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Motor Cortex Stimulation Suppresses Cortical Responses to Noxious Hindpaw Stimulation After Spinal Cord Lesion in Rats

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ABSTRACT

Background: Motor cortex stimulation (MCS) is a potentially effective treatment for chronic neuropathic pain. The neural mechanisms underlying the reduction of hyperalgesia and allodynia after MCS are not completely understood.
Objective: To investigate the neural mechanisms responsible for analgesic effects after MCS. We test the hypothesis that MCS attenuates evoked blood oxygen-level dependent signals in cortical areas involved in nociceptive processing in an animal model of chronic neuropathic pain.
Methods: We used adult female *Sprague–Dawley* rats ($n = 10$) that received unilateral electrolytic lesions of the right spinal cord at the level of C6 (SCL animals). In these animals, we performed magnetic resonance imaging (fMRI) experiments to study the analgesic effects of MCS. On the day of fMRI experiment, 14 days after spinal cord lesion, the animals were anesthetized and epidural bipolar platinum electrodes were placed above the left primary motor cortex. Two 10-min sessions of fMRI were performed before and after a session of MCS (50 μA, 50 Hz, 300 μs, for 30 min). During each fMRI session, the right hindpaw was electrically stimulated (noxious stimulation: 5 mA, 5 Hz, 3 ms) using a block design of 20 s stimulation off and 20 s stimulation on. A general linear model-based statistical parametric analysis was used to analyze whole brain activation maps. Region of interest (ROI) analysis and paired *t*-test were used to compare changes in activation before and after MCS in these ROI.
Results: MCS suppressed evoked blood oxygen dependent signals significantly (Family-wise error corrected $P < 0.05$) and bilaterally in 2 areas heavily implicated in nociceptive processing. These areas consisted of the primary somatosensory cortex and the prefrontal cortex.
Conclusions: These findings suggest that, in animals with SCL, MCS attenuates hypersensitivity by suppressing activity in the primary somatosensory cortex and prefrontal cortex.

Introduction

Central neuropathic pain is defined as pain initiated or caused by lesions or dysfunction in the central nervous system (CNS) [1]. The pain is persistent, relentless, debilitating, and difficult to treat [2–4]. Motor cortex stimulation (MCS) has emerged as a potential technique for the management of pain in patients who suffer from neuropathic pain [5–12]. Both invasive and noninvasive protocols have been proposed for MCS, these include: electrical stimulation with implanted electrodes, repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) [13–16].

Pain relief occurs progressively after the onset of MCS and persists after the stimulation has ended [17,18]. This intriguing post-stimulation effect can last from minutes to hours and even weeks in some reports [19–23]. However, the outcomes of MCS are

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variable and this modality of treatment is often associated with mixed clinical results. The mixed outcomes are a reflection of the complexity and variability of pain conditions, the lack of well-controlled, randomized clinical trials, and the lack of understanding of the mechanisms underlying MCS-induced analgesia [24,25].

Neuropathic pain, due to injury of the CNS, commonly manifests as spontaneous, ongoing pain. Therefore, imaging studies in patients with central pain focused on investigating how MCS ameliorates ongoing pain. Using positron emission tomography (PET), researchers demonstrated that analgesia produced after the end of MCS was associated with increased regional cerebral blood flow (rCBF) in the thalamus, anterior cingulate cortex (ACC), prefrontal cortex (PFC), anterior insular cortex (IC), and rostral brainstem. They also found no changes in rCBF in motor areas directly beneath the stimulating electrodes or in the primary somatosensory cortex (S1) [26–28]. Based on these findings, it was suggested that MCS attenuates ongoing pain by enhancing the release of endogenous opioids and the activation of the descending inhibitory system [29,30].

In addition to spontaneous pain, neuropathic pain can also manifest as an increased response to noxious stimuli (hyperalgesia) and as pain in response to previously innocuous stimuli (allodynia) [4,31,32]. MCS can also attenuate hyperalgesia and allodynia in patients with neuropathic pain [5,6,22,33]. However, the neural mechanisms, and cortical structures underlying the reduction of hyperalgesia and allodynia after MCS are not entirely clear. One functional magnetic resonance imaging study (fMRI) showed that long-term MCS treatment reduces pain evoked by noxious stimuli in patients with post-stroke pain by suppressing activity in the second somatosensory cortex (S2), IC, and PFC [34].

Here, we perform preclinical experiments in rats to further study post-stimulation effects of MCS by assessing changes in regional blood oxygen-level dependent (BOLD) signals in response to noxious stimuli. Specifically, we hypothesize that MCS attenuates evoked BOLD signals in cortical areas involved in nociceptive processing in animals with spinal cord lesions.

