UNIVERSITY OF BIRMINGHAM

Master's by Research: Psychology

Project Title:

'Investigating Changes in Motor Activation after tDCS'

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Abstract

This study looked to assess whether tDCS over M1 had any significant changes in motor behaviour and activity in the motor areas of the brain in healthy right handed participants. This was done using a within subject design whereby participants received 3 different polarities of tDCS (anodal, cathodal and sham) on 3 separate occasions. A motor task was performed before (Baseline) and after (Post) tDCS was delivered at 1mA for 20 minutes over the Left M1. ROI analysis on the Left M1, Left SMA, Left Thalamus and Right Cerebellum Areas 4, 5 and 6 was conducted on the Baseline and Post fMRI motor task data. Group Baseline analysis for the contrast Move>Rest found the task to successfully activate all ROI motor areas with an FWE corrected p value < 0.05. Contrasting the Baseline<Post scenario found significant changes in brain activity in certain motor ROIs for both Anodal and Cathodal stimulation whilst the contrast of Baseline<Post revealed no significant changes in brain activity for either stimulation polarity. As expected, sham stimulation resulted in no significant changes in brain activity. For the contrast of Baseline<Post, cathodal stimulation resulted in a significant increase in brain activity in the stimulated M1 at an uncorrected p value < 0.001. Anodal stimulation resulted in a significant increase in brain activity in the stimulated M1 at an uncorrected p value < 0.001 and the ipsilateral SMA at an FWE corrected p value < 0.05. Interactional analysis of all stimulation polarities across the Baseline<Post contrast found significant brain activity changes in Cerebellum Areas 4, 5 and 6 at an FWE corrected p value <0.05. Analysing behavioural motion tracking and neurophysiological MEP data it was found that there was no significant effect of tDCS stimulation. This study therefore found tDCS to have widespread effects on the motor network, with anodal tDCS having desired effects of increasing brain activity in motor areas of the brain. This evidence for tDCS modulating brain activity in the motor areas of the brain gives promise towards tDCS being a potential modality to explore with regards to motor rehabilitation in patients, such as those with disorders of consciousness.

Introduction

Acquired brain injury (ABI), defined as brain injury occurring since birth, is a leading cause of death and disability throughout the world. In the UK alone there around 300-350 thousand hospital admissions a year due to ABI (Headway, 2015). These can be due to either a head injury from a traumatic origin e.g. a car accident, or brain damage from a non-traumatic origin e.g. a stroke. Whilst the majority of head injuries have minor consequences such as temporary loss of consciousness, headaches or dizziness and do not require hospital admission or care, in other cases people may be left with a severe brain injury resulting in long lasting effects and disabilities. One such condition that can occur from ABI is a disorder of consciousness (DOC) in which a person has significant disruption to their level of consciousness.

It is generally accepted that consciousness can be split into the two core components: wakefulness and awareness (Owen, 2013). Wakefulness is defined as the 'level of consciousness' and represents how the body goes through periodic sleep-wake cycles along with routine eye opening and closing. Awareness relates to the 'content of consciousness' and encompasses both a person's internal awareness of one self as well as external awareness of ones surroundings (Owen, 2013). Whilst wakefulness can be easily monitored and detected using technology such as electroencephalography (EEG), awareness detection often relies on behavioural assessments using command following. This difficulty in detecting awareness has ultimately led to several issues in correctly diagnosing DOC state.

There are 3 broad diagnostic categories of DOC including: coma, vegetative state (VS) and minimally conscious state (MCS) (Schnakers & Laureys, 2014). Following severe

brain injury the initial temporary state patients fall into is called a coma, which is a state lacking both wakefulness and awareness. Following a coma, patients may then go on to recover some level of consciousness and emerge into a VS, MCS or fully conscious state (Schnakers & Laureys, 2014). Following recovery of consciousness however patients will often be left with impairments of disabilities that can be physical, cognitive or behavioural in nature. A patient in a VS, or more recently suggested to have unresponsive wakefulness syndrome (UWS) (Laureys et al., 2010), is defined as retaining wakefulness but lacking awareness. A MCS patient on the other hand also retains wakefulness but will have a low level of awareness which can be varying or intermittent (Giacino et al., 2002). The MCS has been further separated into MCS+ and MCS- (Bruno, Vanhaudenhuyse, Thibaut, Moonen, & Laureys, 2011), with MCS+ exhibiting more robust behavioural signs of awareness and thus demonstrating a higher level of awareness. Brain injury of this nature often comes with varying levels of cognitive, behavioural, physical and emotional disability, with cognitive impairments and motor impairments being those most commonly arising effects (Clayton, Kinley-Cooper, Weber, & Adkins, 2016). There is therefore a huge drive towards development of rehabilitative options for these patients alongside a range of diagnostic issues arising from these disabilities.

Detecting consciousness for instance involves routine behavioural assessments that look for responses to a variety of stimuli such as visual, auditory and sensory stimuli (Majerus, Gill-Thwaites, Andrews, & Laureys, 2005; Royal College of Psyicians, 2013). The most widely used assessment tool for detecting consciousness is the Glasgow Coma Scale (GCS) (Sternbach, 2000). Independently in research the Coma Recovery Scale Revised (CRS-R) which has been devised in order to better discriminate between VS and

MCS (Giacino et al., 2002; Sattin et al., 2015). A study conducted in 2009 looking to compare standard clinical assessments carried out in non-specialist rehabilitation units to the CRS-R found a disturbingly high rate of inaccuracy, with 40% of patients being incorrectly diagnosed as being in a VS with the standard assessments due to incorrect implementation of assessments or signs of consciousness being missed (Schnakers et al., 2009). Furthermore, this result was replicated more recently in 2015 with the study finding the diagnostic inaccuracy of the VS to be 39% (van Erp et al., 2015). Although the CRS-R is currently the most accurate behavioural assessment for diagnosing conscious state, it ultimately still relies on both the patient being able to demonstrate their awareness through movement in response to command, as well as subjectivity of the assessor.

Motor impairments are a common side effect of brain injury meaning that it could be possible for a subset of patients to retain awareness but be unable to demonstrate this externally. With a lot of patients in a DOC state having either intermittent or varying levels of awareness, it is also possible that behavioural assessments may fall at times where their level of consciousness is reduced. The interplay of these factors could therefore owe to signs of awareness going undetected and a misdiagnosis of conscious state. Research using functional magnetic resonance imaging (fMRI) or electroencephalography (EEG) alongside devised motor imagery paradigms has been able to demonstrate awareness in patients diagnosed as being in a VS from behavioural assessments (Bardin et al., 2011; Cruse et al., 2011; Monti et al., 2010; Owen & Coleman, 2008). From this research it has been suggested that around 17-19% of patients correctly diagnosed as being in a VS using the CRS-R do in fact retain some level of awareness and are in fact more likely to be in a MCS or higher (Fernández-Espejo &

Owen, 2013). Following these results a new category of 'covertly aware' patients has been proposed whereby the patient retains some level of consciousness but lack the ability to demonstrate this through movement in response to command (Fernández-Espejo, Rossit, & Owen, 2015).

One important question for neuroscientists that has come about from these findings is the question: what is the neural basis underlying this covertly aware state and lack of purposeful movement? The brain areas involved in movement are already well characterised, with the motor network of the brain comprising various distinct interacting brain areas including the premotor cortex (PMC), primary motor cortex (M1), supplementary motor area (SMA), cerebellum and thalamus amongst others (Kasess et al., 2008). Of these brain areas it is known that the PMC and SMA are primarily involved in motor planning, M1 is involved in motor execution with projections going via the thalamus, whilst the cerebellum forms a feedback loop with M1 and is involved in error correction (Kasess et al., 2008). It is also evident that performing motor imagery and motor execution involves a highly overlapping brain network (Stephan et al., 1995). Recent research carried out by Fernández-Espejo et al., 2015 has now been able to add to this knowledge and find the neural basis for lack of purposeful movement in a DOC patient. This study used dynamic causal modelling (DCM) connectivity analysis of fMRI to reveal the importance of excitatory coupling between M1 and the thalamus during motor execution. Further to this, fibre tractography was also used to reveal that the integrity of the M1-thalamus connection was essential for voluntary movement production, with these fibres being disrupted in a DOC patient unable to perform purposeful movement. Critically the nature of the damage to these fibres in the patient was only partial as opposed to complete severing

and hence could present a potential target for modulatory neurorehabilitative therapies.

Just as correct diagnosis of DOC state has vast implications for treatment, quality of life and care of these patients; finding a neurorehabilitative option allowing patients to gain enhancement in motor ability would have a vast effect on their day to day life. Even a slight increase in motor ability could potentially enable patients access to brain computer interface and robotic devices that would allow greater communication, movement and task completion. This is particularly an issue for DOC patients who often lack voluntary eye control, so whilst a locked in patient with control of eye movement can use eye gaze technology to communicate, these patient cannot use this technology (Lulé et al., 2013). Use of robotic assistance devices would not only allow the patient to become more independent and social, but would also reduce stress on carers, reduce strain on healthcare resources and boost the mental wellbeing of both the patient and the family through an increased communication and quality of life aspect. DOC patients despite their respective rarity as compared to stroke and cancer patients, pose a huge burden on healthcare resources with the cost of a VS patient estimated to be around £250 a day (Formby, Cookson, & Halliday, 2015).

Brain stimulation has been increasingly used in the rehabilitative context with some promising non-invasive brain stimulation modalities showing positive rehabilitative effects in a wide range of disorders (Allman et al., 2016; Benninger & Lomarev, 2010; Fregni et al., 2006; Koganemaru et al., 2015). These non-invasive modalities include transcranial magnetic stimulation (TMS), often applied as repetitive or rTMS, and transcranial direct current stimulation (tDCS).

