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Multimodal exercises simultaneously stimulating cortical and brainstem pathways after unilateral corticospinal lesion

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ABSTRACT

In the context of injury to the corticospinal tract (CST), brainstem-origin circuits may provide an alternative system of descending motor influence. However, subcortical circuits are largely under subconscious control. To improve volitional control over spared fibers after CST injury, we hypothesized that a combination of physical exercises simultaneously stimulating cortical and brainstem pathways above the injury would strengthen cortico-bulbar connections through Hebbian-like mechanisms. We sought to test this hypothesis in mice with unilateral CST lesions. Ten days after pyramidotomy, mice were randomized to four training groups: (1) postural exercises designed to stimulate brainstem pathways (BS); (2) distal limb-grip exercises preferentially stimulating CST pathways (CST); (3) simultaneous multimodal exercises (BS+CST); or (4) no training (NT). Behavioral and anatomical outcomes were assessed after 20 training sessions over 4 weeks. Mice in the BS+CST training group showed a trend toward greater improvements in skilled limb performance than mice in the other groups. There were no consistent differences between training groups in gait kinematics. Anatomically, multimodal BS+CST training neither increased corticobulbar fiber density of the lesioned CST rostral to the lesion nor collateral sprouting of the unlesioned CST caudal to the lesion. Further studies should incorporate electrophysiological assessment to gauge changes in synaptic strength of direct and indirect pathways between the cortex and spinal cord in response to multimodal exercises.

Abbreviations: PyX, pyramidotomy; CRYM, μ-crystallin; BS, brainstem; NT, non-trained; BDA, biotinylated dextran amine; NgR1, Nogo66-receptor; MW, molecular weight; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; PKCγ, protein kinase C-gamma

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1. Introduction

The corticospinal tract (CST) plays an important role in control over skilled distal limb movements in mammals (Martin, 2005; Starkey et al., 2005). Though direct connections between the CST and spinal motor neurons have received more attention, indirect connections through synapses with brainstem neurons and segmental spinal interneurons also play a role in cortically directed movement (Alstermark and Ogawa, 2004; Alstermark et al., 2004; Matsuyama et al., 2004; Sasaki et al., 2004). We are interested in strengthening indirect corticomotoneuron pathways as a strategy to improve recovery from injuries that damage direct CST connections (Martin, 2012). We propose to achieve this goal using a variation of Hebbian theory – repetitive coactivation of CST and brainstem pathways through a combination of targeted physical exercises should strengthen corticobulbar connections, thereby improving cortical control over subcortical circuits.

We previously reported the results of multimodal exercise training in a mouse model of partial cervical spinal cord injury (Harel et al., 2010). In that injury model, which largely spared the CST, multimodal exercise training improved performance in a skilled climbing task. In the present study, we aimed to assess whether multimodal exercise training improves recovery specifically from CST damage. We therefore conducted an experiment in mice that had undergone unilateral pyramidotomy (PyX) – lesion of the CST just rostral to its brainstem decussation (Cafferty and Strittmatter, 2006; Starkey et al., 2005). Mice in the present study underwent a more finely controlled set of training modalities than our previous study (Harel et al., 2010): combined CST and brainstem exercises; brainstem-targeted exercises; CST-targeted exercises; or no formal exercises. We measured the effects of these training paradigms on both skilled and generalized motor behaviors. Anatomically, we measured two distinct mechanisms of recovery: the ability of the *injured* CST to strengthen detour connections with brainstem tracts *rostral* to the lesion; and the ability of the *uninjured* CST to form detour connections with spinal neurons *caudal* to the lesion.

2. Results

2.1. Experimental design

Experimental steps are depicted in Fig. 1. Behavioral testing was performed prior to surgery (T0), 1 week post-surgery (T1), and at the end of training (T2). PyX was performed contralateral to the preferred forelimb (determined through cylinder exploration at T0). Of 57 PyX mice, two were excluded at the T1 assessment due to functional deficits that were too severe. The remaining 55 PyX mice, as well as 10 sham-lesioned mice, were randomly assigned into cages of three to five animals that underwent different training regimens. Mice underwent 20 exercise sessions (30 min each) over 4 weeks, followed by repeated behavioral testing (T2) blinded to training assignment. To visualize the response of the *injured* CST *rostral* to the lesion, we injected the sensorimotor cortex of all mice on the ipsilesional side with the anterograde tracer

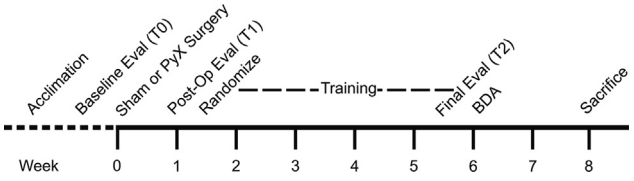

Fig. 1 – Study design. After acclimation, baseline evaluation, and sham or PyX surgery, mice were randomly assigned to four different training groups for a total of 20 sessions. Following the final behavioral evaluation, the lesioned CST was injected with biotinylated dextran amine (BDA) to anatomically visualize the response of the lesioned CST rostral to injury.

biotinylated dextran amine (BDA) prior to sacrifice. In mice carrying the mu-crystallin-GFP (CRYM-GFP) transgene, we exploited the GFP label to visualize the response of the *uninjured* CST *caudal* to the lesion.

2.2. Pyramidotomy causes reproducible unilateral CST lesions

Lesion extent was determined by comparing the staining intensity of CST fibers in the lesioned relative to the unlesioned dorsal column in transverse cervical spinal sections caudal to the lesion. Sham-lesioned animals demonstrated no fiber loss in the targeted CST (Fig. 2A), whereas PyX animals demonstrated nearly complete loss of CST immunoreactivity on the lesioned side (Fig. 2B). Prior to unblinding of training group assignments, eight lesioned animals with greater than 20% CST sparing were excluded from the results (four from the BS+CST group, three from the CST group, and one from the NT group). There were no significant differences in lesion extent between remaining mice in the different training groups, although mice in the BS+CST group tended to have slightly more severe lesions than mice in the other groups ($p=0.11$ on one-way ANOVA) (Fig. 2C).

2.3. Pyramidotomy causes reproducible behavioral deficits

One week after surgery, prior to initiation of training, mice were observed on two behavioral tests of CST function – climbing an inclined ladder with irregularly spaced rungs, and forelimb exploration of a glass cylinder. Compared to sham-lesioned mice, PyX mice demonstrated a significant increase in placement errors of the impaired forelimb on the ladder-climbing task ($p<0.001$ on two-tailed *t*-test), and a dramatic decline in use of the impaired forelimb to explore the walls of a glass cylinder ($p<0.001$ on two-tailed *t*-test) (Fig. 3). Impaired hindlimb placement errors were not significantly different between sham and PyX mice. Post hoc analysis confirmed that mice randomized to the different training groups had similar deficits before the initiation of training (Fig. 3).

2.4. Training assignments

Following behavioral testing at 1 week post-surgery, PyX mice were randomly assigned to four training regimens (Methods