Reliability of tDCS Induced Corticomotor Excitability in Lower Limb Motor Cortex of Healthy Individuals

BY

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THESIS

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ADM- Abductor digiti minimi

ICC- Intra class correlation coefficient

MEP- Motor evoked potential

MT- Motor threshold

MVC- Maximum voluntary contraction

TA- Tibialis anterior

tDCS- Transcranial direct current stimulation

TMS- Transcranial magnetic stimulation

TPE- Typical percentage error

BDNF- Brain derived neurotropic factor

NMDA- N-methyl-D-aspartate

ABSTRACT

Noninvasive brain stimulation techniques, such as transcranial direct current stimulation (tDCS), have emerged as a promising tool to enhance motor function by modulating corticomotor excitability. Anodal tDCS has shown to modulate corticomotor excitability and improve motor function in those with neurological disease. However, there is limited information on the reliability of these effects. In order to increase the potential of tDCS as a clinical tool, it is important to be able to demonstrate that the effects of tDCS are reliable, and individuals respond to the intervention in a predictable manner when retested. The main purpose of this thesis was to explore the test-retest effects of transcranial direct current stimulation (tDCS) applied in conjunction with a skilled motor learning task versus when applied at rest. Fifteen healthy participants were recruited and tested under two stimulation conditions: 1) anodal tDCS during a motor task (tDCS-task) and 2) anodal tDCS at rest (tDCS-rest). The outcome measures evaluated were changes in the corticomotor excitability using single pulse transcranial magnetic stimulation. Results revealed that tDCS application during practice of a skilled motor task resulted in reduced variability in TMS measures compared to tDCS applied in the absence of motor practice. Testing at higher stimulus intensities (140% AMT) and examining input output response curves provided a more reliable method of testing the effects of tDCS.

THESIS OVERVIEW

The main purpose of this thesis was to explore the test-retest reliability of corticomotor excitability changes induced from anodal transcranial direct current stimulation (tDCS) to the lower limb motor cortex under two conditions: 1) anodal tDCS with a motor learning task (tDCS-task) and 2) anodal tDCS applied at rest (tDCS-rest).

Chapter I introduces an overview of tDCS, physiological and behavioral aspects of tDCS, and reliability of non-invasive brain stimulation techniques.

Chapter II presents the main objectives, specific aims, hypothesis and rationale for this study.

In Chapter III, the study is presented in a manuscript format with the following sub-topics:

- Introduction- This section provides an overview of the test-retest reliability of tDCS in previous literature, the significance of this problem followed by hypotheses.
- Methods- This section describes the design of the experiments, selection criteria of participants, experimental procedures, instrumentation, statistical and data analyses.
- Results- This sections summarizes the results of the study.
- Discussion- This chapter presents a detailed explanation of experimental results. A literature review pertaining to experiment results is included.

CHAPTER I- REVIEW OF LITERATURE

1.1 INTRODUCTION

Transcranial direct current stimulation (tDCS) is a neuromodulatory technique that has been widely researched and utilized as an adjuvant to enhance motor learning and functional recovery after neurological impairment such as stroke. tDCS is delivered from a small battery powered constant current stimulator to the scalp by attaching different polarity electrodes to the scalp (Nitsche and Paulus, 2000; Nitsche and Paulus, 2001). The low intensity direct currents (0.5-2mA) are generated through saline soaked sponge electrodes placed on the scalp to modulate neuronal excitability in the targeted brain regions (Webster et al., 2006).tDCS has shown to induce membrane potential shifts that are dependent on stimulation strength, cortical layer and spatial orientation of stimulated neurons (Radman et al., 2009). In general, anodal tDCS which partially depolarizes neurons leads to an increase in corticomotor excitability, and cathodal tDCS that hyperpolarizes neurons leads to a decrease in corticomotor excitability (Bindman et al., 1964; Purpura and McMurtry, 1965).

Anodal tDCS when applied for 15 minutes produces an excitatory effect in the brain that can last up to 90 minutes (Nitsche and Paulus, 2000). This change in cortical excitability is quantified by changes in Motor Evoked Potential (MEP) parameters elicited by single pulse Transcranial Magnetic stimulation (TMS). As the intensity increases, the cortical excitability responds in an analogous fashion (Nitsche and Paulus, 2000). It has been shown in previous research that a 5 min anodal stimulation of the motor cortex using a 1mA current in healthy individuals enhances MEP amplitude by 30% (Nitsche and Paulus, 2000), and a current intensity of 2mA for a 10 min duration can enhance the MEP amplitude by over 40% and the effects last for 60 minutes (Jeffery et al., 2007; Nitsche et al., 2005).

1.2 PHYSIOLOGICAL CHANGES ASSOCIATED WITH tDCS

Depending on the polarity of stimulation, tDCS induces neuronal excitability by a tonic depolarization (anodal stimulation) and hyperpolarization (cathodal stimulation) of resting membrane potential (Purpura and McMurtry, 1965). This pattern of activity was first shown in animals receiving stimulation via intracerebral electrodes (Bindman et al., 1964; Purpura and McMurtry, 1965).The cortical modulation is however not solely dependent on polarity but is also determined by stimulation strength, intensity and spatial orientation of the stimulated neurons (Radman et al., 2009). Creutzfeldt et al, (1962) demonstrated that neurons in the deeper layer of a cat motor cortex were excited by cathode, and inhibited by anode which could be a result of inversion of current flow associated with neuron's spatial orientation. This finding suggests that the orientation of neurons relative to the electric field is very important to their response to stimulation. It is to be noted that different subpopulations of neurons have a different threshold for stimulation. Non pyramidal tract neurons are activated at lower charges than pyramidal tract neurons (Purpura and McMurtry, 1965).

In addition to the physiological effects during the current application, the modulation of neuronal firing is observed to be maximum for a few minutes after the cessation of current (Bindman et al., 1964). In rat motor cortex, a 25mA anodal current for 8 minutes led to an increase in neuronal excitability that lasted for 50 minutes and cathodal current administered for more than 5 minutes led to a decrease in excitability lasting for about the same duration (Bindman et al., 1964). The changes in neuronal membrane potential are responsible for the short term tDCS effects, whereas the long term changes are induced by intracellular calcium levels (Islam et al., 1995). In animals, anodal stimulation has been shown to increase intercellular calcium levels that are important for induction of neuroplasticity (Bennett, 2000). This is

achieved through the modulation of calcium-channel activity due to altering the transmitter release and thereby modifying cortical excitability. At the receptor level, NMDA-receptor modulation is involved in the induction of the short-lasting after-effects of tDCS in humans (Liebetanz et al., 2002), which is important for the induction of neuroplastic mechanisms (Bennett, 2000). Depending on the calcium and NMDA activation, the secretion of a neurotrophin BDNF (Brain derived neurotropic factor) was found to be involved in cortical synaptic plasticity (Akaneya et al., 1996; Lu, 2003). Previous work has suggested that BDNF plays a vital role in synaptic plasticity and motor performance (Cheeran et al., 2008; Kleim et al., 2006; McHughen et al., 2010). Fritsch et al, (2010) further emphasized that BDNF related behavioral alterations are activity dependent. The effects of tDCS on motor learning are more beneficial when BDNF release occurs during training of a motor skill task whereas in the absence of training the effects may not materialize. Another proposed non-synaptic long term mechanism underlying the effects of tDCS are dependent on protein synthesis (Prior et al., 2005). Proteins are used to reorganize the synaptic and dendritic structures (Steward and Schuman, 2001) and help in preserving the motor memory content (Arshavsky, 2003) suggesting that motor skill learning is also dependent on plasticity related protein synthesis (Luft et al., 2004).

As a result of these factors (NMDA receptor strength, intercellular calcium levels and protein synthesis) originates long term potentiation (LTP) and long term depression (LTD) mechanisms. LTP is one of the important factor underlying synaptic plasticity. Increase in synaptic strength leads to a LTP like mechanism LTP-like mechanism is the molecular component that is responsible for learning. When synapses continue to increase in strength as a result of LTP, eventually they reach their maximum level and no learning is possible after this

point. To make the synaptic strength useful, the LTD comes in action to selectively weaken the set of synapses. The NMDA receptor admits calcium into the postsynaptic neuron which begins the LTP process. A rapid rise in calcium levels triggers LTP and conversely a slow release in calcium triggers LTD process. Protein synthesis occurs during the intermediate and final stages of LTP (Malenka and Bear, 2004; Raymond, 2007). These after effects associated with tDCS play a vital role in providing the basis for motor learning and memory.

1.3 BEHAVIORAL CHANGES ASSOCIATED WITH tDCS

As a result of the observed physiological changes mentioned previously, tDCS application over the motor cortex has the potential to facilitate improvements in motor function and learning. Reis et al., (2009) investigated the effects of anodal tDCS on speed and accuracy of task performance within the session (online learning) and during consolidation (offline learning) for a visual isometric pinch task. Subjects received sham, anodal or cathodal tDCS during the motor task and were tested on five consecutive days. A follow up assessment was conducted after 3 months. The anodal tDCS group showed 50% greater learning than the sham and cathodal stimulation group, and the effects lasted for 3 months post intervention. Anodal tDCS also has a positive influence over motor memory (Antal et al., 2004; Galea and Celnik, 2009; Nitsche et al., 2003; Reis et al., 2009). Galea and Celnik, (2009) used an upper extremity movement paradigm to demonstrate that anodal tDCS can not only help initiate initial formation of a motor memory but also assists with retention of the motor memory after training. Anodal tDCS has also been shown to decrease reaction time (Kuo et al., 2008; Nitsche et al., 2003; Stagg et al., 2011). Stagg et al, (2011) examined effects of tDCS on reaction time using an explicit finger sequence learning task. They reported that anodal tDCS during training led to a faster reaction time compared to sham or cathodal.

tDCS has also been shown to be effective in enhancing functional recovery post stroke. Madhavan et al, (2011) examined the effects of anodal and sham tDCS over the lower limb primary motor cortex. tDCS was applied in conjunction with a motor task and reported that improvement with motor practice was observed with anodal stimulation and was not observed with sham stimulation or anodal stimulation of the non-lesioned lower limb M1. Similarly, Tanaka et al, (2011) showed that a single session of facilitatory tDCS is capable of enhancing quadriceps extensor force in chronic patients.

Relative to the timing of tDCS priming, the motor learning period may have an important impact on stimulation induced "gating" of motor learning (Ziemann and Siebner, 2008). Gating occurs by disinhibition or depolarization of intracortical inhibitory circuits which results in an increase in calcium entry and leads to enhancement of postsynaptic response by the same stimulation or learning protocol. Since gating requires an acute increase of cortical excitability during the time of the motor learning period, it is suggested that it may be beneficial for the excitability enhancing priming protocol to be delivered during the period of learning (Ziemann and Siebner, 2008). Another proposed mechanism for priming includes homeostatic plasticity. During homeostatic plasticity, a high level of prior synaptic activity will reduce the facilitatory effects of a concurrent facilitatory neuromodulatory protocol (Bienenstock et al., 1982), and if the preceding postsynaptic neuronal activity was low, it could leads to LTP-like effects (Ziemann and Siebner, 2008). Therefore, the timing of tDCS relative to the learning is very crucial. In previous studies, tDCS has been applied simultaneously with motor practice (Antal et al., 2011; Cuypers et al., 2013; Geroin et al., 2011; Madhavan et al., 2011; Reis et al., 2009; Saucedo Marquez et al., 2013; Stagg and Nitsche, 2011), before a motor practice (Antal et al., 2008; Kuo et al., 2008) and after a motor task (Tecchio et al., 2010). Recently, we conducted a study that

examined the timing dependent priming effects of tDCS where tDCS was applied during an ankle visuomotor skill learning task, prior to the task and sham tDCS (Sriraman et al., 2014). Twelve healthy participants were tested under these three stimulation conditions. Our results showed that tDCS applied during a motor learning task increased motor performance more than when applied prior to the task and sham tDCS. This could possibly be due to the "gating" mechanism.

1.4 RELIABILITY OF tDCS

Although there has been a significant understanding of the physiological and behavioral aspects of tDCS, studies examining the reliability of this priming protocol are limited. Reliability provides an indication of the expected error and statistical power of a measured outcome, and provides confidence that any changes observed in the measure are due to physiological changes within the subjects and not due to the variability in the measure itself (Christie et al., 2007; Malcolm et al., 2006). In order for tDCS to be useful in the clinical setting, this technique must be shown to produce reproducible data over multiple testing sessions.

