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Short Communication

Training the "less-affected" forelimb after unilateral cortical infarcts interferes with functional recovery of the impaired forelimb in rats

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Abstract. *Purpose:* Unilateral lesions of the sensorimotor cortex (SMC) in adult rats cause major behavioral changes in the ipsilesional, "less-affected" forelimb. An increase in function and reliance on this forelimb can aid compensation for contralesional impairments, but may also promote disuse and reduced functionality of the impaired forelimb. We hypothesized that training focused on the ipsilesional forelimb following a unilateral SMC lesion would reduce the efficacy of later motor rehabilitative training of the impaired forelimb.
Methods: Rats with ischemic SMC lesions were trained on a skilled reaching task with the ipsilesional forelimb (PriorT) or received control procedures (Cont) for 10 days. Both groups were then trained with the impaired forelimb on the same reaching task for 10 days.
Results: In comparison with Cont, PriorT rats had little improvement on the reaching task with the impaired forelimb and had a more enduring disuse of the impaired forelimb for postural support behaviors. Lesion sizes were similar between groups.
Conclusions: Behavioral experience with the less-affected forelimb early after unilateral SMC lesions has the potential to increase disuse and dysfunction of the impaired forelimb, consistent with a training-induced exacerbation of learned non-use. These findings are suggestive of competitive processes in experience-dependent neural restructuring after brain damage.

Keywords: Ischemia, caudal forelimb representation area, motor cortex, motor rehabilitation, skilled reaching, learned non-use

1. Introduction

Stroke remains one of the leading causes of disability in the United States [3] and, for many people with strokes, physical therapy is necessary to improve behavioral function. Although there have been major advances in recent years in understanding how rehabilitative training can be used to promote better function and restorative plasticity after brain damage [10,20] an ongoing issue for unilateral strokes affecting the upper extremities is how the two limbs should be treated in order to promote the most effective level of functioning [21,24].

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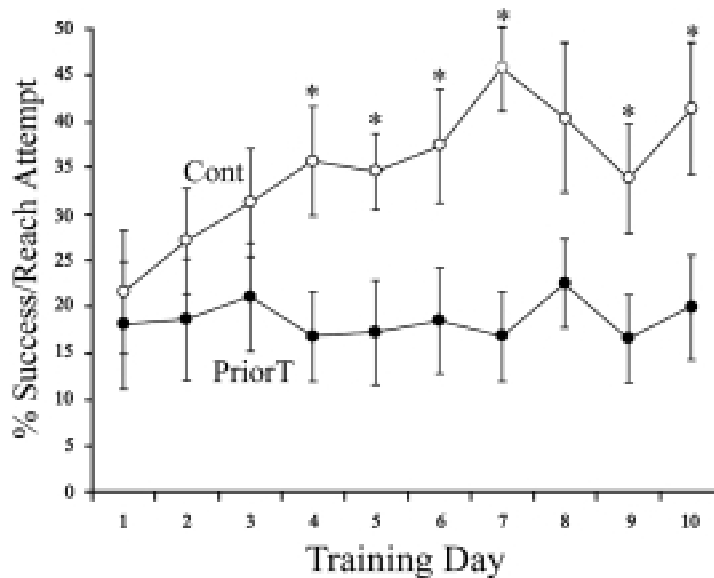

Fig. 1. The percentage of successful reaches with the impaired forelimb on the skilled reaching task after unilateral SMC lesions. Rats that had received prior training of the ipsilesional forelimb (PriorT) performed significantly worse ($*p < 0.05$) with their contralesional (impaired) forelimb compared to control (Cont) rats. Data are means ± S.E.M.

In rats, unilateral lesions in the caudal forelimb representation of the sensorimotor cortex (SMC) cause pronounced sensorimotor impairments in the contralesional forelimb [1,13]. Rats adapt behaviorally by using the less-affected (ipsilesional) forelimb more for postural support behaviors and in compensatory ways, as revealed in tests that require coordinated forelimb movements [4,23]. The degenerative effects of SMC lesions also appear to create a fertile environment for experience-dependent plasticity in the contralesional motor cortex [11,12] which increases in excitability [26], NMDA receptor protein, dendrites and synapses [1,11]. These changes may contribute, in part, to a hyperfunctionality in the ipsilesional forelimb that can be observed in skilled motor tests. When required to learn a novel reaching task, rats with unilateral SMC lesions perform better with the ipsilesional forelimb compared to sham-operated rats [2,4,9,17]. We have previously hypothesized that the enhanced functionality of the ipsilesional forelimb, although good for development of compensatory ways of using the less-affected body side, might occur at the expense of creating greater disuse and dysfunction of the impaired forelimb [4].

