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Research Paper

Motor cortex and spinal cord neuromodulation promote corticospinal tract axonal outgrowth and motor recovery after cervical contusion spinal cord injury

N. Zareen[a], M. Shinozaki[a,1,2], D. Ryan[a], H. Alexander[a], A. Amer[a,b], D.Q. Truong[c], N. Khadka[c], A. Sarkar[a], S. Naeem[a], M. Bikson[c], J.H. Martin[a,b,*]

[a] Department of Molecular, Cellular, and Biomedical Sciences, City University of NY School of Medicine, New York, NY 10031, USA
[b] CUNY Graduate Center, New York, NY 10031, USA
[c] Department of Biomedical Engineering, City College of NY, 10031, USA

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ABSTRACT

Cervical injuries are the most common form of SCI. In this study, we used a neuromodulatory approach to promote skilled movement recovery and repair of the corticospinal tract (CST) after a moderately severe C4 midline contusion in adult rats. We used bilateral epidural intermittent theta burst (iTBS) electrical stimulation of motor cortex to promote CST axonal sprouting and cathodal trans-spinal direct current stimulation (tsDCS) to enhance spinal cord activation to motor cortex stimulation after injury. We used Finite Element Method (FEM) modeling to direct tsDCS to the cervical enlargement. Combined iTBS-tsDCS was delivered for 30 min daily for 10 days. We compared the effect of stimulation on performance in the horizontal ladder and the Irvine Beattie and Bresnahan forepaw manipulation tasks and CST axonal sprouting in injury-only and injury + stimulation animals. The contusion eliminated the dorsal CST in all animals. tsDCS significantly enhanced motor cortex evoked responses after C4 injury. Using this combined spinal-M1 neuromodulatory approach, we found significant recovery of skilled locomotion and forepaw manipulation skills compared with injury-only controls. The spared CST axons caudal to the lesion in both animal groups derived mostly from lateral CST axons that populated the contralateral intermediate zone. Stimulation enhanced injury-dependent CST axonal outgrowth below and above the level of the injury. This dual neuromodulatory approach produced partial recovery of skilled motor behaviors that normally require integration of posture, upper limb sensory information, and intent for performance. We propose that the motor systems use these new CST projections to control movements better after injury.

1. Introduction

Cervical injuries are the most common form of SCI (NSCISC, 2014). People with a cervical injury value hand use as their highest priority for recovery (Anderson, 2004). There is a pressing need for effective therapies for promoting the function of the injured cervical spinal cord and restoring upper extremity use. The motor signs after SCI are due to interruption of descending control signals from the brain and the major source of these signals arise from the motor cortex (M1). Indeed, the loss of voluntary and skillful arm control is largely attributable to the loss of corticospinal tract (CST) control of cervical motor circuits. To restore this control requires reconnecting the damaged CST with the spinal cord below the injury. This is a daunting task, despite the many new directions for neural repair (Benowitz et al., 2017; Park et al., 2010).

Most SCIs are incomplete (Chen et al., 2016), offering the opportunity to foster reconnecting the brain with the spinal cord below the lesion by sprouting of spared CST axon. An effective strategy to restore function after a pyramidal tract lesion is to promote CST sprouting into the denervated side (Carmel et al., 2010; Maier et al., 2008). We have developed a neural activity-based approach in which M1, the principal source of the CST, is electrically stimulated (Brus-Ramer et al., 2007). Previously, we demonstrated that M1 epidural stimulation (which we term multi-pulse stimulation, or MPS) 6 h a day for 10 days promotes

* Corresponding author at: Department of Molecular, Cellular, and Medical Sciences, City University of NY School of Medicine, 160 Convent Avenue, New York, NY 10031, USA.
E-mail address: jmartin@ccny.cuny.edu (J.H. Martin).
[1] Permanent address: Department of Physiology, Keio University School of Medicine, Shinjuku-ku, Tokyo 1608582, Japan.
[2] Co-first authors.

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ipsilateral CST sprouting in naïve animals and, importantly, into the denervated side of the spinal cord after a pyramidal tract lesion. This stimulation therapy fully restored skilled locomotion after pyramidal tract lesion, when applied immediately after the lesion (Carmel et al., 2010) or delayed by 7 weeks (Carmel et al., 2014). By contrast, animals with injury-only do not show any recovery.

The logic of this neuromodulatory strategy derives from our developmental studies showing the importance of activity in steering normal development of the CST (Friel and Martin, 2007; Martin et al., 1999). Postnatal stimulation of the CST—at currents producing muscle activation (Salimi et al., 2008; Salimi and Martin, 2004), in order to ensure activation of spinal cord targets—helped establish spinal connections. This stimulation-based strategy is challenged after serious SCI because few axons remain caudal to the injury to activate damaged spinal motor circuits. Indeed, after SCI or brain injuries that interrupt the CST, the thresholds for evoking motor responses increase substantially and response onset is delayed (for review, see (Oudega and Perez, 2012)).

In this study, we used a moderate C4 bilateral contusion model to interrupt CST projections to the cervical enlargement with minimal forelimb motoneuron involvement. This contusion produces a large central lesion that spares few axons in the rat (Anderson et al., 2009). We chose this model because it closely approximates the pathology of SCI in humans (Anderson et al., 2005; Sharif-Alhoseini et al., 2017) and it represents a highly demanding standard that must be achieved in order to move forward with a therapy. To facilitate the actions of the CST on damaged spinal motor circuits after SCI we used cathodal transspinal direct current stimulation (tsDCS). In contrast to transcranial direct current stimulation, where anodal stimulation is facilitatory, cathodal stimulation strongly enhances M1-evoked response in the intact spinal cord (Song et al., 2015), possibly by facilitating ventral spinal circuits (Song and Martin, 2017). For M1 electrical stimulation, we used an intermittent theta burst stimulation protocol (iTBS). In our recent study (Song et al., 2016), iTBS with cathodal tsDCS 30 min daily for 10 days was as effective as MPS in achieving significant CST outgrowth and functional improvement after pyramidal track lesion. iTBS has the added benefit of requiring a shorter daily stimulation period than MPS and could thus be a more translational neuromodulatory stimulus. Further, since SCIs are not strongly lateralized we electrically stimulated M1 bilaterally.