TMS is a method which allows for activation of the motor cortex in a pain free and safe manner. It works by creating a magnetic current within a hand held coil which creates perpendicular magnetic pulses to the direction of the current in the coil. When held close to the scalp these pulses can penetrate the scalp and induce electric currents that activate the motor cortex below (Kobayashi & Pascual-Leone, 2003). When used over the motor cortex TMS is able to activate the underlying neurons at the stimulation site creating motor evoked potentials (MEPs) in the corresponding muscle. These MEPs can be recorded using electromyography (EMG) and can be used to assess cortical excitability and integrity of the involved pathways. TMS can either be applied as single pulses, as a repetitive trail of pulses known as rTMS or as a paired pulse with two sites of stimulation.

tDCS works by creating a current between two electrodes, an anode and a cathode, placed on the scalp. The electrical current set up between the two electrodes modulates the underlying cortex and causes changes in cortical excitability depending on intensity and polarity of stimulation. When anodal tDCS is applied there is generally a lowering of motor thresholds and thus increasing cortical excitability, whilst cathodal tDCS increases the underlying neuron motor thresholds and causes decreased cortical excitability. tDCS can now also be used in conjunction with MRI so that concurrent fMRI-tDCS experiments can be carried out (Meinzer et al., 2014). This allows for the online effects of tDCS to be assessed without delays and confounding movements.

tDCS has been widely used in neurorehabilitative research on patients with Stroke (Allman et al., 2016; Hummel & Cohen, 2005; Lefebvre et al., 2014), Parkinson's

(Benninger & Lomarev, 2010; Fregni et al., 2006; Subramanian et al., 2016) and Ataxia (Pozzi et al., 2014). Whilst there are promising results coming from Stroke and Parkinson's research into the use of tDCS combined with motor training for rehabilitation (Allman et al., 2016; Subramanian et al., 2016), the transfer of these results into DOC patients is not so straight forward. DOC patients for instance often have no motor ability or very low, intermittent motor ability and so the use of tDCS combined with motor training would not be a viable option for these patients. As there is still a lot of debate regarding the effects of tDCS however, it is necessary to first carry out research into the effects of tDCS on voluntary movements, the motor areas of the brain and movement parameters before looking into effects on patients.

The aim of this study therefore is to use concurrent tDCS-fMRI over M1 to explore the effects of tDCS on the motor network, how this affects motor ability and whether tDCS is a viable modality which could be used in the patient setting to modulate cortical excitability and enhance movements. We used MEP characterisation, motion parameter tracking and brain activity analysis of the motor areas in order to study the effects of tDCS over M1. All 3 different polarities of tDCS: anodal, cathodal and sham were used in a within-participant randomised design meaning participants attended 3 separate sessions, one for each polarity, at least a week apart.

We expected that anodal tDCS applied over M1 would decrease motor threshold and enhance cortical excitability. From analysis of fMRI data we expected to see this through increased brain activity in connected motor areas such as Thalamus, SMA and Cerebellum Areas 4,5, and 6 within the Post scenario. From analysis of MEP and motion tracking data we expected to see this evidenced through enhanced MEP peak-peak

amplitude and enhanced peak acceleration respectively Post stimulation. For cathodal tDCS over M1 however we expected motor threshold to increase and hence cortical excitability to decrease. From analysis of fMRI data we expected to see this through decreased brain activity in connected motor areas such as Thalamus, SMA and Cerebellum Areas 4,5, and 6 within the Post scenario. From analysis of MEP and motion tracking data we expected to see this evidenced through decreased MEP peak-peak amplitude and enhanced peak acceleration respectively Post stimulation. With regards to brain activity we therefore hypothesised that brain activity would decrease in the motor areas of the brain. For sham tDCS we expected to see no significant changes in brain activity, excitability nor task performance across the Baseline-Post scenario.

Method

Participants

We recruited 12 right handed, neurologically healthy participants through the use of the University of Birmingham Psychology Research Participation Scheme. From the 12 participants recruited, 8 completed all 3 sessions of the experiment. We included all 8 data sets within the fMRI analysis, 7 within the motion tracking analysis and 4 within the MEP analysis. We excluded 1 data set from the motion tracking analysis due to an error in the data recording resulting in no data being recorded. We excluded 3 data sets from the MEP analysis due to equipment malfunctioning resulting in inability to acquire data on certain sessions or the loss of data from a scanning session. Finally we excluded 1 data set from MEP analysis due to inconsistency in the data collection procedure (see

Appendix 1 for a full summary of data losses). In order to be eligible, participants must have been right handed, ≥18 years old, have no history of epilepsy, neurological or psychiatric disease (including migraine), not meet any exclusion criteria for brain stimulation and be eligible to enter the MRI environment (see Appendix 2 for full exclusion criteria details).

Recruitment

Participants we asked to fill out the Edinburgh handedness inventory (Oldfield, 1971), MRI and brain stimulation screening forms prior to recruitment in order to check they were suitable to participate. We instructed participants to be well hydrated and well slept, with no alcohol or coffee consumed within 24 hours of the study in order to be in keeping with brain stimulation safety regulations. All participants had the chance to read a participant information sheet, learn about the protocols used and ask questions of the researcher before signing an ethics consent form. Compensation was given in the form of cash or research credits after each session. The University of Birmingham Ethics committee approved this study under ethics code ERN_11-0429.

MRI Acquisition

We used a 3-T scanner (Phillips Achieva) at the Birmingham University Imaging Center (BUIC) to acquire data. We obtained a T1 scan for each participant with the parameters: repetition time (TR) of 7.4ms, echo time (TE) of 3.5ms, matrix size of 256x256mm, field of view of 256x256mm, voxel size of 1x1x1mm and flip angle of 7 degrees. The task-fMRI protocol had the following acquisition parameters: repetition time (TR) of 2000ms; echo time (TE) of 35ms; matrix size of 80x80; field of view of 240x240mm, voxel size of 3x3x3mm and flip angle of 79.1 degrees. There were 34 slices in each

acquisition with slices being acquired in an ascending interleaved fashion. T2, Diffusion Tensor Imaging (DTI) and Resting State fMRI (rs-fMRI) scans were also acquired but not analysed within the study. The scans were recorded in the order: T1, T2, DTI, task-fMRI, rs-fMRI, tDCS, task-fMRI, rs-fMRI for session 1 and: T1, fMRI, resting state, tDCS, fMRI, resting state for session 2 and 3.

Experimental Design

We carried out the study using a within subject design in which participants completed three different sessions: one with anodal, one with cathodal and one with sham tDCS stimulation. Each session was always at least 7 days apart and overall on average sessions were 9.75 days apart with a standard deviation of +-5.2 days and the modal value being 7 days of separation between testing sessions, with the order of the 3 stimulations being counterbalanced across participants. See Table 1 for a summary of stimulation randomisation orders and how many participants received each order of stimulation.

	Session 1	**Session 2**	**Session 3**	**Frequency**
Order 1:	Anodal	Cathodal	Sham	2
Order 2:	Anodal	Sham	Cathodal	2
Order 3:	Cathodal	Anodal	Sham	1
Order 4:	Cathodal	Sham	Anodal	1
Order 5:	Sham	Cathodal	Anodal	1
Order 6:	Sham	Anodal	Cathodal	1

Table 1. Stimulation Randomisation for All Participants. *Summary of randomisation orders and frequencies for all 8 participants included in fMRI data analysis. There was a total of 6 randomisation orders each with a different order of transcranial direct current stimulation (tDCS) polarities - anodal, cathodal and sham - given across the 3 sessions.*

Experimental Procedure

Each session consisted of MEP collection and MRI Acquisition procedure including a Motor Task involving simple thumb movements in response to beeps. The MEP procedure and Motor Task alongside resting state scan were presented both before and after 20 minutes of tDCS delivery at 1mA in order to collect baseline and post data (see Figure 1 for a summary). Following completion of a session participants were asked to complete a post tDCS perceptual scale form (Appendix 3) in which they were asked questions regarding the side effects they experienced from tDCS, their current tiredness state and whether they thought they had received real or sham stimulation. On completion of the session participants were paid for their time.

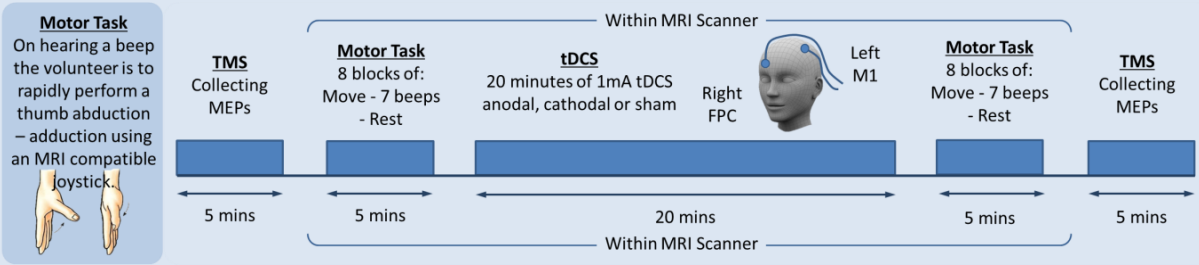

Figure 1. Experimental Procedure for Data Collection. *Healthy volunteers underwent 3 identical testing sessions only differing in the type of transcranial direct current stimulation (tDCS) stimulation applied. These were formatted in the same mirrored fashion with transcranial magnetic stimulation (TMS) motor evoked potential (MEP) collection and Joystick Motor Task being presented.*

fMRI Motion Tracking Task

Participants were audibly instructed to move their thumb towards their index finger and back again to the original start point as fast as they could in response to beeps. These beeps were presented in blocks where each block was cued by the word “move”,

7 irregularly timed beeps (interstimulus intervals: 2 seconds , 2.5seconds and then 3 seconds) were then presented, followed by the word “relax” to end the block. Each of the “move” and “rest” blocks were 20 seconds. There were 8 sets of these blocks played over a span of 5 minutes to complete the task. Throughout the duration of the task participants were requested to keep their eyes on a fixation cross displayed on screen. Participants were given an MRI compatible joystick whilst in the MRI scanner to which their thumb was secured with tape. The participants were given the joystick in the orientation shown in Fig. 2 as this was shown to have the most consistent baseline level based on pilot behavioural trials (data not included). Whilst in the MRI scanner they were given a set of Avotec SS-3100 headphones through which audio commands were issued. Prior to the task a visual projector system (NVIDIA Quadro 5000) was used to present the instructions: *“Start moving your thumb as quickly as you can every time you hear a beep. Stay still when you hear "relax". Make sure you keep looking at the fixation cross at all times”*.

Figure 2. Joystick orientation and positioning

Motor Evoked Potential (MEP) Procedure

TMS was applied over the left M1 hand First Dorsal Interosseous (FDI) area and EMG electrodes were used to record the corresponding MEP response. Recording electrodes were placed over the FDI muscle of the contralateral right hand and the index finger whilst the reference electrode was placed on the elbow bone. Brainsight v2.3 neuronavigation was used to register positions of the TMS coil and tDCS electrode placements for each participant as well as to record the output from the EMG pod

recording. After the first session saved targets were used for each participant in order to locate the same motor hotspot position and place the electrodes in the same locations. Measurements were taken from the nasion to the inion of the participant in order to locate the midpoint Cz. An origin point around 5cm towards the ear and 2.5cm forwards from Cz was then marked as this should be the position of the hand motor area. The TMS coil was initially held at this point at around 45 degrees across the participants head and pulses given. The TMS coil was navigated subtly around this area until a response was seen in the desired FDI muscle and EMG response was also seen on the Brainsight recording. Once the correct area had been located this point was saved as a target for further stimulation in order to first deduce the motor threshold – the intensity at which MEP response was around 50%. The actual stimulation protocol itself was then carried out at 120% this intensity and involved 1 round of 20 pulses each separated by 5 seconds.

