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The effects of bi-hemispheric M1-M1 transcranial direct current stimulation on primary motor cortex neurophysiology and metabolite concentration

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Abstract

Purpose.—The aim of the present study was to assess, in healthy individuals, the impact of M1-M1 tDCS on primary motor cortex excitability using transcranial magnetic stimulation and sensorimotor metabolite concentration using ¹H-MRS.

Methods.—For both experiments, each participant received the three following interventions (20 min tDCS, 1mA): left-anodal/right-cathodal, left-cathodal/right-anodal, sham. The effects of tDCS were assessed via motor evoked potentials (experiment 1) and metabolite concentrations (experiment 2) immediately after and 12 minutes following the end of stimulation and compared to baseline measurement.

Results.—No effect of M1-M1 tDCS on corticospinal excitability was found. Similarly, M1-M1 tDCS did not significantly modulate metabolite concentrations. High inter-subject variability was noted. Response rate analysis showed a tendency towards inhibition following left-anodal/right-cathodal tDCS in 50% of participants and increased GABA levels in 45% of participants.

Conclusion.—In line with recent studies showing important inter-subject variability following M1-supraorbital tDCS, the present data show that M1-M1 stimulation is also associated with large response variability. The absence of significant effects suggests that current measures may lack sensitivity to assess changes in M1 neurophysiology and metabolism associated with M1-M1 tDCS.

Keywords

magnetic resonance spectroscopy; transcranial direct current stimulation; motor cortex; GABA; glutamate

Introduction

Transcranial direct current stimulation (tDCS) is a neuromodulation technique that has gained recognition as a promising clinical tool because of its ability to modulate a large array of behaviors and cognitive functions in both healthy individuals (Jacobson et al., 2011) and clinical populations (Sandrini and Cohen, 2013; Schulz et al., 2013). It is generally assumed that when an anode is placed over the primary motor cortex (M1) and a cathode is positioned over the contralateral supraorbital area (M1-supraorbital montage; anodal tDCS, a-tDCS), corticospinal excitability is enhanced but decreased when the current flow is reversed (cathodal tDCS, c-tDCS) (Nitsche and Paulus, 2000; Lang et al., 2005). Despite a wealth of encouraging results, the apparent potential of tDCS has led to its use in clinical settings without a comprehensive understanding of optimal parameters for treatment, intra- and inter-individual variability of response, individual factors modulating response, and most importantly mechanisms of action underlying its physiological effects (Gomez Palacio Schjetnan et al., 2013; Horvath et al., 2014). For example, recent studies have shown highly variable effects in the motor cortex of healthy individuals following tDCS (López-Alonso et al., 2014; Wiethoff et al., 2014) and limited reliability to modulate cognitive function (Horvath et al., 2015; Tremblay et al., 2014a).

Pharmacological studies and animal models suggest that tDCS modulates corticospinal excitability through its effects on resting membrane potentials (Fritsch et al., 2010; Liebetanz et al., 2002; Stagg & Nitsche, 2011), and synaptic connections via long-term potentiation (LTP) and long-term depression (LTD) mechanisms (Stagg and Nitsche, 2011). In the neocortex, these mechanisms are believed to be partially mediated by GABAergic and glutamatergic neurons (Froc et al., 2000; Trepel and Racine, 2000). Using transcranial magnetic stimulation (TMS), recent studies have suggested that M1-supraorbital tDCS can modulate several indirect measures of GABA and glutamate receptor activity, such as cortical silent period duration (Tremblay et al., 2013) and intracortical facilitation (Di Lazzaro et al., 2012). However, there is currently no consensus on the effect of tDCS on neurophysiological markers of intracortical inhibition and facilitation.

Even though the effects of M1-supraorbital tDCS on M1 excitability have been widely studied using TMS, few studies have assessed the impact of M1-M1 tDCS on corticospinal excitability and current results remain unclear. As such, while some studies have shown the expected polarity-sensitive effects following M1-M1 tDCS (Mordillo-Mateos et al., 2012; Tazoe et al., 2014), one recent study reported no significant effect of M1-M1 tDCS (left anode/right cathode) over bilateral M1 excitability (O'Shea et al., 2013). Although little information is available regarding the neurophysiological mechanism underlying the cortical effects of M1-M1 tDCS, the technique has been used in several clinical studies (Di Lazzaro et al., 2014; Vines et al., 2008). For example, this protocol has shown promising results

among stroke patients for which an interhemispheric imbalance, i.e. an increase of activity in the unaffected hemisphere and a reduction of activity in the affected hemisphere, is linked to poor recovery (Hummel & Cohen, 2006; Murase et al., 2004). A number of recent clinical stroke studies have shown that M1-M1 stimulation, through the combination of a-tDCS over contralesional M1 and c-tDCS over ipsilesional M1, induces greater and longer-lasting effects on motor recovery than M1-supraorbital stimulation (Lindenberg et al., 2013; Sehm et al., 2013; Vines et al., 2008). This suggests that a bi-hemispheric montage can act on the balance between both hemispheres. This montage may therefore induce additive effects on M1 excitability via interhemispheric connections, which are thought to be more focal than those associated with M1-supraorbital stimulation (Di Lazzaro et al., 2014). Despite these positive clinical results, the neurophysiology of M1-M1 stimulation remains poorly understood.

Proton magnetic resonance spectroscopy ($^1\text{H-MRS}$), which allows direct measurement of GABA and glutamate levels, offers a powerful complementary tool to assess the mechanism of action underlying M1 tDCS. Using this method, Stagg and collaborators (2009) showed a significant reduction of GABA and glutamate + glutamine (Glx) levels in sensorimotor cortex following M1-supraorbital c-tDCS. Kim and collaborators (2014), on the other hand, found no evidence of GABA and Glx modulation with a similar stimulation protocol. Anodal M1-supraorbital tDCS has proven to be more robust in showing metabolic effects of tDCS. Indeed, GABA levels were found to be reduced after anodal tDCS in three separate studies (Kim et al., 2014; Stagg et al., 2011; Stagg et al., 2009). To our knowledge, the effect of M1-M1 tDCS on motor cortex metabolism has yet to be investigated.

The primary objective of the present study was to further assess the impact of M1-M1 tDCS on M1 corticospinal excitability using TMS and determine its effect on sensorimotor metabolism using $^1\text{H-MRS}$ in healthy participants. Following recent studies on the effects of M1-supraorbital tDCS, we hypothesized that, when the *anode* was located over left M1, M1-M1 stimulation would increase corticospinal excitability and reduce GABA concentration under the anode. When the *cathode* was located over left M1, we predicted reduced corticospinal excitability and reduced GABA and Glx concentrations under the cathode.

Material and methods

Ethical approval

The experiments described in the current manuscript conformed to the standards set by the *Declaration of Helsinki*, and all of the procedures were approved by the research ethics board of the Comité Mixte d'Éthique de la Recherche du Réseau de Neuroimagerie du Québec (CMER-RNQ). All participants provided written informed consent prior to testing and all participants were aware of the aim of the experiment, including the presence of a sham condition. Both experiments were single-blinded, as only the experimenter was aware of the stimulation condition.

Experiment 1: neurophysiological effects of M1-M1 tDCS

Participants and procedure: Ten right-handed healthy volunteers (5 women; mean age: 25 ± 6 years; age range: 20–40 years) were recruited for this study. Female participants were tested irrespective of their menstrual cycle. Participants were excluded if they had any neurologic or psychiatric disorder or contraindications to tDCS or TMS, including taking drugs that can modify cortical excitability thresholds (Nitsche et al., 2008; Rossi et al., 2009). Each participant took part in the three following experimental sessions: 1) active stimulation left anodal/right cathodal (LA/RC); 2) active stimulation left cathodal/right anodal (LC/RA); 3) sham stimulation (LA/RC). The conditions were counterbalanced across groups and each session was separated by at least 72 hours.

Experimental procedures

Transcranial magnetic stimulation: TMS was delivered through an 8 cm figure-of-eight coil connected to a Magstim 200² stimulator (Magstim Company Ltd, Spring Gardens, UK). The coil was positioned flat on the head of participants at a 45° angle from the midline, with the handle pointing backwards. A monophasic current was induced with a posterior-anterior direction. TMS was delivered over left M1. The optimal site of stimulation was defined as the coil position from which TMS produced motor evoked potentials of greatest amplitude in the FDI muscle of the contralateral hand following the method of the most recent report of the International Federation of Clinical Neurophysiology (Rossini et al., 2015). To achieve this, the coil was moved around a pre-determined spot in 1cm steps in all directions using a starting intensity of 50% maximum stimulator output. Next, the intensity of stimulation was adjusted to elicit MEPs of average amplitude of 1 mV peak-to-peak for 10 consecutive trials. Using this intensity, twenty MEPs were collected immediately before tDCS (pre), immediately after tDCS (post0) and 12 min post-tDCS (post12). TMS pulses were delivered at a frequency of 0.1 to 0.2 Hz to avoid long lasting modulation of M1 excitability (Chen et al., 1997; Rossini et al., 2015).