The purpose of the present study was to test the hypothesis that training focused on the ipsilesional forelimb after SMC infarcts would interfere with later functional improvements of the impaired forelimb. Three to 4 month old male Long-Evans rats were given pre-operative shaping procedures on a skilled reaching task, the single pellet retrieval task, adapted from Miklyaeva and Whishaw [18]. A limb of preference was established after the animal made 15 of 20 reaches with 1 limb. Endothelin-1 (ET-1) [1,5] lesions were then induced in the SMC opposite the animal's preferred-for-reaching forelimb. Lesions were created by removing the skull and dura over the SMC (coordinates relative to bregma: 0.5 mm posterior to 1.5 mm anterior, 3.0 to 4.5 mm lateral) and applying 1.2 μl of ET-1 (96 pmol) to the pial surface. Five days later, rats were trained on the reaching task for 10 days with the ipsilesional/non-preferred forelimb (PriorT group; $n = 8$) while another group (Cont group; $n = 8$) received control procedures. All animals were then trained on the same skilled reaching task with the impaired (pre-operatively preferred) forelimb for 10 days.

As described in more detail previously [2], daily reach training consisted of sessions of 10 minutes or 30 trials, which ever came first. For each trial, a palatable food pellet was placed on a 3 cm high shelf outside of an animal enclosure and rats were permitted to make up to 5 reaches with the trained limb through a narrow window to retrieve it. A Plexiglas wall was inserted into the enclosure ipsilateral to the animal's reaching limb which prevented animals from using the untrained forelimb. A successful retrieval consisted of the rat grasping, retrieving and eating the pellet. Unsuccessful attempts included dropping the pellet or "misses", i.e.,

failing to grasp the pellet or knocking the pellet from its well. Data are reported as the percentage of successful reaches per reach attempt. Cont rats received food pellets placed inside of the enclosure.

The Schallert cylinder test [22,23] was used as an assay of lesion-induced asymmetries in forelimb use for upright postural-support behavior. Animals were filmed in a Plexiglas cylinder (19 cm diameter) for 2 min pre-surgery and on four post-surgery time-points. From slow-motion playbacks of each session, the first 30 instances of sole use of either forelimb (ipsilateral or contralateral to the lesion) or simultaneous bilateral forelimb use for upright support against the cylinder wall were recorded. The forelimb asymmetry score was calculated using the formula: (total ipsilateral limb use + 1/2 bilateral)/total limb use *100. Animal protocols were approved by the University of Texas at Austin Animal Care and Use Committee.

Twenty-two days after surgeries rats were transcardially perfused with fixative, brains were removed and vibratome sectioned coronally and 50 μm thick sections were stained with Toluidine blue. The volume of remaining cortex in the SMC region of the infarcted cortex was obtained by measuring the area of remaining non-necrotic/non-gliotic cortex with Neurolucida software (Microbrightfield Inc.) at a final magnification of X17, as described previously [2]. The Cavalieri method [7] was used to calculate remaining SMC region volume, i.e., the product of the summed areas by the distance between section planes (600 μm). Lesion extent and placement were assessed by reconstruction of lesions onto schematic coronal templates.

Data were analyzed using repeated-measures analysis of variance (ANOVA), one-way ANOVAs or bivariate correlations with SPSS statistical software package (SPSS, Inc.). Post-hoc analyses were performed using SAS (SAS Institute Inc.) general linear models procedure for Scheffe's test.

