Differences in high-definition transcranial direct current stimulation over the motor hotspot versus the premotor cortex on motor network excitability

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Highlights:

- Transcranial direct current stimulation is a tool to modulate brain excitability
- Targeting the motor hotspot leads to unreliable changes in excitability
- Stimulating the premotor cortex produces more reliable changes
- The strength of the premotor corticospinal tract may predict excitability changes
- Functional definitions of the motor hotspot are anatomically variable

Abstract

The effectiveness of transcranial direct current stimulation (tDCS) over the primary descending motor pathway to modulate motor network excitability is highly variable. The premotor cortex and especially the dorsal premotor cortex (PMd) may be a promising alternative target, as it provides high-level motor control using multiple pathways, including one independent of the primary motor cortex (M1) and one with direct, influencing connections to M1. This double-blind, placebo-controlled study aimed to differentially excite motor and premotor regions using high-definition tDCS (HD-tDCS) with concurrent functional magnetic resonance imaging. HD-tDCS applied over both the motor hotspot and the premotor cortex demonstrated high inter-individual variability. However, HD-tDCS over the premotor cortex led to a slightly greater increase in responders and greater changes in local complexity than HD-tDCS over the motor hotspot or sham. Responsiveness to HD-tDCS over the premotor cortex was also positively correlated with the strength of the corticospinal tract originating from the premotor cortex. An MRI analysis of individual motor hotspot anatomical locations revealed that motor hotspots were often located between the anatomical location of M1 and PMd, rather than directly over anatomical M1. This heterogeneity in the stimulation site, despite using a canonical definition of the motor hotspot, may contribute to the variability of tDCS results. Altogether, these findings provide new considerations for improving tDCS effects on motor excitability.

1.INTRODUCTION

Transcranial direct current stimulation (tDCS) is a noninvasive tool that can modulate cortical excitability in human brain regions (Nitsche and Paulus 2000). The effects of tDCS on upper limb motor rehabilitation have been widely studied, and more than 20 studies have shown promising effects of tDCS applied over the primary descending motor pathway (localized as the motor hotspot and thought to represent primary motor cortex (M1)) on post-stroke motor recovery (Lefebvre and Liew 2017; Shaheiwola et al. 2018). However, a major issue that prevents tDCS from being widely adopted in clinical practice is the high inter-individual variability shown in behavioral or cortical physiological changes following M1 stimulation (Ammann et al. 2017; Lopez-Alonso et al. 2018). The reasons for this inter-individual variability have not been fully elucidated (for a review, see Lefebvre & Liew, 2017).

A potential solution to reduce the inter-individual variability is to use alternative stimulation sites and/or more precise tDCS montages. That is, it is possible that stimulating another entry point into the motor network may lead to more reliable changes following tDCS. To date, the majority of tDCS studies for motor rehabilitation have focused on targeting M1 to alter motor network excitability based on two primary reasons. First, the site for M1 stimulation can be easily personalized to each individual using transcranial magnetic stimulation (TMS) to identify the individual's motor hotspot, which is assumed to represent M1 and which is a functional identification of the primary descending motor pathway. Second, since M1 is the primary region associated with descending motor pathways to the spinal cord (i.e., 50% of the corticospinal tract (CST) neurons originate in M1 (Dum and Strick 1991)), it makes sense to target this region to influence motor behavior. However, given the variable responses in stimulating M1, other stimulation sites that have functional connections with M1 should also be considered.

The premotor cortex is a promising alternative neural target for modulating motor excitability. The premotor cortex and particularly the dorsal premotor cortex (PMd) is the second major origin of the CST with 30% of CST projections (Barbas and Pandya 1987; Dum and Strick 1991). Non-human primates studies have shown that, as a higher motor area (Kantak et al. 2012), PMd contributes to motor control of the upper limb using a downstream pathway that is independent of M1 (Dum and Strick 1991; Etkin et al. 2011; Fujiyama et al. 2013), leading to some high-level control of motor commands (Thura and Cisek 2014). Moreover, neural inputs to the hand and forelimb representation in M1 mostly originate from PMd (Dum and Strick 2005; Burman et al. 2014), creating a cortico-cortical circuitry that has influences on motor network excitability (Stinear et al. 2009; Wymbs and Grafton 2013; Neva et al. 2014; Bestmann and Krakauer 2015). Non-human primates studies have also shown that, where these cortico-cortical connections exist, the onset of the movement-related activity occurs sooner in the premotor cortex than in M1 (Umilta et al. 2007; Thura and Cisek 2014). This suggests that cortico-cortical connections between M1 and premotor cortex are initiated from the premotor cortex (Umilta et al. 2007; Thura and Cisek 2014). As such, the premotor cortex may modulate motor output in two ways: first, through its dedicated CST projections to the spinal cord, and second, through its direct controlling connection to M1. Thus, due to its strong involvement in motor learning (Hardwick et al. 2013), motor selection, movement planning, and preparation (Etkin et al. 2011), and its controlling role over M1 excitability, the premotor cortex provides a promising alternative target for modulating motor network excitability. Stimulating the premotor cortex could also make the motor network more susceptible to cortical excitability changes. Only a few studies have examined the use of tDCS to modulate the premotor cortex, but most of them suggest that targeting the premotor cortex and especially PMd with tDCS could enhance motor performance

and motor learning (for a review, see Buch et al., 2017). However, to our knowledge, there are no studies directly comparing the effects of M1 and PMd-tDCS on motor network excitability.

This lack of direct comparison between M1 and PMd as targets for tDCS is likely due to a major drawback of conventional tDCS montages, which is their poor spatial resolution. The distribution of the electrical current using a standard tDCS montage is relatively diffuse, leading to changes far beyond the stimulated regions (Nitsche et al. 2007). Therefore, trying to target M1 with conventional tDCS likely involves stimulation of at least a part of PMd (Kuo et al. 2013). The main reason for diffuse current densities in conventional tDCS montages is the large inter-electrode distance, which is limited by the size of the conventional electrodes (e.g., 5x5 or 5x7 cm). In contrast, recently introduced high-definition tDCS (HD-tDCS) devices provide the ability to increase stimulation focality (Caparelli-Daquer et al. 2012) by delivering current through a 4x1 ring montage using smaller electrodes, making it easier to differentially target M1 and PMd. Although there is still likely a little overlap in the stimulation, the ring configuration helps to focus the peak stimulation over the targeted region.

We assessed changes in motor network excitability between the functionally-defined motor hotspot, thought to represent M1, and the premotor cortex (canonically defined as 2.5 cm anterior to the motor hotspot, thought to represent PMd) using tDCS. We focused on two primary outcomes: (1) changes in cortical physiology using TMS and (2) changes in motor network connectivity and complexity using resting-state fMRI (rs-fMRI). More specifically, TMS-induced motor evoked potentials (MEP) (Nitsche and Paulus 2000) were used to evaluate cortical excitability locally at the motor hotspot, while rs-fMRI (Lefebvre et al. 2017) was used to measure brain activity pre- and post-stimulation more broadly across the motor network. We used rs-fMRI to estimate functional connectivity (functional connectivity) of the motor network as

well as to calculate multiscale entropy (MSE), which reflects the complexity of regional BOLD signals (Liang et al. 2014; Wang et al. 2018). functional connectivity and MSE are complementary measures of network integrity, and MSE has been shown to reflect the dynamics of neural networks across multiple time scales or temporal frequencies (Heisz et al. 2012), with MSE changes reported following tDCS (Liang et al. 2014; Kang et al. 2018). Since the complexity of neural activity fluctuations is associated with the probability of neural firing (Wang et al. 2018), this makes MSE a particularly relevant measure for assessing excitability changes following anodal tDCS, which is thought to increase the probability of neural firing (Giordano et al. 2017).

The present study harnesses recent methodological innovations to examine concurrent HD-tDCS and fMRI. This technique provides the unique opportunity to not only explore whether stimulation of different areas in the motor network (the motor hotspot versus the premotor cortex) may have different effects on motor network cortical excitability but also to see if there were associated local or network changes underlying these effects, allowing us to more carefully examine mechanisms of plasticity. Using both functional and structural brain imaging, we were also able to examine whether there were any neuroimaging predictors of response to HD-tDCS in any of the groups (Motor Hotspot, Premotor, Sham). Finally, an additional benefit of this concurrent method is the ability to localize the stimulation electrodes in relation to the anatomical primary motor and premotor cortices to assess whether the stimulated region matched the presumed anatomical regions.

In this study, we hypothesized that providing HD-tDCS over the left premotor cortex would modulate motor network excitability more reliably than over the left motor hotspot in healthy individuals, due to the additional motor pathways emerging from PMd. We also examined

whether there were concurrent changes in motor network connectivity and complexity related to tDCS responsiveness in each group using both standard functional connectivity and newer MSE approaches. We hypothesized that, based on previous work, MSE might be more sensitive to tDCS-related changes than standard connectivity methods(Wang et al. 2018). We further explored whether there were any functional or structural neuroimaging predictors of HD-tDCS responsiveness in each group. Finally, as an exploratory analysis, we examined each individual's functionally-defined stimulation location in relation to the targeted anatomical stimulation locations to see whether inter-individual variability in the underlying anatomy of the stimulated locations might relate to HD-tDCS responsiveness.

2. MATERIAL AND METHODS

2.1. Population

The protocol was approved by the University of Southern California Institutional Review Board. All subjects gave written informed consent in accordance with the 1964 Declaration of Helsinki. The inclusion criteria were the following: (1) being a healthy volunteer aged 18 – 50 years, (2) being right-handed. The exclusion criteria were the following: (1) having a pacemaker or other piece of metal in the body, (2) pregnancy, (3) previous history of stroke or other brain damage, (4) previous history or family history of epilepsy, (5) previous history of head injury, (6) previous history of skull damage, (7) previous history of migraines, (8) currently taking any medications that may affect cortical excitability (e.g. benzodiazepines), (9) inability to understand the instructions. Forty-six healthy participants took part in this double-blind, placebo-controlled study (neither the participants nor the primary experimenter knew the nature of the stimulation (e.g., real or sham HD-tDCS)). At inclusion, a first experimenter randomly assigned each participant into one of the following 3 groups: HD-tDCS applied over the left TMS-defined motor hotspot (Motor Hotspot), HD-tDCS applied over the left premotor cortex, which was defined as 2.5 cm anterior to the left motor hotspot, (Premotor) or sham HD-tDCS (Sham, in which case, electrodes were evenly randomized over the left motor hotspot or the left premotor cortex). The second experimenter, who performed the data acquisition and analysis, was only aware of the location of the electrodes but remained blinded to whether participants received real or sham tDCS.

2.2. Study design

The study involved a single three-hour visit which included the following 3 sessions (see Figure 1): (1) a pre-MRI TMS session, including the localization of the left motor hotspot and the left premotor cortex as well as the recording of neurophysiological measurements, (2) a concurrent HD-tDCS/MRI session, including the placement of the HD-tDCS cap, acquisition of structural MRI sequences, a pre-HD-tDCS rs-fMRI scan, the double-blinded HD-tDCS stimulation with concurrent acquisition of rs-fMRI scan, as well as a post-HD-tDCS rs-fMRI scan, and (3) a post-MRI TMS session to measure changes in neurophysiological measurements following HD-tDCS. To our knowledge, this is the first known randomized, double-blinded group study using a concurrent HD-tDCS and fMRI paradigm in humans. The safety of these concurrent designs was established in a pilot study from Gbadeyan and collaborators in 2016 using both phantoms and a single participant (Gbadeyan et al. 2016). They also examined the tolerability of their specific double ring HD-tDCS montage in a behavioral session (without fMRI) in 30 participants.

