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Research Article

Transcranial Direct Current Stimulation (tDCS) Can Alter Cortical Excitability of the Lower Extremity in Healthy Participants: A Review and Methodological Study

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Abstract

Objective: Transcranial direct current stimulation (tDCS) has been used to alter cortical excitability of the lower extremity (LE) and to influence performance on LE tasks like ankle tracking accuracy; but no study, to our knowledge, ever reported a significant change in cortical excitability relative to sham-tDCS. Additionally, because several different electrode montages were used in previous studies, it is difficult to know how stimulation should be applied to achieve this effect. Our objective was to determine whether active-tDCS alters cortical excitability of the LE and ankle tracking accuracy relative to sham-tDCS in healthy participants. The efficacy of two electrode montages and two conductance mediums were compared.

Methods: A triple-blind, fully randomized, within-subjects study was conducted with healthy participants (N=18, 24.2 (6.6) years). Cortical recruitment curves and measures of ankle tracking accuracy for the dominant lower extremity were obtained before and after participants received active-tDCS at 2 milliamps for 20 minutes using montage-medium combinations of M_1-SO:Saline, M_1-SO:Gel, C1-C2:Saline, and C1-C2:Gel and a sham-tDCS condition (M_1-SO: Saline).

Results: The motor evoked potential maximum of the recruitment curve was significantly lower for active than sham-tDCS, but only for the M_1-SO:Saline combination. No other significant differences in the recruitment curve parameters or in ankle tracking were found.

Conclusions: This is the first study to our knowledge to demonstrate a significant difference in cortical excitability of the LE between active and sham-tDCS conditions. Given the order in which the experimental procedures occurred, the result is consistent with the concept of a homeostatic plasticity response.

Keywords: *transcranial direct current stimulation, cortical excitability, brain stimulation*

Introduction

Transcranial direct current stimulation (tDCS) stimulates brain tissue by passing a weak electrical current between two or more scalp electrodes. Seminal studies by Nitsche and Paulus revealed changes in cortical excitability recorded from the adductor digiti minimi muscle (ADM) depending on length of tDCS application. The amplitude of transcranial magnetic stimulation (TMS)-motor evoked potentials (MEPs) increased beneath the anodal and they decreased

beneath the cathodal electrode indicating changes in cortical excitability [1,2]. Many studies have now used tDCS with a wide variety of stimulation parameters and experimental conditions [3] to alter cortical excitability of the upper extremity (UE). In actual practice, distinctions between anodal and cathodal stimulation can be complicated depending on stimulation parameters such as electrode placement, dosage amount, and duration. So, in the present study we have used the term active-tDCS to refer to 2 mA stimulation for twenty minutes with the anode electrode placed over the motor cortex and the cathode electrode over either the contralateral supraorbital (SO) region or homologous motor cortex in the opposite hemisphere.

Relatively few studies have used tDCS to alter cortical excitability of the lower extremity (LE). Those that have, failed to show significant differences between active and sham conditions. A review of the literature revealed only six such studies of healthy participants in which it was possible to compare active stimulation to either a baseline or sham condition (Table 1). All of these studies used MEPs from targeted muscles in the LE to measure cortical excitability. While some of the studies reported a significant difference in MEPs between baseline and active-tDCS conditions [4-6], no study has shown a difference between active stimulation and an appropriate control condition [4-9].

Another group of studies used tDCS to alter LE functions such as gait speed, dorsiflexion, reaction times, dynamic balance, and pinch force in healthy participants [5,8,10-20]. These studies have yielded mixed results; some finding differences between sham and active tDCS and others not (Table 1).

Studies of ankle tracking accuracy have revealed differences between sham and active tDCS in healthy participants and at different time intervals lasting 10 minutes, 25 minutes, and 24 hours post-stimulation [9]. Ankle tracking accuracy has also been shown to improve immediately after active tDCS compared to sham in stroke patients [21]. For these reasons, our study evaluated ankle-tracking accuracy as a measure of LE function and particularly because it recruits the tibialis anterior (TA) muscle, the target for MEP recording.

