The effects of anodal transcranial direct current stimulation on cortical and behavioural changes: An investigation of counter-regulatory mechanisms

Maryam Hassanzahraee
B.Sc. & M.Sc. Physiotherapy

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Department of Physiotherapy

“The man tells his story so many times,
that he becomes his stories and
they live on after him.
And in that
he becomes immortal.”

Big Fish Movie (2004) – Tim Burton

Dedicated to

My lovely parents,
My incredible husband, Masoud,
My amazing brothers Ali & Mohammad,
and
My beautiful kids, Taha & Hana

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Publications during Enrolment

Table 1 Publications related to this thesis published in peer-reviewed scientific journals.

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Chapter 2	**Hassanzahraee M.,** Zoghi M., Jaberzadeh S. Safety of TMS as an assessment tool, In S. Jaberzadeh (Ed.), A Closer Look at Motor-Evoked Potential (pp. 217-232). Hauppauge NY USA: Nova Science Publishers.	6 Dec 2018
Chapter 3	**Hassanzahraee M.,** Zoghi M., Jaberzadeh S. 2018. How different priming stimulations affect the corticospinal excitability induced by non-invasive brain stimulation techniques: A systematic review and meta-analysis. **Reviews in Neuroscience**. *29(8):883-899.* *https://doi.org/10.1515/revneuro-2017-0111*. *Journal Impact Factor: 2.157* *Journal Ranking: Q2 (Neuroscience)*	31 Mar 2018
Chapter 4	**Hassanzahraee M.,** Zoghi M., Jaberzadeh S. 2019. Longer TMS inter-trial interval increases size, reduces variability and improves reliability of the motor evoked potentials. **Brain Connectivity**. 9(10):770-776. *https://doi.org/10.1089/brain.2019.0714*. *Journal Impact Factor: 5.263* *Journal Ranking: Q1 (Neuroscience)*	16 Dec 2019
Chapter 5	**Hassanzahraee M.,** Nitsche M.A., Zoghi M., Jaberzadeh S. 2020. Determination of anodal tDCS duration threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms. **Brain Stimulation.** 13(3): 832-839. http://doi.org/10.1016/j.brs.2020.02.027 *Journal Impact Factor: 6.73* *Journal Ranking: Q1 (Neuroscience)*	27 Feb 2020

Chapter 6	**Hassanzahraee M.,** Nitsche M., Zoghi M., Jaberzadeh S. Determination of anodal tDCS intensity threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms. **Scientific Reports** *Journal Impact Factor: 4.15* *Journal Ranking: Q1 (Neuroscience)*	9 Sep 2020
Letter to Editor **(Case report)**	**Hassanzahraee M.,** Zoghi M., Jaberzadeh S. 2018. Significant reduction in the size of motor evoked potentials following transient paraesthesia during TMS measurements in a young healthy male adult. **Journal of ECT**. 35(3): 215. http://doi.org/10.1097/YCT.0000000000000566 *Journal Impact Factor: 2.14* *Journal Ranking: Q2 (Neuroscience)*	15 Sep 2018

Table 2 Publication related to this thesis under peer-review in peer-review journals.

Thesis Chapter	Publication Citation	Journal/ Publication Status
Chapter 7	**Hassanzahraee M.,** Nitsche M., Zoghi M., Jaberzadeh S. Do cortical changes follow the application of anodal-tDCS coincides with behavioral changes: an investigation of counter-regulatory mechanisms.	**Cortex/** Submitted (2020)

Table 3 Conference Poster Presentations throughout candidature related to this thesis.

Presentation	Presenter/ Title of presentation	Date of presentation
Oral	**Hassanzahraee, M.** Determination of anodal tDCS intensity threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms.	*2019. HDR presentations, 1st place, Physiotherapy Department Research Seminar Series. Monash University*
Oral	**Hassanzahraee, M.** The effects of anodal transcranial direct current stimulation on cortical and behavioural changes: An investigation of counter-regulatory mechanisms	*2019. SPAHC Three-Minute Thesis (3MT), Monash University*
Oral	**Hassanzahraee,** M. How different priming stimulations affect the corticospinal excitability induced by non-invasive brain stimulation techniques: A systematic review and meta-analysis.	*2017. SPAHC Research showcase, Monash University.*
Poster	**Hassanzahraee,** M. How different priming stimulations affect the corticospinal excitability induced by non-invasive brain stimulation techniques: A systematic review and meta-analysis.	*2017. Australian Society for Medical Research (ASMR), Student Research Symposium, Melbourne.*

Table 4 Grants and Awards throughout candidature related to this thesis.

Title of Award	Date of achievement
Faculty International Tuition Scholarships **(FITS)**	*2016*
Monash Graduate Scholarship **(MGS)**	*2016*

Thesis Abstract

Transcranial direct current stimulation (tDCS) is a non-invasive brain neuromodulatory technique for the induction of changes in the activity and excitability of the brain. Long-term potentiation- (LTP) like plasticity is induced when anodal tDCS (a-tDCS) is applied over the primary motor cortex (M1). There is a large number of studies providing evidence that within certain limits, the respective stimulation effect on CSE depends linearly on the intensity (up to 1 mA) and duration (up to 13 min) of a-tDCS (Nitsche and Paulus 2000, 2001; Nitsche et al. 2003). The assumption of the generally linear association was challenged by more recent studies, which showed a reduction or even reversal of tDCS-induced excitability alterations with specific current intensities and/or stimulation durations (Monte-Silva et al. 2013; Bastikadze et al. 2013; Lopez-Alonso et al. 2014; Tremblay et al. 2016; Vignaud et al. 2018, Agboada et al. 2019). It has been shown that specific parameters of a-tDCS can induce different effects in different individuals including expected, less or no change, and reversal of the effects on CSE. This is called response variability consisting of responder and non-responder (Wiethoff-off et al. 2014; Lopez-Alonso et al. 2014, 2015; Amman et al. 2017).

While, not much is known about the exact stimulation parameters for reversals, and non-linearities, as well as underlying mechanisms, therefore, the studies introduced in this thesis are motivated by the need to explore if changing duration and intensity of stimulation would affect the effect of a-tDCS on corticospinal excitability (CSE) and even reverse it. Moreover, it was aimed to investigate if changes on CSE following a-tDCS would coincide with reduction or even reversal of the effects in a motor performance task such as sequential visuo-isometric pinch task (SVIPT).

To achieve the above-mentioned aims, two preliminary studies including systematic review and reliability study were conducted and resulted in two published papers. Study 1

systematically reviewed the literature to investigate how different priming-test protocols on M1 affect CSE in healthy individuals. The findings revealed that, based on the homeostatic pattern, the priming stimulation would reduce or reverse the effects of the test protocol if both protocols have the same effect on CSE. However, based on a non-homeostatic pattern, the outcome would be boosted if these protocols reduce the CSE changes or even push it in opposite direction. Then, the reliability and variability of motor evoked potential (MEP), as an index of CSE, were investigated in Study 2. The findings suggested high reliability and low variability when the inter-trial interval (ITI) increased from 5 to 15s. Thereafter, three primary studies were conducted that resulted in three journal submissions, two of which have been published with one under-review. In Studies 3 and 4, it was aimed to determine the a-tDCS duration and intensity thresholds for reversal of the effects on CSE. The results indicated the duration threshold at ≥ 26 min when using a-tDCS of 1mA. Besides, there would be the intensity threshold at ≥ 1mA with a-tDCS used for 26 min. Lastly, Study 5, it was aimed to investigate if duration and intensity threshold for reversal of CSE following a-tDCS also translated into the reversal of the behavioral effects using SVIPT. The present findings demonstrate no reversal of tDCS effects on performance, but improved performance with intensified tDCS protocols. The reversal threshold for tDCS effects on cortical outcome measures might be different compared to the threshold for behavioral outcome measures.

Thesis including published works declaration

In accordance with Monash University Doctoral Regulation 17.2 ‘Doctor of Philosophy and Research Master’s regulations’ the following declarations are made:

‘I hereby declare that this thesis is an original work of my research and contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis’.

This thesis includes six publications with one manuscript under-review in a peer-reviewed journal. The core theme of the thesis is (the determination of duration and intensity thresholds for reversal of the effects on the CSE and motor performance). The ideas, development, and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the Department of Physiotherapy, Faculty of Medicine, Nursing, and Health Sciences under the primary supervision of A/Prof. Shapour Jaberzadeh as well as co-authors Dr. Maryam Zoghi and Prof Michael A. Nitsche.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research. The studies in this thesis were granted ethics approval from the Monash University Human Ethics Committee 10546 (Appendix 1).

In the case of Chapters, 3 to 7 (Studies 1 - 5) my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision, submitted)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
3	*How Different Priming Stimulations Affect the Corticospinal Excitability Induced by Non-Invasive Brain Stimulation Techniques: A Systematic Review and Meta-Analysis*	*Published*	***80%**. Developed study design, review of appropriate literature, and analysis, manuscript synthesis, and preparation.*	*1) Shapour Jaberzadeh, Developed study design, input into manuscript 15%* *2) Maryam Zoghi, input into manuscript 5%*	*No* *No*
4	*Longer TMS inter-trial interval increases size, reduces variability, and improves the reliability of the motor evoked potentials.*	*Published*	***80%.** Developed study design, review of appropriate literature, securing ethics approval, recruitment of participants, data collection and analysis, manuscript synthesis, and preparation.*	*1) Shapour Jaberzadeh, Developed study design, input into manuscript 15%* *2) Maryam Zoghi, input into manuscript 5%*	*No* *No*
5	*Determination of anodal tDCS duration threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms*	*Published*	***80%.** Developed study design, review of appropriate literature, securing ethics approval, recruitment of participants, data collection and analysis, manuscript synthesis, and preparation.*	*1) Shapour Jaberzadeh, Developed study design, input into manuscript 15%* *2) Maryam Zoghi, input into manuscript 5%*	*No* *No*

6	*Determination of anodal tDCS intensity threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms*	*Published*	***80%.*** *Developed study design, review of appropriate literature, securing ethics approval, recruitment of participants, data collection and analysis, manuscript synthesis, and preparation.*	*1) Shapour Jaberzadeh, Developed study design, input into manuscript 15%* *2) Maryam Zoghi, input into manuscript 5%*	*No* *No*
7	*Do cortical changes follow the application of anodal-tDCS coincides with behavioral changes: an investigation of counter-regulatory mechanisms*	*Submitted*	***80%.*** *Developed study design, review of appropriate literature, securing ethics approval, recruitment of participants, data collection and analysis, manuscript synthesis, and preparation.*	*1) Shapour Jaberzadeh, Developed study design, input into manuscript 15%* *2) Maryam Zoghi, input into manuscript 5%*	*No* *No*

I have not renumbered sections of submitted or published papers to generate a consistent presence within the thesis.

Student signature: **Date:** 20th Oct 2020

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author, I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature: **Date:** 20th Oct 2020

Acknowledgments

I would like to thank my supervisors for their support of my Ph.D. research, **Associate Professor Shapour Jaberzadeh**, and **Dr. Maryam Zoghi**.

Special thanks to **Prof. Michael A. Nitsche** of University Medical Hospital Bergmannsheil (Bochum, Germany) whose involvement in these studies has been the highlight of my Ph.D.

I would like to thank my family to whom I owe a great deal.

To my late **Dad**, thank you for showing me that with hard work and determination, anything is possible.

To my **Mum**, thank you for your love and for showing me your strength and resilience.

To my wonderful husband **Masoud**, thank you for your love, support, and encouragement. I am truly grateful for your patience and assistance especially managing increased domestic duties in these last few months.

To my amazing son and daughter, **Taha**, and **Hana**, thank you all for tolerating the ups and downs of my study journey. You have been such an inspiration to me and also an important source of pride, joy, and laughter.

Abbreviations

A-tDCS	Anodal transcranial direct current stimulation
Ag/AgCl	Silver/Silver Chloride
AMPA	Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BCM	Bienenstock-Cooper-Munro
Ca^{2+}	Calcium
Cl^{-}	Chloride
Cm	Centimeter
CSE	Corticospinal excitability
CS	Conditioning Stimulus
c-tDCS	Cathodal transcranial direct current stimulation
cTBS	Continues Theta Burst Stimulation
EMG	Electromyography
EEG	Electroencephalography
ER	Error rate
FDI	First dorsal interosseous
fMRI	Functional magnetic resolution imaging
FPN	frontoparietal network
GA	Grand Average
GABA	Gamma Aminobutyric Acid
Glu	Glutamate
HD	High Definition
HD-tDCS	High-Definition Transcranial Direct Current Stimulation
Hz	Hertz
ICC	Intra-Class Correlation
ICF	Intracortical facilitation
ICI	Intra-cortical inhibition
ISI	Inter-stimulus interval
iTBS	Intermittent Theta Burst Stimulation

ITI	Inter-trial interval
LICI	Long intracortical inhibition
LIF	Long interval facilitation
LTD	Long-Term Depression
LTP	Long-Term Potentiation
M1	Primary motor cortex
mA	Milliampere
MEP	Motor evoked potential
Mg^{2+}	Magnesium
min	Minute
MRI	Magnetic Resonance Imaging
MSO	Maximum stimulator output
MT	Movement time
mV	Millivolt
MVC	Maximum voluntary contraction
Na^{+}	Sodium
NIBS	Non-invasive brain stimulation
NMDA	N-methyl-D-Aspartic acid
NMDAR	N-Methyl-D-aspartic acid Receptor
PAS	Paired associative Stimulation
PEST	Parameter estimation by sequential testing
PMA	Premotor area
PpTMS	Paired pulse transcranial magnetic stimulation
QPS	Quadripulse Stimulation
rmANOVA	Repeated measure analysis of variance
RMT	Resting motor threshold
RT	Reaction time
rTMS	Repeated transcranial magnetic stimulation
s	Second
SD	Standard deviation
SEM	Standard error of the mean
Sham-tDCS	Sham Transcranial Direct Current Stimulation

SICI	Short interval intracortical inhibition
SMA	Supplementary Motor Area
SMD	Standard mean difference
SPSS	Statistical Package for Social Sciences
SpTMS	Single-pulse transcranial magnetic stimulation
SVIPT	Sequential visual isometric pinch task
TBS	Theta Burst Stimulation
tDCS	Transcranial direct current stimulation
TES	Transcranial Electrical Stimulation
TMS	Transcranial magnetic stimulation
tRNS	Transcranial Random Noise Stimulation
TS	Test Stimulus

Thesis Outline

The thesis will provide a body of work investigating a-tDCS duration and intensity thresholds for reversal of the effects on CSE and motor performance (Figure 1).

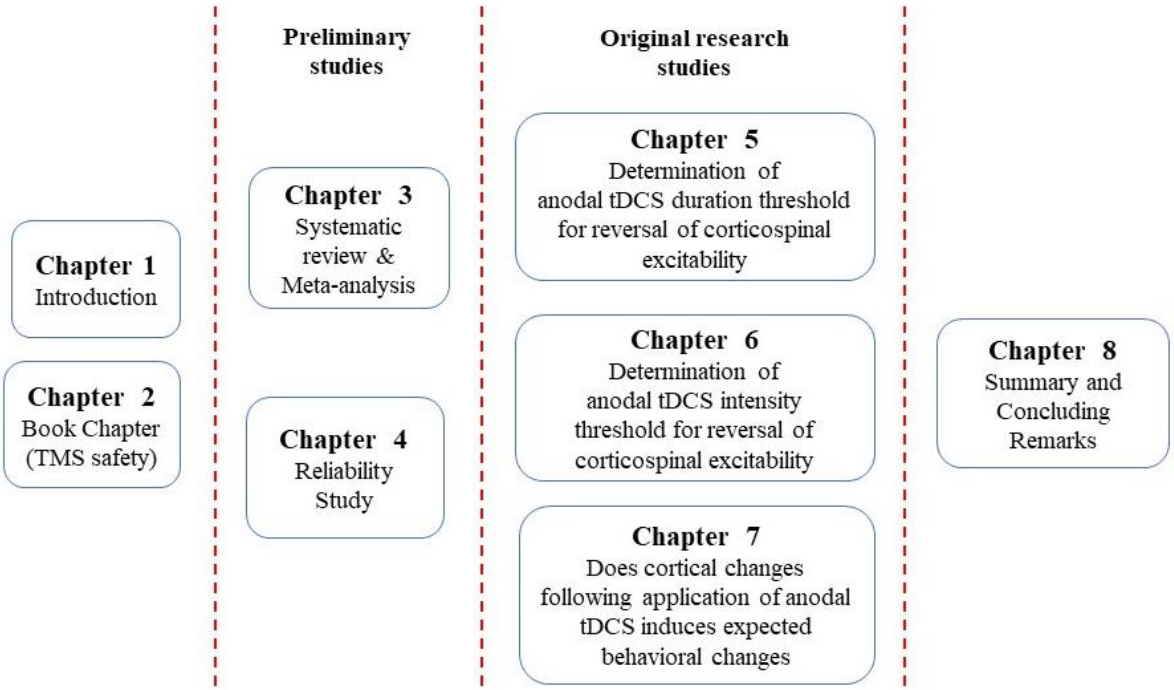

Figure 1 Chapter outline and thesis structure

Chapter 1 presents a background to the topic and important concepts in this thesis to anchor the framework of the research field that this thesis is related to. The introduction provides several sections about the physiology of the cerebral cortex, M1, corticospinal tract, a general description of NIBS techniques with a focus on tDCS as a neuromodulatory technique, and the underlying mechanism behind its effects on M1. It also introduces TMS as an investigational tool for assessment of the CSE, and intracortical excitability mechanisms behind the CSE changes. Moreover, there is a general explanation regarding the motor performance test utilised in the last study of this thesis (Chapter 7).

Chapter 2 presents currently available advice about the safety of TMS as the main assessment tool in the current thesis. TMS was used in all experiments of this thesis to evaluate changes in CSE and intracortical mechanisms behind the changes in CSE.

Chapter 3 presents a systematic review and meta-analysis of current literature about how different priming-test protocols on M1 affect CSE in healthy individuals.

Chapter 4 presents the intra-rater reliability of the assessor (MHZ) for the recording of TMS-induced MEPs as an index for CSE. This study aimed to investigate intra- and inter-session reliability, variability, and changes in the size of peak-to-peak MEP amplitude at different ITIs (5, 10, 15, and 20s) recording from first dorsal interossei (FDI) muscles at rest.

Chapter 5 and **6**, present two double-blinded cross-over randomised experiments that were carried out to determine the "duration" and "intensity" thresholds for reversal of a-tDCS effects on CSE in healthy individuals.

Chapter 7, presents a study using SVIPT to investigate if reversal of CSE following a-tDCS applications is also translated into the reversal of the behavioural effects.

Chapter 8, the final Chapter, presents general conclusions to link the findings of these experiments and provide recommendations for future research.

Chapter 1 - General Introduction

1.1 Overview of Research Problem

Non-invasive brain stimulation (NIBS) techniques are widely used to investigate and modulate cortical/corticospinal excitability (CSE) and plasticity of the M1. These techniques could be used in both healthy populations and those with different neurological or psychological disorders. Among different types of NIBS techniques, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are the most common forms used to investigate mechanisms behind brain neuroplasticity as well as their therapeutic applications. The effects of different NIBS techniques on CSE are measured by the changes in the amplitude of TMS-induced MEPs. These changes in CSE could outlast the period of stimulation, and these after-effects depend on the parameters such as intensity and duration of stimulation (Nitsche and Paulus 2001, 2001). The primary aim of the current thesis is to enhance our understanding of how changes in the parameters of anodal transcranial direct current stimulation (a-tDCS) affect CSE which is a cortical outcome measure. The secondary aim is to investigate if the same effects are also happening in a behavioural outcome measure such as motor performance. Although several studies have successfully reproduced NIBS expected after-effects and the expected changes in CSE, recent studies on these protocols have shown that the responses to these NIBS protocols are rather variable (Wiethoff et al. 2014; Lopez-Alonso et al. 2014, 2015; Hordacre et al. 2015; Vallence et al. 2015; Ammann et al. 2017; Vignaud et al. 2018). Several biological, physiological, and technical factors affect the efficacy and utility of the NIBS techniques that could be considered as probable sources of both inter-and intra-individual

response variabilities (Ridding and Ziemann, 2010). Figure 2, summaries the project conceptual framework explaining factors affecting response variabilities to the tDCS technique.

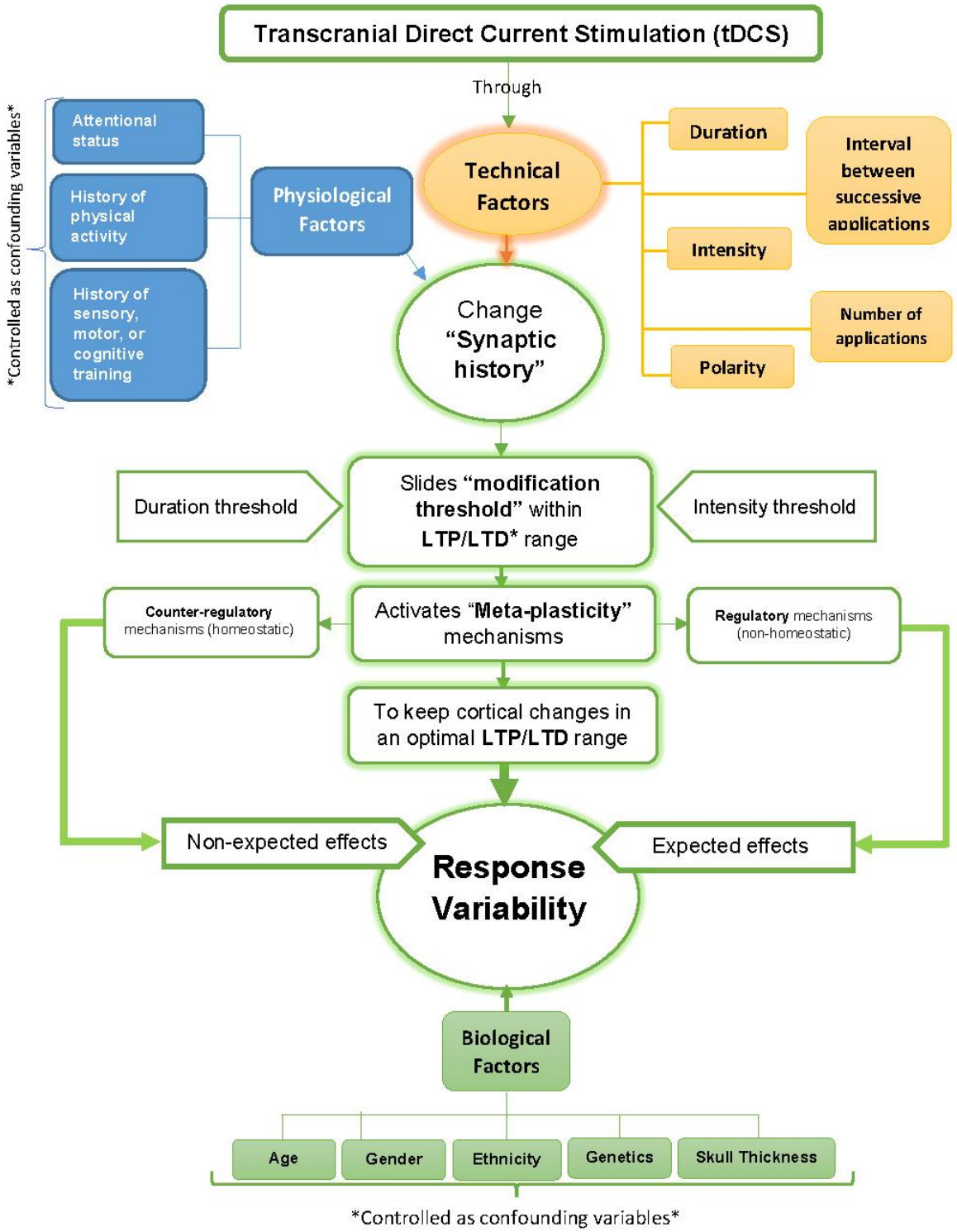

Figure 2 Conceptual framework of the current thesis. TDCS affects response variability through different biological, physiological, and technical factors. These factors modify synaptic history, which in turn slide modification threshold toward more excitation or inhibition depends on the level of synaptic history. The threshold sliding will activate metaplasticity mechanisms to help the brain, keeping its neural activity within an optimal physiological range. These mechanisms include regulatory (result in expected effect) and counter-regulatory (result in non-expected effect) mechanisms. This mechanism activation therefore would result in response variability. In this thesis, two technical factors including stimulation duration and intensity were mainly studied. The two other factors of biological and physiological controlled as confounding variables.

Several important technical factors including stimulation duration, current intensity, polarity, number of NIBS applications, and the interval between these applications can affect this response variability. In this thesis, we mainly focused on the “duration” and “intensity” of stimulation. Among the factors listed above, the "history of the synaptic activity" is affected by both duration and intensity of NIBS techniques and modifies the effect of the stimulation on CSE (Hordacre et al. 2015). The history of synaptic activity is associated with a bidirectional synaptic modification threshold of synaptic plasticity between long-term potentiation (LTP) and long-term depression (LTD) (Karabanov et al. 2015; Muller-Dahlhaus and Ziemann, 2015). Therefore, if the level of the synaptic history is excessively high or low, it may slide the modification threshold, and therefore instead of the expected effect of the stimulation, we may see the reversal of the effects due to the counter-regulatory (homeostatic) mechanisms. For example, the modification threshold decreases at a low level of synaptic activity, thereby favoring the probability of subsequent LTP instead of LTD, and vice versa.

On the other hand, if the modification threshold does not slide excessively toward excitation or inhibition because of a moderate level of synaptic history, the effect of the stimulation will be as expected to regulate through regulatory mechanisms (non-homeostatic) (Bienestock et al. 1982; Abbot and Nelson, 2000; Cooper and Bear, 2012). This sliding modification threshold enables

the brain to keep its neuronal activity within a physiological range avoiding runaway harmful excitation or inhibition (Ziemann and Siebner, 2008).

1.2 Overview of Current State of Literature

NIBS techniques are widely used to probe, modulate, and measure cortical excitability and plasticity in the human cortex. These techniques could be used in both healthy individuals and those with psychological (Gershon et al. 2003; Kincses et al. 2004; Fregni et al. 2005; George et al. 2007) or neurological (Uy et al. 2003; Boggio et al. 2007; Bolognini et al. 2009; Benninger et al. 2010) disorders. NIBS techniques could be used as the alternative therapeutic technique in different pathologies. These techniques show many distinct advantages over pharmacological approaches specifically in inducing therapeutic effects on neurological disorders such as Parkinson's disease and stroke (Floel, 2014; Marquez et al, 2015). The NIBS techniques have achieved significant improvements in the last three decades. These techniques could not only complete the effect of conventional therapies and rehabilitation but also represent an option to the surgical procedures. TMS and tDCS have seen much attention in recent years as the two most common non-invasive and easy to administer techniques. TMS is a neuro-stimulation technique that acts at a supra-threshold level while tDCS is a cost-effective neuro-modulation technique. TMS is used as an assessment tool to investigate the effect of tDCS as an easy-to-use and reusable tool both in research and clinical applications. Application of tDCS over the brain motor areas has become the core of interest for motor recovery and rehabilitation in clinical conditions (Kuo and Nitsche, 2012; Coffman et al. 2014).

1.3 Response variability following NIBS

Recent studies have revealed high response variability following NIBS techniques (Muller-Dahlhaus et al. 2008; Doeltgen and Ridding, 2010; Gamboa et al. 2010; Moliadze et al. 2012; Lopez-Alonso et al. 2014; Wiethoff et al. 2014; Chew et al. 2015; Strube et al. 2015; Tremblay et al. 2016; Amman et al. 2017) with 30-50 % of participants showing no 'expected effect' following stimulation. This response variability, indeed, confirms the non-linear effects of stimulation parameters on changes in CSE. The assumption of a generally linear association between stimulation intensity/duration and LTP-like plasticity was challenged by many recent studies, which showed a reduction and even reversal of tDCS-induced excitability alterations with specific stimulation duration and/or current intensities (Monte-Silva et al., 2013; Bastikadze et al., 2013; Lopez-Alonso et al., 2014; Tremblay et al., 2016).

In 2013, Monte-Silva et al. showed that doubling the duration of a-tDCS with an intensity of 1 mA from 13 to 26 min reverses the excitatory effect of the stimulation (Monte-Silva et al. 2013). Another study in 2013, demonstrated that using c-tDCS with the intensity of 2mA has increased the effect on CSE opposite to the expected reduction (Bastikadze et al. 2013). This finding was confirmed with a crossover study in 2014 using 2mA a- and c-tDCS for 10 min on the left M1. The authors in this study found no reduction of CSE following c-tDCS and only a small overall increase, with high variability, in CSE following a-tDCS (Wiethoff et l. 2014). Also, another study in 2014, comparing three different NIBS techniques of a-tDCS (13min, 1mA), paired alternative stimulation (PAS25), intermittent theta-burst stimulation (iTBS) did not find an effect of any techniques on CSE following the stimulation (Lopez-Alonso et al. 2014). Following these studies, a more recent study utilized two common duration of a-tDCS i.e., 10 and 20 min in combination with two intensities of 1 and 2 mA (Tremblay et al., 2016). The finding did not reveal any differences following these set-ups in post-stimulation measurements.

Over the last two decades, there has been increasing evidence of links between NIBS induced CSE enhancement, skill training (Hummel et al. 2005; Hummel and Cohen, 2005; Fregni et al. 2005; Hunter et al. 2009; Reis et al. 2009; Matsu et al. 2011) and motor performance (Nitsche et al. 2003; Hummel et al. 2010). The functional changes have been indicated to coincide with changes in CSE (Cicinelli et al. 1997; Traversa et al. 1997; Classen et al. 1998; Muellbacher et al. 2001; Lotze et al. 2003; Perez et al. 2004; Jensen et al. 2005) in both healthy individuals (Pascual-Leone et al. 1998; Bütefisch et al. 2004; Kim et al. 2004) and those with different pathological conditions (Uy et al. 2003; Hummel & Cohen 2005; Kim et al. 2006).

Altogether, the optimal parameters of tDCS – such as stimulation duration, and intensity – crucially need to be taken into consideration both in the realm of research and its clinical application in future studies. Optimization of a-tDCS parameters can have a profound impact on its efficacy for enhancement of CSE and possibly motor performance.

Although tDCS has huge potentials for experimental and clinical applications, the response variability has raised serious concerns about the utility of tDCS as a neuromodulatory technique. Current tDCS protocols show high inter-individual variability which means that these protocols with the same parameters do not affect different individuals as expected (Wiethoff et al. 2014; Lopez-Alonso et al. 2014, 2015; Ammann et al. 2017). This, indeed, confirms the need for a comprehensive investigation to determine the modification threshold for both the “duration” and “intensity” threshold for the reversal of a-tDCS effects on CSE. This may lead to the reduction of response variability during the application of tDCS. Moreover, it is worth noting that although this reversal of NIBS-induced plasticity may happen in cortical outcome measures, it is unclear whether behavioral outcome measures might be also similarly affected (reversed).

1.4 Overview of Original Contribution to Knowledge

In the current thesis a book chapter, two preliminary studies (Chapters 3 and 4), and 3 main studies (Chapters 5 - 7) were completed to achieve the thesis aims in the thesis outline. The publications of this thesis that contributed to the current base of knowledge are listed below:

Chapter 1: presents a background to the important concepts in the current thesis. It includes several sections such as the general description of the central nervous system, NIBS techniques with a focus on tDCS as a neuromodulatory technique, and the underlying mechanism behind its effects on M1. It also introduces TMS as an investigational for assessment of the CSE, and intracortical excitability. Moreover, there is a general explanation regarding the motor performance test utilised in the last study of this thesis (Chapter 7).

Chapter 2: provides a comprehensive guideline regarding the safety of the TMS as an assessment tool that is a need in clinical and research streams. Moreover, sometimes regardless of comprehensive safety evaluation, there is still an out-of-control incident that needs the complete awareness of the assessor to all potential risks (refer to a published Letter to Editor, Hassanzahraee, et al. 2019).

Chapter 3: reveals that based on the nature, duration, and magnitude of the priming and test protocols, different effects of stimulation on CSE would be expected from priming-test protocols. It is also concluded that the prior state of neural activity would affect the expected effect of stimulation on CSE changes

Chapter 4: shows that the increase in TMS ITI not only increases the reliability of TMS-induced MEPs but also reduces the chance of MEP variability as well as the improved size of MEP amplitude,

Chapter 5: determines a duration threshold for reversal of the excitability-enhancing effect of a-tDCS with the stimulation durations ≥ of 26 min.

Chapter 6: determines an intensity threshold of ≥ 1mA for 26 min a-tDCS to reverse LTP- into LTD-like plasticity.

It is also discussed that counter-regulatory mechanisms would be a mechanistic foundation for the reversal of effects in both **duration** and **intensity thresholds** to prevent excessive brain activation, and finally,

Chapter 7: demonstrates no reversal of tDCS effects on performance, but improved performance with intensified tDCS protocols. The return points of tDCS effects might thus differ between resting-state physiology and task-related states.

Chapter 8: presents a general discussion to link the findings of the studies within the current thesis, states the limitation of the current thesis, and provides recommendations for future research.

1.5 Anatomy and physiology of the central nervous system

This section provides a brief review of anatomical/ physiological characteristics of the central nervous system (CNS) including the human motor system. It is essential to provide the relevant introductions to cortical structures as the main target of tDCS in the studies of the current thesis. Further sub-sections on the neurophysiology of CNS would also provide the basis of the NIBS mechanisms as both an assessment and intervention tool.

1.5.1 The Cerebral Cortex

The primary motor cortex is the main emphasis of tDCS application in this thesis; therefore, the investigation will be focused on the regions of the cerebral cortex involved in motor control. The cerebral cortex, the 'gray matter' of the brain, is the outer layer of cerebrum neural tissue, and its thickness is about 3 -4 mm (Edelman and Mountcastle, 1978, Taylor, 1999). It contains two-thirds of the brain mass and covers most structures within the brain. It is the most highly developed part of the brain that regulates the most information processing in the brain. The cerebral cortex is divided into four lobes (Figure 3): frontal, temporal, parietal, and occipital. This classification is based on both anatomical landmarks of sulci and gyri, and the distinct functional significance of each lobe such as motor, somatosensory, and visual.

Typically, there are six layers in the cerebral cortex: Layer I, closest to cortex outer layer, to layer VI, preceding the white matter (Figure 4). Each layer is primarily differentiated by the presence or absence of cell types. The neurons of the cerebral cortex are distributed in horizontal and columnar organizations. Each layer thickness depends on the function of the related cortical region (Dinse et al. 2013).

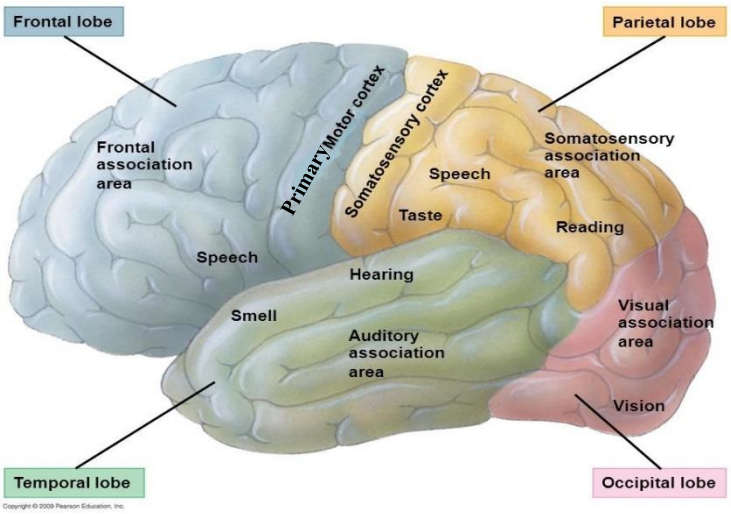

Figure 3. Cerebral Cortex. The illustration of cerebral cortex, depicting four lobes of frontal, parietal, temporal, and occipital. These lobes contain sensory, motor, and association areas. Adapted from: Pearson Education, 2009.

1.5.2 Horizontal Organization

The cerebral cortex layers are numbered in Roman numeral from superficial to deep. Typically, there are six layers in the cerebral cortex: Layer I, closest to cortex outer layer, to layer VI, preceding the white matter (Figure 4). Each layer is primarily differentiated by the presence or absence of cell types. The neurons of the cerebral cortex are distributed in horizontal and columnar organizations. Each layer thickness depends on the function of the related cortical region (Dinse et al. 2013). Layer I (Molecular layer) contain the apical dendrites of pyramidal cells and distal branches of axons located in the thalamus projecting to the cortex. Layer II (External Granular layer) contains the medium size of Stellate cells. Layer III (External Pyramidal layer) contains small Pyramidal cells. Layer IV (Internal Granular layer) contains Stellate cells. Layer V (Internal Pyramidal layer) contains large Pyramidal cells projecting to the corpus striatum, brain stem, and spinal cord. Layer IV (Multiform layer) contains modified Pyramidal cells projecting to the thalamus.

The cerebral cortex layers are functionally divided into three parts: 1) Supra-Granular layers (layers I to III) are the primary origin and termination of intracortical connections, either associational (same hemisphere connections) or commissural (interhemispheric connections via corpus callosum). 2) Internal Granular layer (layer IV) receives the afferents from thalamic relay nuclei and is most prominent in the primary sensory cortices, 3) Infra-Granular layers (layers V & VI) primarily connect the cerebral cortex to subcortical regions and are most developed in motor cortical areas.

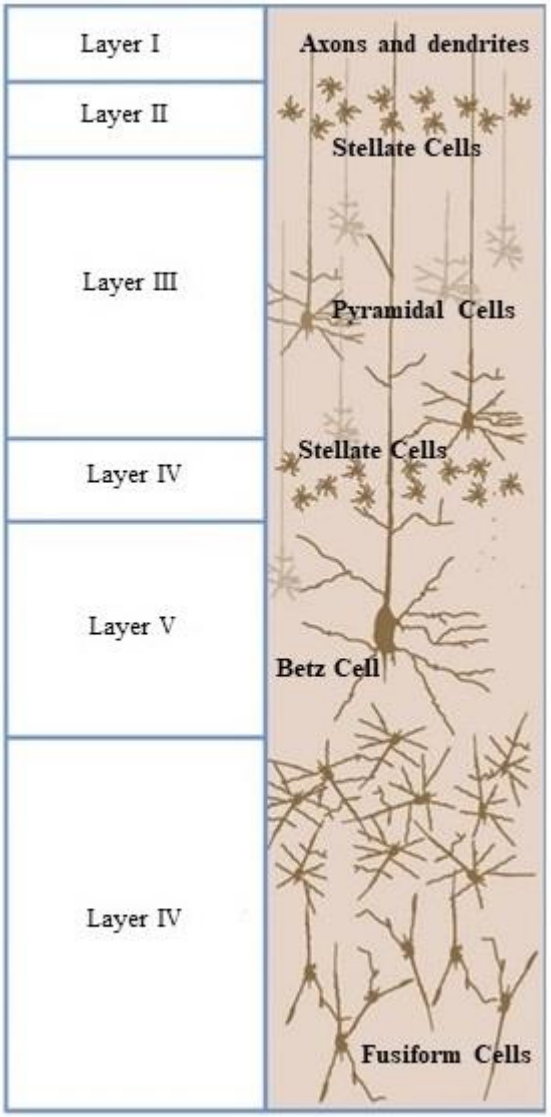

Figure 4. Cerebral cortex layers. The common six layers of cerebral cortex depicting the locations of Axons and denrites (layer I), layer II: the Stellate cells, layer III: the Pyramidal cells, layer IV: the Stellate cells, layer V: the Betz and layer IV: the Fusiform cells. Adapted from: Brodmann K, 1909.

There are two main neuronal cell types in the cerebral cortex: Pyramidal (projection neurons) and non-pyramidal (interneurons) cells. The cell type can determine the layer function of receiving or sending information to other areas: 1) Pyramidal cells with perpendicular orientation are found in layers II-VI but most prevalent in layers III and V (Porter and Lemon, 1993). The dendrites of these cells extend horizontally and vertically to all layers to form an extensive network and are giving rise to the most corticobulbar and corticospinal fibers

(Rothwell, 1991). ‘Betz cells’, the extremely large pyramidal cells, are found in layer V with apical dendrites extended to layer I. The axons of these cells are sent down to the spinal cord to synapse with anterior horn cells that directly synapse to their target muscles. The primary neurotransmitter for pyramidal cells is amino acid glutamate (Kandel et al. 2000) which is a facilitatory neurotransmitter. 2) Non-pyramidal cells (Stellate cells, Granular neurons) form 25% of neurons and act as interneurons in the motor cortex (Rothwell, 1991). These cells have existed in layers II-VI but are prominently found in layer IV. Their dendrites are radially extended with axons that exclusively remain in the cortex. ‘Basket cells’ are the most prevalent stellate cells in the motor cortex that make inhibitory contact to pyramidal neurons. The primary neurotransmitter for stellate cells is gamma-aminobutyric acid (GABA) (Jones, 1982; Meyer, 1987) which is an inhibitory neurotransmitter. As will be further discussed, the main target cells of neurostimualtory TMS pulses are ‘Betz cells’ in the corticospinal tract.

1.5.3 Columnar Organization

Cortical columns are the vertical units of cells that are working together in addition to cortical layers. These columns are consisted of many mini-columns, a narrow chain of neurons, bonding by horizontal connections and extended vertically from layers II-VI, perpendicular to the pia matter (Edelman and Mountcastle, 1978). The cortical columns, with the majority of inhibitory ones, form an extensive synaptic connection between neurons (Jones, 1983). Each column is a complex processing unit that connects to adjacent columns and other cortical regions through extensive horizontal connections (Edelman and Mountcastle, 1978). Although the stimulation of each motor column may activate a single muscle, the activation of several muscles is more common to produce a coordinated movement.

1.6 Excitatory and inhibitory neurotransmitters in Cerebral Cortex

Glutamate is the major excitatory neurotransmitter (Castro-Alamancos and Borrell, 1993; Aoyama and Nakaki, 2013) while, Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS) (Vicario-Abejon et al. 2000; Basile, 2002; Kubota et al. 2003; Szabo et al. 2014). As will be further discussed, the neurostimulatory TMS technologies that assess cortical facilitation do so by measuring the activity of Glutamatergic neurons and measure cortical inhibition via the activity of GABAgeric neurons (Kujirai et al., 1993).

1.6.1 Glutamate in the CNS

Glutamate is released in the synaptic cleft by the depolarized excitatory neurons, pyramidal cells, via calcium-dependent channels (Nicoll et al. 1990; McCormick, 1992) (Figure 5). There are two types of glutamatergic receptors: metabotropic (mGluRs) and ionotropic (iGluRs). Both receptors are activated in postsynaptic plasticity with different speeds and duration of induced-changes (Honore et al. 1982; Zhang et al. 2013). iGluRs have three subtypes: N-methyl-D-aspartate (NMDA), amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainite receptors (Furukawa et al.2005). iGluRs bind to the released glutamate and get activated. The activation of iGluRs results in a postsynaptic depolarizing current. AMPA and kainite receptors respond to the released glutamates by opening Na^+ channels (Perkinton et al. 1999). In NMDA receptors, binding with glutamate removes the Mg^{2+} and increasing the permeability of the membrane to Ca^{2+} (Song and Huganir, 2002; Paoletti and Neyton, 2007). Activation of NMDA and AMPA receptors modifies the strength of the synaptic connection. Prolongation of Ca^{2+} may lead to long-term changes in gene expression (Perkinton et al. 1999), synaptic plasticity, and behavior (Kelley et al. 2003). Changing the number of glutamate receptors may induce long-

term potentiation (LTP) if increased, or long-term depression (LTD), if decreased (Song and Huganir, 2002; Anggono and Huganir, 2012, Bassani et al. 2013, Henley and Wilkinson, 2013).

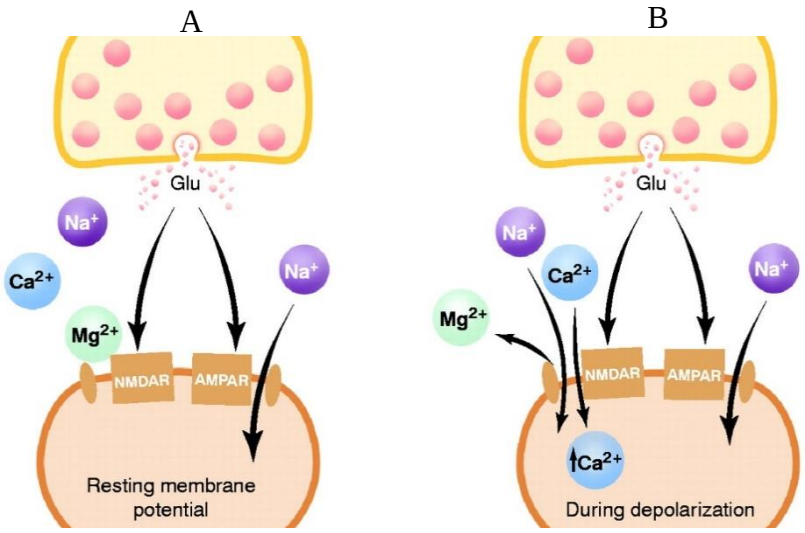

Figure 5. Glutamate Receptors. A simple model of Glutamate receptors and the excitatory effects of glutamergic mechanisms in the formation of long-term potentiation (LTP). **A.** In resting membrane potential state, $^{Na+}$ is just allowed to enter the post-synaptic neuron through AMPAR, while Mg^{2+} bind to NMDAR and prevent any ion enterance. **B.** During depolarization, releasing glutamate affect either AMPAR to increase Na^{+} influx or NMDAR that remove Mg^{2+} and increase Ca^{2+} influx into the post-synaptic neuron/ cell. Adapted from: Malekna et al. 1999.

1.6.2 GABA in the CNS

GABA is synthesized and stored in the neuronal grey matter and plays a major role in motor cortex plasticity (Sheikh et al. 1999) (Figure 6). GABA metabolism is also associated with Glutamate existed in the presynaptic terminals of GABAergic neurons (Sheikh et al, 1999). GABA inhibits pyramidal cells in the motor cortex (Momiyama, 2002), has an essential role in isolating and precision of movements (Ridding et al. 1995), and important in neuroplasticity (Jacobs and Donoghue, 1991; Ziemann et al. 2001). Changes in local GABA concentration seem to be in association with changes in glutamate concentration. This cooperation of GABAergic and glutamatergic neurons maintain excitation/ inhibition balance in the motor cortex. This balance modulation furtherly affects motor learning and plasticity (Krause et al, 2013). GABA has two main receptors: $GABA_A$ and $GABA_B$. Both receptors are involved in the regulation of ion concentrations (Momiyama and Koga, 2001). $GABA_A$ is the most widespread GABA receptor, directly acts on post-synaptic chloride membrane channels and increases the influx of chloride (Cl^-). This causes membrane hyperpolarization and increases the firing threshold. These receptors have a rapid inhibitory effect (Homanics et al. 1997). $GABA_B$ receptors are extensively located in pre-and post-synaptic cells (Misgeld et al. 1995) and act on potassium and calcium channels. These receptors increase the potassium or decrease calcium conductance and have a slow inhibitory effect (Kerr and Ong, 1995).

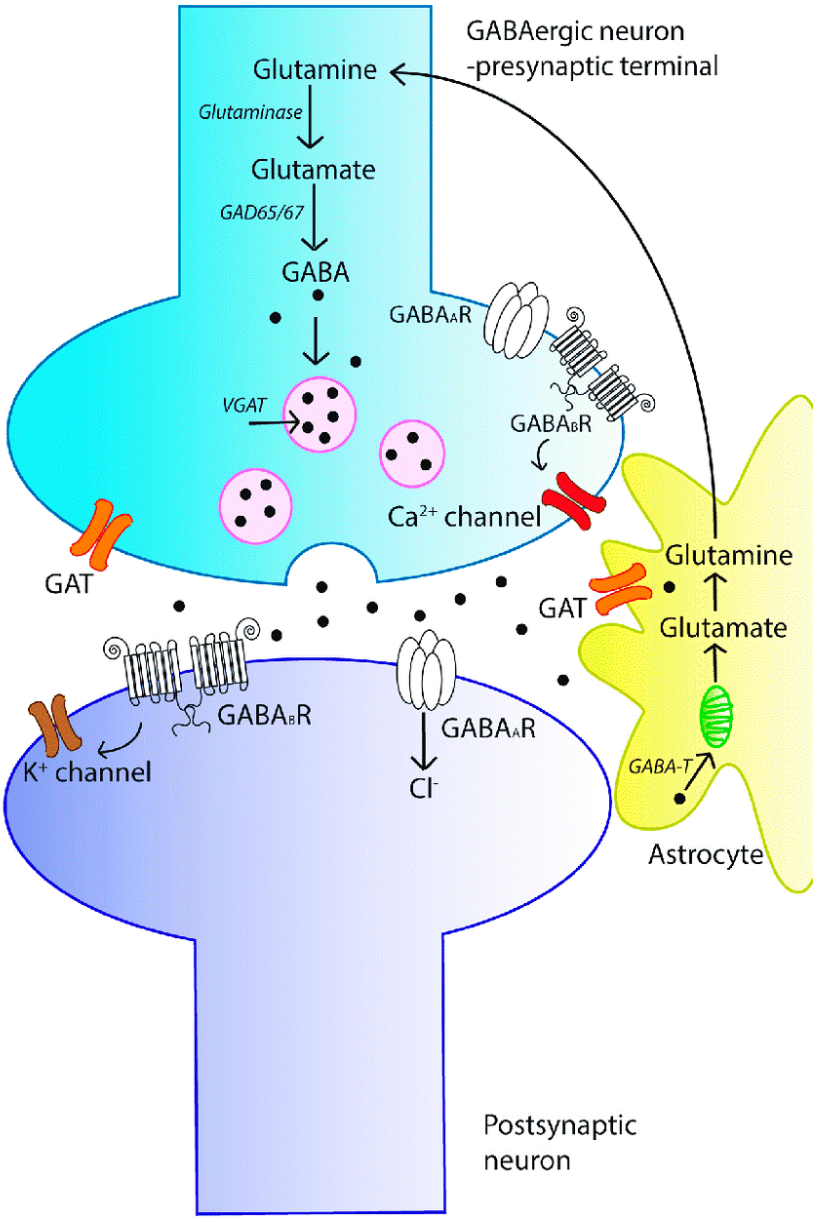

Figure 6. GABA Receptors. A simple model of GABA receptors and the inhibitory effects of GABAergic mechanisms. GABA is synthesized in the pre-synaptic terminals from glutamine. GABA is released into the synapse and would bind to either $GABA_A$ (to increase the influx of Cl^-) or $GABA_B$ (to increase the K^+ conductance). Adapted from: Govindpani et al. 2017.

