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Modulation of cortical activity after anodal transcranial direct current stimulation of the lower limb motor cortex: A functional MRI study

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Background and Objectives
Functional magnetic resonance imaging (fMRI) has shown that transcranial direct current stimulation (tDCS) of the hand motor cortex modulates cortical activity of the healthy human brain. However, few studies have assessed the effects of tDCS on the leg motor cortex. We therefore used fMRI to examine the modulating effects of tDCS on lower limb motor cortex responses.

Methods
In this sham-controlled case-control study, 11 subjects were exposed to active anodal (n = 6) or sham (n = 5) stimulation, with the anode being positioned on the leg motor cortex of the right hemisphere. Each tDCS was delivered for 15 minutes at 2 mA, with each subject receiving a total of four stimulatory sessions on consecutive days. Cortical activity was measured before the first and after the fourth session by fMRI, and changes in cortical activity were calculated.

Results
Anodal tDCS increased activation of the ipsilateral supplementary motor area and lowered the extent of activation of both anterior cingulate gyri, the right middle and superior temporal gyri, the middle and superior frontal gyri, and the primary and secondary somatosensory cortices.

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Conclusions
Anodal tDCS increased corticospinal excitability of the lower limb motor cortex in healthy subjects, suggesting that multiple brain cortical areas may be associated with leg motor performance via involvement of variable corticocortical connections.

Keywords direct current simulation; functional neuroimaging; magnetic resonance imaging

Transcranial direct current stimulation (tDCS), consisting of the application of low-intensity electric current over the scalp area, is used to alter the activity of underlying neurons, with anodal stimulation increasing and cathodal stimulation reducing the excitability of such neurons.[1] tDCS has been shown to affect the hand area of the motor cortex, in that anodal tDCS targeted to the motor cortex changes corticospinal excitability up to 90 minutes after the end of stimulation and improves motor performance.[2,3] Application of either anodal or cathodal tDCS to the leg motor cortex of healthy normal brains increases corticospinal excitability, as assessed by measurements of motor-evoked potentials (MEPs) by transcranial magnetic stimulation (TMS).[4] However, few studies to date have explored whether the motor cortex controls the lower extremities, largely because of difficulties in evaluating tDCS effects on the lower leg.[4,5]

Functional magnetic resonance imaging (fMRI) has been used to estimate changes in cortical activity associated with noninvasive brain stimulation. fMRI detects localized changes in cerebral blood flow during performance of a motor task; such changes serve as surrogates of neuronal activity in the corresponding brain region. fMRI affords good spatial resolution in the cortex and precisely identifies sites of cortical activation.[6] fMRI has also been used to assess changes in cortical activation after application of tDCS.[7-10] The cited studies, most of which focused on the after effects of tDCS application on hand motor function, showed that tDCS modulated the cortical excitability of the underlying motor cortex in healthy human brains. We therefore used fMRI to evaluate changes in both cortical and subcortical activity after application of tDCS to the leg motor cortex of healthy subjects.

Methods

Subjects

In this double-blind, sham-controlled trial, 13 healthy subjects (10 men, 3 women), aged 24-32 years, were randomly divided into active anodal and sham stimulation groups. All subjects were right handed, as assessed using the Edinburgh Handedness Inventory[11]; none had a history of a previous neurologic or psychiatric disorder or was taking any medication. Each subject provided written informed consent to study participation, and the work was approved by the Asan Medical Center Ethics Committee and performed in accordance with the ethical standards of the Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm, 2006).

A baseline fMRI was performed before the first stimulatory session, with the first tDCS session being scheduled for the day after fMRI scanning. Anodal or sham stimulation was applied to each subject for 15 minutes, with each subject undergoing four stimulatory sessions on consecutive days. At the end of the last tDCS session, each subject was assessed by follow-up fMRI. Follow-up fMRI was performed as soon as the tDCS was finished.

tDCS

tDCS was delivered by a Phoresor II Auto Model PM850 (IOMED, Salt Lake City, UT) via two conductive rubber electrodes placed in saline-soaked sponges (5.0×5.0 cm^2). The anode was positioned over the leg area of the right motor cortex, with the optimal cortical area being chosen to represent the leg area (the motor "hot spot"), as determined by evaluation of the position at which the excitatory threshold of the resting tibialis anterior muscle was the lowest, the latency was the briefest, and the average amplitude the highest. The reference electrode was placed above the contralateral orbit of the eye. Before electrode positioning, the skin at the chosen sites was prepared to reduce electrical resistance. The stimulation intensity was 2 mA and each stimulus was applied for 15 minutes. At the beginning of each session, the current was ramped up to 2 mA over 10 seconds and was ramped down at the end at the same rate. The electrode current density was 0.25 mA/cm^2, a density previously shown to be safe[12]; no subject reported any discomfort. For sham tDCS, the electrodes were placed at the same sites used for anodal stimulation. To mimic the skin sensation experienced at the commencement of anodal stimulation, the stimulator was programmed to ramp-up over 10 seconds and immediately ramp down to 0 mA over 10 seconds.