Regardless of whether the tDCS studies focused on changes in cortical excitability or LE function, a major problem is that standard protocols for stimulation have not been used or even investigated. Whereas the primary motor cortex – supraorbital (M_1-SO) montage is most frequently used in tDCS studies investigating LE cortical excitability and function [4,7,9,10,18-20], twelve other montages have also been used to target the LE cortex, including electrodes placed at or near Cz [5,6,11-14,22], C3 [16,17], or C4 [16,17] based on the international electroencephalogram (EEG) 10-20 system, M_1 [7,8,15], supraorbitally (SO) [5,6,11,12,13,14,17], over the inion [5,8,22], at the shoulder [7], and/or on the ipsilateral humerus [15]. Studies comparing the effects of different montages are rare. So, one purpose of this study was to compare the efficacy of two montages– the M_1-SO and C1-C2.

Additionally, electrode conductance mediums can influence the efficacy of tDCS stimulation. While saline was the most commonly used conductance medium in past studies of the LE [4-6,8-12,14-19,22], studies have also used water [7], EEG conductance paste [13], or did not report the conductance medium [20]. Saline as a conductance medium may cause current shunting or a bridging effect between the electrodes if copious amounts of saline are applied to the sponges; whereas a more viscous EEG gel or paste may be less prone to bridging. For these reasons, saline and gel were compared to determine their efficacy.

The main purpose of this methodological study was to determine, in healthy participants, whether and how active and sham-tDCS influences LE cortical excitability and function. We also compared the efficacy of M_1-SO and C1-C2 electrode montages and of the saline solution and gel conductance mediums. Based on previous studies [4,5,7,8,14,16-18,19,22-32], the active tDCS applied at 2 mA for 20 minutes was predicted to increase LE cortical excitability and to improve performance on the ankle-tracking task as a result.

Table 1: Active Transcranial Direct Current Stimulation + Lower Extremity Outcome Measure Studies in Healthy Subject Populations

Montage	Medium	Results	Reference
Corticospinal Excitability			
M_1-SO	Saline	35% motor evoked potential (MEP) increase 60-min post ($p<0.05$) *vs.* baseline.	[4]
M_1-SO	Saline	26.6% Group Mean Modulation (GMM) increase in targeted hemisphere ($p=0.001$), 8.5% GMM decrease in nontargeted hemisphere ($p=0.001$) vs. baseline.	[6]
Cz-Inion	Saline	MEP amplitude increase in right tibialis anterior (TA, $p<0.001$) and left TA ($p<0.001$) *vs.* baseline.	[5]
M_1-SO	Saline	No significant differences in MEP amplitude *vs.* sham.	[9]
M_1-SO & M_1-Shoulder	Water	No significant differences in MEP area *vs.* sham.	[7]
M_1-SO & Inion-Buccinator	Saline	No significant differences in MEP area *vs.* sham.	[8]
Motor Behaviors			
M_1-SO	Saline	Increase pinch force between first and second digits of foot during stimulation ($p<0.05$) *vs.* sham, but not after. No significant difference in reaction time (releasing foot off pedal after visual stimulus) *vs.* sham.	[19]
M_1-SO	Saline	Decrease reciprocal inhibition from TA to Soleus during first ($p=0.03$) and last half ($p=0.002$) of stimulation vs sham. Decrease soleus homonymous recurrent inhibition during last half ($p=0.02$) of stimulation *vs.* sham.	[18]
Cz-Inion	Saline	No significant differences in gait velocity *vs.* sham.	[5]
Cz-SO	Saline	Reduce functional reaction time ($p<0.05$) *vs.* sham. No significant differences in anteroposterior (AP), mediolateral (ML) or overall stability indices *vs.* sham.	[14]
Cz-SO & Inion-SO	Saline	Reduced electromyography (EMG) onset time of TA for M_1-SO ($p<0.05$) *vs.* sham. Increased EMG offset time of TA for M_1-SO ($p<0.05$) *vs.* sham and Inion-SO.	[11]
Cz-SO	Saline	Increase maximal center of pressure (CoP) excursion distance ($p<0.05$) *vs.* sham. No significant differences in functional reaction time vs. sham.	[12]
C3/C4-SO	Saline	Reduce open chain reaction time of ankle dorsiflexion (DF) measured via EMG ($p=0.005$) or accelerometer (0.028) *vs.* sham. Reduced EMG onset time of TA ($p=0.045$) and gastrocnemius (p=0.033) *vs.* sham.	[17]
M_1-SO	Not Given	No significant differences in gait parameters (number of steps, step length, stride length, cadence, double support time, stance phase, swing phase) vs. sham.	[20]
M_1-SO	Saline	Reduce open chain simple reaction time of ankle DF ($p=0.01$) and choice reaction time of ankle DF ($p=0.004$) and plantarflexion (PF, $p=0.001$) *vs.* sham.	[10]
C3-C4	Saline	No significant differences in isokinetic concentric knee flexion/extension peak torque *vs.* sham.	[16]
M_1-SO & Inion-Buccinator	Saline	No significant differences in anterior-posterior (AP) path length, AP peak-to-peak amplitude, and mean power frequency (MPF) post-stimulation *vs.* sham.	[8]
Cz-SO	EEG Paste	No significant differences in sway velocity or sway acceleration *vs.* sham.	[13]
M_1-Humerus	Saline	No significant differences in maximal isokinetic eccentric knee flexion/extension *vs.* sham.	[15]