1.7 Cortical motor regions

The motor cortex is primarily located in the frontal lobes, anterior to the central gyrus, and responsible for motor control of human movements. It comprises three main motor areas (Figure 7):

- The premotor cortex (PMC) is responsible for higher aspects of movement including planning and initiating voluntary movement (Leonard, 1997),
- The supplementary motor area (SMA) is responsible for sequential movement planning and coordinating bilateral movements (Brinkman & Porter, 1979; Tanji & Kurata, 1982),
- The M1 is responsible for generating commands to a specific muscle or muscle groups through the corticospinal tract. It is also highly involved in motor skill learning (Sanes and Donoghue, 2000) and changes in motor representations (Pascual-Leone et al. 1994). In the current thesis, the a-tDCS is used to modulate the excitability of the M1. The M1 will be explained in detail in the following section.

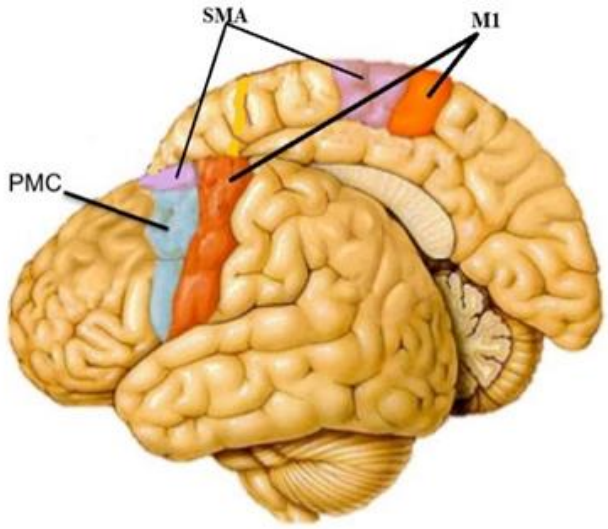

Figure 7. The cortical motor regions of the brain: Premotor Cortex (PMC), Supplementory Motor Area (SMA), Primary Motor Cortex (M1). Adapted from: thebrain.mcgill.ca

1.8 The Primary Motor Cortex (M1)

M1 is one of the principal brain areas that play an essential role in the execution of voluntary movements. M1 is in the precentral gyrus area of the frontal lobe of the cerebral cortex (Garey, 1994). It is characterized by the lack of granule cells in layer IV, and the presence of Betz cells in layer V (Meyer, 1987). Rapid cortical plasticity, including decreased inhibition or increased excitability, occurs following stimulation and/or learning in this area. The extensive cortico-cortical horizontal connections between excitatory glutamatergic pathways that are influenced by GABAergic inhibitory interneurons (Dounghue, 1995; Hess et al, 1994, 1996) provide a basis for cortical plasticity in M1. The M1 receives sensory afferent related to the activity of muscles through the thalamus and primary somatosensory cortex (Ghosh and Porter, 1988). The M1 also has additional afferents from PMC, cingulate motor area, and area 5 of the parietal cortex (Muakkassa & Strick 1979; Ghosh et al. 1987; Tokuno & Tanji 1993). Finally, there are transcallosal afferents from the contralateral M1 (Sloper & Powell 1979), and sparse transcallosal inputs from the contralateral premotor areas (Rouiller et al. 1994). M1 is functionally organized in a somatotopic manner called “Motor Homunculus” (Figure 8); it depicts a disproportionate map of the body in the M1in which each area innervating a part of the body. Larger representations in M1 are related to fine movements including hand and fingers. Smaller parts are related to the body regions characterized by gross movements, such as legs (Geyer et al., 1996).

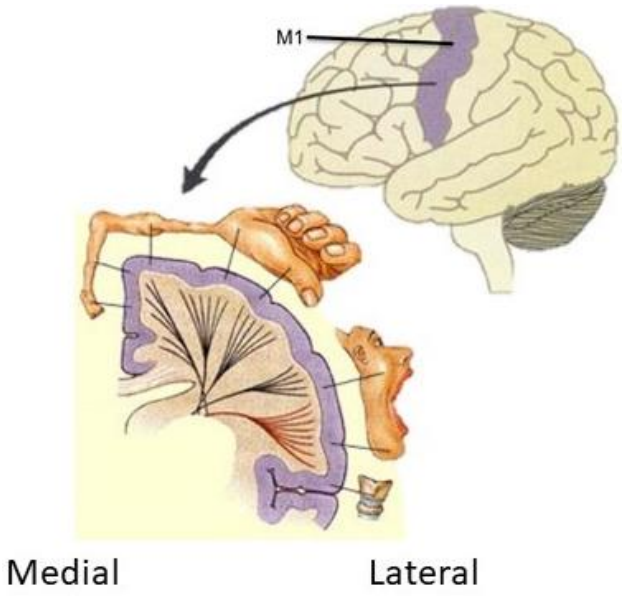

Figure 8. Penfield's motor homunculus. A schematic cross-section though the pre-central gyrus depicting the general principle of somatotopy. Direct electrical stimulation of different points along the pre-central gyrus evokes movements of different body parts. The section is taken at roughly the level indicated by the line through the brain on the left. The representation of the body is distorted, with a disproportionate volume of cortex devoted to the hand

The M1 output projections consist of direct connections from pyramidal cells of layer V to the spinal cord via the corticospinal tract. In the next section, the anatomy of the corticospinal tract is briefly explained.

1.9 The Corticospinal Tract

Axons that comprise the corticospinal tract originate in the frontal lobe, pass through the internal capsule, midbrain, pons, and medullary pyramids. The tract divides into lateral and ventral tracts.

The lateral tract (Figure 9A) has the majority of axons originating from M1 and some from other sensorimotor areas. It crosses at the pyramidal decussation, between the medulla and spinal cord (Chouinard and Paus, 2006). It makes synapses in the ventral horn of the spinal cord with the motor neurons that innervate limb muscles to regulates voluntary movement (Jaillard et al. 2003). The ventral tract (Figure 9B) has the axons from M1 and the PMC that remain uncrossed and descend ipsilaterally. It makes synapses in the ventral columns of the thoracic spinal cord innervate axial and proximal muscles (Nathan et al, 1990) to regulate voluntary movements associated with postural control (Jaillard et al. 2003).

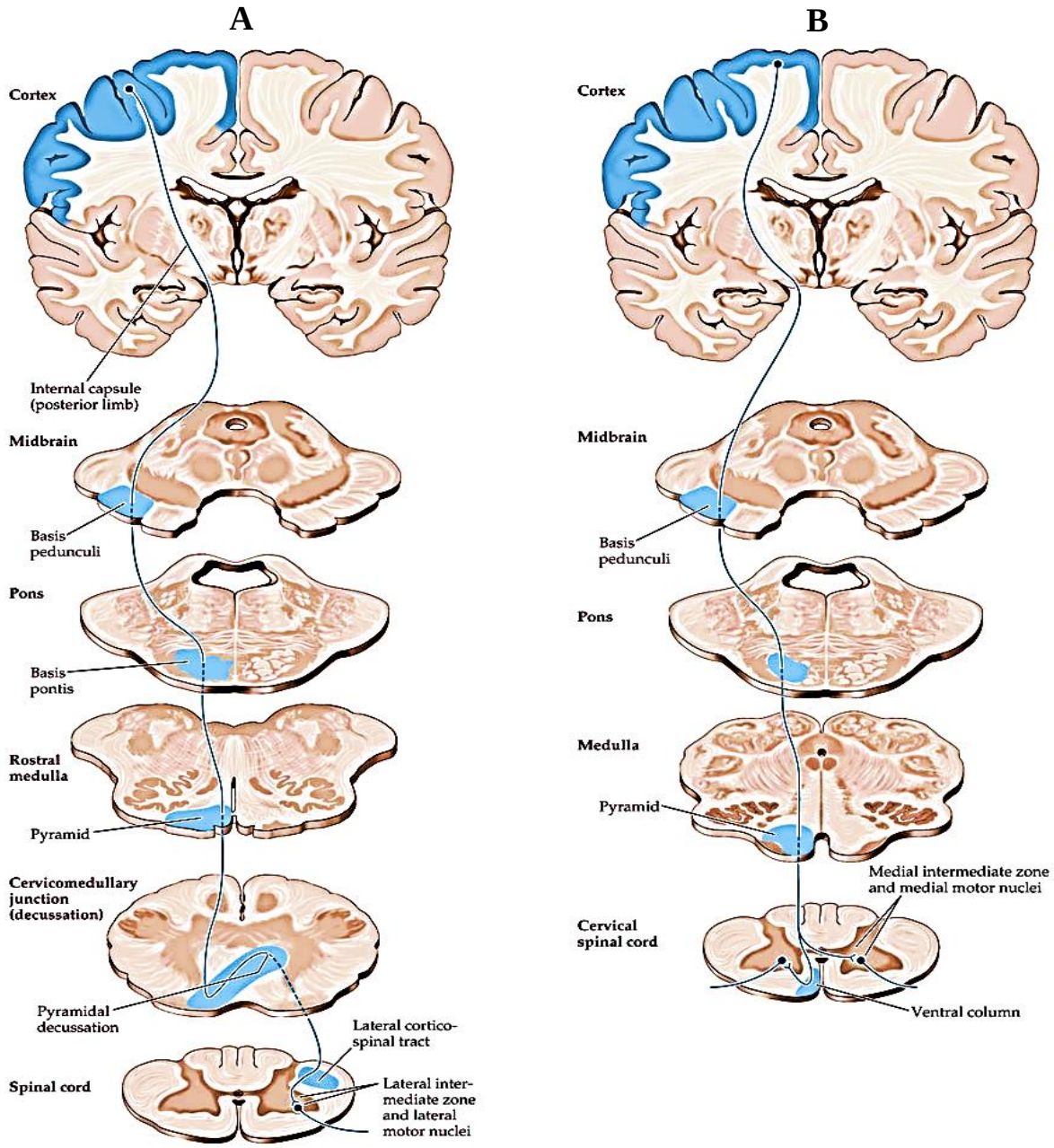

Figure 9. Corticospinal tract. Axons from corticospinal neurons leave the cortex via the internal capsule and project through the midbrain, pons and medulla before decussating to form **A.** the lateral corticospinal tract, or continuing uncrossed to form **B.** the ventral corticospinal tract. Adapted from: Blumenfold, 2010. © Sinauer Associates, Inc.

In chapters 5 to 7, the function of excitatory (glutamatergic) and inhibitory (GABA) mechanisms are evaluated in the motor cortex to investigate the possible mechanisms behind the reversal of the expected CSE effects by a-tDCS. Identification of the duration and intensity thresholds for reversal of the stimulation effect is a novel concept that was investigated in this thesis.

To better understand the function of interneurons in the brain, the next sections briefly explain the neurochemistry of the excitatory and inhibitory mechanisms.

1.10 Neuroplasticity in the CNS

Neuroplasticity is defined as a property of the nervous system to undergo reconfiguration of both structural and functional neural organizations in response to internal and external stimuli (Li et al. 2014). Specifically, neuroplasticity has been demonstrated in the regions of the brain responsible for the execution of voluntary movements (Sanes and Donoghue, 2000). This reorganization can occur under physiological (learning from experience), and pathological (injury or disease) events (Sanes and Donoghue, 2000). This means that the brain reconfigures itself through forming new inter-neuronal connections, modifying the internal structure of existing synapses (cellular modification), and increasing neuronal survival rates following an injury (Karmarkar and Dan, 2006). The ability to induce or optimize neuroplasticity experimentally can be used to have a depth-insight-understanding of neural systems and consequently develop more effective treatment protocols in various neurological disorders (Ridding and Rothwell, 2007). These neuronal properties can be altered through the use of the kind of electrical current stimulation (Nitsche and Paulus, 2001). During the last two decades, the interest in NIBS techniques has been raised as methods for investigation of the neuroplasticity in the intact human brain and also as a therapeutic tool. NIBS techniques are capable of inducing short-lasting plasticity in the brain (Ziemann et al., 2008).

1.10.1 Synaptic plasticity

Synaptic plasticity refers to the changes at a synaptic level as an increase or decrease in strength or efficiency of synapses in response to changes in their activity. Synaptic plasticity includes two broad stages of rapid plastic changes in pre-existing connections and the slower establishment of new connections (Pascual-Leone et al. 2005). Synaptic plasticity can be regulated either pre-synaptically by changing the release of neurotransmitters or post-synaptically by changing the number, types, or properties of neurotransmitter receptors (Kessels and Malinow, 2009). The two most important forms of long-lasting synaptic plasticity are LTP and LTD, characterized by a long-lasting increase (Bliss and Lømo, 1973) or a decrease in synaptic strength (Ito, 1989), respectively. These changes in synaptic strength arise from changes in neurotransmitter release and receptor expression and both LTP and LTD depend on changes in NMDA receptors (Lovinger, 2010; Kullmann and Lamsa, 2011; Hasan et al. 2013).

1.10.2 Metaplasticty

A metaplasticity is a higher-order form of synaptic plasticity that is tightly affected by the history of synaptic activity (Abraham, 2008). Metaplasticity includes homeostatic and non-homeostatic mechanisms helping the brain to stabilize its neuronal activity within an optimal physiological range. Non-homeostatic mechanisms get activated if the level of prior neuronal activity is not very low or high. Indeed, the history of neural activity before the intervention remains within the brain optimal excitation-inhibition (LTP/LTD) limits. Therefore, the plasticity changes would lead to prolongation of the expected effect and result in late LTP- and LTD-like changes on CSE. The activation of these mechanisms would prevent excessive synaptic strengthening or weakening by diminishing and reversing the expected effects of

stimulation (Bienenstock et al, 1982; Zubieta et al, 2005; Benedetti, 2008; Muller-Dahlhaus et al, 2015; Karabanov et al, 2015).

1.10.3 Modification Threshold and synaptic plasticity

The threshold for induction of LTP or LTD (modification threshold) is dynamically adjusted to the level of synaptic activity through activation of different mechanisms (Hebbian rule: Hebb, 1949; Bienenstock-Cooper-Munro (BCM) theory: Bienenstock et al. 1982; Turrigiano and Nelson, 2004). This means that a history of low synaptic activity would slide the modification threshold to induce more excitatory (LTP) effects and a history of high synaptic activity slides it to induce more inhibitory (LTD) effects (Bienenstock et al. 1982). This dynamic sliding of modification threshold is crucial to help the Brain to keep its neuronal activity within an optimal physiological range (Abraham, 2008; Hulme et al. 2013). The sliding of modification threshold activates different mechanisms including counter-regulatory (homeostatic) and non-counter regulatory (non-homeostatic) mechanisms based on the level of synaptic activity (Turrigiano and Nelson, 2004; Ziemann and Siebner, 2008). The activation of these mechanisms will result in non-expected and expected responses, respectively (Muller-Dahlhause and Ziemann, 2015; Karabanov et al. 2015), and this is called response variability.
In this thesis, we are identifying the “duration” (Chapter 5) and “intensity” (Chapter 6) modification thresholds which is a novel step for reduction of inter-individual variability in response to the application of a-tDCS.

NIBS techniques in general and tDCS as the technique used in this thesis will be described in detail in the following sections.

1.11 NIBS techniques

In the past two decades, the NIBS technique has become a prominent tool for both research and clinical applications that non-invasively alter neural activity as well as neural plasticity. **NIBS** techniques can be divided into neurostimulatory and neuromodulatory techniques.

1.11.1 Neurostimulatory techniques

Transcranial magnetic stimulation (TMS) is a neurostimulatory technique used to non-invasively stimulate the human brain to cause action potentials. TMS induces a current flow perpendicularly to the applied magnetic field (Rothwell, 1997) to trigger neuronal action potentials (Wassermann et al. 2008). TMS could depolarize both inhibitory interneurons and cortical excitatory pyramidal cells that contribute to the corticospinal tract (Rothwell et al. 1999). It can be used as an assessment or therapeutic technique. TMS is applied in three different ways: Single-pulse TMS, Paired- and Triple-pulse TMS, and long trains of stimuli (repetitive TMS or rTMS). Single (Laakso et al, 2018) and paired-pulse TMS (Valero-Cabré et al. 2017) is mostly used as an assessment tool for measuring corticospinal and intracortical excitability, respectively. rTMS is used as a therapeutic tool to induce neuroplastic changes within the cortex using different high or low frequencies (Fitzgerald et al. 2006; Rossi et al. 2009).

In chapters, 4 - 6 presented in this thesis, single- and paired-pulse TMS are used as an assessment tool that will be explained in full detail later in this chapter.

1.11.2 Neuromodulatory techniques

Transcranial electrical stimulation (TES) is an umbrella term referring to a group of NIBS techniques that do not stimulate cortical neurons. Indeed, these techniques only modulate the

cortical activity and make it more positive or more negative by manipulating ion channels depends on the characteristics of the applied currents. TES techniques include transcranial random noise stimulation (tRNS), transcranial alternating current stimulation (tACS), and transcranial direct current stimulation (tDCS) (Gebodh et al. 2019). TRNS is a non-invasive electrical stimulation of the brain whereby a weak alternating current oscillating at random frequencies is delivered through the scalp using a pair of electrodes (Chaieb et al. 2009). TACS is the application of a low-intensity alternating electrical current (Paulus, 2011). TDCS is the application of a direct current to the brain (Nitsche and Paulus, 2001).

TDCS is the most popular among different TES techniques. Its effects are dependent on the polarity of the applied current. TDCS devices are portable, inexpensive, and a painless technique with no serious side effects that is feasible for home-based use. It can be used as an adjunct or stand-alone intervention in psychological or neurological disorders.

TDCS is the intervention of interest in this thesis. It will be described in more detail in the following section.

1.12 Transcranial direct current stimulation (tDCS)

TDCS is a relatively easier and cheaper neuromodulatory technique to administer that modulates the neural activity of the cortex compared to other forms of NIBS such as TMS. It is applied through a very weak direct current (typically 1-2 mA) using a pair of saline-soaked surface sponge electrodes to a region of interest in the brain (Nitsche and Paulus 2000, 2001; Nitsche et al. 2008). TDCS is a technique that can affect neuronal excitability and induce neuroplastic changes in the human brain (Priori et al. 1998, Nitsche and Paulus, 2000, 2001; Nitsche et al. 2007; Stagg and Nitsche, 2011; Jamil et al. 2017). Membrane potential changes induced by chemical neurotransmission, either pre-or post-synaptically, may play an important role in tDCS effects (Liebetanz et al. 2002). TDCS modulates neuronal excitability at subthreshold levels and therefore does not directly induce any action potentials (Nitsche et al. 2005). The current comes from an active or target electrode (anode or cathode), passes through the brain tissues, and finally recovered by a reference or return electrode (Bikson et al., 2010). Depending on the stimulation polarity, tDCS increases or decreases the neuronal excitability in the stimulated area (Priori et al. 1998; Wagner et al. 2007; Rowny & Lisanby 2008). Cathodal tDCS (c-tDCS), application of the negatively charged electrode (cathode) over the target area of stimulation, leads to hyperpolarization of cortical neurons, indicating decreased CSE and has an inhibitory effect. On the other hand, anodal tDCS (a-tDCS), application of the positively charged electrode (anode) over the target area of stimulation, results in cortical depolarization, indicating increased CSE and has an excitatory effect (Nitsche & Paulus 2000; Nitsche & Paulus 2001) (Figure 10). For research purposes, sham-tDCS is a third kind of stimulation that consists of a false stimulation used to compare the after-effects of a-and c-tDCS.

The changes in CSE can be measured using TMS-induced MEPs from respective muscles (refer to section 1.14 for further detail).

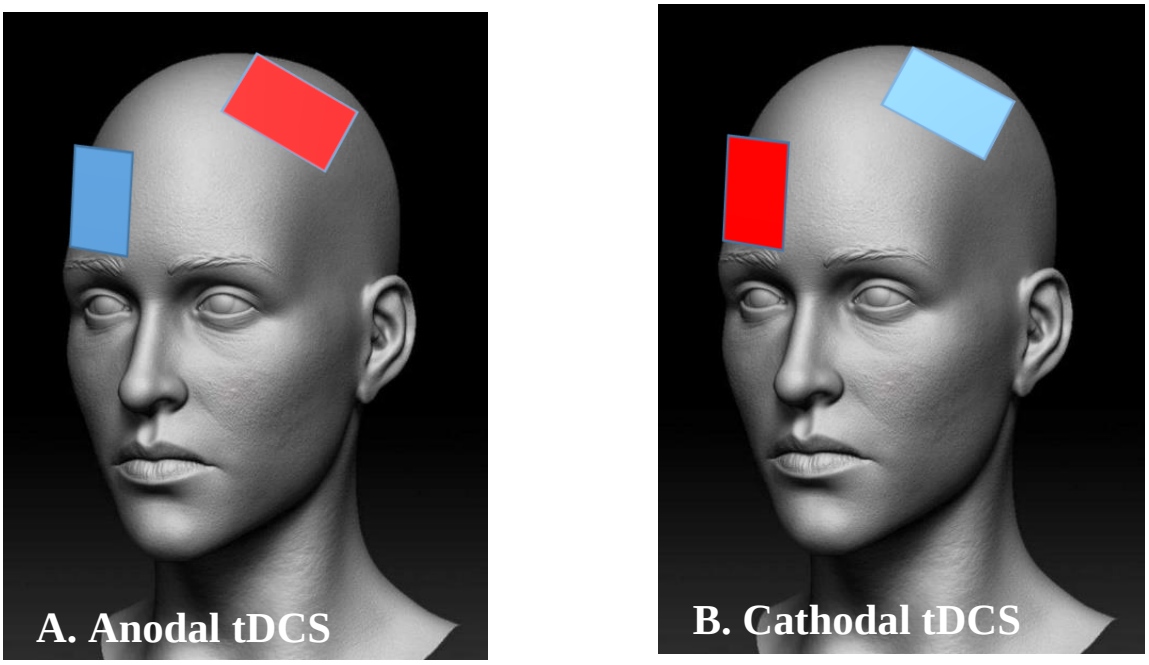

Figure 10. TDCS electrodes placement of M1-supraorbital (SO). A. a-tDCS. The anode is placed over the M1 (the target muscle i.e. FDI), and the cathode is placed over the contralateral supraorbital ridge. **B.** c-tDCS of the M1. The electrodes placement is reversed. (Red electrode: Anode; blue electrode: Cathode)

TDCS is used as an investigatory tool for the understanding of brain function including depression (Brunoni et al. 2016), cognitive enhancement (Hsu et al. 2015), decision making (Fecteau et al. 2007), and chronic pain (Lefaucheur et al. 2008). TDCS is also used as a therapeutic technique. Clinically, tDCS has been introduced as a tool in post-stroke patients to improve limb motor function (Boggio et al. 2007; Elsner et al. 2016) and memory rehabilitation (Jo et al. 2009).

1.12.1 TDCS montages

Conventional vs. High Definition tDCS

There are two common types of tDCS montages: conventional and high definition (HD). In conventional tDCS (Figure 11A), a battery-powered waveform or current generator device is used to deliver a constant electrical current flow through two large electrodes. A constant current is delivered with an intensity of (0.2 – 2mA) for durations of 15 – 40 min with electrode sizes of (25 – 35cm^2) that have been considered to be safe (Nitsche et al. 2008; Stagg and Nitsche, 2011; Woods et al. 2016). At the point of electrode-skin contact, the non-uniformed current diffusion leads to the low current density that would shunt across the scalp (Suh et al. 2009) and stimulate different neuron populations (Radman et al. 2009). On the other hand, near the electrode edges, there will be high current density zones due to the edge-effect (Miranda et al. 2006, 2009; Minhas et al. 2011). Therefore, large surface area electrodes are preferably used to minimize the high currents at the electrode-skin contact and diffuse current zones at the electrode edges. The tDCS electrode montage affects the electric field of the cortex. The most commonly used montage is bicephalic in which both electrodes are placed on the scalp at different regions (Nitsche and Paulus, 2000). As a limitation of this montage is the difficulty on the return electrode effect-isolation (Purpura and Mcmurtry, 1965), and therefore, in some cases, an extra-cephalic return electrode is preferred (Cogiamanian et al. 2007; Im et al. 2012). There is still a controversy regarding the effect of the inter-electrode distance on the brain field strength; while an enhancement was reported following the distance increase (Miranda et al. 2006), another study found the greater effect by the relative electrode's placement (Wagner et al. 2007; Moliadze et al. 2010).

In high definition tDCS (HD-tDCS) (Figure 11B), Datta et al. (2009) have shown that using the arrays of smaller electrodes makes it possible to confine the current flow and enhance brain

targeting by the modulation of limited areas. HD-tDCS has a montage of an active electrode surrounding with multiple (four or more) return electrodes to produce more focused current flow restricted within the ring of return electrodes. The common HD-tDCS configuration is arranged in a 4 x 1 ring in which the center electrode confirms the polarity. The ring radius determines the modulation area due to the purpose of application (Datta et al. 2009). HD-tDCS is diffused effectively in the skin and corticospinal fluid (CSF), not the skull, and can be controlled inside the ring (Minhas et al. 2010). HD-tDCS can deliver a targeted dosage to desired regions using computer models of current flow. Although HD-tDCS was found to have more effective in a pilot study with long-lasting motor cortex excitability (Russowsky et al. 2011; Kuo et al. 2013), and less diffused current (Dmochowski et al. 2011; Muthalib et al. 2017), the impact of electrode configurations, the strength of stimulation and possible modulation mechanisms of this protocol are still under-investigation. Therefore, the main reason for choosing conventional tDCS in this thesis was the fact of the most common use of this montage in the literature, realistic understanding of the mechanisms behind its effects, and to make it possible to compare the findings of both expected and non-expected effects of tDCS.

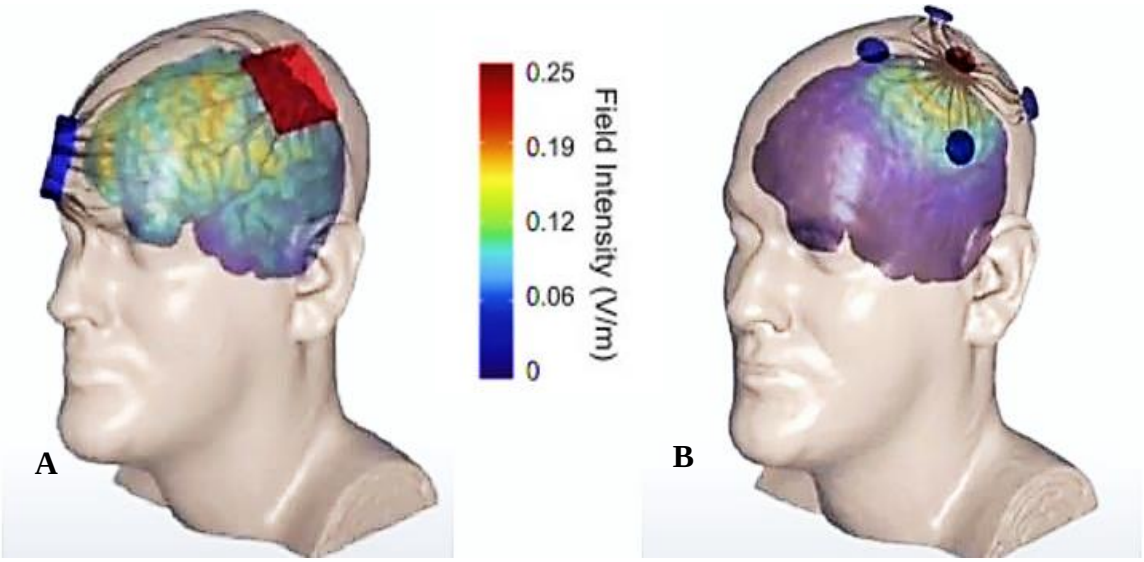

Figure 11. tDCS montages and electrical current flow. A. Conventional tDCS of M1-SO and **B.** 4 x1 HD-tDCS montage on M1. The bar shows the strength of field intensity with red show the maximum mostly near the active electrode to blue as minimum while moving away from the active electrode center. The field intensity is more diffused in conventional tDCS compared to more focused in HD-tDCS. Adapted from: "Neuromodulation for Neuroergonomics" talk by Marom Bikson 2018.

1.12.2 TDCS dosimetry and excitability effects

The number of induced neuromodulatory effects in the brain and brain electrical fields depends on the stimulation duration and current intensity (Priori et al. 1998; Nitsche & Paulus 2000; Nitsche & Paulus 2001; Nitsche et al. 2003; Iyer et al. 2005). The initial concept of linear dose-response effect suggested in earlier studies (Nitsche and Paulus 2000, 2001) was based on the notion of the direct linear relationship between the applied current intensities and/or duration of tDCS application and the size of induced CSE in healthy participants. Recent studies have challenged this concept showing no, minor, or reversal of the tDCS effects on CSE using different stimulation duration and/ or intensities (Monte-Silva et al., 2013; Bastikadze et al., 2013; Lopez-Alonso et al., 2014; Tremblay et al., 2016). It is revealed the more complex and non-linear relationship between electrical dose and stimulus-response that higher electrical doses do not necessarily produce greater increases in CSE. This means that the same tDCS parameters and montage commonly applied among different individuals without the consideration of their anatomical and physiological differences, may consequently affect the response variability to tDCS as it has been shown through recent studies (Wiethoff et al. 2014; Lopez-Alonso et al. 2014, 2015; Li et al. 2015; Amman et al. 2017). According to the increased use of tDCS both in the clinical and experimental application, this starts to make concerns regarding its reproducibility (Jacobson et al. 2011; Berryhill et al. 2014). Therefore, current literature has attempted to optimize the use of tDCS through optimal electrode montage and current dosage for tDCS using different ways such as computational modeling of current flow (Kessler et al., 2013; Turski et al., 2017) however, these efforts are still under investigation. This optimization

of tDCS parameters can have a profound impact on its efficacy on CSE changes and possibly motor performance. This, therefore, confirms the need for a comprehensive investigation to determine the modification threshold for both the “duration” and “intensity” threshold for the reversal of a-tDCS effects on CSE. This may lead to the reduction of response variability during the application of tDCS.

1.12.3 The effects of tDCS on Intracortical excitability

The neuro-modulatory effects of tDCS are predominantly affected by the polarization of intracortical interneurons and not cortico-spinal neurons (Nitsche et al. 2015). These effects are determined by the neuronal orientation and the direction of the electric field.

TDCS affects the membrane potential through voltage-gated sodium and calcium (Ca^{2+}) channels by opening and allowing an inward influx of sodium and calcium to depolarize the neuron by changing the electrochemical gradient. Ca^{2+} is essential in the induction of neuroplasticity and its fluctuations will induce either a potentiation (LTP) or a depression (LTD) at the postsynaptic level (Bennett, 2000; Lisman, 2001).

A-tDCS of the M1 increases intracellular Ca^{2+} concentration (Islam et al. 1995; Bikson et al. 2004). Therefore, a-tDCS over the target area of stimulation results in cortical depolarization, inducing increased CSE (Nitsche and Paulus 2000, 2001).

Also, neuropharmacological studies reveal that the glutamate receptor N-methyl-D-aspartate (NMDA)-dependent mechanisms are mainly involved in the aftereffects of tDCS and controlled the synaptic plasticity (Li and Tsien, 2009). This determines the increase of glutamate (Glu) concentrations following a-tDCS. These changes are accompanied by the local reduction of the neurotransmitter gamma-Aminobutyric acid (GABA) concentrations under the anode over M1 (Stagg et al. 2009). A significant decrease in GABA levels in response to a-tDCS with effects developing during stimulation and persisting for at least 30 min following stimulation (Bachtiar et al. 2015).

Therefore, an increase of CSE following a-tDCS is multifactorial and certainly is driven by modulation of both GABAergic and glutamatergic mechanisms (Nitsche et al., 2004; Stagg et al., 2009; Stagg and Nitsche, 2011).

1.12.4 TDCS protocol used in this Thesis

In 2013, Monte-Silva et al. reported that 26 minutes of a-tDCS (1mA) completely reversed the effects of stimulation on CSE (Monte-Silva et al. 2013). This study has been chosen as the core of Studies 3 – 5 in this thesis to investigate the "duration" and "intensity" thresholds of a-tDCS effects on CSE. Table 5 shows the a-tDCS parameters used in this thesis:

Table 5 A- tDCS parameters used in Studies 3 – 5 in this thesis.

		Study/ Chapter		
Parameter		**Study 3/ Chapter 5**	**Study 4/ Chapter 6**	**Study 5/ Chapter 7**
Stimulation		A-tDCS	A-tDCS	A-tDCS and sham
Intensity		1mA	4 intensities: 0.3, 0.7, 1, 1.5 mA	0.7, 1, 1.5 mA
Duration		5 durations: 22, 24, 26, 28, 30 min	26 min	22, 26, 30 min
Electrode size		5 x 7 cm^2		
Electrode placement	**Target (Anode)**	Left M1		
	Return (Cathode)	Right Supraorbital area		
Current density (mA/cm^2) (Anode and Cathode)		0.028	0.008, 0.02, 0.028, 0.04	0.02, 0.028, 0.04
Total charges (C/cm^2)		0.61, 0.67, 0.72, 0.78, 0.84	0.2, 0.52, 0.72, 1.04	0.52, 0.61, 0.84, 1.04

Most studies have used a conventional electrode montage of two large (7 x 5 cm^2) rectangular rubber-sponge electrodes with the active electrode placed on the M1 and return electrode on the supraorbital ridge (SO) (Nitsche and Paulus, 2000). This montage was also used for the

application of a-tDCS in the studies presented in this thesis utilizing a battery-driven stimulator (NeuroConn, Germany) (Figure 12).

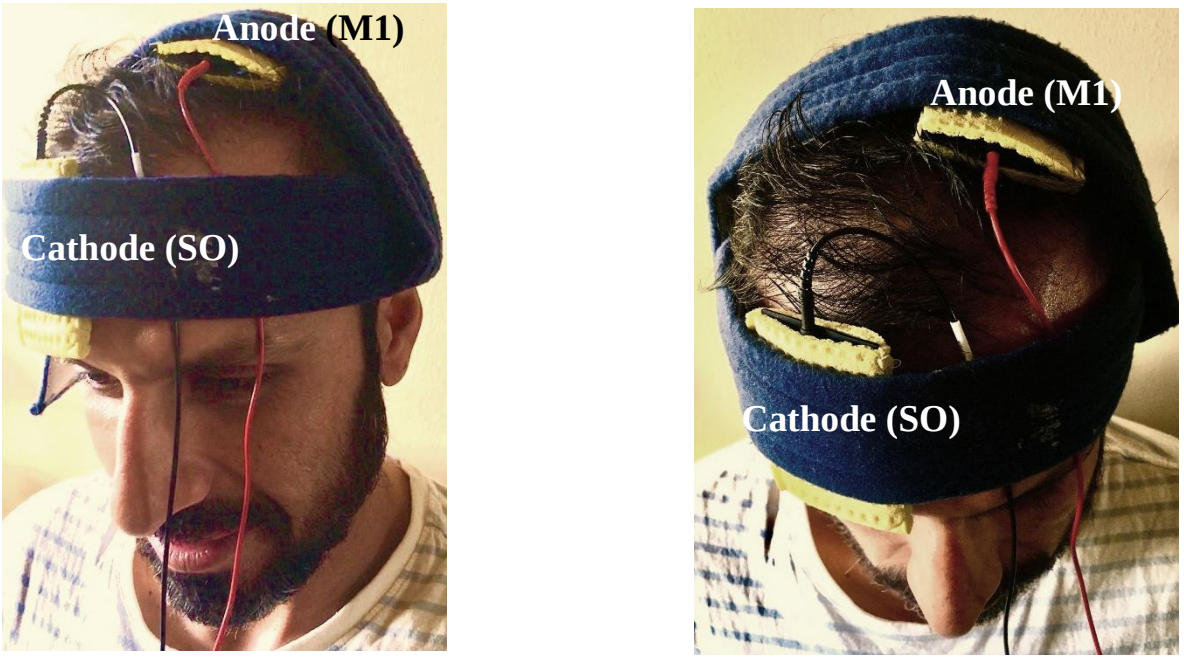

Figure 12. A-tDCS montage (M1 – SO) used in the studies of this thesis. Target electrode (Anode) is placed on M1 of FDI, and return electrode (Cathode) is on supra-orbital ridge. Two perpendicular straps fix the active and return electrodes.

1.12.5 TDCS safety

TDCS is assumed as a very safe method by the safety protocol introduced in 2003 (Nitsche et al. 2003) however, it is critical to developing a precise experimental design to meet the desired safety parameters. The most commonly reported side effects of tDCS are transient cutaneous sensations such as tingling, itching, or a mild burning sensation and in rare cases, reports of slight pain (Poreisz et al., 2007; Fertonani et al., 2015). The recommended safety guideline was determined by McCreery et al. (1990) and Yuen et al. (1981) as 25 mA/cm2 for current density and 216 C/cm2 for the total charge (Yuen et al. 1981; McCreery et al. 1990). The following formulas are used to calculate current density and total charge in this thesis: 1. Current density (mA/cm^2) = stimulus intensity (mA)/ electrode size (cm^2), and 2. Total charge (C/cm^2) = (stimulus intensity (mA)/ electrode size (cm^2)) × stimulation duration. The calculated current densities of a-tDCS applied in this thesis (Chapters 5 – 7) were from 0.008 to 0.04 mA/cm2) and the total charges were between 0.22 to 1.11 C/cm2 which are far below the reported safety limit (216 C/cm2) (Yuen et al. 1981).

Furthermore, research has been done to determine relevant parameters for the safe application of tDCS in humans. The studies on tDCS safety have shown no evidence of harmful effects on patients with frontal lobe disorder (Iyer et al. 2005). Iyer et al. (2005) evaluated 103 subjects in a safety study of tDCS (1 or 2 mA current intensity; 25 cm2 electrode size) and found no adverse effects on cognitive and psychomotor measures and electroencephalography (EEG) changes during or after 20 minutes of treatment (Iyer et al. 2005). Also, in their study on both healthy individuals and patients with stroke Gandiga et al. (2006) have shown that tDCS (1mA current intensity; 25 cm2 electrode size) elicited minimal discomfort, which consisted only of tingling sensations (Gandiga et al. 2006). Moreover, Poreisz et al. (2007) reported the following effects during 567 tDCS administrations (1mA current intensity; 35 cm2 electrode size) in 102

participants (comprised of 75.5% healthy subjects, 9.8% tinnitus patients, 8.8% migraine patients, and 5.9% post-stroke patients) over two years: a mild tingling beneath the electrodes in 70.6% of the subjects, fatigue after treatment in 35.3%, and itching under the electrodes in 30.4%. Headache (11.8%), nausea (2.9%), and insomnia (0.98%) were also reported. The authors concluded that tDCS is safe to use when safety guidelines are followed (Poreisz et al. 2007). Recently, however, it was reported that the use of 2 mA tDCS for 20 min (5 days per week) resulted in skin damages in five patients after 2 weeks. These potential side effects should be explained to participants while using tDCS of 2 mA (Palm et al. 2008), or even with lower intensities in longer tDCS applications. Furthermore, under safe protocols tDCS is suggested to not causing heating effects under the electrodes (Nitsche & Paulus 2000), not increasing the serum neurone-specific enolase level (Nitsche & Paulus 2001; Nitsche et al. 2003b) and not resulting in changes of diffusion-weighted or contrast-enhanced MRI or pathological EEG changes. There is no data in the literature reporting epileptic jerks elicited by tDCS. Furthermore, no cortical oedema, necrosis, or alterations of the blood-brain barrier or cerebral tissue, nor any sign of cell death, were observed (Nitsche et al. 2003b; Nitsche et al. 2004).

In conclusion, the parameters of a-tDCS used in Chapters 5 - 7 were selected based on the tDCS safety guidelines to ensure the safety of the participants. A-tDCS side effects were recorded at the beginning, middle, and end of the stimulation in all experimental sessions. All participants were asked to complete a form to record the side and adverse effects of a-tDCS (Keel et al. 2001) (Appendix 6). The form contained rating scales for the presence and severity of common side effects such as itching, tingling, and burning sensation under the electrodes (Poreisz et al., 2007; George and Aston-Jones, 2010), and other adverse effects including headache and pain during and after stimulation (Brunoni et al., 2011). The unpleasantness of any scalp sensation was rated via numeric analog scales (NAS) (i.e., 0 = no feeling to 10 = worst imaginable

sensation). All participants tolerated the applied currents very well and there was no interruption in experimental procedures due to the side- or adverse-effects of a-tDCS in Studies 3 - 5.

1.13 Tool for assessment of corticospinal and intracortical excitability

TMS was introduced as a painless and non-invasive technique to stimulate the human motor cortex by Barker et al. (1985). TMS generates a magnetic pulse by a magnetic coil that is placed over the brain e.g., M1. This transient magnetic pulse passes through the tissues and scalp and produces an electrical current, known as Eddy current, in nearby conducting tissues (Figure 13). The induced electrical current can depolarize the cell membranes of cortical motor neurons and interneurons and stimulate them if the depolarization exceeds a threshold level. TMS, as an assessment tool, has provided an important window into the neurophysiology underlying the effects of neuromodulatory techniques such as tDCS. TMS evaluates the integrity of the corticospinal tract and intracortical interneurons by applying a direct external stimulus to the brain (Hallet, 2000).

Single and paired-pulse TMS has been widely used to evaluate the corticospinal and intracortical excitability in clinical and physiological studies (Pascual-Leone et al. 2000, Sanger et al. 2001, Anand and Hotson 2002, Chen et al. 2004, Di Lazzaro et al. 2004). In the present thesis, single- and paired-pulse TMS (Mag Pro R30 stimulator, Mag Venture, Denmark) was used to assess the level of CSE, intra-cortical inhibition (ICI), and intra-cortical facilitation (ICF). These two TMS protocols are described in detail in the following sections. TMS is also used to locate the M1 of the target muscle for application of the target electrode during tDCS applications (Nitsche et al. 2008).

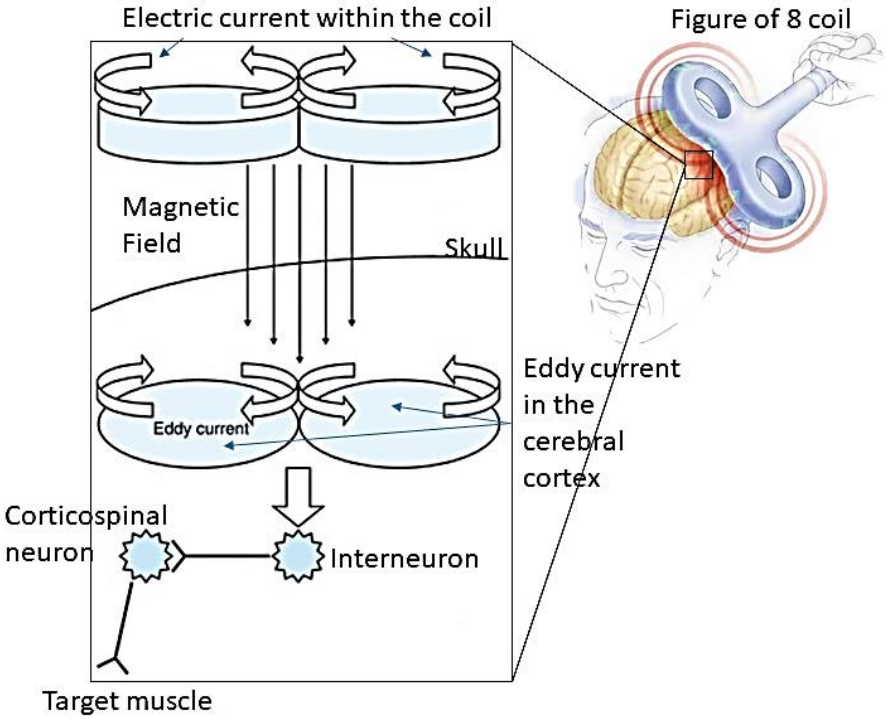

Figure 13. TMS generated electrical field. In TMS, electric current within the coil generates a magnetic field toward the cerebral cortex passed the skull. The induced eddy current is opposite to the electric current in the coil. The eddy current stimulates interneurons and corticospinal neurons which may led to stimulation of the target muscle and induction of MEPs. Adapted from: Abo and Kakuda, 2015.

The corticospinal tract conducts impulses from the M1 area to the spinal cord. These impulses stimulate the anterior horn cells in the spinal cord that in turn, stimulate any muscle triggered by TMS. This recording response from the muscle is called MEP. MEPs can be simply recorded by using surface electromyography (EMG) from the target muscle (Figure 14).

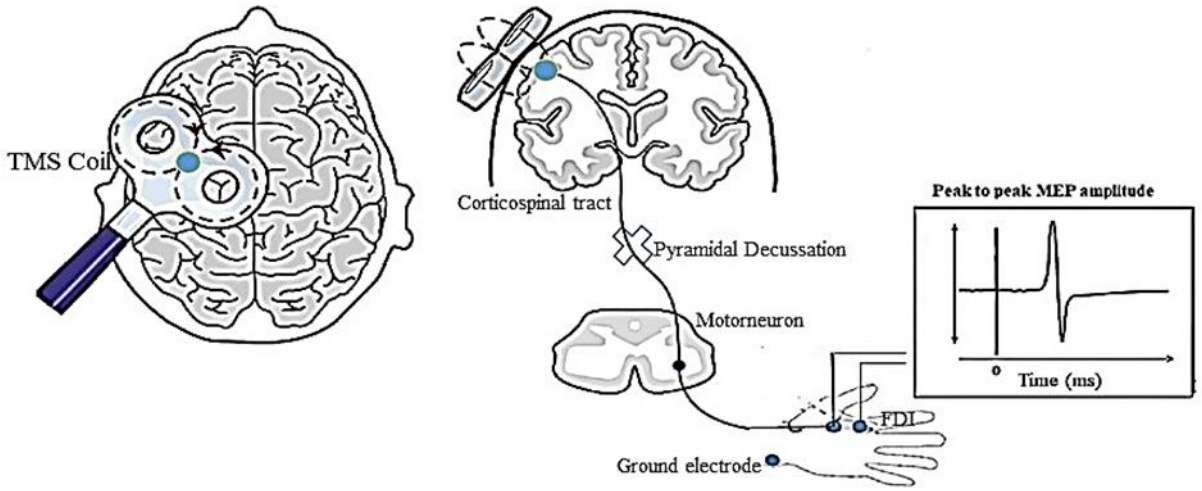

Figure 14. TMS induced MEPs. TMS coil is placed over the FDI hotspot on the scalp. The TMS magnetic filed generates Eddy current crossed via pyramidal Decussation, passed the corticospinal tract and recorded as the induced MEPs from the right FDI through surface EMG. Adapted from: Hui et al. 2020.

1.13.1 TMS coil types

There are different types of TMS coil designed for research and/or clinical use. The shape of the TMS coil affects the induced magnetic field that in turn influences the strength and localization of the generated electrical current into the cerebral cortex. The size of the coil is also important as the smaller coils produce more focused electrical fields (Cohen et al., 1990). The most common-used TMS coils are single round and figure-of-eight/butterfly coils (two round coils) (Figure 15). A single round coil induces the strongest electrical field at the circumference and to the deeper brain regions which are less focal. The Figure-of-eight coil creates a more focal field to the superficial cortical regions and is less stimulating. The peak of field strength is induced at the intersection between two round windings (Cohen et al. 1990). In the Studies of 2 - 5 presented in this thesis, a Mag-Venture figure-of-eight coil (70mm) was used.

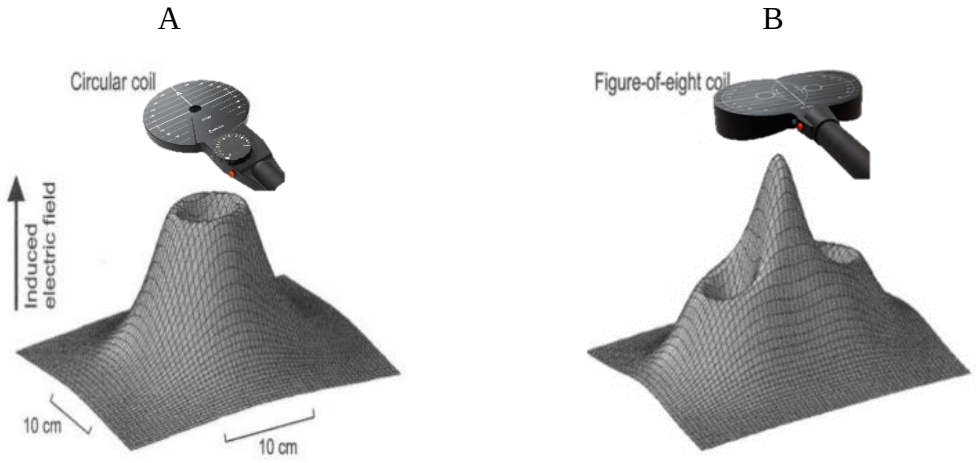

Figure 15. TMS coil types. Circular and Figure-of-eight coils with the electrical field intensity below the coils. Adapted from: Hallett and Chokroverty, 2005.

1.13.2 Single-pulse TMS Protocol used in this Thesis

Assessment of CSE

Single-pulse TMS (spTMS) induces MEPs which are used to measure the direct effect of a-tDCS on the CSE changes of the stimulated area which was the right FDI (Rossini & Rossi, 2007). As the MEP amplitude is an indicator of CSE, spTMS can therefore be used to examine the effects of tDCS on M1 excitability (Thut et al. 2005; Fregni et al. 2006). An electromyogram (EMG) is used to measure the TMS-induced MEP at the target muscle that reflects the excitability within the corticospinal system (Hallet et al. 2007; Di Lazzaro et al. 2008; Groppa et al. 2012; Vallence and Ridding, 2014). Neurophysiological measures obtained by TMS-induced MEPs can provide a measure of neuroplasticity.

