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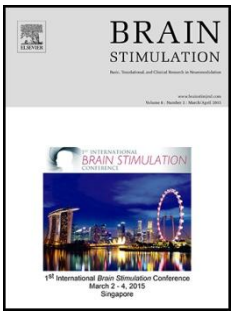

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Intra-subject consistency and reliability of response following 2mA transcranial direct current stimulation

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Highlights

- Investigates intra-subject consistency and reliability of 2mA anodal and cathodal tDCS
- Anodal and cathodal tDCS exhibit poor reliability at an individual level (i.e., they are not consistent across sessions)
- Sham stimulation is moderately reliable across sessions

Abstract

Background

Transcranial direct current stimulation (tDCS) is a popular non-invasive brain stimulation technique that has been shown to influence cortical excitability. While polarity specific effects have often been reported, this is not always the case, and variability in both the magnitude and direction of the effects have been observed.

Objective/ hypothesis

We aimed to explore the consistency and reliability of the effects of tDCS by investigating changes in cortical excitability across multiple testing sessions in the same individuals. A within subjects design was used to investigate the effects of anodal and cathodal tDCS applied to the motor cortex. Four experimental sessions were tested for each polarity in addition to two sham sessions.

Methods

Transcranial Magnetic Stimulation (TMS) was used to measure cortical excitability (TMS recruitment curves). Changes in excitability were measured by comparing baseline measures and those taken immediately following 20minutes of 2mA stimulation or sham stimulation.

Results

Anodal tDCS significantly increased cortical excitability at a group level, whereas cathodal tDCS failed to have any significant effects. The sham condition also failed to show any significant changes. Analysis of intra-subject responses to anodal stimulation across four sessions suggest that the amount of change in excitability across sessions was only weakly associated, and was found to have poor reliability across sessions (ICC=0.276). The effects of cathodal stimulation show even poorer reliability across sessions (ICC=0.137). In contrast ICC analysis for the two sessions of sham stimulation reflect a moderate level of reliability (ICC=.424).

Conclusions

Our findings indicate that although 2mA anodal tDCS is effective at increasing cortical excitability at group level, the effects are unreliable across repeated testing sessions within individual participants. Our results suggest that 2mA cathodal tDCS does not significantly alter cortical excitability

immediately following stimulation and that there is poor reliability of the effect within the same individual across different testing sessions.

Key words

Transcranial Direct Current Stimulation (tDCS), Transcranial Magnetic Stimulation (TMS), Motor Cortex, Cortical Excitability

Introduction

Transcranial Direct Current Stimulation (tDCS) is an increasingly popular non-invasive brain stimulation technique, which is known to alter cortical excitability for periods that outlast the duration of stimulation. These effects have been reported to be polarity specific; specifically cortical areas beneath the anode are reported to increase in excitability whereas the reverse is true for locations below the cathode (Nitsche & Paulus, 2000; Nitsche et al., 2005). Although this polarity specific pattern has been found in many studies, this is not always the case, and variability in the magnitude and direction of the effects are occasionally reported. Some variability between studies is likely caused by parameter selection; for example 20 minutes of 2mA cathodal stimulation has been found to increase cortical excitability rather than decrease it (Batsikadze et al., 2013), and increasing the duration of 1mA anodal stimulation to 26 minutes has led to findings of inhibition (Monte-Silva et al., 2013). The measures used to quantify effects are also likely to influence findings (Horvath et al., 2015; Jacobson et al., 2012), as are factors such as the electrode montage used (Miranda et al., 2006; Nitsche & Paulus, 2000). These aspects are important sources of variability when comparing across studies, however, variability also occurs within studies in which the parameters are held constant. For example, Wiethoff et al. (2014) found substantial inter subject variability in response to both 2mA anodal and cathodal stimulation.

Variability in response to non-invasive brain stimulation is not unique to tDCS, and has also been reported in other techniques such as paired associative stimulation (PAS) and intermittent theta burst stimulation (iTBS) (Fratello et al., 2006; Hamada et al., 2013; Lopez-Alonso et al., 2014; Muller-Dahlhaus et al., 2008). Given individual differences in anatomy including skull shape, thickness and density; and additional factors such as baseline neuronal states, it is perhaps not surprising that these techniques do not yield identical

results across individuals. However, developing an understanding of the factors that may predict this variability is a critical step in increasing the reliability and usefulness of such techniques for use within both research and therapeutic contexts.