Methods

Participants

This study was approved by the University's Institutional Review Board for use with human subjects and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helenski. Twenty participants were recruited via advertisement. Two participants dropped out of the study due to scheduling conflicts. Eighteen participants completed the entire study in the treatment order allocated. Table 2 lists the inclusion/exclusion criteria.

Table 2: Methodological Details

Inclusion Criteria	Exclusion Criteria
18-50 years of age.	Broken or abnormal skin in the area of the electrodes
Completion of the informed consent process and sign a written informed consent form and written HIPPA form approved for the study.	Holes in the skull from trauma or surgery
Complete and pass the Transcranial Magnetic Stimulation Adult Safety Screen (TASS).	A personal history of syncope, epilepsy, head injury, aneurysm, stroke, previous cranial neurosurgery, neurological or psychiatric disorders, or migraines
	Family history of epilepsy
	Metal implants in the head or neck
	Pacemaker
	Pregnancy
	Medications that lower seizure threshold or reduce cortical excitation
Detailed Procedures	
Lower Extremity Dominance: Determined via "ball kick test" where a ball was placed in front of participant. The participant was then instructed to "kick the ball". The foot the participant used to kick the ball was determined the dominant lower extremity.	
Electromyography (EMG) Data: Underwent both an analog band pass filter (10-1000Hz) and a 60 Hz notch filter. Sampled at 5,000 Hz with a National Instruments USB 6215 and read by a custom written data acquisition LabView program on a personal computer (PC). Rectified and smoothed using a root mean square (RMS) calculation using a 50-millisecond (ms) window.	
Footplate: Allowed full ankle dorsiflexion and plantarflexion active range of motion (AROM). Electronic goniometer fastened onto footplate and connected to BioPac MP35 hardware for powering the device and amplifying the voltage output. Voltage signal sampled by a National Instruments USB 6218 at 100 Hz and recorded with a custom-written LabView program.	
Biofeedback Line: Horizontal with the ankle in a neutral position, rose (positive slope) during ankle dorsiflexion, and dropped (negative slope) during ankle plantarflexion. Normalized the height of the waveforms by having participants fully dorsiflex and plantarflex ankle while strapped to the footplate. The maximal height of the computer-generated wave form on the positive (dorsiflexion) and negative (plantarflexion) ends corresponded to each participant's maximal AROM minus five degrees.	
20% Maximal Volitional Isometric Contraction (MVIC) Determination: Participants asked to perform a MVIC of the dominant TA by maximally dorsiflexing their ankle for five seconds while an investigator simultaneously provided manual resistance to the dorsal aspect of the subject's dominant foot and provided verbal encouragement. Three repetitions performed with a minimum rest time of thirty seconds. The peak value (mV) of the three repetitions was used as the MVIC. Biofeedback system used this value to display a 20% contraction.	
M_1/Resting Motor Evoked Potential (MEP) Threshold Algorithm: Beginning at 70% maximal stimulator output (MSO), the region that elicited the highest TA MEP was designated as M_1. To find the resting motor threshold, %MSO was decreased in 3 to 9% increments until the lowest %MSO was found that produced at least 3 MEPs that were ≥ 50 mV out of 6 consecutive stimuli delivered at least 10 seconds apart. Neighboring locations of M_1 were investigated to see if any produced a MEP ≥ 50 mV. If not, the original location was used as M_1 and the resting motor threshold was used to determine the %MSO for establishing the recruitment curve. If so, the investigator repeated the process until the optimal location over the scalp was found that produced a MEP for the TA.	
Boltzman Sigmoidal Function: $y = MEP_{min} + (MEP_{max} - MEP_{min})/(1 + exponent\text{^}(x_{50} - x)/k))$. "k" is a rate parameter used to define the slope of the curve. Is inversely correlated to steepness of the curve.	
Recruitment Curve Algorithm: %MSO was set 3-9% below the resting motor threshold. Because isometric contraction facilitates MEPs, a stepwise decrease in stimulator amplitude occurred until no MEP was achieved. Participants were asked to hold a 20% MVIC (±1%) of the dominant TA. A biofeedback marker was displayed via real-time bar graph on a monitor in view of the subject. Once the 20% MVIC was maintained for 300ms, it triggered the delivery of a single-pulse of transcranial magnetic stimulation (TMS). After the single-pulse TMS, the TA's EMG signal was measured in a 10ms-200ms window post-TMS delivery. Ten repetitions were performed at each %MSO with a minimum rest time of ten seconds between each repetition. The process was continued in a step wise fashion with either increasing or decreasing 3-9% %MSO increments until either 1) 200% of the resting motor threshold had been reached or 2) a plateau in the recruitment curve was visualized or 3) 100% MSO had been reached.	