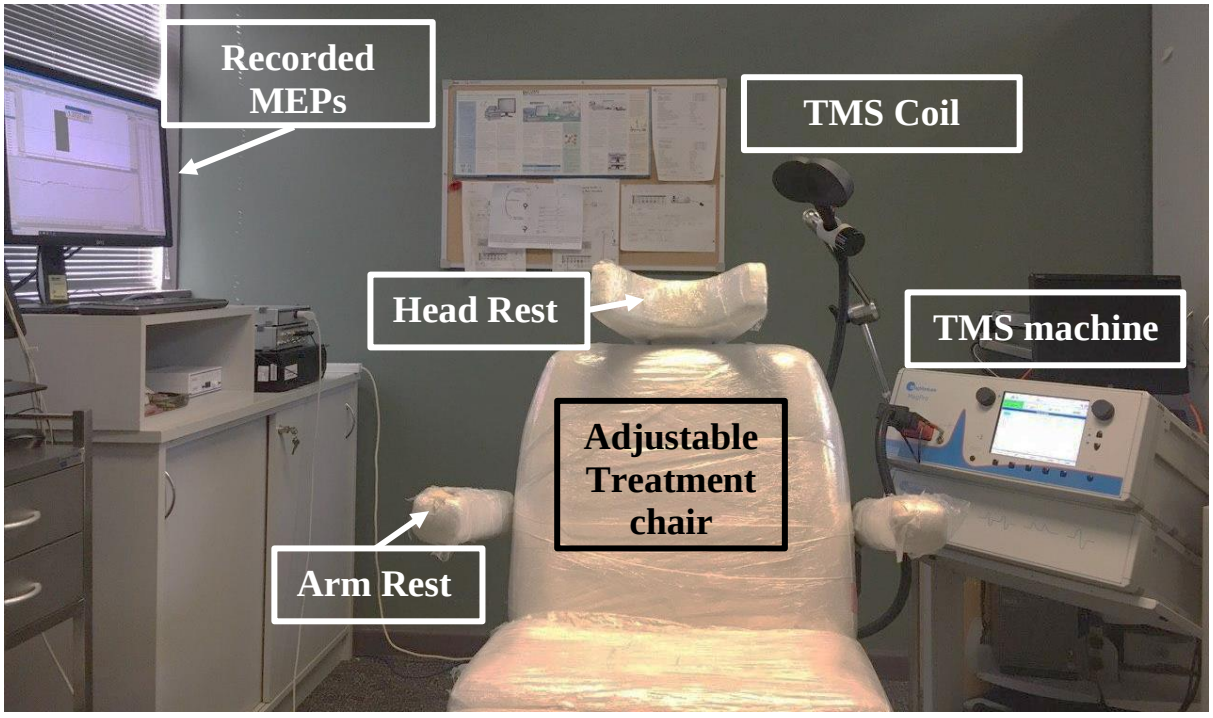

Figure 16. TMS set-up of the current thesis.The participant sat upright in an adjustable treatment chair (MagVenture, Denmark). The TMS coil was positioned on the hotspot for the first dorsal interossei (FDI). When a spTMS pulse was delivered over the M1, surface EMG was recorded from the FDI using bipolar Ag/AgCl disposable surface electrodes. Sp TMS were delivered by a MagPro R30 (MagOption) stimulator (MagVenture, Denmark).

As shown in Figure 17, the peak-to-peak amplitude of MEPs was measured from the right FDI. The average of 25 MEP amplitudes was calculated for the measurement of M1 CSE in Studies of 2 to 5 in this thesis. All raw EMG signals are bandpass filtered (10-500 Hz), amplified (x1000), and collected for offline analysis (PowerLab 8/30, ADInstruments, Australia) on a PC.

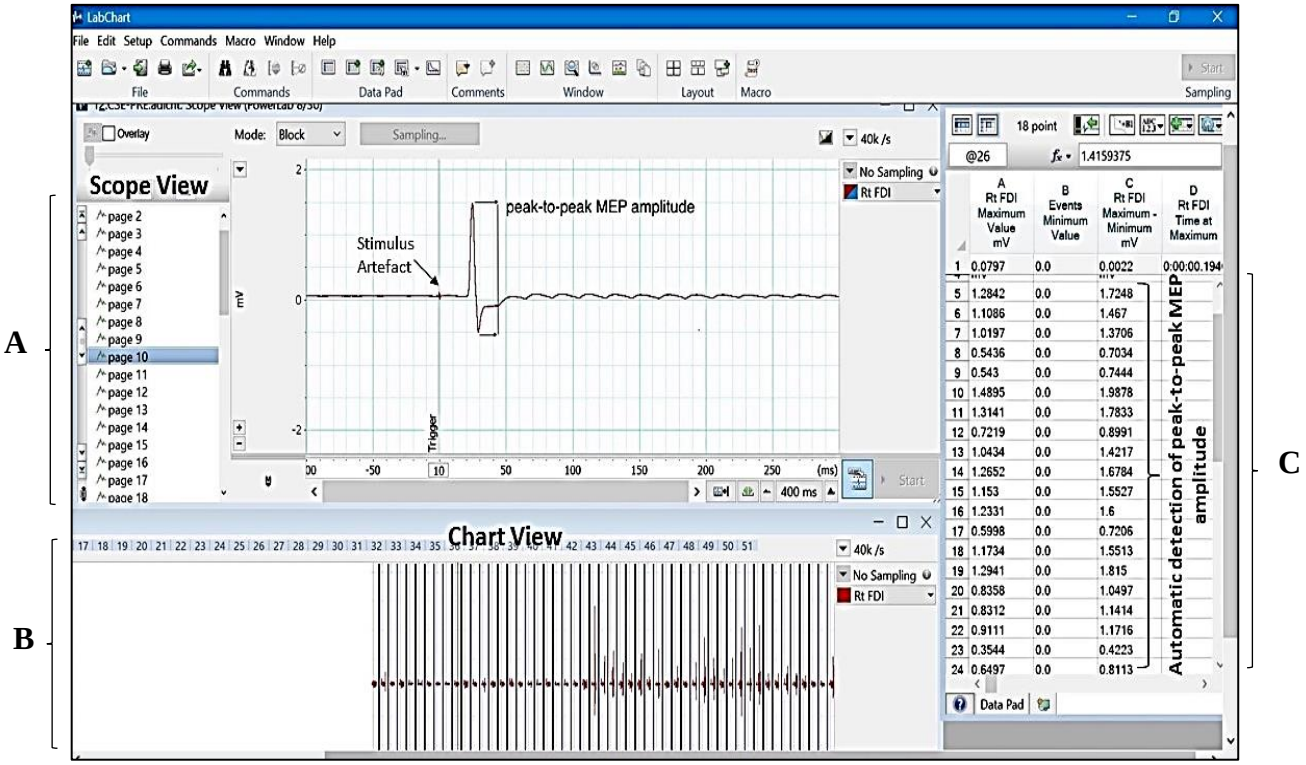

Figure 17. The automatic detection of peak-to-peak MEP amplitudes using LabChart software (ADInstruments). **A.** The **Scope View** provides an additional way of displaying and analysing the capabilities of a digital storage oscilloscope using PoweLab. In Scope View, each sweep is recorded and represented in a single page, creating a list of recorded MEPs that can be averaged and overlaid for analysis. **B.** The **Chart View** is the main window in which data can be dynamically viewed. **C.** The **peak-to-peak MEP amplitudes** are automatically detected and recorded, using a custom designed macro with PowerLab 8/30 software.

1.13.3 Paired-pulse TMS Protocol used in this Thesis

Assessment of intracortical excitability

Paired-pulse TMS (ppTMS) is used to investigate specific inhibitory or facilitatory mechanisms behind the changes in CSE. Paired-pulse protocols combine two consecutive stimuli of a conditioning stimulus (CS) and test stimulus (TS) with different interstimulus intervals (ISIs) (Kujirai et al 1993). The subthreshold CS was set to an intensity of 80% RMT followed by TS adjusted to induce peak-to-peak MEP amplitude of 1 mV (Kujirai et al., 1993; Kobayashi and Pascual-Leone, 2003). Table 6 describes briefly the ppTMS outcomes used in this thesis:

Table 6 ppTMS outcomes used in Studies 3 and 4 in this Thesis.

	ppTMS outcomes			
	Short intracortical inhibition (SICI)	Long intracortical inhibition (LICI)	Intracortical facilitation (ICF)	Long interval facilitation (LIF)
CNS measure	Intracortical inhibition	Intracortical inhibition	Intracortical facilitation	Intracortical facilitation
Neural circuitry	$GABA_A$ receptor-mediated	$GABA_B$ receptor-mediated	Glutamate, other mechanisms	Preventing further GABA release
ISI (msec)	1 – 6	50 - 200	10 - 15	100 - 200
Protocol	Subthreshold CS followed by a suprathreshold TS	Suprathreshold CS and TS	Subthreshold CS followed by a suprathreshold TS	Subthreshold CS followed by a suprathreshold TS

CNS: central nervous system, **ISI:** inter-stimulus interval, **CS:** conditioning stimulus, **TS:** test stimulus, **GABA:** gamma-aminobutyric acid.

Depending on the length of ISI between CS and TS stimuli, ppTMS can stimulate inhibitory (SICI, LICI) (Figure 19A) and/ or excitatory (ICF, LIF) (Figure 19B) intracortical connections to the pyramidal neurons (Kujirai et al., 1993). The sub-threshold conditioning stimulus does not elicit any MEPs and the effect originates from the interneuron activation within the motor cortex (Kujirai et al 1993; Di Lazzaro et al 1998a, b; 1999). The conditioned test MEP is measured

peak-to-peak and expressed as a percentage of the unconditioned test MEP. In SICI, the TS is inhibited by a conditioning stimulus given 1 to 5 msec before; therefore, the conditioned MEPs are smaller compared to unconditioned MEPs (CSE) (Figure 18A1-2). In ICF, the TS is facilitated when the interval gets longer than 10 to 15 msec; therefore, the conditioned MEPs are bigger compared to unconditioned MEPs (CSE) (Figure 18A2-B2). In LIF, the TS is facilitated when the interval gets longer than 50 to 200 msec; therefore, the conditioned MEPs are bigger compared to unconditioned MEPs (CSE) (Vallence et al. 2014) (Figure 18A2-C2). For LICI, the MEP amplitude induced by the second TS is compared to the amplitude of the MEP evoked by the first TS (Figure 18C1) (Valls-Sole´ et al., 1992; Werhahn et al., 1999; McDonnell et al., 2006). ICF is used as an index of excitatory circuits for the investigation of glutamate receptors in the M1 (Ziemann et al., 1996). The LIF has resulted in the activation of presynaptic $GABA_B$ receptors which prevent further GABA release (Cash et al. 2010). While, SICI and LICI have been related to the activity of $GABA_A$ and $GABA_B$ receptors, respectively which are used as an index inhibitory circuit (Kujirai et al., 1993). It has been shown that SICI and LICI have an interaction with each other and it is unclear whether the same population of neurons mediates both these measures, or whether they are mediated by closely interacting interneuronal circuits (Sanger et al., 2001; Chen, 2004).

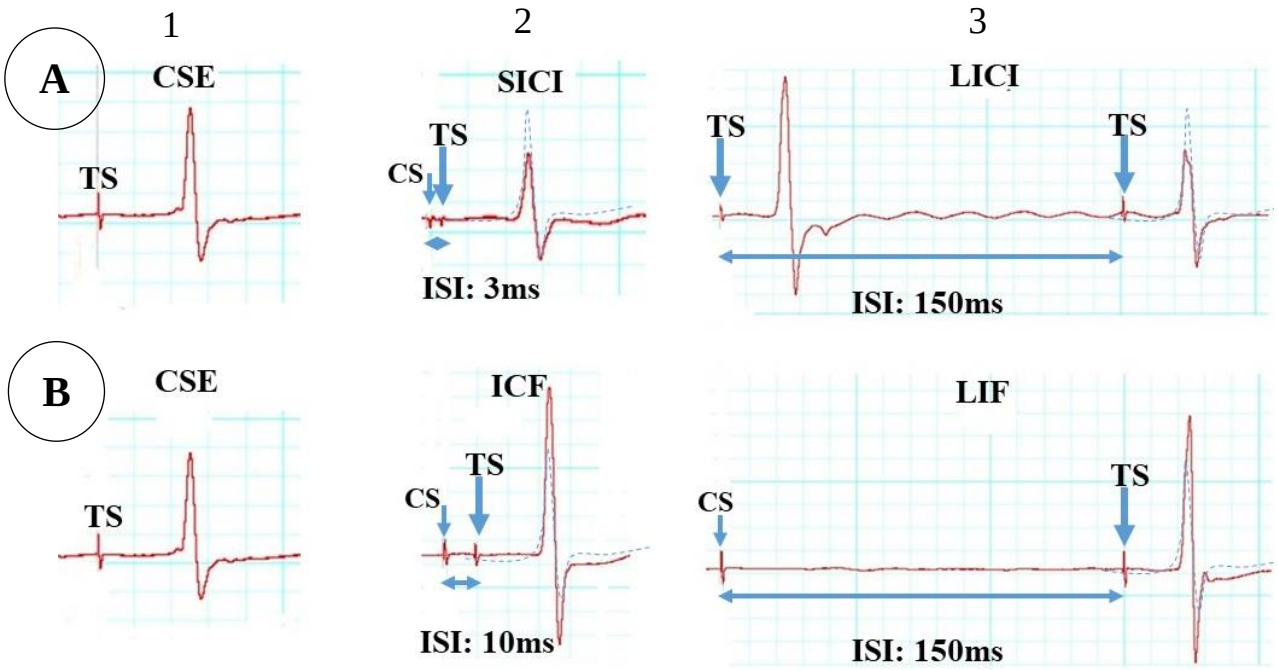

Figure 18. PpTMS and Recorded MEPs from relaxed right FDI. A. 1. CSE, 2. SICI, 3. LICI, and **B.** 1. CSE, 2. ICF, 3. LIF. Representative single-pulse induced MEP using test stimulus **(CSE)**, and when test stimulus **(TS)** conditioned by a subthreshold conditioning stimulus **(CS)** with **ISI**s of 3 ms (SICI), 10 ms (ICF), 150 ms (LIF), and suprathreshold CS and TS with ISI of 150 msec **(LICI)**. PpTMS were delivered by a MagPro R30 (MagOption) stimulator (MagVenture, Denmark).

1.13.4 EMG recording

Surface EMG is recorded by bipolar pre-gelled Ag/AgCl disposable self-adhesive surface electrodes (Figure 19). To ensure good surface contact and to reduce skin resistance, a standard skin preparation procedure of cleaning and abrading will be performed for each electrode site (Gilmore and Meyers 1983, Robertson et al. 2006, Schwartz 2003). The location of the surface electrodes on the target muscle (right FDI muscle) is determined based on anatomical landmarks (Perotto and Delagi, 2005) and observation of muscle contraction in the testing position (Kendall et al. 2005). The accuracy of EMG electrode placement is verified by asking the subject to maximally contract this muscle while the investigator monitors online EMG activity. A ground electrode is placed ipsilaterally on the styloid process of ulnar bone (Basmajian and De Luca 1985, Oh 2003). The electrodes are secured by hypoallergenic tape.

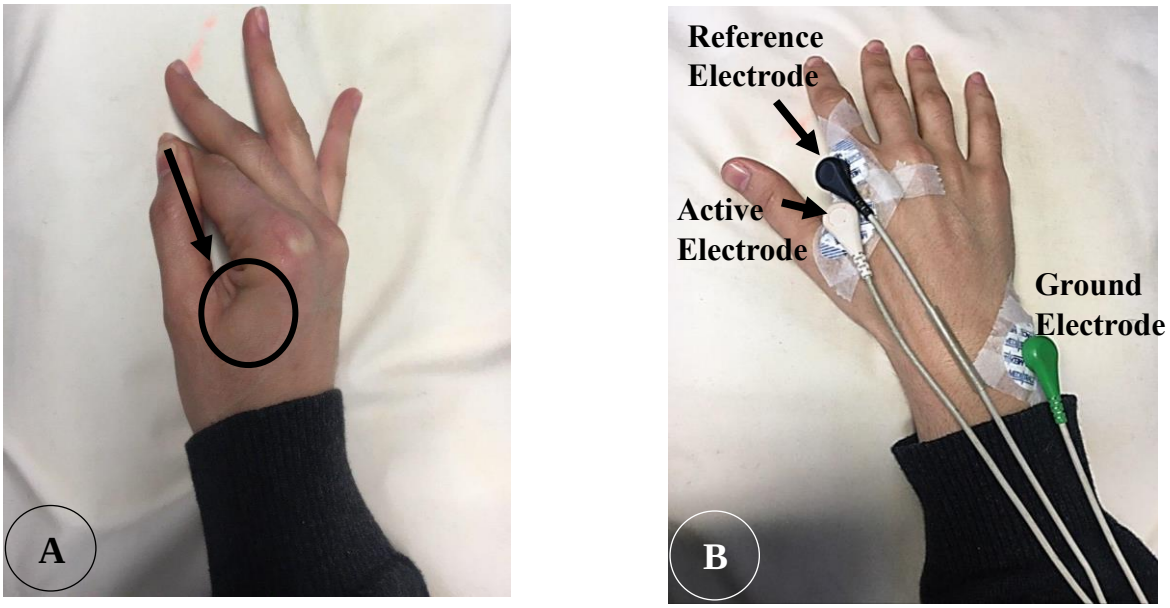

Figure 19. Surface EMG recording of FDI. A. First, the participant is asked to contract FDI (pinch thumb against index finger) to identify FDI belly. **B.** Then, surface EMG electrodes are placed over the right FDI in belly-tendon configuration with a 2 cm inter-electrode space. The ground electrode is taped on the ulnar bone.

1.13.5 Determination of TMS Hotspot

For MEP recording, the participant was seated comfortably in a fully adjustable treatment chair (MagVenture, Denmark) with head and armrests to have easy access to their head for stimulation of the target area. The stimulation site, i.e., M1, contralateral to the target muscle, is first determined by using the international EEG 10 - 20 system (Figure 20). Then to find the optimal site for stimulation, the coil is moved around the M1 until the largest motor MEPs can be recorded from the target muscle. This area is called the 'hotspot' for the target muscle (Neggers et al. 2004). After localizing the hot spot, the coil's position is marked with a soft head marker on the scalp to guide the experimenter during the rest of the testing. The orientation of the coil is set at an angle of 45° to the midline and tangential to the scalp, such that the induced current flows in a posterior-anterior direction in the brain (Brasil-Neto et al. 1992, Rossini et al. 2015).

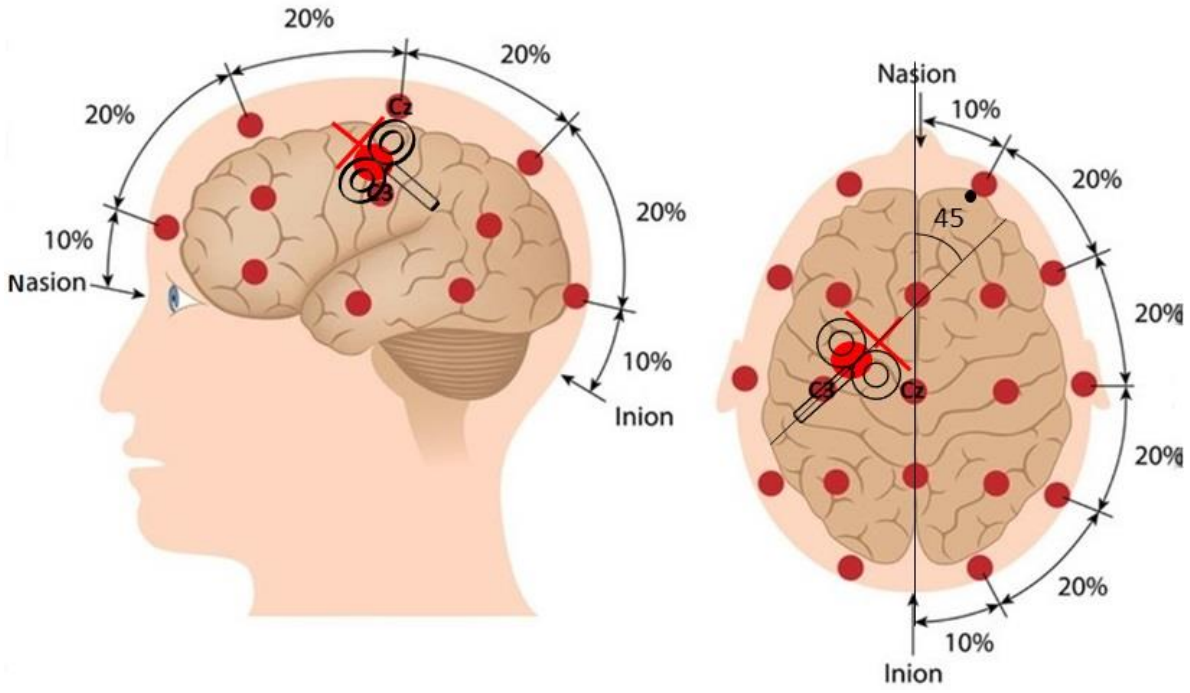

Figure 20. Determination of TMS Hotspot. To determine the stimulation site of M1, 10 - 20 system was used. A representative of the target muscle was marked with a marker in order to keep coil positioning constant in each session. This is called Hotspot that iss an optimal site to record MEPs from target muscle. A TMS coil was held in an angle of 45° to the saggital plane.

1.13.6 Determination of Resting motor threshold

After the determination of the hot spot, the resting motor threshold (RMT) was measured. RMT at FDI hotspot was obtained using parameter estimation by sequential testing (PEST) protocol. This protocol was followed using TMS Motor Threshold Assessment Tool (Freeware, MTAT 2.0; Awiszus and Borckardt, 2011). The program is starting from a TMS intensity of 37% of maximum stimulator output (%MSO). The intensity for the next TMS trial is determined based on the rater interaction with the software. Indeed, the rater indicates whether the trial was successful by clicking on the "Y" key or not (click on the "N" key). It is considered successful if MEP amplitudes ≥ 0.05 μV and then, a new target intensity displays for delivery. The protocol stops after 20 stimuli which provide sufficient accuracy for the RMT estimates within limits imposed by safety guidelines (Awiszus, 2011; Rossi et al, 2009) (Figure 21). The participants were asked to count the number of stimuli they received to minimize the changes in their attention.

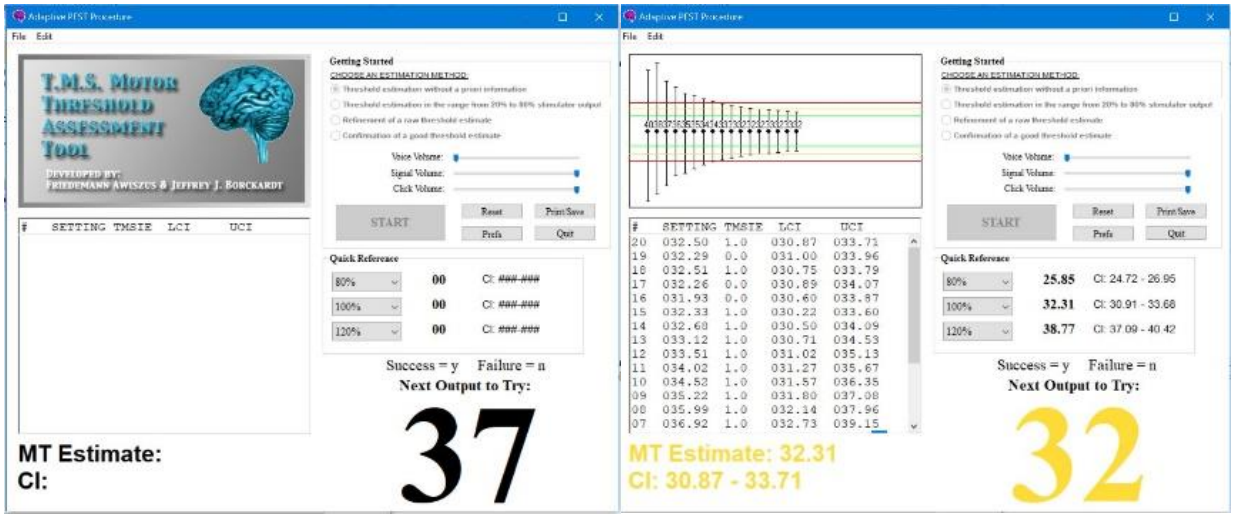

Figure 21. Determination of RMT. The program is started with the number of 37. Each time a successful try (an MEP ≥ 0.05 μV) is replied by pressing 'Y' key and failure by 'N'. The protocol is stopped when the number of trials reach 20. A number at this trial is the TMS intensity (% MSO) for RMT.

1.13.7 Determination of Test Intensity of 1mV (SI_{1mV})

Following the determination of RMT, the test intensity (%MSO) was adjusted to elicit a mean MEP amplitude of about 1mV peak-to-peak (SI_{1mV}) in the resting FDI (Nitsche and Paulus, 2000, 2001; Rossini et al, 2015). Baseline MEP means within the range of 1 mV ± 20% were accepted (Labruna et al, 2016). This is called test intensity (TS) that is used to obtain CSE and cortico-cortical excitability.

1.13.8 Safety of TMS

Generally, TMS is considered a safe and well-tolerated assessment tool for elicitation of MEPs. For a more detailed explanation please refer to **Chapter2** on TMS safety as an assessment tool.

1.14 Tool for assessment of motor performance

To examine the effect of a-tDCS on changes in motor performance, different assessment tests could be used. In this thesis, SVIPT is used as a test of executive function to measure these changes.

SVIPT is a pinch force task in which participants were asked to control their squeeze on the force transducer between their thumb and index finger to move a cursor upward on the computer screen to meet several different target forces as visual cues (Reis et al., 2009; Saucedo Marquez et al., 2013; Schambra et al., 2011) (Figure 22A). At the beginning of each experiment, maximum voluntary contraction (MVC) was individually determined for each participant. Before session one, in a separate short session, two blocks (each block contains 8 trials) were then given to the participants to get familiarized with the task. In each session, three blocks were performed as baseline measurements with the right hand. Each block consisted of eight trials and each trial included seven target forces (10, 15, 20, 25, 30, 35, and 40% MVC) which appeared in random order on the computer screen. The inter-trial interval was set at 1 second. Each target force was only presented once in each trial. The level of each target force was determined by a green line or a numerical number in an indicator box on the computer screen. Participants were instructed to squeeze the force transducer to reach the target force in a range of 5% below or above the target force (5% MVC; Figure 22B). Higher or lower than this range was considered as an over-or under-shoot error.

The following behavioral outcomes were measured in each pre-and post-assessment:

1. The Movement Time (MT) in each trial was defined as the time from movement onset for the first target to the cessation of movement after the final target. The mean movement time for eight trials was taken as the movement time for the given block (Reis et al. 2009).

2. The Reaction time (RT) was defined as the time interval between visual stimulus appearance and the initiation of movement. RT was conducted for each force within a trial, and the average of eight trials counted as the RT of that block. The mean RT was calculated by averaging the RTs of three blocks.

3. The Error Rate was calculated as the proportion of the trials with at least one over-or undershoot (Reis et al. 2009). The mean ER was calculated by averaging the ER of all three blocks at two-time points.

4. The skill index is a combination of movement time and error rate and represents changes in the speed-accuracy trade-off. The following formula was used to calculate skill development (Reis et al. 2009). Skill index was calculated within each block and then the average of the three blocks at two-time points is representing the mean skill.

$$Skill = \frac{1-error\ rate}{error\ rate\ [\ln(movement\ time)^{5.424}]}$$

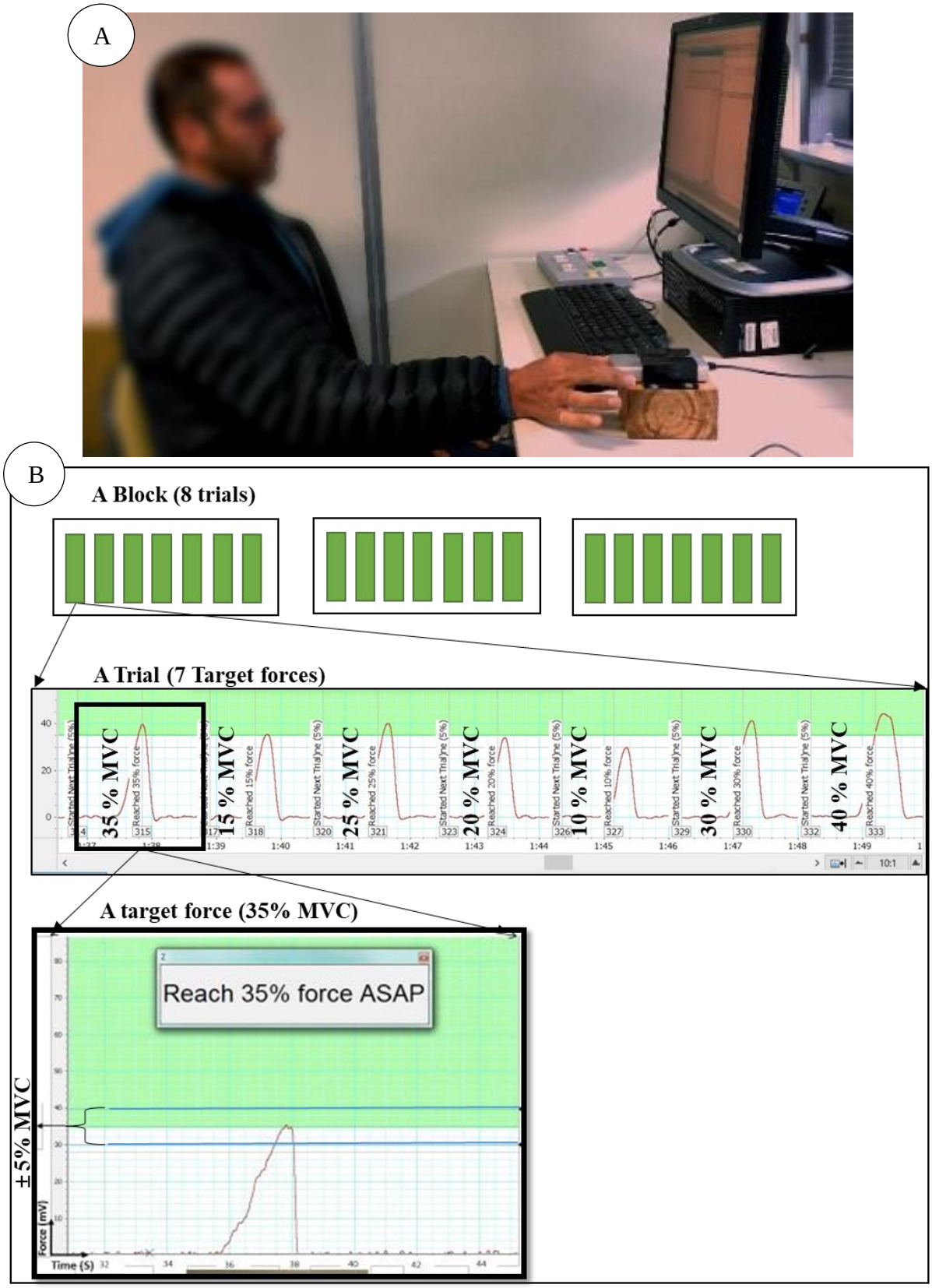

Figure 22. Sequential visual isometric task (SVIPT), **A.** Participant sits infront of screen displaying sequences of target forces and positions the hand to precisely pinch grip the transducer between index and thumb. **B.** Each measurement set consists of three blocks, each block includes eight trials, and each trial has seven target forces (10, 15, 20, 25, 30, 35, 40 %MVC) in a random order.

1.15 Thesis Aims

The following section provides an overview of the present thesis aims that have been investigated:

Thesis Aim 1: To investigate the factors which may affect the safety of TMS as the main assessment tool in this thesis.

Thesis Aim 2: To review the literature for investigating the effects of different priming NIBS protocols on a consequent NIBS test protocol of M1 on CSE in healthy individuals that could potentially contribute to response variability,

Thesis Aim 3. A: To investigate the effects of TMS ITIs (5, 10, 15, and 20s) on intra- and inter-session reliability of MEP amplitude,

B: to explore how different ITIs would affect the variability of TMS-induced MEPs.

Thesis Aim 4 and Aim 5: to determine a-tDCS "duration" and "intensity" thresholds (chapters 5 - 6) for reversal of the effects on CSE and to explore the underlying neurophysiological mechanisms behind these changes. And finally,

Thesis Aim 6: To determine whether the reversal of the CSE changes by a-tDCS coincides with similar changes in behavioral outcome measures such as indices of motor performance.

Aims 4 – 6 were the **primary aims** of this thesis.

Ethical clearance

All procedures were conducted according to the standards established by the Declaration of Helsinki. Copies of the Monash University human ethics certificate of approval, explanatory statement, participant consent form, handedness, TMS, and tDCS safety assessment forms can be found in the appendices (Appendix 1 - 6, pp. 82-89).

Preamble to Chapter 2

TMS is a non-invasive tool used to assess brain function in healthy individuals and those with neurological disorders. Although it is considered a safe technique as an assessment tool, it is not without risks in different individuals. According to wide-range usage of TMS in research and clinical applications in the forms of single- and paired-pulse TMS, it is essential to have a better understanding regarding its potential risks, the mechanisms behind each effect, and the ways to interact with it if happens.

This chapter addresses **Aim 1** in this thesis to investigate the factors which may affect the safety of TMS as the main assessment tool in the studies of this thesis.

A literature review of TMS safety guidelines presents a comprehensive list of common side/ adverse effects of TMS from all available guidelines. The most common side effect is a transient headache that will relieve spontaneously in few hours. Seizure and syncope are the other ones that should be prevented by an accurate screening before using TMS.

Chapter 2 provides a comprehensive insight regarding the TMS side and adverse effects as it was used as an assessment tool to measure changes on CSE in chapters 4 – 6.

Chapter 2. Safety of TMS as an assessment tool

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Chapter 10

SAFETY OF TMS AS AN ASSESSMENT TOOL

Maryam Hassanzahraee*[1,*], *Maryam Zoghi*[2], *PhD* and *Shapour Jaberzadeh*[1], *PhD

[1]Department of Physiotherapy, School of Primary and Allied Health Care, Monash University, Melbourne, Australia
[2]Discipline of Physiotherapy, School of Allied Health, La Trobe University, Melbourne, Australia

ABSTRACT

Transcranial magnetic stimulation (TMS) is a non-invasive tool for assessment of brain function in health and disease. It can also be used as a therapeutic tool in many psychological and neurological conditions. TMS as an assessment tool usually uses single or paired-pulse paradigms, and although it is considered as a safe technique, it is not without risks to the individuals. Hence, due to widespread use of TMS in recent decades, it is essential to better understand its potential risks, the predisposing risk

* Corresponding Author Email: Maryam Hassanzahraee, MSc. Email: maryam.hassanzahraee@monash.edu.

factors, and the ways to minimise these risks. This chapter also provides a list of TMS side and adverse effects with the underlying mechanisms for each effect. Besides, a list of contraindications and recommendations for optimal use of TMS is also presented. This chapter also briefly describes the possible risks of TMS in children, pregnant women, patients with neurological conditions and TMS operators. Finally, ethical and regulatory requirements for application of TMS are also briefly described in this chapter.

Keywords: transcranial magnetic stimulation, TMS, safety, side effects, adverse effects, corticospinal excitability, motor evoked potential, MEP

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a widely used noninvasive technique for stimulation of the brain. TMS uses the electromagnetic field to induce an electrical current which flows in a small region of the brain to stimulate the neural tissues (Barker et al., 1985). TMS could be used as an assessment tool which uses single- (spTMS) or paired-pulse (ppTMS) paradigms (George et al., 2007, Klomaji et al., 2015). It could also be used as a therapeutic device in the form of repetitive TMS (rTMS) (Wassermann et al., 1998, Anand et al., 2002, Rossi et al., 2009). Although TMS safety is an important issue in both applications of TMS, it should be noted that the focus of this chapter is mainly on its use as an assessment tool.

Inherent risks and specialised nature of TMS limited its widespread use in clinical neurophysiology (Wassermann et al., 1998). Single- or paired-pulses used in TMS as an assessment tool are considered safe with minimal side or adverse effects (Groppa et al., 2012) if proper screening of the participants followed before each experiment. The standard practice is to use a short screening checklist (Rossi et al., 2009) to identify the risks and to consider the overall safety of the TMS application in human participants (Green et al., 1997, Wassermann et al., 1998, Anand et al., 2002, Illes et al., 2006, Rossi et al., 2009, Perrera et al., 2016).

This chapter provides a list of potential risks, the predisposing risk factors, and the ways to minimise these risks, side and adverse effects with the underlying mechanisms for each effect and also a list of contraindications and recommendations for optimal use of TMS. This chapter also briefly describes the possible risks of TMS in children, pregnant women, patients with neurological conditions and TMS operators. Finally, ethical and regulatory requirements for application of TMS are also briefly described in this chapter.

Side and Adverse Effects

Although the use of TMS as an assessment tool is generally safe but similar to other non-invasive techniques, its application in research and clinical practice is associated with several sides or adverse effects with different degrees of severity. The most common side effects are transient scalp discomfort and headache (Anand et al., 2002) which are believed to be caused by repeated activation of nerve ending within the scalp and pericranial muscles (Allen et al., 2017). These effects are rare in single- or paired-pulse TMS paradigms (Wassermann, 1998). The primary side or adverse effects associated with the application of the TMS as an assessment tool and a brief description of the mechanisms behind these effects are presented in Table 1.

Although the most severe adverse effects of TMS are the induction of seizure and syncope, their occurrences are very rare during the use of TMS as an assessment tool (Groppa et al., 2012). A seizure is usually manifested by tonic contractions, jerking, vocalisations, incontinence and hallucinations. TMS induced syncope or fainting is more common than the seizure. TMS may cause extreme emotional distress which may lead to vasovagal syncope (VVS) which causes a sudden drop in the heart rate and blood pressure (Groppa et al., 2012). The syncope symptoms may include fainting, transient feelings of lightheadedness, dizziness, fading vision and

Table 1. Side and adverse effects of TMS and the mechanisms behind these side effects

Side or Adverse effects	May caused by
Local pain or discomfort in the scalp (Anand et al., 2002, Groppa et al., 2012) Dependent on the location and the intensity of the stimulation (Perrera et al., 2016).	Scalp irritation or stimulation of the nociceptors within the scalp under the coil.
Headache, usually following the application of TMS over motor or premotor areas (Wasserman et al., 1998)	Repeated stimulation of pericranial muscles (Allen et al., 2017).
Facial Pain (Rossi et al., 2009)	Stimulation of the trigeminal or facial nerve and consequent muscle contractions, especially when holding the TMS coil around the temporal lobe.
Muscle spasms (Anand et al., 2002). Or, Neck pain (Perrera et al., 2016).	Fixation of the participant's head in an uncomfortable position during the TMS sessions which may lead to tiredness, muscle spasms, and neck pain.
Hearing threshold shift (Pascual-Leone et al., 1993, Loo et al., 2008).	Acoustic artefact (repeated click sound) associated with the coil discharges during stimulation.
Seizure (Kratz et al., 2011).	Hypoglycaemia, electrolyte alternation or systemic metabolic disorders (non-neurological medical conditions).
Vasovagal syncope (Riedel et al., 2016) in participants with the history of fainting.	A sudden drop in heart rate or blood pressure.
Eye twitching/pain (Rossi et al., 2014).	Repeated muscle contraction especially when TMS is applied near the orbit.
Visual impairment (Anand et al., 2002).	Application of high-intensity TMS pulses over occipital cortex.
Numbness of the tongue (Groppa et al., 2012).	Application of high-intensity TMS pulses over the Cz (midline) for stimulation of lower limbs corticospinal neurons.
Toothache (Rophl et al., 2004).	Application of TMS over dorsolateral prefrontal cortex (DLPFC). TMS pulses may locally irritate the superficial temporal portion of the trigeminal nerve and its projection via the buccal nerve into the dental region.

pallor. In some cases of VVS, myoclonic muscle jerking is reported which should not be mistaken with motor characteristics of seizure (Gillick et al., 2015). Moreover, VVS occurs more often in women and younger individuals triggered by anxiety, physical discomfort and/or unpleasant stimuli (Romme et al., 2008, Groppa et al., 2012). The critical point in the differentiation between syncope and seizure is the rapid recovery of full consciousness within a few seconds without apparent confusion after syncope episode compared to minutes or longer recovery in a seizure. (Caplan, 2000, Hadar et al., 2012).

Careful screening before the stimulation may reduce the prevalence of these very rare adverse effects; however, sometimes these may happen in individuals without any history of seizure or syncope (Kratz et al., 2011, Hadar et al., 2012, Gillick et al., 2015). TMS operators should carefully monitor the participant's reactions to the TMS to stop its use if a warning sign appears or felt by the TMS recipient.

To manage syncope, the participant should be placed in the supine position with legs elevated to a higher position than the heart. In case of having a seizure, if it is possible, the participant should be positioned in sidelying, and the operator should stay with the participant all the time. If the seizure lasted more than 60 seconds, the operator should follow the emergency protocol in their centre (Kartz et al., 2011).

In general, there is no clear evidence for hormonal and immunological adverse effects, changes in blood pressure, heart rate, EEG, memory, cognition, sensory or motor performances (Bridgers et al., 1991, Chokroverty et al., 1995, Lin et al., 2002).

Recommendations

Recommendations are a list of suggestions or proposals for the best course of action to minimise the side or adverse effects of TMS. In this section, a list of recommendations for optimal use of TMS and the rationale behind these recommendations are provided in Table 2.

Table 2. Recommendations for the use of TMS as an assessment tool

Recommendations	Rationale
Check the integrity of the scalp before any TMS application (Janicak et al., 2002).	May prevent further damage by reducing the number of applied TMS pulses or cancellation of the TMS session if any irritated scalp or scar is detected.
Check the colour of scalp during or after any TMS application (Janicak et al., 2002).	May prevent further redness or irritation (erythema) by reducing the number of applied TMS pulses or cancellation of the TMS session if any changes in the colour of scalp are detected.
Check whether the participants had adequate drinks/food intake before any TMS session (Kirton et al., 2008).	Minimizes the risk of sudden blood pressure reduction and fainting.
Check whether the participant had no history of syncope (Kesar et al., 2016).	May prevents syncope incidence.
Start with low TMS intensities (<30% of maximum stimulator output) followed by a gradual increase of the intensity (Kirton et al., 2008).	Minimizes stress, discomfort and may reduce the chance of adverse effects such as syncope.
Check whether the participants well slept the night before TMS session (at least 6 hours). No recent changes in participants sleep pattern (Kesar et al., 2016).	Minimizes the risk of seizures. Sleep deprivation lowers the seizure threshold (Rossi et al., 2014).
Use ear-plug or other types of hearing protection accessories (Perrera et al., 2016).	Reduces the risk of changes in the auditory threshold.
Avoid TMS intensities over 160% of resting motor threshold (Temesi et al., 2014).	Minimizes TMS intensity related discomfort and possibly vasovagal syncope (Lähteenmäk et al., 2015).
Use a comprehensive explanation of the involved TMS procedure (Perrera et al., 2016).	Minimizes anxiety and helps the participants to become familiar with the TMS procedure.
Use an appropriately worded informed Consent Form (Perrera et al., 2016).	Helps participants to better understand the experimental procedures they attend.
Fill in the TMS Screening Questionnaire before to the first TMS session (Rossi et al., 2009, Green et al., 1997).	Helps the TMS operator to screen high-risk participants.

Recommendations	Rationale
Familiarize the participants with the functioning of TMS device by discharging the TMS coil in the air to let the participants hear the sound of each discharge and also applying it over the operator's forearm to produce a muscle twitch (Rossini et al., 2015).	Helps the participants to become familiar with the functioning of the TMS device and reduces their stress related to being unfamiliar with the procedure.
Use a closed/quiet room for delivery of TMS session (Rossini et al., 2015).	Prevents unnecessary exposure of the magnetic pulses to the surrounding staff.
Monitor participants for any side or adverse effects during and following completion of the TMS sessions (Rossini et al., 2015).	Identifies the high-risk participants and prevents happening of the major adverse effects.
Follow the manufacturer's recommendations for regular maintenance and calibration of the TMS device.	Ensures optimal operational efficacy of the TMS device.
Avoid compressing the TMS coil against the scalp.	Reduces discomfort during TMS applications.
Plug TMS device into GFCI (ground fault circuit interrupter) receptacle.	Prevents the risk of electrocution.

Contraindications

Contraindications related to the TMS as an assessment tool is considered under two categories: "absolute" and "relative." Absolute contraindications refer to the conditions when TMS should not be used under any circumstances because of the severe and potentially life-threatening risks. On the other hand, relative contraindications refer to the conditions when the risk/benefit analysis allows its use. Screening questionnaire and risk/benefit analysis should be carried out before any TMS session to identify the high-risk participants and to weigh the benefits of TMS compared to its possible risks (Groppa et al., 2012). Table 3 summarises absolute and relative contraindications associated with the use of TMS as an assessment tool.

Table 3. Absolute and relative contraindications for using TMS as an assessment tool

Contraindications	Rationale
Absolute	
Over cochlear implant (Rossi et al., 2009).	Risks of interference with electromagnetic fields and malfunctioning of such implanted devices.
Over implanted medical pumps (Rossi et al., 2009).	
Over internal pulse generators (Rossi et al., 2009).	
Over pacemakers (Rossi et al., 2009).	
Intracardiac lines (Electrodes inside the heart) (Rossi et al., 2009).	
Over the eye (Rossi et al., 2009).	Risk of damaging the retina.
Over malignant tumours on the scalp (Rossi et al., 2009) or brain malignancies.	Risk of increasing and spreading tumour.
Relative	
Pre-conditioning (i.e., priming) interventions (Rossi et al., 2009).	Risk of inducing a seizure
TMS applied over more than one single scalp region (Rossi et al., 2009).	
History of seizure (non-treated), taking epileptogenic medications, history of seizures in the first-degree relatives (Rossi et al., 2009).	
History of syncope (Najib et al., 2014).	
Using medications known to lower the seizure threshold (Anand et al., 2002).	
History of head injury or concussion or neurosurgery (Anand et al., 2002) or stroke (Wassermann et al., 1998).	
History of severe heart disease (Wassermann et al., 1998).	
Vascular lesion of the brain, infectious or metabolic disease of the brain (Rossi et al., 2009).	
Implanted cortical or deep brain electrodes (Rossi et al., 2009).	
Ventriculo-peritoneal shunts (Rossi et al., 2009).	
Sleep deprivation (Rossi et al., 2009).	
Alcoholism (Rossi et al., 2009).	
Pregnancy (Rossi et al., 2009).	Not enough evidence on the effect of TMS in this population to rule out any possible harm to the mother or the developing fetus.

TMS RISKS IN DIFFERENT CONDITIONS

Safety of TMS in Children

No severe side or adverse effects of TMS as an assessment tool, other than the ones listed earlier in this chapter, are reported in children above two years old (Frye et al., 2008, Rossi et al., 2014). Literature indicates some side effects including scalp discomfort, hand weakness, headache, neck pain and arm pain/tingling in this group of participants (Garvey et al., 2008). However, it is necessary to keep in mind that the risk of adverse effects in children may be influenced by rapid developmental changes, such as the closure of the fontanelle (Rossi et al., 2009), the growth of the ear canal and maturation of cortical excitability. Any small increases in the length of the external auditory canal cause greater resonance (Kruger et al., 1987) and increase the risk of injury from the high amplitude and high-frequency acoustic noises (Rossi et al., 2014). Besides, cortical excitability is unusually high in infants (Rossi et al., 2014); therefore, it is critical to be aware of these differences until the age of 18 months old. In this age, the fontanelles are still open, and the possible mechanical injuries may occur due to excessive coil pressure (Groppa et al., 2012). Despite the aforementioned side or adverse effects, Gilbert et al., (2003) classified the use of TMS in children as a technique with minimal risk.

Safety of TMS in Pregnancy

It has been reported that the peak magnetic field is minimal (about 1 Gauss) at 46 cm below the coil, (around the sternum or nearest point of a full term pregnant uterus) compared to 9000 Gauss at 1cm away from the coil (Dodick et al., 2010). Even though the induced electromagnetic field decreases rapidly with distance and disappears at a distance of 70 cm away from the discharging coil (Rossi et al., 2009), the effects of minute exposure to the electromagnetic pulses on the fetus are unknown. Therefore, due to possible risks to the fetus, pregnancy is a relative contraindication for the

use of TMS as an assessment tool (Anand et al., 2002, Rossi et al., 2009). It should be noted that, the TMS can only be used in this group if risk/benefit analysis supports the use (Sayer et al., 2014). Having said that, no side effects to the fetus are reported yet (Nahas et al., 1999, Klirova et al., 2008, Dodick et al., 2010). Additional data are needed to assess the effects of TMS in pregnancy adequately.

Safety of TMS in Patients with Neurological Conditions

The use of TMS as an assessment tool in patients with neurological disorders does not induce any permanent side or adverse effects but raises some concerns about induction of seizure in this group of participants (Anand et al., 2002). Single or paired-pulse may rarely induce seizures in patients with neurological conditions such as stroke, amyotrophic lateral sclerosis (ALS) (Green et al., 1997, Illes et al., 2006) and those with medically intractable epileptic seizures (Claus et al., 1993, Classen et al., 1995).

Safety of TMS over the Cerebellum

Safety of TMS over the cerebellum has not been studied as comprehensively as the TMS safety of the motor cortex or other cortical areas. However, no adverse effects have been reported for cerebellar TMS in healthy participants or individuals with neurological disorders (Anand et al., 2002, Dodick et al., 2010) such as ataxia (Ugawa et al., 1997).

Safety of Electromagnetic Radiation in TMS Operator

Even though TMS operators are exposed to electromagnetic radiation, no known risks are detected in TMS operators. Literature indicates no

potential risk to the unborn baby of the pregnant TMS operators (Klirova et al., 2008).

Over-exposure to electromagnetic radiation is an issue which should be avoided (Millerlikken et al., 2016). Therefore, it is recommended that TMS operators at risk should keep a distance of at least 40-70-cm from the TMS coil to avoid risks of accidental over-exposure to magnetic fields (Karlstorm et al., 2006).

ETHICAL AND REGULATORY REQUIREMENTS

In both research and clinical applications of TMS, all ethical and regulatory requirements should be rigorously followed. First, informed consent is an absolute requirement. This means that the participants should be informed fully about the procedures and all known and potential risks. Overall, they should be volunteers and feel free to participate in a TMS study or not. They should be notified that they can quit the study without any penalty (Green et al., 1997, Rossi et al., 2009). Second, the risk/benefit analysis should be carried out and discussed with the participants. In any application of TMS, the potential benefit of TMS should outweigh the risks (Rossi et al., 2009). Third, it is necessary to have an equal distribution of the risks and benefits of the TMS applications among the study populations especially when TMS applied on different groups of participants (Rossi et al., 2009).

CONCLUSION

This chapter presents a comprehensive list of TMS side or adverse effects, contraindications and recommendations for the optimal use of TMS. Additionally, risks of TMS in children, pregnancy, TMS operators, and patients with neurological conditions are also briefly discussed. Finally, the ethical and regulatory requirements for application of TMS were also briefly

presented. TMS as an assessment tool is a safe technique in both adults and children older than two years. The most common side effect of TMS is a headache which is transient and usually subsides after a few hours. The most severe side effects of TMS are seizures and syncope which proper screening may significantly reduce their rare occurrence.

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Post-amble to Chapter 2

The following missed article should be also considered for this book chapter:

(Krishnan, C., Santos, L., Peterson, M. D., & Ehinger, M. (2015). Safety of non-invasive brain stimulation in children and adolescents. Brain stimulation, 8(1), 76-87.).

