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Regular Article

Plasticity and alterations of trunk motor cortex following spinal cord injury and non-stepping robot and treadmill training

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ABSTRACT

Spinal cord injury (SCI) induces significant reorganization in the sensorimotor cortex. Trunk motor control is crucial for postural stability and propulsion after low thoracic SCI and several rehabilitative strategies are aimed at trunk stability and control. However little is known about the effect of SCI and rehabilitation training on trunk motor representations and their plasticity in the cortex. Here, we used intracortical microstimulation to examine the motor cortex representations of the trunk in relation to other representations in three groups of chronic adult complete low thoracic SCI rats: chronic untrained, treadmill trained (but 'non-stepping') and robot assisted treadmill trained (but 'non-stepping') and compared with a group of normal rats. Our results demonstrate extensive and significant reorganization of the trunk motor cortex after chronic adult SCI which includes (1) expansion and rostral displacement of trunk motor representations in the cortex, with the greatest significant increase observed for rostral (to injury) trunk, and slight but significant increase of motor representation for caudal (to injury) trunk at low thoracic levels in all spinalized rats; (2) significant changes in coactivation and the synergy representation (or map overlap) between different trunk muscles and between trunk and forelimb. No significant differences were observed between the groups of transected rats for the majority of the comparisons. However, (3) the treadmill and robot-treadmill trained groups of rats showed a further small but significant rostral migration of the trunk representations, beyond the shift caused by transection alone. We conclude that SCI induces a significant reorganization of the trunk motor cortex, which is not qualitatively altered by non-stepping treadmill training or non-stepping robot assisted treadmill training, but is shifted further from normal topography by the training. This shift may potentially make subsequent rehabilitation with stepping longer or less successful.

Introduction

Injuries such as limb amputation (Cohen et al., 1991; Ojemann and Silbergeld, 1995; Pascual-Leone et al., 1996; Sanes et al., 1990; Schieber and Deuel, 1997; Wu and Kaas, 1999) peripheral nerve transection (Franchi, 2000; Rijntjes et al., 1997; Sanes et al., 1988, 1990; Toldi et al., 1996), stroke (Chelette et al., 2013; Harrison et al., 2013; Nishibe et al., 2010; Nudo and Friel, 1999) or spinal cord injury (SCI) (Ghosh et al., 2009; Jain et al., 1997; Kokotilo et al., 2009; Nardone et al., 2013) can alter the somatotopic organization of the sensory and motor cortices. Alterations occur on both rapid (Aguilar et al., 2010; Sanes et al., 1988) and longer time scales (Aguilar et al., 2010; Franchi, 2000; Sanes et al., 1990; Tandon et al., 2013; Toldi et al., 1996). It is known that in animals and humans, following a complete low thoracic SCI, there is an expansion of the sensory map of spared proximal areas into the deafferented cortex (Aguilar et al., 2010; Chau and McKinley, 1991; Endo et al., 2007; Henderson et al., 2011; McKinley and Smith, 1990) and a shift in motor representations of proximal muscles in the motor cortex (Bruehlmeier et al., 1998; Laubis-Herrmann et al., 2000; Lotze et al., 2006; Topka et al., 1991). Trunk control and representations are likely to be fundamental for highly skilled motions (Anders et al., 2007; Bronner, 2012; Sung et al., 2012). Trunk motor control is also crucial for postural stability and propulsion after SCI in both humans and animals (Bjerkefors et al., 2009; Desroches et al., 2013; Giszter et al., 2008; Yang et al., 2006). Several gait rehabilitative strategies are aimed at trunk stability and control in animals (Dominici et al., 2012; Udoekwere et al., 2006) and humans (Dobkin and Duncan, 2012; Dobkin et al., 2003; Hidler and Sainburg, 2011; Hussain et al., 2011). However little is known about the effect of SCI and rehabilitation on trunk motor representations and their plasticity in the cortex. In clinically complete low thoracic SCI patients, Topka et al. observed that transcranial magnetic stimulation (TMS) activated a large fraction of

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motorneuron pools and evoked motor evoked potentials (MEPs) with shorter latencies from a large number of scalp positions in the abdominal muscles immediately rostral to the level of the SCI (Topka et al., 1991). Cariga et al. observed that TMS elicited motor responses in paravertebral muscles in all segments above the lesion and also in varying ranges of segments below the lesion in clinically complete thoracic SCI patients (Cariga et al., 2002). However, a systematic examination of how the trunk motor cortex reorganizes after adult thoracic SCI and rehabilitation of the paralyzed adults in an animal model is missing from the current literature.

Plastic changes in the cortex can arise spontaneously after injury or depend on use and skill (e.g. Dancause and Nudo, 2011). Prolonged exercise training increases blood flow to the cortex, induces angiogenesis (Seifert and Secher, 2011; Swain et al., 2003) and results in upregulation of neurotrophic factors, and these can promote neuronal survival and differentiation in the cortex (Klintsova et al., 2004; Vaynman and Gomez-Pinilla, 2005). However, representational changes in motor areas are usually thought to instead be associated with skill acquisition and precisely practiced improvements, not with endurance practice of an existing skill (Kleim et al., 1998, 2002a, 2002b, 2004; Perez et al., 2004; Plautz et al., 2000). It is known that treadmill training of adult rats spinalized as neonates (Kao et al., 2009, 2011) or passive hindlimb bike exercise of adult SCI rats (Graziano et al., 2013) induces plasticity in the somatosensory cortex. However, it is not clear whether prolonged exercise training when coupled with the effects of potentially differing skills, strength and endurance alters, improves, or exacerbates, plastic changes in the motor cortex after adult SCI.