2.2.1. Pre-MRI TMS session

Briefly, we used single- and paired-pulse transcranial magnetic stimulation (TMS) (Magstim 200² device with BiStim module; Magstim Inc., UK) with a figure-of-eight coil, along with the Brainsight neuronavigation system (Rogue Resolutions Ltd, UK) and surface electrodes to record muscle activity (electromyography, or EMG; Delsys Trigno wireless sensors, Delsys Inc, MA). To localize the motor hotspot, we recorded EMG at the right first dorsal interosseous (FDI). We then recorded pre-HD-tDCS cortical excitability, including each individual's resting motor threshold (RMT) and single-pulse motor-evoked potentials (MEPs) with a test stimulus at the intensity needed to generate an MEP of 0.75mV peak-to-peak amplitude. Notably, this test

stimulus is in accordance with previous studies showing a range of test stimuli between 0.2mV and 4mV (Wagle-Shukla et al. 2009; Schambra et al. 2015a). We also measured short-interval intracortical inhibition (SICI) using a paired-pulse TMS paradigm (see Brain Stimulation Intervention section below for details on the TMS paradigms). MEPs were recording using Signal 6.05 software (Cambridge Electronic Design Ltd., UK).

2.2.2. MRI session

Immediately following the first TMS session, participants were fitted with the HD-tDCS cap and began an MRI session including the acquisition of an anatomical image (T1w MPRAGE), a T2-weighted anatomical MRI, a diffusion-weighted MRI, a 7-minute baseline rs-fMRI (pre-HD-tDCS rs-fMRI), a second 7-minute rs-fMRI during which participants received sham or HD-tDCS applied over the motor hotspot or premotor cortex, depending on their group, and a third 7-minute rs-fMRI to assess the after effects of HD-tDCS (see MRI section below for more details). During the 3 rs-fMRI runs, participants were asked to look at a fixation cross and to not focus on any specific thoughts. The instructions were repeated at the beginning of each rs-fMRI run. Before the second rs-fMRI run, we informed the participants that the HD-tDCS stimulation was about to start, and we checked with them after the ramp-up to see if they were fine with the associated scalp sensations. Before the third rs-fMRI run, we let them know that the HD-tDCS had ended and that it would be the final rs-fMRI run. An additional experimenter, who was unblinded to the stimulation condition (real or sham), turned the tDCS stimulation device on and off. In this way, the primary experimenter and participant remained blinded to the stimulation condition.

2.2.3. Post-MRI TMS session

Immediately following the MRI session, the participants took part in a second TMS session to quantify the HD-tDCS-induced changes on cortical excitability. The cortical excitability measurements of this second TMS session started less than 10 minutes after the end of the third resting state fMRI scan (i.e., time for the participant to leave the MRI, walk to the adjacent TMS experimental room and have the HD-tDCS equipment removed and the TMS coil repositioned on their motor hotspot).

2.3. Brain stimulation intervention

2.3.1. TMS paradigm

We defined the left motor hotspot, using a canonical definition, as the spot at which the maximal MEP peak-to-peak amplitude was recorded in the FDI muscle using a single TMS pulse at a given suprathreshold intensity (starting at 50% of the maximum stimulator output) (Rothwell et al. 1999; Pellicciari et al. 2013; Rroji et al. 2015; Ah Sen et al. 2017; Rich et al. 2017; Rawji et al. 2018). We defined the RMT as the intensity of the stimulator that induced an MEP of at least 50 µV peak-to-peak amplitude in 5 out of 10 trials (Rossini et al. 1994). We defined the test stimulus for the current study as the intensity of the stimulator that induced an MEP of at least 0.75mV ($TS_{0.75mV}$) peak-to-peak amplitude in 5 out of 10 trials (Wagle-Shukla et al. 2009; Schambra et al. 2015a).

After defining these thresholds, we used them in an MEP-SICI paradigm which consisted of 40 trials in a randomized order with a 7s ± 10% inter-trial duration. This paradigm included 20 single-pulse trials using the $TS_{0.75mV}$ (the peak-to-peak amplitude of these trials will be referred to as MEP amplitude), and 20 paired-pulse trials using a conditioning stimulus (CT) at 80% of the RMT 2.5ms before the $TS_{0.75mV}$ (the peak-to-peak amplitude of these trials will be referred to as

MEP amplitude in SICI trials). We anticipated that the SICI trials should result in a decrease of MEP amplitude in SICI trials compared to MEP trials. Therefore, to analyze HD-tDCS effects on intracortical inhibition, we compared the ratio of MEP amplitude divided by MEP amplitude in SICI trials between pre and post HD-tDCS. (For SICI results, see *Inline Supplementary Data 1*).

For 6 participants (2 in each group), we were unable to reach the intensity needed to elicit an MEP of 0.75mV, or even 0.5mV. In these cases, we used the RMT as the test stimulus to acquire MEP amplitude. For one additional participant in the Motor Hotspot group, we were only able to localize the motor hotspot but did not acquire any other neurophysiological measurements due to the participant's reported discomfort. This participant was included only in the resting-state analyses.

2.3.2. HD-tDCS experiment

We used the Soterix MRI-compatible HD-tDCS system (4x1 ring configuration) to deliver tDCS (1mA) for 7 minutes during an fMRI session (see Figure 1). We used 5 small gel-filled electrodes (base diameter: 2.4 cm, gel-skin contact area of ~4.5 cm^2) positioned in adjacent electrode locations within the cap, and we placed the center electrode on the cap over the location of the neural target (as defined below). Each of the 4 cathodal ring electrodes delivered a -0.25mA current leading to a 1mA stimulation over the center anodal electrode.

2.3.3. Localization of the HD-tDCS targets

For the Motor Hotspot group, we positioned the center electrode over the left motor hotspot. The motor hotspot is the brain area that controls the hand's movement and is defined as the spot at which the maximal MEP peak-to-peak amplitude was recorded using TMS in the FDI muscle

(see section 2.3.1). For the Premotor group, we positioned the center electrode 2.5cm anterior to the motor hotspot, which is the standard way to localize the premotor cortex and thought to represent PMd (Gerschlager et al. 2001; Rizzo et al. 2004; Mir et al. 2005; Suppa et al. 2008; Neige et al. 2018). The use of this MRI-compatible device also gave us the unique opportunity to obtain an anatomical MRI with the exact position of the electrodes on the participant's own head. We therefore also report the location of individual stimulation sites across participants in the Results section.

2.4. MRI session acquisition and preprocessing

2.4.1. MRI acquisition

MRI data were acquired on a 3T Prisma MRI scanner (Siemens, Germany) with a 32-channel head coil, using protocols from the Human Connectome Project (HCP) (*http://protocols.humanconnectome.org/CCF/;https://www.humanconnectome.org/storage/app/media/documentation/lifespan-pilot/LSCMRR_3T_printout_2014.08.15.pdf)*. The MRI sequences acquired included the following: T1-weighted MPRAGE scan (208 sagittal slices, 0.8mm thick, TR = 2400ms, TE = 2.22ms, flip angle = 8°, voxel size 0.8 x 0.8 x 0.8mm); T2-weighted turbo spin-echo scan (208 sagittal slices, 0.8mm thick, TR = 3200ms, TE = 523ms, flip angle = 8°, voxel size 0.8 x 0.8 x 0.8mm); diffusion MRI scan (92 slices, 1.5 mm thick, TR = 3230ms, TE = 89.20ms, multi-band factor = 4, flip angle = 78°, voxel size 1.5 x 1.5 x1.5mm, with a gradient protocol with 7 scans at b=0 s/mm2, 47 at b=1500 s/mm2, 46 at b=3000 s/mm2, and a complete repetition with reversed phase encoding in the A-P direction); and three rs-fMRI sequences (7 minutes and 6 seconds covering 520 volumes of 72 slices per scan; TR = 800ms, TE = 37ms, flip angle = 52°, voxel size 2 x 2 x 2mm).

2.4.2. Resting-state fMRI processing

We analyzed the rs-fMRI data using SPM12, the CONN toolbox (functional connectivity toolbox (Whitfield-Gabrieli and Nieto-Castanon 2012)*;* *http://www.nitrc.org/projects/conn*) and the Complexity Toolbox (http://loft-lab.org/index-5.html). The preprocessing steps included slice-timing correction, motion realignment, noise correction using white matter, CSF and motion parameters as regressors, and band-pass filtering (0.01-0.1 Hz). We also performed co-registration between the functional scans and 3D-T1w MPRAGE scans of each participant. Finally, we normalized the functional scans to MNI space and smoothed them using a Gaussian filter of 6mm.

To measure functional changes in the motor network, we compared the functional connectivity between regions of interest (ROI-to-ROI analyses) and analyzed the neural complexity by computing the MSE of the BOLD time series in each ROI (defined below) before and after HD-tDCS.

2.4.2.1. ROI-to-ROI analyses

We defined 4 ROIs as the left M1, right M1, left PMd and right PMd. We used a meta-analysis from Hardwick and collaborators (Hardwick et al. 2013) to define the location of the ROIs in the left hemisphere and then flipped them to the right hemisphere. The exact coordinates of the center of each ROI, which was defined as a sphere with a radius of 10mm, were: left M1 (x = -38, y = -24, z = 56), right M1 (x = 38, y = -24, z = 56), left PMd (x = -26, y = 2, z = 60), right PMd (x = 26, y = 2, z = 60).

In addition, as an exploratory analysis, we also defined an additional ROI to represent the whole motor network, which was defined from a spatial map from an independent component analysis (ICA) on the rs-fMRI data before HD-tDCS was administered (Figure 2). Although we primarily use the main 4 Hardwick ROIs (left and right M1 and PMd) for most analyses, when analyzing the difference between each ROI and the whole network, we use this ROI as the definition of the whole network. Additional methods on the ICA analysis are presented in *Inline Supplementary Methods 1*.

We extracted the cross-correlations of these 4 ROIs (left/right M1, left/right PMd) and ordered them for each group and each condition based on the correlation intensity. To compare the organization of the motor network following each stimulation session, we compared these orders using Spearman's rank correlations coefficient.

We also used RM-ANOVAs (one RM-ANOVA for each ROI as a seed region) in the CONN toolbox to compare combinations of pairs between the 4 ROIs (the 6 functional connectivity pairs were: left M1-right M1, left PMd-right PMd, left M1-left PMd, left M1-right PMd, left PMd-right M1, right M1-right PMd) using "Time" (pre-HD-tDCS, post-HD-tDCS) and "Group" (Motor Hotspot, Premotor and Sham) as factors.

2.4.2.2. Multiscale-entropy

MSE was used to evaluate changes in neural complexity following HD-tDCS in each of the 4 ROIs (left M1, right M1, left PMd, right PMd). The MSE metric quantifies the regularity or complexity of biological signals across a range of coarse-grained timescales or temporal frequencies. Complex systems with $1/f$ power spectra exhibit constant entropy over various timescales (due to their fractal properties), whereas random noise shows a marked decrease in

entropy at longer time scales (as random fluctuations are smoothed out) (Wang et al. 2018). We used a pattern matching threshold (r) of 0.5 and a pattern length (m) of 2 (McDonough and Nashiro 2014a; Wang et al. 2018; Yang et al. 2018). In total, we obtained 20 coarse-sampled scales. Based on the band-pass filter applied, we kept only 9 scales for the analyses (scales 12 to 20: frequency range [1/(TR*scale)]: 0.06-0.1 Hz).

2.5. Structural Correlates of MRI Responsiveness

In addition to measuring HD-tDCS-induced changes in rs-fMRI (connectivity and complexity), we also performed additional analyses to check for any potential anatomical factors that could explain or predict HD-tDCS-induced effects. Therefore, we also measured the strength of the corticospinal tract (using diffusion MR tractography) and the cortical thickness of the ROIs to see if any of these measures influence HD-tDCS effects.

2.5.1. Diffusion MRI

For the diffusion MRI preprocessing, we followed the HCP processing pipeline (*https://github.com/Washington-University/HCPpipelines/tree/master/DiffusionPreprocessing*). Then, we modeled each voxel with a multi-compartment fiber orientation distribution (FOD) approach (Shi and Toga 2017). Finally, we obtained the diffusion tensor fractional anisotropy (FA) values using FSL and completed the deterministic FOD fiber tracking (Cabeen et al. 2016) using the Quantitative Imaging Toolkit (QIT) (Cabeen RP et al. 2018). For each participant, we created three tracts: a global corticospinal tract (CST) in the left hemisphere, a CST section that originated from the left M1, and a CST section that originated from the left PMd (see Figure 3 for an example in one participant). For each participant, we first drew the global left CST from an

ROI covering the left posterior limb of the internal capsule. Following this, we created two different sub-tracts by defining the overlap between the individual's global CST with M1 and PMd template tracts based on 100 subjects, as defined in Archer et al., 2018 (Archer et al. 2018). This resulted in a total CST tract, an M1 tract, and a PMd tract for each individual. Finally, we extracted the FA value of each tract for each individual to perform a correlation between FA and the physiological outcomes.

