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Epidural cortical stimulation enhances motor function after sensorimotor cortical infarcts in rats

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Abstract

This study examined whether epidurally delivered cortical electrical stimulation (CS) improves the efficacy of motor rehabilitative training and alters neuronal density and/or cell proliferation in perilesion cortex following ischemic sensorimotor cortex (SMC) lesions. Adult rats were pre-trained on a skilled reaching task and then received partial unilateral SMC lesions and implantation of electrodes over the remaining SMC. Ten to fourteen days later, rats received daily reach training concurrent with anodal or cathodal 100 Hz CS or no stimulation (NoCS) for 18 days. To label newly generated cells, bromodeoxyuridine (BrdU; 50 mg/kg) was administered every third day of training. Both anodal and cathodal CS robustly enhanced reaching performance compared to NoCS controls. Neuronal density in the perilesion cortex was significantly increased in the cathodal CS group compared to the NoCS group. There were no significant group differences in BrdU-labeled cell density in ipsilesional cortex. Staining with Fluoro-Jade-B indicated that neurons continue to degenerate near the infarct at the time when cortical stimulation and rehabilitation were initiated. These data indicate that epidurally delivered CS greatly improves the efficacy of rehabilitative reach training following SMC damage and raise the possibility that cathodal CS may influence neuronal survival in perilesion cortex.

Keywords: Ischemia; Motor cortex; Skilled reaching; Cortical stimulation; Behavioral recovery; Bromodeoxyuridine (BrdU); Epidural electrical stimulation; Stroke; Motor learning

Introduction

Motor rehabilitation following cortical injury improves motor function and drives restorative neuroplastic changes in denervated brain areas (e.g., Nudo et al., 1996; Jones et al., 1999; Chu and Jones, 2000; Biernaskie and Corbett, 2001). Electrically stimulating the motor cortex also may facilitate recovery of function. In humans, transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) of the infarcted hemisphere have been found to acutely improve motor function in patients with chronic motor impairments (Pascual-Leone et al., 1996; Cantello, 2002; Hummel et al., 2005; Fregni and Pascual-Leone, 2005). In a small clinical trial, epidural cortical stimulation (CS) combined with physical therapy improved motor performance in chronic stroke patients compared to patients who received only physical therapy (Brown et al., 2006). Recent animal studies have demonstrated that cortical stimulation (CS) combined with rehabilitative training (skilled reaching) enhances motor functional outcome compared to rehabilitation alone. Following SMC lesions in rats (Adkins-Muir and Jones, 2003; Kleim et al., 2003; Teskey et al., 2003) and monkeys (Plautz et al., 2003), subdural stimulation of the motor cortex, via chronically implanted electrodes, combined with skilled reaching enhanced forelimb behavioral recovery compared to rehabilitation alone. Enhanced behavioral function in the CS and rehabilitation groups also coincided with increased dendritic plasticity

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(Adkins-Muir and Jones, 2003), enlarged microstimulation-evoked motor maps (Kleim et al., 2003; Plautz et al., 2003), and enhancement in the polysynaptic component of evoked potentials (Teskey et al., 2003) in ipsilesional cortex compared to rehabilitation alone.

Although animal studies support the benefit of subdurally delivered CS, projected clinical trials will use epidurally placed electrodes. Therefore, it seemed essential to determine if current delivered through the dura would produce similar results as found with subdurally implanted electrodes. The primary aim of this study was to determine the effectiveness of combining epidural cathodal or anodal 100 Hz monopolar CS with reach training of the impaired forelimb following sensorimotor cortex (SMC) lesions compared to rehabilitation alone. The second aim was to evaluate whether CS plays a role in cellular proliferation or survival sufficient to affect neuronal density in the remaining SMC. Electrical stimulation applied to different brain regions can affect neuronal survival (Maesawa et al., 2004), reduce infarct volume (Glickstein et al., 2003), and enhance cell proliferation (Bengzon et al., 1997; Parent et al., 1998; Scott et al., 1998; Nakagawa et al., 2000). Brain injury alone has been found to alter cellular proliferation in the subventricular zone and hippocampus (for review, see Parent, 2003), and in some studies, these new cells have migrated to the site of injury in striatum and cortex (Magavi et al., 2000; Arvidsson et al., 2002; Parent et al., 2002). These new cells, including neurons, may integrate into existing networks and become functionally active (e.g., Nakatomi et al., 2002). Thus, it seemed reasonable to suspect that, following cortical lesions and the administration of cortical electrical stimulation, there would be an increase in the density of neurons in perilesion cortex as a result of greater survival and/or as a result of production of new neurons.