We studied the effect of combined tsDCS and iTBS, delivered 30 min daily for 10 days, on CST axonal sprouting and recovery of motor function. We examined performance in the horizontal ladder task (Metz and Whishaw, 2002) and the Irvine, Beattie and Bresnahan forepaw manipulation task (Irvine et al., 2010; Irvine et al., 2014), both of which display a strong dependence on corticospinal control. We used Finite Element Method (FEM) modeling to position the tsDCS electrodes in order to maximize the density of applied currents in the cervical enlargement and, in turn, the potential for facilitatory neuromodulation of cervical motor circuits (Song et al., 2015). We show that this combined spinal-M1 neuromodulatory approach significantly promotes recovery of skilled locomotion and forepaw manipulation skills and enhances injury-dependent CST axonal outgrowth below and above the level of the injury. Our findings show that a short period of daily M1 stimulation, together with tsDCS to augment M1-evoked motor responses, is an effective neuromodulatory approach to promote forelimb skilled functions after cervical SCI. We propose that the motor systems use these new CST projections to control movements better after injury.

2. Methods

Thirty-two adult female Sprague-Dawley rats (250 to ~300 g) were used in this study (for the chronic study: 14 injury-only; 13, injury plus stimulation; for acute electrophysiology: 3 injury-only; for computer modeling: n = 2, injury only). Experiments were approved by the Institutional Animal Care and Use Committee of the City College of New York and the CUNY Advanced Science Research Center. All CST and behavioral analyses were conducted by laboratory personnel blinded to the animal's condition (injury-only versus injury plus stimulation). For the injury plus stimulation condition, motor cortex stimulating electrodes were implanted 2–3 weeks before the animals were trained in the horizontal ladder and IBB behavioral assessment (Fig. 1A; see below). Injury-only animals did not receive electrode implantation.

2.1. Bilateral M1 electrode implantation

Animals were anesthetized using a mixture of Ketamine (90 mg/kg) - Xylazine (10 mg/kg). Animals were placed in a stereotaxic frame and a craniotomy was made bilaterally over the left and right M1 to expose the forelimb representations. We used the same cortical epidural stimulating electrode as in our earlier studies (PlasticsOne, Inc.; e.g., (Brus-Ramer et al., 2007)). Bilateral electrodes were placed over the M1 forelimb areas epidurally (with respect to bregma: AP 1.5–2.0; ML ± 3–3.5). We verified that the electrode evoked a contralateral forelimb movement (or muscle contraction) and not ipsilateral forelimb movements or hind limb movements, indicating correct placement over the M1 forelimb representation.

2.2. C4 laminectomy and contusion

We performed a C4 midline contusion. A midline incision was made from T2 to the base of the skull and the dorsal neck muscles bluntly dissected to expose the cervical vertebrae from C2 to T2. A laminectomy was performed at C4 sufficiently large so that the impactor probe cleared the bone margins. The rats were suspended on the stabilization platform of the Infinite Horizon (IH) spinal impactor. The spinal cord was held in a level position without any twists. The lateral edges of the vertebral bodies at C3 and C5 were grasped with the fixation forceps of the impactor to stabilize the cord for impact. The impact probe (3.5 mm diameter; tip configuration; inset Fig. 1B) was lowered to the dura at C4. A dissecting microscope was used at 200 × magnification to verify that the probe was positioned correctly at the C4 midline. Stability of the vertebral column was verified by gently tapping the C4 vertebra. The probe was raised 3.9 mm (3 turns on the Infinite Horizon impactor elevation dial) before initiating the hit. We produced a 200 kdyn impact with zero dwell time. After surgery, animals were returned to a holding cage that was set on a heating pad, and observed until ambulatory. An analgesic (Carprofen; 4 mg/kg) and antibiotic (Baytril; 5 mg/kg) were administered.

2.3. Lesion confirmation

During the contusion procedure we verified that the probe was not impeded by bone or other tissues during impact. A small petechial hemorrhage and bruising parallel to the midline were evident at the impact site. Postmortem histological assessments were made using Hematoxylin and Eosin (H & E) staining in all animals. To measure lesion volume, the section of maximal lesion area and adjoining rostral and caudal sections were chosen for analysis. Using Neurolucida (MBF Bioscience), a contour of the lesion was drawn, as well as a contour of the entire section. Neurolucida Explorer was used to compute the average lesion area for each animal. We constructed a lesion overlap image for each animal group (see Fig. 1C, E).

2.4. iTBS and tsDCS

An epidural electrode was used to deliver the phasic iTBS electrical stimulation (Fig. 1F). The stimulation pattern was the same as in our previous study (Song et al., 2016). The electrical iTBS consists of delivering a burst of 3 pulses (interstimulus interval: 50 ms), repeated 10 times, for 2 s followed by 8 s without stimulation; this was repeated 20 times, for a total of 600 pulses. In our previous study, we systematically evaluated stimulation parameters. Over a range from 3 to 100 ms, 50 ms produced the strongest facilitation. The basic electrical iTBS