2.5.2. Cortical thickness analysis

We used FreeSurfer software version 6 (*http://surfer.nmr.mgh.harvard.edu*) to automatically create a 3D model of the cortical surface for cortical thickness measurements on the T1-3D scans of all our participants (Fischl and Dale 2000). We manually inspected the individual surfaces for potential segmentation errors between gray and white matter, and we manually corrected segmentations as needed. We computed cortical thickness measurements on each of the 4 ROIs (left M1, right M1, left PMd and right PMd) for each participant.

2.6. Statistical analyses

We used R (*https://www.r-project.org/about.html*) to perform all the statistical analyses, except for the analyses performed using the CONN toolbox (rs-fMRI functional connectivity). We used the CONN toolbox for ROI-to-ROI analysis, and the results are presented at a qFalse discovery rate = 0.05 (corrected for multiple comparisons).

We performed a repeated measures RM-ANOVAs to compare changes in MEP amplitude across groups, and separate RM-ANOVAS to compare changes in MSE for each of the 4 motor ROIs. We used factors of "Time" (pre-HD-tDCS, post-HD-tDCS) and "Group" (Motor Hotspot,

Premotor and Sham) in all RM-ANOVAs. To evaluate HD-tDCS-induced changes within each group, we also performed a priori analyses of pre/post-HD-tDCS differences in MEP amplitude for each of the 3 groups using two-way paired t-tests. We report the test statistics and p-values, along with the effect size, using partial eta squared ($\square_p^2$) for the ANOVA main effects, and Cohen's d for the t-tests. Any post-hoc tests are corrected for multiple comparisons using the Tukey HSD. In addition, given the large inter-individual variability in the data, we also reported the Bayes Factor (BF) for the group level changes over time in MEP amplitude and MSE analyses using the R package "BayesFactor". The BF is used to report a ratio of the strength of the experimental effect compared to the null hypothesis and has recently been used with highly variable experimental data, including in brain stimulation studies (Apsvalka et al. 2018; Liew et al. 2018). In our data, we are using the anovaBF to determine which model explained the best the variation observed in the results. We tested 3 models: impact of "Time", impact of "Group" and impact of the interaction Group*Time. For each anovaBF, the model with the highest BF value will be the best combination of models among these. The strength of the impact of this model on the data is determined by the size of the BF value. Briefly, a BF of 0-3 should be considered as weak evidence, a BF of 3-10 as substantial evidence, a BF of 10-30 as strong evidence and a BF >30 as very strong evidence (Schonbrodt et al. 2017).

We estimated Pearson's correlation coefficients relating pre-HD-tDCS measurements (rs-fMRI functional connectivity, MSE, FA, cortical thickness) with the MEP changes in each of the Motor Hotspot and Premotor groups to determine which parameters might influence the HD-tDCS effects. We reported correlation p-values that are both uncorrected (p_{uncorr}) and corrected for multiple comparisons (p_{corr}) using the Bonferroni correction for the 4 comparisons (rs-fMRI functional connectivity, MSE, FA, cortical thickness). Finally, we performed two-way

independent samples t-tests to compare each of these 4 measurements between responders and non-responders. For the t-tests, we also reported both p_{uncorr} and p_{corr}. We defined the "responders" subgroup as participants in the Motor Hotspot or Premotor group who showed an increase in MEP amplitude following HD-tDCS [(averaged MEP amplitude over the 20 trials post-tDCS) / (averaged MEP amplitude over the 20 trials pre-tDCS) > 1)]. The "non-responders" subgroup refers to participants in the Motor Hotspot or Premotor group who demonstrated a decrease or no change in MEP amplitude following HD-tDCS [(averaged MEP amplitude over the 20 trials post-tDCS) / (averaged MEP amplitude over the 20 trials pre-tDCS) ≤ 1)]. Although there is a fine boundary between the two, we note that this definition has been consistently used to define responders/non-responders in the literature (Guerra et al. 2017; Lopez-Alonso et al. 2018). Due to the large variability observed in the TMS outcomes, however, we also explored different methods to classify the responders/non-responders. This additional analysis is summarized in *Inline Supplementary Data 2*, *Inline Supplementary Table 1*.

3. RESULTS

3.1. Neurophysiological measurements

3.1.1. Baseline thresholds

We first examined whether there were any differences within the Sham group for individuals with electrodes placed either over the left motor hotspot or the left premotor cortex. Using Student t-tests, we did not find any differences between these two subgroups across any measures, including in RMT ($t(14) = 0.68$, $p = 0.50$), $TS_{0.75mV}$ ($t(12) = 0.57$, $p = 0.58$), MEP amplitude pre-tDCS ($t(14) = 0.05$, $p = 0.96$), or MEP amplitude post-tDCS ($t(14) = 0.29$, $p = 0.78$). Given this, we merged the data in a single Sham group for all of the following analyses (Table 1).

We then checked to see whether the initial cortical excitability between the 3 groups was similar by comparing the pre-HD-tDCS RMT and $TS_{0.75mV}$ across groups (Table 1). Using two separate one-way ANOVAs with "Group" (Motor Hotspot, Premotor group, Sham) as a factor, we found that there was no significant difference between the 3 groups in the stimulator intensity used to reach either the RMT (n = 45, Group effect: $F(2) = 0.50$, $p = 0.61$) or the $TS_{0.75mV}$ threshold (n = 39, Group effect: $F(2) = 0.49$, $p = 0.62$).

3.1.2. Changes in MEP amplitude

3.1.2.1. Inter-individual variability in HD-tDCS response

We then assessed inter-individual variability in response to HD-tDCS between the 3 groups, focusing on the number of individuals showing changes in MEP following HD-tDCS in each group. As noted in the Methods, a 'responder' was characterized as anyone with an increase in MEP amplitude compared to baseline, as per previous research (Guerra et al. 2017; Lopez-Alonso et al. 2018).

We found considerable inter-individual variability in MEP changes following HD-tDCS in the 3 groups (Figure 4). In the Motor Hotspot group, 7 out of 14 (50%) participants showed an increase in MEP amplitude, while in the Premotor group, 11 out of 15 participants (73.3%) showed an increase in MEP amplitude. Importantly, in the Sham group, 5 out of 16 participants (31.25%) showed an increase in MEP amplitude following sham HD-tDCS, suggesting that some individuals will show some level of change in cortical excitability without stimulation. The number of participants with increased MEP amplitude post-HD-tDCS showed a trending difference across the 3 groups (χ^2 (2) = 5.497, $p = 0.06$). Additional, more stringent definitions of 'responders' were also explored (see *Inline Supplementary Data 2*), with generally similar results.

Due to the large inter-individual variability, especially in the Motor Hotspot group, we ran an outlier detection analysis, using the Quantile Range Outliers method to identify any outliers beyond 3 interquartile ranges (calculated on MEP amplitude across the entire cohort). In doing so, we identified and removed one participant with an outlier response in the Motor Hotspot group from further excitability analyses (an increase in MEP amplitude following HD-tDCS of more than 3 interquartile ranges, or 4.2mV; see Figure 4).

3.1.2.2. Group level changes in MEP amplitude

We next evaluated the magnitude of HD-tDCS-induced changes in cortical excitability across the 3 groups by comparing the MEP amplitudes before and after HD-tDCS (Table 1, Figure 5).

A two-way RM-ANOVA was performed on MEP amplitude, with "Time" (pre-HD-tDCS, post-HD-tDCS) and "Group" (Motor Hotspot, Premotor group, Sham) as factors. There were no significant interaction or group effects (RM-ANOVA: n = 44, Interaction Group*Time F(2,41) = 1.88, $p = 0.17$, $\square_p^2 = 0.31$, BF = 0.5 ± 1.29 %; Group: F(2,41) = 0.52, $p = 0.59$, $\square_p^2 = 0.04$, BF = 0.22 ± 0.54%; Time: F(1,41) = 1.83, $p = 0.18$, $\square_p^2 = 0.0030$, BF = 0.48 ± 1.13 %). The BF

analysis confirmed that the model exploring the effect of "Interaction Group*Time" on MEP amplitude is the one explaining the best the results; however, these results are still very weak. We then examined a priori comparisons of pre versus post HD-tDCS for each group. This demonstrated a significant increase in MEP amplitude only following HD-tDCS over the premotor cortex (*Premotor: post > pre-HD-tDCS t(41) = 2.56, p = 0.03, Cohen's d = 0.73;* Motor Hotspot: *post > pre-HD-tDCS t(41) = 0.55, p = 0.58, Cohen's d = 0.20; Sham post > pre-HD-tDCS t(41) = 0.44, p = 0. 66, Cohen's d = 0.12).*

Finally, we examined whether there was a difference in the magnitude of MEP changes within the individuals who responded to HD-tDCS over the motor hotspot versus HD-tDCS over the premotor cortex (i.e., the responders). That is, even though more individuals showed an increased MEP following HD-tDCS in the Premotor group than in the Motor Hotspot group, we wanted to evaluate whether the amplitude of the MEP changes was different between these two groups. Using just responders from the Motor Hotspot group (n=6) and the Premotor group (n=11) groups, a two-way RM-ANOVA with "Time" (pre-tDCS, post-tDCS) and "Group" (Motor Hotspot and Premotor) as factors showed an increase in cortical excitability following HD-tDCS, but no group effect or interaction (RM-ANOVA: n = 17, Interaction Group*Time F(1,27) = 0.13, p = 0.72, $\square_p^2$=0.002, BF = 270.8 ± 7.38 % ; Group: F(1,15) = 2.74, p = *0*.12, $\square_p^2$ =0.12, BF = 0.87 ± 1.76 %; Time: F(1,15) = 26.72, p = 0.0001, $\square_p^2$=0.30, BF = 499.7 ± 1.2 %). The BF analysis confirmed that the model exploring the effect of "Time" on MEP amplitude is the one that best explains the results. Then, we used post-hoc tests to compare MEP amplitude pre versus post HD-tDCS, again just for the responders in each group: Premotor *post* > Premotor *pre-HD-tDCS* t(15) = 4.37, p = *0*.0005, Cohen's d=1.12*;* Motor Hotspot *post* > Motor Hotspot *pre-HD-tDCS* t(15) = 2.72, p = *0*.014, *Cohen's d=2.14.* This demonstrates that when individuals

responded to HD-tDCS, there were no significant differences in the magnitude of MEP amplitude change between Motor Hotspot and Premotor responses. Also, the increase in MEP amplitude for responders was significantly greater than at baseline.

3.1.2.3. Impact of baseline excitability on tDCS-induced MEP improvements

As observed in Figure 5, tDCS effects on MEP amplitude reported in the Premotor group could be due to the fact that the lowest pre-tDCS MEP amplitudes were observed in some participants in this group. This occurred despite the fact that the stimulator intensity was set to induce an MEP of at least 0.75mV for all participants. However, due to intra-individual variability in MEPs, the average MEP amplitude was sometimes higher or lower than the targeted amplitude. Thus, we performed an additional analysis to explore the potential impact of individuals' initial excitability levels on MEP amplitude changes after tDCS. The correlation between pre-MEP amplitude and change in MEP after HD-tDCS over either the motor hotspot or premotor cortex suggests a non-significant trend ($n = 28$, $r = -0.35$, $p = 0.06$). This could be interpreted to suggest that the less excitable the motor cortex is at baseline, the greater the change in MEP amplitude may be after tDCS. Preliminarily, this weak effect seems to be limited to the actual stimulation groups (Motor Hotspot and Premotor). In looking at the six individuals from the Sham with a low baseline MEP amplitude (<0.75mV; Table 1), half show an increase in the post-MEP measurement, while the other half show a decrease. This preliminary result suggests that a low initial MEP may not necessarily be linked to a higher increase in the second MEP, in the absence of an intervention.