Transcranial Direct Current Stimulation (tDCS) Application

An MRI compatible tDCS kit comprising a battery driven constant current stimulation (NeuroComm), electrodes and MRI interface boxes was used in order to administer tDCS stimulation to the participants. Data acquisition and analysis were performed in a double blind manner in which neither the participant nor the researcher analysing the data set knew the stimulation applied. The active electrode was placed over the left M1 area previously located by the TMS procedure, whilst the return electrode was placed over the right occipitofrontal area above the eyebrow. The positioning of each corner of the tDCS electrodes was recorded using Brainsight and saved as a reference for the following sessions. Ten20 conducting gel was used in order to reduce risk of sensation or side effects to the participant and aid in conductance of current. During the anodal

and the sham condition the anodal electrode was placed over M1 with the other electrode over the occipitofrontal area; during the cathodal condition the cathodal electrode was placed over M1 with the other electrode over the occipitofrontal area. During the anodal and the cathodal condition 20 minutes of tDCS stimulation at 1mA was applied within the MRI scanner. During the sham condition only 30 seconds of stimulation was applied before stimulation ceased in order to emulate the sensation of real stimulation (Woods et al., 2016). Post tDCS perceptual scales were filled out following the procedure in order to assess participants perceptions of the stimulation they received and compare across sessions (Attached in Appendix 3).

fMRI Pre-processing

Analysis was carried out using SPM12 on MATLAB R2015b (www.fil.ion.ucl.ac.uk/spm). Spatial pre-processing included realignment to correct for the participants motion, co-registration between the structural and functional data sets, spatial normalization and smoothing with a 8mm full width at half maximum Gaussian kernel.

General Linear Model Analysis

		Stimulation Type		
		Anodal	**Cathodal**	**Sham**
Presentation	**Baseline**	1,1	2,1	3,1
	Post	1,2	2,2	3,2

Table 2. Second Level GLM Contrast Design. *Summary of the two different factors: Stimulation Type and Presentation along with their corresponding levels: anodal, cathodal, sham and post, baseline respectively. The table shows the combination of the different levels which were entered into the GLM analysis.*

1st Level GLM analysis was run on the fMRI Task data whereby T contrasts were run on the conditions of '*Move*' and '*Rest*' with Move>Rest weighted as 1 and Rest>Move weighted as -1. For each participant, realignment factors were entered as effects of non-interest to account for motion related variability. For 2nd Level GLM Analysis contrast files were created for each participant for the two factors: Stimulation Type and Presentation Time (see Table 2). 2nd Level analysis was first run on the Baseline data to establish the normative pattern of activity for the task. Full factorial analysis was carried out across the participants in a region of interest (ROI) design. Regions of interest included: left supplementary motor area (SMA), left precentral gyrus (M1), thalamus, cerebellum region 4 and 5 as well as cerebellum region 6. These were obtained using the Automated Anatomical Labeling (AAL) atlas through the WFU PickAtlas extension in SPM12 (http://www.gin.cnrs.fr/AAL). This atlas was also used to label areas of significant brain activity on fMRI results. Further GLM analysis was then run contrasting Pre vs. Post for each Stimulation type: Anodal, Cathodal and Sham along with an F statistic Stimulation*Presentation interaction analysis across all conditions.

Motion Tracking Data Processing and Analysis

Joystick Data was plotted across each participant with a custom MATLAB script enabling correct selection of responses and calculation of peak acceleration. From the script onset times for the beeps were plotted on the graph with any non-responses to beeps being manually removed on visual inspection so as not to skew average calculations of acceleration. A low pass 5Hz filter was applied to calculations of Euclidian distance from the joystick motion data. Peak acceleration was calculated from Euclidian distance data as the maximum acceleration within a 2 second time frame after

the beep onset. Once the data had been fully assessed calculations of average peak accelerations were entered into an SPSS spreadsheet for all 7 participants included within the analysis for each condition. GLM analysis was then run on this data in order to look for an interaction between stimulation and time point condition using the contrasts detailed in Table 2.

TMS MEP Data Processing and Analysis

GLM analysis was carried out using contrasts as detailed in Table 2 on TMS data from the 4 included participants. Original analysis was carried out on the raw MEP data returned from the Brainsight software which automatically calculated EMG Peak to Peak values from the raw data. Averages were taken from all 20 pulses for each different level combination and transferred into an SPSS spreadsheet where GLM analysis was carried out. To account for missing MEP responses results were plotted within MATLAB using an in-house custom script which enabled manual selection and checking of the MEP Peak Amplitude results. In order to prevent data skewing from pulses for which no MEP was returned, the 10 first clear usable MEPs were selected for further analysis and averaging whilst the rest were discarded. GLM analysis was then run on these 10 selected MEPs.

Results

Motion Tracking Behavioural Analysis

Motion tracking analysis was undertaken on 7 out of the 8 participants included in the study, with exclusions of 1 participant data sets due to data losses. This resulted in all of the possible 6 randomisation orders being utilised within this data set which has been summarised in Table 3.

	Session 1	**Session 2**	**Session 3**	**Frequency**
Order 1:	Anodal	Cathodal	Sham	2/(2)
Order 2:	Anodal	Sham	Cathodal	1/(2)
Order 3:	Cathodal	Anodal	Sham	1/(1)
Order 4:	Cathodal	Sham	Anodal	1/(1)
Order 5:	Sham	Cathodal	Anodal	1/(1)
Order 6:	Sham	Anodal	Cathodal	1/(1)

Table 3. Stimulation Randomisation for All Participants included in Motion Tracking Analysis. *Summary of randomisation orders and frequencies for all 8 study participants ( ) and the 7 participants included in Motion Tracking data analysis. There was a total of 6 randomisation orders each with a different order of transcranial direct current stimulation (tDCS) polarity - anodal, cathodal and sham - given across the 3 sessions.*

No significant main effects or interaction were found from group general linear model analysis of motion data for Stimulation (Anodal, Cathodal and Sham) and Presentation Time (Baseline and Post). Mean and standard deviation data for motion tracking data across all sessions and participants is summarised in Table 4.

Stimulation	Anodal		Cathodal		Sham	
Presentation	Baseline	Post	Baseline	Post	Baseline	Post
Mean Peak Acceleration (mm/s^2)	1.46 ± 0.53	1.36 ± 0.40	1.13 ± 0.44	1.11 ± 0.38	1.19 ± 0.53	1.18 ± 0.50

Table 4. Mean Peak Amplitude and Standard Deviations of Motion Tracking Data.

Individual responses across all 7 included participants were plotted for each of the stimulation conditions: Anodal, Cathodal and Sham to visually assess the responses to each polarity of tDCS (Figure 3).

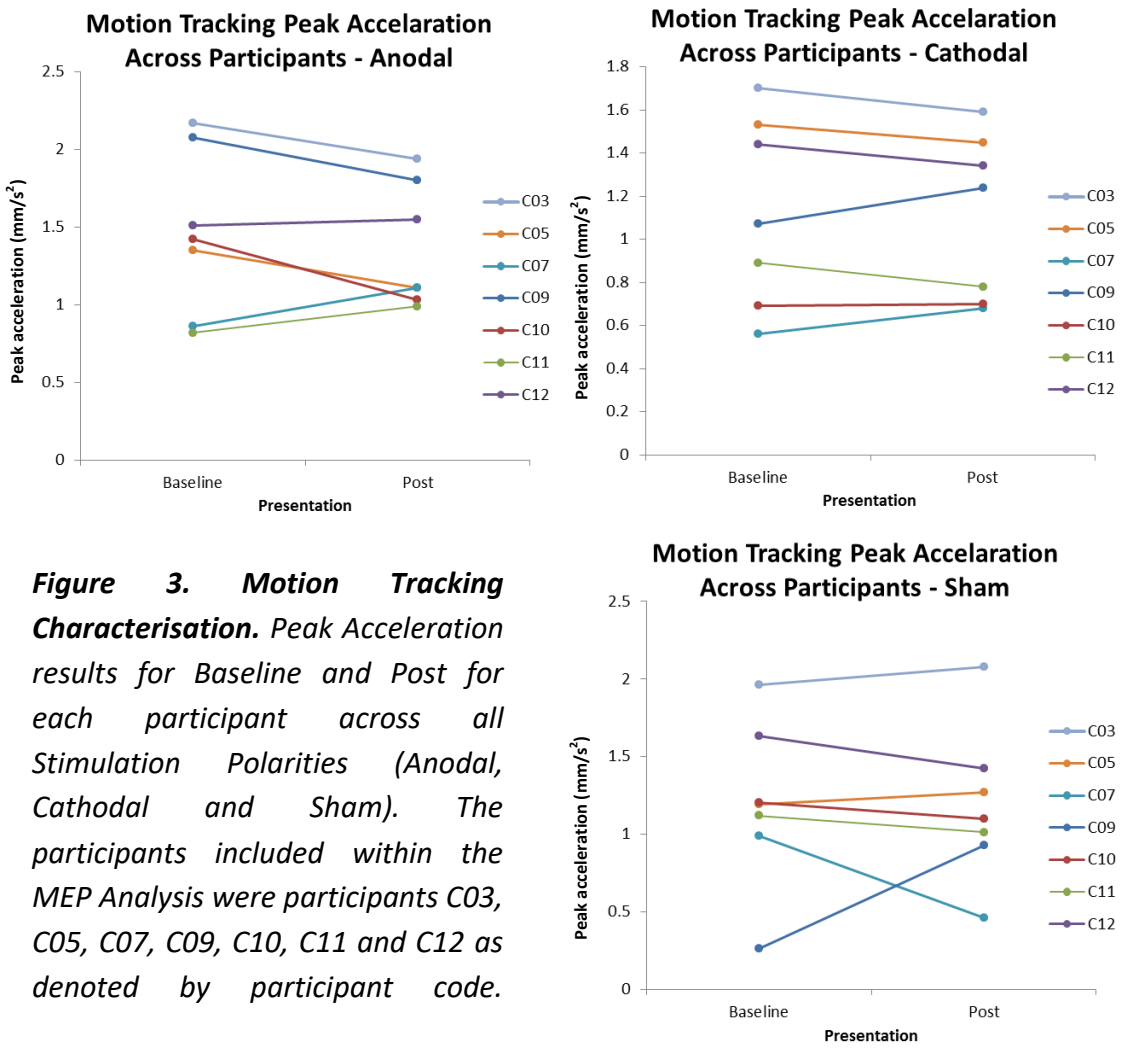

Figure 3. Motion Tracking Characterisation. *Peak Acceleration results for Baseline and Post for each participant across all Stimulation Polarities (Anodal, Cathodal and Sham). The participants included within the MEP Analysis were participants C03, C05, C07, C09, C10, C11 and C12 as denoted by participant code.*

MEP Response Characterisation

MEP response characterisation analysis was undertaken on 4 out of the 8 participants included in the study, with exclusions of participant data sets due to data losses. This resulted in 4 of the possible 6 randomisation orders being utilised within this data set which has been summarised in Table 5.