fMRI

Blood oxygenation level-dependent (BOLD) fMRI was performed before stimulation (baseline), and immediately after the fourth stimulation, using an echo-planar imaging (EPI) technique using a 3-T Achieva MR system (Philips

Medical Systems, Best, the Netherlands). One hundred whole-brain images were collected via T2-weighted EPI sequencing (repetition time, 3000 milliseconds; echo time, 35 milliseconds; flip angle, 90°; number of slices 30; slice thickness 4 mm; slice gap 0.5 mm; matrix size 128 × 128; pixel spacing 1.719 × 1.719 mm).

We used unilateral active toe flexion. Before entering the scanner, each subject practiced the paradigm and was corrected for any synkinetic movement of the opposing limb during familiarization. Once in the fMRI scanner, each subject was permitted to perform a single pretest practice sequence, over 30 seconds.

Each subject lay in the scanner in a relaxed, supine position, with the entire head, trunk, and pelvis, and both arms and legs, immobilized, to eliminate motion artifacts during scanning. Each subject was directed to view an obvious monitor, and, when a visual cue was presented, to flex the toes at a metronome-guided frequency of 1 Hz. Each subject was also instructed to cease motion when the visual cue disappeared. Cycles, consisting of 30 seconds of cued toe flexion movement, followed by 30 seconds of rest, were repeated to analyze movement of the foot of interest; the total scan time per subject was approximately 300 seconds.

fMRI data were processed using SPM8 software (Wellcome Institute of Cognitive Neurology, London, UK). Preprocessing steps included spatial realignment to the mean volume of a series of images; normalization to the same coordinate frame as that of the MNI-template brain, use of transformation parameters derived from segmentation of the high-resolution structural image coregistered with the mean EPI image, and smoothing using an 8-mm full-width half-maximum Gaussian filter.

Statistical analysis

All statistical analyses were performed using SPSS, version 14.0. The Mann-Whitney *U* test was used to compare between-group baseline differences in dependent variables.

Nonparametric random effects were analyzed using SnPM5b software (School of Public Health, University of Michigan, Ann Arbor, MI). Otherwise, preprocessed data were analyzed using SPM8 software. To construct our fixed-effects model, we initially built a single large general linear model (GLM) using preprocessed data obtained from multiple observations of both subject groups. This model was used to describe interactions in terms of analysis of variance (ANOVA) with respect to both group (tDCS versus sham) and time (pre- versus poststimulation) factors. Significance was determined in clusters containing more than 30 voxels (240 mm^3), using an uncorrected *P* value less than 0.0001.

Results

Of the 13 subjects originally enrolled, two were excluded because of contraindications to fMRI or fear of confined spaces. Ultimately, 11 subjects (eight males, three females), of mean ± standard deviation (SD) age 24.88 ± 2.03 years, completed the study. No participant experienced any adverse side effects of tDCS. All subjects reported that they felt a tingling sensation during both actual and sham tDCS sessions.

fMRI

Six participants underwent fMRI scanning after anodal tDCS, and the other five underwent fMRI after sham tDCS. The representative fMRI scans shown in Figure 1 display regions of increased or reduced activation after anodal tDCS relative to sham scanning. After 4 days of anodal stimulation, increased activation was most prominent in the supplementary motor area (SMA) of the right cerebral hemisphere, to which tDCS had been applied ($P < 0.0001$). Compared with the sham procedure, anodal stimulation decreased the extent of activation in the left primary motor cortex (SM1) (i.e., in the hand area of the unstimulated hemisphere), in both anterior cingulate gyri, in the right middle and superior temporal gyri, in the middle and superior frontal gyri, and in the primary and secondary somatosensory cortices ($P < 0.0001$ for each comparison). These results are summarized in Table 1.