To ensure stable coil positioning, a stereotaxic neuronavigation system (Brainsight; NeuroConn GmbH, Ilmenau, Germany) was used. To quantify muscle contractions, two self-adhesive electrodes were positioned over the FDI muscle of the right hand and a ground electrode was positioned over the right wrist. EMG signal was filtered with a bandwidth of 20–1000 Hz and digitized at a sampling rate of 4 kHz using a Powerlab 4/30 system (ADInstruments, Colorado Springs, USA). MEPs were recorded using LabChart7 software (ADInstruments, Colorado Springs, USA) and stored offline for analysis.

Transcranial direct current stimulation: Electrical current was delivered by a Magstim DC Stimulator (Magstim Company Ltd, Spring Gardens, UK) through a pair of rectangular conductive rubber electrodes (35 cm²) inserted into saline-soaked sponges. The electrode center was positioned over the left and right FDI muscle M1 representation, previously determined using TMS. The electrodes were oriented parallel to the central sulcus. For both active conditions, a constant electric current of 1 mA was applied for 20 min, and the current was gradually increased and decreased during the first and last 15 s of stimulation. For the sham condition, the current was ramped up for 15 s and then no current was delivered for 20 min.

Experiment 2: neurometabolic effects of M1-M1 tDCS

Participants and procedure: Eight right-handed healthy volunteers (4 women; mean age: 29 ± 6 years; age range: 24–40 years) participated in this study. The same exclusion criteria as in Experiment 1 were used, in addition to standard magnetic resonance (MR) contraindications. The experimental protocol consisted of three sessions of 2 h duration, separated by at least 72 h and counterbalanced across groups. Each session consisted of a first ^1H -MRS acquisition, 20 min of M1-M1 tDCS that was administered inside the scanner (same conditions as for Experiment 1), and two consecutive ^1H -MRS acquisitions post-stimulation.

Experimental procedures

Transcranial direct current stimulation: Electrical current was delivered using a MR-compatible NeuroConn DC-stimulator Plus (NeuroConn GmbH, Ilmenau, Germany) through a pair of rectangular conductive rubber electrodes (35 cm^2). Electrodes were entirely covered with an EEG-type conductive paste. They were positioned over C3 and C4, according to the 10/20 international system, which corresponds to the left and right primary motor regions, respectively. The electrodes were oriented parallel to the central sulcus and eyebrows. Once the electrodes were properly positioned, the impedance level was tested outside the scanning room prior to testing. If an adequate impedance level was reached ($< 20 \text{ k}\Omega$), participants were positioned comfortably in the scanner and were instructed to lie at rest for the entire scanning session. The electrodes were plugged into the MR-compatible tDCS box, which was positioned inside the scanner. Active and sham condition parameters were identical to Experiment 1. No MR data was acquired during tDCS. See Tremblay et al. (2014b) for a comprehensive description of the protocol.

Proton magnetic resonance spectroscopy: MR acquisitions were performed using a 3 T whole-body system (MAGNETOM Trio, Siemens, Erlangen, Germany) at the *Unité de Neuroimagerie Fonctionnelle, Centre de recherche de l'Institut Universitaire de Gériatrie de Montréal*. Radiofrequency transmission was performed with the built-in body coil, and signal was received with a 32-channel receive-only head coil. The prescription of sensorimotor voxel and detection of potential structural abnormalities were performed using anatomical images of the brain obtained with a T_1 -weighted MPRAGE sequence ($T_R = 2300 \text{ ms}$; $T_E = 2.91 \text{ ms}$; FA: 9° ; FOV = $256 \times 256 \text{ mm}$; 256×256 matrix; T_1 : 900 ms; 176 slices; orientation: sagittal; acquisition time: 4 min 12 s). The voxel of interest ($30 \times 30 \times 30 \text{ mm}^3$) was manually positioned over the left precentral knob without any angulation relative to the scanner reference space (Figure 1). The positioning of the voxel was based on the anatomical landmarks visible on the three orientation slices described by Yoursy and collaborators (1997). First and second order shims were adjusted using FAST(EST)MAP (Gruetter and Tkác, 2000). ^1H -MRS data were then acquired using a MEGA-PRESS sequence (Mescher et al., 1998) as previously described (Tremblay et al., 2014b). The MEGA-PRESS acquisition consisted of four blocks of 64 metabolite scans (32 editOff and 32 editOn, interleaved) each with frequency update between blocks ($T_R = 3 \text{ s}$, $T_E = 68 \text{ ms}$, total acquisition time: 12 min), as well as single blocks of unsuppressed-water reference scans (4 editOff and 4 editOn, interleaved; acquisition time: 42 s; same parameters as for metabolite scans, but MEGA and VAPOR water suppression off). Free

induction decays were stored separately in memory for individual frequency and phase correction using tCr and choline signals between 2.85 and 3.40 ppm. The final spectra were obtained by subtracting (for metabolite scans) or averaging (for unsuppressed-water reference scans) the signal from editOff and editOn scans as described previously (Tremblay et al., 2014b) (Figure 1). The $^1\text{H-MRS}$ metabolite acquisition was performed prior to tDCS ($^1\text{H-MRS}$ pre) and repeated twice following tDCS ($^1\text{H-MRS}$ post1, $^1\text{H-MRS}$ post2), and the $^1\text{H-MRS}$ unsuppressed-water reference acquisition followed the acquisition of the pre and post 2 metabolite spectra. A localizer scan was performed prior to the $^1\text{H-MRS}$ session and following the last metabolite acquisition to visually compare both scans for head movement.

Analysis of $^1\text{H-MRS}$ data: Both editOff and difference spectra were analyzed using LCModel 6.3–1 (Provencher, 1993, 2001), which estimated the best fit of the experimental spectrum as a linear combination of model spectra. The basis set for editOff spectra included an experimentally measured metabolite-nulled macromolecular spectrum from the occipital region (average from 11 subjects) and metabolite spectra simulated with home-written software based on density matrix formalism (Henry et al., 2006) in MATLAB, using known chemical shifts and J couplings (Govindaraju et al., 2000) as described previously (Tremblay et al., 2014b). From LCModel's default simulations of lipid and macromolecular resonances, only "Lip13a" (modeling a broad peak at 1.28 ppm) was allowed during the LCModel fitting that was performed over the spectral range from 0.2 to 4.0 ppm, and LCModel spline model of the baseline was deactivated using the NOBASE = T input parameter. The basis set for difference spectra included an experimentally measured metabolite-nulled macromolecular spectrum from the occipital region (average from 11 subjects) and the experimentally measured spectra from 100 mM phantoms of NAA, GABA, Glu and Gln at 37°C and with pH adjusted to 7.2. No LCModel default simulations of lipid and macromolecular resonances were allowed during the LCModel fitting that was performed over the spectral range from 0.5 to 4.0 ppm, and LCModel spline model of the baseline was also deactivated using the NOBASE = T input parameter. No baseline correction, zero-filling, or apodization functions were applied to the *in vivo* data prior to LCModel analysis. tCr (Cr-CH₃ + PCr-CH₃), mIns, and tNAA (sNAA+NAAG) concentrations were obtained from editOff spectra, and GABA and Glx concentrations were obtained from difference spectra. A scaling factor between the simulated and measured basis sets was calculated using the group average of tNAA measured from editOff spectra and the group average tNAA from difference spectra. The spectra were visually inspected for contamination from subscapular lipid signals and Cramér-Rao lower bounds (CRLB) and linewidth of water spectra were examined for outliers. Given the current methodological issues related to the use of CRLB for quality control of data when decreases in concentration are expected (see Kreis et al. 2015), the CRLB values were also examined for outliers ($M \pm 3$ SD). This led to rescanning of 8 $^1\text{H-MRS}$ sessions. For each time point, the final CRLB were < 30% for Glx, tNAA, and tCr. For each GABA time point, CRLB were generally 40% ($M = 18.13 \pm 8.04$). However, the CRLB value of the "post2" time point of the cathodal condition was 46% (slightly over 3 SD) for participant 1, and the three time points were > 52% (over 3 SD) for participant 4 (sham condition). After a visual inspection of the $^1\text{H-MRS}$ data and localizer images for head motion, data from participant 1 were not excluded, but GABA concentrations from participant 4 (sham condition) were excluded from further analyses. The linewidth of

each water spectra was < 10 Hz. Metabolite concentrations were quantified using the water reference and in secondary analysis using tCr.