To date, a number of inter-subject factors have been identified as influencing tDCS including anatomical structure (Bikson et al., 2012; Datta et al., 2012) age (Fujiyama et al., 2014; Moliadze et al., 2014), and potentially even genetic profile (for review see Li et al. (2015)). Until recently very little research has been conducted which systematically investigates the reliability of tDCS effects within individuals. A few notable studies have explored these issues for anodal stimulation at 1mA (Horvath et al., 2016; Lopez-Alonso et al., 2015) and at 0.5mA (Chew et al., 2015). Interestingly, although 1mA anodal tDCS was found to have a reasonable level of inter-subject reliability (Lopez-Alonso et al., 2015), the same was not true of 0.5mA (Chew et al., 2015). Furthermore, even when reliability has been explored using the same intensity the results are conflicting. Horvath et al. (2016) found that reliability across sessions was poor which contrasts with the findings of Lopez-Alonso et al. (2015). It is highly possible that the differences between the studies reflect the different methodologies used. A few notable and potentially influential differences include electrode size, duration between testing sessions, duration of stimulation, use of neural-navigation and the amount of session's tested. Less research exists exploring the reliability of cathodal stimulation although poor reliability across sessions has been reported when 1mA intensity is used Horvath et al. (2016).

To our knowledge no studies have yet been conducted using 2mA intensity. As these higher intensities are often used by researchers based upon the assumption that these higher intensities will be more effective (Batsikadze et al., 2013), it is particularly important that these factors are explored.

The present study aimed to explore inter and intra subject variability using a repeated measures design in which participants experienced multiple sessions of 2mA tDCS applied to the motor cortex for 20 minutes. Electrodes measuring 35cm² each were attached in a standard electrode montage with the reference placed on the contralateral orbit and testing sessions were separated by a minimum of 3 days to allow for 'wash out'. Changes in cortical excitability were assessed with reference to changes in transcranial magnetic stimulation

(TMS) recruitment (Input Output, IO) curves and resting motor thresholds (RMT). TMS IO curves are thought to reflect the strength of corticospinal projections (Chen, 2000), and have previously been reported to increase following anodal and decrease following cathodal stimulation (Nitsche et al., 2005), however this is not always found to be significant (Batsikadze et al., 2013). Unlike IO curves RMT is thought to reflect the excitability of the main corticospinal projections to the target muscle with the lowest excitation threshold (Hallett, 2007), and have not been found to alter following tDCS (Batsikadze et al., 2013; Nitsche et al., 2005). By testing participants multiple times we aimed to explore the reliability of 2mA anodal and cathodal stimulation at both the group and individual levels.

Method

Subjects

Ten subjects were enrolled in the study, of which 7 were female. The mean age was 24 ± 4 years. The anodal and cathodal stimulation conditions were completed by all ten participants. The sham stimulation condition was completed by all participants bar one (F, 23.4years) who was unable to complete the sham condition due to relocating. All subjects were healthy, free from medication and counter indications to TMS. Subjects were deemed right handed using an adapted, shortened, version of the Edinburgh Handedness Inventory (Oldfield, 1971).

tDCS of the motor cortex

tDCS was delivered using a NeuroConn DC- stimulator (GmbH, Ilmenau, Germany) with a maximum stimulation output of 4.5mA. Stimulation was delivered using saline soaked surface sponge electrodes, each measuring 35 cm^2, to the area representing the first dorsal interosseous (FDI) muscle for the right hand (identified using TMS). The reference electrode was located over the contralateral right orbit. For anodal and cathodal stimulation the current was ramped up to 2mA over 15 seconds, held constant for 20 minutes and then ramped down over a further 15 seconds. By contrast, in the sham condition the current was ramped up to 2mA over a period of 15 seconds, sustained at this intensity for 30 seconds

and then ramped down over a further period of 15 seconds. Electrodes were removed during TMS.

TMS measurements and EMG recording of motor cortical excitability

TMS was delivered using a BiStim TMS system (Magstim, Whiteland, Dyfed, UK) with a figure of 8 coil (diameter of one winding 70mm). The coil was held tangentially to the scalp and positioned 45° from the midline resulting in a posterior to anterior current flow. Neural navigation software (Brainsite, Rogue Research Inc., Montreal Quebec, Canada) was used in conjunction with each participant's anatomical brain scan to aid accurate coil placement over the left primary motor cortex. The coil was moved in small increments within the anatomical target to locate the optimal stimulation site ('hot spot'), which was identified as the location which when stimulated produced the largest MEP amplitude. The optimal location was tracked using the software and was also marked onto a cap which the participant wore during stimulation. Participants were asked to remain as still as possible during testing with the aid of a chin rest, but were offered frequent breaks to stretch and adjust their position. The coil was held stable over the hot spot using a Manfrotto mechanical arm (Vitec Group, Italy) and adjusted as necessary.