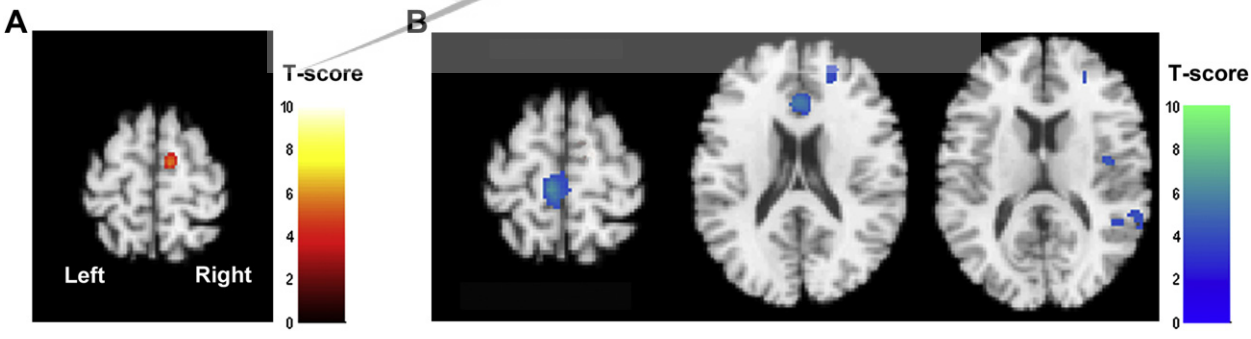

Figure 1 Group-by-time interactions showing (**A**) increased and (**B**) decreased activation within the stimulated group, compared with the sham-treated group, after intervention. **B,** Clusters of > 30 voxels (240 mm^3) with uncorrected *P* values less than 0.0001 were considered significant. The slices are arranged in neurologic orientation.

Table 1 Brain regions that showed group-by-time interactions

Brain region	Brodmann area	Side	Peak MNI coordinates (mm) x	y	z	Volume (mm^3)	T-score
Increased activation in the stimulation group after intervention in comparison with the sham group							
Supplementary motor area	6	Right	10	−10	66	528	5.7287
Decrease activation in the stimulation group after intervention in comparison with the sham group							
Primary motor cortex	4	Left	−6	−28	68	2336	6.4109
Anterior cingulate gyrus	24	Right	2	30	20	1768	5.7847
		Left	0	30	20		5.6228
Middle temporal gyrus	22	Right	62	−44	10	944	4.8250
Superior temporal gyrus	42	Right	60	−44	14		4.2316
Middle frontal gyrus	46	Right	28	46	14	504	4.2985
Superior frontal gyrus	46	Right	20	50	20		4.2912

MNI = Montreal Neurological Institute.
Uncorrected *P* value less than 0.0001 were considered as significant.

Discussion

We have shown here that application of serial tDCS to the leg motor cortex modulates cerebral cortical activity in healthy individuals. In contrast to previous observations on hands,[7,9,10] we observed no fMRI evidence of prominent regional activation of the stimulated right SM1. Rather, we noted increased activation of the right SMA and decreased activation of bilateral multifocal brain cortical areas, although some of these cortical areas have previously been associated with lower leg motor performance. These findings suggest that anodal tDCS of the lower leg motor cortex may increase the excitability of the corticospinal pathway because of the presence of many interconnections among brain cortices, interconnections that may be more significant than direct activation of the SM1.

In the first study exploring the ability of tDCS to change the excitability of the lower leg corticospinal tract, 10 minutes of anodal tDCS increased MEP amplitude both at rest and during background contraction, with these effects lasting for at least 60 minutes.[4] As found in previous studies of hands,[3,12,13] anodal tDCS may alter the excitability of the corticospinal pathway in the lower extremities. However, TMS can determine only an average of tDCS after effects on a target muscle, despite the regulation of motor performance by multiple brain areas, including the SM1, the premotor cortex (PMC), the SMA, and the cingulate cortices. Thus, although TMS can reveal an increase in MEP, TMS cannot identify the cortical or subcortical area involved in activation of the corticospinal system.

To overcome these limitations, fMRI has been increasingly used to assess the effects of noninvasive brain stimulation. fMRI is valuable because of the sensitivity of the technique to relative changes in task-related regional synaptic activity and the relatively high spatial resolution afforded. fMRI can also be used to detect multifocal activation of brain cortical and subcortical areas, which may be helpful in analyzing brain cortical connectivity during motor performance.[6] Several recent fMRI studies have explored the effect of tDCS on brain activation. For example, fMRI performed before and after tDCS of the left SM1 has been used to investigate movement-related changes in BOLD signals during performance of a sequential finger-opposition task.[8] In the cited study, application of 5 minutes of cathodal tDCS resulted in a lasting decrease in the mean number of pixels activated on SMA, whereas application of anodal tDCS was without effect.[8] In addition, increases relative to sham tDCS values were observed in terms of cerebral blood flow (CBF) in the left SM1, the right frontal pole, the right primary sensorimotor cortex, and posterior brain regions, irrespective of polarity, after anodal (on the left SM1) or cathodal (on the right frontopolar cortex) tDCS. However, fMRI examination of direct tDCS cortical effects showed that CBF in underlying hand SM1 was significantly increased during anodal tDCS.[9] Together, these results demonstrated that anodal tDCS increased, whereas cathodal tDCS decreased, CBF in several brain regions, most (but not all) of which are associated with motor performance. The mechanism of activation of unrelated cortical regions remains unclear, although this may reflect indirect interactions between motor cortical and nonmotor cortical areas. It is thus likely that changes in excitability after application of tDCS are mediated not only via a direct corticospinal pathway, but also via triggering of various corticocortical and corticosubcortical connections within the brain.