	Session 1	**Session 2**	**Session 3**	**Frequency**
Order 1:	Anodal	Cathodal	Sham	1/(2)
Order 2:	Anodal	Sham	Cathodal	1/(2)
Order 3:	Cathodal	Anodal	Sham	0/(1)
Order 4:	Cathodal	Sham	Anodal	0/(1)
Order 5:	Sham	Cathodal	Anodal	1/(1)
Order 6:	Sham	Anodal	Cathodal	1/(1)

Table 5. Stimulation Randomisation for All Participants included in MEP Analysis. *Summary of randomisation orders and frequencies for all 8 study participants ( ) and the 4 participants included in MEP data analysis. There was a total of 6 randomisation orders each with a different order of transcranial direct current stimulation (tDCS) polarity - anodal, cathodal and sham - given across the 3 sessions.*

No significant main effects or interaction were found from group general linear model analysis of MEP data for Stimulation (Anodal, Cathodal and Sham) and Presentation Time (Baseline and Post). Mean and standard deviation data for MEP responses across all sessions and participants is summarised in Table 6.

Stimulation	Anodal		Cathodal		Sham	
Presentation	Baseline	Post	Baseline	Post	Baseline	Post
Mean Peak Amplitude (μV)	261 ± 402	246 ± 358	976 ± 1764	360 ± 532	239 ± 359	446 ± 762

Table 6. Mean Peak Amplitude and Standard Deviations of MEP Results.

Individual responses across all 7 included participants were plotted for each of the stimulation conditions: Anodal, Cathodal and Sham to visually assess the responses to each polarity of tDCS (Figure 4).

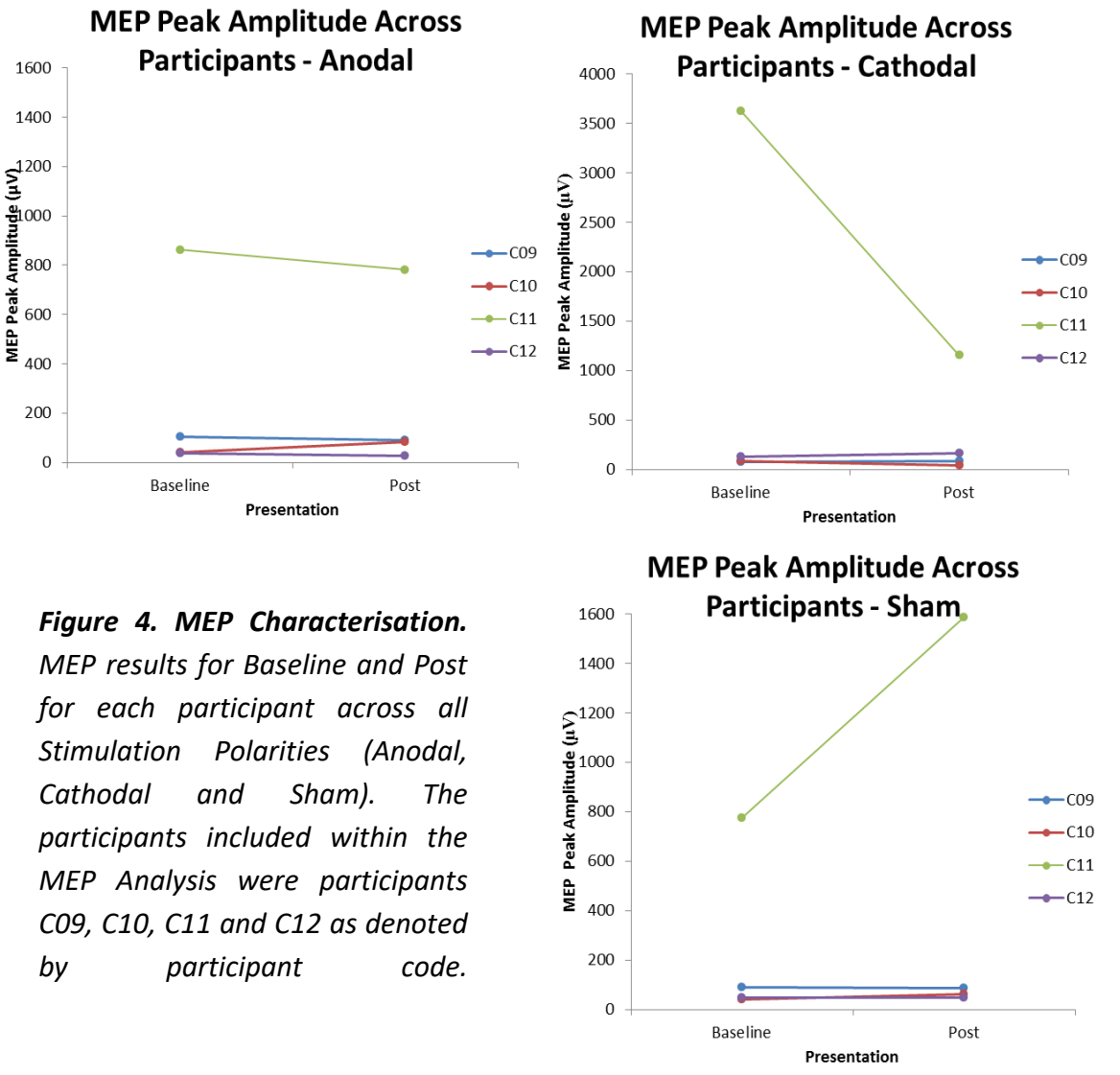

Figure 4. MEP Characterisation. *MEP results for Baseline and Post for each participant across all Stimulation Polarities (Anodal, Cathodal and Sham). The participants included within the MEP Analysis were participants C09, C10, C11 and C12 as denoted by participant code.*

fMRI Group Level Baseline Analysis

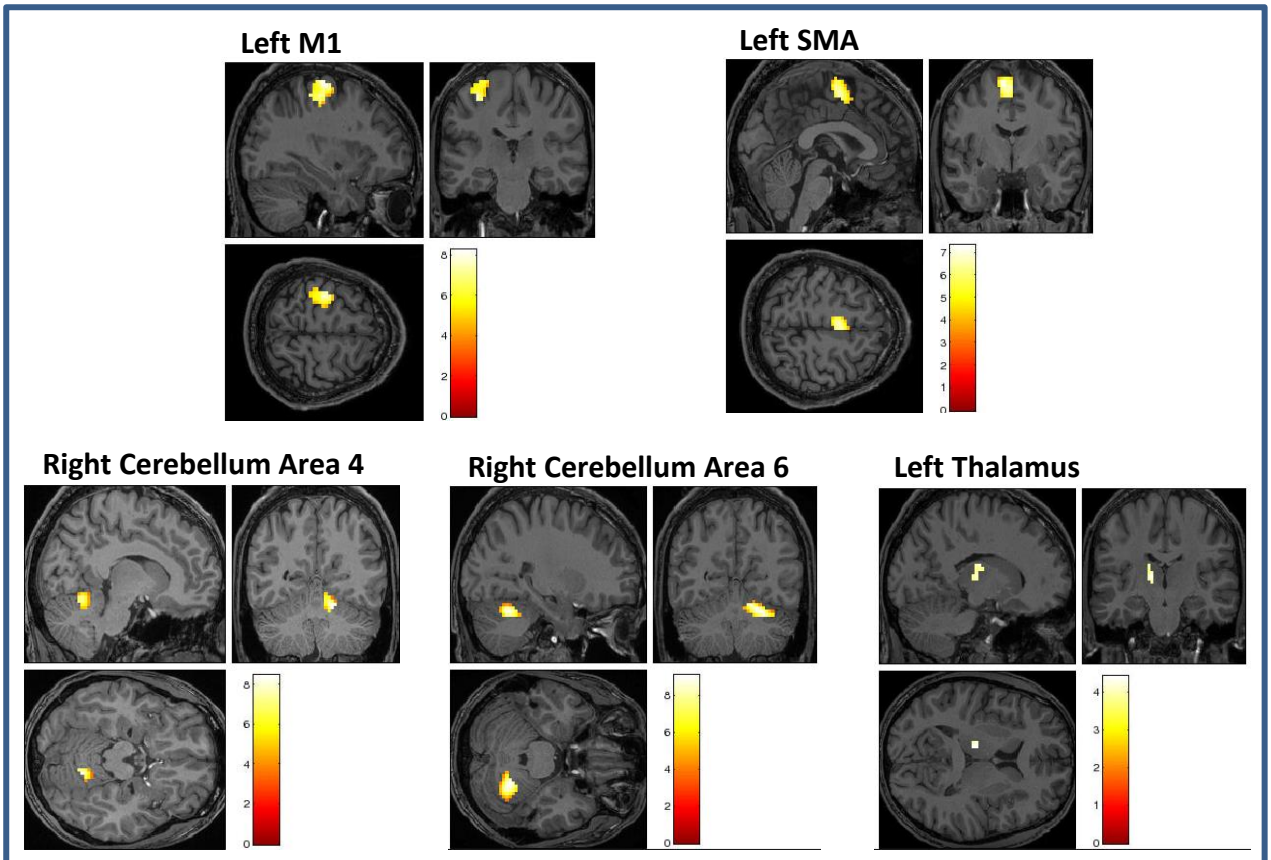

Figure 5. ROI Analysis of Group Baseline Data for the contrast of Move>Rest. *ROI analysis showing significant brain activity in all selected ROIs: Left M1, Left SMA, Left Thalamus and Right Cerebellum Areas 4-6 at an FWE corrected p value < 0.05. M1; Motor Cortex, SMA; Supplementary Motor Area.*

Baseline analysis of normative response to the motion tracking task found no significant activation in the ROI brain areas for the contrast of Move<Rest. Analysis on the contrast Move>Rest found significant brain activity in all the prior hypothesised regions at a FWE corrected p value of <0.05 (Table 7, Figure 5).