All participants completed an approved informed consent process. Participants were assigned to each of five different treatment conditions by a statistician using a Latin square block randomization procedure. The five conditions were as follows: 1) M_1-SO:Saline 2) M_1-SO:EEG Gel 3) C1-C2:Saline 4) C1-C2:EEG Gel and 5) Sham. Depictions of the two electrode montages used are shown in Figure 1. A pre-test, post-test repeated measures design with a minimum of one week between each condition was used. Participants, investigators, and persons conducting data analysis were blinded to treatment condition. In each test session, the MEP threshold was determined prior to tDCS. MEP recruitment curves were obtained twice; before subjects received tDCS and after they had received tDCS and completed the ankle tracking accuracy task (Table 2).

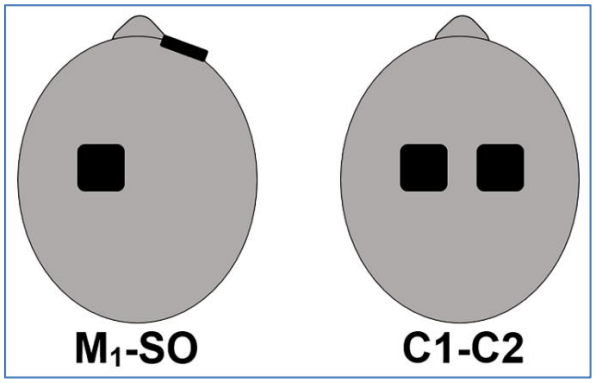

Figure 1. Top-down transverse view of the M_1-SO and C1-C2 electrode montages. The top aspect of the figure corresponds to the anterior aspect of the scalp

Determining M_1 and the MEP threshold

Participants were seated comfortably in a chair that reclined and provided support of the LEs. Single-pulse TMS was delivered using a Magstim 110mm Double Cone Coil and a Magstim Super Rapid2 stimulator (Magstim, UK). Wired electromyography (EMG) data was recorded from the dominant TA using a World Precision Instruments amplifier (Model #ISDB-2, Sarasota, FL, USA) with an input impedance of 10^{12} ohms and a gain of 104. For EMG electrode placement, the skin overlying the dominant TA muscle belly and ipsilateral patella were cleaned and lightly abraded with isopropyl alcohol. Two Ag/AgCl dry-gel EMG surface electrodes were placed on the most prominent aspect of the dominant TA muscle belly 2 cm apart and parallel to the muscle fiber orientation. Another surface Ag/AgCl EMG electrode was placed on the central aspect of the ipsilateral patella, which served as the reference electrode.

The motor "hot spot" for MEPs recorded from the TA was found by delivering single pulses of TMS and marked as M_1 on a template MRI using the BrainSight neuronavigation system software (BrainSight, Rough Research) at the beginning of each treatment session. The resting MEP threshold of M_1 was then determined and quantified by the peak-to-peak value (μV) after a TMS stimulus (see Table 2 for more details).

Procedures for the ankle tracking task

Participants were seated on a moveable height table so the participant's hips could be positioned in approximately 90 degrees flexion, the knees in approximately 75-90 degrees of flexion, and the ankles in an approximate neutral position. The dominant foot was fastened into a footplate that was custom built for this experiment and secured via Velcro straps over the dorsal aspect of the foot. A feedback monitor was placed at eye-level. A custom LabView program created continuous sinusoidal waveforms with varying amplitudes and frequencies displayed from left to right on the monitor. The feedback window also showed a line dependent upon the subject's ankle movement, or the biofeedback line, that was simultaneously displayed on the monitor. During the task, participants were asked to match the biofeedback line with the wave pattern created by the computer program to the best of their ability.