Amongst the few studies that have aimed to examine the effects of multiple sessions of tDCS, there exists extensive variability in the obtained results. In a study conducted by Fricke et al., (2011) they reported data from two similar groups of healthy participants who underwent an identical tDCS protocol for a duration of 5 minutes (anode over the M1). One group demonstrated an average MEP amplitude increase of 93.2% following tDCS whereas the other group demonstrated an increase of only 9.2%. This extensive variability suggests that tDCS generates different responses at the individual level. Possible explanation of inter-individual variability could be due to the anatomical differences such as skull thickness, cerebrospinal fluid quantity, subcutaneous fat levels and surface topography that could have an influence on current

density and flow during stimulation (Datta et al., 2012; Truong et al., 2013). Another possible explanation for the variability associated with tDCS can be due to inconsistency in outcome measurements possibly due to TMS coil positioning resulting in variability of MEPs obtained rather than the variability of tDCS effects (Ahdab et al., 2010; Herwig et al., 2001).Therefore to understand the reliability of tDCS induced effects, data from the same group must be compared on multiple days to rule out the possible drawback of inter-individual differences.

In a study conducted by Galvez et al.,(2013) to examine the effect of constant tDCS versus an increasing tDCS stimulation protocol over five consecutive days in the same group of participants, variable baselines values were reported throughout the week in both the conditions but the amount of change induced by tDCS was similar suggesting test-retest reliability of tDCS (10-40%). Similarly, Alonzo et al, (2012) conducted a study to examine the group effects of multiple sessions of tDCS consecutively applied for five days over a week versus three alternative sessions of tDCS (2mA for 20 minutes) application. In the daily tDCS condition, an increase in excitability was observed between the consecutive sessions suggesting that excitability was preserved between sessions but they did not notice any carry over effects when the sessions were on alternative days. Additionally, they also suggested that the intersession changes, post-tDCS values taken as a ratio of the pre were similar across days in both the conditions (variability between 10-30%). In contrast to these findings, in a study conducted by (Monte-Silva et al., 2013) to examine the two consecutive sessions of anodal tDCS separated by 24 hours, they reported a significant reduction in MEP amplitude on the second session. In all these studies (Alonzo et al., 2012; Galvez et al., 2013; Monte-Silva et al., 2013), tDCS sessions were conducted on consecutive days which could have necessarily lead to cumulative effects.

Future studies must explore the test-retest reliability of tDCS that are independent of effects associated with consecutive sessions.

1.5 CLINICAL IMPLICATIONS OF tDCS

tDCS has been gaining increasing interest as an adjuvant to enhance function and recovery following various neurological diseases. tDCS has been used extensively in stroke rehabilitation where anodal tDCS increases excitability of the lesioned cortex and cathodal tDCS is used to inhibit the non-lesioned cortex (Hummel and Cohen, 2005). Anodal and cathodal tDCS applied over paretic and non-paretic M1 respectively have been shown to be effective in the motor recovery of arm function in stroke patients when combined with robot-assisted arm training (Hesse et al., 2007; Ochi et al., 2013). Geroin et al,(2011) found that applying anodal tDCS in combination with robotic gait training enhanced the effects of training, anodal tDCS applied over the M1 of the paretic cortex and cathodal tDCS applied over the corresponding non-paretic improved the performance of arm function in stroke patients during the Jebsen-Taylor Hand function test (Boggio et al., 2006). Apart from stroke, tDCS has also been effectively used in the treatment of depression. In a study conducted by (Loo et al., 2012) they examined the effects of tDCS for the treatment of depression. Significant greater improvement in mood was observed in the tDCS active group compared to sham session. Similarly, Boggio et al., (2007) conducted a study of anodal tDCS in major depression and reported that anodal tDCS led to an improvement in emotional processing. Effects of anodal tDCS have also been explored in chronic pain (Antal et al., 2010; Ngernyam et al., 2014). In a clinical trial conducted by Antal et al., (2010) to analyze the effects of tDCS on chronic pain syndrome, they reported that anodal tDCS led to a greater improvement in reduction of pain than sham tDCS, evident even three to four weeks post-treatment. The effects of tDCS on motor function and recovery in individuals with Parkinson's

disease has also been explored and found to result in increase in function following anodal tDCS (Doruk et al., 2014; Fregni et al., 2006).

Considering the increasing interest in using tDCS as a potential rehabilitative tool, it is important to be able to demonstrate that tDCS is a reliable device and can reliably produce improvements in motor function and corticomotor excitability when tested on multiple days.

1.6 RELIABILITY OF TRANSCRANIAL MAGNETIC STIMULATION (TMS)

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique to study the human motor cortex. With TMS, a double cone coil is placed over the scalp, that when triggers causes a rapidly changing magnetic field to induce an electric current in the underlying brain tissue. This electric current causes depolarization of nerve cells and the depolarization is transmitted via the corticospinal tract to contralateral peripheral muscles where a motor response can be monitored through surface EMG electrodes. TMS is used to evaluate corticomotor excitability and to measure central nervous system (CNS) adaptation and its relationship to changes in neural control and function (Wheaton et al., 2009). Different aspects of corticomotor excitability are assessed with TMS by evaluating active motor threshold (AMT), resting motor threshold (RMT), motor evoked potentials (MEP), cortical silent periods (CSP) and motor latency (MT) (Lefebvre et al., 2004; Martin et al., 2009; Truong et al., 2013) There is a plethora of studies that have examined the test-retest TMS reliability in upper limb musculature (Carroll et al., 2001; Christie et al., 2007; Lefebvre et al., 2004; Lopez-Alonso et al., 2014; Malcolm et al., 2006) compared to studies pertaining to lower limb musculature. A study conducted by Malcolm et al., (2006) used TMS to determine the test-retest reliability of motor threshold, map tomography, and stimulus-response curves for the hand muscles in healthy subjects. Data were analyzed using an inter class correlation coefficient (ICC) to assess

reliability of outcome measurements. Results showed ICC values of 0.97 and 0.90 for optimal position of the coil and motor threshold respectively, while stimulus response curve results yielded only moderate test-retest reliability with ICC values ranging from 0.60 in the FCR to 0.83 in the EDC muscle. Christie et al., (2007) looked at intra-session reliability for the abductor digiti minimi (ADM) muscle. MEPs were evoked at rest at intensities of 110%, 130%, and 150% times motor threshold (MT). High ICCs were found at all three intensities for the overall group. This study also concluded that the number of trials per intensity was very important when determining reliability. When using only two averaged trials per intensity, the ICC was only 0.07, and when averaging five trials per intensity the ICC increased dramatically to 0.97. This investigation demonstrates that it is important to elicit at least five MEPs at a given intensity to achieve a reliable measure for that participant.

Another intra and inter-investigatory study conducted by (Cacchio et al., 2009) aimed to determine the reliability of TMS measures for tibialis anterior muscle in healthy individuals. The TMS measures evaluated were MEP amplitude, MEP area, silent period and MEP latency. They observed high ICC values indicating a good intra- and inter-investigator reliability for MEP amplitude (ICC= 0.94-0.98), MEP latency (ICC= 0.79-0.93), silent period (ICC= 0.89-0.95) in healthy subjects suggesting that the TMS-related measurements for a lower limb muscle are reliable in healthy subjects.

As we are using TMS as a tool to quantify reliability of tDCS, we will need to establish that our TMS measures are reliable before we can explain the variability of the effects of tDCS. Hence, in this study we will establish the reliability of our TMS experimental technique by looking at baseline measures between the two testing days.

CHAPTER II- THESIS AIM AND HYPOTHESIS

Briefly, we recruited fifteen participants who were tested under two stimulation conditions 1) anodal tDCS applied simultaneously with a lower limb visuomotor skill task (tDCS-task) and 2) anodal tDCS in the absence of motor activity (tDCS-rest). Each participant was tested twice under each stimulation condition. The two testing days for both stimulation conditions were separated by at least three days and the two conditions were separated by at least seven days. The outcome measures evaluated were the changes in corticomotor excitability induced by tDCS immediately after the stimulation (POST0), 10 minutes after the end of stimulation (POST10) and 30 minutes after the end of stimulation (POST30) using single pulse TMS. An input-output recruitment curve was generated at seven TMS intensities. Eight MEPs were recorded and stored at each of these intensities for offline analysis.

2.1 MAIN OBJECTIVE

The main objective of this thesis was to examine the test-retest reliability of corticomotor excitability changes induced by tDCS on the lower limb motor cortex in healthy participants under the two above-mentioned stimulation conditions. The following questions were addressed:

1) Are TMS related measures (MEP Amplitude and MEP Area) obtained before and after tDCS during the first testing session similar to the same measures obtained on the second day under the same stimulation condition?
2) Which is the optimum TMS testing parameter that has the potential to probe the effectiveness of tDCS?
3) Is test-retest reliability of tDCS different for tDCS-task and tDCS-rest conditions?

2.2 SPECIFIC AIMS

This study addressed the following three specific aims in Chapter III.

Specific Aim 1:

a. To examine the reliability of the PRE-tDCS MEP amplitude and area measures obtained on *Day1* vs the same measures obtained on *Day2* for the same stimulation condition at all time points.
b. To examine reliability of POST tDCS induced MEP Amplitude and MEP Area measures obtained on *Day1* vs the same measures obtained on *Day2* for the same stimulation condition at all time points.

Specific Aim 2:

To compare the reliability of MEP amplitude and MEP area measures obtained at 120%, 130%, 140% AMT and slope of recruitment curve (RC slope) in order to examine the most ideal TMS testing parameter.

Specific Aim 3:

To examine which of the stimulation conditions (tDCS-task or tDCS-rest) yields the stronger reliability of tDCS-induced changes.

2.3 HYPOTHESES

The hypotheses for each of the specific aims are as follows:

Hypothesis 1a:

PRE TMS related measures (MEP amplitude and MEP area) obtained on *Day 1* testing session will demonstrate test-retest reliability for the same measures obtained on *Day 2* testing session for the same stimulation condition.

Rationale:

TMS has been shown to be a reliable tool in measuring MEP amplitude and area specifically for the tibialis anterior muscle in both the healthy population and with stroke survivors. In a test-retest reliability study of tibialis anterior muscle, Cacchio et al., (2009) and Cacchio et al., (2011) showed that MEP area and amplitude were found to be reliable (ICC= 0.75- 0.88) among the healthy population. Similarly another study by van Hedel et al., (2007) showed that the MEP amplitude is a reliable measure (ICC=0.7) for the tibialis anterior muscle in healthy population. In this respect, we expect to observe strong reliability for MEP amplitude and area as we have adopted similar testing methods.

Hypothesis 1b:

tDCS induced POST MEP amplitude and MEP area obtained on *Day 1* testing session will demonstrate test-retest reliability for the same measures obtained on *Day 2* testing session for the same stimulation condition.

Rationale:

In this study, we adopted a robust testing technique by locating the representation of tibialis anterior muscle on the lower limb motor cortex using TMS before the application of tDCS. Detailed measurements of this hotspot were recorded and reproduced on the subsequent sessions. Because the hotspot and tDCS parameters were maintained constant, we hypothesized that the POST- tDCS MEP measures obtained on D*ay1* would be similar to the same measures obtained on *Day 2*.

Hypothesis 2:

We hypothesize that the POST-tDCS MEP Amplitude and MEP area measures obtained at the higher intensities of 130% AMT and 140% AMT and RC slope would be the optimal testing parameters in yielding reliable results when compared to measurements obtained at 120% AMT.

Rationale:

Previous studies have shown a trial to trial variability in TMS related measures present at lower stimulus intensities which could be due to the less and uncertain number of corticospinal neurons and motor neurons firing at that intensity. In another study conducted by Wiethoff et al., (2014) to examine the variability in response to tDCS, they reported that only 50% of the participants responded to tDCS when analyzing the POST tDCS MEPs collected at 110% AMT. In this respect we hypothesized that MEP amplitude and area obtained at higher than 120% AMT, like 130% AMT, 140% AMT and RC slope will produce higher test-rest reliability.

Hypothesis 3:

We hypothesize that MEP amplitude and area obtained when tDCS is applied during a motor task (tDCS-task) will produce greater test-retest reliability compared to tDCS applied at rest (tDCS-rest).