As shown in Fig. 1, PriorT rats performed significantly worse with their impaired forelimb on the reaching task compared to animals without prior training. There was a significant effect of Group (F(1,14) = 5.41, $p < 0.05$), Day (F(9,126) = 2.71, $p < 0.01$) and a Group by Day interaction effect (F(9,126) = 2.49, $p < 0.05$), reflecting the greater improvement in reaching performance of the Cont group over days of training the impaired forelimb compared to the PriorT group. The reduction in successful retrievals in the PriorT group compared to the Cont group was due to a significant increase in the percentage of missed reach attempts (F(1,14) = 5.31, $p < 0.05$), in which the pellet was not retrieved after 5 attempts or in which the pellet was knocked from its well, and not due to an increase in the percentage of dropped pellets, in which the pellet was successfully grasped, but dropped before eating (F(1,14) = 0.34, $p > 0.05$).

All animals had increased asymmetrical forelimb use following the lesions (Fig. 2), resulting from an increased reliance on the ipsilesional forelimb and decreased use of the impaired forelimb. Additionally, animals in the PriorT group had a significantly greater asymmetry score during the time-period corresponding to impaired limb training (D18 and D24) compared to the Cont group. A repeated-measures ANOVA revealed a significant effect of Group (F(1,14) = 4.77, $p < 0.05$) but no Day or Group by Day interaction effects ($p's > 0.05$) during the impaired limb training period. There were no significant group differences in the asymmetry score in the time periods preceding the onset of impaired forelimb training.

All lesions produced damage to the caudal forelimb representation of the SMC (see Fig. 3) and lesion placement and extent was similar between groups as evidenced in lesion reconstructions. One-way ANOVA revealed no significant difference in remaining cortical volume between the two groups (means $\pm$ SEM volume in mm^3: PriorT = 86.07 $\pm$ 1.47, Cont = 87.81 $\pm$ 1.77, F(1,15) = 0.17, $p > 0.05$). In the Cont group, there was a significant, and in the PriorT group a marginally significant positive correlation between average reaching performance with the impaired limb and remaining cortical volume (Cont: $r = 0.76$, $p < 0.05$; PriorT: $r = 0.60$, $p = 0.059$, 1-tailed), revealing that animals with larger lesions tended to perform worse with their impaired limb compared to animals with smaller lesions.

In summary, rats with ischemic SMC lesions which were trained on a skilled reaching task with the ipsilesional/less-affected forelimb later performed significantly worse with the impaired forelimb compared to animals without prior training. The PriorT group also demonstrated a more enduring decreased use of the impaired forelimb in postural support behaviors compared to animals in the Cont group. Training focused on the ipsilesional forelimb may therefore contribute to more prolonged disuse of the impaired forelimb. The greater disuse and reduced reaching ability of the impaired forelimb in PriorT animals can not be explained by lesion size effects as both groups had similar volumes of remaining cortex in the SMC region. These data indicate that training focused on the less-affected forelimb early after a SMC lesion conveys detrimen-

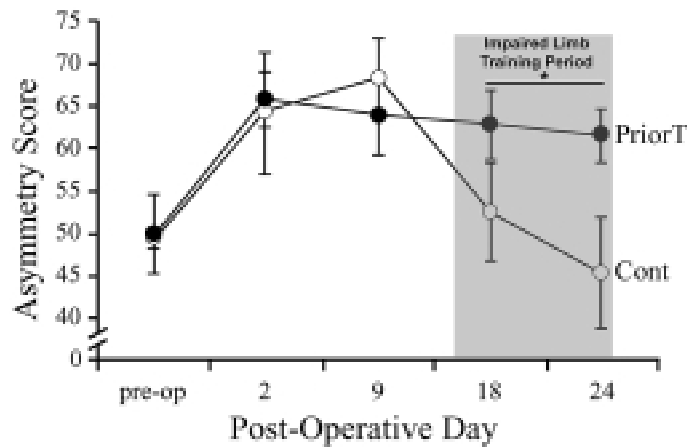

Fig. 2. Forelimb asymmetries in postural support behaviors as measured in the Schallert cylinder test. In both group, SMC lesions resulted in increased use of the ipsilesional forelimb and disuse of the impaired forelimb. PriorT rats continued to show significantly more ($p < 0.05$) reliance on their ipsilesional forelimb compared to Cont rats during the impaired forelimb training period (D18 and D24). Data are means $\pm$ S.E.M.