3.2. Resting-state fMRI

3.2.1. Resting-state functional connectivity

We then examined whether there were overall changes in the functional organization of the motor network by comparing cross-correlation rankings for each of the ROI-to ROI pairs before versus after HD-tDCS. However, the global organization of the motor network did not change after HD-tDCS in any of the groups, as demonstrated by a strong rank correlation within each group between pre and post-HD-tDCS measurements (Motor Hotspot: n = 15, r = 0.88, $p = 0.03$, Premotor : n = 15, r = 0.88, $p = 0.03$, Sham: n = 16, r = 0.94, $p = 0.02$). In addition, we compared changes in rank correlations between groups using a Fisher's Z test (Diedenhofen and Musch 2015), which also showed no significant differences in correlation ranks between groups (Motor Hotspot > Premotor : z = 0.0, p =1; Motor Hotspot > Sham: z = 0.78, $p = 0.43$, Premotor > Sham: z = 0.78, $p = 0.43$). This suggests that there was no global reorganization of the relative ROI-to-ROI connectivity strengths in the motor network following HD-tDCS over either the motor hotspot or the premotor cortex.

We then examined changes in connectivity for each ROI-to-ROI pair using an ANOVA with Time and Group as factors (n = 46, thresholded at qFDR = 0.05; CONN toolbox). Here we also found no significant changes post-HD-tDCS in any of the 3 groups. Using left PMd as the seed ROI, there were no significant changes in functional connectivity post-HD-tDCS in the Motor Hotspot group ($F(3,41) = 1.47$, $p = 0.67$), the Premotor group ($F(3,41) = 0.18$, $p = 0.91$) or the Sham group ($F(3,41) = 0.34$, $p = 0.94$). Using right PMd as the seed ROI, there were no significant changes in functional connectivity post-HD-tDCS in the Motor Hotspot group ($F(3,41) =1.16$, $p = 0.67$), the Premotor group ($F(3,41) = 0.18$, $p = 0.91$) or the Sham group ($F(3,41) = 0.21$, $p = 0.94$). Using left M1 as the seed ROI, there were no significant changes in functional connectivity post-HD-tDCS in the Motor Hotspot group ($F(3,41) = 0.55$, $p = 0.73$), the Premotor group ($F(3,41) = 0.23$, $p = 0.91$) or the Sham group ($F(3,41) = 0.33$, $p = 0.94$).

Using right M1 as the seed ROI, there were no significant changes in functional connectivity post-HD-tDCS in the Motor Hotspot group (F(3,41) = 0.43, *p = 0*.73), the Premotor group (F(3,41) = 0.39, *p = 0*.91) or the Sham group (F(3,41) = 0.13, *p = 0*.94)

Additional exploratory analyses focusing on ROI-to-ROI comparisons and ICA network comparisons at a less stringent threshold (cluster-level corrected p<0.01) are reported in *Inline Supplementary Data 3 & 4, Inline Supplementary Figures 1 & 2, Inline Supplementary Table 2, and Inline Supplementary Discussion*.

3.2.2. Multi-scale entropy of resting-state fMRI

We then examined HD-tDCS-associated changes in MSE to examine potential changes of motor network excitability (Figure 6).

RM-ANOVAs with "Time" (pre-HD-tDCS, post-HD-tDCS) and "Group" (Motor Hotspot, Premotor group, Sham) as factors were performed for each of the 4 regions of interest (left/right M1, left/right PMd) and demonstrated a significant interaction between group and time and significant main effect of group, with a significant increase in MSE only in the left PMd following HD-tDCS over the premotor cortex (Left PMd RM-ANOVA: n = 46, Interaction Group*Time F(2,43) = 9.06, *p = 0*.0005, $\square_p^2$= 0.14, BF = 498 ± 6.4 %; Group: F(2,43) = 7.5 *p = 0*.002, $\square_p^2$ 0.19, BF = 11.19 ± 0.61 %; Time: F(1,43) = 2.33, *p = 0*.13, $\square_p^2$ = 0.02, BF = 0.59 ± 1.91 %. The BF analysis confirmed that the model exploring the effect of the "Interaction Group*Time" on the left PMd MSE changes is the one that best explains the results. Then, we used post-hoc tests to compare left PMd MSE pre versus post HD-tDCS for each group: *Premotor post > pre-HD-tDCS* t(43) =4.09, *p = 0*.0002, Cohen's d=1.31, *Motor Hotspot post > pre-HD-tDCS t(43) =-1.94, p = 0.06, Cohen's d=-0.61, Sham post > pre-HD-tDCS t(43) = 0.50,*

p = 0.61, Cohen's d= 0.17). There was also a trend towards a decrease in MSE in the left PMd following stimulation over the motor hotspot, but this was not significant.

There was also a significant interaction and main effect of group, with a decrease in MSE only in the Premotor group in the right PMd after HD-tDCS (Right PMd RM-ANOVA: n = 46, Interaction Group*Time F(2,43) =3.89, *p = 0*.03, $\square_p^2$= 0.09, BF= 0.41 ± 2.85 %; Group: F(2,43) = 0.02, *p = 0*.9, $\square_p^2$ = 0.0003, BF= 0.14 ± 1.34 %; Time: F(1,43) =1.14, *p = 0*.29, $\square_p^2$= 0.009, BF= 0.45 ± 1.3 %. The BF analysis suggested that the model exploring the effect of "Time" on the right PMd MSE changes best explained the results, although the results are weak. Then, we used post-hoc tests to compare right PMd MSE pre versus post HD-tDCS for each group: *Premotor post > pre-HD-tDCS* t(43) =-2.43, *p = 0*.02, Cohen's d =-0.78, *Motor Hotspot post > pre-HD-tDCS t(43) =-0.71, p = 0.48, Cohen's d=-0.28, Sham post > pre-HD-tDCS t(43) =1.48, p = 0.14, Cohen's d= 0.60.* There were no significant findings in either left or right M1, and there were no significant correlations between changes in MSE with changes in MEP amplitude in either the Motor Hotspot or Premotor groups.

3.3. Correlation between MEP changes and baseline functional/structural brain measures

We next calculated correlations between the changes in MEP amplitude after HD-tDCS in the Motor Hotspot and Premotor groups with a number of baseline functional connectivity and neuroanatomical measures to determine if any initial functional or structural measurements were predictive of changes in cortical excitability following HD-tDCS. Specifically, we assessed correlations between MEP changes and the following factors: (1) baseline rs-fMRI functional connectivity between M1/PMd and the whole motor network ROI, (2) the initial MSE complexity levels in M1/PMd, (3) the strength of the CST originating from M1 or PMd (as measured by

fractional anisotropy), and (4) the cortical thickness in the M1/PMd. As a reminder, the whole motor network ROI was defined using an ICA spatial map representing the motor network.

In the Motor Hotspot group, MEP changes did not correlate with either initial rs-fMRI functional connectivity between left M1 and the whole motor network (n = 13, r = 0.48, P_{uncorr} = 0.09, P_{corr} = 0.36), initial MSE in left M1 (n = 13, r = 0.18, P_{uncorr} = 0.56, $P_{corr} > 0.9$), FA in the left M1 tract (n = 13, r = 0.29, P_{uncorr} = 0.38, $P_{corr} > 0.9$), or cortical thickness in left M1 (n = 13, r =-0.25, $P_{uncorr} = 0.39$, $P_{corr} > 0.9$).

In the Premotor group, MEP changes showed a positive correlation with the FA in the left PMd tract (n = 15, r = 0.62, t(13) = 2.89, P_{uncorr} = 0.012, $P_{corr} = 0.048$), but it did not correlate with the initial resting-state functional connectivity between the left PMd and the whole motor network (n = 15, r = -0.02, $P_{uncorr} = 0.9$, $P_{corr} > 0.9$), initial MSE in left PMd (n = 15, r = 0.19, P_{uncorr} = 0.49, $P_{corr} > 0.9$), or cortical thickness in left PMd (n = 15, r =-0.13, P_{uncorr} = 0.65, $P_{corr} > 0.9$).

We also evaluated whether any of the above factors could explain the distinction between responders and non-responders, and thus compared them between responders and non-responders in the Motor Hotspot and Premotor groups (for full analyses, see *Inline Supplementary Data 5*). Here, we also only found that FA in the left PMd tract was higher in responders (0.55 ± 0.01) compared to non-responders (0.52 ± 0.003; t(12.73) = 6.09, n = 15, $P_{uncorr} < 0.001$, $P_{corr} < 0.001$).

3.4. Comparison of the stimulation site location

Finally, as we collected both TMS and MRI with tDCS stimulation sites localized on them, we performed an additional exploratory analysis to examine if the individual locations of the left motor hotspot and the left premotor cortex, defined using standard TMS and tDCS brain

stimulation protocols (see *Methods*), matched the functional/anatomical definitions for these regions based on the MNI-normalized MRIs. We used the same ROIs from the Hardwick meta-analysis (Hardwick et al. 2013) to define the standard functional M1 and PMd locations (see Figure 2). The standard functional M1 location matched the anatomical hand knob (Yousry et al. 1997), which is commonly thought to represent the anatomical location of the motor hand area. The standard functional PMd location was within the anatomical PMd boundaries (but due to the large extent of the anatomical PMd the functional ROI only covers a small portion of it). In all the participants, the stimulation ROI was defined by a cortex-projection of the center electrode of the 4*1 ring. We projected the coordinates of the center of this electrode on the MNI-normalized scans to the cortex using an in-house script. This script takes in a coordinate on the scalp as input (e.g., under the central anode-electrode of an HD-tDCS montage) and projects this coordinate onto the nearest point ("P") on the cortex. The distance of each voxel of the cortex from "P" is calculated (in mm), enabling the creation of a spherical ROI centered at "P" (10mm diameter). Here “P” was the considered as the cortex-projection of the middle of the center electrode of the 4*1 HD-tDCS ring. This resulted in each participant with a cortex-projected stimulated region. We quantified the minimal distance of each individual’s cortex-projected stimulated region from the standard M1/PMd ROIs by computing the minimum Hausdorff’s distance (i.e., the minimum distance between the two ROI boundaries) (Fedorov et al. 2008).

In the Motor Hotspot group, the average minimal Hausdorff’s distance between individual’s cortex-projected stimulated region and the standard M1 ROI was 3.79±4.64mm, and with the standard PMd, it was 3.46±4.89mm. In the Premotor group, the average Hausdorff’s distance between the anatomical location of the individual stimulation site and the standard PMd was 4.93±4.80mm, and with the standard M1, it was 15.44±4.78mm.

In terms of the number of individuals with overlap with each ROI, in the Motor Hotspot group, the motor hotspot site overlapped with the standard M1 ROI in 6 participants and with the standard PMd ROI in 6 participants. In the remaining 3 participants, the stimulation site was localized between the standard M1 and PMd ROIs. In the Premotor group, the premotor cortex stimulation site overlapped with the standard PMd ROI in 5 participants and between the standard M1 and PMd ROIs in 2 participants. In the other 8 participants, the anatomical location of the stimulation site was localized a little anteriorly to the standard PMd ROI, but still within the anatomical (atlas-based) extent of PMd. However, some studies have found activation associated with motor performance and motor learning in a more anterior part of the anatomical PMd boundaries (Mayka et al. 2006) compared to Hardwick location (Hardwick et al. 2013). Given the wide variability in the anatomical location of the stimulation site in the Motor Hotspot group, in *Inline Supplementary Data 6,* we performed an exploratory analysis to compare the response of the participants who were assigned to the Motor Hotspot group based on the anatomical location of their stimulation site (*Inline Supplementary Figure 3)*. We re-analyzed the pre-post MEP amplitude changes by reallocating the six individuals in the Motor Hotspot group who received stimulation over the anatomical PMd to the existing Premotor group for a total of 20 individuals. We again found a significant increase in MEP amplitude post-tDCS only in the new anatomical PMd group ($t(19)=2.28$, $p=0.03$), confirming previous results.