Table 7. Region of Interest Random Effect Group Analysis Baseline. *T Values and MNI Coordinates are the peak values for the local maximum of each cluster. Abbreviations: FWE, familywise error; M1, Motor Cortex; SMA, Supplementary Motor Area; MNI, Montreal Neurological Institute.*

Region	P Value (FWE corrected)	T Value	MNI x, y, z Coordinates
Left SMA	<0.001	8.24	[-30 -19 68]
Left M1	<0.001	7.3	[-9 -10 65]
Left Thalamus	0.01	4.41	[-12 -19 8]
Right Cerebellum Area 4 & 5	0.001	8.42	[15 -55 -19]
Right Cerebellum Area 6	<0.001	9.07	[18 -55 -22]

fMRI Group Level Main Effects ANOVA Analysis

Analysis of the main effects for Stimulation (anodal, cathodal and sham) found no significant changes in brain activity. Analysis of the main effects for Presentation (Baseline – Post) however found significant changes in brain activity in the stimulated left M1 at an FWE corrected p value < 0.05 (see Table 8, Figure 6).

Table 8. Region of Interest Random Effect Group Analysis Main Effects: Baseline-Post*. F statistic and MNI Coordinates are the peak values for the local maximum of each cluster. Abbreviations: FWE, familywise error; MNI, Montreal Neurological Institute; M1, Motor Cortex.*

Region	P Value (FWE corrected)	F statistic	MNI x, y, z Coordinates
Left M1	0.027	19.89	[-51 -7 32]

Left M1

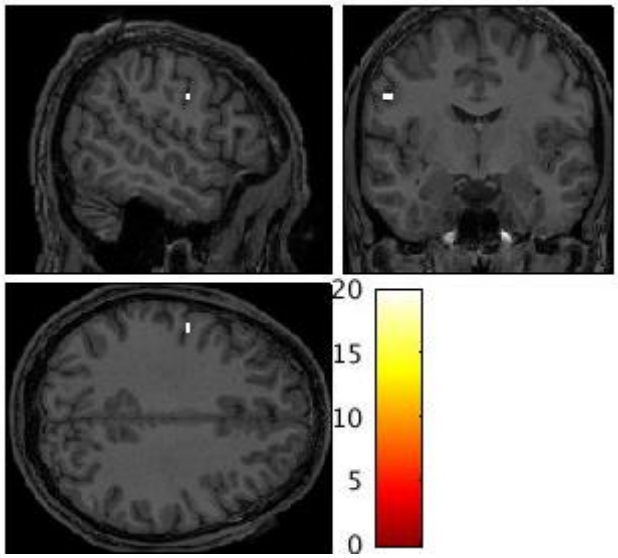

Figure 6. ROI Analysis of Main Effects for Presentation Time. *ROI analysis showing significant brain activity in the left M1 at an FWE corrected p value < 0.05.*

fMRI Group Level Interaction ANOVA Analysis: Stimulation*Presentation Time

ROI analysis of interaction between Stimulation and Presentation Time found significant brain activity in the Cerebellum area 6 at an FWE corrected p value < 0.05 (see Table 8, Figure 7).

Table 8. Region of Interest Random Effect Group Analysis F statistic Interaction*. F statistic and MNI Coordinates are the peak values for the local maximum of each cluster. Abbreviations: FWE, familywise error; MNI, Montreal Neurological Institute.*

Region	P Value (FWE corrected)	F statistic	MNI x, y, z Coordinates
Right Cerebellum Area 6	0.034	10.21	[15 -70 -13]

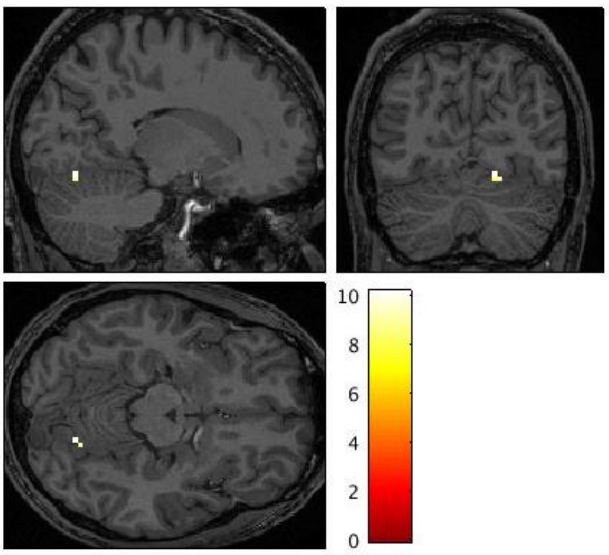

Figure 7. ROI Analysis of Interaction between Stimulation and Presentation Time. *ROI analysis showing significant brain activity in the Cerebellum Area 6 at an uncorrected p value < 0.001.*

fMRI Group Level Sham Condition Post-Hoc Assessment

Contrasting the presentation times of baseline and post for the sham condition returned no significant activation for both the whole brain analysis the ROI Analysis for either of the contrasts Baseline>Post or Baseline<Post.

fMRI Group Level Cathodal Condition Post-Hoc Assessment

Contrasting the presentation times of baseline and post for the Cathodal condition returned no significant activation in the ROIs for the Baseline>Post contrast. Contrasting Baseline<Post returned significant activation in the left Precentral (M1) at an uncorrected p value < 0.001 (see Table 9, Figure 8).

Table 9. Region of Interest Random Effect Group Analysis Cathodal*. T Values and MNI Coordinates are the peak values for the local maximum of each cluster. Abbreviations: FWE, familywise error; MNI, Montreal Neurological Institute; M1, motor cortex.*

Region	P Value (uncorrected)	T Value	MNI x, y, z Coordinates
Left M1	0.019	4.19	[-45 -10 29]

Left M1

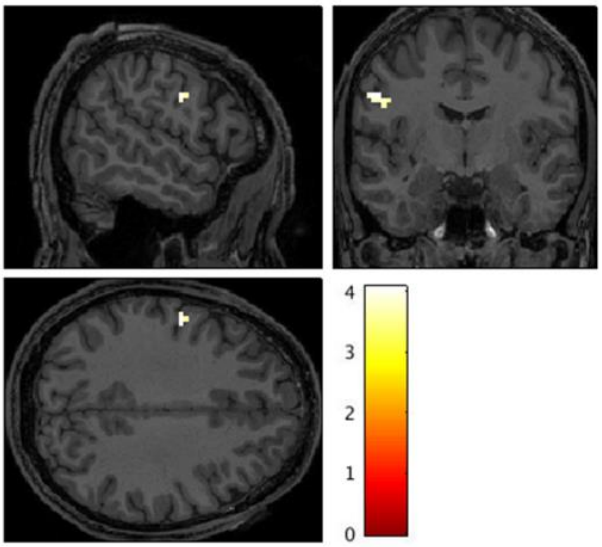

Figure 8. ROI Analysis of Cathodal tDCS Stimulation for the contrast Baseline<Post. ROI analysis showing significant brain activity in Left M1 at an uncorrected p value < 0.001.

fMRI Group Level Anodal Condition Post-Hoc Assessment

ROI analysis on anodal session data revealed no significant activity for the contrast of Baseline>Post. ROI analysis for the contrast Baseline<Post revealed significant activity at an uncorrected p value < 0.001 for the Left Precentral and significant activity in the SMA for an FWE corrected p value < 0.05. (Table 10, Figure 9).

Table 10. Region of Interest Random Effect Group Analysis Anodal*. T Values and MNI Coordinates are the peak values for the local maximum of each cluster. Abbreviations: FWE, familywise error; MNI, Montreal Neurological Institute; M1, motor cortex; SMA, Supplementary Motor Area.*Peak Level Significance.*

Region	**P Value (uncorrected)**	**T Value**	**MNI x, y, z Coordinates**
Left M1	<0.001 *	3.78	[-51 -7 32]
Region	**P Value (FWE corrected)**	**T Value**	**MNI x, y, z Coordinates**
SMA	0.049	3.63	[-6 20 53]

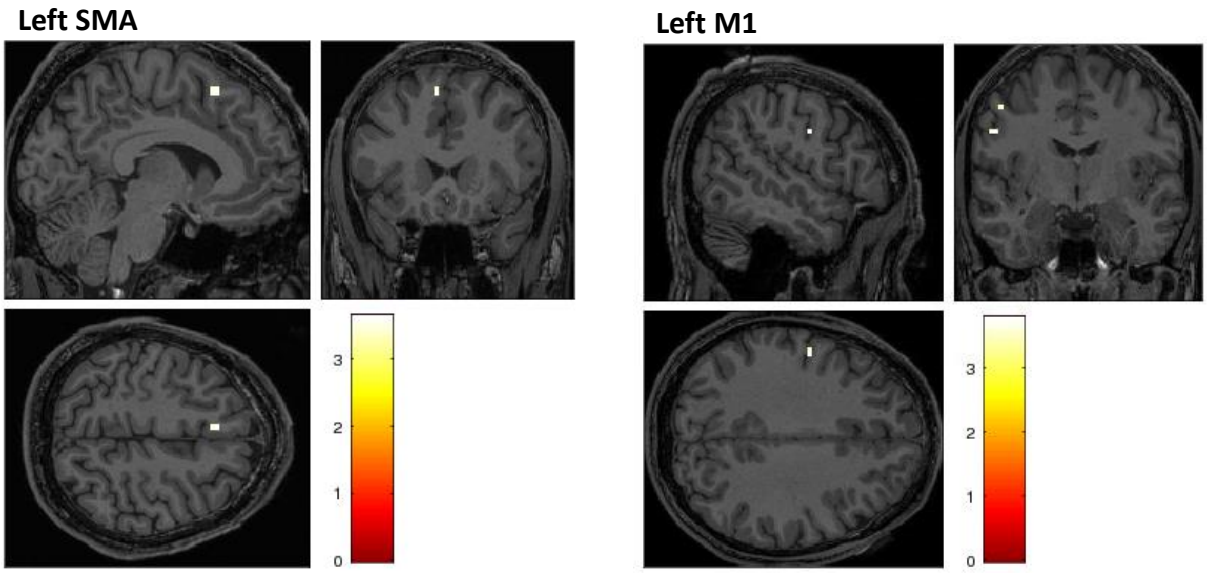

Figure 9. ROI Analysis of Anodal tDCS Stimulation for the contrast Baseline<Post. *ROI analysis showing significant brain activity in Left M1 at an uncorrected p value < 0.001, and the Left SMA at an FWE corrected p value < 0.05.*

tDCS Post Screening Questionnaire Analysis

Analysis from the tDCS Post Screening Questionnaire looked to assess whether participants could correctly identify which type of stimulation they were given – real (anodal or cathodal) or sham. This analysis showed that from a total of 24 testing session scenarios, on 13 occasions participants correctly identified the stimulation they

were given whilst on 11 occasions they were not (Table 11). Regarding sham stimulation, from a total of 8 sham sessions, 2 of these were correctly identified as sham. Regarding actual stimulation, from a total of 16 real tDCS sessions, 11 were correctly identified as real tDCS stimulation.