Rationale:

tDCS applied during the task (Cuypers et al., 2013; Madhavan et al., 2011; Reis et al., 2009) has led to an increase in corticomotor excitability and motor performance. Similarly, in a recent study we reported an increase in motor performance following tDCS applied with a task and a reduced performance following tDCS applied before the task (Sriraman et al., 2014). We suggested that tDCS applied prior to task could be activating different neuronal circuits than when applied concurrently with a task. Likewise, in the present study we expected to observe unreliable effects only for tDCS-rest condition as tDCS may not be directed to the same neuronal circuits every time which could be a potential variability in itself.

CHAPTER III- MANUSCRIPT

TITLE: Reliability of transcranial direct current stimulation (tDCS) induced corticomotor excitability in the lower limb motor cortex of healthy individuals.

3.1 INTRODUCTION

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique used to modulate neuronal excitability of the motor cortex by administering weak direct current through electrodes placed on the scalp (Webster et al., 2006). Depending on the polarity of stimulation, tDCS changes the neuronal excitability by partial depolarization (anodal stimulation) and hyperpolarization (cathodal stimulation) of resting membrane potential (Purpura and McMurtry, 1965). In the last few years, several studies have examined the effects of tDCS on the motor cortex during and prior to motor training (Antal et al., 2011; Cuypers et al., 2013; Kuo et al., 2008; Madhavan et al., 2011; Reis et al., 2009; Sriraman et al., 2014; Stagg et al., 2009; Stagg et al., 2011), examined the differences in current density and electrode size (Bastani and Jaberzadeh, 2013a; Bastani and Jaberzadeh, 2013b) and its effects on neurological diseases (Geroin et al., 2011; Hummel and Cohen, 2005; Madhavan et al., 2011; Zimerman et al., 2012). While these studies have provided information to advance the knowledge of tDCS, there is not much information available on the reliability of this paradigm. Amongst the limited tDCS reliability studies available, the reported results appear to be very inconsistent suggesting that more investigation is required to understand the reliability of the paradigm (Alonzo et al., 2012; Galvez et al., 2013; Monte-Silva et al., 2013; Wiethoff et al., 2014). Galvez et al, (2013) examined the effect of constant tDCS versus an increasing tDCS stimulation protocol over five consecutive days and reported variable PRE-tDCS MEP measures throughout the week in both

the conditions, but the POST to PRE-tDCS MEP measures ratio were similar between groups. Similarly, Alonzo et al., (2012) conducted a study to examine the effects of multiple sessions of tDCS applied for five consecutive days versus three alternative sessions of tDCS (2mA for 20 minutes) application in a week. They also reported variable PRE-tDCS MEP measurements for the consecutive tDCS stimulation but similar baselines measures on the alternate tDCS stimulation sessions. For both these conditions, the changes elicited by tDCS were not significantly different between the testing days, suggesting reliability of tDCS. In contrast to these findings, a study conducted by Monte-Silva et al, (2013) examining effects of two consecutive sessions of anodal tDCS separated by 24 hours reported a significant reduction in MEP amplitude on the second session. It is possible that that consecutive tDCS stimulation leads to a cumulative or abolishment of effects.

Additionally, there is also inter-individual variability that needs to be accounted for when looking at effects of tDCS. Recently a study conducted by Wiethoff et al, (2014) examined the variability in response to single session tDCS for the FDI muscle using three types of TMS current orientation; posterior-anterior, anterior-posterior and lateral-medial cortical current . They reported that for the antero-posterior coil orientation at 110% AMT, the inter-individual variability for change in MEP amplitude was 50%. Similarly another study conducted by Lopez-Alonso et al., (2014) reported that, only 45% of the tested sample responded to anodal tDCS with the expected up regulation.

In all the above mentioned studies, tDCS was applied when the muscle was at rest. In a recent study, we showed that the task associated with tDCS or the timing of the tDCS with respect to the intervention plays an important role in shaping its effects (Sriraman et al., 2014). We reported that the tDCS application during activity enhanced task performance whereas tDCS

application prior to motor task (i.e. when the muscle is at rest) did not significantly alter performance. Additionally none of these studies have examined the reliability of tDCS on a lower limb muscle. Also, the TMS measurement parameters used to examine the effects of tDCS are not comparable amongst the different studies which could possibly be a factor in the high variability reported. Therefore the present study will aim at evaluating the test-retest effects of tDCS on the tibialis anterior muscle in healthy volunteers using various TMS parameters. We will also compare if tDCS applied simultaneously with a motor task is more reliable than tDCS applied at rest. We will specifically address the following questions:

1) Is the amount of change post-tDCS similar between two different days of the same stimulation condition? 2) Are the effects more reliable when tDCS is applied during task compared to when applied in the absence of the task? 3) What are the ideal TMS measurement parameters that produce reliable effects?

3.2 METHODS

Selection criteria

Fifteen healthy participants (5 males, 10 females, age range 22-32 years) were recruited to participate in this study. A description of the study was provided and written consent approved by the University of Illinois Institutional Review Board was obtained from each participant. The inclusion criteria for the study were no neurological disorders, full range of motion of the testing ankle joint and between 18-35 years of age. The exclusion criteria included history of seizures, metal implants, cardiac pacemakers, unexplained headaches, epilepsy and medications likely to alter cortical excitability. Leg dominance was noted by asking participants which leg they used to kick a ball. Thirteen participants reported to be right dominant and two participants reported to be left dominant.

Study design

This was a repeated-measures two factorial study design in which corticomotor excitability related measures were obtained twice (test–retest) under two stimulation conditions separated by at least seven days. The two conditions included- 1) anodal tDCS during a skilled motor task (tDCS-task) and 2) anodal tDCS administered while the participant was not performing any motor activity (tDCS-rest). Each participant was tested on four separate testing days with two testing days (*Day 1* and *Day 2*) under each stimulation condition (tDCS-task and tDCS-rest). The *Day 1* and *Day 2* of the same condition were separated by at least three days and the two stimulation conditions were separated by at least seven days. The two stimulation conditions were pseudo-randomized to avoid order effects.

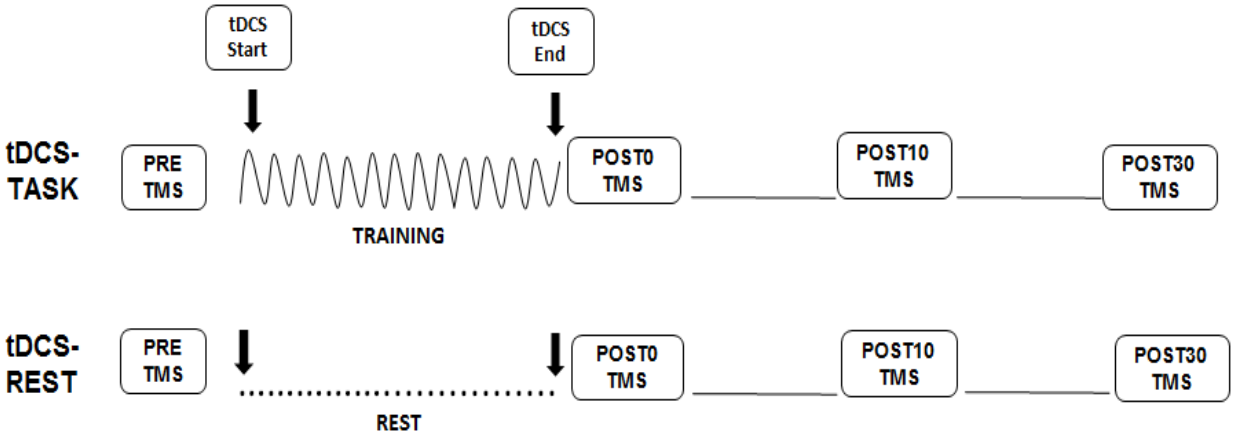

Figure 1: Schematic of the study design

Experimental procedure

During each session, the participant was seated comfortably in a chair with his/her non-dominant leg strapped to a custom built tracking device. Muscle activity was recorded from the

tibialis anterior (TA) muscle belly using surface electromyography. In the tDCS-task condition, tDCS was applied for 15 minutes while the subject practiced a visuomotor ankle motor skill task. During the ankle tracking task, the subjects were instructed to track a computer generated sinusoidal wave pattern as closely as possible with ankle dorsiflexion and plantar flexion. For the tDCS-rest condition, the participant's foot remained strapped into the tracking device but they were instructed not to perform any activity while he/she received tDCS for 15 minutes. Corticomotor excitability before and after stimulation was examined using TMS.

Instrumentation:

Electromyography:

Surface Ag/AgCl electrodes were placed over the muscle belly of the TA. The reference electrode was placed over the spinous process of the seventh cervical vertebrae. Before placing the EMG electrodes, the skin was shaved if needed, and rubbed with alcohol to reduce impedance. All EMG data were sampled at 2000Hz, amplified *1000 and band pass filtered (10-500Hz) with a Delsys EMG system (Bagnoli 8, MA USA). The EMG data collection was performed using Spike2software. (Cambridge Electronic design, Cambridge, UK).

Transcranial direct current stimulation (tDCS):

A simple form of constant current stimulator (Chatanoonga, Ionoto, and Hixson TN, USA) was used to deliver 1mA of direct current for 15 minutes. A 4 cm X 2 cm oblong saline soaked sponge electrode was placed over the leg area of M1, identified by hot spotting for the left (non-dominant) tibialis anterior muscle using single pulse TMS. For most subjects this position was 1cm lateral and 1 cm posterior to the vertex. A 7 cm X 5 cm carbonized electrode

was placed over the dominant supraorbital region. All subjects tolerated the tDCS well and no adverse effects related to the application of tDCS were observed.

Transcranial Magnetic Stimulation (TMS):

TMS was applied using a single-pulse stimulator (Magstim, Dyfed, Wales, and UK) via a 110 cm double cone coil using a posterior-anterior cortical current orientation. Spike2 software was used to trigger the stimulator and also record the trigger pulses. A tight fitting cap was placed on the participant's head above the tDCS electrodes. To maintain consistent coil positioning across sessions, detailed distance recordings were made from the nasion, inion and bilateral pre-tragus to the vertex. For all four testing sessions, TMS related measures were obtained from the same spot to minimize variability in effects observed due to different testing positions for each session (Herwig et al., 2001, Sparing et al., 2008). During TMS, participants were provided with visual feedback of their muscle activity and instructed to maintain a tonic contraction of the TA that represented 20% maximum voluntary contraction (MVC). MVC trials were done at the beginning of each session. During the MVC trials, participants were instructed to pull hard as possible with their foot (dorsiflexion) against resistance and the best of 3 MVCs was noted.

Corticomotor excitability of the non-dominant TA muscle representation was assessed using single pulse TMS at 80, 90, 100, 110, 120, 130, 140% active motor threshold for each participant. Active motor threshold (AMT) was defined as the stimulus intensity resulting in identifiable MEPs of at least 0.4 mV peak to peak in 50% of successive trials from the contralateral TA (Madhavan and Stinear 2010). Eight MEPs were recorded for each intensity

prior to tDCS stimulation (PRE), immediately post stimulation (POST0), ten minutes after the end of stimulation (POST10) and 30 minutes after the end of stimulation (POST30).

Ankle motor task:

We used a custom built manipulandum for ankle motor testing and practice (Madhavan et al., 2010; Madhavan et al., 2011).This device consisted of two adjustable plates and straps to secure the foot and shank in place. Participants performed ankle dorsiflexion and plantar flexion to match a sinusoidal wave on the computer screen as accurately as possible. A computer generated a waveform at a random frequency (0.2- 0.4 Hz) and amplitude (60- 80% of the individual's maximum comfortable range of motion). For tDCS-task condition, the participants performed the motor task with a random waveform sequence for 15 minutes with a one-minute rest interval after every four minutes. For the tDCS-rest condition, participants were instructed to maintain the foot at rest and relax.