Preamble to Chapter 3

Non-invasive brain stimulation techniques (NIBS) are vastly used to induce changes in corticospinal excitability (CSE) for therapeutic purposes. These effects, however, could be affected through different factors including any history of neuronal activity before the main stimulation session (priming-test protocols). The literature review confirms that using a priming protocol before the test protocol could affect the effect on CSE by modifying the level of the synaptic activity.

This chapter addresses **Aim 2** in this thesis to review the literature for investigating the effects of different priming NIBS protocols on a consequent NIBS test protocol of M1 on CSE in healthy individuals that could potentially contribute to response variability.

It is revealed that based on stimulation parameters of priming and test protocols; different results would be seen from expected to non-expected on CSE. It also confirms that different types of neuroplasticity mechanisms could be involved.

Chapter 3 provides a systematic review and meta-analysis on how different priming-test protocols on M1 affect CSE in healthy individuals. This chapter became the basement of the theoretical framework of the current thesis and the main studies of 3 – 5 were designed based on this systematic review.

Chapter 3. How different priming stimulations affect the induced corticospinal excitability by test non-invasive brain stimulation techniques? Systematic review and Meta-analysis

The format of this chapter is consistent with the Journal of *Reviews in Neuroscience (impact factor: 2.157, ranking: Q2 in Neuroscience).* This chapter was published on Mar 31st, 2018.

Hassanzahraee M., Zoghi M., Jaberzadeh S. 2018. How different priming stimulations affect the corticospinal excitability induced by non-invasive brain stimulation techniques: A systematic review and meta-analysis. **Reviews in Neuroscience**. 29(8):883-899. https://doi.org/10.1515/revneuro-2017-0111.

Maryam Hassanzahraee*, Maryam Zoghi and Shapour Jaberzadeh

How different priming stimulations affect the corticospinal excitability induced by noninvasive brain stimulation techniques: a systematic review and meta-analysis

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Abstract: Noninvasive brain stimulation (NIBS) techniques could induce changes in corticospinal excitability (CSE) and neuroplasticity. These changes could be affected by different factors, including having a session of stimulation called the 'priming' protocol before the main stimulation session called the 'test' protocol. Literature indicates that a priming protocol could affect the activity of postsynaptic neurons, form a neuronal history, and then modify the expected effects of the test protocol on CSE indicated by the amplitude of transcranial magnetic stimulation-induced motor-evoked potentials. This prior history affects a threshold to activate the necessary mechanism stabilizing the neuronal activity within a useful dynamic range. For studying the effects of this history and related metaplasticity mechanisms in the human primary motor cortex (M1), priming-test protocols are successfully employed. Thirty-two studies were included in this review to investigate how different priming protocols could affect the induced effects of a test protocol on CSE in healthy individuals. The results showed that if the history of synaptic activity were high or low enough to displace the threshold, the expected effects of the test protocol would be the reverse. This effect reversal is regulated by homeostatic mechanisms. On the contrary, the effects of the test protocol would not be the reverse, and at most we experience a prolongation of the lasting effects if the aforementioned history is not enough to displace the threshold. This effect prolongation is mediated by nonhomeostatic mechanisms. Therefore, based on the characteristics of priming-test protocols and the interval between them, the expected results of priming-test protocols would be different. Moreover, these findings could shed light on the different mechanisms of metaplasticity involved in NIBS. It helps us understand how we can improve the expected outcomes of these techniques in clinical approaches.

Keywords: motor-evoked potentials; plasticity; primary motor cortex; priming; tDCS; TMS.

***Corresponding author: Maryam Hassanzahraee,** Non-invasive Brain Stimulation and Neuroplasticity Laboratory, Department of Physiotherapy, School of Primary and Allied Health Care, Faculty of Medicine, Nursing and Health Science, Monash University, Peninsula Campus, P. O. Box 527, Victoria 3199, Australia, e-mail: maryam.hassanzahraee@monash.edu.
http://orcid.org/0000-0002-8629-4104
Maryam Zoghi: Department of Rehabilitation, Nutrition and Sport, School of Allied Health, Discipline of Physiotherapy, La Trobe University, Bundoora, Victoria 3086, Australia
Shapour Jaberzadeh: Non-invasive Brain Stimulation and Neuroplasticity Laboratory, Department of Physiotherapy, School of Primary and Allied Health Care, Faculty of Medicine, Nursing and Health Science, Monash University, Peninsula Campus, P. O. Box 527, Victoria 3199, Australia

Introduction

During the past decade, noninvasive brain stimulation (NIBS) has become a widely used neuromodulation technique. NIBS can induce neuroplasticity changes in the human primary motor cortex (M1; Goldsworthy et al., 2015). Changes in M1 plasticity can be probed by the assessment of single-pulse transcranial magnetic stimulation (TMS)-induced motor-evoked potentials (MEPs; Siebner et al., 2004). The sizes of these MEPs can be considered as a measure of corticospinal excitability (CSE). CSE is known to be affected by several factors, including prior application of NIBS techniques, prior activity (motor or cognitive learning), mental status, attentional level, and time of day (Ridding and Ziemann, 2010). Among these, having a prior session of brain stimulation by NIBS or training techniques induces changes in the history of involved synapses in the brain and is one of the most important causes of variability in response to NIBS protocols (Hordacre et al., 2015). The application of two successive stimulation techniques in priming-test protocols is a novel approach that is valuable in three respects: for understanding the variability in response to NIBS, for developing more efficient stimulation protocols to induce optimal therapeutic changes with longer-lasting effects, and for studying mechanisms underpinning the stabilization of neuronal activity of the brain within a physiologic range.

Metaplasticity is an important aspect of neuroplasticity, shaping the direction, magnitude, and duration of the induced synaptic changes (Abraham and Bear, 1996).

Several researchers have investigated these mechanisms using different priming-test NIBS protocols (Iyer et al., 2003; Lang et al., 2004; Siebner et al., 2004; Muller et al., 2007; Nitsche et al., 2007; Todd et al., 2009; Gamboa et al., 2010; Iezzi et al., 2011; Cosentino et al., 2012). These studies have shown that the characteristics of the priming and/or test protocols (that is, their excitatory or inhibitory nature, duration, and the interval between the priming and test applications) alter the test protocols' effects on CSE. These different effects can be explained by homeostatic and nonhomeostatic metaplasticity mechanisms (Bienenstock et al., 1982; Abbot and Nelson, 2000). In addition, there is a sliding threshold for bidirectional plasticity induction (Bienenstock et al., 1982; Cooper and Bear, 2012) in favor of long-term potentiation (LTP) and long-term depression (LTD)-like effects. The LTD/LTP-like threshold is dynamically adjusted to the historical level of the postsynaptic activity. Homeostatic metaplasticity is activated in favor of LTD-like effect induction if historical postsynaptic activity is high, whereas it will favor an LTP-like effect if historical activity is low (Ziemann and Siebner, 2008). However, sometimes priming-test protocols are regulated by nonhomeostatic metaplasticity mechanisms. Based on these mechanisms, when historical synaptic activity is not high or low enough to displace the threshold, the priming protocol will intensify the effect of the test protocol. Therefore, the application of a priming protocol can intensify, weaken, prolong, or reverse the effect of the test protocol.

Müller-Dahlhaus and Ziemann (2015) and Karabanov et al. (2015) illuminated the concept of metaplasticity in their reviews of research on the effects of priming interventions on test protocols. The former (Müller-Dahlhaus and Ziemann, 2015) concluded that homeostatic metaplasticity is a mechanism that adjusts the brain activity within a physiologic range and that nonhomeostatic metaplasticity is a mechanism responsible for the prolongation of the after-effects of NIBS on cortical excitability or learning. The latter (Karabanov et al., 2015) reviewed research using NIBS priming-test techniques and concluded that homeostatic metaplasticity occurs at the system level and in interaction with physiologic conditions. Although these authors attempted to explain how different physiologic changes within the motor cortex regulate activities through metaplasticity mechanisms, their reviews were not systematic but narrative and included studies that targeted different sites of stimulation. Hence, the current systematic review and meta-analysis is the first in this area of research. In addition, unlike the aforementioned reviews, which included priming-test techniques including NIBS, motor training, and motor learning, our systematic review and meta-analysis only included studies that used NIBS techniques as both priming and test protocols to investigate how priming affects the outcome of the test protocol in healthy individuals. Moreover, in the current review, only NIBS studies over M1 were included, and the sole outcome measure was TMS-induced MEPs.

The main objective of this systematic review and meta-analysis was to find and evaluate studies that used two successive NIBS as priming and test protocols and investigated the magnitude and direction of the priming technique on the effect of the test protocols on M1 CSE in healthy individuals.

Methods

Literature search

The following seven databases were searched from their inception until February 2017: PubMed, Ovid Medline, Scopus, EMBASE, PROQuest, CINAHL, and Cochrane Library. Reference lists of all retrieved papers were also searched as an additional source. The following key search terms were used: non-invasive brain stimulation, variability, transcranial direct current stimulation (tDCS), corticospinal excitability, CSE, transcranial magnetic stimulation, TMS, repetitive TMS (rTMS), synaptic activity, priming NIBS, conditioning, NIBS, motor-evoked potential, MEP, paired associative stimulation (PAS), theta-burst stimulation (TBS), quadri-pulse stimulation (QPS), plasticity, and motor cortex.

Selection criteria

Inclusion criteria

Papers were included if they met the following criteria: (1) they described applications of tDCS, or different types of TMS over M1, as priming or test NIBS protocols consecutively; (2) the studies involved healthy individuals without pervasive developmental, neurodegenerative, psychiatric, or neurologic conditions; (3) they were published in peer-reviewed journals in English; and (4) measurements of CSE changes by MEP amplitude were the main outcome measures.

Exclusion criteria

Studies were excluded if (1) neither the priming nor test protocols included NIBS and (2) the main outcome

measures were not CSE changes (i.e. not reported as MEP amplitude).

Quality assessment

Two researchers independently reviewed each article and gave a quality score using the modified Downs and Black (D&B) tool (Downs and Black, 1998). The modified D&B tool contains 27 questions, of which 26 are graded on a 0 or 1 basis ('yes'/'no' or 'not determined'); the remaining item is scored on a 0–2 scale. Thus, this scale ranges from 0 to 28, with higher score indicating higher methodologic quality.

Outcome measure

The main outcome measure in this review is the average value of peak-to-peak amplitude of TMS-induced MEPs. This amplitude is an index of CSE (Siebner et al., 2004).

Subgroup analysis and assessment of heterogeneity

We assessed heterogeneity using χ^2 and I^2 statistics. Also, the effects of the different NIBS techniques as the priming-test protocols on CSE were measured in the M1 of healthy individuals.

Data extraction

The following data relevant to the aim of this study were extracted from all papers: sample size, characteristics of parameters of NIBS for both priming and test protocols, expected effect of priming on CSE, expected effect of the test protocol, and overall effects of the priming-test protocols, which were categorized into four different groups:

1. Studies with excitatory priming-test protocols,
2. Studies with inhibitory priming-test protocols,
3. Studies with excitatory priming and inhibitory test protocols, and
4. Studies with inhibitory priming and excitatory test protocols.

For meta-analysis, the number of participants in each of the four groups, their means, and the standard deviations (SDs) of their elicited MEPs were required. The means and SDs of the MEP amplitudes were extracted from each article whenever possible. If the required data were not reported, we contacted the corresponding author(s) to request the original data. If the authors did not respond, a JAVA-based Plot Digitizer (Joseph, 2011) was used to directly estimate mean and SD from the graphs. Data were entered into the effect size calculator using REVMAN 5.3 software (Cochrane Collaboration 2008).

Results

Identification and selection of studies

After the removal of duplicates, the literature search identified 79 studies, of which only 35 were considered appropriate for inclusion in this review. Two papers were excluded because no data could be obtained either from corresponding author or graphs, bringing the total number of studies to 33 (Figure 1).

Method of quality assessment

The D&B scores of included clinical studies ranged between 16 and 18 (with a mean score of 17), indicating good quality.

Participants in included studies

In total, across the included studies, the effects of different NIBS techniques as priming-test protocols (excitatory/inhibitory) were investigated in 378 healthy individuals allocated to four main subgroups.

Pooled data analysis

Excitatory priming-test protocols

Twenty studies that involved two excitatory protocols were divided into two groups based on their priming protocol [anodal tDCS (a-tDCS) or TMS].

a-tDCS as the priming technique: Figure 2 summarizes the pooled MEP amplitude data extracted from studies split into five subgroups using a-tDCS as the priming followed by test protocols including a-tDCS (with or without interval), rTMS, and PAS in healthy individuals.

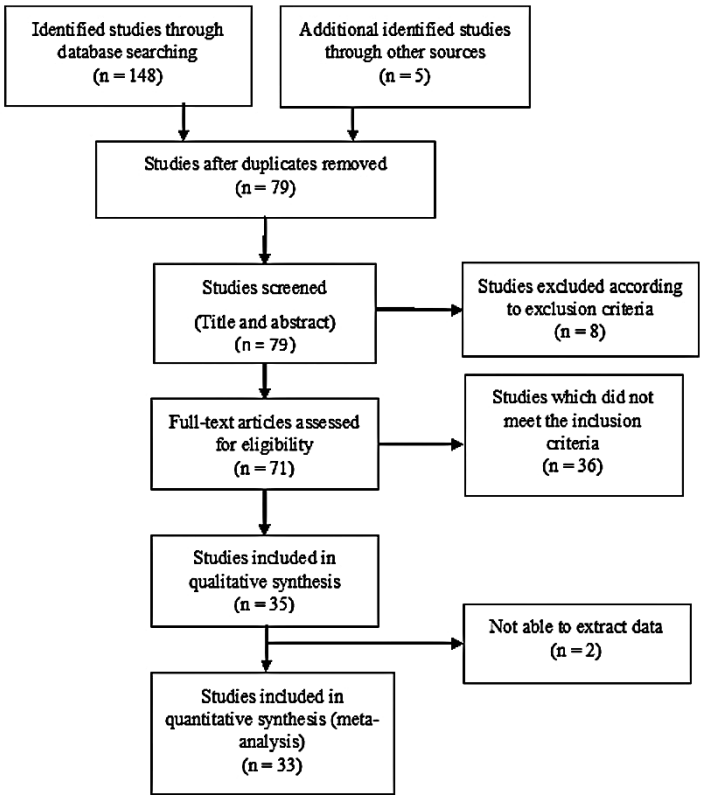

Figure 1: QUORUM flowchart of studies included in this review.

Meta-analysis of four studies showed that using paired a-tDCS (with no, short, and long intervals) increased the excitatory effect of the test a-tDCS significantly ($p = 0.02$; Fricke et al., 2010; Jaberzadeh et al., 2013; Monte-Silva et al., 2013; Bastani and Jaberzadeh, 2014).

Meta-analysis of two studies showed that a-tDCS suppressed the excitatory effect of rTMS significantly ($p = 0.01$; Lang et al., 2004; Cosentino et al., 2012).

In one study, priming a-tDCS increased the expected excitatory effect of PAS (Table 1; Nitsche et al., 2007). It was not pooled with other studies in meta-analysis due to its unique priming-test protocols.

TMS as the priming technique: Figure 3 summarizes the pooled MEP amplitude data extracted from 14 studies using different TMS techniques as the priming and test protocols, including intermittent TBS (iTBS), PAS, rTMS, and QPS.

Forest plot and meta-analysis results from three studies using paired PAS protocols showed that priming either with 10- or 30-min intervals increased the excitatory effect of the test protocol significantly ($p = 0.002$; Muller et al., 2007; Muller-Dahlhaus et al., 2015; Opie et al., 2017a).

Meta-analysis of seven studies using paired iTBS with or without interval showed that the priming iTBS did not suppress the excitatory effect of the following iTBS significantly ($p = 0.56$; Gentner et al., 2008; Gamboa et al., 2010, 2011; Huang et al., 2010; Murakami et al., 2012; Mastroeni et al., 2013; Opie et al., 2017b).

The results of two studies showed that rTMS, regardless of the type of the test protocol (iTBS or PAS), did not suppress the excitatory effect of test protocol. In contrast, in another study using paired QPS, the priming protocol suppressed the expected excitatory effect of the test protocol. These studies were not pooled in the meta-analysis (Table 1; Hamada et al., 2008; Potter-Nerger et al., 2009; Iezzi et al., 2011).

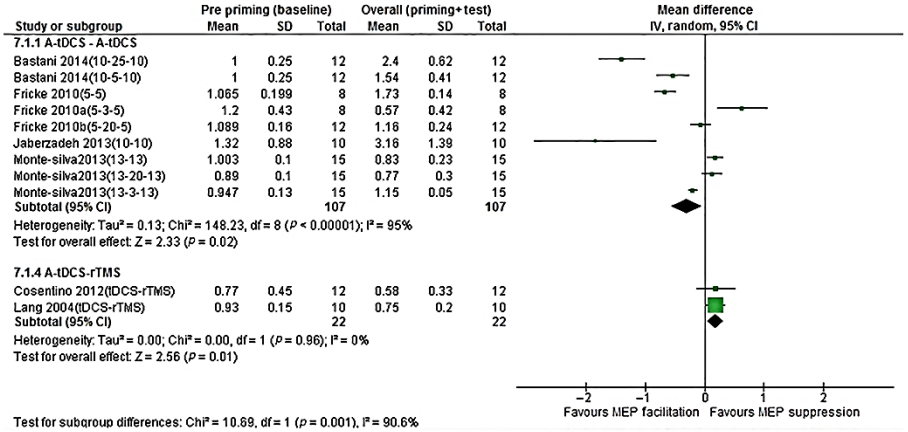

Figure 2: Forest plot of the effects of excitatory priming-test protocols: a-tDCS as the priming technique.
Comparison of baseline MEP and MEP after applying priming-test protocols. ■, effect size for one trial; horizontal line: 95% confidence interval; ♦, pooled effect size for all trials.

Table 1: Excitatory priming-test protocols.

Study	n	Priming technique (tDCS or TMS)	Expected effect of priming protocol	Test protocol (tDCS or TMS)	Expected effect of test protocol	Overall effect (priming+test protocols)
Fricke et al., 2010	8	a-tDCS: 2 mA, 5 min, 35 cm^2	↑ MEP	a-tDCS: 2 mA, 5 min, 35 cm^2	↑ MEP	↓ MEP
Monte-Silva et al., 2013	15	a-tDCS: 1 mA, 5 min, 35 cm^2	↑ MEP	a-tDCS: 1 mA, 5 min, 35 cm^2	↑ MEP	↓ MEP
Moloney and Witney, 2014	15	a-tDCS: 1 mA, 10 min, 25 cm^2	↑ MEP	rTMS: 5 Hz, RMT_{90}	↑ MEP	↓ MEP
Lang et al., 2004	10	a-tDCS: 1 mA, 10 min, 35 cm^2	↑ MEP	rTMS: 5 Hz, AMT_{100}	↑ MEP	↓ MEP
Cosentino et al., 2012	12	a-tDCS: 1.5 mA, 15 min, 35 cm^2	↑ MEP	rTMS: 5 Hz, RMT_{120}	↑ MEP	↓ MEP
Iezzi et al., 2011	10	rTMS: 5 Hz, RMT_{120}	↑ MEP	iTBS: 5 Hz, AMT_{80}	↑ MEP	↓ MEP
Potter-Nerger et al., 2009	11	rTMS: 5 Hz, RMT_{90}	↑ MEP	PAS_{N20+2}: 0.25 Hz	↑ MEP	↓ MEP
Hamada et al., 2008	6	QPS-5 ms: 10 min, AMT_{90}	↑ MEP	QPS-10 ms: 30 min	↑ MEP	↓ MEP
Murakami et al., 2012	9	iTBS: 5 Hz, AMT_{80}	↑ MEP	iTBS: 5 Hz, AMT_{80}	↑ MEP	↓ MEP
Huang et al., 2010	8	iTBS: 5 Hz, AMT_{80}	↑ MEP	$cTBS_{150}$: 5 Hz, AMT_{80}	↑ MEP	↓ MEP
Gentner et al., 2008	9	$cTBS_{300}$: 5 Hz, AMT_{80}, 20 s	↑ MEP	$cTBS_{300}$: 5 Hz, AMT_{80}, 20 s	↑ MEP	↓ MEP
Gamboa et al., 2010	14	iTBS: 5 Hz, AMT_{80}	↑ MEP	iTBS: 5 Hz, AMT_{80}	↑ MEP	↓ MEP
Muller et al., 2007	11	PAS_{LTP}	↑ MEP	PAS_{LTP}	↑ MEP	↓ MEP
Gamboa et al., 2011	10	iTBS: 5 Hz, AMT_{80}	↑ MEP	iTBS: 5 Hz, AMT_{80}	↑ MEP	↑ MEP
Muller-Dahlhaus et al., 2015	12	PAS_{LTP}	↑ MEP	PAS_{LTP}	↑ MEP	↑ MEP
Nitsche et al., 2007	12	a-tDCS: 1 mA, 7 min, 35 cm^2	↑ MEP	PAS_{25}: 7 min	↑ MEP	↑ MEP
Opie et al., 2017a	16	PAS_{LTP}	↑ MEP	PAS_{LTP}	↑ MEP	↑ MEP
Opie et al., 2017b	15	iTBS: 5 Hz, AMT_{80}	↑ MEP	iTBS: 5 Hz, AMT_{80}	↑ MEP	↑ MEP
Mastroeni et al., 2013	29	iTBS: 5 Hz, AMT_{80}	↑ MEP	iTBS: 5 Hz, AMT_{80}	↑ MEP	↑ MEP
Jaberzadeh et al., 2013	10	a-tDCS: 1 mA, 10 min, 42 cm^2	↑ MEP	a-tDCS: 1 mA, 10 min, 42 cm^2	↑ MEP	↑ MEP
Bastani and Jaberzadeh, 2014	12	a-tDCS: 1 mA, 10 min, 16 cm^2	↑ MEP	a-tDCS: 1 mA, 10 min, 16 cm^2	↑ MEP	↑ MEP

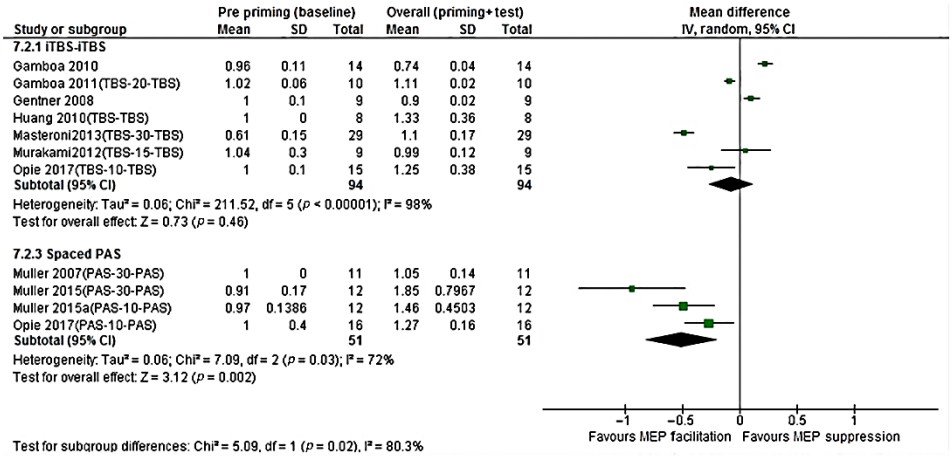

Figure 3: Forest plot of the effects of excitatory priming-test protocols: TMS as the priming technique.
Comparison of baseline MEP and MEP after applying priming-test protocols. ■, effect size for one trial; horizontal line: 95% confidence interval; ♦, pooled effect size for all trials.

Inhibitory priming-test protocols

Fifteen studies using cathodal tDCS (c-tDCS) or TMS techniques as the inhibitory priming protocol were investigated.

c-tDCS as the priming technique: Figure 4 summarizes the pooled MEP amplitude data extracted from four studies using c-tDCS as the priming protocol followed by test protocols including c-tDCS and rTMS.

Forest plot and meta-analysis results of two studies show that using the paired c-tDCS (with or without interval) boosted the inhibitory effect of the test c-tDCS significantly ($p = 0.12$; Fricke et al., 2010; Monte-Silva et al., 2010).

Forest plot and meta-analysis results of two studies show that c-tDCS did not suppress the inhibitory effect of

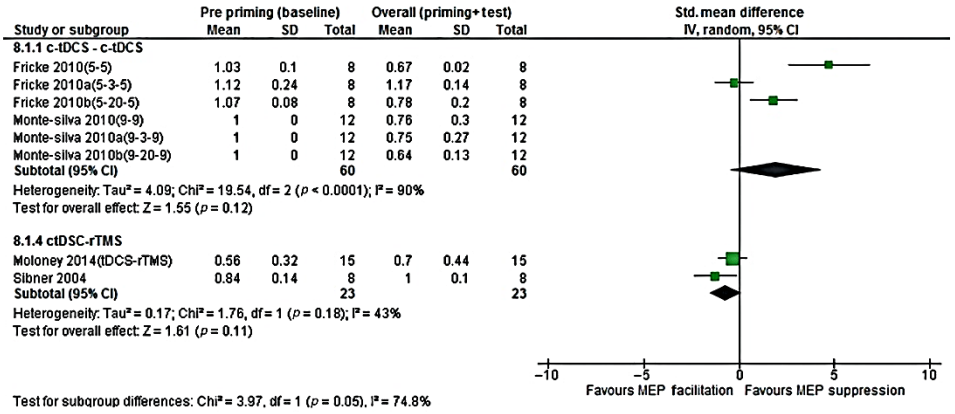

Figure 4: Forest plot of the effects of inhibitory priming-test protocols: c-tDCS as the priming technique.
Comparison of baseline MEP and MEP after applying priming-test protocols. ■, effect size for one trial; horizontal line: 95% confidence interval; ♦, pooled effect size for all trials.

TMS ($p = 0.11$; Siebner et al., 2004; Moloney and Witney, 2014).

TMS as the priming technique: Figure 5 summarizes the pooled MEP amplitude data extracted from 11 studies using rTMS and continuous TBS (cTBS) as the priming protocol followed by test protocols such as PAS and cTBS (with or without interval).

Meta-analysis of three studies showed that priming rTMS and cTBS suppressed the inhibitory effect of the PAS significantly ($p = 0.03$ and 0.05, respectively; Potter-Nerger et al., 2009; Delvendahl et al., 2010; Ni et al., 2014).

Meta-analysis of eight studies using two following cTBS (with or without interval) showed that the priming did not suppress the inhibitory effect of the test protocol significantly ($p = 0.60$; Gamboa et al., 2010, 2011; Huang et al., 2010; Goldsworthy et al., 2012, 2013, 2014; Murakami et al., 2012; Mastroeni et al., 2013).

Excitatory priming and inhibitory test protocols

Nine studies using excitatory priming and inhibitory test protocols were investigated. These studies were split into two groups based on the type of the priming protocol (a-tDCS or TMS).

a-tDCS as the priming technique: Figure 6 summarizes the pooled MEP amplitude data extracted from two studies using a-tDCS as the priming followed by rTMS. Meta-analysis showed that the priming did not

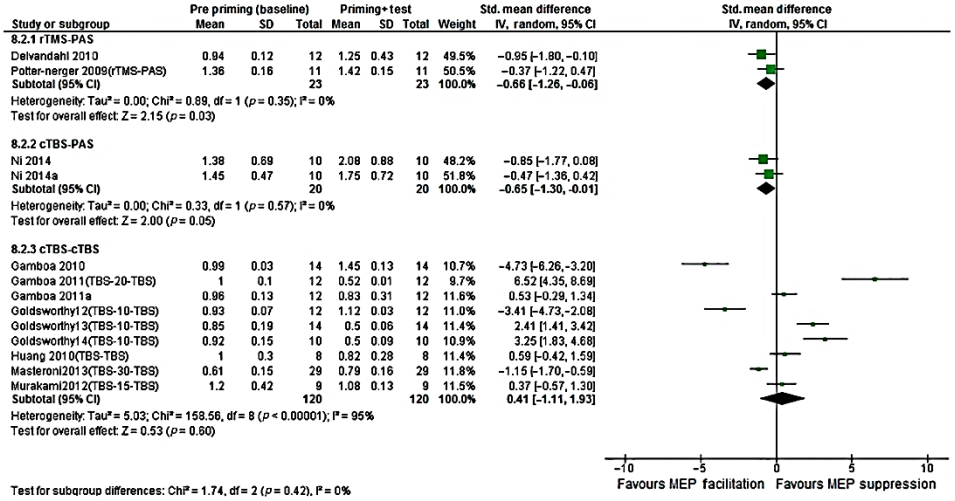

Figure 5: Forest plot of the effects of inhibitory priming-test protocols: TMS as the priming technique.
Comparison of baseline MEP and MEP after applying priming-test protocols. ■, effect size for one trial; horizontal line: 95% confidence interval; ♦, pooled effect size for all trials.

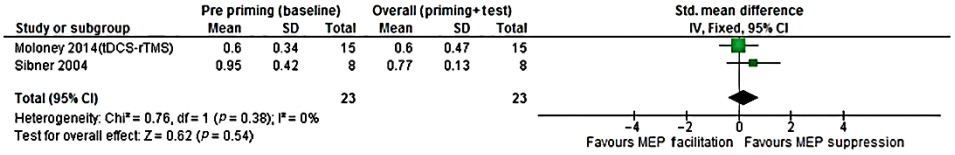

Figure 6: Forest plot of the effects of excitatory priming-inhibitory test protocols: a-tDCS as the priming technique.
Comparison of baseline MEP and MEP after applying priming-test protocols. ■, effect size for one trial; horizontal line: 95% confidence interval; ♦, pooled effect size for all trials.

significantly boost the inhibitory effect of the test protocol ($p = 0.54$; Siebner et al., 2004; Moloney and Witney, 2014).

TMS as the priming technique: Figure 7 summarizes the pooled MEP amplitude data extracted from seven studies using rTMS, iTBS, and PAS as the priming protocol.

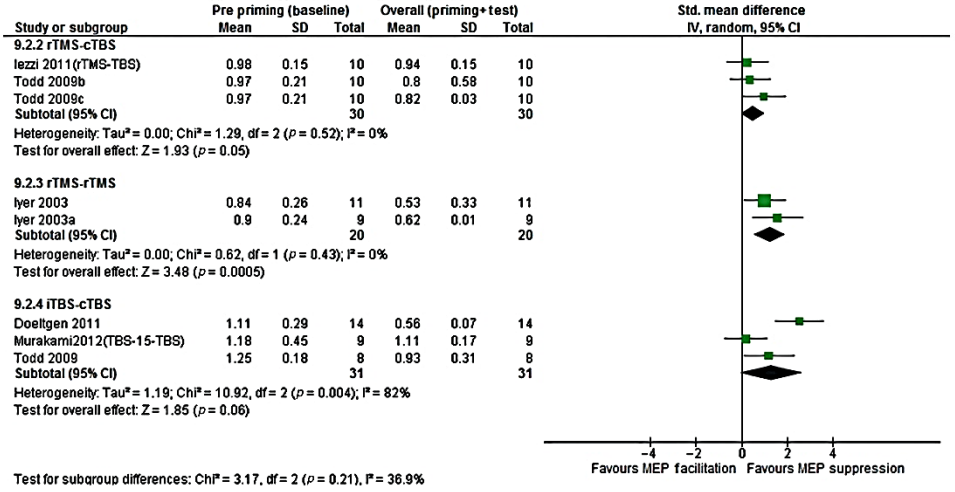

Figure 7: Forest plot of the effects of excitatory priming-inhibitory test protocols: TMS as the priming technique.
Comparison of baseline MEP and MEP after applying priming-test protocols. ■, effect size for one trial; horizontal line: 95% confidence interval; ♦, pooled effect size for all trials.

Table 2: Inhibitory priming-test protocols.

Study	n	Priming protocol (tDCS or TMS)	Expected effect of priming protocol	Test protocol (tDCS or TMS)	Expected effect of test protocol	Overall effect (priming+test protocols)
Delvendahl et al., 2010	12	rTMS: 0.1 Hz, RMT_{80}	↓ MEP	PAS_{10}: 15 min	↓ MEP	↑ MEP
Potter-Nerger et al., 2009	11	rTMS: 1 Hz, RMT_{90}	↓ MEP	PAS_{N20-5}: 0.25 Hz	↓ MEP	↑ MEP
Siebner et al., 2004	8	c-tDCS: 1 mA, 10 min, 35 cm^2	↓ MEP	rTMS: 1 Hz, RMT_{90}	↓ MEP	↑ MEP
Gamboa et al., 2010	14	cTBS: 5 Hz, AMT_{80}	↓ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↑ MEP
Ni et al., 2014	10	cTBS: 5 Hz, AMT_{80}	↓ MEP	PAS_{10}	↓ MEP	↑ MEP
Fricke et al., 2010	8	c-tDCS: 2 mA, 5 min, 35 cm^2	↓ MEP	c-tDCS: 2 mA, 5 min, 35 cm^2	↓ MEP	↑ MEP
Ni et al., 2014	10	PAS_{10}	↓ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↑ MEP
Murakami et al., 2012	9	cTBS: 5 Hz, AMT_{80}	↓ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↑ MEP
Mastroeni et al., 2013	29	cTBS: 5 Hz, AMT_{80}	↓ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↑ MEP
Gamboa et al., 2011	12	cTBS: 5 Hz, AMT_{80}	↓ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↓ MEP
Goldsworthy et al., 2012	12	cTBS: 5 Hz, AMT_{80}	↓ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↓ MEP
Goldsworthy et al., 2013	14	cTBS: 5 Hz, AMT_{80}	↓ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↓ MEP
Goldsworthy et al., 2014	10	cTBS: 5 Hz, AMT_{80}	↓ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↓ MEP
Monte-Silva et al., 2010	12	c-tDCS: 1 mA, 9 min, 35 cm^2	↓ MEP	c-tDCS: 1 mA, 9 min, 35 cm^2	↓ MEP	↓ MEP
Todd et al., 2009	10	rTMS: 2 Hz, RMT_{90}	↓ MEP	cTBS: 5 Hz, AMT_{80}, 40 s	↓ MEP	↓ MEP

Meta-analysis of the included studies using rTMS followed by rTMS or cTBS as the test protocol showed that the priming boosted the inhibitory effect of the test protocol significantly ($p = 0.0005$ and 0.05, respectively; Iyer et al., 2003; Todd et al., 2009; Iezzi et al., 2011).

Meta-analysis of the included studies using iTBS as the priming and cTBS as the test protocol showed that the priming did not significantly boost the inhibitory effect of the test protocol ($p = 0.06$; Todd et al., 2009; Huang et al., 2010; Doeltgen and Ridding, 2011; Murakami et al., 2012).

Another study used PAS as the priming protocol, which boosted the expected inhibitory effect of the test cTBS protocol (Ni et al., 2014). It was not pooled with other studies in the meta-analysis due to its unique priming-test protocol, but it is presented in Table 3.

Inhibitory priming and excitatory test protocols

Thirteen studies involved excitatory priming and inhibitory test protocols. These studies were separated based on their priming protocol: either c-tDCS or TMS.

c-tDCS as the priming technique: Figure 8 summarizes the pooled MEP amplitude data extracted from three studies using c-tDCS as the priming protocol followed by the test rTMS protocol.

Meta-analysis showed that the priming protocol did not boost the expected excitatory effect of the test protocol significantly ($p = 0.26$; Lang et al., 2004; Cosentino et al., 2012).

Another study using priming c-tDCS did not boost the excitatory effect of the test PAS protocol. It was not included in the meta-analysis due to its unique priming-test protocol (Nitsche et al., 2007). This study is reported in Table 4.

TMS as the priming technique: Figure 9 summarizes the pooled MEP amplitude data extracted from nine studies using cTBS, rTMS, QPS, and PAS as the priming protocol.

Meta-analysis of the two studies using c-tDCS-rTMS protocols showed that the priming did not significantly increase the excitatory effect of rTMS protocol ($p = 0.26$; Lang et al., 2004; Cosentino et al., 2012).

Table 3: Excitatory priming and inhibitory test protocols.

Study	n	Priming protocol (tDCS or TMS)	Expected effect of priming protocol	Test protocol (TMS)	Expected effect of test protocol	Overall effect (priming+test protocols)
Iyer et al., 2003	26	rTMS: 6 Hz, RMT_{90}	↑ MEP	rTMS: 1 Hz, RMT_{115}	↓ MEP	↓ MEP
Huang et al., 2010	8	iTBS: 5 Hz, AMT_{80}	↑ MEP	$cTBS_{150}$: 5 Hz, AMT_{80}	↓ MEP	↓ MEP
Doeltgen and Ridding, 2011	14	iTBS: 5 Hz, AMT_{80}	↑ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↓ MEP
Todd et al., 2009	8	iTBS: 5 Hz, AMT_{80}	↑ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↑ MEP
Murakami et al., 2012	9	iTBS: 5 Hz, AMT_{80}	↑ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↓ MEP
Iezzi et al., 2011	10	rTMS: 5 Hz, RMT_{120}	↑ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↓ MEP
Todd et al., 2009	10	rTMS: 6 Hz, RMT_{90}	↑ MEP	cTBS: 5 Hz, AMT_{80}	↓ MEP	↓ MEP
Siebner et al., 2004	8	a-tDCS: 1 mA, 10 min, 35 cm^2	↑ MEP	rTMS: 1 Hz, RMT_{90}	↓ MEP	↓ MEP

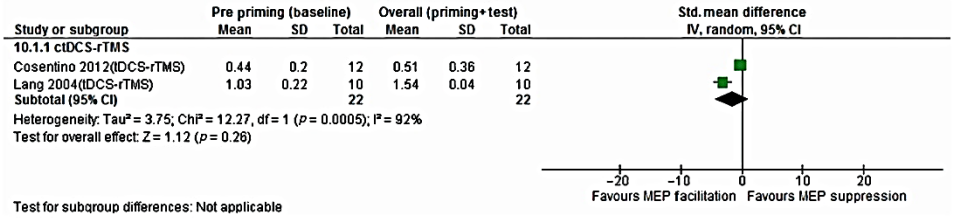

Figure 8: Forest plot of the effects of inhibitory priming-excitatory test protocols: c-tDCS as the priming technique.
Comparison of baseline MEP and MEP after applying priming-test protocols. ■, effect size for one trial; horizontal line: 95% confidence interval; ♦, pooled effect size for all trials.

Table 4: Inhibitory priming and excitatory test protocols.

Study	n	Priming protocol (tDCS or TMS)	Expected effect of priming protocol	Test protocol (TMS)	Expected effect of test protocol	Overall effect (priming+test protocols)
Moloney and Witney, 2014	15	c-tDCS: 1 mA, 10 min, 25 cm^2	↓ MEP	rTMS: 5 Hz, RMT_{90}	↑ MEP	↑ MEP
Lang et al., 2004	10	c-tDCS: 1 mA, 10 min, 35 cm^2	↓ MEP	rTMS: 5 Hz, AMT_{100}	↑ MEP	↑ MEP
Nitsche et al., 2007	12	c-tDCS: 1 mA, 7 min, 35 cm^2	↓ MEP	PAS_{25}: 7 min	↑ MEP	↑ MEP
Cosentino et al., 2012	12	c-tDCS: 1.5 mA, 15 min, 35 cm^2	↓ MEP	rTMS: 5 Hz, RMT_{120}	↑ MEP	↑ MEP
Delvendahl et al., 2010	10	rTMS: 0.1 Hz, RMT_{80}	↓ MEP	PAS_{25}: 15 min	↑ MEP	↑ MEP
Huang et al., 2010	8	cTBS: 5 Hz, AMT_{80}	↓ MEP	$iTBS_{150}$: 5 Hz, AMT_{80}	↑ MEP	↑ MEP
Player et al., 2012	10	cTBS: 5 Hz, AMT_{80}	↓ MEP	iTBS: 5 Hz, AMT_{80}	↑ MEP	↑ MEP
Murakami et al., 2012	9	cTBS: 5 Hz, AMT_{80}	↓ MEP	iTBS: 5 Hz, AMT_{80}	↑ MEP	↑ MEP
Mastroeni et al., 2013	29	cTBS: 5 Hz, AMT_{80}	↓ MEP	iTBS: 5 Hz, AMT_{80}	↑ MEP	↑ MEP
Hamada et al., 2008	6	QPS-50 ms: 10 min, AMT_{90}	↓ MEP	QPS-10 ms: 30 min	↑ MEP	↑ MEP
Opie et al., 2017b	15	cTBS: 5 Hz, AMT_{80}	↓ MEP	iTBS: 5 Hz, AMT_{80}	↑ MEP	↑ MEP
Player et al., 2012	10	cTBS: 5 Hz, AMT_{80}	↓ MEP	PAS_{25}: 0.25 Hz, 13 min	↑ MEP	↑ MEP
Opie et al., 2017a	16	PAS_{LTD}	↓ MEP	PAS_{LTP}	↑ MEP	↑ MEP
Huang et al., 2010	8	cTBS: 5 Hz, AMT_{80}	↓ MEP	$iTBS_{150}$: 5 Hz, AMT_{80}	↑ MEP	↑ MEP

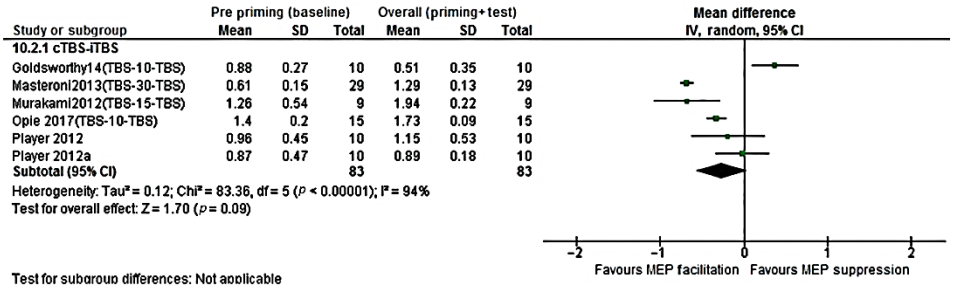

Figure 9: Forest plot of the effects of inhibitory priming-excitatory test protocols: TMS as the priming technique.
Comparison of baseline MEP and MEP after applying priming-test protocols. ■,effect size for one trial; horizontal line: 95% confidence interval; ♦, pooled effect size for all trials.

Meta-analysis of the included studies using cTBS followed by iTBS showed that the priming significantly increased the expected excitatory effect of the test protocol ($p = 0.002$; Murakami et al., 2012; Player et al., 2012; Mastroeni et al., 2013; Opie et al., 2017a).

Analysis of the other three studies in this category showed that the excitatory effect of the PAS was increased regardless of the priming type (cTBS, rTMS, or PAS; Delvendahl et al., 2010; Ni et al., 2014; Opie et al., 2017b). Moreover, a study using a paired QPS protocol showed that the priming increased the excitatory effect of the test protocol (Hamada et al., 2008). In addition, Huang et al. (2010) showed that a priming cTBS protocol suppressed the excitatory effect of the $iTBS_{150}$ as the test protocol (Huang et al., 2010). Due to the uniqueness of their priming-test protocol, the findings of these studies were not pooled with other studies in the meta-analysis but are shown in Table 4.

Discussion

In this systematic review and meta-analysis, we aimed to investigate how different NIBS priming influenced the

effects of test protocols in healthy individuals. Results show that, although metaplasticity mechanisms, either homeostatic or nonhomeostatic, play crucial roles in stabilizing neuronal activity within a physiologic range, these are all adjusted according to the modification of an LTP/LTD-like threshold. Different nature and durations of each priming and test protocol and the interval between them influence the overall effect, which is determined based on this threshold. It is also necessary to keep in mind that this threshold is not fixed but is displaced bidirectionally toward inhibition or facilitation. Indeed, any increase in CSE threshold in one direction is followed by a decrease of threshold in the opposite direction. This displacement occurs in response to the history of synaptic activity to prevent excessive increase or decrease in neuronal activity. This means that if the neuronal activity is historically high or low but does not reach the threshold after the priming, there is still the possibility that the test protocol will achieve the expected effect but not to the same extent as when there is no history of prior stimulation. This happens because the threshold for the same effect increases and needs stronger stimulation than when no priming exists. However, if historical neuronal activity reaches this threshold, the effect of the test protocol will decrease or reverse. Indeed, the history of the neuronal activity affects the threshold displacement, which in turn determines the mechanism that should be activated to stabilize the neuronal activity within the physiologic range.

In the following sections, we discuss four different priming-test protocols and their effects on the expected effect of the test protocol.

Excitatory priming-test protocols

Excitatory NIBS protocols primarily increase CSE, which is evidenced by increased amplitude of TMS-induced MEPs. Twenty studies included in this section were split into two groups based on their priming protocols, including a-tDCS and TMS (Muller et al., 2007; Gentner et al., 2008; Hamada et al., 2008; Potter-Nerger et al., 2009; Gamboa et al., 2010, 2011; Huang et al., 2010; Iezzi et al., 2011; Murakami et al., 2012; Mastroeni et al., 2013; Muller-Dahlhaus et al., 2015; Opie et al., 2017a,b).

a-tDCS as the priming technique

Seven studies involved a-tDCS as the priming protocol followed by another a-tDCS or an rTMS protocol.

Paired a-tDCS protocols

The results of a meta-analysis in this group showed that the application of paired a-tDCS with or without inter-tDCS interval increased the excitatory effect of an a-tDCS protocol in healthy individuals (Fricke et al., 2010; Jaberzadeh et al., 2013; Monte-Silva et al., 2013; Bastani and Jaberzadeh, 2014). Of four included studies, two used paired a-tDCS without an inter-tDCS interval, including the application of two successive 5-min a-tDCS with 0-min interval (5-0-5; Fricke et al., 2010) and application of two successive 10-min a-tDCS with 0-min interval (10-0-10; Jaberzadeh et al., 2013). In both studies, the priming protocol increased the excitatory effect of the test protocol. This finding conflicts with homeostatic metaplasticity mechanisms proposed in the two previous reviews on this topic (Karabanov et al., 2015; Müller-Dahlhaus and Ziemann, 2015). In these reviews, it was claimed that doubling the duration of NIBS application would reverse the effect of the test protocol due to homeostatic metaplasticity. In the current study, excitatory priming makes a history of high synaptic activity displace the modification threshold and facilitate an LTD-like effect (Bienenstock et al., 1982). However, studies by Fricke et al. (2010) and Jaberzadeh et al. (2013) have shown that the excitatory effects of the priming-test protocols do not reach the threshold; therefore, the excitatory effect was increased beyond that seen in individual applications of priming or test protocols. This effect could be explained by nonhomeostatic mechanisms. On the contrary, two successive 13-min a-tDCS with no inter-tDCS interval (13-0-13; Monte-Silva et al., 2013) reversed the overall effect toward inhibition. Indeed, in that study, the first 13-min tDCS provided sufficient history of high neuronal activity to reach the threshold for homeostatic mechanisms, increased induction of LTD-like effect, and therefore suppressed the expected effect of the test protocol to inhibition.

In the other three studies in this group, the priming and test protocols were separated by an interval. This included the application of two successive 5-min a-tDCS with a 3-min interval (5-3-5; Fricke et al., 2010), application of two successive 10-min a-tDCS with a 5-min interval (10-5-10; Bastani and Jaberzadeh, 2014), and application of two successive 13-min a-tDCS with a 3-min interval (13-3-13; Monte-Silva et al., 2013). When priming a-tDCS is applied for 5 min, the after-effects last about 5 min; therefore, if the test a-tDCS is applied with an inter-tDCS interval of 5 min (5-3-5), the expected effect of the test protocol is suppressed (Figure 10A). The likely mechanism behind this finding is that the excitatory effect of the priming protocol reaches a maximum in about 2.5 or 3 min, which

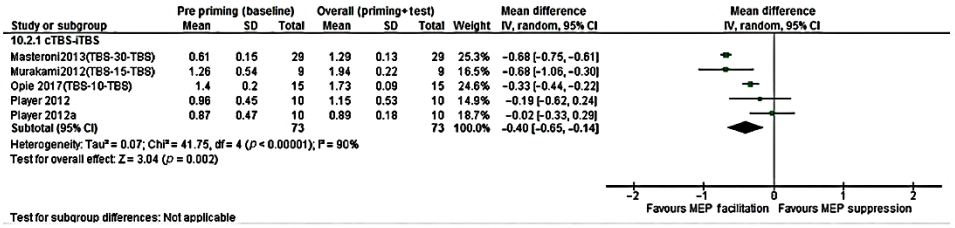

Figure 10: Hypothetical diagram of 'critical time window' of paired tDCS protocols.
(A) The expected effect of the test protocol is prolonged if it is applied at the first one-third of the expected after-effect of the priming protocol. (B) The expected effect of test protocol is reversed if it is applied at the middle one-third of the expected after-effect of priming. It is supposed that the after-effect would be almost at its maximum in this period. (C) The expected effect of test protocol is prolonged if it is applied at the last one-third of the expected after-effect of the priming protocol.

is the 'critical time window' (Fricke et al., 2010), and the effect of the following test protocol decreases or even reverses. However, in 10-5-10 and 13-3-13 protocols, the priming a-tDCS only increased the expected excitatory effect of the test protocol and there was no reversal of the effects. Therefore, it is likely that nonhomeostatic mechanisms were involved in the prolongation of the LTP-like effects. The after-effects of 10- and 13-min a-tDCS lasted about 30 and 60 min, respectively. It can be concluded that this critical time window is located in the middle third of the expected after-effect. Therefore, this 3-min interval between the priming-test protocols was at the aforementioned critical time window and the excitatory effect of the test a-tDCS was suppressed.

In the long-spaced a-tDCS applications, the priming protocols increased the expected excitatory effect of the test protocol and no suppression of the effects occurred. Bastani and Jaberzadeh (2014) applied two successive 10-min a-tDCS with a 25-min interval (10-25-10) to healthy individuals, whereas Fricke et al. (2010) applied two 5-min a-tDCS with a 20-min interval (5-20-5). In both of these studies, the test protocols started outside the abovementioned critical time window (Figure 10C). However, in the study of Monte-Silva et al. (2013), in which a 13-min a-tDCS was followed by another 13-min a-tDCS after a 20-min interval, the excitatory effect of the second a-tDCS application was suppressed (Figure 10B). In this protocol (13-20-13), the test protocol started at the critical middle third of the expected after-effect of the priming application (Monte-Silva et al., 2013).

a-tDCS-rTMS protocols

The findings of our meta-analysis of studies in this category showed that priming a-tDCS protocol suppressed the effect of the test rTMS. In two included studies, when an excitatory rTMS protocol followed a priming a-tDCS protocol with or without an interval, the effect was suppressed (Lang et al., 2004; Cosentino et al., 2012). This result was regulated by homeostatic metaplasticity in which the priming protocol increased historical neural activity, displaced the threshold, and suppressed the excitatory effect of the rTMS and reversed it to inhibition.

