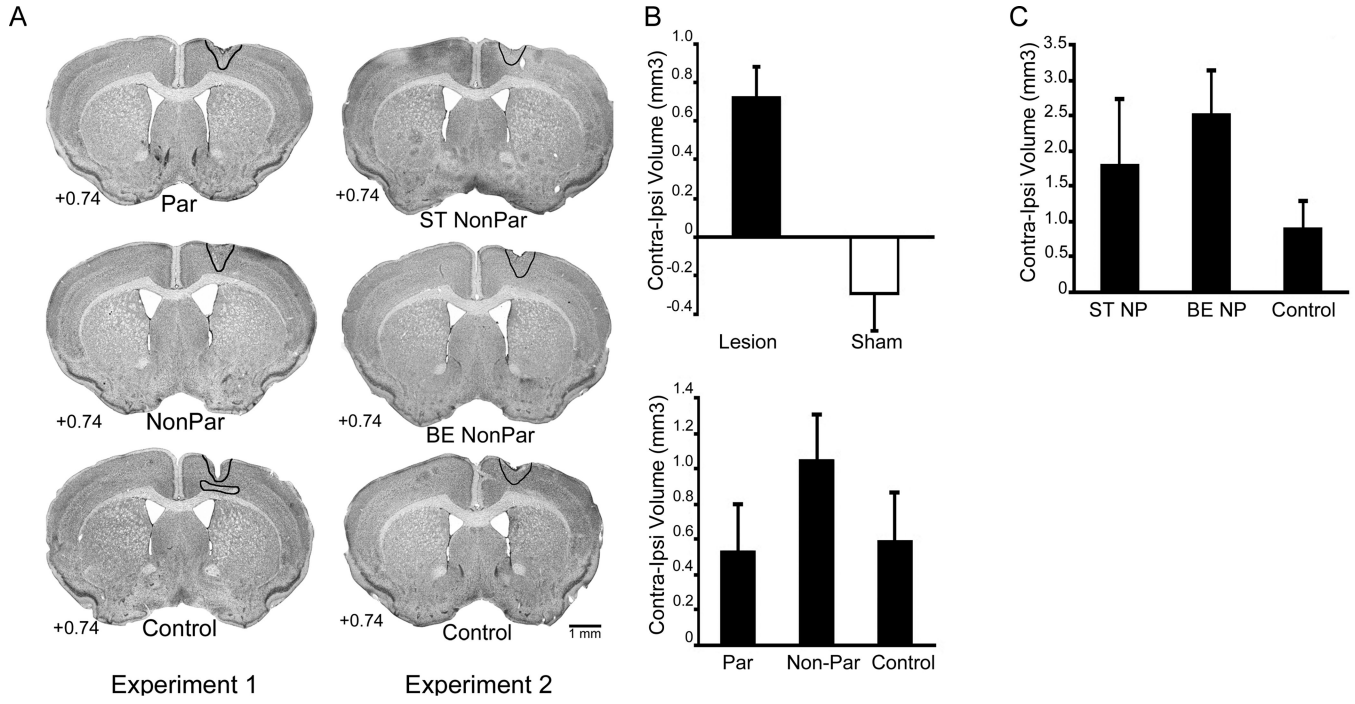

Fig. 4.

Lesion Analysis. Representative images of Nissl stained sections from Experiments 1 and 2 (A). In Experiment 1 (A left) Par, NonPar, and Control mice exhibit similar damage. Mice in Experiment 2 (A right) exhibited lesion sizes that were slightly larger than those in Experiment 1, though lesion sizes between ST NonPar, BE NonPar, and Control groups were similar. Representative lesion sizes in both experiments are indicated in black outline. (B top) ET-1 infusion, as estimated by contralateral-ipsilateral cortical volume difference, resulted in lesion sizes of approximately 0.8 mm^3 in Experiment 1. Sham procedures did not cause cortical damage. Within ET-1 lesioned animals (B bottom), there were no differences between training groups in lesion volume ($p > 0.1$). In Experiment 2 (C), there were no statistically significant differences in lesion sizes between groups ($p > 0.1$).