(A) (B)

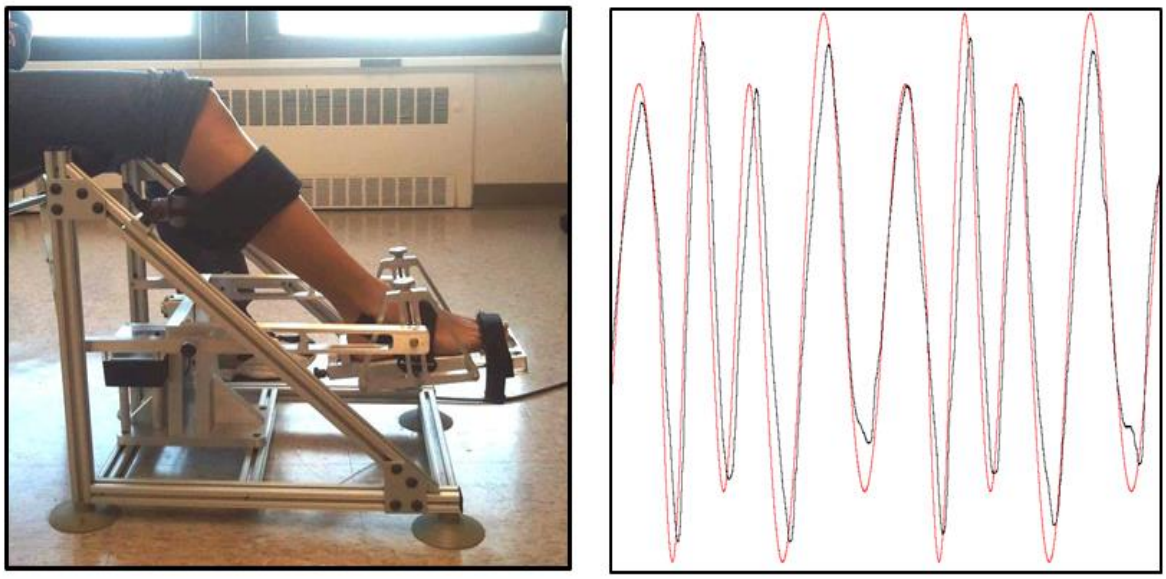

Figure 2: Tracking device and tracking task.

Figure 2(A) provides a pictorial representation of the tracking device and figure 2(B) provides an example of the tracking task. The red sine wave is computer generated and the black sine wave is controlled by the participant in an attempt to match the red sine wave.

Data analyses

All data were imported and analyzed by Spike2 software (Cambridge Electronic design, Cambridge, UK). The TMS related measures evaluated were:

MEP amplitude: The peak to peak MEP amplitude as a measure of change in corticomotor excitability was calculated. MEP amplitude at each TMS intensity was calculated for PRE, POST0, POST10 and POST30 time points.

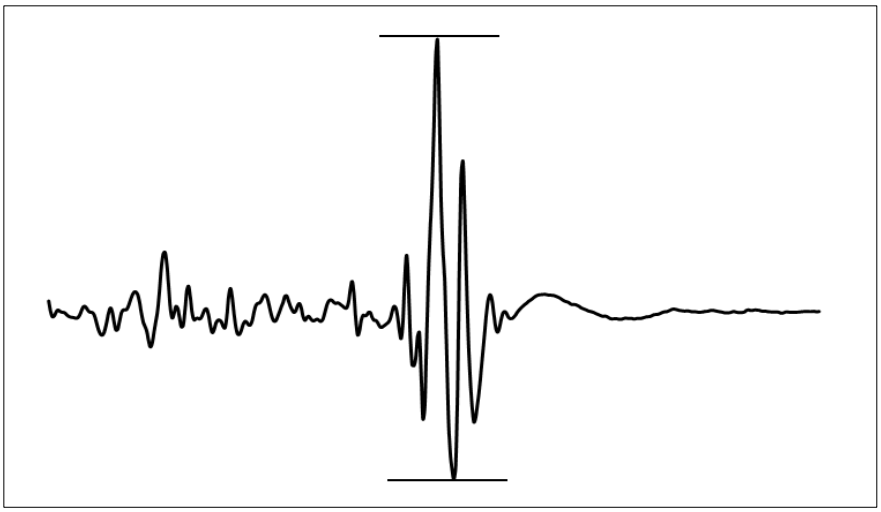

Figure 3: Example of MEP amplitude analysis.

Peak to peak MEP amplitude is denoted by the two horizontal lines.

MEP area: The rectified area under the MEP was also used to evaluate corticomotor excitability changes. MEP area was recorded at each intensity for PRE, POST0, POST10 and POST30 time points.

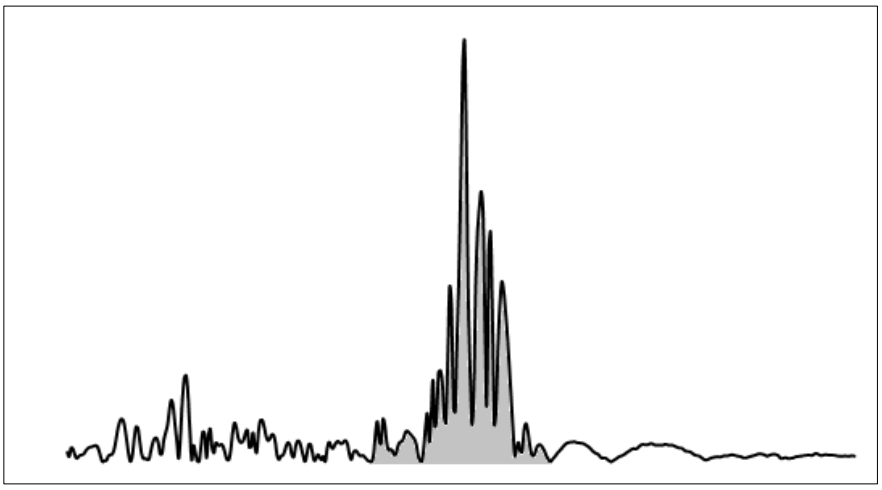

Figure 4: Example of MEP Area analysis

MEP area is denoted by the shaded portion under the rectified MEP

MEP amplitude and area were averaged for each intensity. MEP area was also normalized to the respective baseline values by dividing the average post values by the average baseline value for each participant.

Recruitment curve (RC): Also known as stimulus response curve displays the change in MEP size with increasing stimulus intensity. This stimulus response curve was generated based on the level of AMT that was determined. 80%, 90%, 100%, 110%, 120%, 130% and 140% of AMT stimulus was determined. Eight MEPs were collected at each of the % AMT. The linear slope of recruitment curve that reflects the strength of corticospinal projections or gain of recruitment curve was calculated. The RC slope at each intensity was calculated for PRE, POST0, POST10 and POST30. This assessment was performed for both MEP amplitude and MEP area measures. Below is an example of a raw MEP amplitude (data that have not been normalized) recruitment curve values at each time point for a single representative subject for tDCS-task condition.

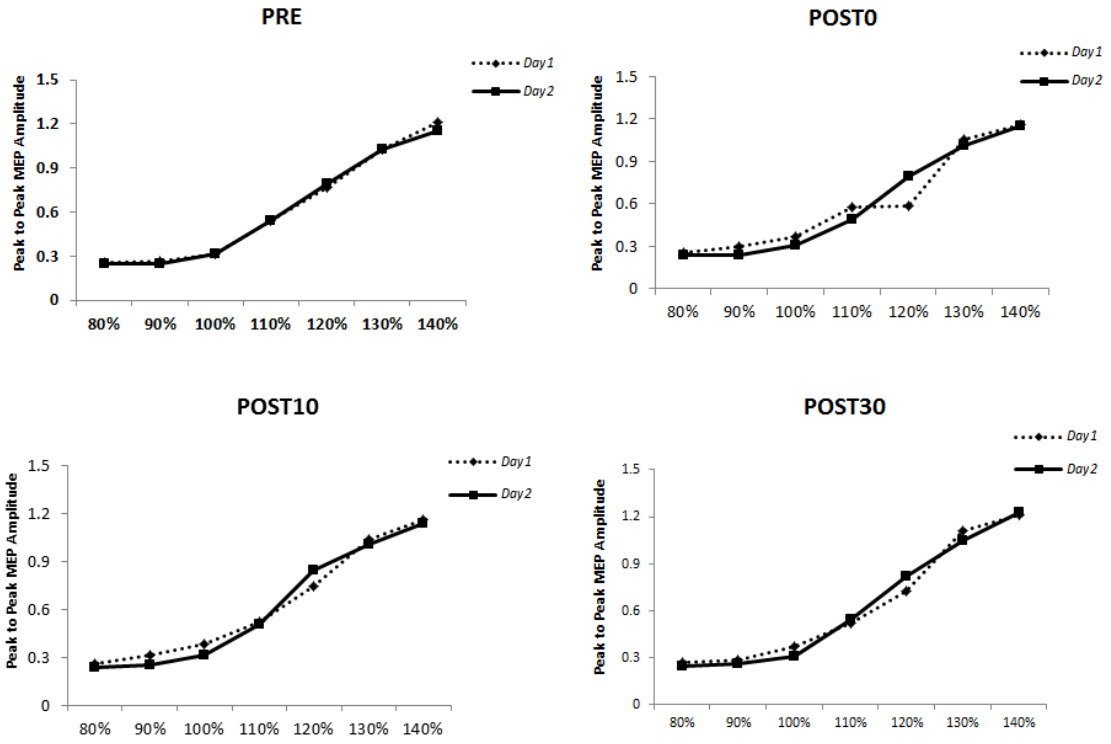

Figure 5: Example of Slope of Recruitment curve for tDCS-task condition at all time points.

Figure 5 depicts the recruitment curve at each of the four time points. X-axis denotes the %AMT and Y-axis denotes the peak-to-peak MEP amplitude. Note the increasing size of the MEP amplitude with increasing intensity.

Statistical analyses

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, IBM software version 22, Armonk, NY). The following analyses were used to examine test-retest reliability of corticomotor excitability after tDCS:

a. Interclass Correlation Coefficient (ICC)

An interclass correlation coefficient was used to determine the test-retest reliability between testing days for both stimulation conditions. ICC was performed to overcome some of

the limitations of the Pearson correlation coefficient. Correlation coefficients provide information about the degree of association between two sets of data however does not account for systemic errors. ICC reflects both degree of consistency and agreement between data sets as it is calculated using variance estimates obtained though partitioning of total variance into within and between subject variance. Thus ICCs were run using a two-way random analysis for absolute agreement (Bartko, 1966). ICC scores ranges from 0-1 with a value of 1 indicating perfect reliability. An ICC value ≥ 0.90 is considered to be excellent, 0.75- 0.89 considered to be good, ≤0.74 considered to be Moderate and below 0.5 explains a poor relationship. ICC's comparisons were made for the MEP amplitude and area measurements for the non-normalized linear slopes of the recruitment curve, and also at each individual intensity of 120, 130, 140% AMT for all time points on Day1 and Day 2 for both the stimulation conditions.

b. Typical Percentage Error (TPE)

To analyze the inter-individual variability associated with these outcome measures, typical percentage error (TPE) was calculated (Hopkins 2000). TPE was calculated using the formula:-

$$100\times[(S_{diff}/\sqrt{2})/\bar{x}]$$

Where S_{diff} is the standard deviation of the difference in scores between Day1 and Day2 (Day2-Day1) of a given outcome measure at a given time point and $\bar{X}$ is the average of Day1 measurement. TPE was calculated between Day 1 and Day 2 for each stimulation condition for the RC slopes and individually for 120, 130 and 140% AMT (for both MEP amplitude and area). It is generally accepted that a TPE of less

than 5% reflects a reliable measure (Hopkins 2000).

c. Change in MEP measures between Day 1 and Day 2 for both the stimulation conditions:

To evaluate reliability of change in corticomotor excitability from baseline at each post time point, a 2×4 repeated measures ANOVA with factors testing days (Day1 vs Day2) and time points (PRE, POST0, POST10 and POST30) was performed. Post hoc analysis (paired t-test) was conducted if ANOVA revealed a significant main effects or interaction between factors.

d. tDCS-task versus tDCS-rest

To examine changes in cortical excitability between tDCS-task vs. tDCS-rest condition, the scores from *Day 1* and *Day 2* in each stimulation condition were averaged and compared. A 2×4 repeated measures ANOVA with factors stimulation conditions (tDCS-task vs tDCS-rest) and time points (PRE, POST0, POST10 and POST30) were performed. Post hoc analysis (paired t-test) was conducted if ANOVA revealed a significant main effects or interaction between factors.

3.3 RESULTS

All participants tolerated the experiment well. Apart from the mild tingling sensation perceived during ramping of the current, the participants reported no adverse effects.

3.3.1 ANALYSIS OF MEP AMPLITUDE MEASURES:

3.3.1.1 Reliability of slopes of recruitment curves

a) tDCS-task condition:

To examine the reliability of peak to peak MEP amplitude with increasing stimulus (i.e., gain of corticomotor excitability), the slope of the recruitment curve was calculated for each day and compared between *Day 1* and *Day 2* for each time point using ICCs (Figure 6). ICC's of the slopes were found to be 0.91 (p=0.001) for PRE, 0.85(p=0.001) for POST0, 0.83 (p=0.001) for POST10 and 0.80 (p=0.003) for POST30.