Quantification using water reference: Quantification was performed using an unsuppressed water signal obtained from the same voxel after eddy current correction (Kolse, 1990) and after averaging editOff and editOn scans. The pre-tDCS water reference scan was used for the quantification of the pre-tDCS metabolite scan, and the post-tDCS water reference scan was used for the quantification of both post-tDCS metabolite scans. Concentrations were corrected for cerebrospinal fluid (CSF) content. The tissue composition was obtained from the high-resolution anatomical MR images of the sham session of each subject, which were segmented to gray matter (GM), white matter (WM), and CSF content using the automated *FreeSurfer* pipeline (V 5.3.0, <http://freesurfer.net>). The fractional volumes of GM, WM, and CSF were obtained for the ¹H MRS voxels. The relative densities of MR-visible water for GM, WM, and CSF were assumed to be 0.78, 0.65, and 0.97 (Gasparovic et al., 2006), respectively. The T₁ and T₂ relaxation times of water used in the calculation of attenuation factors were taken from published reports [T₁(GM) = 1.29 s, T₁(WM) = 0.87 s, T₁(CSF) = 4 s, T₂(GM) = 110 ms, T₂(WM) = 80 ms, and T₂(CSF) = 400 ms] (Rooney et al., 2007; Wansapura et al., 1999). The water attenuation was computed using the fractional volume of each compartment (Gasparovic et al., 2006).

Distance between M1 and the scalp: Given that previous studies have shown an influence of the scalp-to-cortex distance on TMS measures such as motor threshold (McConnell et al., 2001), scalp-to-cortex distances were assessed in all participants taking part in the ¹H-MRS study to determine their impact on individual responses to stimulation. From the sagittal view of the MPRAGE scan (sham condition) of each individual participant, the distance between M1 and the scalp was measured using a previously described procedure (McConnell et al., 2001) (Figure 2).

Statistics: Data were analyzed separately using a standard statistical software package (version 21.0, SPSS inc, Chicago, IL, USA). A *p* value of < .05 was considered statistically significant. When significant effects were observed, *post-hoc* analyses were computed and the *p* value was adjusted for multiple comparisons (Bonferonni correction). For the main analyses, effect size was calculated and expressed as partial eta-squared (η^2_{partial}). When necessary, non-sphericity was adjusted using Greenhouse-Weisser correction. Values that exceeded 3 standard deviations from the mean were excluded from further analyses, which led to the exclusion of one TMS data point (subject 2, post12). For both experiment, power-analyses were conducted using G*Power 3.1 (Faul et al., 2007) to determine the required sample size to obtain a significant main effect or interactions, when results were close to significance.

For Experiment 1, individual peak-to-peak MEP size was calculated offline and averaged for each subject and each condition. The mean latencies of the MEP were also calculated to ensure proper recruitment of cortical excitability. Paired-sample t-Tests were used to compare raw baseline MEPs between conditions. Then, normalized (ratios of change from baseline) MEP data were compared using a general linear model repeated-measure analysis of variance (RM-ANOVA), with factors Polarity (LA/RC, LC/RA, sham) and Time (post0,

post12). To account for differences in time-dependent arousal, the root mean square (RMS) of the EMG signal recorded 50ms prior to the TMS pulse was calculated and data were compared using a repeated-measure ANOVA, with factors Polarity (LA/RC, LC/RA, sham) and Time (pre, post0, post12). To account for within-subject variability in MEP amplitudes within a time-point and therefore use all information available, individual MEPs were included in a generalized estimation equations model (GEE: Hanley et al. 2003). The model included “subjects” as cluster variable, and “time” and “condition” as within-subjects factors. Following a recent study (Feurra et al., 2013), we used an autoregressive-lag1 (AR1) with an M-dependent working correlation (value of 4). MEP values were log-transformed and the Wald test was used to assess significance. Because of the use of all time points (including the baseline) in the analysis, only the interaction time X condition was assessed. Finally, to assess inter-individual variability, classification of response rate was conducted for both active conditions. The standard error of the mean (SEM) was used as an objective criterion (Simeoni et al. 2015). The average of normalized MEPs (post0 and post12) was considered a significant change following stimulation when it exceeded $\pm 95\%$ confidence interval of the grand-average of the individual baseline SEM value. Using this criterion, individuals were divided in three clusters as follows: Responders Excitation (ratio > 1.26), Non Responders ($1.26 > \text{ratio} < .74$) and Responders Inhibition (ratio $< .74$). Finally, two-tailed Pearson correlation coefficients were computed between the baseline raw MEP amplitudes and the average of normalized MEP amplitudes for each active condition to assess prediction of response (see Wiethoff et al., 2014).

For Experiment 2, metabolite concentrations quantified using tCr and water (normalized as ratios of change from baseline) were compared using a repeated-measure ANOVA, with factors Polarity (LA/RC, LC/RA, sham) and Time (post1, post2). Two-tailed Pearson correlation coefficients were computed between scalp-to-cortex measures and metabolite concentrations. As for experiment 1, assessment of inter-individual variability was conducted according to a 95% of confidence interval of the SEM for GABA concentrations. Using this criterion, individuals were divided in three clusters as follows: Responders Excitation (ratio > 1.11), Non Responders ($1.11 > \text{ratio} < .89$) and Responders Inhibition (ratio $< .89$).

Results

Experiment 1

Mean latencies of the MEPs as well as raw MEP amplitudes for each condition are presented in Table 1. Baseline raw MEP amplitudes were not different between conditions, as assessed with paired-sample t-tests (Bonferonni corrected). A repeated-measures ANOVA was computed on normalized MEP amplitudes (post0/pre; post12/pre; see Figure 3a). No significant main effect of Polarity ($F_{(2,9)} = 1.72$; $p = .22$; $\eta^2_{\text{partial}} = .22$) or Time ($F_{(2,9)} = .79$; $p = .41$; $\eta^2_{\text{partial}} = .12$) was observed. The interaction was also not significant ($F_{(2,9)} = .15$; $p = .86$; $\eta^2_{\text{partial}} = .03$). Power analyses revealed a required sample size of 20, 36 and 143 to obtain a significant effect of polarity, time and interaction, respectively. For pre-trigger muscle activity, a repeated-measures ANOVA of RMS data revealed no significant main effect of Time ($F_{(3,9)} = 1.00$; $p = .39$) or Polarity ($F_{(3,9)} = 0.22$; $p = .98$)

and the interaction was not significant ($F_{(3,9)} = 1.32; p = .28$). The GEE analysis revealed a significant interaction between Time and Condition (Wald = 33.08, $p = .0001$). Post-hoc pairwise comparisons (Figure 3b) showed a significant difference between the LA/RC Post0 compared to LA/RC baseline (Mean difference = .28, $p = .007$), indicating a reduction of MEP size. The LA/RC Post0 time point was also significantly different from the baselines measures of the two other conditions (LC/RA: Mean difference = .23, $p = .047$; Sham: Mean difference = .27, $p = .038$).

Response rate for both active conditions, individual changes in normalized MEP amplitudes and rates of response are shown in Figure 4. Pearson correlations showed a significant negative correlation between baseline MEP amplitudes and the mean ratio of change following LC/RA tDCS ($r = -.66; p = .04$; see Figure 5) whereas the correlation was not significant for the LA/RC condition ($r = -.33; p = .35$).

Experiment 2

Water-quantified metabolite concentrations—Average percent change in concentration of metabolites following M1-M1 tDCS are shown in Table 2. Change ratios between pre-tDCS and post-tDCS measures (Post1/Pre; Post2/Pre) were calculated for each metabolite of interest and were used for analysis. To assess the effects of tDCS on metabolite concentration, a 2 X 3 repeated measures ANOVA with Time and Polarity as factors was computed for each metabolite of interest. Figure 6 shows changes in concentration ratios. For GABA, no significant main effect of Time ($F_{(2,6)} = .16; p = .70; \eta^2_{\text{partial}} = .02$) or Polarity ($F_{(2,6)} = .03; p = .97; \eta^2_{\text{partial}} = .005$) was found. A trend towards significance was shown for the interaction between factors ($F_{(2,6)} = 3.51; p = .063; \eta^2_{\text{partial}} = .37$). Power analyses revealed that sample sizes of 212, 859 and 12 would be required to obtain a significant effect of time, polarity and interaction, respectively. Response rate for both active conditions and individual changes in GABA concentration are shown in Figure 7.