	Delivered: Real	**Delivered: Sham**
Thought: Real	**11**	**6**
Thought: Sham	**5**	**2**

Table 11. tDCS Post Screening Questionnaire Results. *Across the rows this table dictates which type of stimulation was actually delivered to participants – Real (anodal or cathodal) and Sham. Down the columns indicates whether participants answered thinking they had received either Real (anodal or cathodal) or Sham stimulation. Across the diagonal from top left to bottom right indicates a correct response. Across the diagonal from top right to bottom left indicates an incorrect response. The total number of scenarios was 24 (8 participants x 3 sessions).*

The questionnaire also asked questions regarding the side effects experienced by the participants including: burning, pain, tingling, itching, dizziness and mental fatigue. These asked about the intensity felt and also required participants to comment on the duration for which they found these effects to occur over. From these the only significantly different effect between stimulation types was the duration of tingling which had a p value of 0.037 and F statistic of 7.121 (Table 12).

Stimulation	Duration Average (mins)
Sham	0.5
Cathodal	1.2
Anodal	4.1

Table 12. Results from tDCS Screening Questionnaire regarding Tingling Duration. *Average results across each session for the duration (mins) of intensity experienced from tDCS for each of the stimulations: Sham, Cathodal, Anodal. NB: Number of participants being 7 from 8 as 1 participant did not answer this field. Significant P value for within subject contrast of 0.037, F statistic 7.121.*

Results from the amount of sleep participants had resulted in participants on average getting equal amounts of sleep across the 3 sessions (Table 13).

Stimulation	Amount of Sleep (hours)
Sham	7.5
Cathodal	7.4
Anodal	7.4

Table 13. Results from tDCS Screening Questionnaire regarding Hours of Sleep. *Average results across each session for the duration (hours) of sleep participants got before each of the sessions: Sham, Cathodal, Anodal. NB: Number of participants being 8 from 8. P Value non-significant at > 0.05.*

Discussion

Within this study a variety of assessment techniques were used in order to investigate the effect of tDCS stimulation over M1 on the motor areas of the brain. These assessments included a behavioural motion tracking analysis using a joystick which was able to give insight into motor performance through measuring parameters such as peak acceleration; neurophysiological MEP characterisation to evaluate changes in cortical excitability as well fMRI in order to investigate changes in brain activity.

Motion Tracking Behavioural Analysis

No significant changes in the motion tracking parameter of peak acceleration were found within the study after tDCS was applied. As can be seen in Table 4, each scenario of anodal, cathodal and sham resulted in the same group average outcome of a slight, statistically non-significant decrease in peak acceleration. It was expected however that anodal stimulation would increase cortical excitability and result in enhanced motor performance and peak acceleration. For cathodal stimulation it was expected that cortical excitability would decrease owing to a decrease in peak acceleration. Sham stimulation was hypothesised to have no effect on performance and would capture any effects of placebo or time e.g. participant fatigue.

As the results from the anodal and cathodal tDCS stimulation were not significantly different from sham stimulation this could suggest that anodal and cathodal stimulation were not having any behavioural effects on motor performance. There was also a similar directional decrease in group peak acceleration across all three stimulation polarities which could be due to time factors such as participant fatigue throughout

scanning, lack of mental stimulation and boredom. Results from the tDCS post stimulation screening questionnaire found that on average participants got on average around 7-7.5 hours of sleep across all sessions (Table 13) which is within the recommended sleep for a young adult (Hirshkowitz et al., 2015). With regards to mental fatigue across all of the stimulation blocks it was found that the average rating for this factor was between 'extremely weak (could not feel/detect side effect)' and 'very weak' [results not shown]. The physical fatigue of the hand was rated on average for all sessions below 'Very little fatigue (no cramping, slight tiredness of the muscles)' [results not shown]. There was no question within the screening form to account for either boredom or tiredness related to the study duration and task which could have played an influence on results between baseline and post. Despite being well slept the night before the study, the simplicity of the task and long wait times between Baseline and Post could have created drowsiness within the participants.

It could also be the case that due to the simplicity of the task there is no room for task improvement for a healthy volunteer on their dominant hand. Previous studies have also shown that tDCS of the dominant hand does not have any significant behavioural effects on motor performance of healthy volunteers (Boggio et al., 2006) whilst other studies have solely tested the non-dominant hand (Rroji, Van Kuyck, Nuttin, & Wenderoth, 2015) There is also the potential to look at other motion parameters, as whilst this study and others have looked at peak acceleration (Koyama, Tanaka, Tanabe, & Sadato, 2015), other studies have looked into other motion parameters such as reaction time and dexterity and found significant behavioural changes (Devanathan & Madhavan, 2016; Koyama et al., 2015). Further analysis of motion data could therefore

look to explore whether tDCS had any significant effects on other motion tracking parameters.

Lack of power due to a small sample group of 7 participants (Table 3) could also contribute to no significant results being returned within the analysis. On an individual level (Figure 3) our data shows high variability across participants and a clear differentiation between non-responders and responders is not inherently obvious. In certain instances however an upward trend of peak acceleration indicated practise effects across sessions, with the worst motor performance in the first chronological session and best performance in the last chronological session (Appendix 4). This was only observed for one participant however and with pseudo-randomisation of sessions it is unlikely that practise effects had any significant impact on either the main effect or the Baseline-Post results.

It is most likely within this study that the motion tracking analysis was not sensitive enough to detect changes in motor function of healthy participants, especially on the dominant hand due to potential ceiling effects. Whilst similar devices and methodologies have been used and designed within the literature (Meinhardt & Müller, 2001) it is possible that increasing task difficulty or looking at alternative motion parameters might enable detection of behavioural motor changes. Regarding healthy volunteers, a more complex task might eliminate any ceiling effect that is evident with this task. With an outlook towards patient rehabilitation however, a simple task easily interpreted and achievable for patients who either fully lack or have very inhibited movement would be more preferable.

MEP Neurophysiological Analysis

No significant changes in MEP peak amplitude were found within the study after tDCS was applied. Overall in the group analysis it was found that anodal and cathodal stimulation non-significantly decreased peak amplitude values whilst sham stimulation increased peak acceleration values (Table 6). It was expected that anodal stimulation would increase cortical excitability and result in increased peak amplitudes of MEPs, cathodal stimulation would decrease cortical excitability and result in decreased peak amplitudes of MEPs whilst sham stimulation would have no significant effect on MEP peak amplitude. With a decreased sample size of only 4 participants (Table 5), this is too low to draw valid statistical conclusions and results should hence be interpreted with caution. Collecting data from more participants would be required in order to characterise accurate responses to stimulation.

It is well characterised within the literature that TMS is a valid method for assessing cortical excitability and integrity (Kobayashi & Pascual-Leone, 2003; Paulus, Peterchev, & Ridding, 2013) with it often being used as a diagnostic tool in assessing corticospinal tracts and motor cortical function (Auriat, Neva, Peters, Ferris, & Boyd, 2015; Lapitskaya et al., 2013; Li et al., 2015).
Within the research environment TMS can be used in order to analyse motor cortex plasticity through obtaining motor threshold levels and stimulating at an upwardly adjusted intensity. Motor threshold is defined as the intensity that gives 50% MEP responses at an amplitude of 50μV.Commonly in the literature this is an intensity of 120% the motor threshold (Jung, Bungert, Bowtell, & Jackson, 2016; Rosenkranz, Nitsche, Tergau, & Paulus, 2000) with a study specifically on DOC patients using a stimulation of 120% the motor threshold to assess cortico-spinal integrity (Lapitskaya

et al., 2013). This method has been used within the literature on the motor cortex in order to analyse effects of tDCS and confirmed the differential effects of tDCS polarity (Rossini et al., 1994).

There is still debate however, around how TMS works on a neurophysiological level, with studies highlighting the impact of many factors such as coil orientation and conductance on MEP amplitude (Souza et al., 2017; Udupa & Chen, 2013). Other factors such as skull thickness and white matter characteristics can also have an effect on individual differences in motor threshold and responsiveness to TMS (Herbsman et al., 2009). These factors are unlikely to influence our study results due to a within subject design which would account for individual response differences. From our study it is clear to see between subject differences in MEP amplitude (Figure 4) with participant C11 having extensively larger MEP amplitudes that the other 3 participants included within analysis. As different motor threshold values were calculated between participants it could be possible that inaccuracies occurred in deducing correct motor threshold values for certain participants. This could have thus resulted in MEP amplitudes being either lower or higher than normal for 120% motor threshold. It is likely therefore that the stimulation intensity for participant C11 was calculated to be too high giving larger than normal MEP responses above those for 120% motor threshold. This and sensitivity of TMS MEP procedures to coil movement and orientation could have contributed to the expected neurophysiological outcome from TMS stimulation not being obtained. Further analysis not able to be conducted within this study could look to investigate errors in certain coil orientation and positioning parameters such as twist area and distance from target (M1 motor hotspot).

Furthermore, it is generally accepted that anodal stimulation enhances cortical excitability and cathodal decreases cortical excitability of the motor cortex (Rosenkranz et al., 2000; Stagg et al., 2009). This is not a fully linear relationship however as the study by Jamil et al., 2017 found that increasing stimulation intensity from 0.5-2mA did not linearly correlate to alterations in motor cortex excitability as measured by TMS. This study found that 20 minutes of anodal stimulation of 1mA was found to have significant differences from sham, whilst 20 minutes of anodal stimulation of 1.5mA was not (Jamil et al., 2017). For cathodal stimulation Jamil et al., 2017 showed that stimulations ≤ 15 minutes and ≥ 60 minutes had significant differences from sham whilst stimulation of durations between these values did not. At 1mA cathodal stimulation for 20 minutes, although non-significant, decreases in MEP amplitude were observed, whilst 20 minutes of cathodal stimulation at 1.5mA showed slight increase in MEP amplitude (Jamil et al., 2017). At our stimulation of 1mA for 20 minutes therefore, it would be expected that anodal stimulation would enhance performance whilst cathodal stimulation would decrease performance but perhaps non-significantly.