TMS as the priming technique

Among 20 studies in this group, 12 used paired iTBS or PAS techniques as the priming-test protocols and their data were pooled in a meta-analysis.

Paired iTBS protocols

Meta-analysis of seven studies demonstrated that the priming iTBS did not suppress the excitatory effect of the test iTBS regardless of the inter-TBS intervals. In three studies in which iTBS was applied with no inter-TBS interval, the priming protocol suppressed the excitatory effect of the test protocol to inhibition (Gentner et al., 2008; Gamboa et al., 2010). Homeostatic mechanisms were considered to regulate this result as the priming protocol increases the historical neuronal activity and displaces the threshold for synaptic changes. In the other four studies, paired iTBS protocols were successively applied with different inter-TBS intervals of 10 min (Opie et al., 2017a), 20 min (Gamboa et al., 2011), and 25 min (Mastroeni et al., 2013). In these studies, the priming iTBS protocol increased the effect of the test iTBS. As the priming after-effects did not reach and displace the threshold, this result was regulated by activating nonhomeostatic

mechanisms. Finally, in another study, the test protocol was applied 15 min after the priming protocol and the expected effect of the test protocol was suppressed as the threshold displaced (Murakami et al., 2012). This result could be explained as the fact that the duration of the effect of iTBS is about 30 min (Huang et al., 2010). Therefore, the application of the test protocol after a 15-min interval would occur at the critical time window, in which historical neuronal activity reached the threshold and activated homeostatic mechanisms.

Paired PAS protocols

Meta-analysis of three studies showed that the priming PAS enhances the excitatory effect of the test PAS regardless of the interval duration (Muller et al., 2007; Muller-Dahlhaus et al., 2015; Opie et al., 2017b). This could be explained by the fact that the PAS after-effect lasts for about 30 min (Stefan et al., 2000). Therefore, test protocols starting 10 or 30 min after the priming protocols may occur before or after the critical time window. After a 10-min interval, the after-effects may be below the critical level, and after 30 min, it seems that the start of the test protocol occurs after the critical window. Therefore, the effect of the test protocol was only enhanced by the priming protocol. This result was regulated by activating nonhomeostatic mechanisms that prolonged the excitatory effect of the test protocol.

Inhibitory priming-test protocols

Inhibitory NIBS protocols commonly affect CSE, as evidenced by decreased TMS-induced MEPs. Fifteen studies were divided into two groups based on the priming protocol, including c-tDCS and TMS (Siebner et al., 2004; Potter-Nerger et al., 2009; Delvendahl et al., 2010; Fricke et al., 2010; Gamboa et al., 2010; Goldsworthy et al., 2012; Murakami et al., 2012; Mastroeni et al., 2013; Ni et al., 2014).

Paired c-tDCS protocols

Meta-analysis of two studies showed that using paired c-tDCS, regardless of the interval, means the priming boosted the inhibitory effect of the following test protocol but not significantly (Fricke et al., 2010; Monte-Silva et al., 2010). According to these studies, when two successive c-tDCS are applied with no or a 20-min inter-tDCS interval, the priming protocol boosted the inhibitory effect of the test protocol. It seems that, in the no interval protocols, including two successive 5-min c-tDCS with no interval (5-0-5; Fricke et al., 2010) and two successive 9-min c-tDCS with a 9-min interval (9-0-9; Monte-Silva et al., 2010), the history of neuronal activity did not reach the modification threshold. In addition, in studies with 20-min intervals between the priming and test c-tDCS protocols, including the studies with two successive 5-min (5-20-5; Fricke et al., 2010) and two successive 9-min (9-20-9) applications (Monte-Silva et al., 2010), the after-effects of the priming were likely to be too weak to displace the threshold. In these cases, the overall effect was not reversed and largely prolonged the expected inhibitory effect of the test protocol (Figure 10C). Based on the metaplasticity concept, this prolongation of the expected effect was probably regulated by nonhomeostatic mechanisms (Karabanov et al., 2015; Müller-Dahlhaus and Ziemann, 2015). Although the expected inhibitory effect was increased when two successive 9-min c-tDCS had a 3-min inter-tDCS interval (9-3-9; Monte-Silva et al., 2010), the enhancement was not comparable to that resulting from the (9-20-9) protocols (Figure 10A). The probable reason behind this difference is the critical time window: for a 3-min inter-tDCS interval the test protocol starts when the after effect is increasing, but in 20-min interval the test protocol starts when the after-effect seems to be almost gone. However, in two successive 5-min tDCS with a 3-min interval (5-3-5; Fricke et al., 2010), the effect of the test was suppressed by the priming. In this study, the priming after-effects lasted 5 min. It is supposed that the test protocol applied at the middle of the critical time window of the priming after-effect suppressed the expected effect to the excitatory effect (Figure 10B). This expected effect suppression seems to be the result of homeostatic mechanisms.

c-tDCS-rTMS protocols

Meta-analysis of the two studies using c-tDCS-rTMS protocols showed that the priming reversed the inhibitory effect of the rTMS, although it was not significant (Siebner et al., 2004; Moloney and Witney, 2014). This could be explained by the fact that the priming protocol lowered the neuronal activity, hit the threshold, and displaced it toward the induction of LTP-like effects. This reversal of the expected effects was regulated by the activation of homeostatic mechanisms.

Paired cTBS protocols

The result of eight studies pooled in meta-analysis showed that in the paired cTBS protocols, regardless of the

inter-TBS interval, the priming did not reverse the inhibitory effect of the test protocol. In the study of Gamboa et al. (2010), it was shown that using paired cTBS with no inter-TBS interval reversed the effect of the test protocol as the threshold was displaced in favor of the LTP-like effect induction. It seems that there is a critical time window during the after-effect of the priming in which the effect is at its maximum, which seems to cover the middle third of the approximately 60-min lasting after-effects of cTBS (Figure 8; Huang et al., 2005). Based on this assumption, with inter-TBS intervals of 2, 10, 15, and 20 min, the priming boosted the inhibitory effect of the test protocol as its after-effects were insufficient to displace the threshold toward LTP-like induction (Huang et al., 2010; Gamboa et al., 2011; Murakami et al., 2012; Goldsworthy et al., 2013, 2014). It seems that nonhomeostatic mechanisms are activated to prolong the effect of the test protocol. However, in another study that employed a 10-min inter-TBS interval, the inhibitory effect was suppressed and reversed to the excitatory effect (Goldsworthy et al., 2012). The probable reason behind this difference is methodologic differences between the studies. In the study of Goldsworthy et al. (2012), the authors used the resting motor threshold, whereas the other TBS studies used the active motor threshold. In other words, muscle contraction before the priming could also have affected the neuronal history and decreased the threshold toward inhibition. Therefore, the application of the inhibitory priming protocol lowers the historical neuronal activity and increases the threshold for the induction of LTD-like effects to prevent more inhibition. Therefore, the inhibitory effect of the test protocol decreased. In contrast, in another study, the expected effect of the test cTBS was applied 30 min after the priming was reversed. It seems that the test protocol started during the critical time window of 60-min duration of cTBS after-effects, hit the threshold, and displaced it (Mastroeni et al., 2013). Homeostatic mechanisms regulated the reversal of the expected inhibition toward facilitation.

(rTMS-PAS) and (cTBS-PAS) protocols

The result of three studies included in the meta-analysis showed that the inhibitory effect of PAS protocol was suppressed significantly regardless of the priming technique (Potter-Nerger et al., 2009; Delvendahl et al., 2010; Ni et al., 2014). It seems that the neuronal history formed by the priming reached the threshold and displaced it toward LTP-like effect induction. The reversal of the effect is regulated by homeostatic mechanisms.

Excitatory priming and inhibitory test protocols

Eight studies used different priming techniques, including a-tDCS and TMS.

a-tDCS-rTMS protocols

Meta-analysis of two studies showed that using a-tDCS did not enhance the inhibitory effect of rTMS significantly (Siebner et al., 2004; Moloney and Witney, 2014). Although the result was not significant, it seems that a-tDCS decreases the threshold for further induction of the inhibitory effect and the overall effect was in favor of suppression. The homeostatic mechanisms were activated to regulate this inhibitory enhancement.

(rTMS-cTBS) and paired rTMS protocols

Meta-analysis of three studies showed that rTMS, regardless of the type of test protocol, enhanced the inhibitory effect of the following cTBS or rTMS test protocols significantly (Iyer et al., 2003; Todd et al., 2009; Iezzi et al., 2011). Indeed, the history of high synaptic activity lowered the threshold in favor of the LTD-like effects. Therefore, the priming protocol intensifies the inhibitory effect of the test protocol compared to a single inhibitory protocol. This prolongation of the effects is likely to be regulated by homeostatic mechanisms.

iTBS-cTBS protocols

Meta-analysis of three studies showed that the priming iTBS protocols did not enhance the effect of the test cTBS significantly (Todd et al., 2009; Doeltgen and Ridding, 2011; Murakami et al., 2012). Although the result was not significant, the excitatory priming protocol lowered the threshold for further inhibition and the overall effect was in favor of inhibition. This expected inhibitory enhancement was regulated by homeostatic mechanisms.

Inhibitory priming and excitatory test protocols

Six studies used different priming techniques, including c-tDCS and TMS.

c-tDCS-rTMS protocols

Meta-analysis of two studies showed that using c-tDCS did not increase the excitatory effect of rTMS significantly (Lang et al., 2004; Cosentino et al., 2012). Nonetheless, c-tDCS decreases the threshold for the induction of further excitatory effect and the overall effect was in favor of facilitation. The homeostatic mechanisms were activated to regulate this expected excitatory intensification.

cTBS-iTBS protocols

Meta-analysis of four studies showed that inhibitory priming protocols intensified the excitatory effects of the test iTBS protocol significantly (Murakami et al., 2012; Player et al., 2012; Mastroeni et al., 2013; Opie et al., 2017a). It seems that the inhibitory priming decreased the historical neuronal activity, displacing the modification threshold in favor of LTP-like effects and intensified the excitatory effect of the test protocols. This intensification of the excitatory effect was regulated by homeostatic mechanisms.

Limitations

The findings of this systematic review and meta-analysis should be interpreted in the context of some limitations. First, the small sample sizes in some included studies were associated with larger effect sizes that may have affected overall results and statistical significance. Second, the literature was limited to articles written in English. Third, all included studies were performed with healthy individuals, making it impossible to generalize these findings to nonhealthy individuals. Finally, both genders were investigated in these studies, limiting the possibility of generalizing the results on a gender-specific basis.

Suggestions for future research

It is important to determine the role of gender in metaplasticity mechanisms and effects in future studies. Furthermore, it is crucial to investigate priming-test protocols in nonhealthy individuals, especially those with neurologic disorders. Further studies should be organized to find how the priming-test protocol could play an effective role in clinical procedures.

Conclusions

This meta-analysis indicates that the characteristics of the priming-test protocols are critically important for the induction of the overall effect by affecting the modification threshold zone. Moreover, it shows that the history of synaptic neuronal activity is a crucial factor in determining the threshold zone. A history of high synaptic activity is in favor of inhibition (LTD-like effect), whereas a history of low synaptic activity is in favor of facilitation (LTP-like effect). Another key factor that should be kept in mind is the concept of the critical time window. Findings suggest that this time window is located in the middle part of the duration of the effect after the priming protocol. If a test protocol starts within this time window, the threshold may be the displaced toward LTP/LTD-like effects. These effects are mediated by different metaplasticity mechanisms.

Therefore, if a priming protocol is followed by a test protocol that induces similar effects, it would lead to the reversion of the effects of the test protocol if it achieves the sliding threshold or induce LTP/LTD-like prolongation if it does not reach the threshold. This prolongation is mediated by nonhomeostatic mechanisms. On the contrary, opposite priming and test protocols would boost the expected effect of the test protocol, making it more effective, again using homeostatic mechanisms.

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Preamble to Chapter 4

Transcranial magnetic stimulation (TMS) is a high-use technique for investigating the changes in corticospinal excitability (CSE). TMS- induced motor evoked potentials (MEPs) are shown to have high variable findings. Therefore, this becomes crucial to evaluate the inherent reliability of the TMS-induced MEP amplitude as an index of CSE.

This chapter addresses **Aim 3** in this thesis **A:** to investigate the effects of TMS ITIs (5, 10, 15, and 20s) on intra- and inter-session reliability of MEP amplitude, and **B:** to explore how different ITIs would affect the variability of TMS induced MEPs.

The findings confirm the MEP reliability through high intra-class correlation (ICC) and agreement (inter-and intra-sessions) for all used ITIs. It is also found that longer ITI up to 15s can significantly induce larger MEPs with lower variability and higher reliability.

Chapter 4 examines the intra- and inter-reliability of recording peak-to-peak MEPs which is an index for CSE as well as amplitude and variability. Any application of transcranial current stimulation involves the measurement of changes before and after the intervention. Therefore, this reliability study makes sure that the changes following anodal tDCS in chapters 5 and 6 are not due to systematic errors and methodological inconsistencies.

Chapter 4. Longer TMS inter-trial interval increases size, reduces variability, and improves the reliability of the motor evoked potentials

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The Ethics approval, consent form, system set-up used in this study, TMS safety, and Edinburg handedness questionnaires are provided in Appendices 1 - 7.

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Longer Transcranial Magnetic Stimulation Intertrial Interval Increases Size, Reduces Variability, and Improves the Reliability of Motor Evoked Potentials

Maryam Hassanzahraee,[1] Maryam Zoghi,[2] and Shapour Jaberzadeh[1]

Abstract

High rates of variability in the amplitude of transcranial magnetic stimulation (TMS)-induced motor evoked potentials (MEPs), a popular method for assessing corticospinal excitability (CSE), make it essential to examine inherent reliability of the MEP amplitude. We aimed to investigate the effects of different intertrial intervals (ITIs) of single-pulse TMS on the amplitude, variability, and test-retest reliability of MEPs. Twenty-five TMS single pulses were recorded at four different ITIs of 5, 10, 15, and 20 sec from 15 healthy participants who attended two experimental sessions. Repeated measures analysis of variance (rmANOVA) and standardized *z*-value standard deviations (SDs) were used to investigate the effects of ITIs on MEP amplitudes and variability. Test-retest reliability of MEP amplitudes was also assessed using rmANOVA and intraclass correlation (ICC). rmANOVA revealed significantly larger MEP amplitudes following ITIs of 10, 15, and 20 sec compared with ITI 5, with no significant increases between ITIs of 15 and 20 sec. Standardized *z*-value SDs revealed variability rate reduction following longer ITIs with significant reductions occurring following ITIs of 10, 15, and 20 sec compared with ITI 5 with no significant difference between ITIs of 15 and 20 sec. rmANOVA showed no significant Time main effect on the MEP changes confirming within- and between-session agreement. ICCs reported significant within- and between-session reliability in all selected ITIs. The findings of the current study indicate that longer ITIs up to 15 sec can significantly induce larger MEPs with lower variability and higher reliability. The increase in ITIs not only reduces the chance of TMS-induced changes in CSE but also helps us to use this assessment tool in studies with smaller sample sizes.

Keywords: corticospinal excitability; inter-trial-interval; motor evoked potential; reliability; single pulse; transcranial magnetic stimulation

Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique. It can be used to investigate the integrity and excitability of the corticospinal pathways for different muscles in the human primary motor cortex (M1) (Barker et al., 1985). Application of suprathreshold TMS pulses over M1 induces motor responses, which can be recorded by surface electromyography (EMG) electrodes from the target muscle. These responses are known as motor evoked potentials (MEPs). The peak-to-peak MEP amplitude is used as an index of corticospinal excitability (CSE) changes (DiLazzaro et al., 2004; Priori et al., 1998). Larger MEPs indicate higher levels of CSE (Nitsche and Paulus, 2000). It is generally believed that single-pulse TMS (spTMS), as an assessment tool that is applied at a defined intertrial interval (ITI), does not change the CSE by itself (Kiers et al., 1993; Pell et al., 2011).

Two common characteristics of the recorded MEPs are amplitude and latency. Amplitude represents the net excitatory and inhibitory changes on corticospinal pathways (Kamen, 2004). It has been shown that changes in MEP amplitudes could exhibit physiological and pathological changes in the corticospinal tract and intracortical circuits (Chen et al., 2008). MEP latency, on the other hand, is an indication of the time needed for the pulse to reach the targeted muscle throughout the pathway. Latency is relatively more stable compared with the highly variable MEP amplitude (Kiers et al., 1993).

[1]Noninvasive Brain Stimulation and Neuroplasticity Laboratory, Department of Physiotherapy, School of Primary and Allied Health Care, Faculty of Medicine, Nursing and Health Science, Monash University, Melbourne, Australia.
[2]Department of Rehabilitation, Nutrition and Sport, School of Allied Health, La Trobe University, Melbourne, Australia.

While TMS has been used for several decades as a reliable measurement tool (Kamen, 2004; Nitsche and Paulus, 2000), some of its fundamental methodological principles have not been sufficiently analyzed. A measurement tool should be reliable and valid, producing accurate and meaningful data (Portney and Watkins, 2015). Reliability refers to similar results of repeated measures in the same individuals. It is a degree to which repeated measurements provide similar results over time (de Vet et al., 2006). TMS, as a reliable measurement tool, must induce comparable MEPs at different testing sessions over time in the absence of an intervention (Christie et al., 2007; Lexell and Downham, 2005). Different physiological and technical factors, including muscle activity, attention, stimulation intensity, waveform, coil placement, and ITI, could affect MEP amplitudes and contribute to variability in CSE measurements (Ridding and Ziemann, 2010). These confounding variables could directly reduce the reliability of CSE measurements (Wassermann, 2002). Therefore, there is always the risk of whether CSE changes are due to real neurophysiological changes induced by an intervention or the result of one or more of the abovementioned factors (Schambra et al., 2015). Thus, these confounding factors may violate common assumptions about the effects of spTMS, delivered in sequence, on CSE as a highly reliable and less variable tool. It has previously been reported that TMS with longer ITIs induced MEPs with higher stability and lower variability, reflected in the input–output curve (Moller et al., 2009). Later, it was explained that despite a linear correlation between MEP and ITI, there was no significant difference between the fixed and random ITI range (<10 sec) (Julkunen et al., 2012; Pellicciari et al., 2016). While an increasing number of studies in this field confirms the importance of the research on the effect of ITIs on the MEP amplitude, there are several shortcomings in this area that have not been systematically investigated. Majority of the studies on this topic only investigated the effects of two ITIs (fixed or random range), mainly focused on the conventional ITI (<10 sec) (Cuypers et al., 2014; Julkunen et al., 2012; Moller et al., 2009; Pellicciari et al., 2016; Stamoulis et al., 2011; Vaseghi et al., 2015). Because it takes about 12 sec for the brain perfusion to return to baseline (Thomson et al., 2012), finding and comparing the effects of ITIs higher and lower than 12 sec on CSE changes seem to be essential. The current study not only tried to find a response to this gap in the literature but also aimed to investigate the MEP variability and reliability following different ITIs systematically. It should be noted that in this study, we controlled the abovementioned physiological and technical factors (Ridding and Ziemann, 2010) to reduce their potential effects on CSE changes.

In summary, the main aim was to compare the effects of four ITIs (5, 10, 15, and 20 sec) on size, variability, and reliability of MEP amplitudes. There were three main hypotheses in this study: (1) longer ITIs would induce larger MEP amplitudes; (2) MEP amplitude variability is lower at longer ITIs; and (3) longer ITIs would induce higher within- and between-session reliability.

Materials and Methods

Participants

Fifteen participants (9 females) aged 19–40 years (mean ± standard deviation [SD]: 24.06 ± 5.37) participated in this study. All participants were right-handed according to the Edinburgh handedness questionnaire (Oldfield, 1971) and screened for any contraindication to TMS, including the history of medical, neurological, psychiatric, or psychological disorders (Keel et al., 2001; Rossi et al., 2009). All participants gave a written consent form before experimental sessions. Each participant attended two main testing sessions with at least a 48-h between-session interval. The study was conducted according to the Declaration of Helsinki and approved by the Human Research Ethics Committee at Monash University.

Electromyography

Surface EMG was recorded from the right first dorsal interosseous (FDI) muscle using a pair of Ag-AgCl electrodes taped with a 2-cm distance in belly-tendon orientation. A ground electrode was placed over the ulnar styloid process at the wrist. The skin of FDI was gently abraded and then cleaned to reduce electrode–skin impedance and improve the recorded EMG responses (Gilmore and Meyers, 1983). Signals with a sampling rate at 1000 Hz were amplified (×1000) before being band-pass filtered (10–500 Hz) (PowerLab; ADInstruments, Australia) and stored using LabChart™ software (ADInstruments) in a laboratory computer for further off-line analysis. During the experiments, complete muscle relaxation was controlled by visual EMG feedback, and participants were instructed to relax their hand (FDI) when necessary.

TMS procedure

Participants were comfortably seated in an adjustable chair with the right forearm and hand resting on the armrest and did not wear earplugs. TMS was performed using a 70-mm figure-of-eight coil (MagVenture, Farum, Denmark) with a biphasic current waveform. The TMS coil was held tangentially to the skull with the handle pointed backward and 45° away from the sagittal plane (Brasil-Neto et al., 1992; Kaneko et al., 1996; Mills et al., 1992). The optimal coil positioning on M1, hot spot, was identified for induction of the largest MEP amplitudes in the relaxed right FDI muscle. The vertex (Cz) location was measured using the international electroencephalography (EEG) 10–20 system and marked to be used as a reference (Schwartz and Andrasik, 2017). Then, an FDI hotspot was marked on the scalp with a soft-tipped marker by moving 5 cm lateral (toward the left external auditory meatus) and slightly anterior to Cz. These markings ensured the reproducible positioning of the TMS coil throughout the experimental sessions. This process was repeated in both testing sessions. Resting motor threshold (rMT) at the FDI hot spot was obtained using parameter estimation by the sequential testing (PEST) protocol. This protocol was followed using the TMS Motor Threshold Assessment Tool (Freeware, MTAT 2.0; Awiszus and Borckardt, 2011). The program started with a TMS intensity of 37% of stimulator output. The intensity for the next TMS trial was determined based on rater interaction with the software. Indeed, the rater indicates whether the trial was successful by clicking on the Y key or not (click on the N key). It was a success if MEP amplitudes ≥0.05 μV and then a new intensity displayed for delivery. The protocol stopped after 20 stimuli, which provide sufficient accuracy for rMT estimates within limits imposed by safety guidelines (Awiszus, 2011; Rossi et al.,

2009). As the second session of assessment was not carried out in more than 48 h from the first session, the traces of markings still remained on the scalp. However, all measurements for identification of the hot spot were repeated to make sure that the marked hot spot induced the largest MEP amplitude compared with nearby points. The consistency for the hot spot was also confirmed with no significant difference between mean rMT values of the two sessions. Moreover, unlike a previous study (Bastani and Jaberzadeh, 2012), which was designed based on a combined hot spot for FDI and extensor carpi radialis (ECR) muscles, the current study used the exact hot spot for FDI muscle. Based on the study by Ridding and Ziemann (2010), it is necessary to control all confounding factors, including attention as it could contaminate the real changes following TMS measurements. For example, if participants even think about hand movements, based on the concept of mirror neurons, it may affect the MEP amplitude. Therefore, participants were asked to count the number of stimuli they received to minimize the changes in their attention.

Experimental procedure

All participants attended (three sets of data collection) two experimental sessions. Session 1 included two sets of data collection (T1 and T2) separated by a 20-min rest, while session 2 only included one set of data collection (T3). The order of four ITIs was pseudo-randomized using computerized randomization software (Randomization.com). Each participant took part at the same time of the day to avoid diurnal variation and was blinded to the experimental procedure. At each session, after determination of rMT, TMS intensity was set at [rMT (% of stimulator output) $\times$ 1.2] to record 25 MEPs with the ITIs of 5, 10, 15, and 20 sec. Within-session reliability of recorded MEPs was calculated using data collected at T1 and T2, while between-session reliability was calculated using data at T1 and T3. Figure 1 summarizes the experimental design of the study.

Data analysis

SPSS 25 (IBM Corporation, NY) was used for data analysis. Peak-to-peak MEP amplitude was measured automatically using LabChart software (ADInstruments). Then, CSE changes were determined by averaging 25 MEPs at each time point (T1, T2, and T3) for each ITI. To measure MEP variability, SDs of mean standardized z-scores of 25 MEPs were used for each participant ($n = 15$), ITI ($n = 4$), and time point ($n = 3$). Normal distribution of MEP amplitudes and z-value SDs were tested using the Shapiro–Wilk test, and where required, log transformations of data were performed to correct the skewness of data.

ITI and MEP amplitude. A two-way repeated measures analysis of variance (rmANOVA) was conducted to test the effect of ITI and Time on log-transformed MEPs. To test sphericity, Mauchly's test was carried out, and in case of sphericity violation ($p < 0.05$), Greenhouse–Geisser correction was used. *Post hoc* analysis using Bonferroni correction was used to assess multiple comparisons wherever a significant main or interactive effect was revealed ($p = 0.05$).

ITI and MEP variability. A two-way rmANOVA was used to investigate the effect of ITI and Time on log-transformed, standardized z-score SDs. When a significant main or interaction effect was found, *post hoc* pairwise comparisons were performed using Bonferroni correction ($p = 0.05$).

ITI and MEP reliability. Within- and between-session reliability values of measured MEPs for each ITI were examined using the intraclass correlation (ICC) coefficient with 95% confidence interval (Portney and Watkins, 2015). A two-way mixed-effects model (ICC3, 1) was used to examine the consistency of estimates (Shrout and Fless, 1979). The reliability coefficient ranges from 0 to 1, with values closer to 1 representing stronger reliability. It has been suggested that coefficients were considered poor for ICC <0.4, fair for 0.4 ≤ ICC ≤0.58, good for 0.59 ≤ ICC ≤0.75, and excellent for ICC ≥0.75 (Cicchetti et al., 1981). ICC tests were used to assess agreement between repeated measurements for each ITI. The significance level was set at the level of 0.05.

Results

All participants completed both experimental sessions. Mean rMT was 36% of stimulator output (36.2 ± 5.3) for session 1 (T1 and T2) and 35% (34.6 ± 4.8) for session 2 (T3). As MEP amplitudes and z-value SD measurements did not follow normal distribution, log transformations were performed to correct data skewness. Figure 2 presents the MEP amplitude (raw and log-transformed), SDs, and reliability plots.

Hereafter, in all remaining sections, MEP(s) and SDs were used instead of their full-term of log-transformed MEP and long-transformed, standardized z-value SDs, respectively.

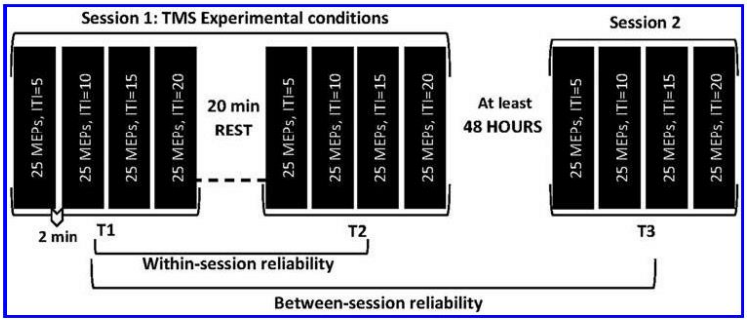

FIG. 1. Experimental design. Twenty-five TMS-induced MEPs were recorded at four selected ITIs. There were two sets of data collected at session 1 separated by a 20-min resting period. There was a 48-h interval between sessions 1 and 2. ITI, intertrial interval; MEP, motor evoked potential; TMS, transcranial magnetic stimulation.

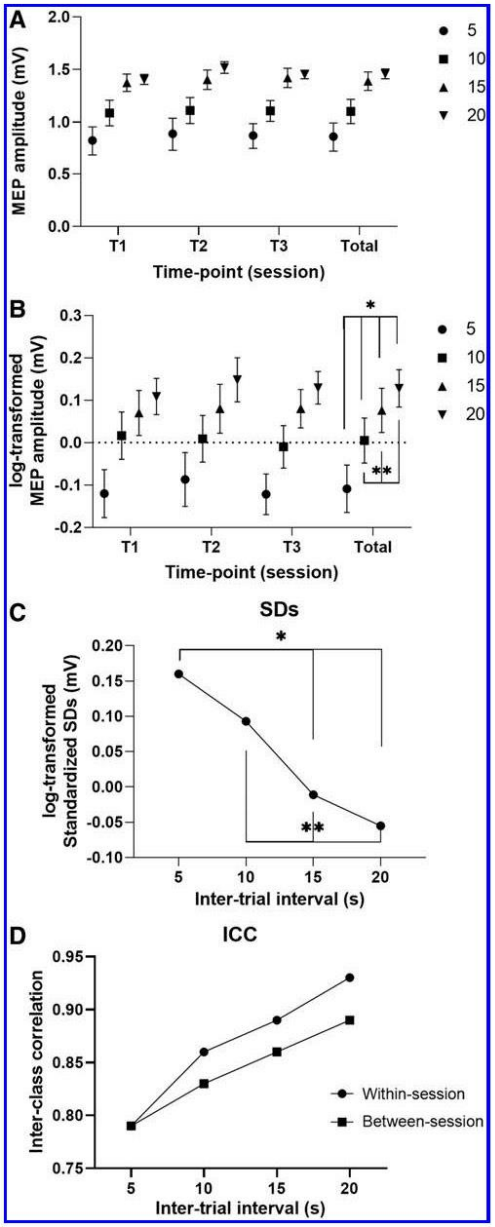

FIG. 2. Effects of ITIs on (**A**) MEP amplitude (raw data), (**B**) log-transformed MEP amplitude, (**C**) log-transformed standardized *z*-value SDs, and (**D**) within/between-session reliability. (Mean ± SEM) and error bars show SEM. *,**Significant difference in log-transformed MEP amplitudes and SDs between ITI 5 with 10, 15, and 20 sec and ITI 10 with 15 and 20 sec ($p < 0.05$). SD, standard deviation; SEM, standard error of mean.

ITI and MEP amplitude

Two-way rmANOVA showed a significant ITI main effect on MEPs ($F_{(3, 42)} = 12.39$, $p < 0.001$, $\eta_p^2 = 0.46$). Moreover, there was no significant Time main effect and ITI × Time interaction on MEPs ($F_{(2, 28)} = 0.13$ and $F_{(6, 84)} = 0.58$). *Post hoc* comparison using the Bonferroni correction indicated that mean MEP amplitude increased as ITI increased up to 15 sec. There was a significantly lower mean score of MEP amplitude at ITI 5 compared with 10, 15, and 20 sec ($p < 0.01$) and at ITI 10 with 15 and 20 sec ($p < 0.01$). However, there was no significant difference between the mean score of ITIs of 15 and 20 sec ($p = 0.25$; Fig. 2B).

ITI and MEP variability

rmANOVA revealed a significant ITI main effect on SDs ($F_{(3, 42)} = 14.27$, $p = 0.002$, $\eta_p^2 = 0.505$) (Fig. 2C). However, there was no significant Time main effect and ITI × Time interaction on SDs ($F_{(2, 28)} = 2.94$, and $F_{(6, 84)} = 0.33$). The *post hoc* comparison showed that while ITIs increased, SDs decreased. SDs did not reduce significantly from 0.16 at ITI 5 to 0.11 at ITI 10 ($p = 1.000$). Then, SDs significantly reduced to −0.012 at ITI 15 ($p = 0.003$). Further reduction was revealed at ITI 20, reducing to −0.055 ($p = 0.002$). However, there was no significant reduction following ITI 15 to 20 sec ($p = 0.491$; Fig. 2C).

ITI and MEP reliability

rmANOVA revealed no significant Time main effect on MEP amplitudes ($F_{(2, 28)} = 1.86$, $p = 0.189$, $\eta_p^2 = 0.11$). A comparison of mean MEP amplitude following different ITIs shows more consistency in MEP amplitudes following longer ITIs (10, 15, and 20 sec). The pairwise comparison did not show any significant difference in mean MEP values following measurements by the same rater on two different days; this reveals agreement of within- and between-session reliability and confirms intrarater reliability.

Within-session reliability. ICC tests showed significant within-session reliability for all ITIs at three time points. By increasing the ITI, reliability gradually improved from good correlation of 0.79 at 5 sec to excellent at 10 sec ($r = 0.86$), 15 sec ($r = 0.89$), and 20 sec ($r = 0.90$).

Between-session reliability. ICC tests showed high inter-session reliability with a good correlation of 0.79 at ITI 5 that increased to the excellent level of 0.83, 0.86, and 0.89 at 10, 15, and 20 sec, respectively.

Figure 2D shows within- and between-session reliability of MEPs using different ITIs.

Discussion

The current study was designed to systematically investigate the effect of ITIs on size, variability, and reliability of MEP amplitude. Our findings indicated that there is a positive linear correlation between the ITI and MEP amplitude. It was also found that variability significantly reduced, while MEP reliability increased, as the ITI was becoming longer. Therefore, all original hypotheses are strongly supported by the findings of this study.

ITI and MEP amplitude

We hypothesized that longer ITIs would induce larger MEP amplitudes. The current findings supported this hypothesis. Our findings were in line with the findings of different studies regardless of methodological differences (Julkunen et al., 2012; Moller et al., 2009; Pellicciari et al., 2016; Vaseghi et al., 2015). Based on previous studies, it was shown that MEP amplitudes increased when ITIs increased from 5 to 20 sec (Moller et al., 2009), 5 to 10 sec (Julkunen et al., 2012), and 4 to 10 sec (Vaseghi et al., 2015). This positive linear relationship between ITI and MEP amplitude revealed that the general assumption regarding the independency of spTMS pulse and ITI is not valid and can be violated under certain conditions, for example, using a shorter ITI, that is, 5 sec (Moller et al., 2009). It has been explained that each TMS pulse could change cerebral hemodynamics of the stimulated area by affecting cerebral vessels through vasoconstriction and reduction of blood flow. It has been shown that it takes about 8–10 sec for the cerebral blood flow to return to the prestimulation baseline state (Thomson et al., 2012). These hemodynamic changes would significantly reduce the Oxy-Hb (HbO) concentration, which takes about 15 sec to return to the baseline (Mochizuki et al., 2006). Indeed, these changes could explain smaller MEPs following shorter ITIs (5 and 10 sec) as there was not enough time (<12 sec) for cerebral hemodynamic changes to return to the baseline level before the next upcoming TMS pulse. In addition, no significant difference between ITIs of 15 and 20 sec on MEP amplitudes would indicate enough time between consecutive pulses (>12 sec) for cerebral changes to return to baseline (Furubayashi et al., 2013). Furthermore, it has also been shown that consecutive pulses could also have a cumulative effect on each other (Pellicciari et al., 2016; Stamoulis et al., 2011), affecting the size of MEP amplitudes. Indeed, each pulse provides a neuronal history for subsequent pulses that could cancel further enhancement in MEP amplitudes following shorter ITIs compared with longer ones. Moreover, this cumulative effect seems to diminish gradually as ITIs increased from 5 to 20 sec, reflecting in the larger size of MEP amplitudes.

ITI and MEP variability

We hypothesized that longer ITIs would induce less variable MEPs compared with the shorter ones. The current findings strongly supported this hypothesis. The results indicated an inverse relationship between the length of the ITI and MEP variability. Findings revealed that the SDs reduced when ITIs got longer from 5 to 20 sec. This finding is supported by several studies regardless of methodological differences (Julkunen et al., 2012; Moller et al., 2009; Pellicciari et al., 2016; Schmidt et al., 2009). It has been shown that compared with ITI 5, MEP variability decreased following ITI 20 (Moller et al., 2009). In another study, less consistency of MEP amplitudes has been confirmed following the use of a short ITI of 3 sec (Schmidt et al., 2009). Moreover, it has been indicated that ITI <10 sec would not necessarily guarantee the stabilization of MEP measurements because of the high variability following ITIs from 1 to 10 sec (Julkunen et al., 2012).

In addition, some studies revealed that MEP amplitude variability could be affected by the carryover effect of each pulse on subsequent pulses, mainly when shorter ITIs are used. It has been shown that spTMS could prime baseline neuronal activities and increase background excitability for subsequent pulses in ITIs ≤5 sec (Pellicciari et al., 2016). This change, therefore, highlights the cumulative effects of each pulse on consequent pulses, especially if the ITI is not long enough for neuronal hemodynamic changes to return to baseline (Furubayashi et al., 2013; Mochizuki et al., 2006; Nilsen, 1996; Schmidt et al., 2009). Therefore, this could increase MEP variability following shorter ITIs compared with longer ones.

Although literature indicates a number of possible mechanisms, including hemodynamic and neurophysiological mechanisms for regulation of changes at the stimulated area following different ITIs, there is still no consensus on the exact mechanisms behind variability changes in MEP amplitude.

ITI and MEP reliability

We hypothesized that the overall within- and between-session MEP reliability would increase following longer ITIs. This hypothesis was strongly supported by the findings in the current study.

Within-session reliability. The current results indicated a linear relationship between the MEP reliability and ITI up to 15 sec. There was good to high within-session reliability for each ITI; however, longer ITIs had a higher level of ICC and agreement. Within-session reliability values in this study are supported with findings of previous studies reporting good to high levels of ICC: 0.65–0.83, Christie and associates (2007); 0.79–0.97, Bastani and Jaberzadeh (2012); and 0.79–0.96, Vaseghi and associates (2015). On the other hand, our findings are negated by the results of Julkunen and associates (2012) as they revealed that MEP changes were not time invariant (Julkunen et al., 2012). The discrepancy may be related to the methodological differences between these studies. While in the current study, we did reliability measurements in different experimental sessions (method section), Julkunen and associates (2012) split 30 recorded MEPs into three blocks of 10 MEPs and did the within and between comparisons in these blocks to investigate the time invariance of MEP amplitudes.

Between-session reliability. The current findings on between-session reliability revealed high ICC values for all ITIs, with the highest level for 20 sec, which supported our hypothesis. Our findings on reliability values, including ICC and agreement, are supported by previous studies showing values ranging from 0.8 to 0.87 in the study by Bastani and Jaberzadeh (2012) and 0.84 to 0.97 in the study by Vaseghi and associates (2015).

Limitations

The findings in the current study should be interpreted considering the following limitations. First, the current sample size was low; therefore, a generalization of the findings to a larger population is hard. Further studies with larger sample sizes are required to ensure generalization of the findings to a larger population. Second, even though the current study showed that variability decreased following longer ITIs, it should be emphasized that the source of variability is multifactorial and

other factors such as age, gender, coil positioning, and attention level (Li et al., 2015; Ridding and Ziemann, 2010) should be considered in future studies. Moreover, the current study was done on young and healthy individuals. Therefore, the findings might not be extrapolated to other age groups and patients with different pathological conditions. Furthermore, while a number of studies suggested that the neuro-navigational systems provide more robust data compared with detection of hot spots using conventional methods (Sparing et al., 2008), other studies have shown that the intrasubject and intersubject variability remains high (Gugino et al., 2001; Julkunen et al., 2009; Jung et al., 2010) in the assessments using neuro-navigational systems. It should be noted that the current study utilized conventional methods for determination of the hot spot and therefore interpretation of data should be considered accordingly. Finally, the findings in this study are only applicable to the use of TMS as a measurement tool. Therefore, we cannot extrapolate these findings into the context of rTMS, which is mainly a therapeutic technique.

Future directions

In the current study, the outcome measure of interest was the MEP amplitude. Future studies should also check the effects of ITI on neurophysiological measures such as indices of intracortical excitation or inhibition, which will shed light on the underlying mechanisms behind changes in MEP amplitude. Due to the differential effects of hormones on males and females, the study of gender effects on the MEP size and their variability seems to be necessary. In addition, further investigations comparing different TMS intensities at both resting and active conditions are recommended. Furthermore, technical factors such as coil shape, coil direction, waveform, frequency, target muscle, and different intersession intervals may also affect the findings. Therefore, further studies are required to investigate the effects of these technical factors. As was also recommended previously (Stamoulis et al., 2011), longer ITIs seem to be safer than shorter ITIs (<10 s). Thus, in some clinical applications, it may be appropriate to use longer ITIs, especially when high numbers of pulses are required. Finally, due to dependency of the MEP amplitude on ITI, it seems to be useful to add the ITI as a modulatory criterion to the international checklist for application of investigational TMS.

Conclusions

The present study concluded that longer ITIs are associated with larger, less variable, and highly reliable MEP amplitudes. The findings confirm the superiority of longer ITIs for assessment of TMS-induced MEPs. The use of longer ITIs is more significant when a higher number of pulses are necessary for the assessment of corticospinal and corticocortical circuits. Indeed, longer ITIs would minimize the possible modulatory effects of TMS pulses on the effects of subsequent interventions. Therefore, it is highly recommended to report ITI in all TMS measurements.

Authors' Contributions

M.H.Z. and S.J. conceived and designed the study and interpreted the findings; M.H.Z. performed data collection, conducted the analysis, and wrote the manuscript; and M.H.Z., M.Z., and S.J edited the drafts.

Author Disclosure Statement

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Address correspondei
Maryam Hassanza
Non-invasive Brain Stimu
and Neuroplasticity Labo
Department of Physioth
School of Primary and Allied Health
Faculty of Me
Nursing and Health Science
Monash University
Melbourne 3800
Australia

E-mail: maryam.hassanzahraee@monash.edu

Preamble to Chapter 5

The application of anodal transcranial direct current stimulation (a-DCS) over the primary motor cortex (M1) is an established technique to enhance M1 excitability. However, recent studies challenge the linear relationship between stimulation duration and induced effects on corticospinal excitability (CSE). It has been shown that prolonged a-tDCS protocols could result in the reduction and even reversal of after-effects however, the important question regarding the threshold for this reversal is still unanswered.

This chapter addresses **Aim 4** in this thesis to determine a-tDCS "duration" threshold for reversal of the effects on CSE and to explore the underlying neurophysiological mechanisms behind these changes.

A systematic investigation using a crossover study design determined the existence of duration threshold for reversal of a-tDCS-induced effects on CSE. Assessing the mechanisms behind these changes using the transcranial magnetic stimulation (TMS) paired-pulse paradigm, also revealed the intracortical inhibition and facilitation regulating this reversal on CSE.

Chapter 5 presents a double-blinded cross-over randomised experiment that was carried out to investigate the duration threshold for reversal of a-tDCS effects on CSE. The findings of on duration threshold in this chapter provide the structure of study designs in the following chapter to find the intensity threshold for reversal of CSE and the effect of both thresholds on motor performance following a-tDCS for studies 4 and 5.

Chapter 5. Determination of anodal tDCS duration threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms.

The format of this chapter is consistent with the Journal of *Brain Stimulation (impact factor: 6.73, ranking: Q1 in Neuroscience).* This chapter was published on Feb 27th, 2020.

The Ethics approval, consent form, study setup system used in this study, TMS and tDCS safety, and Edinburg handedness questionnaires and are provided in Appendices. 1 - 9.

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Determination of anodal tDCS duration threshold for reversal of corticospinal excitability: An investigation for induction of counter-regulatory mechanisms

Maryam Hassanzahraee [a, *], Michael A. Nitsche [b, c], Maryam Zoghi [d], Shapour Jaberzadeh [a]

[a] Non-invasive Brain Stimulation & Neuroplasticity Laboratory, Department of Physiotherapy, School of Primary and Allied Health Care, Faculty of Medicine, Nursing and Health Science, Monash University, Melbourne, Australia
[b] Department of Neurology, University Medical Hospital Bergmannsheil, Bochum, Germany
[c] Dept. Psychology and Neurosciences, Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany
[d] Department of Rehabilitation, Nutrition and Sport, School of Allied Health, Discipline of Physiotherapy, La Trobe University, Melbourne, Australia

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ABSTRACT

Background: Transcranial direct current stimulation (tDCS) is used to induce neuroplasticity in the human brain. Within certain limits of stimulation duration, anodal tDCS (a-tDCS) over the primary motor cortex induces long term potentiation- (LTP) like plasticity. A reversal of the direction of plasticity has however been described with prolonged a-tDCS protocols.

Objective: We aimed to systematically investigate the intervention duration threshold for reversal of a-tDCS-induced effects on corticospinal excitability (CSE) and to determine the probable mechanisms involved in these changes.

Methods: Fifteen healthy participants received a-tDCS of 1 mA for five different durations in pseudo-random session order. Transcranial magnetic stimulation (TMS) was delivered over the left M1, and motor evoked potentials (MEPs) of a contralateral hand muscle were recorded before, immediately and 30 min following intervention to measure CSE changes. Short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and long interval facilitation (LIF) were assessed via paired-pulse TMS protocols.

Results: A-tDCS significantly increased CSE as expected at stimulation durations of 22 and 24 min. However, this effect of a-tDCS on CSE decreased and even reversed when stimulation duration increased to 26, 28, and 30 min. Respective alterations of ICF, LIF, and SICI indicate the involvement of glutamatergic, and GABAergic systems in these effects.

Conclusions: These results confirm a duration threshold for reversal of the excitability-enhancing effect of a-tDCS with stimulation durations $\geq$ 26 min. Counter-regulatory mechanisms are discussed as a mechanistic foundation for these effects, which might prevent excessive brain activation.

Introduction

Modulation of corticospinal excitability (CSE) by transcranial direct current stimulation (tDCS) is directly influenced by the duration, intensity, and polarity of the applied currents. Anodal tDCS (a-tDCS) of the primary motor cortex (M1) increases, while cathodal tDCS (c-tDCS) decreases CSE. Early studies by Refs. [1,2] have shown a linear relation between CSE enhancement and a-tDCS intensity (up to 1 mA, electrode size 35 cm^2), and duration for up to 13 min [1,2]. This observation has been supported by a large number of studies [3–7], and led to the assumption of polarity-depended excitatory effects of a-tDCS on CSE, independent from stimulation duration, and intensity.

This assumption was however challenged by several other studies [8–11] which indicated no change or even a reduction of CSE following application of a-tDCS. The results of these studies led to the conclusion that a more complex interaction does exist between the applied a-tDCS parameters, direction, and size of CSE changes. Indeed, it has been suggested that stimulation parameters

* Corresponding author.
E-mail addresses: maryam.hassanzahraee@monash.edu, mzahraee88@gmail.com (M. Hassanzahraee).

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such as polarity, duration, and intensity of the applied current, but also other factors such as the history of synaptic activity, training, consumption of certain foods such as caffeine or energy drinks, and poor sleep may affect the response to neuromodulatory intervention effects of non-invasive brain stimulation, including those of tDCS [12]. Out of these factors, the focus of the present study was the history of synaptic activity of the target area of the respective intervention, which is closely related to the dynamic online effects of stimulation duration on synaptic activity, and neuronal excitability. Synaptic plasticity is an activity-dependent form of plasticity [13,14]. Indeed, changes in the level of synaptic activity can affect the induction and direction of synaptic plasticity [15], which could destabilize neuronal networks [16]. Therefore, counter-regulatory, homeostatic mechanisms have been described by the Bienenstock-Cooper-Munro (BCM) rule (1982). According to this rule, the bidirectional modification threshold of synaptic plasticity is not static, but dynamically slides based on the history of synaptic activity between long-term potentiation (LTP) or depression (LTD). This sliding threshold is important to keep neuronal activity of the brain within an optimal physiological range to prevent excessive excitation or inhibition [17,18]; [16]. Therefore, depending on the inhibitory or excitatory nature of previous brain activity, the respective modification threshold will slide towards LTP or LTD, and change the effects of a given stimulation protocol on CSE [13,14,19]. Likewise, several studies using different intervention protocols have shown that this rule is applicable to non-invasive brain stimulation of the human motor cortex [20–22]; [55]; [8]. For tDCS [8], revealed that when the duration of 1 mA a-tDCS was doubled from 13 to 26 min (13 + 13), the excitatory effect on CSE reversed into excitability diminution [8]. In accordance with the BCM rule, it can be speculated that for the prolonged application, the initial part of the stimulation changed the history of the synaptic activity of the target area towards facilitation (LTP-like effects as obtained for 13 min a-tDCS). This initial increase of excitability, and spontaneous activity would then reduce the modification threshold in favour of LTD induction for the remaining duration of the intervention. These homeostatic mechanisms would reverse the directionality of later tDCS effects on CSE. Although several studies shed light on probable mechanisms underlying such a counter-regulation of plasticity [23,24]; [55]; [8], the duration threshold for this effect has not been determined yet.

The main aim of the current study was thus to systematically determine the intervention duration threshold for reversal of M1 a-tDCS (1 mA) effects on CSE. Moreover, we aimed to explore the respective mechanisms underlying this reversal effect. Based on the foregoing studies, we hypothesized that application of a-tDCS (1 mA) over M1 for $\geq$ 26 min might reverse the excitatory effects of stimulation on CSE due to a calcium-dependent mechanism [25]. A-tDCS after-effects depend on voltage-dependent calcium channels ([25]), and glutamatergic receptor activation, specifically, N-methyl-D-asparate receptor (NMDAR) efficiency, which also have calcium channel properties ([56] [25]. Reduction of GABA activity seems to have a gating effect on the respective glutamatergic plasticity [26]. For the excitability-reducing effects of 26 min a-tDCS, it was shown that calcium channel block prevented this effect, and it was suggested that this might be caused by calcium overflow-induced counterregulatory mechanisms, which might include the activation of hyperpolarizing potassium channels [8,24] Therefore, we expected that this neuronal counter-regulation would be associated with an increase of inhibitory and a decrease of facilitatory brain activity, whereas stimulation duration below 26 min should reduce intracortical inhibition, but enhance facilitation [27].

Material and methods

Participants

A total of 15 healthy non-smoking volunteers (8 female) aged between 19 and 39 years (mean age ± SD: 24.66 ± 7.5) were recruited. The sample size was calculated (power of 0.8 and $\alpha = 0.05$) based on the critical effect size generated from a pilot study on eight participants. All participants were right-handed according to the Edinburgh Handedness Inventory [28] and screened for contraindications to transcranial magnetic stimulation (TMS) [29] and tDCS [30]. None of the participants reported any neurological or psychiatric disease. Participants were asked not to consume any caffeine or alcohol from the day before the experimental sessions, and sleep about 6–7 h at night before the session. Ethical approval was obtained from the Human Ethics Committee at Monash University, Melbourne, Australia, and the study protocol was conducted in accordance with the Declaration of Helsinki. Before the experiments, all participants provided informed consent.

Study design

A randomised double-blinded crossover design was applied in this study. Each volunteer participated in five experimental sessions, which were pseudo-randomly ordered. Order of sessions was counterbalanced, and the respective sessions were separated by at least seven days [31]. All experimental sessions started at the same time of the day for each individual to reduce the risk of circadian influences [32,33]. Due to the nature of this study, no sham condition was included. Participants were blinded to a-tDCS conditions and the purpose of the study. The selection of stimulation parameters was based on the study of Monte-silva et al. (2013); where a-tDCS with 1 mA and 35 cm^2 electrodes for 26 min and showed a reversal of CSE alterations [8]. In the present study, these parameters (1 mA, 35 cm^2) were applied to explore the effects of a-tDCS on CSE for 22, 24, 26, 28, and 30 min stimulation duration.