Three practice trials of the tracking task were conducted by the participants at the beginning of each treatment condition. The practice trials lasted 20 seconds each with a 30-second break in between each trial. Five separate waveforms were utilized that lasted 20 seconds with a 15-second break given between each waveform. The first two seconds and last 2.5 seconds were removed and the remaining 14.5 seconds were used for data analysis. Participants received the same waveforms before and after tDCS application in the same order. The error score was calculated with the following equation: Error = [Target Waveform – Actual Waveform]. The average error score pre-TDCS and post-TDCS for all five trials was calculated for data analysis.

Procedures for recording recruitment curves

TMS pulses were triggered when a subject held a contraction of the TA muscle at 20% of the maximal voluntary isometric contraction (MVIC). A biofeedback system, a bar graph in real-time showing the target for muscle contraction, was displayed in front of the subject. MVIC was measured before and immediately after tDCS for each treatment session.

Cortical excitability was operationally defined by parameters of the recruitment curves. A recruitment curve was calculated by recording MEPs at varying levels of the maximum stimulator output (MSO) and with regard to the motor threshold expressed in terms of the percent of (% MSO) (Table 2). To calculate parameters of the recruitment curve, a Loess function was used to smooth the data. Smoothed data were then processed using the Boltzmann Sigmoidal Function. The maximal MEP value (MEP_{max}) and slope were used for data analysis. To normalize the data, difference scores and ratios were calculated and used in data analysis via the following equations:

- MEP_{max} Difference = Post-tDCS MEP_{max} – Pre-tDCS
- MEP_{max} Ratio = Post-tDCS MEP_{max}/Pre tDCS MEP_{max}
- Slope Difference = Pre-tDCS slope – Post-tDCS slope
- Slope Ratio = Pre-tDCS slope/Post-tDCS slope

A positive value for the difference scores or a value greater than 1.0 for the ratios indicated an increase in cortical excitability.

tDCS application

tDCS was administered using a Soterix constant current stimulator (Model# 1300A, New York, NY, USA). For all active treatment conditions, 2.0 millamps (mA) of current was delivered for twenty minutes. The stimulator utilized a 30-second ramp-up and 30-second ramp-down period that preserved the 20-minute "on" time. During sham stimulation, an M_1-SO montage was used with saline solution as the conductance medium. Following standard procedures for sham stimulation, a ramp-up of 30 seconds to 2.0 mA and ramp-down of 30 seconds to 0 mA occurred at the beginning and end of the 20-minute stimulation period with no stimulation in between. For active treatment, the anode was placed over M_1 as determined by TMS MEP recording. For the treatment conditions using an M_1-SO montage, the cathode was placed on the contralateral SO region and for the C1-C2 montage (using International EEG 10/20 System) the cathode was placed contralateral to the anode.

When saline solution was used as the conductance medium, 10 milliliters of 0.9% saline solution was evenly dispersed via syringe onto each Soterix 5 × 5 cm (25 cm^2) sponge electrode. Any excess saline that seeped from the electrode after application was wiped away. The EEG gel treatment conditions used OneStep EEG Gel as the conductance medium. The gel was placed onto one side of a 5 × 5 cm (25 cm^2) carbon-rubber electrode (Covidien 664 REFX 2 × 2) so the entire side was covered. The gel side of the electrode was placed onto the scalp. Any excess gel that seeped from underneath the electrode was removed.

Data analysis

Histograms revealed that some dependent variables were not normally distributed. So, a Friedman test was used to compare difference scores and ratios of the MEP_{max} and slope between the active conditions. If any active conditions were significantly different than each other, they were compared to sham-tDCS with a Friedman test and Wilcoxon signed-rank tests (SRT) used for post hoc comparisons.

To normalize the ankle tracking accuracy scores across participants, both ratio and difference scores were assessed. For the ratio scores, the Post-tDCS mean error score was divided into the mean Pre-tDCS mean error score. This was known as the accuracy ratio (AR) where a value greater than 1.0 indicated increased accuracy. For difference scores, the Post-tDCS mean error score was subtracted from the Pre-tDCS mean error score where a larger difference correlated to increased accuracy. A one-way repeated-measures analysis of variance was used to compare both the AR and difference scores between the M_1-SO:Saline, C1-C2:Gel, and Sham conditions.