Variation in stimulation effects, whilst heavily dependent on stimulation and duration, can also be attributed to the site of stimulation and electrode configuration (Salvador, Wenger, Nitsche, & Miranda, 2015). As there are many different methodologies for carrying out tDCS stimulation, it can often make results between studies trickier to compare, with a multitude of factors paying influence to a final outcome. Motor cortex tDCS is however repeatedly confirmed within the literature to have significant neurophysiological effects (Antal, Terney, Kühnl, & Paulus, 2010; Boggio et al., 2006; Rroji et al., 2015). Whilst the neural mechanisms of action of tDCS are still unknown and

under debate therefore (Venkatakrishnan & Sandrini, 2012), effects on the underlying cortex when stimulating M1 are well characterised (Kwon et al., 2008). It is likely therefore that confounding factors in carrying out MEP data collection or electrode configuration could have influenced the finding of non-significant MEP responses and more data collection would be required to draw valid statistical conclusions from the study.

fMRI Group Level Baseline Move>Rest Analysis

From Group Level random effects analysis of baseline data it was found that no brain areas were significantly more active in the Move<Rest contrast. It was found from Move>Rest analysis however that all of the predicted motor brain areas had significantly increased activity at an FWE corrected p value < 0.05, with the largest p value being p < 0.01 for the Thalamus (Table 7). When carrying out a motor task there are several brain areas that have been well defined and characterised to be involved in movement production (Weiller et al., 1996). These include the Motor Cortex (M1), Supplementary Motor Area (SMA), Thalamus and Cerebellum – specifically areas 4 and 6 (Groiss & Ugawa, 2013; Lindenberg, Nachtigall, Meinzer, Sieg, & Floel, 2013). These findings therefore confirm the efficacy of the task in eliciting correct and expected motor responses within the brain. This also replicates previous studies by Fernández-Espejo et al., 2015 and Osborne, Owen, & Fernández-Espejo, 2015 which found corresponding effects using similar tasks and statistical analysis. Follow on analysis could then look to compare the Baseline and Post conditions to see what effect tDCS was having on this motor activity.

fMRI Group Level Interaction Analysis Stimulation*Presentation Time

Analysis of the interaction between stimulation polarity and the time points of baseline and post enables assessment of significant activity changes in the tested brain areas. From the ROI analysis it was found that significant brain activity changes were found in the Cerebellum Area 6 at an FWE corrected p value of <0.05 (Table 8). As there are cortical projections going from the Thalamus to the Cerebellum Areas 4 and 6 in the motor network (Groiss & Ugawa, 2013) this result indicates that the stimulation given over M1 had widespread network effects which has been previously confirmed within the literature (Polanía, Paulus, & Nitsche, 2012). The cerebellum itself has been targeted as a potential site of stimulation for improving motor function (Hahn, Paik, & Ph, 2015; Hiraoka, Horino, Yagura, & Matsugi, 2010) due to its role in the motor network and crucial involvement in motor ability (Guldenmund et al., 2016; Lemon & Edgley, 2010).

Whilst significant changes in brain activity were seen for the Cerebellum Area 6, no significant changes in brain activity were observed for the M1, SMA, Thalamus nor the Cerebellum Areas 4 and 5. As shown by Kwon et al., 2008 tDCS over M1 elicits changes in the underlying motor cortex detectable with fMRI analysis and MEP analysis. Our results however did not show significant increase in M1 brain activity following tDCS stimulation for the Baseline<Post comparison. It did however show significant changes in brain activity for a distal brain area involved in motor movements. This was an unexpected result which could be explained through lack of power to show significant results. Looking at individual brain activity data, it did occur that for some participants no significant brain activity was found in either the baseline or the post scenario. Whilst in a larger study non-responders to a stimulation protocol would not greatly effect

overall results, our study with only 8 participants could be greatly impacted by this factor. It is also possible that brain activity may have significantly altered across only 1 stimulation polarity, whilst we were looking to compare the interaction of all 3 polarities across the presentations of baseline and post. Further analysis could also look closer at the raw responses in BOLD signal for each stimulation polarity to see if the BOLD signal was modulated and to what extent. I could be for instance that there was a change in the BOLD signal in expected ROIs however this was not large enough to be statistically significant at cluster level.

Post-hoc analysis of each of the stimulation polarities – anodal, cathodal and sham - is therefore necessary to understand exactly what type of interaction this is and directionality of brain activity responses between baseline and post conditions. Post hoc analysis may also give insight into how brain activity changed in the other hypothesised ROIs across the different stimulations and give insight into why there was no significant interaction for other ROIs.

fMRI Group Level Post-Hoc Stimulation Analysis

From comparison of baseline and post contrasts it became apparent that there was no significant cluster level activation for either the Baseline>Post nor the Post>Baseline contrasts. This is in line with the hypothesis that sham stimulation should elicit no significant changes in brain activity as there is no stimulation applied. Sham stimulation was administered in a confirmed manner which has been successfully shown to blind participants to the type of stimulation given (Woods et al., 2016) For 1mA tDCS with an electrode size of 25 cm2, this method has been shown to reliably blind participants

(Gandiga et al., 2006; Ambrus et al., 2012).. The way this is done is by administering 30 seconds of anodal stimulation before turning off the stimulation entirely. Results from the tDCS post stimulation screening questionnaire confirm that participants were successfully blinded as participants were not able to correctly identify whether they were indeed receiving real or sham tDCS. Only 2 of 8 participants correctly identified application of sham stimulation and even with this correct response they were 'completely uncertain' and 'uncertain' as to whether they had responded correctly [results not shown]. This was also shown in the stimulation scenario with around the same ratio answering 'Yes to having had stimulation, and responses certainty averaging in the response range between 'uncertain' and 'certain' [results not shown]. These results therefore confirm that sham stimulation was able to successfully blind participants and hence could aid as a comparative means for anodal and cathodal stimulation.

From analysis of cathodal stimulation it was found that there were no significant changes in brain activity for the contrast of Baseline>Post. For the contrast of Baseline<Post however it was found that activity within the Left M1 (Precentral Left) area had increased brain activity at an uncorrected p value of 0.019 for cluster level. This was the opposite way to expected as it is shown within the literature that cathodal stimulation decreases cortical excitability and decreases brain activity (Rosenkranz et al., 2000).

As shown in the study conducted by Jamil et al., 2017 however there was no significant change in MEP responsiveness at a stimulation duration of 20 minutes at 1mA which

was used in our study. It could be the case therefore that the cathodal stimulation was no significantly modulating underlying cortical excitability nor does it do so in a linear predictable manner and our result at an uncorrected p value could purely be a false positive result. This might have contributed to there being no significant changes in brain activity at an FWE corrected p value < 0.05 and only returning one significant effect in the area underlying the electrode at an uncorrected p value < 0.001. Whilst the neural workings for anodal stimulation are fairly well characterised, cathodal stimulation is less known about and less well characterised (Kwon et al., 2008). It is also found to be the case that although the majority of motor tDCS studies for cathodal stimulation to be inhibitory, there are exceptions to this with studies occasionally finding the hypothesis that anodal stimulation is excitatory, and cathodal stimulation is inhibitory to be falsified (Jacobson, Koslowsky, & Lavidor, 2012). It is worth noting also that although this activity was observed, the p value was set to an uncorrected p value < 0.001 with a sample size of only 8 participants. More participant data would be necessary in order to reach an adequate study power and confirm statistically significant and relevant results.

From the analysis of anodal stimulation there was found to be no significant changes in brain activity for the contrast of Baseline>Post stimulation. This fits with our hypothesis that anodal has excitatory effects on the underlying brain area and thus should have no significant decreases in brain activity going from the baseline to the post condition. Significant changes in brain activity were found for the Baseline<Post condition however in the Left M1 and Left SMA. For the Left M1 significant brain activity was found at an uncorrected p value < 0.001 whilst activity in the SMA was found to be

significant at an FWE corrected p value < 0.05. This activity in M1 was as expected as this was the brain area directly under the stimulation electrode which is shown to cause increased cortical excitability (Kwon et al., 2008). With increased cortical excitability it thus follows that a task involving activation of this brain area will result in an increased brain activity response as detected by fMRI.
The SMA is the motor area neighbouring M1 which has been shown to be involved in movement preparation and motor planning (Kasess et al., 2008).

It is possible also that movement within the scanner could have offset results and created significant results where there were none. Through running a movement regressor code, 3 participants were shown to have significant head movements more than 2.5 mm and more that 0.035 radians [results not shown]. This totalled 3 from 24 scanning sessions with 2 of these movements occurring in anodal testing sessions. Further analysis, not able to be completed within this study, could look to remove the specific returned volumes that had large head movements in Independent Component Analysis which is able to remove regressors from the GLM. With these movements only occurring over 1-2 volumes from these few sessions however it is unlikely that this would have a significant effect on overall group results. Effects may not have been detected in other ROI areas such as the Thalamus or Cerebellum due to a lack of power to detect these changes or activity changes not being large enough to detect.

Whilst results from this study are underpowered, there are promising results in anodal stimulation increasing brain activity in the stimulated M1 and ipsilateral SMA for the Baseline<Post condition as well as the task eliciting correct brain activity in all expected

ROIs. Whereas the cathodal results are less confirmed and occur in the opposite direction to expected, cathodal tDCS is less characterised within the literature and for patient rehabilitation in the context of DOC, it would be enhancement of motor cortex excitability that would be desired.

tDCS has also already been safely and effectively used in DOC patients (Angelakis et al., 2014; Thibaut, Bruno, Ledoux, Demertzi, & Laureys, 2014). This study would hence benefit from increasing participant sample size in order to better characterise statistically significant results in order to fully assess whether tDCS could be a potential avenue to explore for DOC patient motor rehabilitation. It is also important to characterise effects of tDCS on motor activity in healthy participants as most tDCS studies on healthy participants have used motor training (Hashemirad, Zoghi, Fitzgerald, & Jaberzadeh, 2016). A confirmed motor task not involving motor training used alongside tDCS in healthy volunteers would therefore be a valuable resource that could give promise to motor rehabilitation in DOC patients unable to partake in motor training regimes.

Conclusion

This study found that whilst tDCS over M1 elicited no significant interactional effects on either behavioural motion tracking nor MEP data, significant interactional and condition specific effects were found within the fMRI brain imaging data. From analysis of 'Move' vs 'Rest' contrasts on group baseline data it was able to replicate previous results which produced increased brain activity in all expected ROI motor areas for the 'Move' condition. It also found significant interaction effects in the Cerebellum motor areas for the two factors of stimulation (anodal, cathodal, sham) and presentation (baseline, post). Through looking at each stimulation set separately and contrasting Baseline vs. Post it was further found that significant changes in brain activity were experience with anodal and cathodal tDCS but not with sham tDCS. For cathodal stimulation significantly increased brain activity in the stimulated left M1 was found for the contrast Baseline<Post, which was opposite to the expected decrease in activity going from Baseline to Post condition (Baseline>Post). For anodal stimulation it was found that brain activity significantly increased in the Post condition (Baseline<Post) in the stimulated left M1 and corresponding ipsilateral left SMA. No significant changes in brain activity were found in the other ROIs of the Thalamus or Cerebellum (except in the interactional analysis) which could have been due to low sample sizes decreasing power or modulatory effects being too weak to be detected. These results show promise in the notion that tDCS brain stimulation has network wide effects and could possibly be a potential technique to explore for motor rehabilitation in DOC patients.