Two researchers were involved in the current study, one as an assessor and the other as a-tDCS administrator. The assessor, responsible for data collection and analysis, was blinded to all experimental conditions. The administrator, who was responsible for delivering a-tDCS interventions, was not involved in any data collection or analysis.

Experimental procedures

Electromyography (EMG)

Surface EMG was recorded from the right first dorsal interosseus (FDI) with pre-gelled self-adhesive Ag/AgCl electrodes (inter-electrode distance 2 cm) in a belly-tendon montage. The reference electrode was placed on the styloid prominence of the ipsilateral ulna. The skin over the FDI was gently abraded and then cleaned to reduce electrode-skin impedance and improve the recorded EMG responses [34]. EMG signals were filtered (bandwidth 10–500 Hz), amplified (× 1000), and digitized at a sampling rate of 1 kHz, using a Powerlab 4/35 system (ADInstruments, Australia). MEPs were recorded using LabChart 8 software (ADInstruments, Australia), and stored in a PC for offline analysis.

Transcranial magnetic stimulation (TMS)

TMS was applied via an angulated figure-of-eight coil connected to a MagPro R30 stimulator (MagVenture, Denmark). The coil was positioned over the left M1 with an angle of 45 from the midline and the handle pointing backwards (posterior-anterior current orientation). The "motor hotspot" was defined as the coil position

from which TMS-induced MEPs of maximum amplitude could be recorded in the target muscle with a given medium TMS intensity. The spot was marked on the scalp for exact repositioning of the coil throughout each session. Resting motor threshold (RMT) at the M1 hot-spot was obtained using the parameter estimation by sequential testing (PEST) method [35]. MEP amplitudes were recorded to monitor intervention-generated CSE changes. The TMS intensity (as a percentage of maximum stimulator output, %MSO) was adjusted to elicit a mean MEP amplitude of about 1 mV peak-to-peak (SI $_{1mV}$) in the resting FDI [1,2,36]. Baseline MEP means within the range of 1 mV ± 20% were accepted [7]. All TMS procedures were done by the same experimenter (MHZ), who was well-trained in TMS.

Assessment of CSE: single-pulse TMS induced MEPs (1 mV)

Twenty-five single-pulse TMS induced MEPs were recorded using the SI $_{1mV}$ before (T_{pre}), immediately (T_0) and 30min (T_{30}) after the application of a-tDCS. The same intensity was used for all time bins to monitor tDCS-induced changes of CSE.

Assessment of intracortical excitability: paired-pulse TMS induced MEPs

Intracortical excitability changes were assessed by a TMS paired-pulse protocol, including 75 stimuli, and interstimulus intervals (ISIs) of 3, 10, and 150 ms. In this protocol, short intracortical inhibition (SICI, 3 ms), intracortical facilitation (ICF, 10 ms), and long interval facilitation (LIF, 150 ms [37]; were assessed by combining a subthreshold conditioning stimulus (CS: 80% of RMT) with a suprathreshold test stimulus (TS: SI $_{1mV}$) ([59]). TS intensity was adjusted to achieve a baseline MEP of about 1 mV (SI $_{1mV}$) and readjusted after the application of a-tDCS in order to compensate for effects of the intervention on the MEP amplitude if required [27].

Anodal-transcranial direct current stimulation

A-tDCS was delivered through a battery-driven stimulator (NeuroConn, Germany). The current was applied through a pair of saline-soaked surface sponge electrodes (5 × 7 cm, 35 cm^2). The active electrode (anode) was centred over the FDI hotspot of the left M1 as identified by TMS. The return electrode (cathode) was positioned over the right supraorbital area. Current intensity of 1 mA was applied for five durations (22, 24, 26, 28, and 30 min) in randomised order on different days. There was a 15s ramp-up/down at the beginning and end of the stimulation to minimize any potential discomfort. During stimulation, participants were instructed to keep their hands in a relaxed position.

Fig. 1 summarizes the experimental design of the current study.

Monitoring of side effects

All participants were asked to complete a questionnaire during all experimental conditions to record side or adverse effects of a-tDCS. The questionnaire contained rating scales for the presence and severity of some common side effects such as itching, tingling or burning sensation under the electrodes [38,39], and other adverse effects, including headache and pain during and after stimulation [30]. All participants rated the unpleasantness of any scalp sensation by a numeric analogue scale (NAS; e.g. 0 = no sensation to 10 = worst sensation imaginable) during and after stimulation. Finally, at the end of each experiment, participants were asked to indicate if they distinguished any difference between received stimulation compared to the previous session(s). They replied, choosing 'Yes', 'No', or 'cannot say' as the answer.

Statistical analyses

To exclude baseline differences between the five tDCS-sessions, a one-way repeated measure ANOVA (rmANOVA) was used for all dependent variables (RMT, SI $_{1mV}$, MEP amplitude). Peak-to-peak amplitudes of 25 single-pulse MEPs were calculated and averaged online for each time point of measurement, using a custom-designed macro. The size of the conditioned MEP was expressed as a percentage of the unconditioned test MEPs for SICI, ICF, and LIF. The Shapiro-Wilk test was applied to explore the normality of each dataset. The post-intervention values were normalized, and are given as ratios of the respective baselines. A rmANOVA was conducted to assess the effects of two repeated measure factors, 'Experimental conditions' (a-tDCS durations of 22, 24, 26, 28 and 30 min) and 'Time' (T_{pre}, T_0, and T_{30}) on CSE, SICI, ICF, and LIF. Mauchly's test was used to assess the validity of the sphericity assumption for the rmANOVA; it requires that the variances of each set of difference scores are equal. Greenhouse-Geisser-corrected significance values were used when the sphericity assumption did not apply [40]. In case of significant results of the ANOVA, Bonferroni-corrected post hoc paired-sample t-tests were conducted to test whether the baseline value of each experimental condition differed significantly from post-intervention time points (T_0 and T_{30}).

An analysis of covariance (ANCOVA) with session order as co-variate was performed, to exclude that this factor had an impact on the results, which would hint for instability of the results due to this methodological aspect.

For side effect analysis, mean intensity values were calculated based on the numerical analogue scale ratings. A one-way ANOVA was carried out on the rating scale data recorded to assess any significant differences between sessions. To determine whether participants were successfully blinded to the experimental conditions, after completion of each experiment, participants were asked whether they could differentiate between stimulation they received at each session. Data were analysed using Pearson's chi-square. Means are reported ± standard error of the mean (SEM). Statistical analyses were computed using SPSS 25 (IBM, NY, USA), and the critical level of significance was set to $p = 0.05$.

Results

All fifteen participants completed all experimental sessions. The Shapiro-Wilk test confirmed normality of all data sets. The results of the respective one-way rmANOVAs revealed no significant difference of baseline RMT, SI $_{1mv}$ (CSE), and MEPs (SICI, ICF, LIF) between all experimental sessions, Table 1.

Moreover, the results of the respective ANCOVAs show no significant impact of this co-variate on the outcome ($F = 0.05$, $df = 4$, $Sig = 0.94$ for CSE, please see Table 2 for the remaining results).

Effects of different A-tDCS durations on CSE

The two-way rmANOVA conducted for single pulse amplitudes showed a significant main effect of 'Experimental condition' ($F_{(4, 56)} = 19.19$, $P < 0.001$, $\eta_p^2 = 0.60$, $1\text{-}\beta = 0.98$) and a significant 'experimental conditions × time' interaction ($F_{(8, 112)} = 11.39$, $P < 0.001$, $\eta_p^2 = 0.46$, $1\text{-}\beta = 0.96$). However, the results showed no significant main effect of 'time' ($F_{(2, 28)} = 3.88$, $P = 0.33$, $\eta_p^2 = 0.21$, $1\text{-}\beta = 0.65$). Fig. 2 (A1-E1) shows the respective CSE changes of all participants for the five a-tDCS durations. Bonferroni-corrected post-hoc t-tests revealed that peak-to-peak MEP amplitudes significantly increased (T_0, T_{30}) following tDCS durations of 22 and 24 min, as compared to baseline ($p < 0.01$). On the other hand, MEP amplitudes were significantly reduced following stimulation

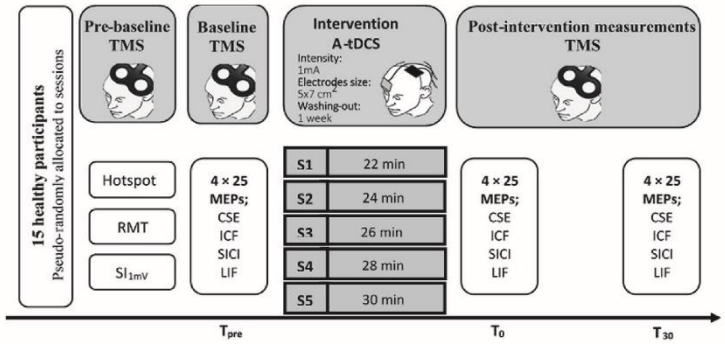

Fig. 1. Schematic representation of the experimental procedure for each session. The timeline shows the order of the procedures from left to right.
TMS: Transcranial magnetic stimulation, S: session, MEPs: Motor evoked potentials, CSE: Corticospinal excitability, ICF: intra-cortical facilitation, LIF; long interval facilitation, SICI: Short latency intra-cortical inhibition, A-tDCS: Anodal-transcranial direct current stimulation, RMT: resting motor threshold, SI1mV: Stimulator intensity required for peak-to-peak MEP amplitude of approximately 1 mV, Tpre: Baseline, T0: Immediately after, and T30: 30 min after the intervention.

durations of 26 (T_0), 28, and 30 min (T_0, T_{30}), as compared to baseline ($p < 0.01$).

Effects of different A-tDCS durations on SICI

The rmANOVA revealed a significant main effect of 'Experimental conditions' ($F_{(4, 56)} = 8.55$, $P < 0.001$, $\eta_p^2 = 0.37$, 1-β = 0.94), and a significant 'experimental conditions × time' interaction on SICI ($F_{(8, 112)} = 10.01$, $P < 0.001$, $\eta_p^2 = 0.41$, 1-β = 0.97). However, there was no significant main effect of 'time' ($F_{(2, 28)} = 0.25$, $P = 0.77$, $\eta_p^2 = 0.01$, 1-β = 0.08). Pairwise comparisons revealed significant differences for tDCS durations of 22, 28, and 30 min. Specifically, SICI decreased significantly in the 22 min a-tDCS condition (T_0, T_{30}), while it increased significantly after 28 min (T_{30}), and 30 min (T_0, T_{30}) a-tDCS, as compared to the respective baseline values (Fig. 2; A2-E2).

Effects of different A-tDCS durations on M1 ICF and LIF

The rmANOVA showed a significant main effect of 'Experimental conditions' on ICF ($F_{(4, 56)} = 18.55$, $P < 0.001$, $\eta_p^2 = 0.56$, 1-β = 0.98), and LIF ($F_{(4, 56)} = 4.47$, $P < 0.01$, $\eta_p^2 = 0.24$, 1-β = 0.84), and a significant 'experimental conditions × time interaction' for both, ICF ($F_{(8, 112)} = 8.45$, $P < 0.001$, $\eta_p^2 = 0.37$, 1-β = 0.95), and LIF ($F_{(8, 112)} = 4.85$, $P < 0.001$, $\eta_p^2 = 0.25$, 1-β = 0.89). However, there was no significant main effect of 'time' resulted for both ICF ($F_{(2, 28)} = 1.90$, $P = 0.16$, $\eta_p^2 = 0.36$, 1-β = 0.12) and LIF ($F_{(2, 28)} = 0.04$, $P = 0.94$, $\eta_p^2 = 0.004$, 1-β = 0.05). Pairwise comparisons revealed that ICF increased significantly following a-tDCS for 22 min (T_0, T_{30}), but was significantly reduced after 26 (T_0), 28, and 30 min (T_0, T_{30}) a-tDCS (Fig. 2; A3-E3). Moreover, LIF increased significantly after 22 min a-tDCS (T_0, T_{30}), while it significantly decreased following 30 min a-tDCS (T_{30}) (Bonferroni-corrected post hoc t-tests, $p < 0.05$) (Fig. 2; A4-E4).

Safety and side effects of A-tDCS

No adverse effects were reported after a-tDCS, except tingling sensations and light itching under the electrodes during stimulation reported by some of the participants in all experimental conditions. Side effects were recorded at the beginning, middle and end of stimulation. Table 3 summarizes the means ± SEM for reported side effects under the anode and cathode for each of the experimental sessions. No reports of burning sensations, headaches, or pain were recorded during or after stimulation.

Moreover, the Chi-square test conducted to control for successful blinding showed no significant differences between the experimental conditions [χ^2 (4, n = 15) = 7.52, P = 0.12], demonstrating that participants were not able to identify the respective stimulation protocol. The percentage of participants who could not guess the a-tDCS condition they had been received correctly, and replied 'No' was 96% (excluding 'cannot say' responders) and 92% (including 'cannot say' responders). Blinding of the participants of the present study was therefore successful.

Discussion

The results of the current study confirm the existence of a duration threshold for the reversal of excitability-enhancing effects of a-tDCS (1 mA) on CSE at 26 min. This finding is in line with the

Table 1
Baseline TMS measurements. **Means ± Standard error of mean** (SEM). **SI1mV:** stimulus intensity required for induction of 1 mV MEP. **CSE:** corticospinal excitability, **SICI:** short latency intracortical inhibition (% conditioned MEP/Test MEP), **ICF:** Intracortical facilitation (% conditioned MEP/Test MEP), **LIF:** long interval facilitation (% conditioned MEP/Test MEP).

	Experimental conditions Application of a-tDCS at different durations							
Baseline Measurements	22 min	24 min	26 min	28 min	30 min	df	F value	P value
SI_{1mV} (%)	46.53 ± 1.91	44.33 ± 2.13	45.53 ± 2.18	46.11 ± 1.89	45.17 ± 2.01	4	1.5	0.28
CSE (mV)	1.01 ± 0.04	1.14 ± 0.05	1.06 ± 0.02	1.10 ± 0.03	1.08 ± 0.02	4	1.02	0.41
SICI (%)	33.63 ± 1.51	36.58 ± 2.06	37.10 ± 1.24	33.35 ± 1.88	36.55 ± 1.23	4	1.22	0.31
ICF (%)	110.13 ± 4.54	117.5 ± 4.81	120.73 ± 4.6	110.84 ± 2.11	114.34 ± 5.28	4	2.03	0.13
LIF (%)	104.51 ± 2.09	108.26 ± 3.02	106.55 ± 4.12	112.42 ± 4.69	108.12 ± 3.79	4	2.21	0.08

Table 2
Analysis of covariance (ANCOVAs). **CSE:** corticospinal excitability, **SICI**: short latency intracortical inhibition, **ICF:** Intracortical facilitation, **LIF:** long interval facilitation, **df**: degree of freedom, **Sig:** significance.

Parameter	df	F value	Sig.
CSE	4	0.05	0.94
SICI	4	1.77	0.14
ICF	4	1.96	0.11
LIF	4	1.33	0.26

study of [8]; in which the excitability-enhancing effects of a-tDCS reversed after doubling stimulation duration from 13 to 26 min [8]. Accordingly, unlike shorter (<26 min) applications, longer (≥26 min) applications of a-tDCS (1 mA) reversed the excitatory effects of stimulation on CSE. Moreover, the results of the present study confirm that the CSE reversal at longer intervention durations (≥26 min) is associated with an increase of inhibitory and decrease of excitatory intracortical mechanisms. TDCS was well tolerated in all experimental sessions, and the blinding procedure was successful.

Effects of A-tDCS durations on CSE and intracortical excitability

A-tDCS durations <26 min

We hypothesized that application of a-tDCS < 26 min would increase CSE and assumed that this enhancement would be accompanied by reduced SICI, and increased ICF, and LIF. Our findings support these hypotheses. In detail, our results show that CSE was significantly increased following a-tDCS, in line with the results of previous studies [27]; [43]; [3–7,41]. Furthermore, the results are in line with the hypothesized SICI reduction, which also aligns with respective results of the previous findings [27,42]. As expected, the results also show an increase of ICF and LIF after a-tDCS. In addition, these results confirm those of previous findings [27,43] which are indicative for an involvement of facilitatory mechanisms in the respective results.

TDCS affects the stimulated area by different mechanisms and can induce changes in different brain areas ([57]). The primary mechanisms are assumed to be calcium-dependent and mainly related to glutamatergic activity [25,44] with a gating effect on $GABA_A$ receptors [25–27,42], which is supported by the current results of the paired-pulse protocols. Indeed, glutamate receptors and specifically the NMDAR are involved in ICF ([54],;[58] [45]. Therefore, it can be concluded that the activity of NMDARs in M1 and therefore, glutamatergic activity was intensified following a-tDCS < 26 min. This would result in an increase of intracellular Ca^{2+} in the postsynaptic neuron that enhances ICF and increases CSE ([23]).

SICI is considered to be primarily depended on GABAergic interneuronal activity [26,44]; [42]). A reduction of GABAergic activity by a-tDCS has been already shown in previous studies [26,46,47]. This reduction of GABA activity would indirectly enhance NMDAR responses and intracellular Ca^{2+} concentration [48], and therefore contribute to the observed CSE enhancement. Finally, the LIF enhancement observed for a-tDCS < 26 min, as an index of late cortical disinhibition, fits well with the resulting increase of CSE caused by the respective tDCS protocols.

A-tDCS durations ≥26 min

We hypothesized that application of a-tDCS ≥ 26 min would reduce and may even reverse the facilitatory effect of the intervention on CSE. The current findings support this hypothesis. These findings are in principle agreement with other studies showing the non-linear effect of a-tDCS [8,10,11]. They furthermore support the assumption of a duration and intensity window for anodal tDCS that results in linear effects, and that exceeding stimulation parameters beyond respective limits results in non-linearities. We also assumed that the hypothesized CSE reduction would involve respective intracortical excitability alterations. The current findings, showing a gradual decrease of ICF and LIF following a-tDCS ≥ 26 min, support the respective hypothesis with respect to a reduction of intracortical facilitatory mechanisms in case of increased stimulation duration. Moreover, the gradual increase of SICI following a-tDCS ≥ 26 min in the current study suggests an enhancement of inhibitory mechanisms involved in the non-linear effects induced by prolonged a-tDCS. Although SICI was not enhanced at 26 and 28 min significantly, we observed a respective trend-wise effect. Moreover, the significant enhancement of SICI for the a-tDCS duration of 30 min supports this hypothesis and confirms increasing inhibitory activities.

It seems that neuronal counter-regulatory mechanisms are activated by prolonged stimulation duration, which reverses CSE. The already above-mentioned Ca^{2+} overflow induced by prolonged stimulation [8], might activate counteracting potassium channels [24] which would limit Ca^{2+} influx [49], and might convert effects.

ICF reduction and SICI enhancement, revealed by current results, is in accordance with proposed mechanisms of synaptic scaling, which opposite scaling directions of inhibitory and excitatory synapses of respective neuronal circuits [50,51]. This suggests that a high level of synaptic activity induced by prolonged a-tDCS enhances activation of intracortical inhibitory interneurons on excitatory interneurons and decrease NMDA currents. This mechanism would then scale down synaptic strengths. These hypothesized mechanisms are however speculative at present and should be confirmed by future studies.

Safety and side effects of a-tDCS

All participants tolerated the applied currents in the different experimental conditions well. There were no dropouts due to adverse or side effects of a-tDCS. Itching sensations were reported by all participants in all sessions. No reports of burning sensations, headache, or pain were mentioned during or after stimulation.

Limitations of the study

Our findings should be interpreted in the context of some limitations. First, the data was obtained from a healthy population; therefore, the results may not necessarily be extrapolated to the patients with neurological, or psychiatric disorders. Second, the effects were evaluated in young participants (under 40 years); older individuals may respond differently to the applied a-tDCS conditions. Finally, in the current study, the effect of a-tDCS was assessed only for up to 30 min post-stimulation, which limits our understanding with respect to possible further lasting effects or delayed developing changes.

Suggestions for future studies

Future experiments should conduct additional excitability measures during stimulation to receive more profound knowledge about the temporal dynamics of the development of plasticity by tDCS, and add mechanistic information via exploration of the contribution of ion channels and neurotransmitters to the effects of stimulation. Moreover, investigations exploring the duration threshold of M1 a-tDCS in older adults and patients with neurological disorders would be valuable to enhance the transferability of the findings. Studies applying different stimulation intensities, electrode sizes, and stimulation montages in both healthy

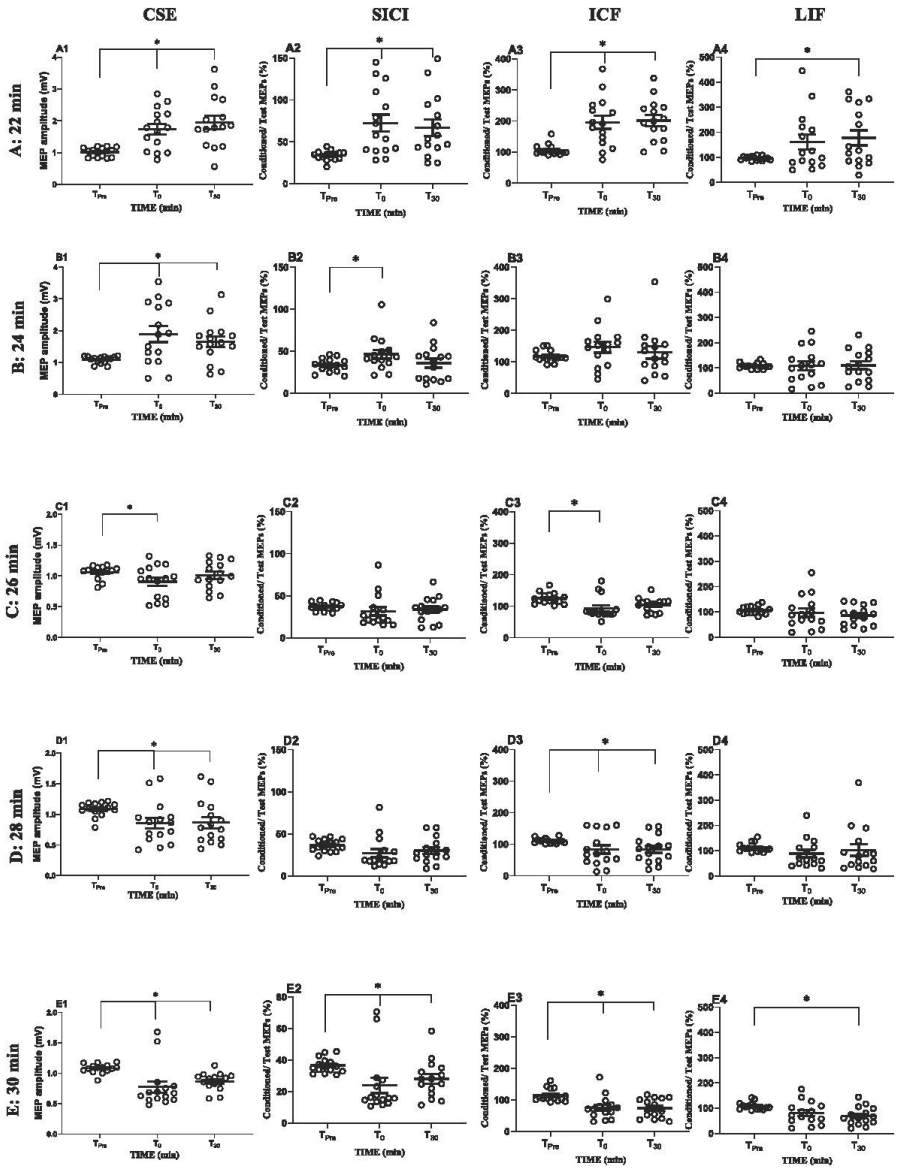

Fig. 2. The effects of different durations of a-tDCS on corticospinal excitability (CSE; A1-E1), short intracortical inhibition (SICI; A2-E2), intracortical facilitation (ICF; A3-E3), and long interval facilitation (LIF; A4-E4). A1-4: 22 min, B1-4: 24 min, C1-4: 26 min, D1-4: 28 min, E1-4: 30 min (*) shows significant differences, $p < 0.05$. Each dot represents one participant. Lines show the means. Error bars show SEM.

participants and patients in a systematic manner would provide valuable information about the parameter range of a-tDCS. In addition, behavioural outcome measures would be worthwhile to investigate if reversal of the CSE effects also affects the relevant motor or cognitive behaviours. Finally, we observed a higher variability of responses to a-tDCS with shorter durations in the present study. Future systematic studies should disentangle possible causes for the differences of variability, which might be methodological, because higher MEP amplitudes allow for larger variability, or physiological, because of instability of effects in transition zones between excitability-enhancing, and -reducing effects.

Table 3
The values are based on ratings via a Numeric Analogue Scale (NAS). 0 is representing no sensation, and 10 as the worst sensation imaginable. The sensations were recorded during three phases of stimulation: Beginning (0 min–1/3 of stimulation duration), Middle (1/2 to 2/3 of stimulation duration), End (last 6 min to end of stimulation). Sensations under both active (anode) and return (cathode) electrodes were recorded during different durations of a-tDCS. Scores are reported as mean ± SEM.

Side effect		Anode (Active electrode)					Cathode (Return electrode)				
		22min	24min	26min	28min	30min	22min	24min	26min	28min	30min
Tingling sensation	Beginning	4.6 ± 0.28	5.1 ± 0.42	4.3 ± 0.48	4.8 ± 0.26	4.1 ± 0.16	1.8 ± 0.11	2.7 ± 0.21	1.7 ± 0.09	2.1 ± 0.26	2.6 ± 0.11
	Middle	3.6 ± 0.23	3.9 ± 0.34	2.7 ± 0.19	2.9 ± 0.31	3.0 ± 0.21	1.6 ± 0.12	2.0 ± 0.06	0.9 ± 0.10	1.0 ± 0.09	0.8 ± 0.07
	End	1.7 ± 0.15	1.3 ± 0.11	1.8 ± 0.22	1.8 ± 0.12	1.9 ± 0.16	0.5 ± 0.09	0.5 ± 0.11	0.6 ± 0.12	0.4 ± 0.08	0.6 ± 0.08
Itching sensation	Beginning	3.0 ± 0.17	3.2 ± 0.17	3.1 ± 0.18	3.2 ± 0.11	3.6 ± 0.28	2.7 ± 0.21	3.0 ± 0.13	2.9 ± 0.10	2.8 ± 0.11	3.0 ± 0.17
	Middle	1.8 ± 0.13	1.5 ± 0.11	2.1 ± 0.28	2.2 ± 0.35	2.1 ± 0.18	1.2 ± 0.12	1.1 ± 0.18	1.4 ± 0.1	1.1 ± 0.20	1.0 ± 0.08
	End	0.9 ± 0.11	1.2 ± 0.12	1.0 ± 0.09	1.1 ± 0.10	1.2 ± 0.12	0.8 ± 0.12	0.9 ± 0.08	0.8 ± 0.10	0.6 ± 0.11	0.9 ± 0.09
Burning sensation	Beginning	–	–	–	–	–	–	–	–	–	–
	Middle	–	–	–	–	–	–	–	–	–	–
	End	–	–	–	–	–	–	–	–	–	–
Not tolerated	Beginning	–	–	–	–	–	–	–	–	–	–
	Middle	–	–	–	–	–	–	–	–	–	–
	End	–	–	–	–	–	–	–	–	–	–

Conclusions

The results of this study show that increasing the duration of a-tDCS does not necessarily enhance its efficacy to induce LTP-like plasticity, but might even convert the direction of effects. Moreover, the results show that respective corticospinal effects are mirrored at the level of intracortical circuits. These findings stress an essential role of metaplastic mechanisms for the effects of a-tDCS. The a-tDCS duration threshold for the reversal of the effects identified in this study confirms the assumption of a 'ceiling effect' of stimulation protocols in healthy participants, which might not be easily overcome with the application of prolonged interventions, but might require sophisticated adaptation.

Declaration of competing interest

This manuscript is based on research conducted by Maryam Hassanzahraee, PhD candidate at Monash University, Melbourne, Australia. This project had no external funding, and no financial or other relationships pose a conflict of interest. MAN is member of the scientific advisory boards of Neuroelectrics, and Neurodevice.

CRediT authorship contribution statement

Maryam Hassanzahraee: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration. **Michael A. Nitsche:** Data curation, Writing - review & editing. **Maryam Zoghi:** Conceptualization. **Shapour Jaberzadeh:** Conceptualization, Methodology, Data curation, Writing - review & editing, Supervision.

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Preamble to Chapter 6

The stimulation intensity is another important parameter contributing to the after-effect of a-tDCS on CSE changes. In Chapter 5, the duration threshold for the reversal of anodal transcranial direct current stimulation (a-DCS) effects on corticospinal excitability (CSE) was investigated. To assess the mechanisms behind the efficacy of this novel technique, intracortical inhibition and facilitation are also measured by the paired-pulse TMS paradigm. Therefore, based on the findings of the previous study, Study 4 was designed to find out if there is an intensity threshold for reversal of a-tDCS effect son CSE while keeping the duration unchanged.

This chapter addresses **Aim 5** in this thesis to determine the a-tDCS "intensity" threshold for reversal of the effects on CSE and to explore the underlying neurophysiological mechanisms behind these changes.

Chapter 6 provides a double-blinded cross-over randomised experiment that was carried out to investigate the intensity threshold for reversal of a-tDCS effects on CSE.

The findings of this chapter determined the intensity threshold for reversal of a-tDCS effect on CSE and the underlying mechanisms behind the changes. Studies 3 and 4 improve our understanding regarding the importance of a-tDCS parameter selection and how it would affect and even reverse the expected effect of a-tDCS if they reach the thresholds. Moreover, these studies become the core of Study 5 design to investigate how these thresholds would interfere with the effects of a-tDCS on motor performance changes.

Chapter 6. Determination of anodal tDCS intensity threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms.

The format of this chapter is consistent with the Journal of *Scientific Reports (impact factor: 4.525, ranking: Q1 in Neuroscience).* This chapter was published on Sep 30th, 2020.

The Ethics approval, consent form, study setup system used in this study, TMS and tDCS safety, and Edinburg handedness questionnaires and are provided in Appendices. 1 - 9.

Hassanzahraee M., Nitsche M., Zoghi M., Jaberzadeh S. Determination of anodal tDCS intensity threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms.

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Determination of anodal tDCS intensity threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms

Maryam Hassanzahraee[1✉], Michael A. Nitsche[2,3], Maryam Zoghi[4] & Shapour Jaberzadeh[1]

Transcranial direct current stimulation is applied to modulate activity, and excitability of the brain. Basically, LTP-like plasticity is induced when anodal tDCS (a-tDCS) is applied over the primary motor cortex. However, it has been shown that specific parameters of a-tDCS can induce a plasticity reversal. We aimed to systematically assess the intensity threshold for reversal of the direction of plasticity induced by a-tDCS, monitored by corticospinal excitability (CSE), and explored mechanisms regulating this reversal. Fifteen healthy participants received a-tDCS in pseudo-random order for 26 min with four intensities of 0.3, 0.7, 1, and 1.5 mA. To measure CSE changes, single-pulse TMS was applied over the left M1, and motor evoked potentials of a contralateral hand muscle were recorded prior to a-tDCS, immediately and 30-min post-intervention. Paired-pulse TMS was used to evaluate intracortical excitation and inhibition. CSE increased significantly following a-tDCS with an intensity of 0.7 mA; however, the expected effect decreased and even reversed at intensities of 1 and 1.5 mA. ICF was significantly increased while SICI and LICI decreased at 0.7 mA. On the other hand, a significant decrease of ICF, but SICI and LICI enhancement was observed at intensities of 1, and 1.5 mA. The present findings show an intensity threshold of ≥ 1 mA for 26 min a-tDCS to reverse LTP-into LTD-like plasticity. It is suggested that increasing stimulation intensity, with constant stimulation duration, activates counter-regulatory mechanisms to prevent excessive brain excitation. Therefore, stimulation intensity and plasticity induced by a-tDCS might non-linearly correlate in scenarios with prolonged stimulation duration.

Anodal transcranial direct current stimulation (a-tDCS) is a non-invasive brain stimulation tool which induces long-term potentiation (LTP)-like plasticity of the human brain via application of weak direct currents, it enhances corticospinal excitability (CSE)[1]. Within certain limits, the respective stimulation effect depends linearly on the intensity (up to 1 mA) and duration (up to 13 min) of a-tDCS, as shown by earlier studies[1–3]. This finding has been supported by a number of studies stating polarity-dependent excitatory effects of a-tDCS on CSE[4–7], and more recent studies, which explored an extended range of stimulation intensity and duration[8,9]. TMS-EEG studies offer valuable adjunctive information, because they allow for a relatively specific measure of cortical excitability. Indeed, a couple of respective studies have shown excitability-enhancing effects of anodal tDCS, which is in agreement with relevant cortical effects of this intervention[10–13].

[1]Non-Invasive Brain Stimulation and Neuroplasticity Laboratory, Department of Physiotherapy, School of Primary and Allied Health Care, Faculty of Medicine, Nursing and Health Science, Monash University, Melbourne, Australia. [2]Department of Neurology, University Medical Hospital Bergmannsheil, Bochum, Germany. [3]Department of Psychology and Neurosciences, Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany. [4]Department of Rehabilitation, Nutrition and Sport, School of Allied Health, Discipline of Physiotherapy, La Trobe University, Melbourne, Australia. ✉email: maryam.hassanzahraee@monash.edu; mzahraee88@gmail.com

Baseline Measurements	Experimental conditions (a-tDCS intensities				df	F value	P value
	0.3 mA	0.7 mA)	1 mA	1.5 mA			
CSE (mV)	1.06 ± 0.05	1.09 ± 0.04	1.04 ± 0.02	1.08 ± 0.06	3	1.62	0.19
SICI (%)	33.59 ± 1.32	30.04 ± 1.54	35.60 ± 1.03	32.87 ± 2.24	3	0.51	0.67
ICF (%)	115.70 ± 3.21	119.10 ± 2.90	113.73 ± 3.05	117.83 ± 4.72	3	2.46	0.10
LIF (%)	119.92 ± 6.09	111.83 ± 4.12	110.62 ± 3.99	114.76 ± 5.90	3	0.31	0.76
LICI (%)	30.36 ± 3.56	30.29 ± 1.96	31.13 ± 1.69	28.39 ± 2.34	3	0.61	0.63

Table 1. Baseline TMS measurements. Mean ± Standard error of mean (SEM). A one-way ANOVA was calculated for inter-session differences of the average baseline CSE (1 mV), SICI, LICI, ICF, and LIF. There was not significant difference across experimental sessions for all baseline measurements. *CSE* corticospinal excitability, *SICI* short-latency intracortical inhibition, *ICF* Intracortical facilitation, *LIF* long interval facilitation, *LICI* long-latency intracortical inhibition.

Experimental conditions	TMS stimulus intensity (%)					
	RMT			SI_{1mV}		
	Baseline	Post-intervention	P value	Baseline	Post-intervention	P value
A-tDCS intensities						
0.3 mA	37.46 ± 1.17	37.13 ± 1.32	0.49	46.13 ± 2.88	45.33 ± 1.54	0.017*
0.7 mA	38.66 ± 1.37	36.53 ± 1.59	0.001*	47.21 ± 2.66	45.28 ± 1.38	0.004*
1 mA	37.60 ± 1.49	38.40 ± 1.35	0.25	46.87 ± 1.81	48.04 ± 1.72	0.008*
1.5 mA	38.13 ± 1.08	40.26 ± 1	0.001*	45.12 ± 2.98	47.85 ± 1.31	0.001*
P value	0.87	0.30		0.82	0.2	

Table 2. TMS Stimulus intensity (in percentage MSO) of baseline and post-intervention (mean ± SEM). There was not significant difference across experimental sessions for baseline measurements. *SI1mV* stimulus intensity (for an average motor evoked potential (MEP) of 1 mV), *RMT* resting motor threshold. (*) shows significant difference.

The assumption of a generally linear association between stimulation intensity/duration and LTP-like plasticity was however challenged by other studies, which showed a reduction or even reversal of tDCS-induced excitability alterations with specific current intensities and/or stimulation durations[9,14–19]. Other a-tDCS studies have shown no significant CSE-difference following different stimulation intensities for stimulation durations of 10 min[20,21], 15 min[8], 20 min[9,15], and 30 min[9]. These findings suggest a more complex interaction between stimulation parameters, and the induced plasticity, as already shown for cathodal tDCS[15,22]. The exact boundary conditions of respective effect reversals, and non-linearities, as well as mechanisms, have however not been explored in detail. These could be caused by counter-regulatory effects which might be driven by alterations of GABAergic, and glutamatergic feedback loops, and involve calcium-dependent mechanisms, including the activation of potassium channels induced by calcium overflow[14,23,24]. In accordance, reduction of calcium influx by pharmacological block of voltage-gated calcium channels abolished the conversion of LTP- to LTD-like plasticity induced by a 26 min/1 mA a-tDCS protocol[14]. Moreover, higher intensity, and longer duration of stimulation might enhance calcium influx in a larger number of neurons to a sufficient degree to induce plasticity, which could explain why beyond reversal of the after-effects of stimulation, with even longer, and/ or stronger stimulation the primary plasticity effect—in case of a-tDCS LTP-like plasticity—can re-emerge[9].

In our foregoing study, we have systematically explored the critical stimulation duration required for reversal of the after-effects of a-tDCS, and kept current intensity constant[19]. The results revealed a duration threshold for reversal of the excitability-enhancing effect of a-tDCS with stimulation durations ≥ 26 min[19]. In the present study we were interested to explore if beyond duration also the intensity of stimulation is relevant for the reversal of a-tDCS after-effects. Based on the calcium-dependency hypothesis, we expected an intensity-dependency for reversal of tDCS after-effects, with only higher stimulation intensities (based on the results of previous studies equal or above 1 mA[14,20]) reversing LTP- into LTD-like plasticity. In addition, we aimed to investigate neurotransmitter-dependent mechanisms responsible for this reversal effect. We hypothesised that with higher stimulation intensities, which were expected to cause a reversal of CSE, intracortical facilitation driven by glutamatergic *N*-methyl-D-aspartate (NMDA) receptors would be reduced, while gamma-aminobutyric acid (GABA)-dependent cortical inhibition would be enhanced.

Results

All participants completed all experimental sessions and tolerated all experimental conditions well. The Shapiro–Wilk test confirmed normality of all data sets. The respective one-way rmANOVAs revealed no significant difference between baseline values of MEP amplitudes (sp- and pp-MEPs) (Table 1), and baseline and post-intervention TMS stimulus intensity (SI_{1mV}) (Table 2) between the respective sessions with different current

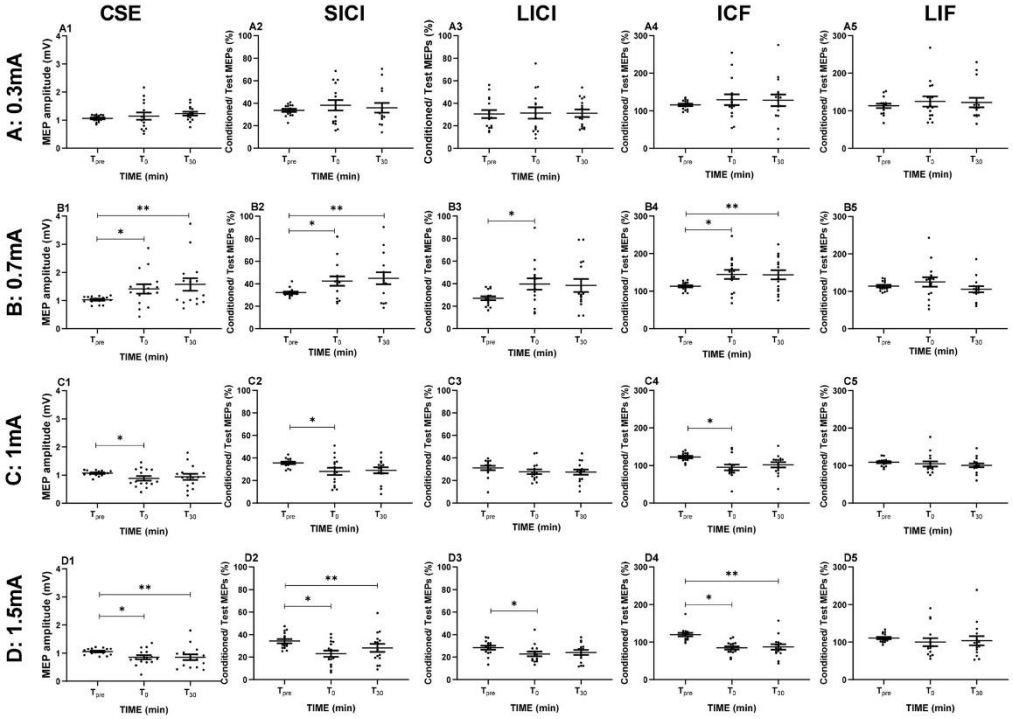

Figure 1. The effects of different intensities of a-tDCS on corticospinal excitability (CSE; **A1–D1**), short latency intracortical inhibition (SICI; **A2–D2**), long latency intracortical inhibition (LICI; **A3–D3**), intracortical facilitation (ICF; **A4–D4**), and long interval facilitation (LIF; **A5–D5**). **A1–A5**: 0.3 mA, **B1–A5**: 0.7 mA, **C1–C5**: 1 mA, **D1–D5**: 1.5 mA. (*) shows significant differences between baseline (T_{pre}) and T_0, T_{30} ($P < 0.05$). CSE and ICF were enhanced at a stimulation intensity of 0.7 mA, and decreased at 1 and 1.5 mA. In contrast, SICI and LICI decreased at 0.7 mA and increased at 1 and 1.5 mA. Each dot represents one participant. Lines show the means. Error bars represent standard error of the mean (SEM).

intensities. There was also no significant difference between the baseline and post-intervention single pulse MEPs for paired-pulse protocols ($P > 0.05$).

The effects of a-tDCS intensity on CSE. The two-way rmANOVA showed a significant main effect of 'intensity' ($F_{(3, 42)} = 9.77$, $P > 0.01$, $\eta_p^2 = 0.48$) and a significant 'intensity × time' interaction ($F_{(6, 84)} = 4.69$, $P < 0.01$, $\eta_p^2 = 0.25$). The Bonferroni-corrected post-hoc analysis revealed significant differences for tDCS intensities of 0.3 vs. 1.5 mA ($P = 0.01$), and 0.7 vs. 1.0 ($P = 0.01$), and vs. 1.5 mA ($P = 0.007$). The peak-to-peak MEP amplitudes significantly increased (T_0, T_{30}) following a-tDCS with 0.7 mA, as compared to baseline. On the other hand, MEP amplitudes showed a significant reduction following tDCS intensities of 1 (T_0) and 1.5 mA (T_0, T_{30}), as compared to T_{pre}. Figure 1A1–D1 shows CSE changes of all participants for the four a-tDCS intensities.

The effects of a-tDCS intensity on SICI and LICI. The two-way rmANOVA revealed a significant main effect of 'intensity' ($F_{(3, 42)} = 4.65$, $P < 0.01$, $\eta_p^2 = 0.28$), and the 'intensity × time' interaction on SICI ($F_{(6, 84)} = 4.27$, $P < 0.01$, $\eta_p^2 = 0.23$). Pairwise comparisons revealed significant differences for tDCS intensities of 0.7 vs. 1.5 mA ($P = 0.009$). SICI decreased significantly following stimulation with 0.7 mA (T_0, T_{30}), while it increased significantly following stimulation intensities with 1.0 (T_0), and 1.5 mA (T_0, T_{30}), as compared to baseline values (Fig. 1A2–D2). The rmANOVA conducted for LICI showed a significant main effect of 'intensity' ($F_{(3, 42)} = 2.75$, $P < 0.05$, $\eta_p^2 = 0.23$), and the 'intensity × time' interaction ($F_{(6, 84)} = 2.30$, $P < 0.05$, $\eta_p^2 = 0.32$). LICI decreased significantly following tDCS with an intensity of 0.7 mA (T_0), while it increased significantly following tDCS intensity of 1.5 mA (T_0), as compared to baseline measures (Fig. 1A3–D3).

The effects of a-tDCS intensity on ICF and LIF. The rmANOVA conducted for ICF showed a significant main effect of 'intensity' ($F_{(3, 42)} = 6.10$, $P < 0.01$, $\eta_p^2 = 0.32$) and 'intensity × time' interaction ($F_{(6, 84)} = 6.09$, $P < 0.001$, $\eta_p^2 = 0.30$). Pairwise comparisons revealed significant differences for tDCS intensities of 0.7 vs. 1.0 mA

	Anode (target electrode)				Cathode (return electrode)			
Side effect	0.3 mA	0.7 mA	1 mA	1.5 mA	0.3 mA	0.7 mA	1 mA	1.5 mA
Tingling sensation								
Beginning	2.7 ± 0.14	3.9 ± 0.23	4.3 ± 0.48	4.8 ± 0.24	2.3 ± 0.21	2.7 ± 0.09	2.1 ± 0.26	2.6 ± 0.11
Middle	2.4 ± 0.23	2.3 ± 0.14	2.7 ± 0.19	2.6 ± 0.22	0.8 ± 0.15	1.0 ± 0.10	1.0 ± 0.21	1.2 ± 0.17
End	1.7 ± 0.15	1.3 ± 0.21	1.8 ± 0.22	2.0 ± 0.19	0.5 ± 0.21	0.6 ± 0.12	0.4 ± 0.18	0.6 ± 0.2
Itching sensation								
Beginning	2.7 ± 0.27	2.6 ± 0.17	3.1 ± 0.28	3.7 ± 0.21	1.8 ± 0.13	1.9 ± 0.10	2.2 ± 0.16	2.7 ± 0.27
Middle	1.8 ± 0.13	1.5 ± 0.11	2.1 ± 0.28	2.2 ± 0.12	1.1 ± 0.18	1.4 ± 0.1	1.1 ± 0.25	1.5 ± 0.18
End	0.9 ± 0.11	1.2 ± 0. 12	1.0 ± 0.19	1.1 ± 0.12	0.9 ± 0.08	0.8 ± 0.10	0.6 ± 0.11	0.9 ± 0.09
Burning sensation								
Beginning	–	–	–	–	–	–	–	–
Middle	–	–	–	–	–	–	–	–
End	–	–	–	–	–	–	–	–
Not tolerated								
Beginning	–	–	–	–	–	–	–	–
Middle	–	–	–	–	–	–	–	–
End	–	–	–	–	–	–	–	–

Table 3. Side effects are based on ratings on a Numeric Analogue Scale (NAS). 0 is representing no sensation, and 10 as the worst sensation imaginable. The sensations were recorded during three phases of stimulation: Beginning (0 min to 1/3 of stimulation duration), Middle (1/2 to 2/3 of stimulation duration), End (last 6 min to end of stimulation). Sensations under both, target (anode) and return (cathode) electrodes were recorded. Scores are reported as mean ± SEM. (–) indicates that no sensations were reported.

(P = 0.01), and 0.7 vs. 1.5 mA (P = 0.001). ICF increased significantly after tDCS with 0.7 mA (T_0, T_{30}), but was significantly reduced after stimulation with intensities of 1.0 (T_0), and 1.5 mA (T_0, T_{30}) (Fig. 1A4–D4). The rmANOVA conducted for LIF showed no significant main and interaction effects (P > 0.05; Fig. 1A5–D5).

Safety and side effects of A-tDCS. No side and/ or adverse effects were reported after a-tDCS, except tingling and light itching under the electrodes during stimulation reported by some of the participants in all experimental conditions. No burning sensations, headaches, or pain were recorded during or after stimulation. Side effect means ± SEM are reported in Table 3, and did not differ significantly between sessions (P > 0.05).

The Chi-square test conducted to control for blinding showed no significant differences between conditions [χ^2 (3, n = 15) = 5.37, P = 0.09]. This result demonstrates that participants were unable to distinguish between the experimental conditions in this study. The percentage of participants who replied 'No' was 89%. Therefore, the blinding procedure was successful in the present study.

Discussion

The results of the current study suggest the existence of an intensity threshold for the reversal of the excitability-enhancing effects of a-tDCS applied for 26 min on CSE. This finding confirms that of our previous study on a stimulation duration threshold[19] in which the excitability effects of 1 mA a-tDCS reversed at stimulation durations ≥ 26 min. Accordingly, unlike a-tDCS at lower intensities (< 1 mA), higher intensities (≥ 1 mA, 26 min) reversed the excitatory effects of a-tDCS on CSE. The results of the present study also confirm that the reversal of CSE at higher intensities (≥ 1 mA) is associated with specific alterations of intracortical physiology, including an increase of inhibitory and decrease of excitatory mechanisms. TDCS was well tolerated in all experimental sessions, and the blinding procedure was successful.

The effects of A-tDCS intensity on CSE and Intracortical excitability. *26 min a-tDCS with intensities < 1 mA.* We hypothesized that a-tDCS with intensities < 1 mA would increase CSE and assumed that this increase would be accompanied by reduction of SICI and/or LICI, an increase of ICF and/or LIF. Our findings partially support these hypotheses. Indeed, our results showed that CSE was significantly increased at a-tDCS of 0.7 mA in line with the results of previous studies using a-tDCS < 1 mA[1,8,20,21,38]. However, the missing increase of CSE following a-tDCS of 0.3 mA was in contrast with studies of Bastani and Jaberzadeh (0.3 mA)[38], and Chew et al. (0.2 mA)[21], probably related to smaller electrodes used in those studies (24 cm^2 and 16 cm^2) compared to the present study (35 cm^2), which result in larger current densities. The findings of the present study show furthermore a reduction of SICI and LICI following 0.7 mA a-tDCS, confirming reduced inhibition associated with excitability-enhancing a-tDCS shown in some previous studies[20,34,39,40]. Furthermore, the results reveal an increase of ICF and LIF, in accordance with our hypothesis, and other studies[15,34].

The findings of the paired-pulse protocols show thus an increase in intracortical facilitation, and decrease in inhibition in case of excitability-enhancing effects of tDCS. The increase in ICF supports the main involvement of calcium-dependent mechanisms, since ICF is mainly controlled by glutamatergic NMDA receptor activity,

which has calcium channel properties[3,41–44]. The observed disinhibition shown by reduced SICI, and LICI suggests the presence of a gating effect by reduced GABA activity, which controls for these TMS parameters[3,34,40,45].