A family-wise alpha of 0.05 was used for all analyses and all values were reported as means and standard deviations. Statistical analyses were conducted by an investigator blinded to the treatment conditions using the SPSS 22.0 computer program.

Results

No adverse effects due to tDCS or TMS were reported during the course of the study. Eighteen participants (10 female, 18 Right LE dominant, mean age = 24.2 years) participated in the study. Data from one subject was not included in the analysis of cortical excitability due to being an outlier. An outlier was defined as a value that was greater than two standard deviations from the mean.

Primary analysis

Regarding cortical excitability, there was a significant effect of condition on the MEP_{max} difference score ($x^2(3) = 7.941$, $p=0.047$). The MEP_{max} difference score was significantly higher post-stimulation for the C1-C2:Gel condition (0.97 (1.32)) compared to the M_1-SO:Saline condition (-0.82 (1.27), $p=0.004$). A Friedman test comparing the C1-C2:Gel, M_1-SO:Saline, and Sham conditions was significant ($x^2(2) = 8.824$, $p=0.012$). Whereas, post hoc comparisons using the Wilcox SRT revealed no significant difference between the C1-C2:Gel and Sham stimulation (0.92 (1.75) $p=0.906$); MEP_{max} difference values were significantly lower post stimulation for the active M_1-SO:Saline condition when compared to sham stimulation ($p=0.006$). No other significant differences were found between any of the montage/medium combinations for either the MEP_{max} ratio ($x^2(3) = 6.106$, $p=0.107$), slope difference ($x^2(3) = 4.059$, $p=0.255$), or slope ratio scores ($x^2(3) = 3.988$, $p=0.263$) (Figure 2).

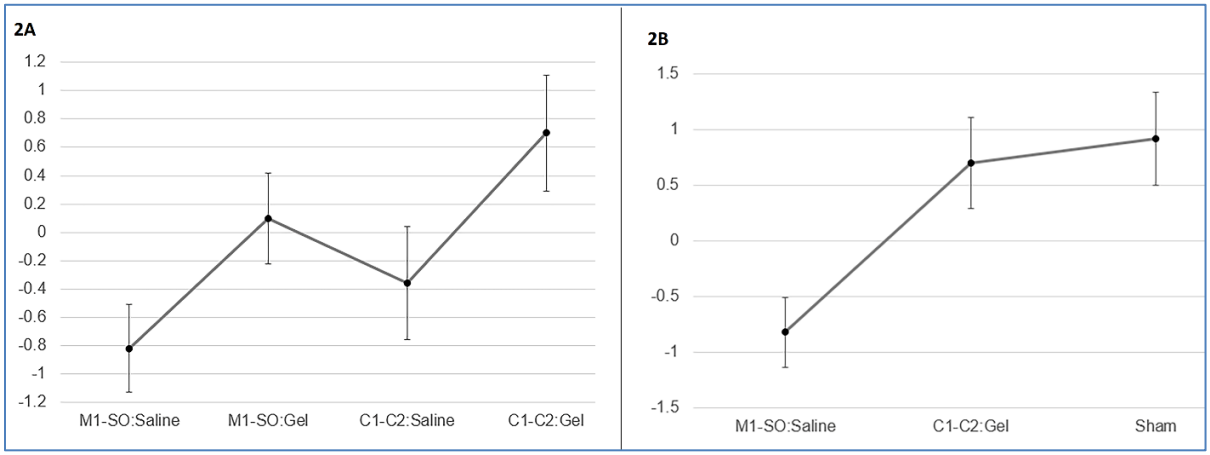

Figure 2. Mean motor evoked potential maximum (MEP_{max}) difference scores between **a)** the M_1-SO:Saline, M_1-SO:Gel, C1-C2:Saline, and C1-C2:Gel conditions and between **b)** the M_1-SO:Saline, C1-C2:Gel, and Sham conditions. Bars represent standard errors

Regarding ankle tracking, the AR and difference scores were normally distributed for each treatment condition; however, there was no effect of condition on the AR [M_1-SO:Saline 1.025 (0.082), C1-C2:Gel 1.020 (0.067), Sham 1.017 (0.075) (Wilks' Lambda=0.991, F(2,16)= 0.069, p=0.933, partial eta squared=0.009)] or on the difference scores [M_1:SO:Saline 77.30 (269.78), C1-C2:Gel 61.00 (228.74), Sham 57.65 (280.17) (Wilks' Lambda=.994, F (2,16)= 0.049, p=0.952, partial eta squared=0.006)].