Previous studies have used fMRI to assess changes resulting from hand and finger movements. To the best of our knowledge, this is the first functional neuroimaging study to demonstrate the effects of tDCS on the leg motor cortex area of the human brain. We found that application of anodal tDCS caused a significant increase in the CBF of the SMA area without significantly changing the CBF of other cortical and subcortical areas. SMA activation may be attributable, at least in part, to modulation of an interaction between the SM1 and the SMA; the latter region has been implicated in planning of motor neuron action and in bimanual control, and exhibits a strong functional

connectivity with both the primary and secondary motor cortices.[14] Via such corticocortical connections, SMA activation may be induced by excitation of the SM1, which, in turn, is stimulated by ipsilateral anodal tDCS. However, we found that such stimulation did not significantly activate the SM1; the precise effects of the SM1 on the SMA thus remain unclear. Similarly, cathodal tDCS has been reported to induce a decrease in CBF in the ipsilateral SMA, without any changes in SM1, suggesting that the mechanism underlying cathodal tDCS effectively eliminated activation stimulated by any processing event that resulted from a corticocortical connection, but could not suppress a representation made by a direct sensorimotor input.[8] This may partially explain why SMA alone is activated. A limitation of our work, shared by previous studies,[9,10] is that we assessed only the after effects of anodal tDCS; had fMRI scanning been performed during tDCS stimulation, it may have been possible to demonstrate activation of the ipsilateral SM1.

Alternatively, anodal tDCS may not affect CBF in the SM1 area of the lower leg motor cortex, at least not to an extent greater than the effect thereof on the SMA. The SMA is part of the sensorimotor cerebral cortex of Brodmann area 6, which lies just in front of the SM1.[14] However, the SM1 of the lower leg is located in a deep anatomic location and is vertically oriented in the midsagittal brain cortical area. Accordingly, it may simply be more difficult to activate the lower leg motor cortex than to activate the area controlling the hands. SMA activation without SM1 activation has been also observed,[9,15] suggesting that such activation is linked to both sensory stimulation and attention development, with other mechanisms being involved in the invocation of SMA. To date, however, no comprehensive mechanism has been defined, indicating the need for further studies.

Using fMRI, we observed decreased activity in several cortical regions, including the right frontal gyri and both anterior cingulate gyri. Although the specific mechanism involved in the decrease of CBF in these regions is unclear, the frontal gyri of the dorsolateral prefrontal cortex (DLPFC) also became rather quiescent. The DLPFC is involved in general cognitive demands and spatial processing,[16] controls coordination with other task-related areas, and can authorize increased visual search time.[17] As task difficulty increases, cognitive demands also rise, and fMRI has shown that the elevated assistance requirement of the latter function is associated with DLPFC activation.[16,18] Repetitive anodal tDCS may decrease the extent of cognitive demand, via a mechanism as yet unknown, modulating dorsolateral prefrontal cortical activity. Thus, after tDCS application, less cognitive effort may be necessary to achieve the same level of task performance. However, only a few studies support this hypothesis. Although anodal tDCS over the DLPFC enhances working memory,[19,20] no study has yet found that anodal tDCS applied to the primary motor cortex modulates the DLPFC. Further studies of the effects of tDCS on variable corticocortical pathways are needed.

Our study had several limitations. First, the number of study subjects was relatively small. Second, we measured only the after effects of anodal tDCS; we did not evaluate either the direct effects of anodal tDCS or the direct effects or after effects of cathodal tDCS on the leg motor cortex. Anodal tDCS performed during fMRI scanning may allow SM1 activation to be observed.[4] Although cathodal tDCS has not been shown to affect MEP responses, further contralateral cathodal tDCS experiments using fMRI may detect changes in cortical connections or cortical spinal pathways not detected by TMS.

In conclusion, we found that anodal tDCS modulated the activity of the leg motor cortex in healthy subjects. Using fMRI, we demonstrated that the SMA, which is involved in motor performance of the lower leg, was excited by tDCS, but that several other cortical areas were inhibited. Thus, excitability of the corticospinal pathway projected to the lower extremities appears to be increased by activation of multiple brain cortical areas, mediated via corticocortical connections, and anodal tDCS applied over the leg primary motor cortex may modulate the actions of such pathways.

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