For Glx, no significant main effect of Time ($F_{(2,7)} = .10; p = .78; \eta^2_{\text{partial}} = .02$) or Polarity ($F_{(2,7)} = .50; p = .61; \eta^2_{\text{partial}} = .07$) was found. The interaction was also not significant ($F_{(2,7)} = .30; p = .75; \eta^2_{\text{partial}} = .04$). For mIns, no significant main effect of Time ($F_{(2,7)} = 3.31; p = .11; \eta^2_{\text{partial}} = .32$) or Polarity ($F_{(2,7)} = 1.32; p = .30; \eta^2_{\text{partial}} = .16$) was found. The interaction was also not significant ($F_{(2,7)} = .38; p = .69; \eta^2_{\text{partial}} = .05$). For tNAA, no main effect of Time ($F_{(2,7)} = 3.63; p = .10; \eta^2_{\text{partial}} = .34$) or Polarity ($F_{(2,7)} = 3.08; p = .08; \eta^2_{\text{partial}} = .31$) was found. The interaction was also not significant ($F_{(2,7)} = 2.26; p = .14; \eta^2_{\text{partial}} = .24$). Ratios of Glx over GABA were also computed to measure the interaction between the two metabolites (Figure 8). Change ratios were computed as for previous metabolite measurements. No significant main effect of Time ($F_{(2,7)} = 3.90; p = .10; \eta^2_{\text{partial}} = .36$) or Polarity ($F_{(2,7)} = .004; p = .99; \eta^2_{\text{partial}} = .001$) was found. The interaction was also not significant ($F_{(2,7)} = 2.44; p = .13; \eta^2_{\text{partial}} = .26$).

tCr quantified metabolite concentrations—Prior to tCr scaling, a 3 X 3 repeated measures ANOVA with *time* and *polarity* as factors was computed on raw tCr concentrations to confirm the stability of the reference metabolite. No significant main effect of Time ($F_{(2,7)} = .49; p = .63$) or Polarity ($F_{(2,7)} = .74; p = .49$) was found. The interaction was also not

significant ($F_{(2,7)} = .90$; $p = .48$). Metabolite concentrations were then computed as ratios over tCr for secondary analyses. Results were highly similar to those of water-quantified metabolites. Statistical analyses are presented in Table 3.

Scalp-to-cortex measures—The average scalp-to-cortex distance was 17.36 mm (SD = 2.73 mm), which is comparable to previous studies using the same protocol (Lepage et al., 2011; McConnell et al., 2001). Bivariate Pearson's correlations were performed between scalp-to-M1 distance and absolute percent change (water-scaled) following both active conditions. Bonferroni corrections were applied for multiple comparisons. No significant correlation was found for any metabolite of interest. Given the exploratory nature of this analysis, absolute percent change for all metabolites and all conditions was also computed in a single correlation ($N = 128$) to increase statistical power. The correlation was not significant ($r = .10$, $p = .25$).

Discussion

The present set of experiments investigated the effects of M1-M1 tDCS on corticospinal excitability, as well as the effects of M1-M1 tDCS on sensorimotor cortex metabolism. The two active tDCS interventions (LA/RC and LC/RA) did not significantly modulate left M1 corticospinal excitability compared to sham stimulation. Similar results were obtained with $^1\text{H-MRS}$, where M1-M1 tDCS failed to modulate GABA, Glx, tNAA or mIns concentrations.

Neurophysiological effects of M1-M1 tDCS

The failure of M1-M1 tDCS to modulate corticospinal excitability in the present study is in line with a previous report where identical stimulation parameters were used (O'Shea et al., 2013). In that study, M1-M1 tDCS (1 mA, 20 min, 35 cm² electrode size) resulted in no MEP size difference between LA/RC stimulation and sham tDCS. These results contrast with previous studies that reported “classical” effects of M1-M1 tDCS (increased excitability under the anode and decreased excitability under the cathode) (Kidgell et al., 2013; Mordillo-Mateos et al., 2012; Tazoe et al., 2014). In fact, examination of individual responses of the present sample shows that corticospinal excitability was *reduced* in 50% of the sample following LA/RC stimulation and *increased* in 40% of the sample after LC/RA stimulation. The reduction in MEP amplitude was significant immediately after LA/RC stimulation, as revealed by the GEE analysis, but not different from sham. Although caution should be taken in the interpretation of the present data given small sample sizes and non-significant group effects, they suggest that our sample showed a tendency towards opposite polarity-dependent effects.

A possible explanation for this discrepancy may reside in recently reported non-linear effects of duration and intensity of M1-supraorbital tDCS on corticospinal excitability (Batsikadze et al., 2013; Fricke et al., 2011). A systematic evaluation of M1-M1 stimulation parameter efficacy is needed to determine whether changing stimulation duration or intensity, for example, can reverse polarity-dependent effects or increase efficacy. This has significant importance considering the use of M1-M1 tDCS in clinical populations (e.g. Lindenberg et al. 2013; Sehm et al. 2013; Vines et al. 2008).

In addition, fine changes in the balance between excitation and inhibition within the motor system could account for our contrasting results. It was recently suggested that the excitatory/inhibitory balance differs between individuals and may contribute to inter-individual variability following supraorbital-M1 stimulation (Krause, 2013). Due to strong interactions between primary motor cortices, the effects of M1-M1 tDCS on corticospinal excitability may be more complex than those of M1-supraorbital tDCS. In fact, by stimulating both M1 simultaneously, it is likely that the effects of tDCS not only occur in each M1 separately, but also in the balance of inhibitory/excitatory interactions within and between both areas. For example, Tazoe et al. (2014) found reduced interhemispheric inhibition (IHI) from the left M1 to the right M1 and increased IHI from the right to the left M1 in a LC/RA stimulation protocol. In another study, short-interval intracortical inhibition (SICI) was found to be significantly reduced under the anode following M1-M1 stimulation (Kidgell et al., 2013). Consequently, the absence of MEP modulation following M1-M1 tDCS does not necessarily imply a lack of stimulation effects. In fact, MEP amplitudes may not be the most reliable measure of bi-hemispheric stimulation because of these interactions. Other measures of M1 excitability, such as IHI and SICI, may be more sensitive to M1-M1 stimulation effects than MEPs..

M1-M1 tDCS effect on sensorimotor metabolism

In line with neurophysiological results, M1-M1 tDCS did not significantly modulate metabolite concentrations in sensorimotor cortex. Although this is the first study to report ¹H-MRS-derived metabolite levels following M1-M1 tDCS, previous studies investigating the effects of M1-supraorbital tDCS have reported metabolite concentration modulation following stimulation. Cathodal stimulation has been associated with reduced Glx concentration (Stagg et al., 2009), while no effect was observed in another study (Kim et al., 2014). Anodal stimulation, on the other hand, failed to modulate Glx levels (Kim et al., 2014; Stagg et al., 2009). For GABA, four studies have reported reduced concentrations following anodal stimulation (Bachtiar et al., 2015; Kim et al., 2014; Stagg et al., 2009;2011), which were associated with motor learning (Stagg et al., 2011), whereas cathodal stimulation has been associated with both a reduction (Stagg et al., 2009) and an absence of effect (Kim et al., 2014). Taken together, these studies suggest an involvement of GABA in the physiological effects of M1-supraorbital a-tDCS. In the present study, examination of response rates revealed a tendency towards increased GABA concentration in 45% of participants following LA/RC stimulation, whereas no clear pattern of responders/non-responders emerged following LC/RA tDCS. In line with results from the TMS experiment, these trends are opposite to our hypotheses based on M1-supraorbital studies and further suggest that M1-M1 stimulation may induce more complex effects on excitatory and inhibitory mechanisms within primary motor cortex.

Very few studies have directly compared the effects of uni-hemispheric and bi-hemispheric tDCS protocols. Using TMS, Mordillo-Mateos et al. (2012) found no significant difference in the induced corticospinal excitability changes between the two protocols. Interestingly, Linderberg and collaborators (2013) reported *greater* BOLD activity in bilateral M1 during a reaction time task following M1-M1 tDCS compared to M1-supraorbital tDCS, which may be partly explained by state-dependent effects (Silvanto et al., 2008). As a result, coupling

M1-M1 tDCS with a motor task may possibly prove more effective in producing robust neurophysiological, metabolic and behavioral effects.