Secondary analysis

Since the M_1-SO:Gel and C1-C2:Saline combinations were not compared to Sham in our primary analysis in regards to MEP_{max} difference scores, a Friedman test that included all five stimulation conditions was conducted. Pairwise comparisons in this secondary analysis confirmed no differences between the M_1-SO:Gel (p=0.092) and C1-C2:Saline (p=0.054) conditions relative to Sham.

Discussion

We found that active-tDCS delivered at 2 mA for 20 minutes over the TA "hot spot" of M_1 significantly changed cortical excitability recorded from the TA muscle when compared to sham-tDCS, but only when using an M_1-SO electrode montage with saline as the conductance medium. Using gel as a conductance medium failed to produce any difference in cortical excitability relative to sham. Additionally, the C1-C2 electrode montage failed to produce a significant difference relative to sham regardless of the conductance medium used.

Saline as a conductance medium produced a significant change in cortical excitability in the LE motor cortex whereas gel did not. We hypothesized gel would improve overall impedance compared to saline and decrease the possibility of bridging. It is yet unclear why saline was superior to gel but the finding demonstrates a need for more tDCS studies comparing medium-based effects of cortical excitability.

Contrary to our original prediction, cortical excitability decreased rather than increased post-tDCS; however, we think this result can be explained by the order of the experimental procedures - specifically, the time at which recruitment curves were obtained; after the ankle tracking task rather than immediately following tDCS. In short, we think that active-tDCS with the M_1-SO saline montage did increase the activation state of the motor cortex for the LE immediately after tDCS delivery but that subsequent, intervening neural activation induced by the ankle tracking task elicited a homeostatic plasticity response mechanism, described below, that resulted in a long term depression (LTD) like response. Similar results have been observed in tDCS studies of the UE in humans [33-35]. In the remainder of the discussion, we will examine the strengths and weaknesses of this study, compare our findings to those of other studies, and explore the validity of our main finding and interpretation.

The main strength of this methodological study of LE stimulation was to compare two different electrode montages and conductance mediums to a valid sham condition while holding dose constant. For anyone who reviews the available literature in this topic area, it is obvious the studies are simply not comparable in terms of participants and stimulation dose, duration, or method of delivery because so many different stimulation parameters are used (Table 1).

In contrast, our within-subject comparisons were not ambiguous in showing which electrode montage and medium combination led to a significant difference in cortical excitability between the sham and active-tDCS conditions. For example, in the first statistical comparison, when the sham condition was not considered, the C1-C2:Gel combination appeared to increase cortical excitability of the LE motor cortex compared to the M_1-SO:Saline combination. However, when conditions were compared to Sham, the C1-C2:Gel condition actually had no effect. In fact, it was similar to sham stimulation, whereas the M_1-SO:Saline combination led to a significant decrease in the MEP_{max} relative to sham. To our knowledge, this is the only study to find a significant difference in cortical excitability of the LE between an active and a sham-tDCS condition. Further, it shows the M_1-SO saline combination was uniquely effective at altering cortical

excitability of the LE. These findings reveal that more focused, methodological studies of this technique are needed to determine which stimulation parameters are effective at achieving a change in cortical excitability of the LE.

Several limitations of the study may hinder the generalizability and interpretation of our main finding. We only assessed short-term changes in cortical excitability and function of the dominant LE of young, healthy participants, so we do not know how the results will generalize beyond this sample. Also, a possible ceiling effect may have rendered tDCS ineffective at further improving ankle tracking accuracy. Some limitations of the experimental design were that, to reduce subject burden, we did not include a cathodal-tDCS condition. Additionally, recruitment curves were not measured immediately following tDCS delivery. Both limitations hinder our ability to fully interpret how the M_1-SO saline montage led to a decrease in the MEP_{max} for the active-tDCS condition. We now turn to a possible explanation for that result.