MEPs were recorded using disposable Ag-AgCl surface electrodes attached to the right FDI muscle in a belly tendon montage. The signals were amplified, bandpass filtered (10Hz-2kHz, sampling rate 5kHz), and digitized using Brainamp ExG (Brain Products GmbH, Gilching, Germany) controlled by Brain Vision Recorder (Brain Products GmbH, Gilching, Germany). Participants were encouraged to maintain their hand in a relaxed position throughout testing.

Resting motor threshold (RMT) was determined as the lowest intensity needed to yield an MEP with a peak-to-peak amplitude of >50μV in the relaxed FDI muscle in a minimum of 5 of 10 trials. IO curves were measured using TMS intensities set at 100, 110, 120, 130, 140 and 150% of RMT. Ten pulses at each of the 6 intensities were delivered in a randomized order with 5 seconds occurring between each pulse. All trials were triggered using an in-house Matlab program (Mathworks, MA, USA).

Experimental procedures

All participants completed anodal and cathodal tDCS conditions; nine also completed the sham condition.

The study was initially designed to investigate the stability of 2mA anodal tDCS but was extended to include cathodal and sham conditions. As a result participants always completed the anodal session first followed by completion of sham and cathodal conditions, the order of which was counterbalanced between participants. On average the study took 6 months for participants to complete all 10 testing sessions. Anodal and cathodal sessions were separated by a minimum of one month (mean separation period 5 months). The participants were blind to the experimental condition, however for practical reasons the researcher was not.

Each testing session started with identification of the hand motor hot spot and measurement of RMT. The TMS coil was then placed over the hotspot and IO curves were measured. Following this, tDCS electrodes were then placed on the scalp and stimulation was applied for 20 minutes. Immediately after tDCS stimulation the electrodes were removed, the TMS coil was replaced, and TMS coil location and RMT were checked. If necessary small adjustments to RMT were made prior to measuring IO curves the second time. The same procedure was completed four times for each polarity. Each testing session was separated by 3-4 days (i.e., a minimum of 3 and a maximum of 4 days), and where possible the time of testing was kept constant within subjects. To maintain relatively constant levels of alertness and arousal throughout testing, subjects watched wildlife documentaries throughout the testing sessions.

Data analysis

Peak-to-peak MEP amplitudes were estimated using in-house Matlab software (Mathworks, MA, USA). All trials in the 500ms period prior to MEP were carefully visually inspected and any trials in which there was evidence of pre-contraction of the FDI muscle were excluded (resulting in a maximum of 3% trials being excluded for each given condition).

IO curve measurements were estimated by calculating the median intra-individual MEP amplitudes for each TMS intensity value (i.e., 100-150% of RMT), linear fits were then applied to the resultant values (mean $R^2 = 0.89$). Median values were calculated rather than the mean in order to limit the effect of non-standard distribution of individual data. For one participant 150% RMT could not be tested. Slopes were therefore fitted to the available values (i.e. 100-140% RMT).

Individual slope values were entered into a repeated-measures ANOVA, with time of testing (pre/post) and experimental session (1-4) entered as within-subject independent factors. Mauchleys test of sphericity was performed and was found to be non-significant, therefore no further corrections were made. *A priori* assessment of baseline threshold differences were carried out using Students *t* tests (paired samples, two tailed, *P <0.05).*

In order to investigate intra-subject reliability of tDCS induced changes, ratios of slope change were calculated by dividing each individual's post-tDCS IO slope by their baseline (pre tDCS) slope for each session. Intra-class correlation coefficient (ICC (2,1)) analysis was then used to explore the reliability of tDCS induced changes over the four sessions for anodal, cathodal and sham data. ICC results are reported based upon Lahey et al. (1983), ICC values of <0.4 are considered to indicate poor intra-class reliability, values >0.4 and <0.59 are fair, values >0.6 and <0.74 are good, and values >0.74 are excellent. Negative ICC values are taken to indicate a lack of reliability within the measure.

Results

Paired samples t-tests confirmed that baseline values for RMT did not significantly differ between sessions ($p > 0.05$) for anodal (all $t(9) < 1.63$, all $p > 0.14$), cathodal (all $t(9) < 1.13$, all $p > 0.29$) or sham conditions ($t(8)=.610$, $p=0.56$). See table 1.