In non-motor regions, a recent study using a bilateral prefrontal tDCS montage reported elevations in prefrontal NAA and striatal Glx concentrations during stimulation, which were no longer present following stimulation (Hone-Blanchet et al., 2016). This suggests that modulation of GABA and Glx could have been present during stimulation in the current study. In line with this, a recent study showed a slight decrease of GABA levels during M1-supraorbital anodal tDCS (Bachtiar et al., 2015). Online effects on sensorimotor metabolism remain to be assessed using a M1-M1 montage.

Variability of tDCS effects

A series of recent studies have highlighted the fact that large inter-individual variability in the response to M1-supraorbital tDCS (López-Alonso et al., 2014; Strube et al., 2015; Wiethoff et al., 2014) is one of the main factors driving inconsistent or negative results in the response to M1-supraorbital tDCS, with an average of half of the sample showing the expected changes in M1 cortical excitability. The present study suggests that a M1-M1 stimulation montage does not reduce the inherent variability associated with tDCS.

It is unlikely, however, that variability in ¹H-MRS measures *per se* can explain this result. Indeed, the within-session reproducibility of four MEGA-PRESS acquisitions over the dorso-lateral prefrontal region at 3 T was recently reported (O’Gorman et al., 2011). High reproducibility was found, with low coefficients of variation between the four acquisitions: 0.07 for GABA and 0.06 for Glx. Similar coefficients of variation were observed in the present study for sham tDCS (pre, post1, post2; GABA = 0.09; Glx = 0.03). Coefficients of variation, however, were much more elevated for TMS measures: in the sham condition (pre, post1, post2), the coefficient of variation was 0.25. Intra-individual variation of TMS-induced MEP amplitudes is a well-documented phenomenon (Darling et al., 2006; Kiers et al., 1993; Pitcher et al., 2003), with MEP trial-to-trial coefficients of variation reaching upwards of 0.5 depending on TMS intensity (Darling et al., 2006; Pitcher et al., 2003).

The present data suggest that the above-mentioned inter-individual variability should be taken into account in clinical studies using a bi-hemispheric M1-M1 montage, such as with a population of stroke patients. Further studies are needed to investigate predictors of response to M1-M1 tDCS, such as I-wave recruitment (Hamada et al., 2013; Wiethoff et al., 2014), synchronicity in brain oscillations as revealed by electroencephalography (Ferreri et al., 2014) and sensitivity to TMS (Labruna, 2015) that may help identify responders. Exploratory correlational analyses from the present study suggest that individuals with a lower MEP baseline show a tendency towards higher ratios of change, a result that was also reported by Wiethoff and collaborators (2014). This raises important questions regarding the intensity of TMS stimulation used to determine baseline excitability, which may contribute to inter-subject variability (Vallence et al., 2015). Indeed, the findings suggest that smaller MEPs may be associated with higher response rates. As a result, a 1mV criterion for determining stimulation intensity may be too high for some individuals and lead to ceiling effects and reducing sensitivity. It should however be noted, conversely, that a recent study showed that higher stimulation intensities at baseline (150% of the resting motor threshold)

induce stronger suppression of MEPs following continuous theta burst stimulation (Vallence et al., 2015).

Finally, it is important to note that numerous approaches have been proposed to maximize the effects of tDCS on cortical excitability (e.g. Dmochowski et al. 2013). In clinical populations where interhemispheric balance is compromised, such as the case of stroke, *monopolar* bilateral stimulation could be a viable alternative to bi-hemispheric M1-M1 tDCS. In monopolar bilateral stimulation, M1-supraorbital stimulation is applied to each hemisphere (either anodal or cathodal). This type of stimulation has been used successfully in healthy individuals (Xu et al., 2015) and, in stroke patients, may theoretically be more effective in modulating activity independently in each M1. This is also relevant in healthy individuals, where transcallosal connections may be dynamically involved in updating the excitatory/inhibitory balance between the two hemispheres.

Also, generalization of the present data may be limited by the lack of a double-blind design, as well as the small sample sizes in both studies. Sample size was based on current literature reporting significant MEP and metabolite concentration modulation with an average of 8 and 10 participants for ¹H-MRS and TMS studies, respectively. Power calculations showed that considerable increases in sample size would be required to obtain a significant effect of active vs. sham stimulation for both ¹H-MRS and TMS experiments. In light of the high cost and time-intensive analyses associated with MR spectroscopy, the present data suggest that ¹H-MRS may not be an effective tool to assess tDCS treatment response in clinical studies where eligible patients are limited, such as stroke survivors. As a result, the present study suggests that small exploratory studies of tDCS response and mechanism are probably underpowered to account for the large inter-subject variability associated with tDCS.

Conclusion

The present study suggests a limited impact of M1-M1 tDCS on both TMS and ¹H-MRS measures of neurophysiology and metabolism. These negative results are in line with recent studies showing highly variable inter-individual responses to M1-supraorbital tDCS and therefore suggest that both techniques may lack sensitivity to reliably quantify the neurophysiological and metabolic effects of M1-M1 tDCS. It remains to be determined whether specific behavioral outcomes are more sensitive to the effects of M1-M1 tDCS than TMS and ¹H-MRS alone. Importantly, these findings further highlight the importance of uncovering the factors that underlie inter-individual variability in response to non-invasive brain stimulation protocols. Multimodal studies that combine behavioral outcome with neurophysiological and metabolic measures, that systematically evaluate stimulation parameters effects, and that identify factors predicting outcome are greatly needed to support its use in clinical settings.

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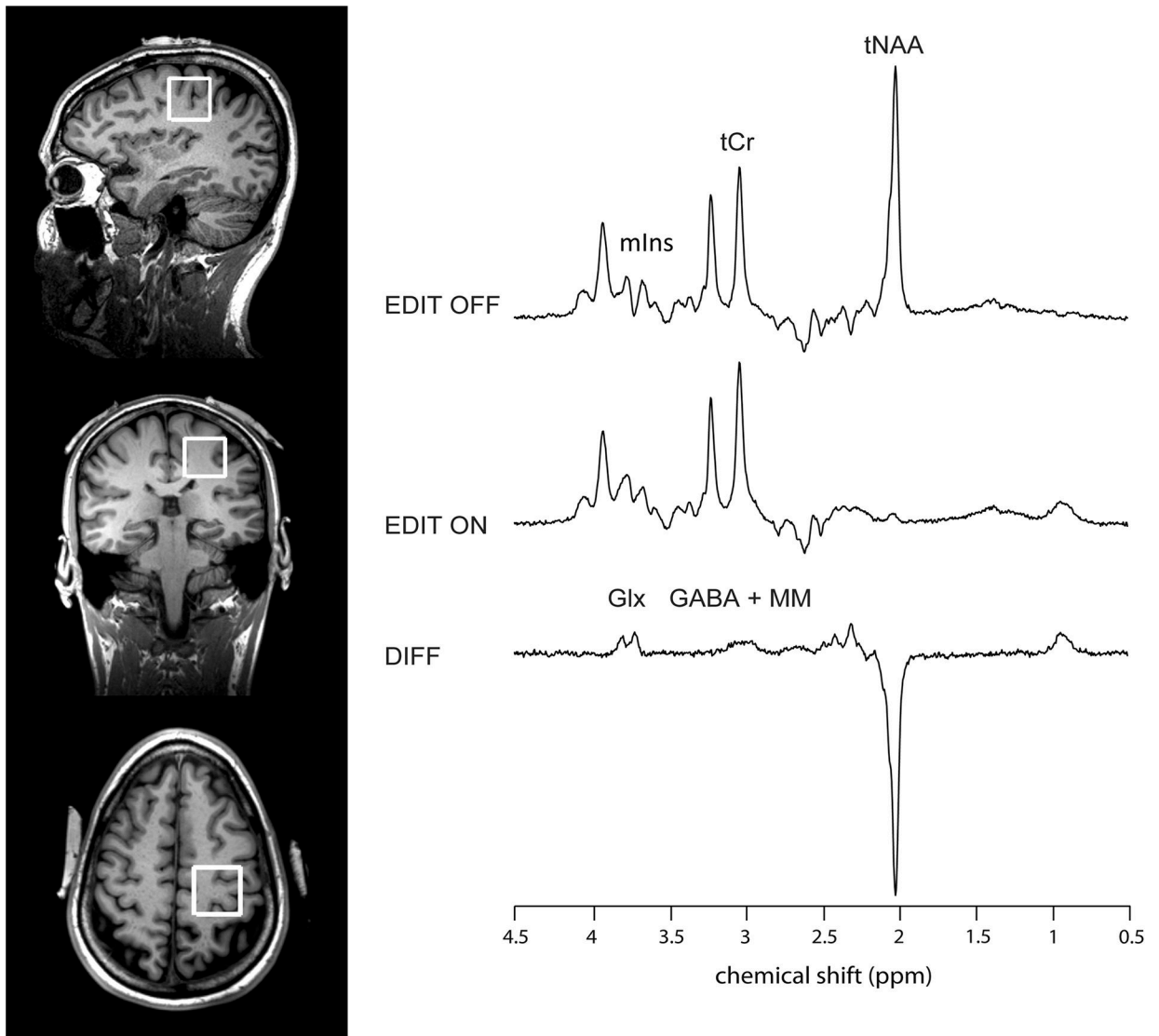


Figure 1. Representative ^1H -MRS spectrum.