Our interpretation is that active-tDCS with the M_1-SO saline was effective at increasing cortical excitability of the LE motor cortex and that subsequent stimulation provided by the ankle tracking task produced an LTD-like response. The LTD-like response was measured during the recruitment curve as a significant decrease in the MEP_{max} compared to sham stimulation. This explanation is based on the concepts of activity dependent changes in synaptic plasticity, like LTD and long-term potentiation (LTP). Regulatory mechanisms such as homeostatic plasticity, which is synergistic with synaptic plasticity, serve to establish a set point based on neural activity and to keep LTD and LTP like responses within a physiologically reasonable dynamic range [36]. Seminal studies of homeostatic plasticity found that a prolonged increase in postsynaptic activity made neurons less excitable while a prolonged decrease in postsynaptic activity made the neurons more excitable. Observations like these culminated in the Bienenstock, Cooper, and Munro (BCM) rule of synaptic plasticity in which prolonged increases in postsynaptic activity are thought to raise a modification threshold, or set point, and favor the induction of LTD upon further stimulation. In contrast, a prolonged decrease in postsynaptic activity lowers the modification threshold and favors the induction of LTP with further stimulation [37].

While homeostatic plasticity has been investigated in many studies of human participants [36], we will focus on two tDCS studies of humans [34,35] because they show how tDCS may establish a set point for homeostatic plasticity. Both investigators examined how tDCS preconditioning prior to rTMS delivery altered cortical excitability recorded from the upper extremity (UE) motor cortex in healthy participants. In each of the respective studies, tDCS was applied using an M_1-SO:Saline combination at 1 mA for 10 minutes while targeting the right FDI representation of the motor cortex (or left FDI motor cortex). Participants performed in anodal, cathodal, and sham-tDCS conditions. Ten minutes after tDCS was delivered, active rTMS (at either 1Hz [35] or 5Hz [34]) was provided over the left FDI motor cortex. Cortical excitability of the left FDI motor cortex was assessed immediately after tDCS (INTER), immediately after rTMS (Post 1), and 10 minutes after rTMS (Post 2). At the INTER period, both studies found that anodal-tDCS tended to increase MEPs and that cathodal-tDCS tended to decrease MEPs in comparison to baseline. In contrast, at the Post 1 assessment, the polarity of the MEP response switched relative to the INTER period where anodal-tDCS followed by rTMS lead to significantly decreased MEPs and cathodal-tDCS followed by rTMS led to significantly increased MEPs, regardless of rTMS frequency, which persisted into the Post 2 period [34,35].

The results of our study are consistent with the results in the Seibner and Lang studies [34,35]. In our study, we measured cortical excitability, not immediately after tDCS delivery (i.e., the INTER period), but rather after the ankle-tracking task (i.e., the POST 1 period). Not surprisingly, our results resemble those for the POST 1 phase of the Seibner and Lang studies [34,35]. Similarly, we think the active-tDCS delivered using an M_1-SO:saline combination was effective at increasing cortical excitability (i.e., at raising the modification threshold during the INTER period), and the

ankle tracking task provided subsequent stimulation (like the rTMS in the Seibner and Lang studies), which drove a LTD like synaptic plasticity response and caused the MEP_{max} to be decreased relative to sham-tDCS. Other studies have shown that motor tasks are effective at priming homeostatic responses [36]. Unfortunately, we did not include a cathodal stimulation condition, which could have produced opposite results, nor did we obtain recruitment curves immediately after tDCS, which could prove or disprove our interpretation. It will be important to include additional control conditions in future studies.

By examining our results in light of previous studies, it is reasonable to conclude that active-tDCS applied alone may not lead to significant differences in MEPs recorded from the LE relative to a sham-tDCS condition. Every study that has measured cortical excitability of the LE immediately after tDCS has failed to find a significant difference between active and sham-tDCS [7-9]. We too might have failed to detect a difference between active and sham-tDCS had we assessed cortical excitability immediately and only after tDCS delivery. Taken together, these studies suggest that, in order to produce significant changes in cortical excitability, it may be best to use tDCS as a precondition to set the modification threshold at either a high or low level prior to a subsequent behavioral or neurostimulation procedure. In that way, tDCS may be used to set up the desired LTD or LTP like response.

Conclusion

When combined with a behavioral task, active-tDCS at 2 mA for 20 minutes using an M_1-SO montage and saline as a conductance medium was effective at significantly changing cortical excitability of the LE relative to sham-tDCS. Gel as a conductance medium did not appear to work as well as saline. Active-tDCS using the C1-C2 electrode montage did not alter cortical excitability relative to sham-tDCS. Ankle tracking accuracy was not affected by tDCS, possibly due to a ceiling effect on the task.

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