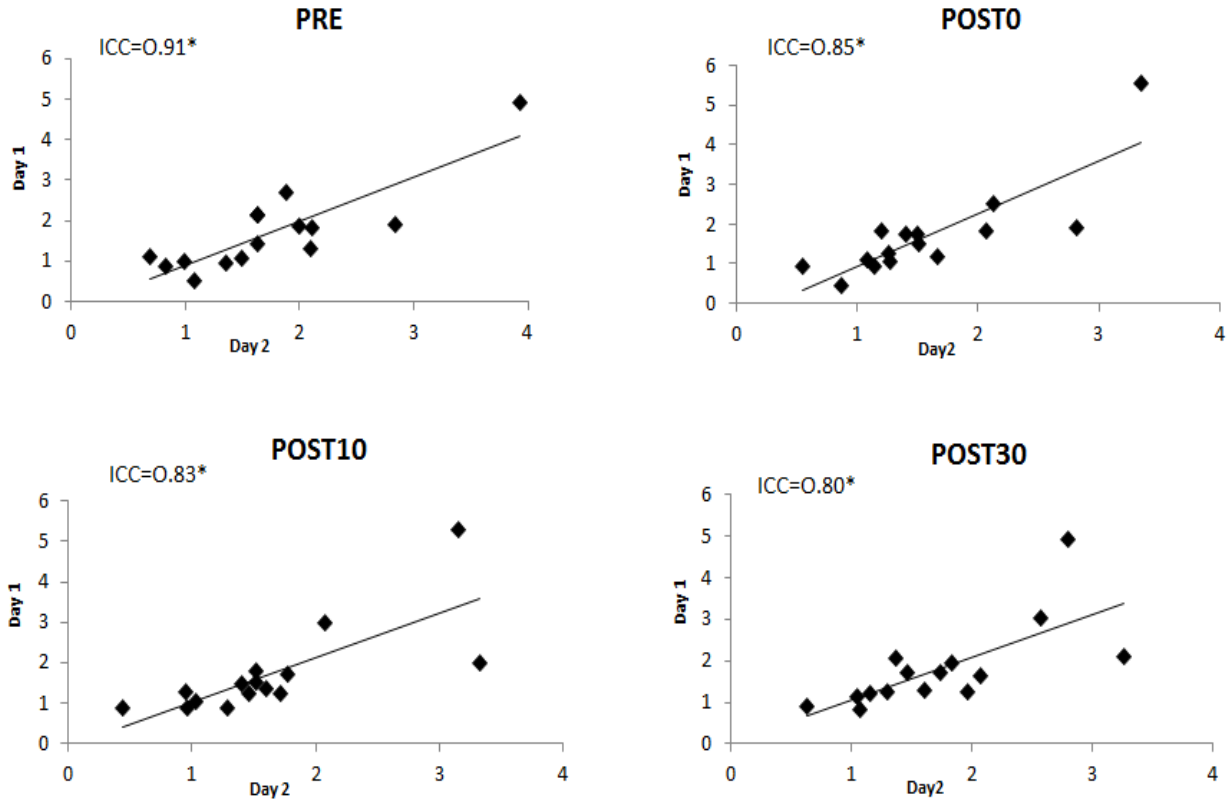

Figure 6: ICCs of slopes of recruitment curves for the tDCS-task condition

Shows the scatter graphs of relationship between Day1 vs Day2 for the slope of recruitment curve at four time points (PRE,POST0,POST10 and POST30). The ICC values at each time points are shown on each graph. Datapoints represent each subject (n=15).

b) tDCS-rest condition:

Similarly, to examine the reliability of the gain of corticomotor excitability for tDCS-rest condition, the slope of recruitment curve was calculated for each day, and compared between *Day 1* and *Day 2* for each timepoint (Figure 7). ICCs of the slopes were found to be 0.80 (p=0.002) for PRE, 0.80(p=0.002) for POST0, 0.63 (p=0.042) for POST10 and 0.72 (p=0.014) for POST30.

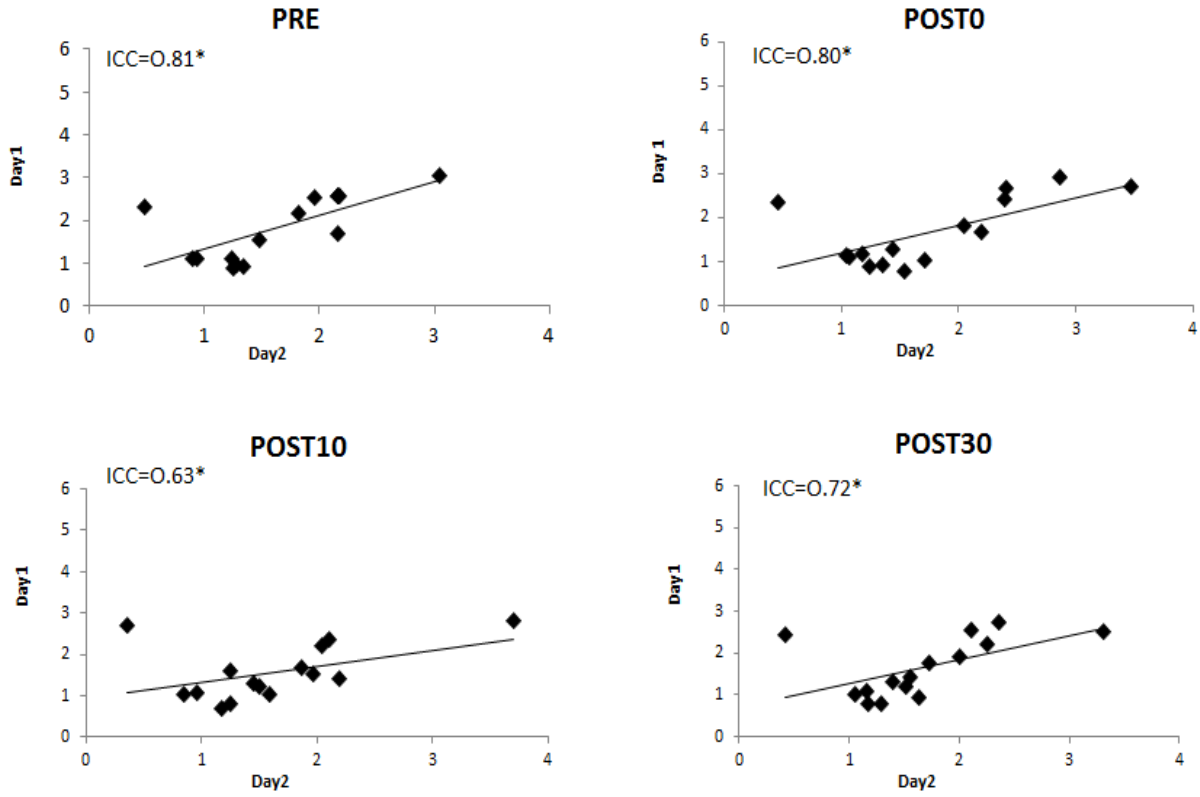

Figure 7: ICCs of slope of recruitment curve in tDCS-rest condition

Shows the scatter graphs of relationship between Day1 vs Day2 for the slope of recruitment curve at four time points (PRE, POST0, POST10 and POST30). The ICC values at each time points are shown on graph. Data points represent each subject (n=15).

3.3.1.2 ICCs at individual intensities

ICC's at 120, 130 and 140% AMT were individually calculated for the tDCS-task and tDCS-rest condition (Figure 8A and Figure 8B).

a) tDCS-task condition:

At 120% AMT, ICC values for PRE, POST10 and POST30 were 0.63, 0.86, 0.60 and p=0.03, 0.001 and 0.04 respectively. The ICC value for POST0 time point (0.34) was not significant (p=0.20). At 130% AMT, ICC values for all time points (PRE, POST0, POST10 and POST30) were 0.97, 0.84, 0.85, 0.76 and p=0.001, 0.001, 0.001 and 0.006 respectively. At 140% AMT, the ICC values for all time points were 0.94, 0.94, 0.88, 0.86 and p= 0.001 at all time points.

b) tDCS-rest condition:

At 120% AMT, ICC values for PRE, POST0 and POST10 were 0.65, 0.71, 0.69 and (p=0.032, 0.016, 0.017 and 0.014 respectively. The ICC value for POST30 time point (0.45) was not significant (p=0.14). At 130% AMT, ICC values for all the time points were 0.68, 0.88, 0.63, 0.76 and p= 0.023, 0.001, 0.003 and 0.007.At 140% AMT, the ICC values for all the time points were 0.88, 0.80, 0.63, 0.72 and p= 0.001, 0.002, 0.008 and 0.007.

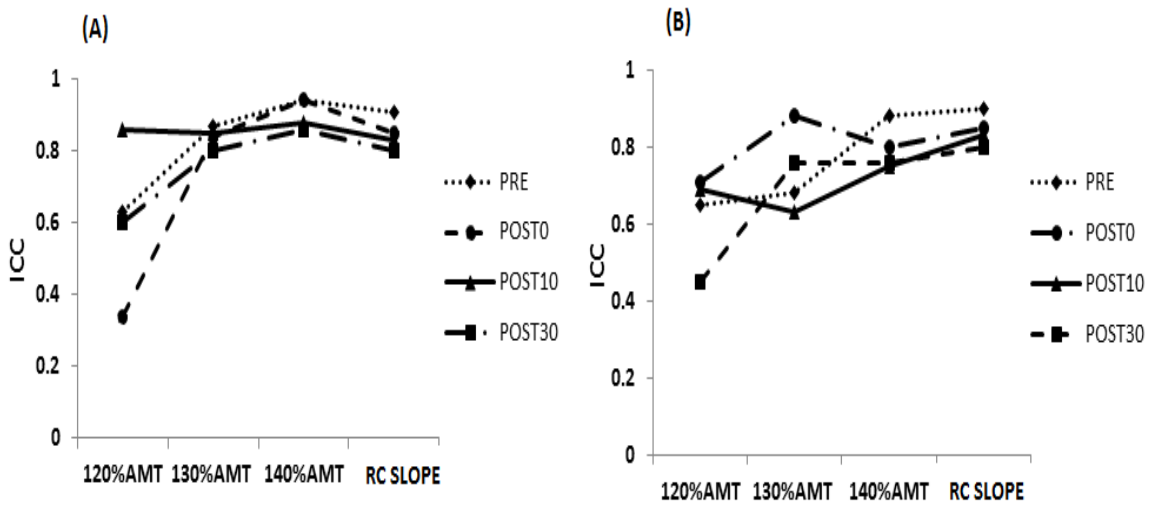

Figure 8: Graphical representation of MEP amplitude ICCs for the tDCS-task and tDCS-rest conditions.

Depicts the ICC values at 120, 130, 140% AMT and R-C slope for all the time points for tDCS-task (Figure 8A) and tDCS-rest (Figure 8B). All the ICC values are tabulated in Table 1 (tDCS-task) and Table 2 (tDCS-rest).

3.3.1.3 TPE

TPE, which represents the associated inter-individual variability, was calculated from the differences in MEP amplitude for *Day 1* and *Day 2* at each time point at 120, 130 and 140% AMT and the RC slope for the tDCS-task and tDCS-rest condition.

a) tDCS-task condition:

At 120% AMT, TPE data for PRE and all the POST time points ranged from 27-36%. At 130% AMT, TPE ranged from 19-22%. At 140% AMT, TPE ranged from 16-19%. TPE data for the RC slope ranged from 29-37%. TPE data for tDCS-task condition is provided in Table 1.