Word Count: 10,224 (excl. figures, textboxes, references)

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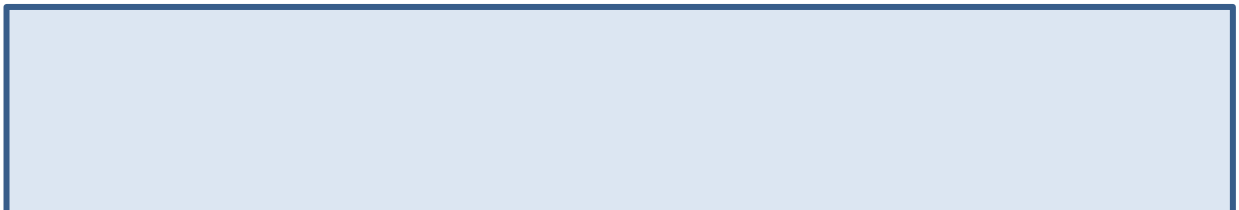
Appendix



Appendix Figure 1. Summary of Data Losses Across throughout study. *This table summarises where data was lost across each of the study sessions across participants. A red X indicated where data is lost and written beside is for which study methodology. MEP: Motor Evoked Potential Data, Motion: Motion tracking Data.*

	Sham		Cathodal		Anodal	
Participant	**Baseline**	**Post**	**Baseline**	**Post**	**Baseline**	**Post**
C03	**X MEP**					
C04			**X Motion**	**X Motion**	**X MEP**	**X MEP**
C05	**X MEP**	**X MEP**				
C07				**X MEP**		
C09						
C10						
C11						
C12						

Appendix Figure 4. Evidence for Practise effects for Participant C09*. Baseline and Post Peak acceleration (units: μV) values for participant C09 across all stimulation sessions here ordered chronologically showing a general trend increase from around 300μV* to *800μV.*

	Session 1	**Session 2**	**Session 3**
Baseline	298.1	485.3	828.2
Post	454.7	531.7	744.0

Appendix Figure 2. MRI and Brain Stimulation (tDCS and TMS) Screening forms given to participants before recruitment for testing sessions.

MRI SAFETY SCREENING QUESTIONNAIRE

EVERYONE must fill out this form BEFORE entering the MRI suite. The MRI suite has a very powerful magnetic field that may be hazardous to those with metallic, electronic, magnetic or mechanical implants or devices. All information will be kept strictly confidential.

BUIC
Birmingham University Imaging Centre

Name:		**Date of Birth:**	
Email:		**Tel No:**	

Section A – To be completed by EVERYONE entering the MRI suite

Please indicate if you have any of the following:	YES	NO	If yes please explain
Cardiac Pacemaker, pacing wires or defibrillator			
Aneurysm clip (metal clips put around blood vessels during surgery)			
Electrical Stimulator for nerves, bone or brain			
Ear or Eye implants (e.g. cochlear implants)			
Implanted insulin, drug or infusion pump			
Stent, catheter, coil or filter in any blood vessel			
Orthopaedic hardware (e.g. artificial joints, metal plates, screws)			
Any other type of prosthesis or implant			
Gun pellets, shrapnel, bullets or metal fragments			
Any surgery or an operation			

Section B – Complete ONLY if you are being scanned or intend to go inside the scanner room

Please answer the following questions carefully	YES	NO	Staff Notes
Have you had an MRI scan before?			
Are you claustrophobic?			
Have you ever been a welder, machinist, grinder or worked with metal without eye protection?			
Do you suffer from any medical condition that may be relevant (e.g. epilepsy, diabetes, asthma)?			
Do you have any tattoos or body piercings (other than earrings)?			
Do you wear dentures, a dental plate or a brace (not fillings)?			
Do you have any transdermal skin patches (e.g. nicotine patch)?			
(*Females only*) Are you or could you be pregnant?			
Please state your weight (kg)			
Other information (e.g. spectacle prescription)			

Please tick the boxes before being scanned or going inside the scanner room

I confirm that the above information is accurate to the best of my knowledge. ☐

I will remove all metal including mobile phones, keys, watches, coins, credit cards, body piercings, jewellery, false teeth, hearing aids etc before entering the scanner room. (Lockers available in waiting room.) ☐

I acknowledge that BUIC has taken reasonable precautions to screen for potential difficulties and is not liable for any event that might result from incorrect answers to the above. ☐

Signed:		Date:	

Form verified by (*Authorised Personnel only*):

Print Name:		Signed:		Date:	

Staff Note: This form is only valid for six months from date of initial screening

School ology, University of Birmingham
Edgba ingham B15 2TT
Teleph (0) 121 414 4949

If you agree to take part in this study, please answer the following questions. The information you provide is for screening purposes only and will be kept completely confidential.

CIRCLE or CROSS OUT

Have you ever suffered from any neurological or psychiatric conditions? . . . . . YES / NO
If YES please give details (nature of condition, duration, current medication, etc)

. . . . . .

Have you ever suffered from epilepsy or febrile convulsions in infancy or had recurrent fainting spells? . . . . . YES / NO

Have you ever lost consciousness or fainted? . . . . . YES / NO

If YES when did this (last) happen and what caused it:

. . . . . .

Does anyone in your immediate or distant family suffer from epilepsy? . . . . . YES / NO
If YES please state your relationship to the affected family member.

. . . . . .

Do you suffer from migraine? . . . . . YES / NO

Have you ever undergone a neurosurgical procedure (including eye surgery)? . . . . . YES / NO
If YES please give details.

. . . . . .

Do you currently have any of the following fitted to your body? . . . . . YES / NO
- Heart pacemaker
- Cochlear implant
- Medication pump
- Surgical clips

Are you currently taking any unprescribed or prescribed medication? . . . . . YES / NO
If YES please give details.

. . . . . .

Have you ever suffered from brain injury or brain trauma? . . . . . YES / NO

If YES please give details.

. . . . . .

Have you drunk more than 3 units of alcohol in the last 24 hours? . . . . . YES / NO

Have you drunk alcohol already today? . . . . . YES / NO

Have you had more than one cup of coffee, or other sources of caffeine, in the last hour? . . . . . YES / NO

Have you used recreational drugs in the last 24 hours? . . . . . YES / NO

Did you have very little sleep last night? . . . . . YES / NO

Have you already participated in a TMS/TDCS experiment today? . . . . . YES / NO

Have you participated in more than 2 TMS/TDCS experiment in the last 6 months? . . . . YES / NO

Are you taking any prescribed (prescribed by your GP or a hospital) or unprescribed drugs? . . . . . YES / NO

If YES please give details.

. . . . . .

School
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Teleph
ology, University of Birmingham
ingham B15 2TT
(0) 121 414 4945

Is there any chance that you could be pregnant?	YES / NO
Are you currently undergoing anti-malarial treatment?	YES / NO
Are you left or right handed?	Left / Right
Date of Birth ____/____/____	

I confirm that the above information is accurate to the best of my knowledge.

Name in capital letters:	
Signature:	Date:
This form has been verified by (Staff only): Print Name: Signature:	Date:

Appendix Figure 3. The tDCS Perceptual Scale Questionnaire given to participants at the end of each testing session.

tDCS Perceptual Scale

We would like to know how you experienced the task and tDCS. You may have received real tDCS or a placebo version that mimics the sensations evoked by real tDCS.

1) Please rate the difficulty level of the task from 0-10, where:

0= Extremely easy
1 =
2 = Very Easy
3 =
4 = Easy
5 =
6 = Challenging
7=
8= Very challenging
9=
10= Extremely challenging

2) Please rate your attention level (how focused you were on the task) from 0-10, where:

0= Extremely unfocused
1 =
2 = Very unfocused
3 =
4 = Unfocused
5 =
6 = Focused
7=
8= Very focused
9=
10= Extremely focused

3) Please rate the fatigue level of your hand from 0-10, where:

0= No fatigue (no pain, no cramping, no tiredness of muscles)
1 =
2 = Very little fatigue (no cramping, slight tiredness of muscles)
3 =
4 = Little fatigue (slight cramping, tiredness of muscles)
5 =
6 = Fatigue (cramping, tiredness of muscles, slight pain)
7=
8= High fatigue (cramping, pain, hand use difficult)
9=
10= Very high fatigue (cramping, pain, hand use very difficult)

4) Please rate the overall intensity level of tDCS (how strong it felt to you) from 0-10, where:

0= Extremely weak (could not feel/detect anything)
1 =
2 = Very weak
3 =
4 = Weak
5 =
6 = Intense
7=
8= Very intense
9=
10= Extremely intense (intolerable)

5) Please rate the distraction level due to tDCS from 0-10, where:

0= Not distracted at all due to tDCS
1 =
2 = Minimally distracted due to tDCS
3 =
4 = Somewhat distracted due to tDCS
5 =
6 = Distracted due to tDCS
7=
8= Very distracted due to tDCS
9=
10= Extremely distracted due to tDCS

6) Please rate your level of discomfort due to tDCS from 0-10, where:

0= No discomfort
1 =
2 = Very little discomfort
3 =
4 = Little discomfort
5 =
6 = Discomfort
7=
8= High discomfort
9=
10= Very high discomfort

7) tDCS can be associated with side effects, such as itching and burning. The most common side effects are listed in the table below. For these side effects, please write the intensity to which you experienced that side effect in the table below from 0-10 and specify how long this side effect lasted in minutes. Please use a fraction if the effect lasted less than 1 minute (e.g. write '0.5' to specify 30 seconds).:

0= Extremely weak (could not feel/detect side effect)
1 =
2 = Very weak
3 =
4 = Weak
5 =
6 = Intense
7=
8= Very intense
9=
10= Extremely intense (intolerable)

Side effect	**Rating**	**Duration**
Tingling		
Pain		
Burning		
Itching		
Dizziness		
Mental Fatigue		

8) Did you receive REAL tDCS? If you believe that you received real tDCS, please select 'YES'. If you believe that you did not receive real tDCS (i.e. received the placebo version) please select 'NO'.

YES NO

9) How certain are you that your response to the question above is correct? (Please rate from 0-10, as below)

0= I am completely uncertain.
1 =
2 = I am very uncertain.
3 =
4 = I am uncertain.
5 =
6 = I am certain.
7=
8= I am very certain.
9=
10= I am completely certain.

10) Regardless of whether you thought that you received real tDCS or not, what effect do you expect that real tDCS would have on performance in this task? (Please rate from 0-10, as below)

0= tDCS will severely hurt performance
1 =
2 = tDCS will hurt performance
3 =
4 = tDCS will slightly hurt performance
5 = tDCS will have no effect on performance
6 = tDCS will slightly benefit performance
7=
8= tDCS will benefit performance
9=
10= tDCS will highly benefit performance

11) Have you ever had brain stimulation (including transcranial magnetic stimulation and / or transcranial direct current stimulation) before today? Circle one. If yes, please report how long ago it was and what kind of brain stimulation it was.

Yes No

12) How many hours of sleep did you have last night? _____