Our goal in this study was first to examine the effect of adult SCI on trunk motor cortex representations and second to test whether prolonged treadmill or robot assisted treadmill training executed *without* any induced hindlimb stepping (i.e., 'non-stepping' treadmill training) can influence trunk motor cortex plasticity, and if so, how. Given that central or peripheral nerve injury results in a significant cortical expansion of proximal (to injury) regions we hypothesized that chronic adult SCI results in significant reorganization and expansion of trunk motor cortex and given that plasticity of motor cortex is typically associated with novel skill learning we further hypothesized that non-stepping treadmill and robot training that do not induce any functional recovery will not alter cortical reorganization on top of that caused by adult SCI alone. To test this, we used intracortical microstimulation to examine the motor cortex representations in three groups of chronic adult SCI rats after completion of three treatments: untrained, treadmill trained (but 'non-stepping') and robot assisted treadmill trained (but 'non-stepping'). All transected groups were also compared with a group of normal rats. The results we will present show that adult complete low thoracic SCI induces a significant reorganization of the trunk motor cortex compared to the normal group, which remains largely similar across the three tested SCI groups. However, we also saw these changes were further exaggerated by the non-stepping treadmill or robot assisted treadmill training, changes which might then impact any other subsequent rehabilitation treatments.

Materials and methods

Overview

A total of 44 adult female Sprague–Dawley rats (250–325 g) were used in this study. 36 rats received complete spinal cord transection at T9/10 level (ATX). The rats were subdivided into four groups: normal (uninjured), spinalized-untrained (ATX-U), spinalized-treadmill trained (ATX-TM) and spinalized-robot assisted treadmill trained (ATX-R). The latter trained groups were not given any special step promoting interventions such as epidural stimulation or pharmacotherapy, and as adult spinal complete rats thus showed little to no stepping (Rossignol and Frigon, 2011). We used intracortical microstimulation (ICMS) to examine the representation of motor cortex in all rats. All surgical and experimental procedures were carried out with the Institutional Animal Care and Use Committee (IACUC) guideance and approval.

Adult complete spinal transection at T9–10 (ATX)

36 rats received complete spinal cord transection at T9/10 level similar to that described in Hsieh and Giszter (2011) and Udoekwere et al. (2006). Rats were anesthesized intraperitoneally with 1.0 ml/kg KXA cocktail (2.0 ml Ketamine (100 mg/kg):1.0 ml Xylazine (20 mg/kg):0.15 ml Acepromazine (10 mg/kg)). Supplemental doses of KXA (0.38 ml/kg) were administered intraperitoneally as needed to maintain deep level of anesthesia throughout the procedure. A mid dorsal skin incision spanning approximately from T6 to T12 segments was made. Thoracic vertebrae were exposed; fat pad and paraspinal muscles were deflected. Laminectomy was performed on arches T9/11. A full segment of the spinal cord was gently removed at T9/10 using iridectomy scissors and aspiration after opening a slit in the dura. The cavity was filled with gelfoam and the incision was closed in layers. Core body temperature was maintained at 37 °C throughout the surgery using a heating pad.

Pelvic orthosis implantation

12 rats received pelvic orthosis implantation surgery along with spinal transection. Orthosis design and surgical procedure were similar to that described in Hsieh and Giszter (2011), Song and Giszter (2011) and Udoekwere et al. (2006, 2014). Briefly, after spinal transection a sterile pelvic orthosis was inserted by making bilateral angled incisions (45°, approximately 1 cm caudal to the iliac crest) separating the gluteus muscle through blunt dissection and with minimal tissue damage. The orthosis was then clamped to the iliac processes on both sides of the rat's pelvis and the sides of the implant were fastened with screws and epoxy cement (J–B weld) was applied to the joint where the pelvic implant parts were connected.

Post-operative care

Post-operatively rats were given 1.0 ml/kg of 0.05 mg/ml buprenorphine subcutaneously for analgesia, every 8–12 h for 48 h, 0.5 ml/kg prophylactic antibiotics (dilute 3.4 ml of sterile water with 1 g of ampicillin vial) subcutaneously once a day for 7 days and 5–10 ml lactated ringer subcutaneously for 7 days. Spinalized rats were monitored twice daily for skin lesions, autophagia or other health concerns. Bladders were expressed at least twice daily until automatic voiding returned, as happens routinely in spinal rats.

Training

Spinalized rats (ATX) were divided into three groups, untrained (U), treadmill trained (TM) and robot trained (R). Untrained rats were left sedentary in their cages but handled and checked twice daily. The other two groups began training 7–10 days post-surgery. Training did not involve any additional (e.g., perineal) stimulation that would actively recruit locomotor pattern generators, as our goal was to examine cortical plasticity due to training with *non-stepping* hindlimbs. The treadmill trained group was trained (unassisted) daily on a motorized treadmill at 8–12 cm/s for 20 min/day, 5 days/week for 4–5 weeks. Periodically rats were also video recorded using a digital camera for assessment of locomotor function. Robot trained rats were trained on a robot that applied elastic forces during treadmill locomotion for 20 min/day, 5 days/week for 4–5 weeks similar to that described in Hsieh and Giszter (2011) and Udoekwere et al. (2006). Briefly, a cantilevered phantom haptic robot (Sensable Devices Inc.) is connected via a gimbal to the pelvic orthosis. Isotropic elastic force fields were applied ($kx = ky = kz = 45$ N/m) with equilibrium position set such that the equilibrium trunk posture and carriage height of the spinalized