Shows placement of the ^1H -MRS voxel over the left sensorimotor region in a single subject, with a representative spectrum obtained with the MEGA-PRESS sequence. tCr = total creatine; tNAA: *N*-acetyl-aspartate + NAAG; Glx: glutamate + glutamine; GABA + MM: γ -aminobutyric acid + macromolecules; mIns: *myo*-inositol.

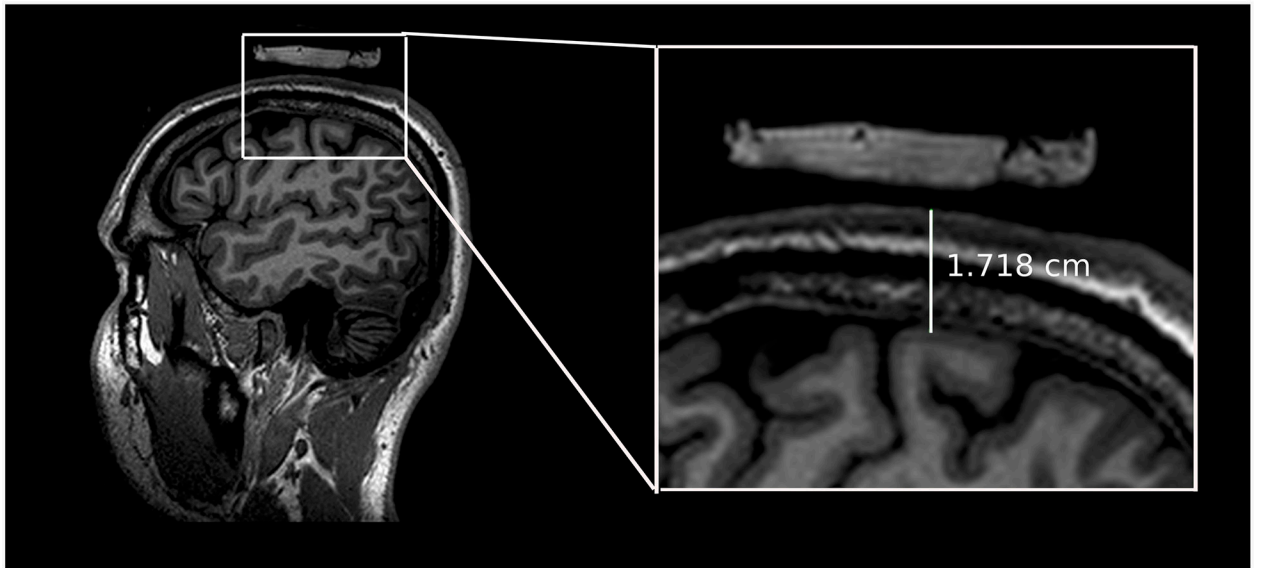


Figure 2. Scalp-to-M1 measurements.

Shows an example of a measurement of the scalp-to-M1 distance for a single participant.

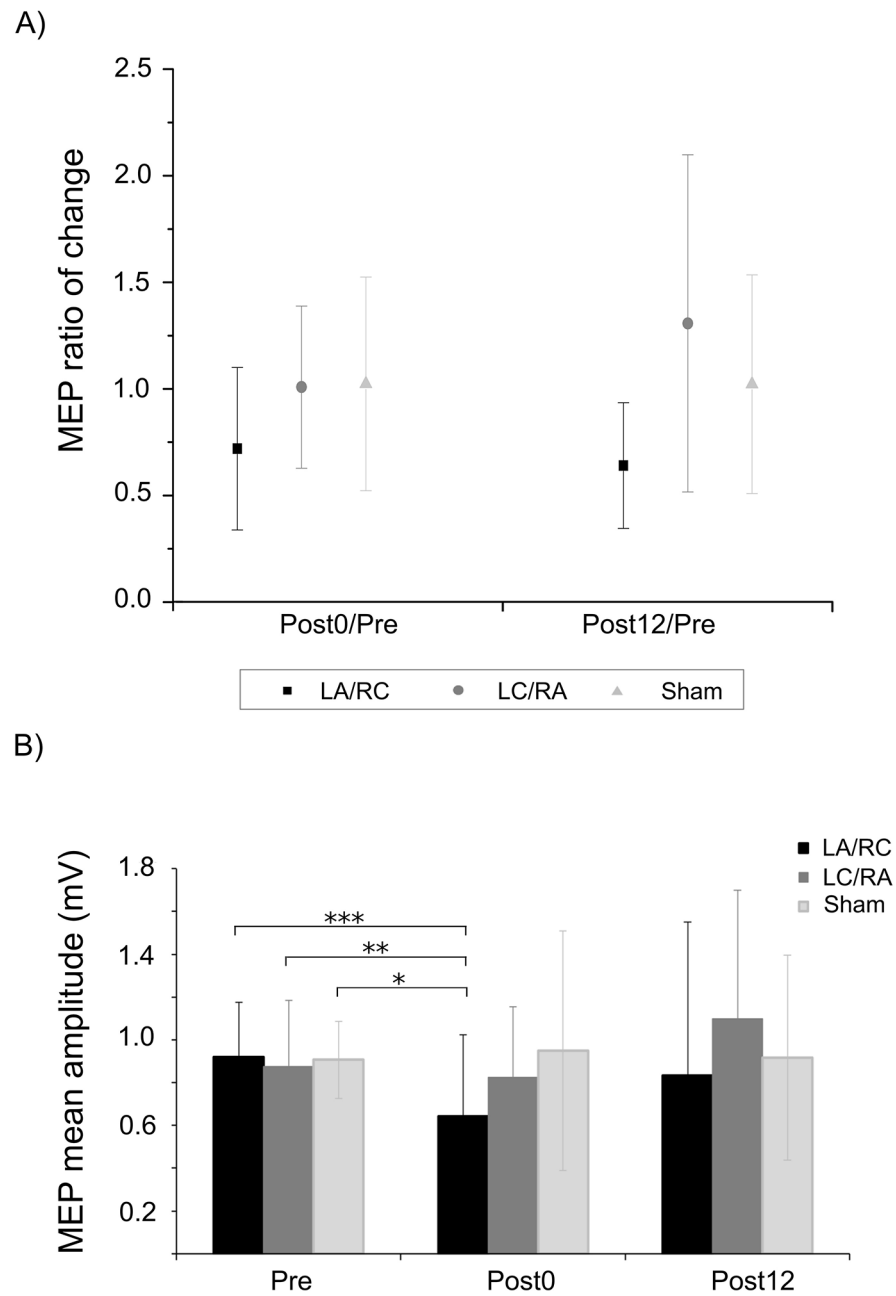


Figure 3. Effects of M1-M1 tDCS on MEPs

A) Shows average change ratio (\pm SD) at the two time-points following the three M1-M1 tDCS conditions. No modulation is observed for both active M1-M1 conditions when compared to sham. B) Shows average raw MEP amplitudes (\pm SD) at the three time points. A significant reduction of MEP amplitude is observed at Post0 following the LA/RC condition when compared to all baseline values. Note that the analysis was conducted on non-averaged MEPs. *** $p < .001$; ** $p < .01$; * $p < .05$