Day 1 Vs Day 2	PRE		POST0		POST10		POST30	
	ICC	TPE %	ICC	TPE %	ICC	TPE %	ICC	TPE %
120% AMT	0.63* (-0.1-0.87)	28	0.34 (-0.697 – 0.769)	36	0.86** (0.613-0.954)	27	0.60* (-0.142-0.867)	35
130% AMT	0.87** (0.62-0.93)	19	0.84** (0.526-0.947)	21	0.85** (0.562-0.952)	20	0.76* (0.295-0.921)	22
140% AMT	0.94** (0.81-0.91)	18	0.94** (0.823-0.981)	16	0.88** (0.656-0.962)	19	0.86** (0.656-0.962)	19
RC SLOPE	0.91** (0.76-0.97)	29	0.85** (0.583-0.953)	37	0.83** (0.489-0.943)	36	0.80** (0.416-0.935)	30

Table 1: ICCs and TPE for MEP amplitude in tDCS-task condition

*Presents ICCs (95% confidence interval) and TPE for 120, 130 ,140% AMT and RC slope between Day 1 and Day 2 for tDCS-task condition. * denotes a significant $p<0.05$ and ** denotes a significant $p\leq0.005$.*

b) tDCS-rest condition:

At 120% AMT, TPE data for PRE, POST0, POST10 and POST30 ranged from 25-31%. At 130% AMT, TPE ranged from 23-34%. At 140% AMT, TPE ranged from 23-34%. TPE data for the RC slope ranged from 22-32%.

TPE data representing the inter-individual variability is provided in Table 2.

Day 1 Vs Day 2	PRE		POST0		POST10		POST30	
	ICC	TPE %	ICC	TPE %	ICC	TPE %	ICC	TPE %
120% AMT	0.65* (-0.43-0.88)	26	0.71* (0.11-0.90)	25	0.69* (0.11-0.89)	26	0.45 (-0.74-0.82)	35
130% AMT	0.68* (-0.43-0.88)	34	0.88** (0.67-0.96)	23	0.63* (-0.12-0.89)	32	0.76* (0.29-0.92)	22
140% AMT	0.88** (0.62-0.96)	34	0.80** (0.42-0.93)	23	0.73* (0.25-0.91)	29	0.76* (0.27-0.92)	19
RC SLOPE	0.81** (0.47-0.93)	26	0.80** (0.42-0.93)	26	0.63* (-0.56-0.87)	32	0.72* (0.13-0.90)	30

Table 2: ICCs and TPE for MEP amplitude in tDCS-rest condition

*Presents ICCs (95% confidence interval) and TPE for 120, 130, 140% AMT and RC slope between Day 1 and Day 2 for tDCS-task condition. * denotes a significant $p<0.05$ and ** denotes a significant $p\leq0.005$.*

3.3.1.4 Change in MEP measures between the two testing days in each condition

The change in corticomotor excitability from baseline at each post time point was quantified by normalizing the POST values to the PRE for each day and compared between days for each stimulation condition.

a) tDCS-task condition:

A 2×4 repeated measures ANOVA with two factors- days (*Day 1* versus *Day 2*) and time points (PRE, POST0, POST10, POST30) was performed at 120, 130, 140% AMT and for RC slopes to evaluate changes in corticomotor excitability. The ANOVA revealed no significant

interactions between the two factors and no significant main effects at any of the TMS parameters, suggesting no differences in the amount of change in corticomotor excitability between the *Day 1* and *Day 2*.

b) tDCS-rest condition:

Similarly, a 2×4 repeated measures ANOVA with two factors - testing days (*Day 1* versus *Day 2*) and time points (PRE, POST0, POST10, POST30) was performed at 120, 130,140% AMT and for RC slopes to evaluate changes in corticomotor excitability. The ANOVA revealed no significant interactions between the two factors and no significant main effects at any of the TMS parameters, suggesting no differences in the amount of change in corticomotor excitability between the *Day 1* and *Day 2*.

3.3.1.5 tDCS-task vs tDCS-rest

To compare the effects of tDCS between the two stimulation conditions (tDCS-task and tDCS-rest), the change in MEP amplitude was examined using a 2 ×4 factorial repeated measures ANOVA for 120% AMT, 130% AMT and 140% AMT and the RC slopes. The factors were stimulation condition (tDCS-task vs tDCS-rest) and time points (PRE, POST0, POST10, and POST30). The MEP amplitudes from *Day 1* and *Day 2* were averaged for each time point under each stimulation condition respectively (Figure 9).

ANOVA revealed no significant interaction effects and main effects of condition and time at 120% AMT and 130% AMT. The interaction effect of stimulation × time was significant for 140% AMT ($F_{1, 14}$=4.3, p=0.022) and for RC slopes ($F_{1, 14}$=3.5, p=0.044). Post hoc analysis (paired t-test) was performed for 140% AMT and RC slope data. At 140% AMT, paired t-test revealed a significantly higher mean (12% increase) for tDCS-task (1.12) compared to tDCS-rest

(0.99) condition for POST30 time point ($t_{1, 14}$=2.36, p=0.033). Similarly, paired t –test revealed a significantly higher mean (7% increase) for tDCS-task (1.067) compared to tDCS-rest (0.97) for RC slope ($t_{1, 14}$=1.71, p=0.05).

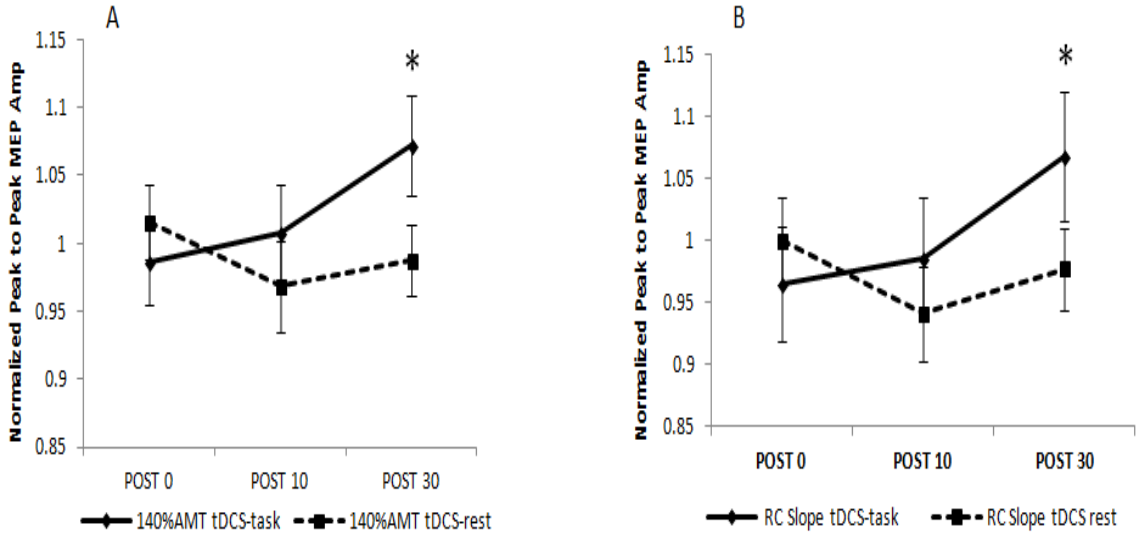

Figure 9: MEP amplitude comparisons between tDCS-task and tDCS-rest

*Figure 9(A) depicts the difference in normalized MEP amplitude between tDCS-task and tDCS-rest for post time points at 140% AMT. Figure 9(B) depicts the difference in normalized RC slope between the two conditions for post time points. * denotes a significant difference between the two conditions (p<0.05).*

3.3.2 ANALYSES OF MEP AREA MEASURES:

3.3.2.1 Reliability of Slope of recruitment curve

a) tDCS-task condition:

To examine the reliability of MEP area measure with increasing stimulus (i.e) gain of corticomotor excitability, the slope of the recruitment curve was calculated for each day and compared between *Day 1* and *Day 2* for each timepoint using ICC (Figure 10).The ICCs of the

slopes were found to be 0.85 (p=0.001) for the PRE, 0.76 (p=0.03) for POST0, 0.78 (p=0.04) for POST10 and 0.80, (p=0.003) for POST30 respectively.

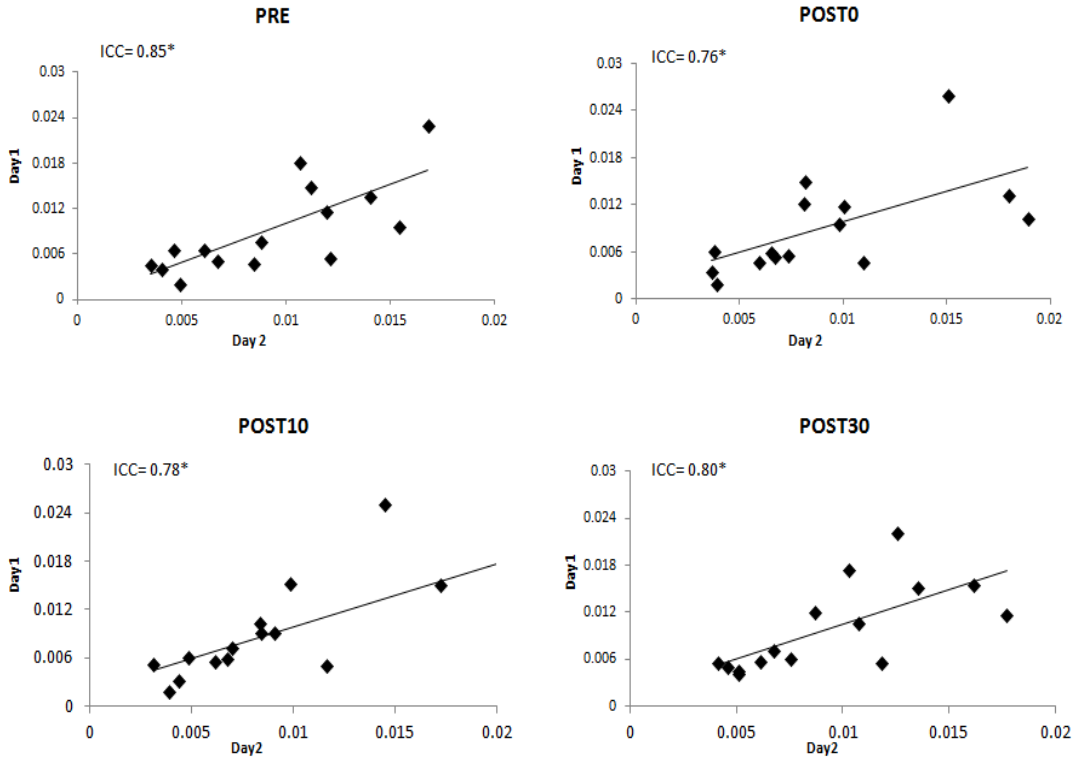

Figure 10: ICCs of Slope of Recruitment curve in tDCS-task condition

Shows the scatter graphs of relationship between Day 1 vs Day 2 for the slope of recruitment curve at four time points (PRE, POST0, POST10 and POST30). The ICC values at each time point are shown on each graph. Data points represent each subject (n=15).

b) tDCS-rest condition:

Similarly, to examine the reliability of the gain of corticomotor excitability for tDCS-rest condition, the slope of recruitment curve was calculated for each day, and compared between *Day 1* and *Day 2* for each timepoint using ICCs (Figure 11). The ICCs of the slopes were found

to be 0.93 (p=0.001) for the PRE, 0.80 (p=0.003) for POST0, 0.55 (p=0.14) for POST10 and 0.86 (p=0.004) for POST30 respectively.