In this study, adult male rats were pre-trained to proficiency on a skilled reaching task, the single pellet retrieval task. They then received unilateral endothelin-1 (ET-1; a vasoconstrictor) induced SMC lesions and epidural implantation of monopolar electrodes over remaining SMC. Ten to fourteen days after surgery, animals underwent 18 days of rehabilitative training with concurrent delivery of either cathodal or anodal 100 Hz stimulation or no stimulation. Animals received injections of bromodeoxyuridine (BrdU) every third day of rehabilitative training. The density of BrdU+ cells in perilesion cortex and striatum was examined using stereological methods, and the phenotype of cortical BrdU cells was analyzed with immuno-fluorescence and confocal microscopy. Neuronal density was estimated in Nissl-stained sections of the ipsilesional cortex adjacent to and underlying the site of electrical stimulation. In a subset of animals, Fluorojade-B was used to assess the possibility of ongoing neural degeneration at the time of onset of CS and rehabilitative training.

Methods

Animals

Sixty-three adult (3 to 4 months old) male Long–Evans hooded rats were used. Rats were made tame by gentle handling beginning after weaning and were housed on a 12:12-h light: dark cycle. Thirty-one rats were used to assess the effects of CS after unilateral SMC lesions. Rats were moderately food restricted (13–15 g/day) to motivate performance on the reaching task. Animals were randomly assigned to the following groups with the exception that they were matched as closely as possible for pre-operative and pre-rehabilitation performance: (1) 100 Hz cathodal stimulation during training (CSCath, n = 10), (2) 100 Hz anodal stimulation during training (CSAnod, n = 11), and (3) training with no stimulation (NoCS, n = 10). An additional 32 rats were used for a Fluoro-Jade B (FJB) neurodegeneration labeling study. These rats received SMC lesions or sham operations and were sacrificed 24 h (lesion: n = 3), 3 days (sham: n = 6, lesion: n = 9), or 14 days (sham: n = 5, lesion = 9) following surgery. All animal use was in accordance with a protocol approved by the Animal Care and Use Committee of the University of Texas at Austin.

Surgical procedures

Ischemic damage to the sensorimotor cortex (SMC) was created using endothelin-1, a vasoconstricting peptide, applied to the cortical surface (Macrae et al., 1993; Fuxe et al., 1997). Rats were anesthetized with a cocktail of ketamine (100 mg/kg) and xylazine (10–13 mg/kg). Rats were placed in a stereotaxic apparatus, received an incision at midline of the scalp, and the skull was removed between 0.5 mm posterior and 2.5 mm anterior to Bregma and 3 to 5 mm lateral to midline. Dura was cut with a scalpel parallel to midline from the anterior to posterior edge of the craniectomy. Then, 240 pmol endothelin-1 in 3 μl of sterile saline (0.2 μg/μl) was applied to the cortical surface at a rate of 1 μl/3 min and was left undisturbed for 10 min after the last application. This lesion method has previously been found to produce focal damage to cortical layers I-VI underlying the craniectomy (Fuxe et al., 1997; Adkins-Muir and Jones, 2003; Adkins et al., 2004; Adkins and Jones, 2005).

Electrode implantation

Ten minutes after the final endothelin-1 application, the craniectomy was enlarged by ~ 1 mm each at the anterior and medial edges to expose perilesion motor cortex for epidural electrode implantation. Each electrode consisted of 0.4 mm wide by 2 mm long parallel platinum wire strip contacts mounted on a 3 mm by 3 mm supporting plate extending from an electrode connector pedestal (Plastics One Inc., Roanoke, VA). The platinum contacts were placed on dura and were orientated approximately parallel to midline. Extensive preliminary data indicated that this electrode placement reliably enables post-operative stimulation-evoked contralesional forelimb and/or upper body and face movement. The electrode was configured so that one current polarity was delivered to the contacts on cortex and the other polarity's current flowed through a small metal disk placed below the skin (contact side up) near Lambda to ground the current (see Fig. 1; Northstar Neuroscience, Inc., Seattle, WA). The craniotomy was covered with gel foam and sealed with SDI Wave dental acrylic (SDI Inc., Bensenville, IL).