26 min a-tDCS with intensities ≥ 1 mA. We hypothesized that the facilitatory effect of a-tDCS on CSE would decrease and even reverse into LTD-like plasticity when stimulation intensities of ≥ 1 mA are used. Our results support this hypothesis, and are in line with previous studies showing non-linear effects of a-tDCS[8,14,17–19]. On the other hand, these findings are not in line with other studies showing a CSE increase following higher intensities of stimulation of 1.2, 1.5, and 2 mA probably due to shorter stimulation duration (10 min) compared to the current study[20,21,38]. We also hypothesized that specific intracortical excitability changes would accompany the CSE reduction induced by higher intensity a-tDCS, including the reduction of facilitatory and enhancement of inhibitory intracortical mechanisms. The current findings support this assumption. ICF significantly decreased, whereas SICI and LICI were significantly enhanced at a-tDCS of ≥ 1 mA.

Although the current findings support the assumption of a-tDCS intensity and duration windows for linear effects, they also reveal that exceeding stimulation parameters beyond specific limits results in non-linear effects. Increasing stimulation intensity/duration therefore does not necessarily improve the efficacy of a-tDSC, in principle accordance with previous studies[9,14,18,20,38].

Taken together, tDCS has been shown to induce plasticity via calcium dependent mechanisms, and at the synaptic level NMDA receptors, and GABA are involved[3,34,40,45]. In addition to reduced glutamatergic NMDA receptor activity with higher stimulation intensities, which is indicated by reduced intracortical facilitation, enhancing GABA activity[45] might also contribute to this after-effect conversion. This is suggested by increased GABA-dependent inhibition, as shown by increased SICI, and LICI. Hereby, enhancement of inhibition regulated by both, $GABA_A$-, as revealed by enhanced SICI[46], and $GABA_B$-receptor activity, as suggested by enhanced LICI[47,48], may suggest a global enhancement of GABA activity following higher stimulation intensity. Such mechanistic concepts, however, are actually theoretical, and should be more specifically investigated in future studies.

Based on the results of the present, and other studies, with respect to mechanisms it can be assumed that within certain windows of stimulation parameters, a-tDCS induces LTP-like plasticity via calcium enhancement, supposedly driven by NMDA receptor activation, and GABA reduction. Beyond this window, enhancing stimulation intensity/duration likely results in counter-regulative mechanisms, which—as suggested by the results of the present and other studies—depend at the cellular level on calcium dynamics, and at the synaptic/neuronal network level on glutamatergic/GABAergic neurons[14,19].

Interestingly, a secondary conversion of after-effects of tDCS with even higher stimulation intensities and duration was found in other studies (for a-tDCS:[9], and cathodal tDCS:[22]). Respective mechanisms are not well explored, and not easily explained within the above-mentioned framework. One explanation might be that stronger, and longer stimulation will result in a calcium increase sufficient for a larger pool of neurons to develop plasticity, which would then counteract respective reversal effects.

These mechanistic explanations are however speculative at present, and should be explored more directly in future studies. In addition, it might be advantageous to add TMS-EEG in future studies, because it is a more direct measure of cortical excitability compared to TMS-induced MEPs.

Methods

Participants. Fifteen non-smoking healthy right-handed volunteers [8 female, mean age of 26.95 ± 6.3 (SD) years] participated in this study. The sample size was calculated (power of 0.8 and $\alpha = 0.05$) based on the critical effect size generated from a pilot study on eight participants. None of the participants reported contraindications to tDCS[25] and TMS[26] including history of seizure, intake of CNS-acting medications, psychiatric or neurological disorders[27,28]. The study was conducted in a double-blinded crossover design with at least 7 days wash-out period between sessions[29]. Each participant was pseudo-randomly assigned to four different experimental sessions in counterbalanced order. To reduce the risk of circadian influences for each individual, all experimental sessions started at the same time of the day[30,31]. Each participant gave written informed consent before attending the study. The Human Research Ethics Committee of Monash University approved the study and we conform to the Declaration of Helsinki (1991, BMJ, 302, 1194).

Experimental procedures. Participants were comfortably seated in a fully adjustable treatment chair (MagVenture, Denmark) with their head and arms at rest. Two pre-gelled self-adhesive Ag/AgCl electrodes (inter-electrode distance 2 cm) were placed on the right first dorsal interosseus (FDI) muscle in a belly-tendon montage to record surface electromyography (EMG). The ground electrode was placed over the styloid process of the ulna. EMG signals were filtered (bandwidth 10–500 Hz), amplified (1000 ×), and digitized at a sampling rate of 1 kHz, using a Powerlab 4/35 system (ADInstruments, Australia). MEPs were recorded using LabChart 8 software (ADInstruments, Australia) and stored on a PC for offline-analysis[19].

Transcranial magnetic stimulation (TMS). TMS was applied using a MagPro R30 stimulator (MagVenture, Denmark) with a butterfly 70 mm figure-of-eight coil (max. initial dB/dt 28 KT/s near the coil surface). The coil was positioned over the left M1 with the handle pointing posterolateral. The optimal site of stimulation, which was defined as the coil position resulting in the largest MEP amplitudes elicited in the target muscle with medium TMS intensity, was marked with a soft marker as "motor hotspot". This spot on the scalp was used for exact repositioning of the coil throughout each session. The induced current had a biphasic waveform. Resting motor threshold (RMT) was defined via the parameter estimation by sequential testing (PEST) method[32]. The current study was not conducted by aid of a neuronavigation system.

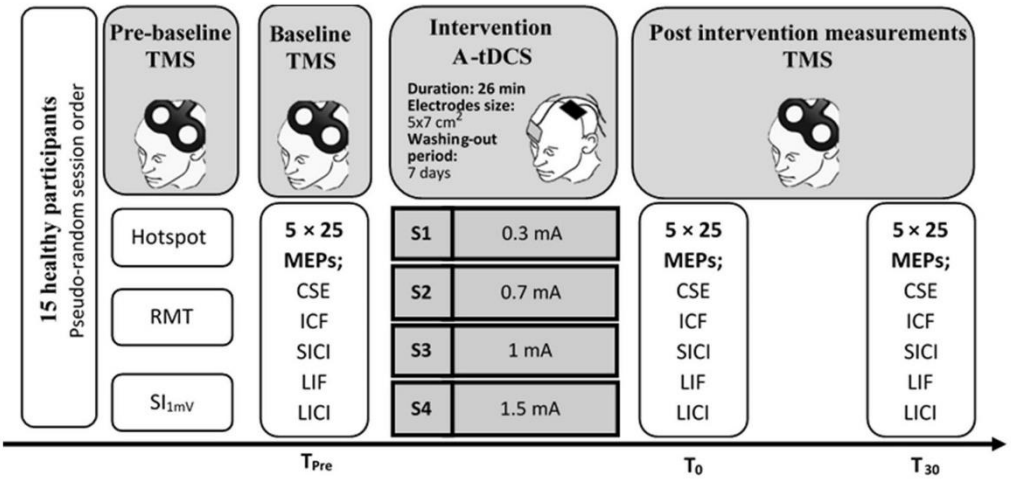

Figure 2. Schematic representation of the experimental procedure for each session. The time course is from left to right. *TMS* transcranial magnetic stimulation, *S* session, *MEPs* motor evoked potentials, *CSE* corticospinal excitability, *ICF* intra-cortical facilitation, *LICI* long interval intra-cortical inhibition, *SICI* short latency intra-cortical inhibition, *LIF* long interval facilitation, *A-tDCS* anodal-transcranial direct current stimulation, *RMT* resting motor threshold, SI_{1mV} stimulator intensity required for a peak-to-peak MEP amplitude of approximately 1 mV.

Single-pulse TMS-induced MEPs (1 mV): CSE assessment. Twenty-five single pulse (sp)-elicited MEP amplitudes were recorded to monitor CSE before, and after intervention. The TMS intensity was adjusted as the stimulator output (%MSO) of spTMS to elicit a 1 mV peak-to-peak amplitude ($SI_{1\,mV}$) in the resting FDI on average[1,2,28]. Twenty-five single-pulse TMS (spTMS) -elicited MEPs were recorded at baseline (T_{pre}), immediately (T_0) and 30 min (T_{30}) post-intervention. The mean baseline MEP was accepted if it was within the range of 1 mV ± 20%[7]. To obtain CSE changes following a-tDCS, stimulation intensity was kept constant throughout the session.

Paired-pulse TMS-induced MEPs: intracortical excitability assessment. A paired-pulse TMS protocol (ppTMS) was used to investigate intracortical excitability, including interstimulus intervals (ISIs) of 3, 10, and 150 ms. The protocol contained 25 MEPs for short and 25 MEPs for long latency intra-cortical inhibition (SICI: 3, LICI: 150 ms), 25 MEPs for intracortical facilitation and 25 MEPs for long interval facilitation (ICF: 10 ms, LIF: 150 ms). In SICI, ICF, and LIF protocols, a subthreshold conditioning stimulus (CS: 80% of RMT) followed by a suprathreshold test stimulus (TS: $SI_{1\,mV}$)[33] was applied. TS intensity was adjusted to achieve baseline MEPs of about 1 mV, and re-adjusted after applying a-tDCS to compensate for the effects of intervention on the MEP amplitude[34], if required. The long interval inhibition (LICI) protocol was carried out by two consecutive identical suprathreshold CS and TS ($SI_{1\,mV}$) at an ISI of 150 ms).

Anodal-transcranial direct current stimulation. A-tDCS was administered using a battery-driven stimulator (NeuroConn, Germany) through a pair of rubber electrodes enclosed in saline-soaked sponge pockets (5 × 7 cm; area: 35cm²). The selection of the stimulation parameters was based on our previous study, in which 1 mA a-tDCS reversed the excitability-enhancing effect of a-tDCS on CSE when applied for ≥ 26 min[19]. The target electrode (anode) was centered over the left M1 on the FDI hotspot, and the return electrode (cathode) over the right supraorbital area. In four pseudo-randomly ordered sessions, a-tDCS was applied with current intensities of 0.3, 0.7, 1, and 1.5 mA for 26 min. The current densities of a-tDCS under the electrodes were 0.008 (0.3 mA), 0.02 (0.7 mA), 0.029 (1 mA), and 0.04 mA/cm² for 1.5 mA.

To minimize any potential discomfort, a 15 s ramp-up/down was applied at the beginning and end of the stimulation. During stimulation, participants were instructed to remain relaxed and keep their hands in a relaxed position. The experimental design of the current study is summarised in Fig. 2.

Measurement of the side and adverse effects. Side effects were recorded at the beginning, middle and end of the stimulation in all experimental sessions. All participants were asked to complete a form to record the side and adverse effects of a-tDCS. The form contained rating scales for the presence and severity of common side effects such as itching, tingling, and burning sensation under the electrodes[35,36]and other adverse effects including headache and pain during and after stimulation (Brunoni et al.[25]). The unpleasantness of any scalp sensation was rated via numeric analogue scales (NAS) (i.e. 0 = no feeling to 10 = worst imaginable sensation).

To test the efficacy of blinding, participants were asked at the end of each session to tell if they did perceive any difference between the present stimulation compared to previous session(s). They answered choosing 'Yes', 'No'.

Statistical analyses. The peak-to-peak amplitudes of 25 single-pulse MEPs (sp-MEPs) were calculated and averaged for the three time points (T_{pre}, T_0, and T_{30}). The size of each conditioned MEP was expressed as percentage of the unconditioned test MEPs for paired-pulse MEPs (pp-MEPs). The means of SICI, LICI, ICF, and LIF (each 25 MEPs) were calculated for each time point separately. A one-way repeated measures ANOVA (rmANOVA) was used on baseline values to rule out carry over effects between the experimental conditions. The Shapiro–Wilk test was applied to explore the normality of each outcome. Separate repeated measure (rm) ANOVAs were calculated to assess how main effects of 'intensity' with 4 levels (0.3, 0.7, 1, and 1.5), and 'time' with 3 levels (T_{pre}, T_0, and T_{30}) and the interaction between the effects may have affected CSE, SICI, LICI, ICF, and LIF. Mauchly's test was used to assess the validity of the sphericity assumption for the rmANOVAs. Greenhouse–Geisser-corrections were applied for non-spherical data[37]. If a significant effect was found, Bonferroni-corrected post hoc paired-sample t tests were conducted to explore whether the baseline value of each experimental condition differed significantly from post-intervention time points (T_0 and T_{30}).

To assess whether participants were successfully blinded to the experimental conditions, Pearson's chi-square test was used. In addition, a one-way ANOVA was carried out on the mean scores recorded in the numerical analogue scale ratings to assess differences of side effects between sessions. Statistical analyses were performed using SPSS 25 (IBM, NY, USA) with a level of significance of $P = 0.05$ for all statistical tests. All results are presented as mean ± standard error of means (SEM).

Suggestions for future studies. It would be interesting to monitor excitability measures also during stimulation, to receive more profound knowledge about the temporal dynamics of the development of plasticity by tDCS. Furthermore, additional experiments should be conducted to add mechanistic information via more direct exploration of the contribution of ion channels and neurotransmitters to the effects of stimulation, which might also require animal experiments. Coil positioning was performed manually based on physiological data without the aid of a neuronavigation system. This procedure might result in larger MEP variability, as compared to a navigation-supported procedure, which should be taken into consideration for future studies. Moreover, the intensity threshold of M1 a-tDCS should be studied in older adults and patients with neurological disorders to enhance the transferability of the findings, which might be relevant for clinical application of this intervention. Studies using behavioural outcome measures would provide valuable information to investigate if the reversal of CSE effects revealed in the present study also affects motor or cognitive behaviour accordingly.

Conclusions

The results of this study show that increasing the intensity of a-tDCS does not necessarily enhance its efficacy to induce LTP-like plasticity, but might even reverse the direction of the effects, especially for specific prolonged stimulation durations, and that this conversion critically depends on the intensity of stimulation. Moreover, the results show that respective corticospinal effects are mirrored at the level of intracortical circuits.

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Author contributions

M.HZ., M.Z., and S.J. designed the study. M.HZ. performed the experiments. M.HZ. processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. M.HZ. wrote the main manuscript text. M.HZ, MA.N., & S.J. reviewed the manuscript.

Competing interests

This manuscript is based on the research conducted by Maryam Hassanzahraee, Ph.D. candidate at Monash University, Melbourne, Australia. MAN is in the Scientific Advisory Boards of Neuroelectrics, and NeuroDevice. The other authors declare no conflict of interest.

Additional information

Correspondence and requests for materials should be addressed to M.H.

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Preamble to Chapter 7

In Chapters 5 and 6, the duration and intensity thresholds for reversal of anodal transcranial direct current stimulation (a-tDCS) effects on corticospinal excitability (CSE) and the mechanisms behind this reversal are investigated. Besides the effects of a-tDCS duration and intensity thresholds on CSE changes, these thresholds would possibly affect the changes in motor performance following the stimulation. Sequential visual isometric pinch task (SVIPT) is utilized as an assessment tool to examine the changes following a-tDCS on motor performance.

This chapter addresses **Aim 6** to determine whether the reversal of the CSE changes by a-tDCS coincides with similar changes in behavioural outcome measures such as indices of motor performance.

Chapter 7 provides a single-blinded, randomized sham-controlled crossover design to assess the effect of a-tDCS duration and intensity threshold on motor performance.

The findings demonstrate that a-tDCS duration and intensity thresholds have a significant effect on motor performance using SVIPT. It shows that although higher intensities and longer durations would reverse the effect of a-tDCS on CSE (Studies 3 and 4), this indeed could improve motor performance outcomes.

Chapter 7: Does cortical changes following application of anodal-tDCS induce expected behavioral changes: an investigation of counter-regulatory mechanisms?

The format of this chapter is consistent with the *Journal of Physiology (impact factor: 4.54, ranking: Q1 in Neuroscience).* This chapter was submitted on Nov 23rd, 2020.

The Ethics approval, consent form, and Edinburg handedness questionnaires and are provided in Appendices. 1, 2, 5, and 6.

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Title: Does plasticity following application of anodal-tDCS induce the expected behavioral changes? An investigation of counter-regulatory mechanisms

Authors: Maryam Hassanzahraee
Michael Nitsche
Maryam Zoghi
Shapour Jaberzadeh

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Does plasticity following application of anodal-tDCS induce the expected behavioral changes? An investigation of counter-regulatory mechanisms

Maryam Hassanzahraee[a]*, Michael A. Nitsche[b,c], Maryam Zoghi[d], Shapour Jaberzadeh[a]

[a] Non-invasive Brain Stimulation & Neuroplasticity Laboratory, Department of Physiotherapy, School of Primary and Allied Health Care, Faculty of Medicine, Nursing and Health Science, Monash University, Melbourne, Australia.

[b] Department of Neurology, University Medical Hospital Bergmannsheil, Bochum, Germany

[c] Dept. Psychology and Neurosciences, Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany

[d] Department of Rehabilitation, Nutrition and Sport, School of Allied Health, Discipline of Physiotherapy, La Trobe University, Melbourne, Australia

*Corresponding Author: maryam.hassanzahraee@monash.edu

Abstract

Background: Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation tool to modulate brain activity, and induce neuroplasticity. Long-term potentiation-like plasticity-inducing tDCS applied over the primary motor cortex is associated with motor performance improvements in healthy individuals. It has however been shown that tDCS-induced plasticity can change its direction if dosage exceeds certain limits.

Objective: We aimed to assess if the duration and intensity threshold for the reversal of the direction of plasticity induced by anodal tDCS (a-tDCS) from long-term potentiation- to long-term depression-like plasticity changes also the direction of its impact on sequential visual isometric pinch task (SVIPT) performance in healthy individuals.

Methods: Twenty healthy participants attended a sham-controlled single-blind crossover study. They received a-tDCS in five pseudo-randomized sessions: 1) a-tDCS of 1mA for 22 min (excitatory effect), and 2) 30 min (reversal of excitatory effect), 3) a-tDCS of 0.7mA (excitatory effect), 4) 1.5mA for 26 min (reversal of excitatory effect), and 5) sham a-tDCS. SVIPT was conducted before and after application of a-tDCS over the primary motor cortex. Behavioural outcomes, including movement time, error rate and skill calculated as speed/accuracy trade-off were assessed for all blocks assessed across these two time points.

Results: Movement and reaction times (MT and RT) significantly decreased following a-tDCS with intensities of 0.7, 1.5 mA (for 26 min), and longer duration of 30 min (1mA) ($p < 0.01$). MT and RT were not altered by sham stimulation. Error rate and skill did not change significantly in all intervention conditions.

Conclusions: The present findings demonstrate no reversal of tDCS effects on performance, but improved performance with intensified tDCS protocols. The return points of tDCS effects might thus differ between resting-state physiology, and task-related states.

Keywords: primary motor cortex, transcranial direct current stimulation, current intensity, stimulation duration, sequential visual isometric pinch task (SVIPT)

Introduction

Anodal transcranial direct current stimulation (a-tDCS) is a non-invasive brain stimulation technique, which induces long-term potentiation (LTP) -like plasticity of the human brain via application of weak direct currents. Previous studies revealed that motor cortex a-tDCS enhances corticospinal excitability (CSE), as shown by an increase in amplitude of motor evoked potential (MEP) (Nitsche & Paulus, 2000, 2001). At the behavioral level, in general accordance several studies showed that excitability changes induced by a single session of a-tDCS over the primary motor cortex (M1) associated with reduction of reaction time and improvement of motor performance (Fregni et al. 2005; Hummel et al. 2006), pinch force (Hummel et al. 2006; Tanaka et al. 2011), motor control (Hummel et al. 2005; Madhavan et al. 2011), motor learning and adaptation (Nitsche et al. 2003; Boggio et al. 2006; Nitsche and Turner, 2009; Reis et al. 2009; Galea and Celnik, 2009; Tecchio et al. 2010; Fritsch et al. 2010). Hereby, the behavioural effects of a-tDCS are associated with stimulation and task timing. Whereas online stimulation improved motor learning (Nitsche et al. 2003), offline stimulation before task performance had no effects on the same task (Kuo et al. 2008; Stagg et al. 2011). The rationale for these timing-specific effects on motor performance could be synergistic strengthening of task-related excitability and plasticity, as it has been suggested that the same groups of synapses and circuits may be involved in a-tDCS-induced LTP-like plasticity and motor learning (Stagg et al. 2011), including not only glutamatergic plasticity, but also the decreasing effect of a-tDCS on GABA within the stimulated motor cortex (Stagg et al. 2009, 2011; Stagg and Nitsche, 2011), which is related to motor learning-related dynamics (Floyer-Lea et al. 2006).

In the sequential visual isometric pinch task (SVIPT), improvements of sequence motor learning by application of online anodal transcranial direct current stimulation (a-tDCS) have been reported based on trial-based measurements (Reis et al., 2009; Schambra et al., 2011;

Saucedo Marquez et al., 2013). In the SVIPT, a series of trials (consisting of different target forces) are clustered into sequence blocks and participants are required to precisely squeeze a force transducer to reach different target forces as quickly and accurately as possible.

Whereas behavioral studies showed so far relatively uniform, and linear results with different dosages of tDCS, at the physiological level it has been shown that the directionality of tDCS effects critically depends on stimulation intensity, and duration. TDCS with increased duration and intensity during rest induces non-linear physiological effects (Monte-Silva et al. 2013; Bastikadze et al. 2013). In our foregoing studies, we have systematically explored the critical stimulation duration and intensity for reversal of the after-effects of a-tDCS (Hassanzahraee et al, 2020a, b). The results revealed a duration threshold for reversal of the excitability-enhancing effect of a-tDCS of 1mA with stimulation durations $\geq$ 26 min (Hassanzahraee et al, 2020a) and, and a respective intensity threshold for intensities of $\geq$ 1mA with a stimulation duration of 26 min (Hassanzahraee et al. 2020b). This conversion is likely caused by calcium overflow above the LTP-inducing limits induced by too strong, and long stimulation, which results in counterregulatory mechanisms (Lisman et al. 2001; Misonou et al. 2004; Monte-Silva et al. 2013).

For the dosage-dependency of stimulation effects on motor learning, numerous sequence motor learning, and motor reaction time studies showed an improvement of motor performance with a-tDCS applied online (Nitsche et al. 2003; Boggio et al. 2006; Vines et al. 2088a, b; Reis et al. 2009; Tecchio et al. 2010; Fritsch et al. 2010; Schambra et al. 2011; Kantak et al. 2012; Seudo-Marquez et al. 2013; Prichard et al. 2014; Butts et al. 2014). Cuypers et al. (2013) moreover showed intensity-dependent effects with improved efficacy of 1.5, as compared with 1 mA stimulation. These results suggest tDCS-intensity-dependent effects on motor performance. However, no study has been conducted so far to explore if an

upper dosage-dependent limit of performance-improving tDCS effects does exist, similar to the conversion of the directionality of plasticity.

In the present study, we aimed to explore if such a non-linearity of tDCS effects on motor performance does exist. We explored offline a-tDCS effects on motor performance in order to be able to modulate not only stimulation intensity, but also duration. We hypothesized that the physiological reversal of a-tDCS effects would be mirrored by behavioral outcome measures of the SVIPT.

Methods

Study Design

The study was conducted in a single-blinded crossover sham-controlled design with at least seven day washout period between the sessions (Woods et al, 2016). To reduce the risk of circadian influences on experimental outcomes, all experimental sessions carried out at the same time of the day (Krause and Cohen Kadosh, 2014; Li et al, 2015).

Participants

Twenty non-smoking healthy right-handed volunteers (12 females mean age 26.95 ± 6.3 (SD) years) participated in this study. Each participant was pseudo-randomly assigned to five different experimental sessions in counterbalanced order. The parameter selection of a-tDCS was based on the results of our previous neurophysiological studies (Hassanzahraee et al. 2020 a, b) in which excitability-enhancing effects of a-tDCS emerged with (1) 1mA for 22min, and (2) a-tDCS of 0.7mA for 26min, but an excitability-diminishing effect of a-tDCS was observed by stimulation with (3) 1mA for 30min, (4), and 1.5mA for 26min. The participants underwent also a session of sham a-tDCS.

None of the participants had contraindications to tDCS (Brunoni et al, 2011), including history of seizure, intake of CNS-acting medications, psychiatric or neurological disorders (Nitsche et al, 2008; Rossini et al, 2015), any disability affecting finger, hand, or wrist movements, and all had normal or corrected-to-normal vision. All participants were naive to the purpose of the experiments. Each participant gave written informed consent before attending the study. The Human Research Ethics Committee of Monash University approved the study and we conform to the Declaration of Helsinki (1991, BMJ, 302, 1194).

Sequential visual isometric pinch task (SVIPT)

The SVIPT is a pinch force task in which the participants hold a force transducer (ADinstrument MLT004/ST, NSW, Australia) between the thumb and middle phalanx of the index index finger of the right hand. They were asked to squeeze the force transducer to reach different target forces displayed on the computer screen (Figure 1). The force transducer is an isometric dynamometer that converts biological signals into electrical analogue signals digitized using PowerLab data acquisition hardware.

Experimental Procedure

At the beginning of each session and after stimulation, maximum voluntary contraction (MVC) was determined for each participant to calibrate the force transducer. Each participant conducted two familiarization blocks before the first session. In all sessions, the participants performed three baseline blocks (pre-intervention measurement). The inter-block interval was set at 1 min. Each block consisted of eight trials and each trial included seven forces (10, 15, 20, 25, 30, 35, and 40% of MVC) in random order. The random order was chosen to exclude any probability of sequence learning. The inter-trial interval was set at 1s. Each target force was only shown once in each trial. Participants were asked to squeeze the force transducer as quickly and accurately as possible to move the cursor towards the target force. A green line and a number displayed on an indicator box on the computer screen determined the level of each target force. Each target force within the range of ± 5% MVC of the target level was counted as hit. Any movement above and below this 5% MVC range was considered over- and under-shoot, respectively, categorized as an error and calculated for each block. After reaching each target, the participants released the transducer. As soon as the cursor returned back to the starting point, the next target appeared on the screen with visual feedback. There was no feedback given by the investigator. Participants performed three blocks at baseline and immediately after tDCS. (Figure 1). <<Please insert Figure 1 in here>>

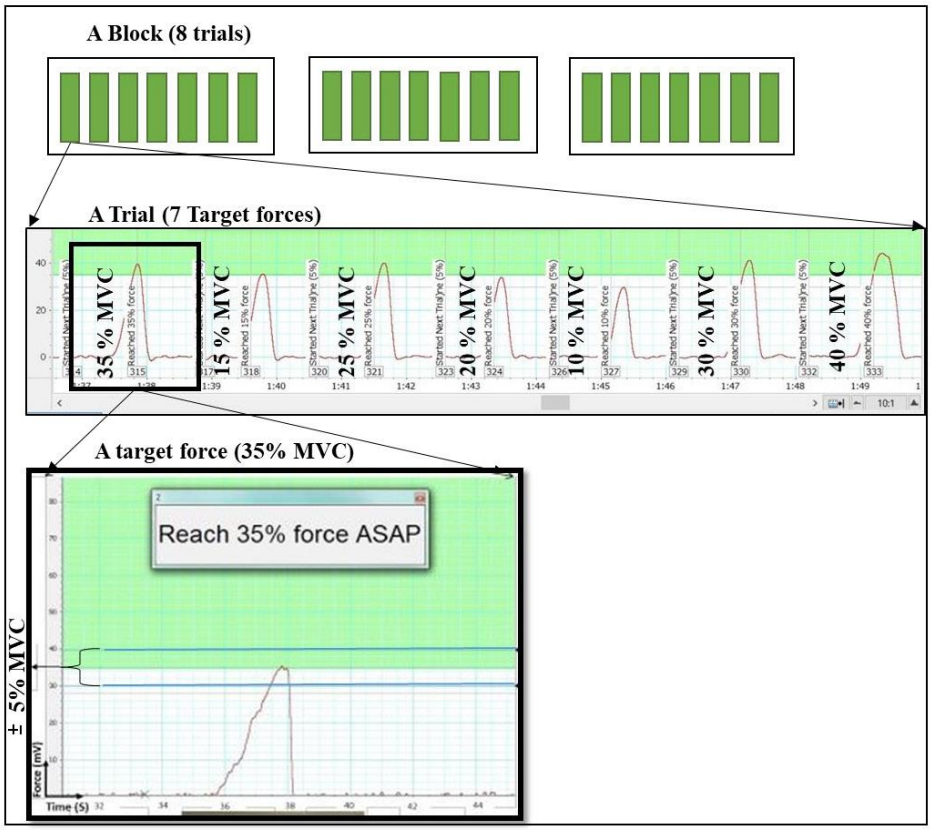

Figure 1. Sequential visual isometric pinch task (SVIPT). Each participant was instructed to squeeze a force transducer as quickly and precisely as possible to reach each target force that appeared on the computer screen. Each sequence block consisted of eight trials, which included seven different target forces from 10 to 40 % of the individual MVC.

Anodal transcranial direct current stimulation

A-tDCS was administered by a battery-driven stimulator (NeuroConn, Germany) through a pair of rubber electrodes enclosed in saline-soaked surface sponge (5×7 cm; area: 35cm^2). The selection of the stimulation parameters was based on the results of previous neurophysiological studies (Hassanzahraee et al, 2020a, b). The target electrode (anode) was placed over the left M1 over the hand motor area (C3) following the international 10-20 system, and the return electrode (cathode) over the right supraorbital area. In five pseudo-randomly ordered sessions, a-tDCS was applied with current intensities of 0.7 and 1.5 mA for 26 min, durations of 22 and 30 min for 1mA intensity, and Sham a-tDCS. To minimize any potential discomfort, a 15s ramp-up/ down was applied at the beginning and end of the stimulation. In the sham condition, the electrodes were placed in the same position as with a-tDCS, but the stimulator was turned off after 30 seconds of stimulation.

The experimental design of the study is summarised in Figure 2.

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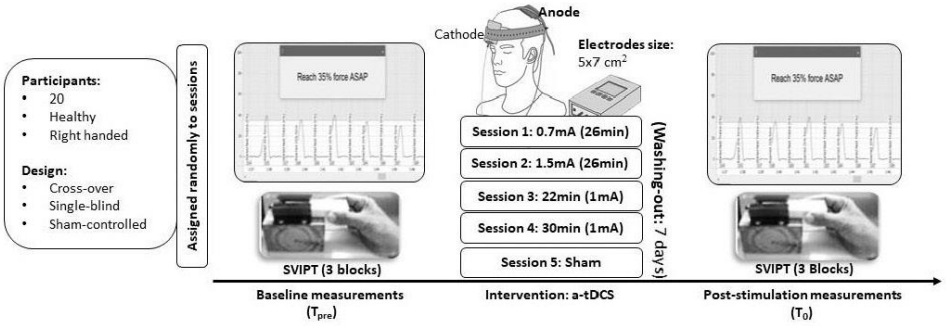

Figure 2. Schematic representation of the experimental procedure for each session. The SVIPT was measured at baseline and following a-tDCS to track performance changes following stimulation. The time course is from left to right. **A-tDCS**: Anodal transcranial direct current stimulation, **SVIPT**: sequential visual isometric pinch task.

Outcome measures

In each trial, movement time (MT) was measured from the start of the first target movement to the return to baseline of the last target movement. The mean MT of eight trials in each block was considered as MT for that given block (Reis et al. 2009). The mean MT before and after tDCS was calculated by averaging the MTs of the respective three blocks. Reaction time (RT) was defined as the time interval between visual stimulus appearance and the initiation of movement. RT was measured for each target force within a trial, and the average of RTs from eight trials counted as the RT of that block. The mean RT before and after tDCS was calculated by averaging the RTs of the respective three blocks. The error rate (ER) was calculated as the proportion of trials with at least one under -or overshooting movement (Reis et al. 2009). The mean ER was calculated by averaging the ER of all three blocks at two time points. The skill index is a combination of movement time and error rate, and represents changes in the speed-accuracy trade-off. The following formula was used to calculate skill development (Reis et al. 2009). Skill index was calculated within each block, and the average of the three blocks before, and after tDCS represents the mean skill.

$$Skill = \frac{1 - error\ rate}{error\ rate\ [\ln(movement\ time)^{5.424}]}$$

Statistical analyses

The Shapiro-Wilk test was applied to explore the normality of data. A one-way ANOVA was conducted to exclude baseline performance differences between experimental conditions in normal distributed data, while a Kruskal-Wallis H test one-way analysis by rank was used for non-normally distributed data. Separate repeated measure (rm) ANOVAs were conducted to assess how the main factors of 'condition','time' and the interaction between these affected movement, reaction time, and skill changes: **1)** an rmANOVA with duration as the main factor of 'condition' with 3 levels (22, 30 min, and sham), and 'time' with 2 levels (T_{pre} and T_{post}). **2)** an rmANOVA with intensity as the main factor of 'condition' with 3 levels (intensities of 0.7, 1.5mA, and sham) and 'time' with 2 levels (T_{pre} and T_{post}).

Mauchly's test was used to assess the validity of the sphericity assumption for the rmANOVAs. Greenhouse-Geisser-corrections were applied for non-spherical data (Meyers et al, 2005). If a significant effect was found, Bonferroni-corrected post hoc paired-sample t-tests were conducted to explore the presence of significant differences between tDCS conditions over time (T_{pre} vs T_{post}), and compared to sham.

For non-normally distributed data, a log transformation was performed in order to achieve normal distribution of the data. After transformation, if the skewness of the log data was still > 1, non-parametric tests were conducted. In this case, the Friedman two-way analysis of variance by ranks was used to assess differences in the mean rank of non-parametric variables across the two time points. A K-independent median test was conducted to evaluate whether the median differed between groups and time points. Data analysis was performed via MATLAB (R2018b) and SPSS 25 (IBM, NY, USA) with a level of significance of $P = 0.05$ for all statistical tests. All results are presented as mean ± standard error of means (SEM).

Measurement of side effects

Side effects were recorded at the beginning, middle and the end of stimulation in all experimental sessions. All participants were asked to complete a questionnaire to record their sensations and any side effects. The questionnaire contained rating scales for the presence and severity of common side effects such as itching, tingling, and burning sensations under the electrodes (Poreisz et al, 2007; George and Aston-Jones, 2010), and other adverse effects including headache and pain during and after stimulation (Brunoni et al, 2011). The unpleasantness of any sensation was rated via numeric analogue scales (NAS) (i.e. 0 = no sensation to 10 = worst imaginable sensation). To test blinding integrity, participants were asked were asked to indicate the nature of stimulation (active or sham) they received. They answered choosing 'Yes', 'No'.

Results

All participants completed all experimental sessions and tolerated the experimental conditions well. The Shapiro-Wilk test confirmed the normal distribution of the data for movement and reaction times ($p > 0.05$) but not for error rate and skill ($p < 0.05$). Non-parametric analyses were performed for the latter variables, since error rate and skill data were not corrected to normal distribution by log-transformation ($p < 0.05$).

The one-way parametric and non-parametric ANOVAs conducted for baseline performance comparisons of different stimulations durations (intensity: 1mA), and intensities (duration: 26min) revealed no significances for the dependent variables movement time, reaction time, error rate, and skill (Table 1). <<<Please insert Table 1 in here>>>

		Baseline Measurement						
		Movement time (s)	Reaction time (s)		Error rate		Skill	
Intensity Group	0.7mA	12.49 ± 0.20	0.90 ± 0.03		31.73		28.45	
	1.5mA	12.40 ± 0.21	0.89 ± 0.03		29.33		31.30	
	sham	12.48 ± 0.19	0.88 ± 0.01		32.25		31.75	
	ANOVA	F	0.26	0.27	H Test	H	0.88	0.52
		P	0.76	0.75		P	0.24	0.77
Duration Group	22min	12.44 ± 0.18	0.87 ± 0.02		29.2		31.05	
	30min	12.46 ± 0.25	0.87 ± 0.03		30.05		30.25	
	sham	12.48 ± 0.19	0.88 ± 0.01		32.25		31.75	
	ANOVA	F	0.05	0.04	H Test	H	0.82	0.03
		P	0.97	0.98		P	0.38	0.98

Table 1. Baseline measurements. A one-way ANOVA was calculated for inter-session differences of the average baseline movement and reaction time. The parametric data are reported as **mean ± standard error of mean** (SEM). A Kruskal-Wallis test was used for error rate, and skill. The non-parametric data are reported as **mean rank**. There was no significant difference across experimental sessions for all baseline measurements.

Effects of different a-tDCS durations (intensity of 1mA for 22 and 30 min)

Movement time

The results of the two-way rmANOVA showed a significant main effect of 'Time' ($F_{(1, 19)}$ = 10.78, P = 0.004, η_p^2 = 0.36), and the 'condition × Time' interaction ($F_{(2, 38)}$ = 3.76, P = 0.04, η_p^2 = 0.17) on movement time. However, no significant effect was found for the main effect of 'condition'. Pairwise comparisons revealed that movement time significantly improved by application of 30 min a-tDCS (1mA) as compared to baseline (P < 0.001), 22 min (P < 0.01) and sham a-tDCS (P < 0.01) (Figure 2).

Reaction time

The results of the two-way rmANOVA showed a significant main effect of 'condition' ($F_{(2, 38)}$ = 3.38, P = 0.043, η_p^2 = 0.16) and 'Time' on reaction time ($F_{(1, 19)}$ = 7.65, P = 0.012, η_p^2 = 0.28). Pairwise comparisons revealed that reaction time significantly improved by application of 30 min a-tDCS (1mA) as compared to baseline (P < 0.001) and sham (P < 0.05) conditions (Figure 3).

Error rate and skill

Error rate and skill were not normally distributed; therefore, the non-parametric Friedman's test was conducted. No significant difference was found for error rate, and skill (P > .05) for application of different durations (intensity 1mA) of a-tDCS (Figure 4 and 5).

Effects of different a-tDCS intensities (0.7 and 1.5 mA for 26 min)

Movement time

The results of the two-way rmANOVA showed a significant main effect of 'Time' ($F_{(1, 19)} = 12.85$, $P = 0.002$, $\eta_p^2 = 0.40$) and the 'condition × time' interaction ($F_{(2, 38)} = 3.22$, $P = 0.046$, $\eta_p^2 = 0.14$). No significant effects were observed for the main effect of 'condition', and. Pairwise comparisons revealed that movement time significantly decreased after a-tDCS with an intensity of 0.7 and 1.5 mA compared to baseline ($P < 0.05$ and $p < 0.01$, respectively). Movement time was significantly reduced at an intensity of 1.5 mA compared to 0.7 mA ($P < 0.05$) and sham ($P < 0.01$) (Figure 2).

Reaction time

The results of the two-way rmANOVA showed a significant main effect of 'Time' ($F_{(1, 19)} = 5.97$, $P = 0.02$, $\eta_p^2 = 0.25$) on reaction time. No significant effects were observed for the main effect of 'condition', and the 'condition × time' interaction. Pairwise comparisons revealed that movement time significantly decreased after a-tDCS with an intensity of 0.7 and 1.5 mA compared to baseline ($P < 0.05$ and $p < 0.01$, respectively) (Figure 3).

Error rate and skill

No significant difference was found for error rate, and skill ($P > 0.05$) using different intensities (duration 26 min) of a-tDCS (Figures 4 and 5).

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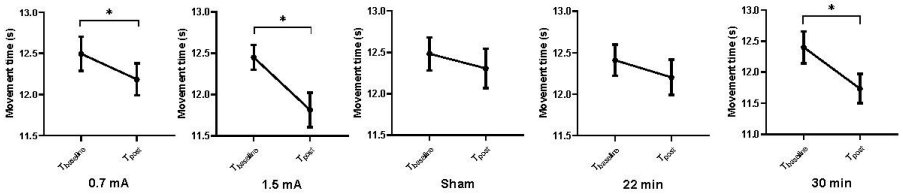

Figure 2: Effects of a-tDCS on **movement time**. The mean MT was calculated by averaging the MTs (of eight trials) for each block. Each shape presents the mean MT of all three blocks for each group at each time point. Values are shown as means ± SEM. There was a significant improvement using a-tDCS for 30 min (intensity of 1mA) for movement time compared to baseline. There was significant difference in MT between duration of 30 min with duration of 22 min and sham. Significant differences were found at a-tDCS intensities of 0.7 and 1.5 mA (duration 26min) compared to baseline. There was significant difference between 1.5 mA with intensity of 0.7 mA and sham. (*) shows the significant difference.

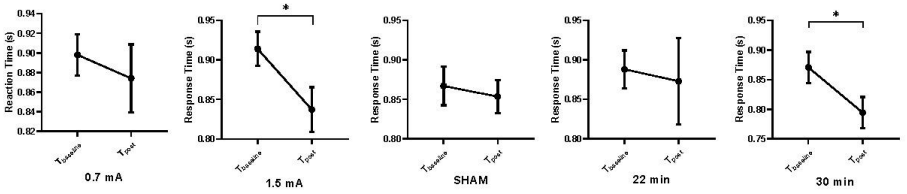

Figure 3. Effects of a-tDCS on **Reaction time**. The mean RT was calculated by averaging the RTs (of eight trials) for each block. Each shape presents the mean RT of all three blocks for each group at each time point. Values are shown as means ± SEM. There was significant differences at a-tDCS with duration of 30 min (intensity of 1mA) compared to baseline, duration of 22 min and sham. There was significant improvement following intensity of 1.5mA compared to baseline. (*) shows the significant difference.

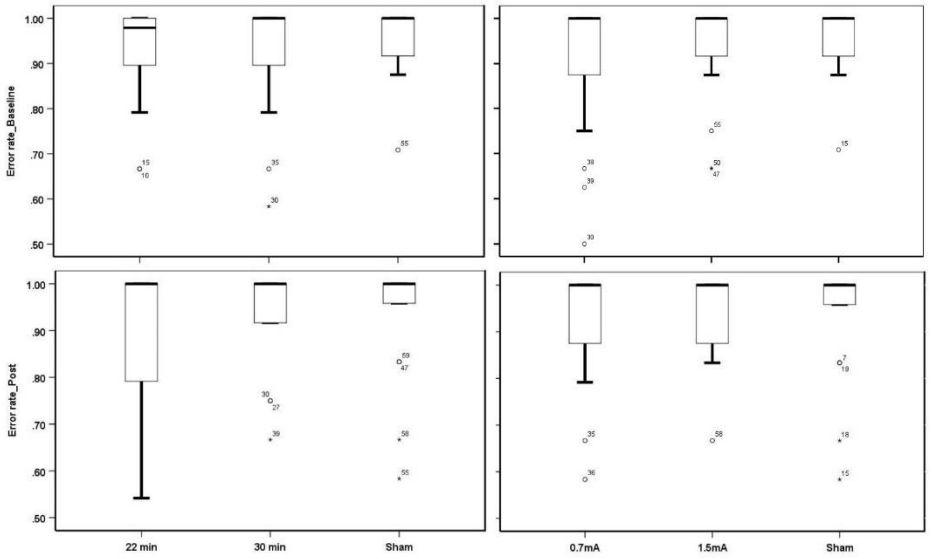

Figure 4. Effects of a-tDCS on error rate in the the different conditions before, and after tDCS. A. Different durations (intensity of 1mA) and sham a-tDCS. B. Different intensities (Duration of 26min) and sham a-tDCS. No significant effects between all stimulation conditions were found. The boxes show the interquartile range (IQR) of data (25 – 75%). The lower whiskers (bars) show the lower extreme (at most 1.5 IQR). The horizontal bar in the boxes shows the Median. The dot shows the outlier.

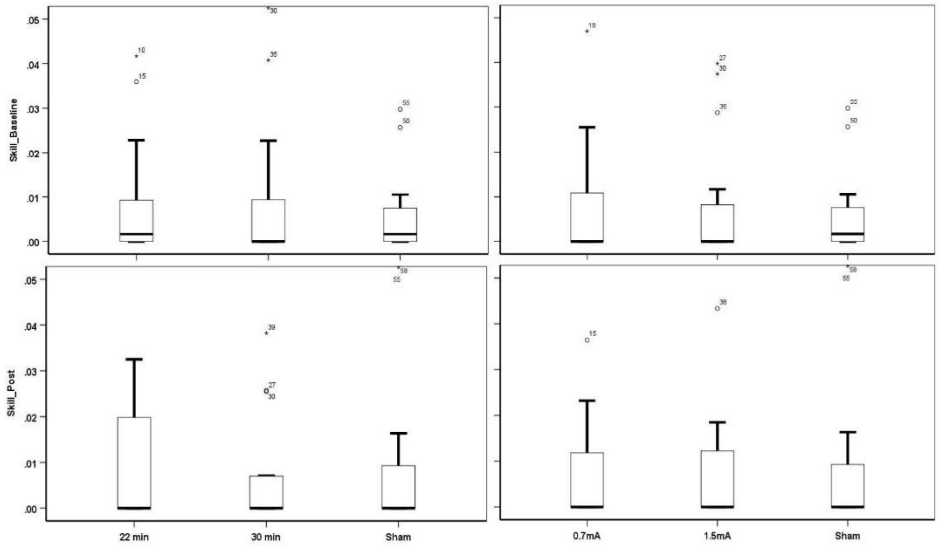

Figure 5. Effects of a-tDCS on skill between the different conditions at two time points. **A.** With different durations (intensity of 1mA) and sham a-tDCS. **B.** With different intensities (Duration of 26min) and sham a-tDCS. No significant effects were found between all stimulation conditions. The boxes show the interquartile range (IQR) of data (25 – 75%). The upper whiskers (bars) show the upper extreme (at most 1.5 IQR). The horizontal bar in the boxes shows the Median. The dot shows the outlier.

Safety and Side Effects of A-tDCS

No adverse effects were reported after a-tDCS, except tingling and light itching under the electrodes during stimulation reported by some of the participants in all experimental conditions. The results of the one-way ANOVA indicated that sensations did not differ between conditions ($P > 0.05$). No burning sensations, headache, or pain were reported during or after stimulation. The Pearson's chi-square test conducted to control for blinding showed no significant differences between conditions [$\square^2$ (4, n = 20) = 2.76, P = 0.22]. The percentage of participants who replied 'No' ask for the presence of stimulation was 85%. This result demonstrates that participants were unable to distinguish between the experimental conditions and active versus sham a-tDCS in this study. Therefore, blinding integrity was successful in the present study.

Discussion

The present study was designed to explore whether different electrophysiological effects of a-tDCS based on stimulation duration, and intensity (Hassanzahraee et al. 2020a, b) are reflected by related behavioural outcomes as assessed by SVIPT. We measured behavioural outcomes including movement time, reaction time, error rate and skill, the main outcome parameters of the SVIPT (Reis et al., 2009, 2015; Schambra et al., 2011; Saucedo Marquez et al., 2013). The findings showed that movement and reaction times improved using longer a-tDCS duration (1mA, 30 min) compared to baseline and a stimulation duration of 22 min. For the effect of stimulation intensity, a-tDCS with 0.7 and 1.5 mA (stimulation duration of 26 min in both conditions) improved movement and reaction time significantly compared to baseline. Respective improvements of movement times were moreover significant versus sham stimulation for both, longer durations, (30min). The results did not show any difference of stimulation on error rate and skill.

Although these findings show an improvement of movement and reaction times by a-tDCS, , these changes were relatively minor, as compared to other studies in the field. Moreover, error rate and skill were not altered by a-tDCS. In this connection, it is relevant to discern the current study from previous studies on sequence motor learning with respect to at least two parameters. In difference to other studies in the field, (Nitsche et al. 2003; Kuo et al. 2008; Vines et al. 2008; Reis et al. 2009, 2015; Stagg et al. 2011; Schambra et al., 2011; Saucedo Marquez et al. 2013; Kuo and Nitsche, 2015) we did not explore the effect of a-tDCS on learning. We aimed to eliminate the potentially confounding effects of motor learning, and explored how the physiological effects of different a-tDCS durations and intensities would affect simple motor performance. Moreover, he majority of previous studies revealed greater online improvement resulting from stimulation compared to sham (Nitsche et al. 2003; Stagg et al. 2011a, b; Amadi et al. 2015). Therefore, the lack of significant changes in this study on

behavioural outcomes might be due to offline stimulation (Kuo et al. 2008). The minor, but still observable effect of offline stimulation in the present study, might be a hint that offline stimulation indeed might work, but require intensified stimulation protocols. In a study in 2013 however, it was reported the significantly improved motor performance improved both online and offline in healthy participants as compared to sham when a-tDCS of 1.5 mA (20 min) combined with motor training (Cuypers et al. 2013). They however, revealed that there was no significant differences between a-tDCS of 1.5 with 1 mA, and 1 mA with sham. Although their study were on the combination of stimulation and training, their findings on offline and online stimulation were in line with ours confirming an intensity-dependent effect of stimulation. It may because of less relevant learning-related plasticity to pure reaction time and raise the need of excitability alteration. The lower efficacy of offline stimulation might be caused by the lack of synergistic effects of simultaneous stimulation- and task-related activation on the respective target neurons.

Alternatively, due to the relatively simple task used in this study, which did not include learning, the lack of significant results might be related to a ceiling effect of task performance not stimulation. Although longer/ stronger stimulation result in minor improvement on the effects of stimulation on behaviour, this is not due to the ceiling of stimulation effects as no effects were induced at the lowest dosage of a-tDC.

The results on the error rate did not show significant changes neither for longer durations nor for stronger currents. If this is affected by a ceiling effect, then an intensified stimulation would not work. If it is no ceiling effect, intensified protocols might however increase efficacy further and the improvement in accuracy and speed may be required repeated intervention (Camus et al. 2009).

With respect to mechanisms of the effects, it is obvious that the tDCS-induced performance alterations differ from physiological effects of these stimulation protocols, which were obtained in previous studies (Hassanzahraee et al. 2020a, b). In the current study, prolonged stimulation (26 and 30 min) and stronger intensities improved movement and reaction times. In previous brain physiology studies, prolonged stimulation (1mA for durations ≥ 26min) and higher intensities (≥ 1mA for 26 min) reversed the excitability-enhancing effects of a-tDCS on CSE into an excitability diminution (Hassanzahraee et al. 2020a, b). Thus one might speculate that the reversal threshold for task-related plasticity is higher than that for resting state plasticity induced by tDCS. This might be partially due to the relatively lower efficacy of offline, as compared to online tDCS. This argument is supported by previous studies on motor learning, showing no effect of tDCS applied before task performance (Kuo et al. 2008; Stagg et al, 2011). However, results are not completely homogeneous, since in other motor tasks, offline stimulation improved performance (Antal et al. 2004). An alternative explanation might be homeostatic effects of the intensified stimulation protocols, assuming that these induced LTD-like plasticity. This would be in accordance with the results of the study conducted by Antal and co-workers (2004). But since lower stimulation dosages, which should have enhanced cortical excitability, had no effects, this explanation seems unlikely.