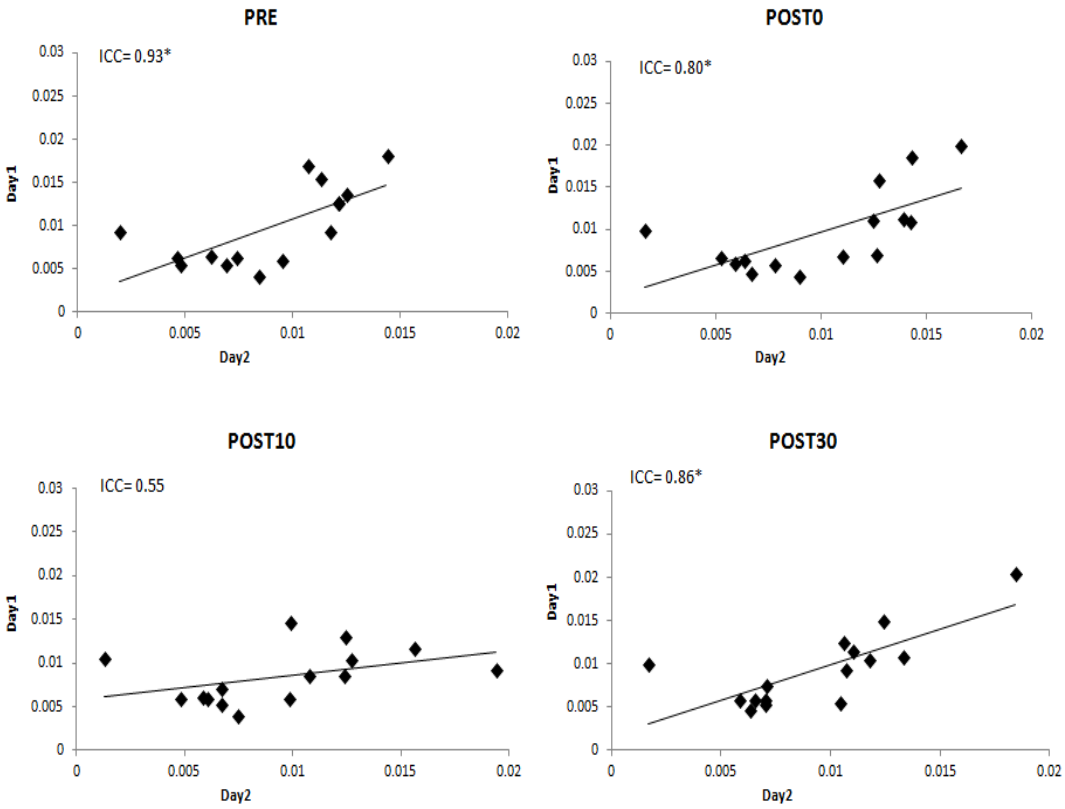

Figure 11: ICCs of Slope of Recruitment curve in tDCS-rest condition

Shows the scatter graphs of relationship between Day1 vs Day2 for the slope of recruitment curve at four time points (PRE, POST0, POST10 and POST30). The ICC values at each time point are shown on graph. Data points represent each subject (n=15).

3.3.2.2 ICCs at individual intensities

ICC's at 120, 130 and 140% AMT were also individually calculated for the tDCS-task and tDCS-rest condition (Figure 12A and Figure 12B).

c) tDCS-task condition:

At 120% AMT, ICC values for all time points (PRE, POST0, POST10 and POST30) were 0.66, 0.62, 0.79, 0.61 and p= 0.048, 0.044, 0.003 and 0.042 respectively. At 130% AMT, the ICCs for all the time points were 0.75, 0.68, 0.76, 0.77 and p=0.003, 0.022, 0.006, 0.006. . At 140% AMT, the ICCs for all the time points were 0.93, 087 0.85, 0.87 and p= 0.001 for all the time points.

d) tDCS-rest condition:

Similarly, at 120% AMT, ICC values for all time points (PRE, POST0, POST10 and POST30) were 0.85, 0.82, 0.86, 0.71 and p=0.001, 0.002, 0.001 and 0.016 respectively. At 130% AMT, the ICCs for all the time points were 0.69, 0.89, 0.67, 0.81 and p=0.017, 0.001, 0.025 and 0.002. At 140% AMT, the ICCs for all the time points were 0.87, 0.78, 0.75, 0.89 and p=0.001, 0.005, 0.006 and 0.001.

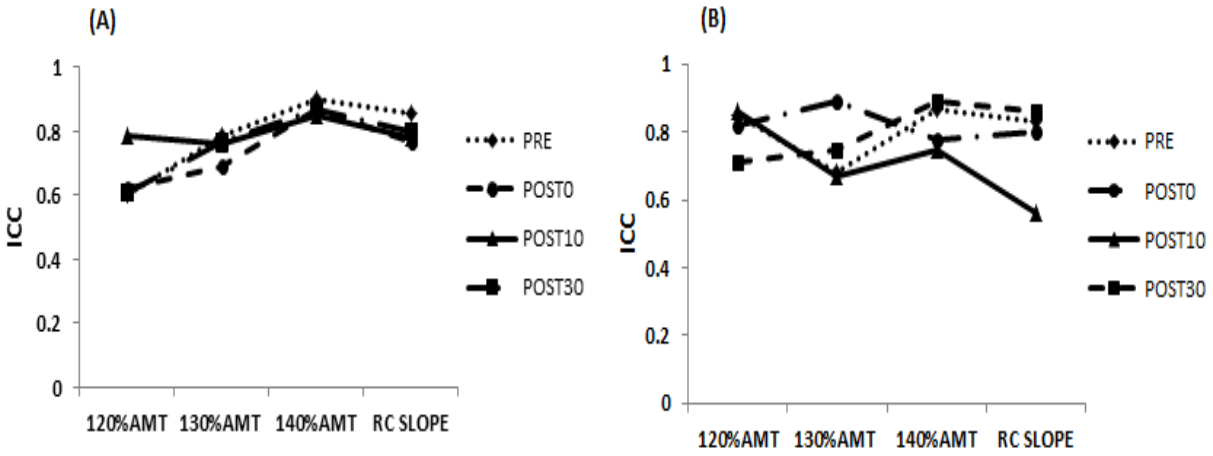

Figure12: Graphical representation of MEP area ICCs for tDCS-task and tDCS-rest condition.

Depicts the ICC values at 120,130,140% AMT and the RC slope for all the time points for tDCS-task (Figure 12A) and tDCS-rest (Figure 12B).All the ICC values are tabulated in Table 3(tDCS-task) and Table 4(tDCS-rest).

3.3.2.3 TPE

TPE, which represents the associated inter-individual variabilty, were calculated from the differences in MEP area for Day 1 and Day 2 at each time point at 120% AMT,130% AMT, 140% AMT and RC slope for both tDCS-task and tDCS-rest condition.

a) tDCS-task condition:

At 120% AMT, TPE data for PRE and POST time points ranged from 33-44%. At 130% AMT, TPE ranged from 28-35%. At 140% AMT, TPE ranged from 20-24%. The TPE data for the RC slope ranged from 29-37%. TPE data for the tDCS-task condition is provided in Table 3.

Day 1 Vs Day 2	PRE		POST0		POST10		POST30	
	ICC	TPE %	ICC	TPE %	ICC	TPE %	ICC	TPE %
120% AMT	0.66* (-0.1-0.87)	35	0.62* (-0.16-0.87)	44	0.79* (0.39-0.93)	33	0.61 (-0.74-0.82)	33
130% AMT	0.68* (0.62-0.93)	30	0.68* (0.04-0.89)	35	0.76* (0.28-0.92)	31	0.77* (0.30-0.92)	28
140% AMT	0.88** (0.81-0.91)	20	0.87** (0.63-0.95)	24	0.85** (0.56-0.95)	24	0.87** (0.62-0.95)	21
RC SLOPE	0.85** (0.76-0.97)	29	0.76* (0.28-0.92)	37	0.78* (0.34-0.92)	36	0.80** (0.40-0.93)	30

Table 3: ICCs and TPE for MEP area in tDCS-task condition

*Presents ICCs (95% confidence interval) and TPE for 120,130,140% AMT and RC slope between Day1 and Day2 for tDCS-task condition. * denotes a significant $p<0.05$ and ** denotes a significant $p\leq0.005$.*

b) tDCS-rest condition:

At 120% AMT, TPE data for PRE, POST0, POST10 and POST30 time points ranged from 20-28%. At 130% AMT,TPE ranged from 19-33%. At 140% AMT, TPE ranged from 19-22%. TPE data for the RC slope ranged from 22-32%. TPE data for the tDCS-rest condition is provided in Table 4.

Day 1 Vs Day 2	PRE		POST0		POST10		POST30	
	ICC	TPE %	ICC	TPE %	ICC	TPE %	ICC	TPE %
120% AMT	0.85* (-0.1-0.87)	20	0.82** (0.46-0.93)	22	0.86** (0.58-0.95)	22	0.71* (0.11-0.90)	28
130% AMT	0.69* (0.62-0.93)	33	0.89** (0.67-0.96)	19	0.67* (-0.68-0.89)	29	0.81** (0.43-0.93)	23
140% AMT	0.87** (0.81-0.91)	19	0.78** (0.33-0.92)	24	0.75* (0.30-0.91)	24	0.89** (0.67-0.96)	19
RC SLOPE	0.93** (0.76-0.97)	26	0.80** (0.41-0.93)	26	0.55 (0.30-0.85)	32	0.86** (0.58-0.95)	22

Table 4: ICCs and TPE for MEP area in tDCS-rest condition

*Presents ICCs (95% confidence interval) and TPE for 120, 130, 140% AMT and RC slope between Day1 and Day2 for tDCS-task condition. * denotes a significant $p<0.05$ and ** denotes a significant $p\leq0.005$.*

3.3.2.4 Change in MEP measures between the *Day 1* and *Day 2* for each condition

The change in corticomotor excitability from baseline at each post time point was quantified for each day, compared between days for each stimulation condition. The average POST0, POST10 and POST30 MEP area was normalized by dividing by the respective PRE.

a) tDCS-task condition:

A 2×4 repeated measures ANOVA with factors- testing sessions (*Day 1* vs *Day 2*) and time points (PRE, POST0, POST10, POST30) were performed at 120,130,140% AMT an RC slopes to evaluate the change in corticomotor excitability. The ANOVA revealed no significant interactions between the two factors and no significant main effects at any of the TMS

parameters, suggesting no differences in the amount of change in corticomotor excitability between the *Day 1* and *Day 2*.

b) tDCS-rest condition:

Similarly, a 2×4 repeated measures ANOVA with factors- testing sessions (*Day1* vs *Day2*) and time points (PRE, POST0, POST10, POST30) were performed at 120, 130, 140% and RC slope to evaluate the change in corticomotor excitability. The ANOVA revealed no significant interactions between the two factors and no significant main effects at any of the TMS parameters, suggesting no differences in the amount of change in corticomotor excitability between the *Day 1* and *Day 2*.

3.3.2.5 tDCS-task versus tDCS-rest

To compare the effects of tDCS between the two conditions, the change in MEP area measures between the two conditions (tDCS-task and tDCS-rest) were examined using 2×4 factorial repeated measures ANOVA at 120,130 and 140% AMT and the RC slopes. The factors were stimulation condition (tDCS-task vs tDCS-rest) and time points (PRE, POST0, POST10, and POST30). The MEP area from *Day 1* and *Day 2* were averaged for each time point under each stimulation condition respectively (Figure 13).

ANOVA revealed no significant interactions effects and main effects of condition and time at 120 % and 130% AMT. The interaction effect of stimulation condition × time was significant for 140% AMT ($F_{1, 14}$=10.06, p=0.001) and RC slopes ($F_{1, 14}$=3.3, p=0.048). Post hoc analysis (paired t-test) was performed for 140% AMT and RC slope data. At 140% AMT, paired t-test revealed a significant higher mean (4%) for tDCS-rest (1.04) compared to tDCS-task condition (0.98) at the POST0 ($t_{1, 14}$=2.34, p=0.014). At POST30 time point, tDCS task (1.08)

revealed a significant higher mean (8%) compared to tDCS-rest (0.99) condition ($t_{1, 14}$=-1.75, p=0.05). Similarly, paired t-test revealed a significant higher mean (5%) for tDCS-rest (1.05) than tDCS-task (0.98) for tDCS-rest condition only at POST0 time point ($t_{1, 14}$=1.70, p=0.05).

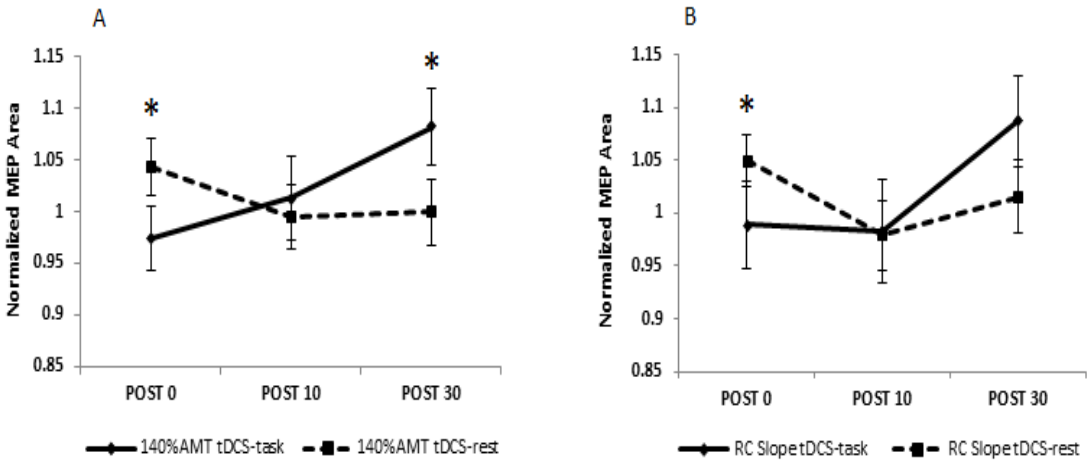

Figure 13: MEP area comparison between tDCS-task and tDCS-rest

*Figure 13(A) depicts the difference in normalized MEP area between tDCS-task and tDCS-rest for post time points at 140% AMT and, Figure 13(B) depicts the difference in normalized RC slope between the two conditions for Post time points. *denotes a significant difference between the two conditions (p<0.05).*

3.4 DISCUSSION

To the best of our knowledge, this is the first study that aims at evaluating the test-retest reliability of tDCS induced corticomotor excitability to the healthy lower limb M1 during motor activity (tDCS-task) versus the absence of motor activity (tDCS-rest). Participants were tested twice under each of these stimulation conditions to examine reliability of effects. The primary outcome measure evaluated was change in corticomotor excitability at different TMS

parameters. Our results suggest that tDCS is a reliable tool when applied with a motor task or in the absence of motor task, and the reliability is higher when tested at a higher TMS intensity (140% AMT) or when evaluating recruitment curve slopes.