Finally, we computed Pearson correlations between the distance from each ROI and MEP changes to see if distance from a specific ROI related to MEP response. This showed only non-significant trends for each group. In the Motor Hotspot group, participants with the greatest MEP changes also tended to have the largest distance between the anatomical location of the stimulation site and the standard PMd ROI ($t(13) = 2.3$, $n = 15$, $r = 0.53$, $p = 0.06$). In contrast, in

the Premotor group, participants with the greatest MEP changes also tended to have the shortest distance between the anatomical location of the stimulation site and the standard PMd ROI ($t(13) = 1.8$, $n = 15$, $r = -0.48$, $p = 0.07$). There were no significant correlations between these distances and changes in MSE post-HD-tDCS in any group. To see a visualization of the overlap of each individual stimulation site with the standard ROIs, see Figure 7. For more information and correlation plots, see *Inline Supplementary Figure 4*.

Overall, this suggests that there is a large inter-individual variability in the left motor hotspot and the premotor cortex anatomical localization using canonical methods (e.g., TMS-defined motor hotspot and 2.5 cm anterior to the motor hotspot). In particular, many individuals in the Motor Hotspot group had stimulation sites that were bordering or overlapping with the standard PMd ROIs, or somewhere between the standard M1 and PMd ROIs. This heterogeneity in the stimulation site could contribute to the variability of responsiveness to HD-tDCS. We, therefore, compared the location of the stimulation sites between responders and non-responders. As shown in Figure 8, more than half of the responders, including participants in both the Motor Hotspot and Premotor groups, received stimulation over the same area inside PMd anatomical boundaries. We found that 10 responders had an overlapping stimulation site within PMd, regardless of group allocation (see *Inline Supplementary Figure 5*), while the anatomical location of the stimulation site in non-responders across groups was more widespread and no stimulation sites were shared by more than 4 participants. This suggests that even if participants responded to tDCS over the left motor hotspot or somewhere between left anatomical M1 and PMd, the highest and most consistent number of responders was observed when stimulation was applied over left PMd. However, there is still large inter-individual variability in the stimulated anatomical location in responders, and some individuals in the non-responder group also were stimulated within PMd,

suggesting that there is likely not a single target across all participants that would effectively increase excitability.

4. DISCUSSION

In this study, we performed a systematic, double-blind investigation using concurrent HD-tDCS and fMRI to examine changes in brain network connectivity and complexity before and after HD-tDCS stimulation of either the left motor hotspot or left premotor cortex. To our knowledge, this is the first known group study using concurrent HD-tDCS and fMRI to compare the left motor hotspot and left premotor cortex stimulation. In addition to concurrent HD-tDCS and fMRI, we examined changes in cortical excitability using TMS before and after HD-tDCS. We also investigated whether any baseline measures of cortical connectivity, complexity or neuroanatomy correlated with response to HD-tDCS in either group. Finally, we explored the inter-individual variability in the stimulation site using canonical localization techniques and reported the distance between anatomical versus expected stimulation sites.

Overall, we observed that HD-tDCS applied over both the left motor hotspot or left premotor cortex, produced highly variable changes across individuals. Due to the large inter-individual variability in response to tDCS, the magnitude of changes in cortical excitability was not significantly different between the 3 groups. However, despite this, we found that HD-tDCS over left premotor cortex produced more slightly reliable increases in cortical excitability than HD-tDCS over the left motor hotspot or sham (i.e., more participants showed increased MEP amplitudes following HD-tDCS over left premotor cortex than over the left motor hotspot or Sham), and we found that MEP amplitude increased significantly after HD-tDCS only in the Premotor group (not in the Motor Hotspot or Sham groups). The slight increase of cortical excitability in the Premotor group was also accompanied by modulations in motor network complexity, observed only after HD-tDCS over premotor cortex and not after HD-tDCS over the motor hotspot or Sham.

We also explored whether any functional or structural brain characteristics could influence responsiveness to tDCS. We observed that the strength of the CST originating from left PMd demonstrated a significant positive correlation with the magnitude of MEP changes following HD-tDCS over the premotor cortex and was significantly different between responders versus non-responders in the Premotor group. In addition, motor cortex excitability slightly predicted the magnitude of the response to HD-tDCS over the motor hotspot or the premotor cortex, such that a less excitable motor cortex at baseline correlated with a greater change in MEP amplitude after tDCS.

Finally, we showed that there was significant variability in the location of the anatomical stimulation sites compared to the expected stimulation sites based on canonical electrode placement – a factor which may introduce great variability into studies of brain stimulation. Specifically, we showed that many participants in the Motor Hotspot group received stimulation over anatomical PMd or between anatomical M1 and PMd. In addition, more than half of the participants who responded to tDCS, across both Motor Hotspot and Premotor groups, received stimulation in the anatomical PMd boundaries, whereas the participants who did not respond to tDCS received stimulation across the motor network with no consistent location. Put another way, stimulation over PMd (regardless of the group assignment) produced the greatest number of individuals with increased MEP amplitude following tDCS.

Altogether, these results suggest that HD-tDCS over the premotor cortex may lead to more reliable changes in cortical excitability and complexity than HD-tDCS applied over the motor hotspot, but also that individual brain structural and functional characteristics, such as corticospinal tract integrity, baseline motor cortex excitability and the location of the functional motor hotspot, likely play a strong role in tDCS responsiveness.

4.1. HD-tDCS applied over premotor cortex may lead to small but more reliable changes in cortical excitability than HD-tDCS applied over the motor hotspot

As previously observed, there was large variability in the MEP measurement even in the Sham group, suggesting that this measure is highly variable over time and across individuals. However, in line with our initial hypothesis, we observed a trend of a slightly more reliable increase of MEP amplitude in the Premotor group, resulting in 75% responders (i.e., participants who demonstrated an increase in MEP amplitude after HD-tDCS) compared to only 50% responders in the Motor Hotspot group (and 31% in the Sham group). Moreover, the comparison between pre and post-tDCS MEP amplitude demonstrated that only the Premotor group showed a slightly significant increase post-tDCS.

However, when examining just the participants who responded to the stimulation, HD-tDCS over both the motor hotspot and the premotor cortex led to significant increases in MEP amplitude after HD-tDCS, with no significant differences in amplitude between the groups. This suggests that changing the entry point into the motor network to the premotor cortex increases the reliability of HD-tDCS effects on cortical excitability by increasing the number of participants who responded to HD-tDCS, but not the amplitude of changes in the responders. Similar to what has been previously suggested (Lopez-Alonso et al. 2018), here we showed that the motor hotspot may not be the best target to consistently enhance cortical excitability, even in healthy volunteers. Moreover, in line with recent studies from stroke rehabilitation, targeting the premotor cortex and especially, PMd may be an effective option to enhance the excitability of the motor network (Cunningham et al. 2015). Notably, this study used a modest sample of 15-16 participants per group and had relatively modest effect sizes; future studies with more participants are needed to

confirm this finding. However, we also observed that higher changes in MEP amplitude following tDCS tend to be related to an initial lower excitability level of the motor cortex. Thus, participants with a less excitable motor cortex may benefit the most from tDCS, suggesting that the magnitude of tDCS effects may be related to an individual's basic cortical excitability at rest. Interestingly, previously work has also shown that responders to anodal tDCS had lower baseline MEPs than non-responders (Lopez-Alonso et al. 2014). However, in this previous study, the baseline MEP characteristic did not show a significant correlation with response using logistic regression. Overall, this finding is preliminary given our limited sample and mixed previous results and should be confirmed with larger samples.

4.2. Network changes following HD-tDCS

4.2.1. Functional connectivity

HD-tDCS over both the motor hotspot and premotor cortex led to no differences in motor network functional connectivity. Although previous work has suggested that tDCS may induce changes in functional connectivity of the motor network (Sehm et al. 2013; Amadi et al. 2014), our analyses showed no significant changes. Our exploratory analyses, at a lower threshold, presented in *Inline Supplementary Data 3 and 4*, *Inline Supplementary Figures 1 and 2, and Inline Supplementary Discussion*, demonstrated some trends towards modifications in functional connectivity occurred after HD-tDCS but these modifications are only significant at lower thresholds. It is possible that the short duration of the HD-tDCS session (7 minutes) and low amplitude of current (1 mA) might not have been sufficient to induce reliable and significantly different changes in the functional connectivity of the motor network, which had been previously observed after 10 to 20 minutes of stimulation (Sehm et al. 2013; Amadi et al. 2014).

4.2.2. Multiscale Entropy

On the other hand, we did find changes in multiscale entropy in left PMd following HD-tDCS over left premotor cortex. MSE, a method for measuring the complexity of finite length time series, quantifies the regularity of a time series by calculating the complexity of a signal at different time scales (Costa et al. 2005). This measure is used as a complexity measure for neuroimaging signals (McDonough and Nashiro 2014b; Smith et al. 2014; Li et al. 2018) and reflects the dynamics of the neural networks (Heisz et al. 2012). Previous work has shown that increasing brain complexity is associated with brain adaptation (Liang et al. 2014; Wang et al. 2018). Importantly, MSE is thought to measure fine-grained changes in brain function following tDCS (Liang et al. 2014; Lafontaine et al. 2016; Kang et al. 2018). Despite a large variability in the baseline MSE measurements, both the RM-ANOVA and the Bayesian Factor analysis suggested that MSE measurement in left PMd presented changes following tDCS that were different across the 3 groups, while the changes in right PMd were relatively weak. However, the post-hoc analyses confirmed that as hypothesized, HD-tDCS over premotor xortex led to significant and more reliable changes in MSE than HD-tDCS over the motor hotspot (i.e., a strong increase of the MSE in the left PMd along with a weak decrease of the MSE in the right PMd). On the other hand, no significant MSE changes were observed after HD-tDCS over the motor hotspot. Given that HD-tDCS over premotor cortex led to more reliable changes in cortical excitability, it is likely this was also captured in the MSE changes found in both hemispheres (i..e., an enhancement of the excitability in the stimulated hemisphere associated with a decrease of the excitability in the second hemisphere (Shin and Pelled 2017; Mondini et al. 2018)). In line with previous studies (Wang et al. 2018), MSE and functional connectivity seemed to be complementary metrics: MSE may be more sensitive and able to capture changes in a node of the

motor network whereas traditional rs-fMRI functional connectivity would capture changes between nodes.

4.3. Fractional anisotropy of the corticospinal tract corresponds to HD-tDCS response when applied over the premotor cortex

Previous research has shown difficulty finding reliable biomarkers that predict responsiveness to HD-tDCS. Examples of proposed but variable biomarkers include neurochemical or genetic variants (Antal et al. 2010; Brunoni et al. 2013; Puri et al. 2015) and EEG-based measures of brain activity (Nuzum et al. 2016). However, recent studies have suggested that functional connectivity (Hordacre et al. 2017), as well as the integrity of the white matter fiber tracts originating from the targeted area (Lin et al. 2017), could predict the response to HD-tDCS. In the present study, none of the measured parameters tended to correlate with the change in MEP amplitude after HD-tDCS over the motor hotspot. On the other hand, in the Premotor group, similar to what was observed previously (Lin et al. 2017), higher FA in the part of the CST that originated from left PMd was related to increased MEP amplitude after HD-tDCS over the premotor cortex. Moreover, stronger FA values in the left PMd-portion of the CST were observed in the participants who responded to HD-tDCS over the premotor cortex. This suggests that an individual's structural integrity of the descending corticospinal tract, as measured by the fractional anisotropy of the corticospinal tract emerging from the stimulated region, may be a predictor of HD-tDCS response. This should be further examined in a large sample.