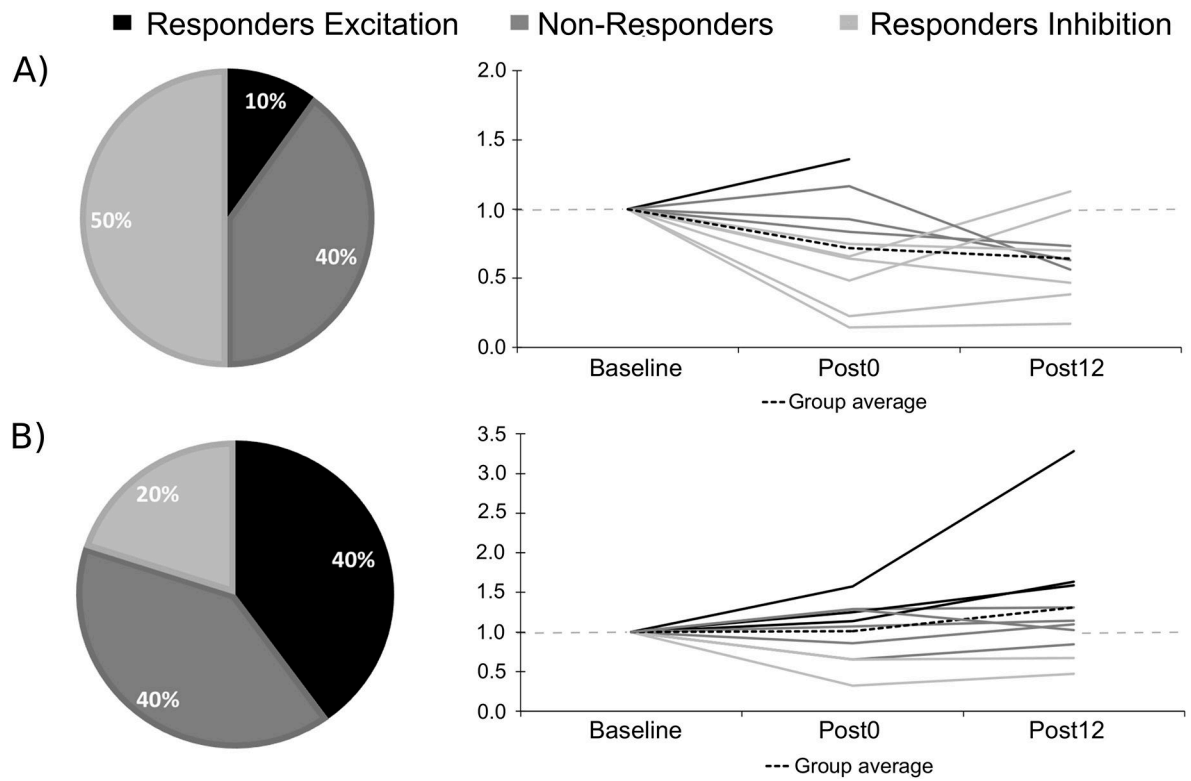


Figure 4. Response rate following the two active tDCS conditions and correlations with baseline MEP values

Shows the response rate for the three clusters and the individual MEP ratios of change for A) LA/RC; B) LC/RA. Note that the dotted grey line indicates a ratio of 1.00 (no change).

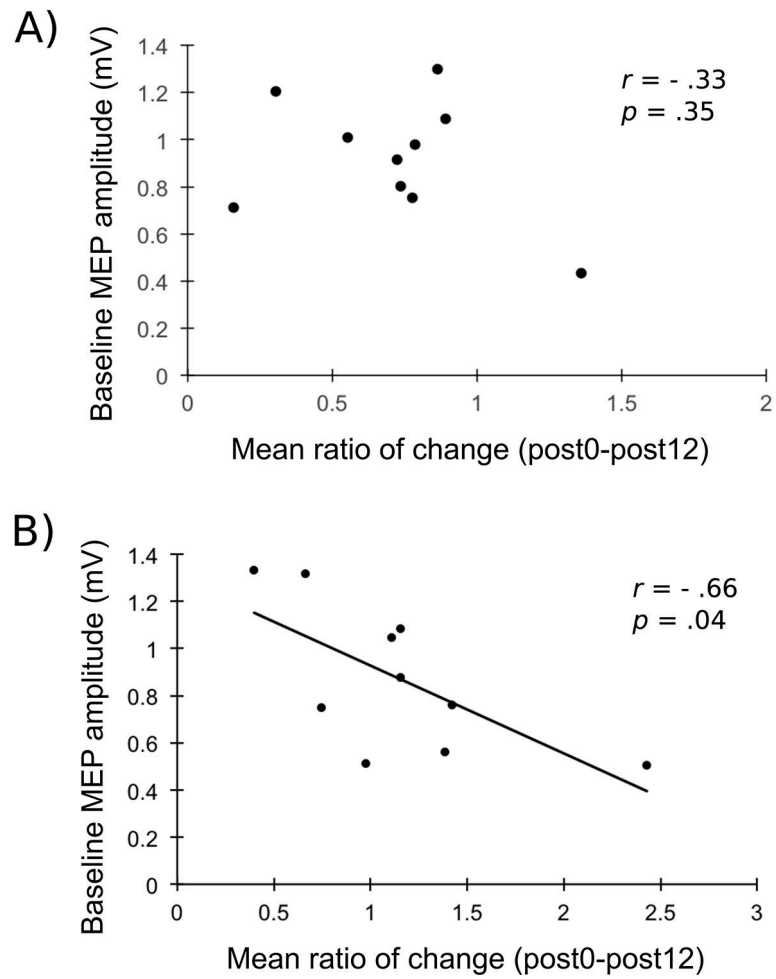


Figure 5. Relationship between baseline MEP values and mean MEP ratio of change Shows the correlation between the raw baseline average MEP amplitudes and the mean ratio of change (Post0 and Post12 averaged) for A) LA/RC; B) LC/RA. A significant relationship is observed for the LA/RC condition.

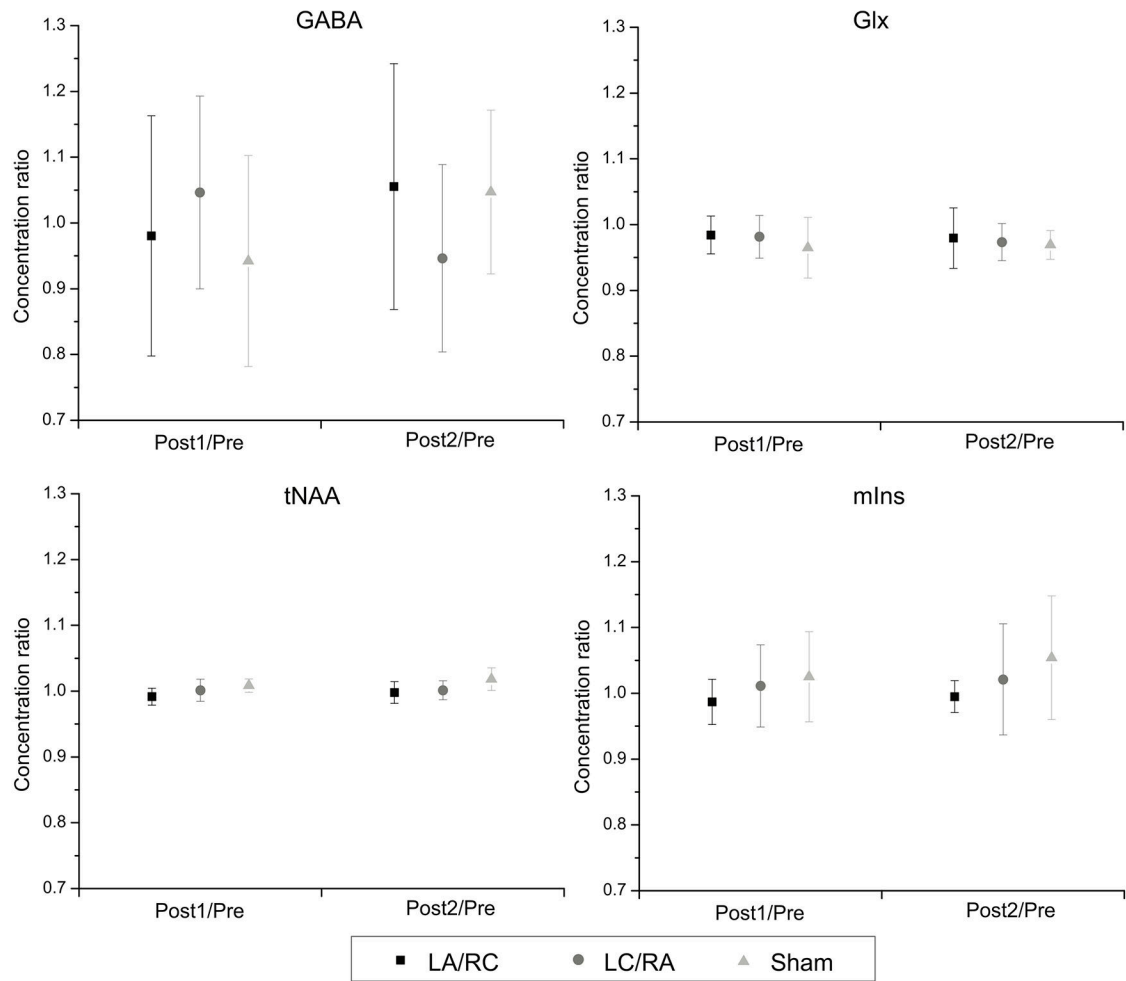


Figure 6. Effects of M1-M1 tDCS on water-quantified concentrations of metabolites
Shows average change ratios (\pm SD) of water-quantified metabolite concentrations between pre-tDCS and post-tDCS measures. No significant modulation is observed for any metabolite concentration ratios at all time points.