In this study, we assessed the reliability of MEP amplitude and area measurements of the TA muscle in healthy individuals. ICCs were performed comparing similar time points between the two testing days for both the stimulation conditions, and TPE analyses were performed to explore the inter-individual variability associated with the reliability.

Reliability of TMS as an experimental tool

TMS has shown to be a reliable tool for evaluating corticomotor excitability in both with the upper limb and lower limb musculature (Cacchio et al., 2011; Carroll et al., 2001; Christie et al., 2007; Malcolm et al., 2006; van Hedel et al., 2007).In a test-retest reliability study of the tibialis anterior muscle, Cacchio et al., (2011) showed that MEP area and amplitude was found to be reliable (0.75-0.88) among the healthy population. Similarly another study by (van Hedel et al., 2007) showed that the MEP amplitude is a reliable measure (0.7) for the tibialis anterior muscle in healthy population. In the present study, our baseline values for RC slope, MEP amplitude and area showed a moderate to high ICC between the two testing days of the same condition indicating an overall high reliability for the TA muscle. The strong reliability of the PRE data suggests any changes noted in the POST measures can be attributed to changes evoked by tDCS and not due to measurement variability of TMS.

Reliability of individual intensities and RC slope

To examine the reliability of POST-tDCS MEP measures, comparisons between the two testing days of the same condition were performed. The MEP amplitude and area for individual

intensities (120%, 130% and 140%AMT) and RC slopes at all the post time points from *Day 1* were compared with *Day 2* for each stimulation condition separately. The ICCs' comparison between 120% AMT, 130% AMT, 140% AMT and RC slope showed moderate to excellent reliability for tDCS-task condition and (Table 1-4) for tDCS-rest conditions at all time points suggesting strong test-retest reliability of outcome measures between *Day 1* and *Day 2*. These results suggest that the amount of change demonstrated by each individual was consistent between the two days. An interesting finding of this study is that the ICC values increased with increasing stimulus intensity with moderate to good reliability at 120% AMT, 130% AMT and RC slope, and excellent reliability at 140% AMT for both the stimulation conditions. The POST-tDCS MEP data suggest that at 140% AMT, 11 out of 15 participants (73%) showed an increase in corticomotor excitability. At 130%, 9 out of 15 participants (60%) showed an increase and 120% AMT, 8 out of 15 participants (53%) showed an increase in corticomotor excitability. The 53% increase in excitability observed at 120% AMT partly supports (Wiethoff et al., 2014) who reported only a 50% increase in POST-tDCS MEPs collected at 110% AMT. A reason for the variation at the lower stimulus intensities could be due to the trial to trial variability associated with the TMS related outcome measures (Ngomo et al., 2012; Pitcher et al., 2003). Pitcher et al, (2003) conducted a study to evaluate the age and sex differences associated with TMS measures suggested that the variation associated at lower stimulus intensities could be due to the less and uncertain number of corticospinal neurons and motor neurons firing at that intensity. Similarly, in a study conducted by (Ngomo et al., 2012) to evaluate the reliability of MEP amplitude and area suggested that, the measures obtained at 110% AMT showed more variability compared to the measures obtained at 120% AMT. Our low reliability for the lower intensities are in agreement with the above mentioned studies (Ngomo et al., 2012; Pitcher et al., 2003). In

addition to the reliability associated with the individual stimulus intensities and RC slope, this study also provides information on the inter-individual variability associated with each of these stimulus intensities. In general, TPE below 5% is considered to reflect a reliable measure between variables (Hopkins, 2011). However, due the inherent biological variability associated with each individual, (Lewis et al., 2014) suggested that the intersession changes in TMS measures need to be greater than 35% in order to observe real changes in healthy individuals. Our TPE data for 130% AMT (19-22%) and 140% AMT (16-19%) have been consistently less for MEP amplitude and MEP area for both conditions. The TPE for RC slope ranged from 22-37% in both conditions, suggesting that the RC slope may better represent the inter-individual variability associated with tDCS.

Reliability of changes in tDCS-induced corticomotor excitability between *Day 1* and *Day 2* in tDCS-task and tDCS-rest condition

We were also interested in examining the corticomotor excitability changes induced by tDCS for MEP measures between *Day 1* and *Day 2* for both the stimulation conditions. No significant differences in the changes were noticed between days or for between sessions, suggesting that the amount of modulation was similar between subjects.

There are a few studies that have examined the effects of multiple sessions of tDCS on the motor cortex (Alonzo et al., 2012; Galvez et al., 2013; Reis et al., 2009). Reis et al., (2009) conducted a study to examine the effects of consecutive five day sessions of tDCS to the motor cortex on motor skill learning. The outcomes were measured in terms of performance and showed that the improvements in motor skill learning were cumulative between the sessions. Alonzo et al., (2012) conducted a study to test the effects of tDCS consecutively applied for five days in a

week's time versus three alternative sessions of tDCS application in a week. They reported variable baseline measures for the 5-day tDCS session vs. similar baseline values for the alternate day's stimulation. Similarly, in a study conducted by Galvez et al., (2013) to examine the effect of constant tDCS versus an increasing tDCS stimulation protocol over five consecutive days reported variable baselines throughout the week in both the conditions. All these three studies have reported variable baseline values for a consecutive tDCS stimulation approach. The variability in baseline values could be due to the result of cumulative increase in cortical excitability that could have been preserved between stimulation sessions. Alonzo et al., (2012) suggested that a time interval of 1 day between testing days was within the window for consolidative cumulative tDCS effects compared to a 2 day time interval between testing days. We attribute this reason for the reliability of our PRE-tDCS MEP measures as our testing days within each condition were three days apart which eliminates the possibility of retention of these cumulative effects associated with consecutive sessions. This may also explain the high consistency we report for the tDCS-induced modulations.

Examination of inter-session change in MEP amplitude and area for tDCS-task and tDCS-rest conditions revealed that the change in MEP amplitude and area between *Day 1* and *Day 2* ranged from 3-15% for tDCS-task condition and 7-23% for tDCS-rest condition. Our results for the amount of change in excitability between the two testing days is similar to Alonzo et al., (2012) and Galvez et al., (2013), where the pre-post ratio remained similar between days, with the variability of measurements ranging from 10-30% in the former study and 10-40% in the latter study. Regardless of the wide range in the expected up regulation, our results suggests that amount of up-regulation is consistent among our subjects.

tDCS-task vs tDCS-rest

In addition to looking at reliability at different the different TMS parameters, we were also interested in comparing the effects of tDCS between the two stimulation conditions. Several studies have demonstrated that anodal tDCS leads to an increased corticomotor excitability evaluated by TMS (Geroin et al., 2011; Jensen et al., 2005; Perez et al., 2004; Saucedo Marquez et al., 2013). tDCS has been applied both during a motor task (Cuypers et al., 2013; Madhavan et al., 2011; Reis et al., 2009) and prior to a motor task (Antal et al., 2011; Kuo et al., 2008; Stagg et al., 2009). Recently, we conducted a study that examined the timing dependent priming effects of subsequent vs concurrent tDCS on ankle visuomotor skill learning. Our results suggested that tDCS applied concurrently with a motor learning task increased motor performance better than when applied prior to the task (Sriraman et al., 2014). The excitability inducing tDCS delivered during motor training resulting in an increased performance could be an example of priming by "gating" mechanism. Similarly tDCS applied prior to a task could be activating different neuronal circuits than when applied concurrently with a task. Based on our previous findings, we expected to observe lower test-retest reliability of tDCS for the tDCS-rest condition.

In this study, we did not observe any differences between the tDCS-task and tDCS-rest group at 120% and 130% for both the MEP amplitude and MEP area measures. At 140% AMT and RC slope, the tDCS-task condition showed a significant increase in excitability at POST30 time (~8%) for MEP amplitude compared to tDCS-rest. For the MEP area measures at 140% AMT, we noticed a significant increase at POST30 (12%) for the tDCS-task condition. For the RC slope, none of the time points reached significance. The differences manifested primarily in the tDCS-task condition could be due to the number of participants who exhibited an increase in corticomotor excitability at that time point. Approximately 10 out of 15 participants exhibited an

increase in excitability in tDCS-task condition for 140% AMT and RC slope at POST30 time point whereas only 6 out of 15 participants showed an increase in excitability in tDCS-rest condition for the same. This variability in number of participants responding to the stimulation condition supports our ICC and TPE data which depicts good to excellent ICC and an associated reduced TPE value for tDCS-task condition in comparison to tDCS-rest condition.

3.5 LIMITATIONS

As with any research investigation, this study is not without its limitations. The *first* limitation of this study was the small sample size. Nevertheless we were able to demonstrate moderate to excellent reliability in some of our measures with a sample size of 15 healthy individuals. Our *second* limitation was that the above results are pertaining to reliability of corticomotor excitability of anodal tDCS specifically for the tibialis anterior muscle in the healthy population, and therefore no extrapolations can be made regarding the reliability of anodal tDCS in individuals with damage to the nervous system or for other muscles. As the TA is the most commonly tested muscle in studies examining corticospinal excitability of the lower limb M1, our findings will still provide new knowledge in this area. Our study is also limited to the fact that we did not examine the reliability of cathodal tDCS. Cathodal tDCS has been widely used in the neurological disease population and future studies examining the reliability of cathodal tDCS induced corticomotor excitability changes would make it a clinically beneficial tool. Our *third* limitation was that we did not account for diurnal variations, as assessing activity levels of participants throughout the day is an aspect to consider in a reliability paradigm. We tried to overcome this limitation by testing each participant at the same time of the day. However, different participants were tested at various times of the day which may

contribute to come of the inter-individual variability. In the tDCS-rest condition while the participants were receiving tDCS stimulation, they were instructed to remain quiet and try to relax. If the participant was stressed or wandered with their thoughts it could affect their neural activity. This was difficult to monitor which could potentially be a *fourth* limitation of this study. Our *fifth* limitation was that we did not account for sweat and hair thickness between participants. Sweat may influence current flow by accumulating in pores of the scalp and leading to alterations in current entering the cortex. Hair thickness may affect the current density and effective penetration of current into the scalp which could have been a reason for some amount of variation we observed between individuals (Horvath et al., 2014). Finally, our *sixth* limitation was that our study has a majority of woman of reproductive age. It has been shown in previous studies that woman of reproductive age are subject to menstrual variation which accounts for variability in cortical excitability (Inghilleri et al., 2004; Smith et al., 2002). We did not take this factor into consideration.

3.6 CONCLUSIONS

In conclusion, the results of the present study provide a comparison between the reliability of tDCS induced corticomotor changes under two stimulation conditions, tDCS-rest and tDCS-task. In the present study, tDCS induced TMS measures obtained at higher stimulus intensities (such as 140% AMT) and the recruitment curve slopes yielded good to excellent reliable results and were associated with a reduced inter-individual variability. Therefore, future studies should consider testing at higher intensities or performing an input-output response curve in order to obtain higher reliability. Additionally, the present study also suggests that applying tDCS concurrently with a motor task resulted in high reliability and reduced inter-individual variability

and the participants exhibited these changes even 30 minutes after the end of stimulation. It is important to keep in mind that although we showed high reliability of tDCS-induced modulations in corticomotor excitability, only 73% of individuals showed the desired up regulation. Future studies will need to explore other parameters of tDCS, such as current dosage, electrode montages etc., to decrease inter-individual responses to this plasticity inducing protocol.

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