4.4. Inter-individual variability is observed after tDCS

In the present study, we observed high inter-individual variability in HD-tDCS-induced changes in cortical excitability. This variability is similar to what has been recently reported, with up to 50% of participants showing no response to tDCS (Wiethoff et al. 2014; Lopez-Alonso et al. 2015; Ammann et al. 2017). Here, we found only a modest enhancement of the number of participants who responded to HD-tDCS when applied over premotor cortex. However, when we reallocated individuals according to their anatomical stimulation location, we found a consistent increase in MEP amplitude only for the Premotor group (for those that received HD-tDCS over premotor cortex). We also consistently found the PMd to be the most-stimulated anatomical location in responders. This suggests that stimulating the premotor cortex to modulate the motor network might help reduce inter-individual variability compared to stimulating the motor hotspot, as long as it is actually the PMd that is being stimulated.

The position of the electrodes may also introduce considerable variability. In accordance with the canonical methods for electrode placement, we localized the motor hotspot using TMS and the premotor cortex as 2.5 centimeters anterior to the motor hotspot (Cunningham et al. 2015). As displayed in Figure 7, despite using this established localization method, the position of the stimulated area was highly variable across individuals when compared with their expected anatomical location. Specifically, in the Motor Hotspot group, in more than half of the participants, the anatomical location of the stimulation did not overlap with the standard anatomical or functional M1 location (Hardwick et al. 2013). In contrast, as the premotor cortex was slightly wider and defined anteriorly to the motor hotspot, more individuals fell within the PMd ROI definition or within its anatomical boundaries (slightly anterior to the PMd ROI). Therefore, it seems that the one-size-fits-all method to localize the target area may not be effective in everyone. It also suggests that the motor hotspot, which is thought to reflect the brain

area that controls the FDI muscle in our case, may not always be localized at the anatomical M1 location or the hand knob, but may bleed into the adjacent premotor cortex, as previously observed (Ahdab et al. 2016). This is also supported by studies with non-human primates showing that both M1 and the adjacent PMd are involved in maps of motor control, without any clear functional boundaries between the regions (Godschalk et al. 1995; Graziano et al. 2002a; Graziano et al. 2002b). This suggests that the motor hotspot localization using TMS cannot be assumed to be M1 (hand knob) but may also be a location within the premotor cortex. Moving forward, these data suggest that researchers should keep track of where they are stimulating and report where they ultimately find the motor hotspot instead of assuming it is always over the anatomical M1. This would help to show the variability in hotspot locations across individuals. Moreover, this also suggests that there is an individualized brain organization which, similar to fingerprints, may be slightly different across individuals.

Also, since the premotor cortex stimulation site location was dependent on the location of the motor hotspot, this lead to variation in the anatomical location of the premotor cortex stimulation site as well. In our study, the anatomical location of the premotor cortex stimulation site did not always overlap with the functional PMd location (as obtained from the Hardwick meta-analysis (Hardwick et al. 2013)) but it still overlapped with the anatomical PMd boundaries. In future work, if it is possible to first obtain an MRI, it may be advisable to use neuroanatomical constraints on the motor hotspot localization using the individual's MRI and neuronavigation-guided TMS to find the spot within the anatomical area that provides the strongest response. This might help to reduce inter-individual variability in experimental parameters. In the practical constraint that an MRI cannot be obtained prior to TMS, registering all participants to a template brain to guide and record motor hotspot location may be an alternative solution to at least note

whether there is a common stimulation site for responders.

Finally, our results suggest there is no single brain area that would ensure a positive response to tDCS across all participants. Although more than half of the participants who did respond to tDCS received stimulation over an overlapping PMd spot, for the other half, the stimulation site was somewhere between the anatomical M1 and PMd. Moreover, in the participants who did not respond to tDCS, the stimulation site was all over the motor network, including in PMd, with no strong overlap across participants. Again, this suggest that more than the absolute location, differences in brain structure and function (Valizadeh et al. 2018) should be taken into account to determine the responsiveness of each individual to tDCS. Future studies should determine which stimulation parameters (neural target, nature of stimulation, duration, etc.,) will be the most effective to induce changes in motor network excitability and function at the individual level instead of following a one-size-fits-all method.

4.5. Limitations and future directions

First, although the size of our groups (n=15-16 per group) is relatively large for studies looking at cortical excitability following brain stimulation, these groups are still relatively small, especially given the large variability within each group. Replicating these results in a larger sample would be useful. A second limitation is that the current Sham group included half motor hotspot and half the premotor cortex electrode positions, which showed no significant differences with one another, and which we therefore combined into a single Sham group. However, to provide proper control for each experimental group, a matched full Sham group for each experimental group should be used in the future. Third, in the present study, we used a short 7-minute stimulation duration at 1 mA current, which may not have been enough to induce a strong

effect in cortical excitability or network changes. However, as noted previously, this is in line with previous studies showing effects of 5-7 minutes of anodal tDCS on cortex excitability (for up to 30 min after the end of the stimulation) {Fricke, 2011 #387}{Uy, 2003 #388}{Brunoni, 2016 #389}. In addition, this study demonstrated that stimulating a brain region other than the motor hotspot (in this case, the premotor cortex) even for a short period can lead to an enhancement of cortical excitability and complexity, suggesting it was strong enough to elicit some changes. However, future work may examine whole-brain network changes following longer durations or higher intensities of HD-tDCS. In addition, as the MRI-compatible tDCS, when turned on, introduced noise into the BOLD signal with some undesirable, hard to remove, artifacts, even in a cadaver (Antal et al. 2014), we were not able to analyze rs-fMRI data during brain stimulation. However, it would be very useful in future research to examine also BOLD signal changes during brain stimulation. Fourth, individual anatomy could have influenced the tDCS observed effect, as our anatomical findings suggested. Future studies that more carefully target stimulation based on individual anatomy would help address this issue. Fifth, we used a parallel study design, as opposed to a cross-over design. Each participant received HD-tDCS over only one neural target. Consequently, we cannot affirm that the participants who responded to HD-tDCS over premotor cortex would have not also responded to HD-tDCS over the motor hotspot, or vice-versa. Future studies involving a larger sample size and a cross-over design are needed to answer this specific question. Sixth, one could argue that alertness or drowsiness could have happened during the rs-fMRI runs and that could have impacted both the rs-fMRI connectivity and the excitability measurements. Even if we did not measure alertness and drowsiness between the runs, we did communicate with the participants between the scans. Moreover, at the end of the study, we did ask the participants about their sensations and effects

that both the tDCS and MRI had on them. None of the participants reported alertness or drowsiness during the rs-fMRI runs. Seventh, the present study did not include any electrical field modelling. However, in future studies, the impact of the anatomical location of the stimulation area in the non-normalized brain of each individual could be used to explore the deeper impacts of the anatomical stimulation location on motor network excitability. Finally, in our study, the main outcome (MEP amplitude over time as measured with TMS) suffers from both large intra- and inter-individual variability. Previous reports (Schambra et al. 2015b) suggested that this variability was diminished at the group-level, suggesting TMS measures may be best suited for detecting group-level changes. Thus, due to the high variability in the TMS outcomes, the definition of "responders" as those with any increase in MEP amplitude compared to the baseline measurement could be criticized as being part of measurement noise. Accordingly, we tested several other thresholds for "responders" that required more stringent thresholds (see *Inline Supplementary Data 2*). Based on these additional methods, we demonstrated that the responders/non-responders distinction was generally reliable across the different methods, especially for the Premotor group (which had consistently more responders in it than the other groups). This confirmed the accuracy of the method we choose and also confirmed that changes observed in the Premotor group were more reliable than in the Motor Hotspot group.

5. Conclusion

To our knowledge, this is the first large study employing HD-tDCS with fMRI in a double-blind design with multiple stimulation sites. Using this design, we investigated the neurophysiological effects of tDCS on the motor network immediately after stimulation. We compared HD-tDCS over the motor hotspot versus the premotor cortex and showed that the premotor cortex and especially PMd may be a potential alternative neural target to modulate

motor network excitability and neural complexity with less variability that what was observed following HD-tDCS over the motor hotspot. These results also suggest that multiscale entropy may be a sensitive measure of changes in neuronal excitability following noninvasive brain stimulation within a single region and may be able to detect changes not detected by more standard functional connectivity measures. We further showed that response to HD-tDCS over the premotor cortex may be predicted by the integrity of the cortical spinal tract originating from the targeted brain area. Finally, we demonstrate that conventional methods for defining tDCS targets may be influenced by large inter-individual variability in the anatomy of the motor hotspot location. Future work should carefully consider and account for the anatomical, versus assumed, stimulated location. Altogether, these results open new considerations for improving the efficacy of tDCS on modulating the motor network and suggest that individualized targeting based on each individual's brain structure and function may result in more reliable excitability changes.

Data availability

The unthresholded statistical maps used to generate the motor network (i.e., ICA analyses) are stored on the NEUROVAULT repository (https://neurovault.org/collections/UTZRKARR/). The raw data supporting these analyses will be made available following reasonable requests to the corresponding author. These data are not publicly available due to IRB restrictions.

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Author contributions

S-L L and S.L. conceived and designed the study with input from D.J.J.W., K.J., and N.S.,

S.L., M.J. K.I., A.S. acquired the data,

S.L. M.J., K.J., Y.Q., R.C., Y.S., and S.L.L. contributed to the data analyses and interpretation,

S.L. and S-L.L. wrote the paper with contributions from all the authors,

All the authors agreed with the submitted version or the paper.

Competing interests

The authors declare no competing interests.

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Figures

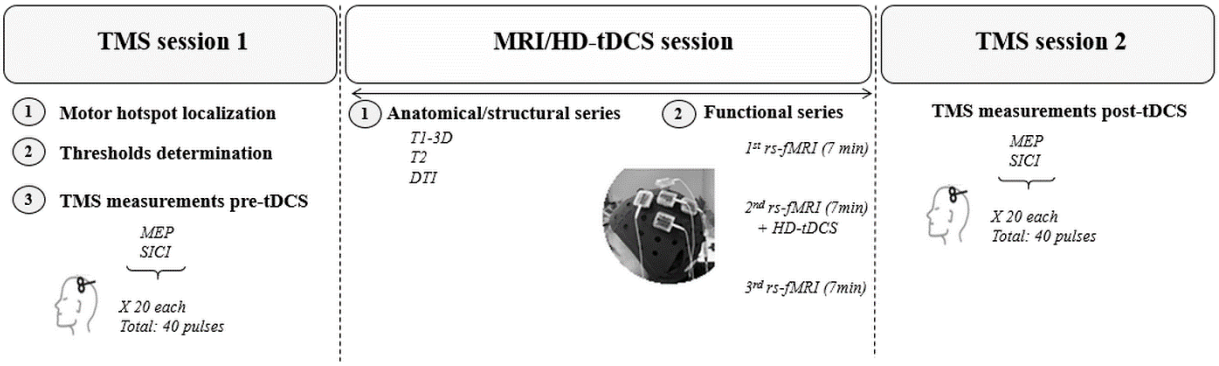

Figure 1: Study design. Each participant will take place in 3 sessions as follows: a first TMS session, then an MRI/HD-tDCS session, and finally a second TMS session. *HD-tDCS: high-definition transcranial direct current stimulation, MEP: Motor Evoked Potential, RMT: Resting Motor Threshold, rs-fMRI: resting-state functional magnetic resonance imaging*

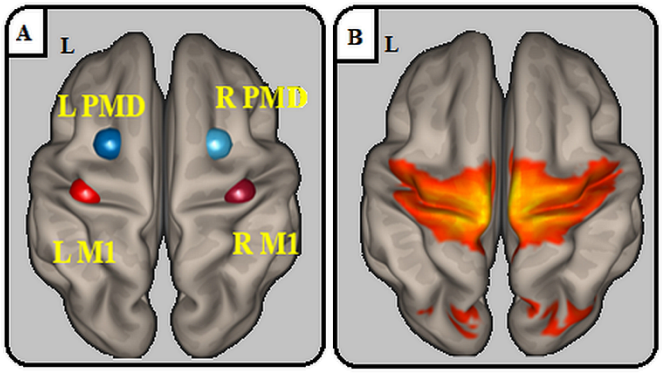

Figure 2: Location of the regions of interest. Panel A: Location of the 4 ROIs based on the meta-analysis from Hardwick and collaborators[(Hardwick et al. 2013)]. Each ROI was defined as a sphere with a radius of 10mm around the reported peak coordinates for each region. These were: left M1 (x = -38, y = -24, z = 56), right M1 (x = 38, y = -24, z = 56), left PMd (x = -26, y = 2, z = 60), right PMd (x = 26, y = 2, z = 60). Panel B: The whole motor network component as generated from the pre-HD-tDCS spatial map from the ICA analysis. *L: left, M1: primary motor area, PMd: dorsal premotor cortex, R: right, ROI: region of interest*