Table 1 about here

Paired samples t-tests also confirmed that RMT did not significantly alter from baseline for any session following anodal stimulation (all p>0.168), cathodal stimulation (all p>0.343) or sham (all p>0.347).

IO curve slope

For the anodal condition a repeated-measures ANOVA revealed a significant main effect of time of stimulation (i.e., Pre vs. Post tDCS), $F(1,9)=5.232$, $p = 0.048$. There was no significant effect of testing session $F(3,27)=2.5$, $p=0.08$, and no significant interaction between these two factors $F(3,27)=0.58$, $p=0.63$. Figure 1 shows mean MEP amplitudes at each TMS intensity before and after anodal stimulation.

Figure 1 about here

For the cathodal condition a separate repeated-measures ANOVA was calculated. The ANOVA revealed no significant main effects of time of stimulation $F(1,9)=3.491$, $p=0.095$ or of testing session $F(3,27) < 1.0$, $p=0.99$, and the interaction between these two factors was not significant $F(3,27) < 1.0$, $p=0.67$. Figure 2 shows mean MEP amplitudes at each TMS intensity before and after cathodal stimulation.

Figure 2 about here

For the sham condition a repeated-measures ANOVA revealed no significant main effect of time of stimulation, $F(1,8)=.218$, $p=.653$. There was no significant effect of testing session $F(1,8)=.424$, $p=0.533$, and no significant interaction between these two factors $F(1,8)=0.186$, $p=0.678$. Figure 3 shows mean MEP amplitudes at each TMS intensity before and after sham stimulation

Figure 3 about here

Intra-subject reliability

To investigate the intra-subject reliability of the effect of anodal, cathodal and sham tDCS on the slope of each participant's IO curve, we conducted an intra-class correlation coefficient (ICC) analysis, based upon the ratio of post-tDCS and pre-tDCS slopes, separately for each stimulation. For anodal stimulation, the change in IO curve slope was found to be poorly

related across the four separate testing sessions, *ICC(2,1)* = 0.276 . Relevant data are presented in Figure 4A. When the same analyses were conducted for cathodal tDCS the ICC analysis revealed poor interclass reliability , *ICC(2,1)* = 0.038, Relevant data are presented in Figure 3B. ICC analysis of the sham condition revealed fair interclass reliability across the two testing sessions ICC(2,1)=.439. Relevant data are shown in figure 4C.

To allow for more accurate comparisons to sham ICC was also calculated using the first two sessions for the 9 participants who completed all conditions. For anodal stimulation this revealed a poor reliability ICC(2,1)=0.098, poor reliability was also found for the cathodal condition ICC(2,1)=0.35.

Figure 4 about here

Discussion

This study investigated the reliability and consistency of the effects of 2mA anodal and cathodal tDCS by assessing any changes induced in the slope of each individual's TMS IO curve over repeated testing sessions carried out on the same individuals. Our results demonstrate that at a group level the slope of IO curves increased following anodal tDCS, but there was no significant change in IO slope following cathodal or sham stimulation. This indicates that anodal tDCS may increase motor cortical excitability as previously reported (Nitsche & Paulus, 2000; Nitsche et al., 2005), however cathodal tDCS failed to induce a statistically significant influence on motor excitability in either direction.

Importantly, analysis of the reliability of the observed tDCS effect across four sessions using interclass correlation coefficient (ICC) analysis revealed that the response to 2mA anodal stimulation is poorly reliable within individuals. The effects of cathodal tDCS were also found to show poor reliability across sessions, whereas the sham condition revealed moderate stability. These results are discussed below.

It should be noted that the above findings and conclusions are restricted to the effects occurring immediately after 2mA tDCS stimulation was applied to the motor cortex for 20 minutes. Interestingly, the same parameters of cathodal stimulation have been reported previously to significantly *increase* cortical excitability (Batsikadze et al., 2013) . However, in that study the effect was not measured as a change in TMS IO curves, and it was not observed immediately after stimulation, but was instead apparent as a change in MEP amplitudes occurring 90 and 120 minutes post stimulation. The difference in TMS measures used (i.e., TMS IO curves vs. MEP amplitude) is not necessarily problematic, as IO curves measure the change in MEP amplitude over a range of TMS intensities, and would therefore encompass the measure used by Batsikadze et al. (2013).