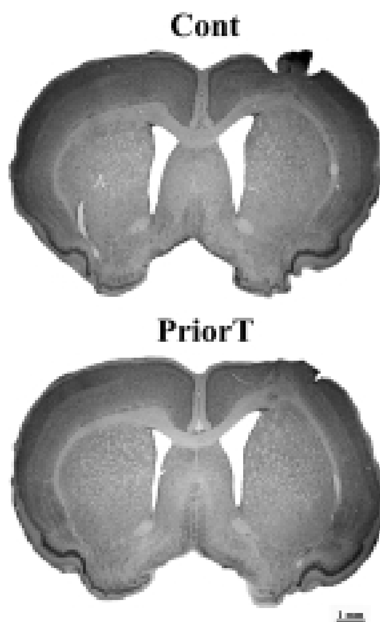

Fig. 3. Nissl stained coronal sections depicting representative lesions in each group.

tal effects on the recovery of function of the impaired forelimb on a skilled reaching task.

Other studies have demonstrated that forced overuse of the *impaired* forelimb after unilateral SMC lesions worsens behavioral outcome of the impaired forelimb [8,15,16]. This effect was linked to a use-dependent exaggeration of tissue loss from the lesion and requires both a major amount of use and that the manipulations be performed during an early sensitive time window. In the present study, there was no difference between groups in the amount of tissue loss; therefore the mechanisms underlying the exaggeration of impairment resulting from focused training of the ipsilesional forelimb are likely to be fundamentally different than the effects of overusing the impaired forelimb.

One issue is the contribution of motor learning interference effects to the present results. The single pellet retrieval task used in this study is not a truly unilateral task because, while animals are reaching with one forelimb, the other forelimb is used for postural support. It could be posited that learning to use the impaired forelimb for postural support during training reduces the ability for rats to learn to use it for reaching. This however, seems unlikely given that rats in the PriorT group actually relied less on their impaired forelimb for postural support behaviors, as measured in the Schallert cylinder test. The configuration of the reaching apparatus used in this study also minimizes the postural support requirements of the non-trained forelimb [18]. Preliminary unpublished data also indicates that, in intact animals, reach training with one forelimb (the non-preferred limb) does not interfere with later reaching performance of the other forelimb.

Many issues remain to be addressed in future studies. The inclusion of sham-operates in this experimental design would more clearly identify any motor learning interference effects due to sequential training of the 2 forelimbs. The lesions in this study were rather small and, in the Cont group, produced relatively transient forelimb asymmetries in the Schallert cylinder test. It

remains to be determined whether the effect found in this study will generalize to rats recovering from more substantial motor system damage, such as that producing detectable ipsilesional impairments, and whether they generalize to other measures of forelimb sensorimotor function. It is unknown whether there are sensitive post-lesion time windows for the effects of training focused on the less-affected forelimb. It also remains to be determined if the worsening of function of the impaired forelimb can be prevented using prior bilateral, rather than focused ipsilesional, forelimb training.

While several issues remain to be addressed, this study provides insight into problems associated with focusing rehabilitative training efforts after unilateral motor system injury. Animals, including humans, spontaneously learn to use the less-impaired body side to compensate for lesion-induced impairments. We have previously found that, after small unilateral cortical lesions, learning with the ipsilesional forelimb can even be facilitated relative to intact animals, presumably as a result of degeneration-triggered growth promoting processes that facilitate the synaptic changes underlying learning [2,4,9,11,17]. Although the development of this compensation with the less-affected forelimb appears to be functionally adaptive, the present results indicate that making this limb the focus of rehabilitative training efforts can impair or delay functional recovery of the affected forelimb. Such focused training may have reduced brain recovery processes for movements of the impaired forelimb and reduced the capacity to activate these recovery processes later. This is consistent with an exacerbation of learned non-use, the reduced use of the impaired limb resulting from repeated negative reinforcement associated with its ineptitude [24]. The neural bases of the present effects are as yet unknown, but transhemispheric inhibitory influences of the contralesional SMC have been found to be exaggerated in humans with unilateral strokes [25], and it is possible that training focused on the less-affected forelimb intensifies this effect. Furthermore, learning a skilled reaching task involves plasticity of synaptic activity and structure in the motor cortex opposite the trained forelimb [14,19,20,24] and there is some evidence to support a bilateral contribution to skilled reaching with one forelimb [6]. It is possible that early training focused on the less-affected forelimb confiscates neural circuits in the contralesional and/or infarcted hemisphere that could be used to mediate better recovery of the impaired forelimb.

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