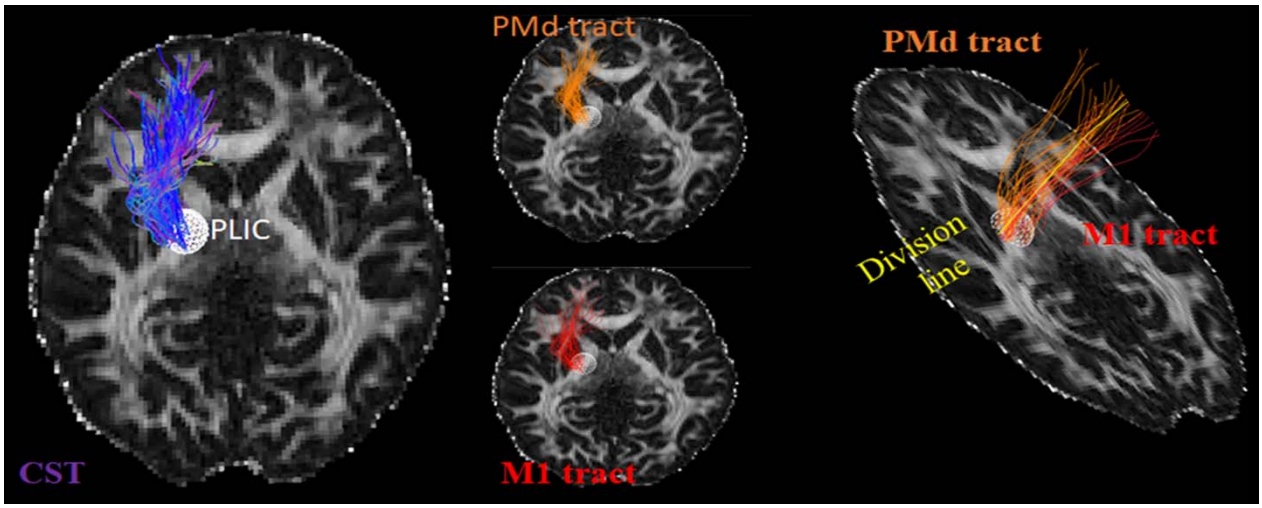

Figure 3: Example of the DTI tracts in one participant. Left panel: After positioning the PLIC ROI, we extracted the CST of the participant. Middle panel: By overlapping the CST obtained and the M1 or PMd template tract, we obtained the M1 and PMd tract for each participant. Right panel: Superposition of the M1 and PMd tracts for one participant. Note that the M1 tract is localized at the posterior part of the CST whereas the PMd tract is more anterior. The yellow division line is shown to visualize the separation between the two tracts. *PLIC: posterior limb of the internal capsule, CST: corticospinal tract, M1: primary motor area, PMd: dorsal premotor cortex*

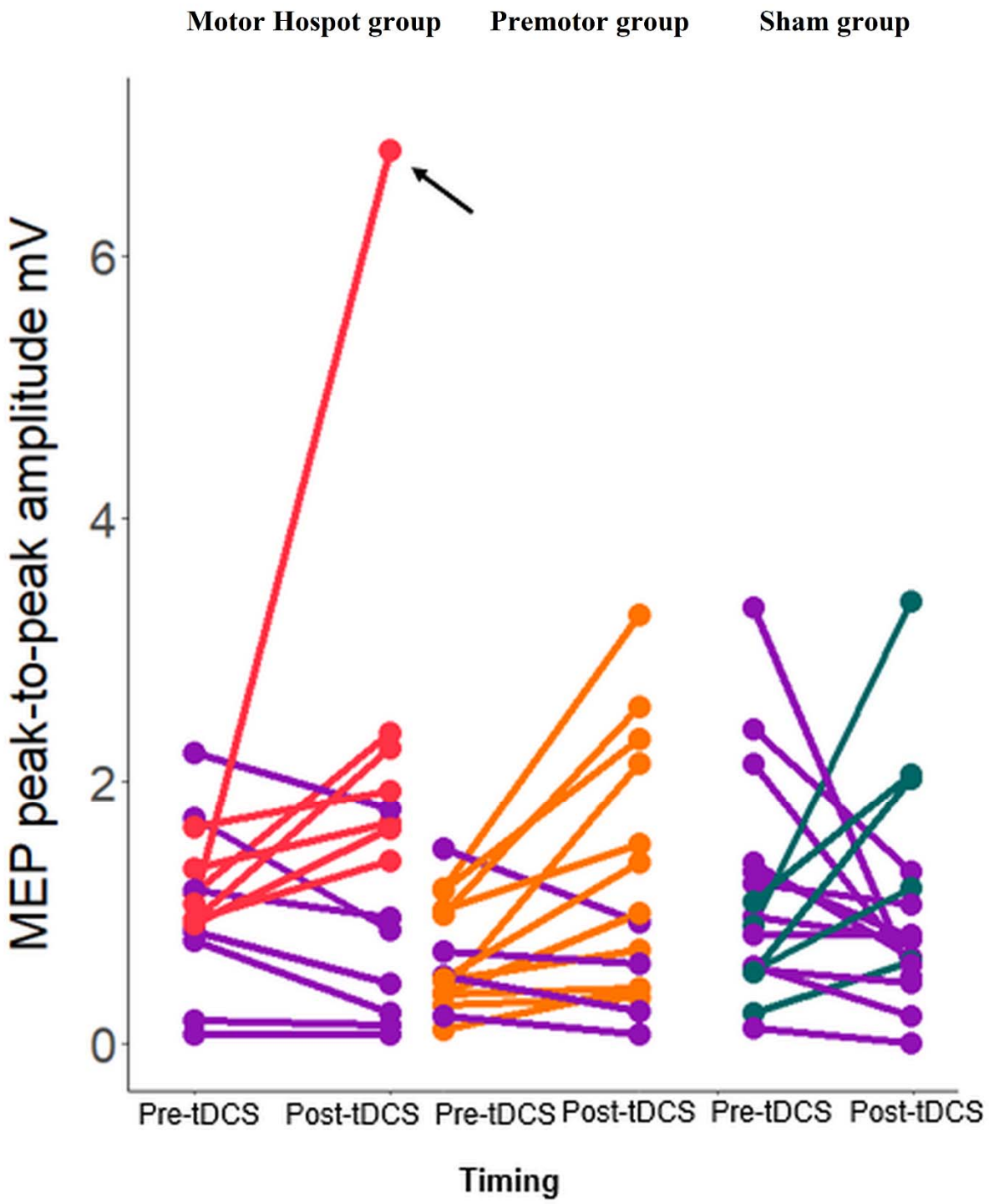

Figure 4: Individual changes in MEP amplitude over time. The dot pairs reflect MEP amplitude pre and post-tDCS for each participant in each of the 3 groups. Participants who showed a decrease in MEP between pre and post-tDCS are displayed in purple. *In the Motor

Hotspot group, one participant presented a very strong effect post-tDCS. After running an outlier detection, this participant was removed from the rest of the analyses (indicated by the black arrow). *M1: primary motor area, MEP: motor evoked potential, mV: millivolts, PMd: dorsal premotor cortex, tDCS: transcranial direct current stimulation.*

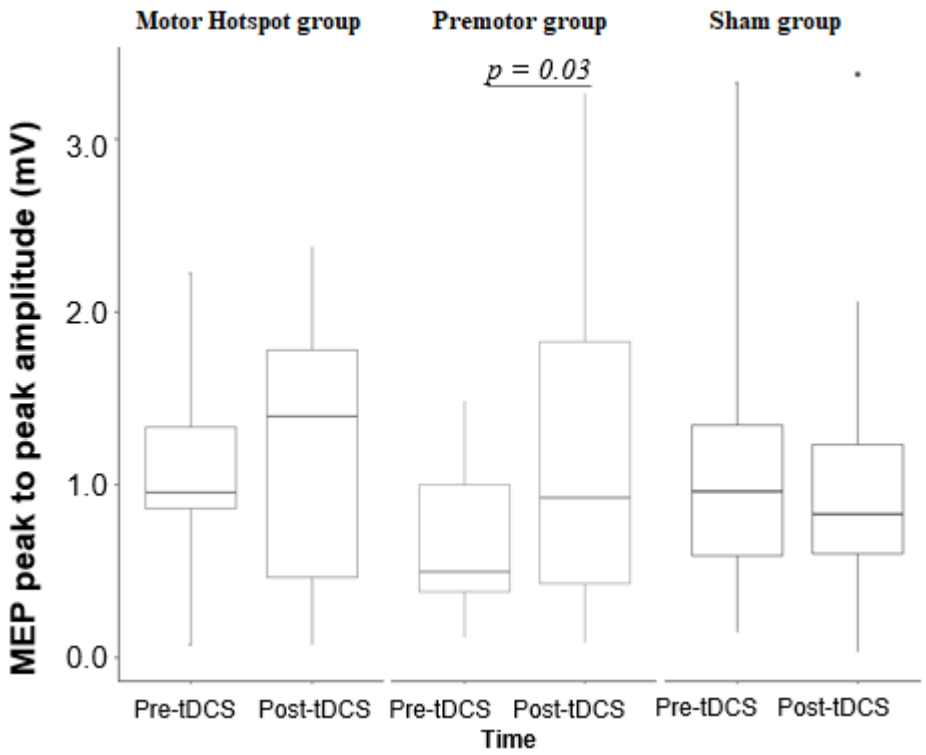

Figure 5: Changes in MEP amplitude for each group across time. Box-and-whisker plots of MEP amplitude per group over time (pre/post-tDCS). The center line represents the median value, the lower bound of the box represents the 25th percentile, the upper bound of the box the 75th percentile, and the whiskers represent 3 times the interquartile range. Any outliers are indicated by dots. Note that the only statistically significant difference is observed in the Premotor group reflecting an increase of MEP amplitude post-tDCS compared to pre-tDCS. The p-values refer to the

statistically significant post-hoc contrasts. *M1: primary motor area, MEP: motor evoked potential, mV: millivolts; PMd: dorsal premotor cortex, tDCS: transcranial direct current stimulation.*

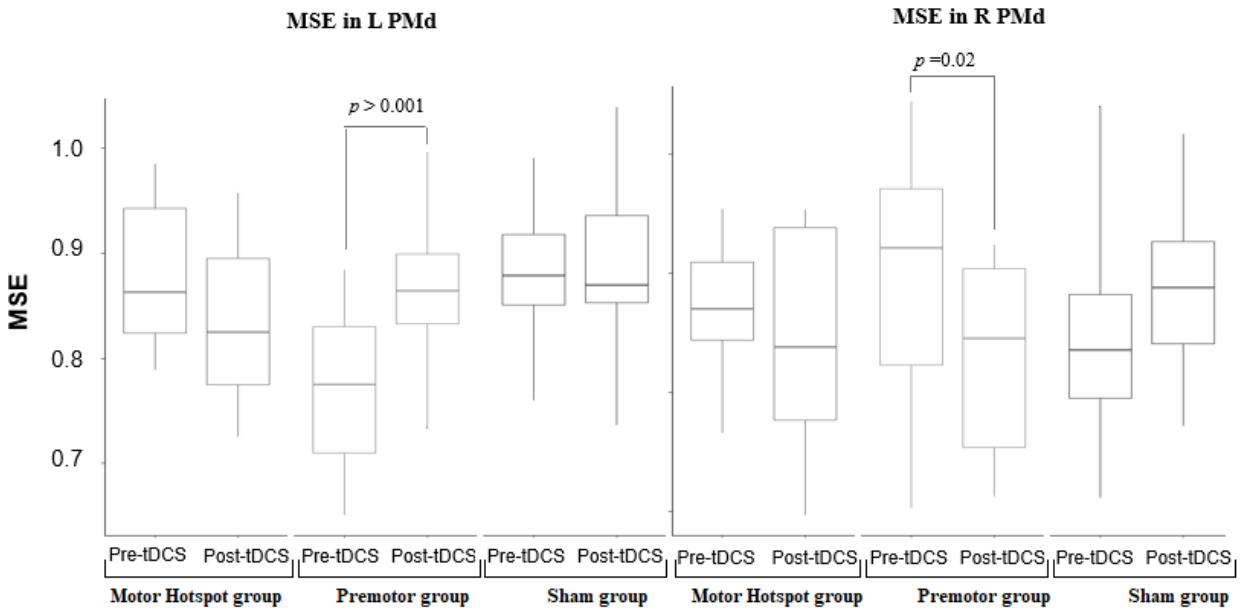

Figure 6: Changes in MSE across groups. Box-and-whisker plots of the MSE per group over time (pre/post-tDCS) in the left PMd ROI (in the left panel) and in the right PMd ROI (in the right panel). The center line represents the median value, the lower bound of the box represents the 25th percentile, the upper bound of the box the 75th percentile, and the whiskers represent 3 times the interquartile range. Any outliers are indicated by dots. The p-values refer to the statistically significant post-hoc contrasts. *L: left, M1: primary motor area, MSE: multiscale entropy, PMd: dorsal premotor cortex, R: right, tDCS: transcranial direct current stimulation.*