The results reported by Batsikadze et al. (2013) are counter to previous studies reporting the effects of cathodal tDCS on motor excitability insofar as they reported that cortical excitability, as indexed by MEP amplitude, was increased following 2mA cathodal tDCS whereas previous studies of cathodal tDCS at lower stimulation intensities had reported that cathodal tDCS decreases cortical excitability (Nitsche et al., 2003; Nitsche & Paulus, 2001). Inspection of Figure 4B suggests that our findings are broadly consistent with the findings of Batsikadze et al. (2013) in that many participants showed an *increase* rather than a decrease in motor excitability and this effect was close ($p=0.08$) to conventional statistical significance thresholds. However, this effect was variable across participants and more importantly it was variable within participants,as indicated by ICC analysis. It should be noted that we did not measure TMS IO curves 90-120 minutes post stimulation, and we therefore acknowledge that we must interpret the null effects of 2mA cathodal tDCS with some caution as it may be the case that the effects of 2mA cathodal tDCS become more stable and consistent after a sizeable delay (90-120 minutes).

The finding that the effects of 2mA anodal stimulation were not reliable within individuals despite significant group level effects is particularly interesting as it suggests that group level analysis may hide substantial variability which occurs both between and within subjects. Previous evidence regarding stability of anodal effects has been mixed. Our results are more in line with the recent findings of Horvath et al. (2016) than those of Lopez-Alonso et al. (2015). However, as previously noted there are methodological differences between the studies which may in part contribute to the findings. In particular the study by Lopez-

Alonso et al. (2015) was conducted with sessions separated by 6-12 months and only two sessions were compared, although it should be noted that this work was conducted with a larger sample. Our finding of moderate stability following sham stimulation is also in agreement with previous work (Horvath et al., 2016).

Although the reliability of anodal and cathodal induced change in IO curve slope was found to be poor, ICC analysis suggests that this was moderate for the sham condition. This was also found by Horvath et al. (2016).

Limitations

The counterbalancing of the conditions tested in this experiment was not optimal, and we accept that ideally the experimenter would be blind to the stimulation used. Unfortunately, for practical reasons this was not feasible. We do feel however that sufficient experimental controls were in place (such as the use of neuro-navigation systems to guide TMS coil placement) to offset these limitations.

Conclusions

In summary, we investigated the reliability and consistency of the effects of 2mA anodal and cathodal tDCS on motor excitability by examining how the slope of TMS IO curves was influenced by tDCS. We found that anodal tDCS significantly increased the slope of TMS IO curves and that this effect was consistent at a group level across repeated testing sessions. Using ICC analysis these effects were not found to be reliably consistent within individuals. Sham stimulation failed to significantly influence IO curve slope, however the effects of this intervention were found to be moderately reliable within subjects.

As previously discussed variability with non-invasive brain stimulation techniques is not uncommon. However, in order to develop techniques such as tDCS into more powerful methods in both research and therapeutic contexts understanding the sources of this are likely to be critical. In particular furthering our understanding regarding the reliability & consistency of these effects may allow us to better identify responders and non-responders to particular paradigms. This could be particularly useful in furthering the development of tDCS as an effective treatment alternative to conventional approaches.

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Table and Figure Captions

Figure 1. Group mean MEP amplitude and SEM in response to varying degrees of stimulator intensity before and after 20 minutes of 2mA **anodal** stimulation for (A) Sessions 1; (B) Session 2; (C) Session 3; and (D) session 4.

Figure 2. Group mean MEP amplitude and SEM in response to varying degrees of stimulator intensity before and after 20 minutes of 2mA **cathodal** stimulation for (A) Sessions 1; (B) Session 2; (C) Session 3; and (D) session 4.

Figure 3. Group mean MEP amplitude and SEM in response to varying degrees of stimulator intensity before and after 20 minutes of **sham** stimulation for (A) Sessions 1 and (B) Session 2.

Figure 4. Amount of change in IO curve slope (pre/post) for each participant following (A) anodal, (B) cathodal and (C) sham stimulation. Each coloured data point represents a single session, black diamonds indicate mean change. Horizontal line indicated no change from baseline.

Table 1: Mean RMT values expressed as absolute stimulator intensity ± SD at baseline for all testing sessions and condition.

	Anodal RMT †	**Cathodal RMT †**	**Sham RMT †**
Session 1	45.8 ± 4.6	45.8 ± 4.3	45.6 ± 5.8
Session 2	46 ± 4.4	45.9 ± 4.8	45.8 ±5.8
Session 3	46.2 ± 4.7	46 ± 5.0	Na
Session 4	46.3 ± 4.6	47.1 ± 5.0	Na