Methods

This study was carried out in strict accordance with the Animal Welfare Act regulations and Public Health Service guidelines and the International Association for the Study of Pain guidelines. All experimental procedures were approved by the University of Maryland Baltimore, Institutional Animal Care and Use Committee.

Twelve adult female *Sprague–Dawley* rats weighing 260 ± 30 g were used in this study. Two naïve rats were used in initial pilot experiments to optimize and test fMRI and noxious electrical stimulation parameters. The remaining rats ($n = 10$) underwent a surgery to lesion the spinal cord and were used in fMRI experiments to study the effect of MCS on evoked cortical BOLD signals.

Spinal cord lesion (SCL)

To lesion the spinal cord, we used similar procedures to those described previously [35–37]. Briefly, under aseptic conditions, the rats were anesthetized with ketamine/xylazine (80/10 mg/kg, i.p.) and placed on a thermo-regulated heating pad to maintain body temperature. A laminectomy was performed to expose the spinal cord between C5 and T2 and the dura was removed. A quartz-insulated platinum electrode (5 μm tip) was targeted to the anterolateral quadrant in the right side of the spinal cord (1.8 mm lateral to the midline). Direct current (10 μA for 10 s, repeated 4 times) was delivered through the electrode to produce an electrolytic lesion in the area of C6. After surgery, the muscles and skin were sutured in layers to approximate incision sites. We have shown previously that these unilateral lesions produce ongoing pain and bilateral "below-level" (relative to spinal lesion site) hypersensitivity, and bilateral aberrant activity in the thalamus and cortex [35,36,38,39].

Behavioral testing

Animals were habituated for two weeks prior to behavioral testing. The behavioral tests were conducted on three consecutive days before the SCL surgery (baseline) and at days 7 and 14 after surgery. A dynamic plantar aesthesiometer (Ugo Basile, Comerio, Italy) was used to assess mechanical withdrawal thresholds of the hindpaws as described previously [40]. The difference in mechanical withdrawal thresholds at days 7 and 14 from baseline was calculated and divided by baseline thresholds to estimate the percent change in mechanical withdrawal thresholds after SCL. Repeated measures ANOVA on Ranks was used to test for statistically significant changes in mechanical withdrawal thresholds. A $P < 0.05$ was considered significant. Only animals that exhibited significant reduction in hindpaw withdrawal thresholds were included in the study.

fMRI

Animal preparation

On the day of fMRI, 14 days after SCL, the animal was initially anesthetized with isoflurane (2%). The femoral vein contralateral to the spinal lesion was catheterized and connected to an infusion pump (Kent Scientific Corp., MA, USA) to administer α-chloralose anesthesia (an initial i.v. bolus of 60 mg/kg and then at a constant rate of 30 mg/kg/h) for the duration of the experiment [41]. Once α-chloralose was administered, isoflurane anesthesia was discontinued.

The animal was attached to a stereotaxic frame and the bone overlying the motor cortex was removed and custom made epidural bipolar platinum electrodes (diameter: 70 μm, exposed tip: 50 μm, distance between the electrodes: 500 μm) were placed above the left primary motor cortex using stereotaxic coordinates (A: −3.6 mm, L: 2.8 mm, D: 7.3 mm relative to Bregma) [18,39], and stabilized using a thin layer of cyanoacrylate followed by acrylic resin.

In our SCL animals, the lesion disrupts the continuity of the spinothalamic tract and the flow of nociceptive information from the hindpaw contralateral to the lesion to the brain. Therefore, we electrically stimulated the right hindpaw ipsilateral to the lesion site because these animals develop bilateral below level hypersensitivity [18]. Two custom-made needle electrodes (160 μm diameter) were inserted under the skin of the right hindpaw, one between digits 1 and 2, and another between digits 3 and 4. These electrodes were later connected to a pre-programed constant current stimulator (ISO-Flex and Master 8, A.M.P.I, Jerusalem) and then secured using surgical tape. Due to the large distance between the stimulation electrodes and the center of the MRI head coils, the stimulation current did not introduce artifacts in the fMRI signal.

After placement of the electrodes, the α-chloralose anesthetized animal was moved to a 7.0 T Bruker Biospin/BioSpec 70/30 USR 30 cm horizontal bore animal MRI scanner (Bruker Biospin MRI GmbH, Germany). The animal was placed in prone position in the Bruker semi cylindrical cradle equipped with warm circulating water channels. The head was carefully secured to a stereotaxic head holder by means of a bite-bar and ear pins to prevent head motion and improve head position consistency through the whole experiments. A Bruker ^{1}H 4-channel animal brain surface coil array was used as the receiver and a Bruker ^{1}H 72 mm linear-volume coil as the transmitter. An MR-compatible small animal monitoring and gating system (SA Instruments, Inc., Stony Brook, NY, USA) was used to continuously monitor the animal body temperature and