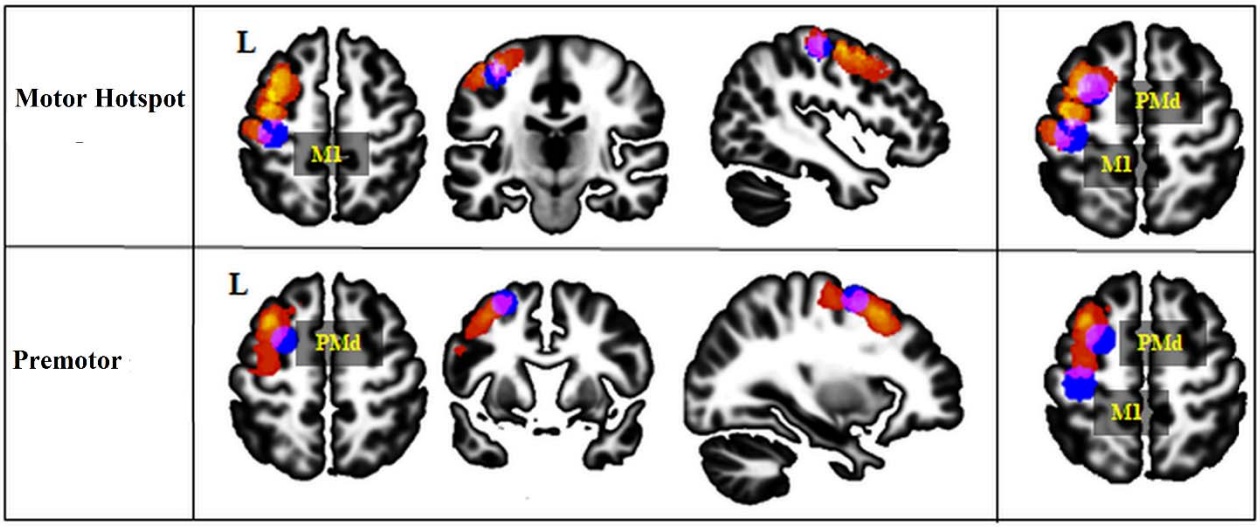

Figure 7: Location of the stimulation site. This figure displays the anatomical stimulation location sites in all the participants and shows an overlap of these sites with the standard left M1 or left PMd location for both groups. The anatomical stimulation sites are presented in a heat map, with individual subjects plotted in red and overlap between participants reflected in warmer colors (with yellow representing the surface shared by most of the participants). The standard M1 and PMd ROIs are displayed in blue. The overlap between the anatomical stimulation site location and the standard ROIs location is colored in purple. *M1: primary motor area, PMd: dorsal premotor cortex, L: left.*

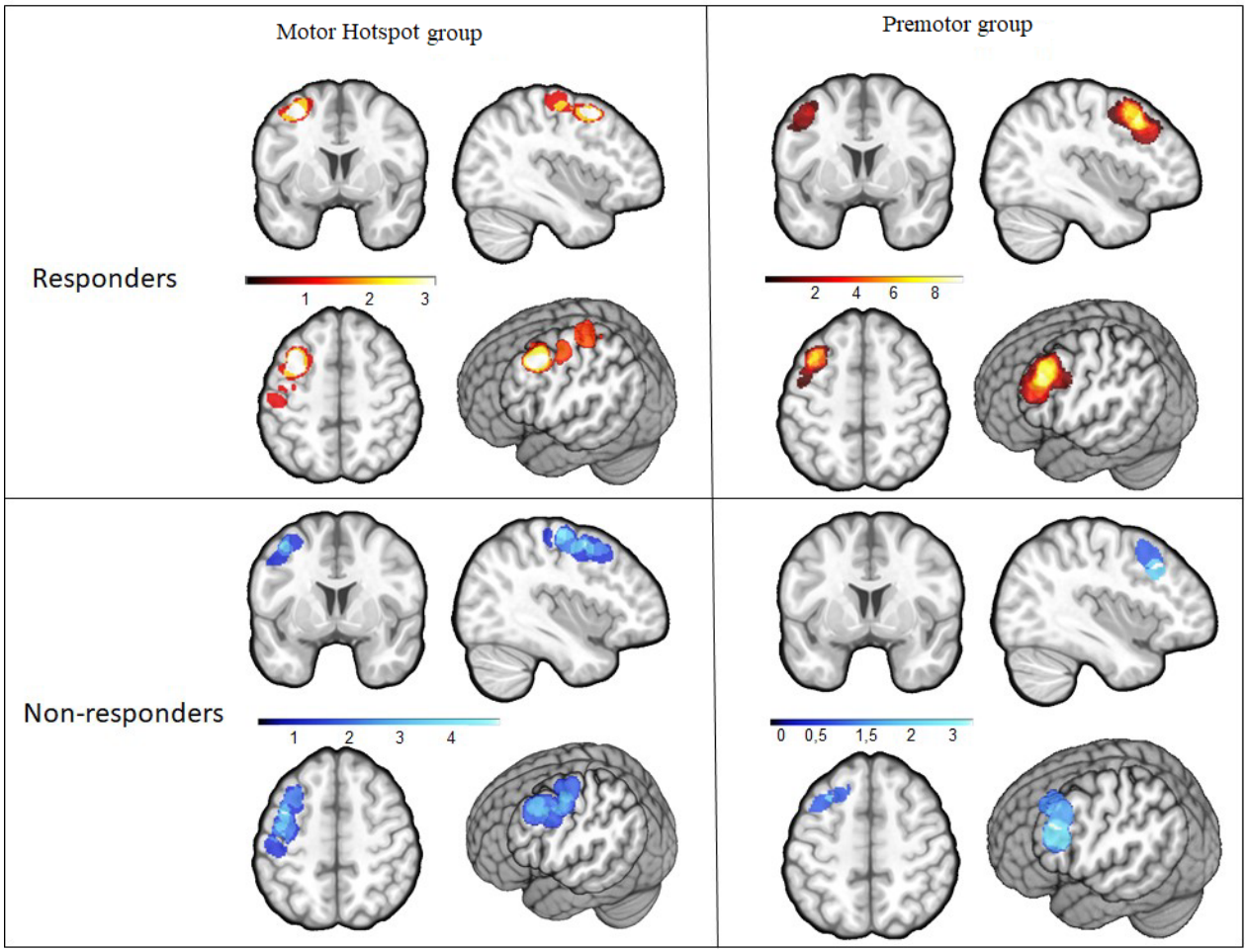

Figure 8: Heat map of the stimulation site between responders and non-responders in the Motor Hotspot and Premotor groups. This figure displays the location of the anatomical stimulation sites in the participants allocated to the Motor Hotspot group (n= 14) for the 7 responders (hot heat map) and the 7 non-responders (cool heat map), and to the Premotor group (n=15) for the 11 responders and the 4 non-responders. The majority of the responders in both Motor Hotspot and Premotor groups received stimulation over the same spot within the PMd anatomical boundaries (see also *Inline Supplementary Figure 5*).

Table

Subject	Group	RMT	TS0.75mV	MEP pre-tDCS (mV)	MEP post-tDCS (mV)
s01	Motor Hotspot	48	55	1.14	2.37
s02	Motor Hotspot	--	--	--	--
s03	Motor Hotspot	47	65	1.72	0.87
s04	Motor Hotspot	47	55	1.33	1.68
s05	Motor Hotspot	50	57	2.22	1.78
s06	Motor Hotspot	50	69	1.17	0.96
s07	Motor Hotspot	41	47	0.86	0.46
s08	Motor Hotspot	43	70	0.94	2.25
s09	Motor Hotspot	46	54	0.79	0.24

s10	Motor Hotspot	59	--	0.07	0.07
*s11**	Motor Hotspot	*38*	*48*	*1.06*	*6.8*
s12	Motor Hotspot	47	--	0.18	0.15
s13	Motor Hotspot	58	75	1.64	1.92
s14	Motor Hotspot	46	66	0.91	1.63
s15	Motor Hotspot	37	64	0.95	1.39
Mean		*46.93*	*60.42*	*1.07 (*1.07)*	*1.21(*1.61)*
SD		*6.32*	*8.99*	*0.58 (*0.56)*	*0.80(*1.68)*
s16	Premotor	38	57	1.18	3.26
s17	Premotor	45	80	0.48	0.71
s18	Premotor	50	63	0.38	0.49
s19	Premotor	42	51	1.48	0.92
s20	Premotor	44	53	0.98	2.56
s21	Premotor	48	72	0.38	0.99
s22	Premotor	38	44	1.17	2.32
s23	Premotor	63	73	0.3	0.41
s24	Premotor	54	--	0.11	0.43

s25	Premotor	35	52	1.02	1.52
s26	Premotor	51	--	0.21	0.08
s27	Premotor	41	80	0.39	2.13
s28	Premotor	38	39	0.52	0.25
s29	Premotor	37	41	0.7	0.61
s30	Premotor	39	56	0.49	1.38
Mean		*44.20*	*58.54*	*0.68*	*1.20*
SD		*7.74*	*14.06*	*0.41*	*0.96*
s31	Sham-HS	60	78	1.39	0.68
s32	Sham-HS	50	--	0.26	0.65
s33	Sham-HS	40	50	2.14	0.62
s34	Sham-HS	33	44	0.99	0.82
s35	Sham-HS	48	60	0.61	0.23
s36	Sham-HS	35	40	2.41	1.33
s37	Sham-HS	49	53	0.92	3.37
s38	Sham-HS	43	52	0.58	1.2
s39	Sham- PM	48	72	0.85	0.85
s40	Sham- PM	48	54	0.59	0.49
s41	Sham- PM	56	72	1.33	0.83
s42	Sham- PM	65	--	0.14	0.03
s43	Sham- PM	48	55	1.24	0.87
s44	Sham- PM	44	65	3.32	0.16
s45	Sham- PM	38	50	0.56	3.63

s46	Sham- PM	36	36	1.1	1.87
Mean sham		*46.31*	*55.79*	*1.15*	*1.05*
SD sham		*8.94*	*12.39*	*0.84*	*0.83*

Table 1: Initial cortical excitability measurements. SD: standard deviation; RMT: resting motor threshold, *outlier, -- indicates the measurement could not be elicited. For the Motor Hotspot group, MEP mean and SD are presented excluding the outlier and with the outlier (*). *Motor Hotspot: HD-tDCS applied over the left TMS-defined motor hotspot, Premotor: HD-tDCS applied over the left premotor cortex, Sham-HS: Sham group with electrodes over the left motor hotspot, Sham- PM: Sham group with electrodes over the left premotor.*