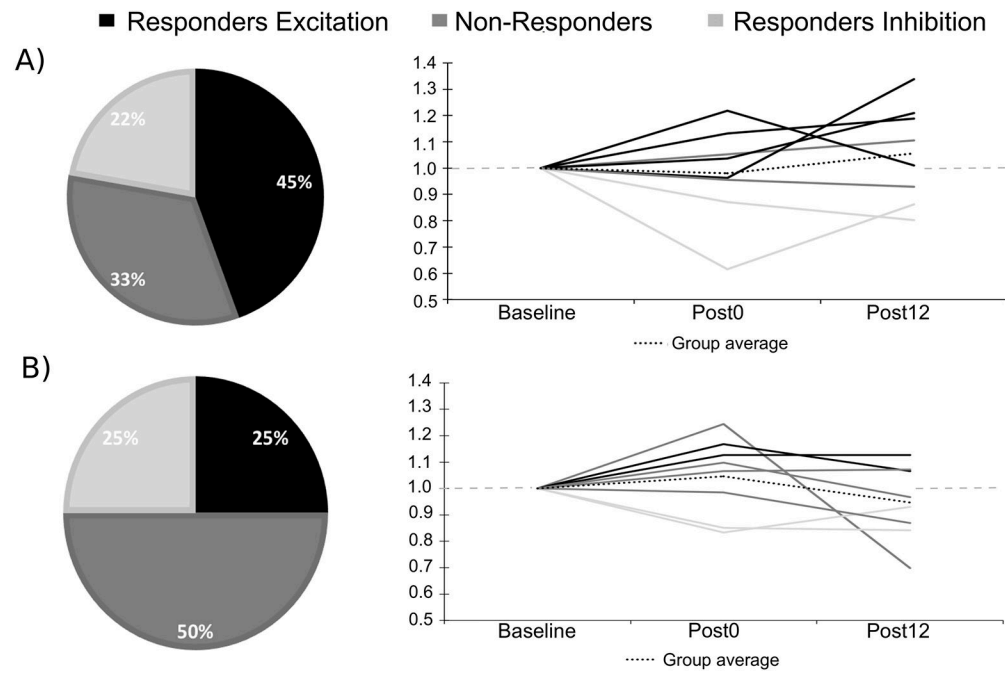


Figure 7. Individual change in GABA concentrations and response rate following the two active tDCS conditions

Shows the response rate for the three clusters and the individual GABA ratios of change for A) LA/RC; B) LC/RA. Note that the dotted grey line indicates a ratio of 1.00 (no change).

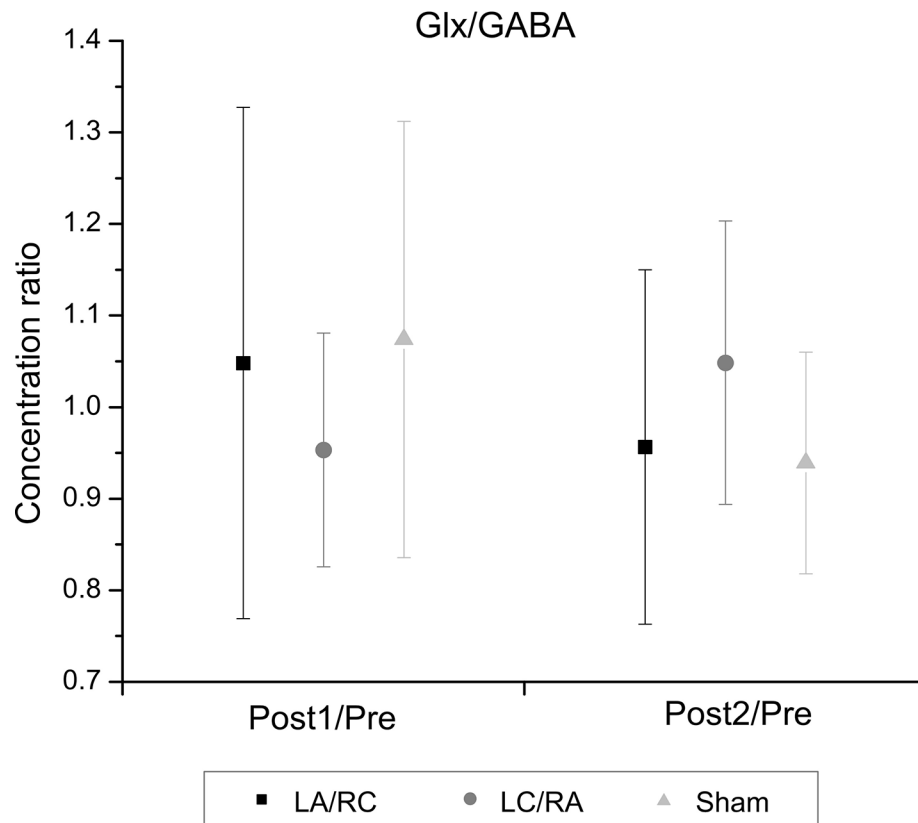


Figure 8. Effects of M1-M1 tDCS on Glx/GABA ratios

Average change ratios (\pm SD) following M1-M1 tDCS for the ratio of water-quantified Glx over GABA levels are shown. No significant change is observed.

Table 1.

Mean latency and amplitude of motor evoked potentials for each condition and time point

| tDCS condition | Pre | | Post0 | | Post12 | |
|----------------|--------------|----------------|--------------|----------------|--------------|----------------|
| | Latency (ms) | Amplitude (mV) | Latency (ms) | Amplitude (mV) | Latency (ms) | Amplitude (mV) |
| LA/RC | 21.78 (1.61) | .91 (.26) | 22.20 (1.29) | .64 (.37) | 22.25 (1.70) | .62 (.30) |
| LC/RA | 22.10 (1.35) | .87 (.31) | 22.67 (1.50) | .82 (.33) | 22.62 (1.78) | 1.09 (.60) |
| Sham | 21.90 (1.33) | .91 (.18) | 22.00 (1.53) | .95 (.56) | 22.40 (1.52) | .92 (.48) |

Data presented as: Mean (SD).

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Table 2.

Percent change in water-quantified concentrations of metabolites following M1-M1 tDCS in 8 participants

| Metabolite | Sham tDCS Percent change (M ± SD) | | LA/RC tDCS Percent change (M ± SD) | | LC/RA tDCS Percent change (M ± SD) | |
|------------|--------------------------------------|---------------|---------------------------------------|---------------|---------------------------------------|--------------|
| | Post1 | Post2 | Post1 | Post2 | Post1 | Post2 |
| | GABA | -5.78 ± 16.04 | 4.71 ± 12.44 | -1.96 ± 18.27 | 5.53 ± 18.67 | 4.65 ± 14.65 |
| Glx | -1.53 ± 8.32 | -3.07 ± 2.17 | -1.56 ± 2.85 | -2.03 ± 4.58 | -1.84 ± 3.26 | -2.64 ± 2.81 |
| tNAA | 0.85 ± 1.01 | 1.82 ± 1.73 | -0.82 ± 1.28 | -0.19 ± 1.65 | 0.14 ± 1.68 | 0.12 ± 1.44 |
| mIns | 2.51 ± 6.84 | 5.40 ± 9.39 | -1.30 ± 3.44 | -0.50 ± 2.42 | 1.12 ± 6.24 | 2.11 ± 8.44 |

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Table 3.

Repeated measures ANOVA for concentration of metabolites quantified using tCr.

| Metabolite | Main effect: Time | | Main effect: Polarity | | Interaction | |
|------------|-------------------|---------|-----------------------|---------|-------------|---------|
| | F value | p value | F value | p value | F value | p value |
| GABA | .16 | .70 | .02 | .98 | 2.89 | .10 |
| Glx | .57 | .47 | .67 | .53 | .07 | .93 |
| tNAA | .20 | .67 | 2.89 | .09 | .05 | .95 |
| mIns | 1.58 | .25 | 1.16 | .34 | .31 | .74 |
| GABA/Glx | 2.85 | .14 | .04 | .96 | 2.35 | .14 |

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