Limitations of the study

The current findings should be interpreted in the context of some limitations. First, the target population was healthy adults; therefore, the results may not extrapolate one-to-one to patients with neurological, or psychiatric disorders. Furthermore, data were obtained in young participants (under 40 years); older individuals may respond differently to the applied a-tDCS conditions. Moreover, in the current study, the a-tDCS effect was only assessed immediately after the stimulation, which limits our understanding with respect to possible longer lasting effects or changes developing with a delay. This study investigated offline stimulation which is less efficient than online stimulation thus, we do not know what happen in online stimulation. The studied outcomes in this paper were purely behavioural therefore, the mechanisms have to be clarified in future studies. As tDCS is a neuromodulatory technique, the effects are state-dependent, and inter-individually variable. This therefore, might gradually differ between individuals and make it difficult to believe that the plasticity turning point are identical between the present and previous studies (Hassanzahraee et al. 2020a, b). Indeed, inter-individual differences might make it complicated to anticipate individual turning points based on the group data and this could limit the interpretation.

Suggestions for future studies

Further studies are required to explore the mechanisms behind changes of motor performance induced by a-tDCS, including optimal dosages, by combining behavioral with physiological outcome measures. This includes the investigation of the existence of and dosages for turning points of performance, and the exploration of online stimulation effects, as well as dosage-dependent stimulation effects on motor learning.

Conclusions

Our results show that a-tDCS at a dosage which did not induce LTP-like plasticity, but reduced motor cortex excitability in the resting state, improved motor task performance when applied before conduction of the task. This might hint for different thresholds for plasticity converting tDCS dosages in resting, and active conditions. Alternatively, performance-improving homeostatic effects cannot be ruled out. The improvements where relatively minor however, which might be caused by the offline stimulation, and thus it might be speculated that for efficient offline stimulation, intensified protocols might be required.

Conflict of interest

This manuscript is based on research conducted by Maryam Hassanzahraee, PhD candidate at Monash University, Melbourne, Australia. This project had no external funding, and no financial or other relationships pose a conflict of interest. MAN is member of the scientific advisory boards of Neuroelectrics, and NeuroDevice.

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Chapter 8: Concluding Remarks

8.1. Remarks on the findings addressing the Thesis aims

Based on the Thesis aims explained in Chapter 1 – Section 1.15, different studies have been carried out in this thesis. To explore Thesis aims, and provide concluding remarks on how I addressed these aims, I have divided the studies in this thesis into four categories:

1. A literature review to address aim one (book chapter),
2. A systematic review of the literature to address aim two (Study 1),
3. A reliability study to address aim three (Study 2),
4. A determination of anodal transcranial direct current stimulation (a-tDCS) duration and intensity thresholds for reversal of the effects on corticospinal excitability (CSE) to address aims four and five (Studies 3 and 4) and finally,
5. An investigation if the reversal of the effects could be generalised to non-cortical outcomes such as changes in motor performance (Study 5).

In these concluding remarks, a brief summary of the findings from each of the studies will be provided with reflections on their novelty, significance, comparison to previous studies and importance to the tDCS literature. This will include the findings from all studies addressing all thesis aims. Additionally, overall thesis limitations will be discussed as well as the implications the results of these studies will have for future research in the tDCS literature.

8.1.1 A literature review (Book chapter)

The first thesis aim was to review the literature providing a list of TMS side and adverse effects as an assessment tool to better understand them and the ways to minimise these risks.

TMS as an assessment tool is a safe technique in both adults and children older than two years. The most common side effect of TMS is a headache which is transient and usually subsides after a few hours. The most severe side effects of TMS are seizures and syncope which proper screening may significantly reduce their rare occurrence. This chapter presents a comprehensive list of TMS side or adverse effects, contraindications and recommendations for the optimal use of TMS. Additionally, risks of TMS in children, pregnancy, TMS operators, and patients with neurological conditions are also briefly discussed.

The significance of chapter two in relation to the remaining chapters is it comprehensively investigated previously TMS safety guidelines. By carefully summarising available literature about TMS safety, it provided a guideline of TMS side/ adverse effects and how to solve if it happens to be used in the main studies of this thesis (chapters 5 and 6).

8.1.2 A systematic review of the literature (Study 1/ Chapter 3)

The second thesis aim was to review literature for investigating the effects of different priming non-invasive brain stimulation (NIBS) protocols on a consequent NIBS test protocol of primary motor cortex (M1) on CSE in healthy individuals that could potentially contribute to response variability.

In study 1, a systematic review and meta-analyses were carried out to find and evaluate studies that used two successive NIBS as priming and test protocols and investigated the magnitude and direction of the priming technique on the effect of the test protocols on M1 CSE in healthy individuals. Based on the meta-analysis findings in chapter 3, it can be assumed that according to the homeostatic mechanisms, priming would reduce or even reverse the expected effects of the test protocol, if both protocols had the same effect on excitability. However, the effects of test protocol would be boosted if the priming has an opposite effect on excitability (Muller-Dahlhause and Ziemann, 2015; Karabanov et al. 2015). This effect, indeed, confirms the non-homeostatic patterns that may interact to increase the effect lasting of the test protocol.

This systematic review reveals that the efficiency of priming-test protocols of M1depends on the stimulation, duration and magnitude (intensity, frequency) of the both protocols. In addition, it shows how different plasticity mechanisms would regulate the effects based on the types of consecutive protocols. This improves our understanding regarding how the expected outcomes of an intervention (NIBS techniques) could be intensified in the clinical applications.

This systematic review also provides an in-depth insight regarding how different levels of synaptic history such as applying priming protocols, stimulation longer durations, and/ or higher intensities would reduce or even reverse the a-tDCS effect on CSE. This concept shapes the core designs of Studies 3 and 4 to investigate how systematic changes of a-tDCS parameters would modify the a-tDCS effect by sliding the modification threshold based on the level of synaptic

history. According to the high I-squared values found in the meta-analysis of the data, it should be considered that some systematic influences on the results have to be explored in the future researches.

8.1.3 Reliability study (Study 2/ Chapter 4)

The third aim of this thesis had two parts that was primarily to investigate the effects of TMS inter-trial intervals (ITIs: 5, 10, 15 and 20s) on intra- and inter-session reliability of MEP amplitude, and secondarily to explore how different ITIs would affect the variability of TMS induced MEPs. Any tDCS application includes pre- and post-intervention measurements to investigate changing following the stimulation on CSE changes. Therefore, these measurements were planned to assess the effects of a-tDCS on CSE changes in studies 3 and 4. The study two was carried out to ensure that post-intervention changes are not because of systematic errors and/ or methodological inconsistencies in the recorded TMS-induced MEPs. In comparison to previous similar studies on ITI (Moller et al. 2009; Schmidt et al. 2009; Julkunen et al. 2012; Pelliciari et al. 2016), this study was the first series designed to find out the effects of different ITIs using single-pulse TMS on the averaged size of MEP amplitudes, MEP variability, as well as intra- and inter-session reliability.

This study also showed that longer ITIs increases the size of MEP amplitudes with higher inter- and intra-session reliability and lower variability. It could be concluded that longer ITIs not only reduces the chance of TMS-induced changes in CSE, but also help us to use this assessment tool in studies with smaller sample sizes. As was also recommended previously (Stamoulis et al, 2011), longer ITIs seem to be safer than shorter ITIs (< 10 s). Thus, in some clinical applications, it may be appropriate to used longer ITIs especially when high numbers of pulses are required.

The significance of this chapter in relation to the remaining chapters of the current thesis was the revealed statistically significant reliability of both within- and between –session intra-rater reliability. Therefore, this finding ensured that the TMS-measurements following stimulation

would reveal the changes following intervention used in the main studies of the current thesis (chapters 5 and 6) rather than intra-rater/ technical inconsistencies.

8.1.4 Determination of a-tDCS duration threshold for reversal of CSE (Study 3/ Chapter 5)

The fourth aim of this thesis was to determine a-tDCS "duration" threshold for reversal of the effects on CSE and to explore the underlying neurophysiological mechanisms behind these changes.

In study three**,** the a-tDCS duration threshold for reversal of stimulation effect was investigated. A significant number of studies, over the last few years, have done using a-tDCS as a non-invasive neuromodulatory technique. A large number of these studies showed the polarity-dependent excitatory effects of a-tDCS on CSE and motor performance. However, recent studies have challenged this assumption by revealing no change or even reduction of CSE following the use of a-tDCS. These findings confirm a more complex dose-response relationship between the a-tDCS parameters and the size of CSE changes (Wiethoff et al. 2014; Lopez-Alonso 2014, 2015, Tremblay et al. 2016). The study three reveals the a-tDCS (1mA) duration threshold of ≥ 26 min for reversal of excitatory effects on CSE changes. The findings also demonstrate the contribution of intracortical circuits in the expressed corticospinal changes. All of these changes illustrate a crucial significance of metaplastic mechanisms for the a-tDCS effects.

This study made a novel contribution to the tDCS literature by suggesting that prolonged applications of a-tDCS will not simply increase its efficiency to cause LTP-like plasticity, but also might reverse the effect direction. This threshold for the effect reversal of a-tDCS in healthy participants also confirms the concept of 'ceiling effect' in these simulation techniques. This

effect cannot be simply overcome by using of prolonged applications and may involve a more complex adjustment (Monte-Silva et al. 2013).

8.1.5 Determination of a-tDCS intensity threshold for reversal of CSE (Study 4/ Chapter 6)

The fifth aim of this thesis was to determine a-tDCS "intensity" threshold for reversal of the effects on CSE and to explore the underlying neurophysiological mechanisms behind these changes.

In study four**,** the a-tDCS intensity threshold for reversal of stimulation effect was investigated. The current findings support the assumption of a-tDCS intensity and duration windows for linear effects however, they also reveal that exceeding stimulation parameters beyond specific limits results in non-linear effects. Increasing stimulation intensity/ duration therefore does not necessarily improve the efficacy of a-tDCS, in principle accordance with previous studies (Monte-Silva et al., 2013; Bastani and Jaberzadeh, 2013, Kidgell et al., 2013; Vignaud et al., 2018; Agboada et al., 2019). The findings determine the intensity threshold of ≥ 1 mA for the duration of 26 min that reverse the direction of excitatory effect. The findings also show that higher intensities would activate counter-regulatory mechanisms to avoid excessive brain excitation. It is suggested that these mechanisms might reverse the LTP-like to LTD-like plasticity and have a relationship with the prolonged stimulation. A secondary conversion of after-effects of tDCS with even higher stimulation intensities and duration was found in other studies (for a-tDCS: Agboada et al., 2019, and cathodal tDCS: Mosayebi et al., 2019).

This study made a novel contribution to the tDCS literature by suggesting that stronger applications of a-tDCS will not simply increase its efficiency to cause LTP-like plasticity, but also might reverse the effect direction. The findings also demonstrate that beyond a certain

windows of stimulation parameters would activate counter-regulative mechanisms and result in expressed reversed corticospinal changes.

8.1.6 The effects of a-tDCS duration and intensity thresholds on motor performance (Study 5/ Chapter 7)

The sixth aim of this thesis was to determine whether the reversal of the CSE changes by a-tDCS coincides with similar changes in behavioural outcome measures such as indices of motor performance.

The growing numbers of studies on SVIPT suggested it as a fine motor task required strong coordination of visual and motor systems (Reis et al, 2009, 2015; Schambra et al., 2011; Saucedo Marquez et al., 2013). A respective muscle engaged in this task is FDI which is the target cortical zone for stimulation by tDCS. Precise pinch force is an essential part of most daily life activities and may be affected following some of the brain lesions such as stroke.

The findings of study 5 showed an improvement of movement and reaction times by a-tDCS, as compared to baseline and sham conditions, but no alternations in error rate and skill. Although the effects on behaviour measures using a-tDCS were minor, there was higher efficacy with higher dosage. Indeed, a reversal of physiological effects following intensified a-tDCS (longer/ stronger stimulation) (study 5 and 6) may be related to the improvement of movement and reaction times, which were absent with a-tDCS with excitability-enhancing effects on targeted regions (lower duration and intensity). The minor effect of offline stimulation in this study, which is in accordance with other studies (Kuo et al. 2008; Stagg et al. 2011), might be a hint that offline stimulation indeed might work, but require intensified stimulation protocols. Other studies also revealed a lack of correlation between stimulation-induced plasticity, and motor learning (Li Voti et al. 2011; Vallence et al. 2013, Lopez-Alonso et al. 2015).

This study made a novel contribution to the tDCS literature by suggesting that return points of tDCS effects might thus differ between resting-state physiology, and task-related states. The present study reveals no reversal of tDCS effects on performance, but improved performance with the intensified tDCS protocols.

8.2 Thesis Limitations

Limitations of each study in the thesis have been previously presented in each chapter (Chapters 4 - 7) and therefore will not be repeated in this section. It is however important to recognize that there are limitations to the overall interpretations that can be drawn from the findings of the studies that comprise this thesis. These will be briefly discussed in this section.

Thesis limitation 1: The participants in the studies (2 – 5) of this thesis were selected from young individuals under the age of forty-five among university students and staff as a sample of convenience. This was in the effort to minimize the influence of older-age-related changes (Tecchio et al. 2008; Fujityama et al. 2014; Heise et al. 2014) on changes in CSE and cortico-cortical excitability following tDCS. It is also important to be noted that the neural activity levels between young adults significantly vary with older adults, adolescents, and/ or the "more-plastic" brain of children. Therefore, the investigation into the relationship between different age groups and changes in CSE and motor performance following tDCS duration and intensity thresholds appears a logical next step for future research looking to provide an in-depth understanding of differences in responses to tDCS between different age groups.

Thesis limitation 2: All attended participants in the studies (2 – 5) of this thesis were healthy. In neurological patients, brain function and reaction to stimulation might be different. Therefore, our findings cannot be generalized to populations of different ages other than young adults and/ or patients with neurological conditions and this offers the opportunity for further investigation about tDCS effects turning points on CSE and motor performance in neurological patients.

Thesis limitation 3: The participants attended in the studies of this thesis were from both genders and the gender differences were not explored. Although it is important to split and study

the participants in the gender-specific group as a factor of variability, this was not possible in this thesis due to the limited population, budget, and time. Therefore, the investigation into the relationship of gender-specific and changes (reversal) in the effect of a-tDCS duration and intensity threshold on CSE and motor performance appears a logical next step for future research looking to develop a deeper understanding of differences in responses to tDCS between genders.

Thesis limitation 4: A-tDCS was used in Studies 3 - 5 of this thesis; therefore, the findings could not be translated to cathodal tDCS (c-tDCS) or other brain stimulation techniques with expected excitatory effects. It does, however, offer the opportunity for further research into the effect of c-tDCS duration and intensity thresholds on CSE and motor performance and their interaction with each other.

Thesis Limitation 5: The after-effect in the main studies of this thesis was assessed only for only up to 30 min post-stimulation. Due to time constraints in conducting experimental sessions and data collection, the further assessment of tDCS effects to obtain deeper insight into the long-lasting after-effects of a-tDCS was not feasible. This, therefore, provides the opportunity for the investigation to look to an understanding for possible further lasting effects or delayed developing changes on CSE and motor performance.

8.3 Recommendations for future research

As with the thesis limitations above, recommendations on future directions of research have been previously addressed in each chapter (Chapters 4 - 7). They will therefore not be repeated in this section. What will be briefly discussed however is the recommendations for future research based on the overall findings of the thesis as a body of work.

Recommendation 1: Investigations exploring the duration and intensity threshold of M1 a-tDCS and its effects on CSE and motor performance in different age ranges (children/ adolescents, older adults) and patients with neurological disorders would be valuable to enhance the transferability of the findings to different age groups.

Recommendation 2: As discussed above in limitation two, only healthy adults were selected in the main studies of this thesis in an attempt to increase the participant homogeneity and reduced potential inter-individual variability. Therefore, it is recommended that similar studies be systematically conducted on neurological patients to provide information about the parameter range of a-tDCS and thresholds turning points effects on CSE and motor performance.

Recommendation 3: As discussed in limitation three, due to physiological differences between males and females and the potential effect of gender on the delivered current into the brain (Russel et al. 2014), it would be highly recommended to develop new independent studies on the different genders. This will enable investigation into the interaction between gender and thresholds turning point effect of tDCS on CSE and motor performance to check if the findings in the main studies of this thesis are gender-specific or not.

Recommendation 4: It is recommended that similar study designs be implemented in future studies to determine the c-tDCS duration and intensity thresholds, their effects on CSE, and the relationship between cortical changes and motor performance.

Recommendation 5: It is recommended for future tDCS studies to check when the observed changes in current studies will return to the pre-stimulation state and implement the longer follow-up measurements. This will enable investigation into the long-lasting and delayed effects of tDCS on CSE and motor performance and understanding the mechanisms that encode for key regulators of motor cortical plasticity.

Other Recommendations: It is also recommended that future research include neuroimaging and current flow modelling components to further verify the physiologic effects of stimulation. The neuroimaging techniques such as electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) can provide more exact details regarding functional brain connectivity, the involved motor network, and brain-tissue effect during the reversal of effects on CSE (Zheng et al., 2011; Amadi et al., 2014; Muthalib et al., 2015; Sood et al., 2016). Additionally, neuroimaging may determine factors that affect individual differences in the outcome of tDCS, such as baseline brain state. It would be also interesting to shift the focus from studying results of tDCS at a group level and showing that some respond and others do not, to finding the reasoning behind the inter-individual variability in response to tDCS either as the responders or non-responders that make it possible to move the findings toward clinical translation of tDCS. Moreover, some modelling or optimisation technology could be the step forward in future studies to explore any possible parameter space and enhance the accuracy of data if needed. Furthermore, it would be helpful to use a combination of data (duration and intensity) across different experiments in future exploration to find out any parameter space

relationship across the experiments. It is also suggested to use a regression-based approach or LME to combine data across experiments into a global statistical model.

8.4 Implications for future research

The implications of the findings from the studies in this thesis are that they raise questions about the efficiency of tDCS application in the clinical setting on individuals with neurological and psychological disorders. The main studies of this thesis provide further evidence challenging the linear tDCS dosage-response relationship assumption in healthy young participants. The main studies of this thesis reported a-tDCS duration and intensity thresholds of the effects on CSE. These thresholds did reverse the effect of a-tDCS as on CSE, there were not found any changes as expected on motor performance. This adds to the growing body of work within the tDCS literature of studies reporting high response variability following tDCS in young healthy individuals (Wiethoff et al. 2014; López-Alonso et al., 2014, 2015; Chew et al., 2015; Strube et al., 2015, 2016; Tremblay et al., 2016; Viguad et al. 2019). The reversal of a-tDCS effects on CSE following prolonged and stronger stimulation as reported in this study does once again challenge its reliable application in the clinical setting, as an ultimate goal of the tDCS literature. The main studies in this thesis were in the attempt to understand the dosage-response relationship in a-tDCS and improve the expectations regarding its widespread usage. These suggestions for future research are in the endeavor to progress the use of tDCS to more consistent use in the clinical setting. The tDCS in particular, and NIBS literature, in general, discussed personalized medicine or in other words, individualized treatment (Koch & Hummel, 2017; Yavari et al. 2017; Cocchi & Zalesky, 2018). These recommendations, therefore, can implement key first steps to achieve effective and reliable future application in pathological populations and provide personalized and tailored tDCS protocols to optimize the treatment to all individuals.

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APPENDICES

Appendix 1. Human Ethics Certificate of Approval

MONASH University

Monash University Human Research Ethics Committee

Approval Certificate

This is to certify that the project below was considered by the Monash University Human Research Ethics Committee. The Committee was satisfied that the proposal meets the requirements of the *National Statement on Ethical Conduct in Human Research* and has granted approval.

Project Number: 10546
Project Title: Effects of anodal and cathodal tDCS on cortical excitability and neural activation: the effects of priming protocols
Chief Investigator: Assoc Professor Shapour Jaberzadeh
Expiry Date: 01/09/2022

Terms of approval - failure to comply with the terms below is in breach of your approval and the *Australian Code for the Responsible Conduct of Research*.

1. The Chief Investigator is responsible for ensuring that permission letters are obtained, if relevant, before any data collection can occur at the specified organisation.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by MUHREC.
4. You should notify MUHREC immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. The Explanatory Statement must be on Monash letterhead and the Monash University complaints clause must include your project number.
6. Amendments to approved projects including changes to personnel must not commence without written approval from MHUREC.
7. Annual Report - continued approval of this project is dependent on the submission of an Annual Report.
8. Final Report - should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected completion date.
9. Monitoring - project may be subject to an audit or any other form of monitoring by MUHREC at any time.
10. Retention and storage of data - The Chief Investigator is responsible for the storage and retention of the original data pertaining to the project for a minimum period of five years.

Thank you for your assistance.

Professor Nip Thomson

Chair, MUHREC

Appendix 2. Explanatory statement

MONASH University

EXPLANATORY STATEMENT

Project Title:
The effects of anodal transcranial direct current stimulation on cortical and behavioural changes: An investigation of counter-regulatory mechanisms

Chief Investigator's name

A/Prof Shapour Jaberzadeh
Department of Physiotherapy
School of Primary Health Care
Faculty of Medicine, Nursing & Health Sciences
Monash University
Phone: 9904 4827
Email: shapour.jaberzadeh@monash.edu

Student's name

Maryam Hassanzahraee
Department of Physiotherapy
School of Primary Health Care
Faculty of Medicine, Nursing & Health Sciences
Monash University
Phone: 9904 4827
Email:
maryam.hassanzahraee@monash.edu

You are invited to take part in this study. Please read this Explanatory Statement in full before deciding whether or not to participate in this research. If you would like further information regarding any aspect of this project, you are encouraged to contact the researchers via the phone numbers or email addresses listed above.

What does the research involve?

The primary aim of this project is to determine intra-session and inter-session reliability of single pulse TMS induced MEPs.

Why were you chosen for this research?

You have been invited to participate in this study because you have responded to the related advertisement and have met the following criteria:
- You are at least 18 years old
- You are able to understand and follow instructions in English
- You met the inclusion criteria as outlined by the screening questionnaire

Why were you not chosen to participate?

You were not chosen to participate in this research as you met at least one of the following criteria:
- Over the age of 40
- Psychiatric or neurological illness (e.g. brain injury)
- History of seizures, epilepsy
- Metal implants in the head excluding the mouth
- Implanted cardiac pacemaker
- Implanted neuro-stimulator
- Experiences frequent severe headaches and a history of migraines
- Pregnant
- Taking any medications or excessive intake of caffeine or energy drinks
- Sleep deprived
- Unable to interpret and communicate in English
- Past history of nerve conduction issues (e.g. Carpal Tunnel Syndrome, nerve grafts, nerve entrapment syndromes)
- Experiencing neurological symptoms (e.g. numbness, tingling, pins and needles, overnight pain)

- Skin conditions prone to irritability

What does this research involve?
This study will be carried out at the Non-Invasive Brain Stimulation and Neuroplasticity Laboratory in the Department of Physiotherapy (Room B1.09, Building B, Peninsula Campus, Monash University) and participants will attend four sessions of 120-minute and four sessions of 60-minute for following up. Prior to taking part in this study, a screening questionnaire will be completed to assess for suitability to the use of Transcranial Magnetic Stimulation. Each intervention will involve electrodes placed over the scalp and forehead and non-invasive brain stimulation administered for 16-22 minutes. Once the intervention is complete, the same pre-intervention outcome measures of brain excitability and motor behaviour will be repeated at 0, 30 and 120 minutes and 24 hours post-intervention.
Transcranial Magnetic Stimulation will be used to record outcome measures of brain excitability. It is considered a safe and painless assessment technique commonly used in non-invasive brain stimulation laboratories for research purposes. It will be applied at rest in a comfortable sitting position through a magnetic coil which the assessor will hand-hold above your head. Measures of brain excitability to be recorded via surface electrodes that are placed on the intrinsic muscles of your hand in between your thumb and index finger.

How long will this research take?
Each of the four sessions will take approximately 120 minutes with an overall time commitment of 4-5 hours across the four sessions. Each session will have 24-hour follow up.

Will I be reimbursed for my participation in this research?
Each participant will be reimbursed a total of $20 per session. Therefore each participant will receive $80 at the conclusion of the four 120-minute session. Participants will be reimbursed in full at the completion of the forth 120-minute session. The reimbursement will be in the format of a Coles/Myer gift card to the value of $80. Participants will not be reimbursed in cash or direct bank account transfer.

Are there any risks in participating in this study?
As stated above, the use of Transcranial Magnetic Stimulation is considered safe and painless and is a commonly used technique for this form of research. Despite this, while chances of experiencing adverse effects are low, the potential risks associated with Transcranial Magnetic Stimulation in this study are as follows:
- Headaches. Participants may experience slight headache symptoms following transcranial magnetic stimulation

- Seizures. There is very small potential that participants may experience seizure following transcranial magnetic stimulation
Additionally, while receiving the non-invasive brain stimulation technique Transcranial Direct Current Stimulation, you may experience different sensations, including:
- Mild itching, tingling or burning during the stimulation

- Mild headache symptoms following the stimulation

- Mild skin reactions under the electrode placement following the stimulation

Can I withdraw from this study?
Participation in this study is voluntary. You are free to withdraw from this study at any time.

What will happen to my information?
You will be assigned a code number for this study. All information about you will be coded with this number. All forms of information sheets will be stored in a locked filing cabinet in a locked office for the duration of the study. Data stored electronically will be protected by security passwords. The results of this study will form the basis of a PhD thesis that may become accessible via the internet. Papers arising from the thesis will be submitted for publication in scientific journals and will also be presented at conferences.
Despite this, no publications arising from this work will enable any individual participant identification.
At the completion of the study, all forms and questionnaires will be filed in a locked cabinet in a locked office for 5 years as per Monash University policy. After this, they will be destroyed in a confidential manner. Nobody other than members of the research team will have access to these files at any stage. You may request a copy of personal information collected in the course of the research at any stage of the study.

Where will my information be stored?
Storage of the data for this research project will comply with Monash University regulations. Data will be kept on Monash University property in a secure location for 5 years.

What if I require counselling as a result of this research?
Should you experience any issues with participation in this research and require counselling, please do not hesitate to contact the Monash University Counselling Services at the following addresses:
Berwick Campus: Monash Connect, Building 930
Caulfield Campus: University Health Services, Building B, Level 1
Clayton Campus: University Health Services, 21 Chancellor's Walk, Campus Centre, Ground Floor
Parkville Campus: Monash Connect, Sissons Building, Ground Floor
Peninsula Campus: University Health Services, Building U, Level 1
Phone: +61 3 9905 3020 (for all campuses)
Hours: 9am – 5pm Monday – Friday
Cost: Free of charge
Website: http://www.monash.edu/health/counselling/counselling-appointments

What if I have any complaints?
Should you have any complaints concerning the manner in which this research is conducted, please do not hesitate to contact the Monash University Human Research Ethics Committee at the following address:
Executive Office, Human Research Ethics
Monash University, Human Research Ethics Committee (MUHREC)
Room 111, Building 3E
Research Office
Monash University VIC 3800
Tel: 9905 2052
Fax: 9905 3831
Email: muhrec@adm.monash.edu.au

Thank you again for your participation.

A/Prof Shapour Jaberzadeh

Mrs. Maryam.Hassanzahraee

Appendix 3. TMS safety assessment form

MONASH University
Medicine, Nursing and Health Sciences

Project Title: .....

Screening questions for initial telephone contact

Inclusion criteria: Participant

☐ Is an adult aged 18 years or older?

☐ Is right handed?

☐ Is able to speak, read and write English comprehension

Exclusion criteria:

Please circle your response. Have you ever:

1. Had an adverse reaction to Transcranial Magnetic Stimulation (TMS)?	Yes/No
2. Had a seizure or epileptic fit?	Yes/No
3. Had an Electroencephalogram (EEG)?	Yes/No
4. Had a stroke?	Yes/No
5. Had a head injury or neurosurgery?	Yes/No
6. Do you have any metal in your head (outside of the mouth,) such as shrapnel, surgical clips, or fragments from welding or metalwork?	Yes/No
7. Do you have any implanted devices such as cardiac pacemakers, medical pumps, or intracardiac lines?	Yes/No
8. Do you suffer from frequent or severe headaches?	Yes/No
9. Have you ever had any other brain-related condition?	Yes/No
10. Have you ever had any illness that caused brain injury?	Yes/No
11. Are you taking any medications?	Yes/No
Please specify:	
12. If you are a woman, are you pregnant or is it possible that you may be pregnant?	Yes/No
13. Does anyone in your family have epilepsy?	Yes/No
14. Do you need further explanation of Transcranial Magnetic Stimulation and its associated risks?	Yes/No

If you answered yes to any of the above, please provide details (use reverse if necessary):

.....

.....

I certify that the above information is correct to the best of my knowledge. I have read and understand all of this form and I have had the opportunity to ask questions regarding the information on this form.

Participant's name: .....

Participant's signature: .....

Date: .....

Appendix 4. Administration and data collection checklist

Name:

Participant No.	Start	PRE-RMT	80% RMT: **A**	120% RMT	PRE-1mV: **B**	PRE-**B/A** ratio	End	SICI, LICI, ICF TIME	End	Stimulation End	**Immediately** after Measurements of **CSE with PRE – RMT and 1mV INTENSITY (25 +25 MEPs)**	POST-RMT	80% RMT: **A**	POST-1mV: **B**	POST-**B/A** ratio	End Immediately	Post 30 min Start
Session one																	
Session two																	
Session three																	
Session four																	
Session five																	

Appendix 5. A-tDCS safety assessment form

MONASH University
Medicine, Nursing and Health Sciences

Side effects: Day 1

Subject name/code _____ Dominant side _____

Date _____ Gender _____

Numerical Analog Scale (NAS)

		Tingling Itching Burning sensation Warmness Discomfort	Headache and/or nausea	Fatigue	Others (specify)	Skin redness
Active electrode (M1)	Beginning (2 min)					
	Middle A (7-10 min)					
	Middle B (16-19 min)					
	End (Last min)					
Return electrode (Right supra orbital)	Beginning (2 min)					
	Middle A (7-10 min)					
	Middle B (16-19 min)					
	End (Last min)					

Distraction attributable to tDCS (0:10)	
Detectability of tDCS status (yes or no)	

Appendix 6. Consent form

CONSENT FORM

Project title: The effects of anodal transcranial direct current stimulation on cortical and behavioural changes: An investigation of counter-regulatory mechanisms
Study title: Determination of anodal tDCS duration threshold for reversal of corticospinal excitability: an investigation for induction of counter-regulatory mechanisms
Chief investigator: Dr. Shapour Jaberzadeh, Senior Lecturer Physiotherapy, School of Primary Health Care, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne - Peninsula Campus, Tel: 9904 4827
Email: shapour.jaberzadeh@monash.edu
PhD student: Maryam Hassanzahraee, Physiotherapist, PhD Candidate, Physiotherapy Department, School of Primary Health Care, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne – Peninsula Campus
Email: maryam.hassanzahraee@monash.edu
NOTE: This consent form will remain with the Monash University researcher for their records

☐ I agree to take part in the Monash University research project specified above. I have had the project explained to me, and I have read the Explanatory Statement, which I can keep for my records. Any questions I have asked have been answered to my satisfaction.

☐ I agree to take part in the following experimental procedures:

a. Transcranial Magnetic brain Stimulation (TMS) and Transcranial direct current stimulation (tDCS)

b. Recording of muscle activity using surface electrodes (EMG)

☐ I understand that I can withdraw all records of my participation in the study up until completion of the final exercise session for the study.
☐ I understand the possible risks of TMS stimulation, such as seizure.

☐ I understand that my participation is voluntary, that I can choose not to participate in part or all of the project, and that I can withdraw at any stage of the project without being penalized or disadvantaged in any way.

☐ I understand that any information I provide is confidential and that no information that could lead to the identification of any individual will be disclosed in any reports on the project, or to any other party.

☐ I understand that data from this study will be kept in secure storage and access to the research team. I also understand that the data will be destroyed after 5 years.

☐ I understand that any data that the researcher uses from the study reports or in published findings will not, under any circumstances, contain names or identifying characteristics.

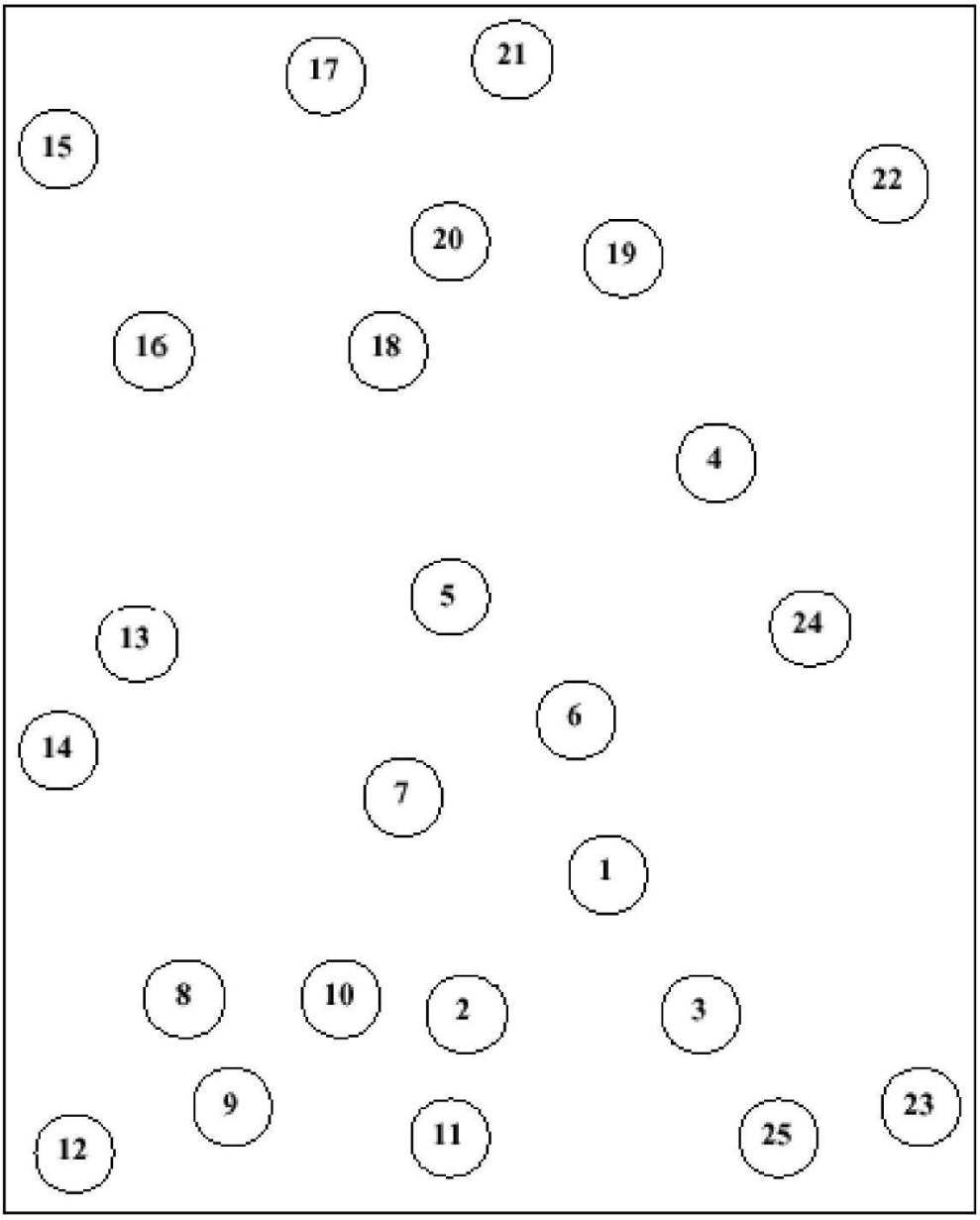
Participant Name: **Signature:**

Researcher's name: **Signature:** **Date:**

Appendix 7. Trail making test

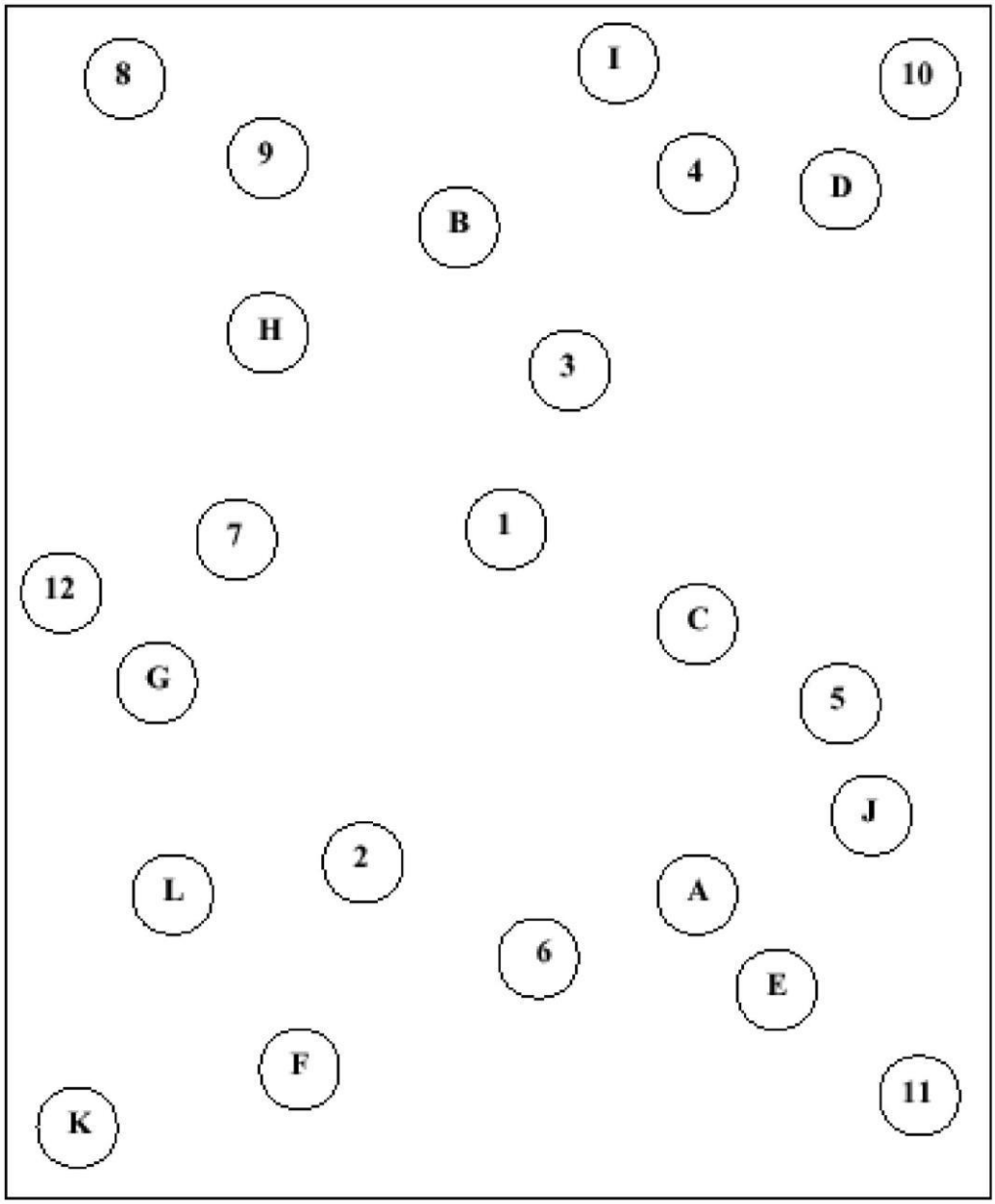
Trail Making (Part A)

Patient's Name: _____ Date: _____



Trail Making (Part B)

Patient's Name: _____ Date: _____



Appendix 8. Edinburgh Handedness Questionnaire

Subject's Initials: Age: Height (cm): Weight (kg):

Please indicate with a check (✓) your preference in using your left or right hand in the following tasks.

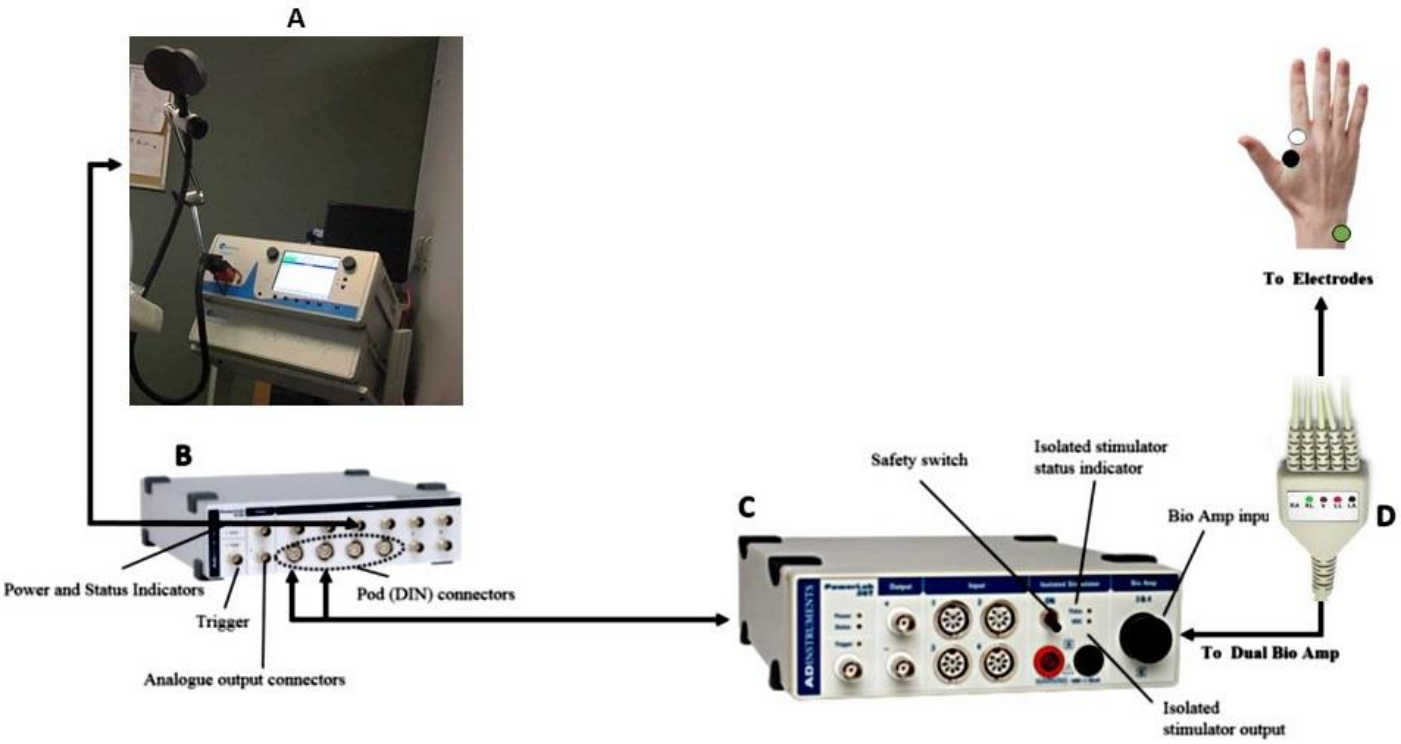
Where the preference is so strong you would never use the other hand unless forced to, put two checks (✓✓).

If you are indifferent, put one check-in in each column (✓/✓).

Some of the activities require both hands. In these cases, the part of the task or object for which hand preference is wanted is indicated in parentheses.

Task / Object	Left Hand	Right Hand
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking a Match (match)		
10. Opening a Box (lid)		
Total checks:	LH =	RH =
Cumulative Total	CT = LH + RH =	
Difference	D = RH – LH =	
Result	R = (D / CT) × 100 =	
Interpretation: (Left-Handed: R < -40) (Ambidextrous: -40 ≤ R ≤ +40) (Right-Handed: R > +40)		

Appendix 9. TMS set-up system used in the studies 3 - 5



A) MagVenture TMS machine, B) The PowerLab 8/30 has three indicators at the left frontal panel, one BNC connector for the external trigger, two BNC connectors for analog output, and eight BNC connectors (marked input 1-8) with four alternative pods (DIN) connectors for inputs 1 – 4, for recording external signals C) Dual Bio amp/stimulator D) Cables for recording EMG of the target muscle.

Appendix 10. Sample-size considerations in this Thesis

Power analysis for the analysis of variance

This appendix describes statistical procedures for power analysis and sample size estimation for studies of this thesis using analysis of variance. Sample size could be easily determined based on the effect size of the pilot study. The SPSS reports the effect size index as eta (η2). The below table gives power estimates for different values of the effect size index, *f*, at df_b = 1 to 6, 8, 10 at α = 0.05.

The sample size needed for the ANOVA for α = 0.05 (Adapted from Cohen J. (1988))

	f											
Power	**0.05**	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**	**0.40**	**0.50**	**0.60**	**0.70**	**0.80**
$df_b = 1$												
0.70	1235	310	138	78	50	35	26	20	13	10	7	6
0.80	1571	393	175	99	64	45	33	26	17	12	9	7
0.90	2102	526	234	132	85	59	44	34	22	16	12	9
$df_b = 2$												
0.70	1028	258	115	65	42	29	22	17	11	8	6	5
0.80	1286	322	144	81	52	36	27	21	14	10	8	6
0.90	1682	421	188	106	68	48	35	27	18	13	10	8
$df_b = 3$												
0.70	881	221	99	56	36	25	19	15	10	7	6	5
0.80	1096	274	123	69	45	31	23	18	12	9	7	5
0.90	1415	354	158	89	58	40	30	23	15	11	8	7
$df_b = 4$												
0.70	776	195	87	49	32	22	17	13	9	6	5	4
0.80	956	240	107	61	39	27	20	16	10	8	6	5
0.90	1231	309	138	78	50	35	26	20	13	10	7	6
$df_b = 5$												
0.70	698	175	78	44	29	20	15	12	8	6	5	4
0.80	856	215	96	54	35	25	18	14	9	7	5	4
0.90	1098	275	123	69	45	31	23	18	12	9	7	5
$df_b = 6$												
0.70	638	160	72	41	26	18	14	11	7	5	4	4
0.80	780	195	87	50	32	22	17	13	9	6	5	4
0.90	995	250	112	63	41	29	21	16	11	8	6	5
$df_b = 8$												
0.70	548	138	61	35	23	16	12	9	6	5	4	3
0.80	669	168	75	42	27	19	14	11	8	6	4	4
0.90	848	213	95	54	35	24	18	14	9	7	5	4
$df_b = 10$												
0.70	488	123	55	31	20	14	11	8	6	4	3	3
0.80	591	148	66	38	24	17	13	10	7	5	4	3
0.90	747	187	84	48	31	22	16	13	8	6	5	4
Adapted from Cohen J. (1988)												

Appendix 11. Checklist for reporting the quality assessment by Downs & Black scale

Appendix

Checklist for measuring study quality

Reporting

1. *Is the hypothesis/aim/objective of the study clearly described?*

yes	1
no	0

2. *Are the main outcomes to be measured clearly described in the Introduction or Methods section?*
   If the main outcomes are first mentioned in the Results section, the question should be answered no.

yes	1
no	0

3. *Are the characteristics of the patients included in the study clearly described ?*
   In cohort studies and trials, inclusion and/or exclusion criteria should be given. In case-control studies, a case-definition and the source for controls should be given.

yes	1
no	0

4. *Are the interventions of interest clearly described?*
   Treatments and placebo (where relevant) that are to be compared should be clearly described.

yes	1
no	0

5. *Are the distributions of principal confounders in each group of subjects to be compared clearly described?*
   A list of principal confounders is provided.

yes	2
partially	1
no	0

6. *Are the main findings of the study clearly described?*
   Simple outcome data (including denominators and numerators) should be reported for all major findings so that the reader can check the major analyses and conclusions. (This question does not cover statistical tests which are considered below).

yes	1
no	0

7. *Does the study provide estimates of the random variability in the data for the main outcomes?*
   In non normally distributed data the inter-quartile range of results should be reported. In normally distributed data the standard error, standard deviation or confidence intervals should be reported. If the distribution of the data is not described, it must be assumed that the estimates used were appropriate and the question should be answered yes.

yes	1
no	0

8. *Have all important adverse events that may be a consequence of the intervention been reported?*
   This should be answered yes if the study demonstrates that there was a comprehensive attempt to measure adverse events. (A list of possible adverse events is provided).

yes	1
no	0

9. *Have the characteristics of patients lost to follow-up been described?*
   This should be answered yes where there were no losses to follow-up or where losses to follow-up were so small that findings would be unaffected by their inclusion. This should be answered no where a study does not report the number of patients lost to follow-up.

yes	1
no	0

10. *Have actual probability values been reported(e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?*

yes	1
no	0

External validity

All the following criteria attempt to address the representativeness of the findings of the study and whether they may be generalised to the population from which the study subjects were derived.

11. *Were the subjects asked to participate in the study representative of the entire population from which they were recruited?*
    The study must identify the source population for patients and describe how the patients were selected. Patients would be representative if they comprised the entire source population, an unselected sample of consecutive patients, or a random sample. Random sampling is only feasible where a list of all members of the relevant

population exists. Where a study does not report the proportion of the source population from which the patients are derived, the question should be answered as unable to determine.

yes	1
no	0
unable to determine	0

12. *Were those subjects who were prepared to participate representative of the entire population from which they were recruited?*
The proportion of those asked who agreed should be stated. Validation that the sample was representative would include demonstrating that the distribution of the main confounding factors was the same in the study sample and the source population.

yes	1
no	0
unable to determine	0

13. *Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?*
For the question to be answered yes the study should demonstrate that the intervention was representative of that in use in the source population. The question should be answered no if, for example, the intervention was undertaken in a specialist centre unrepresentative of the hospitals most of the source population would attend.

yes	1
no	0
unable to determine	0

Internal validity - bias

14. *Was an attempt made to blind study subjects to the intervention they have received ?*
For studies where the patients would have no way of knowing which intervention they received, this should be answered yes.

yes	1
no	0
unable to determine	0

15. *Was an attempt made to blind those measuring the main outcomes of the intervention?*

yes	1
no	0
unable to determine	0

16. *If any of the results of the study were based on "data dredging", was this made clear?*
Any analyses that had not been planned at the outset of the study should be clearly indicated. If no retrospective unplanned subgroup analyses were reported, then answer yes.

yes	1
no	0
unable to determine	0

17. *In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls ?*
Where follow-up was the same for all study patients the answer should yes. If different lengths of follow-up were adjusted for by, for example, survival analysis the answer should be yes. Studies where differences in follow-up are ignored should be answered no.

yes	1
no	0
unable to determine	0

18. *Were the statistical tests used to assess the main outcomes appropriate?*
The statistical techniques used must be appropriate to the data. For example non-parametric methods should be used for small sample sizes. Where little statistical analysis has been undertaken but where there is no evidence of bias, the question should be answered yes. If the distribution of the data (normal or not) is not described it must be assumed that the estimates used were appropriate and the question should be answered yes.

yes	1
no	0
unable to determine	0

19. *Was compliance with the intervention/s reliable?*
Where there was non compliance with the allocated treatment or where there was contamination of one group, the question should be answered no. For studies where the effect of any misclassification was likely to bias any association to the null, the question should be answered yes.

yes	1
no	0
unable to determine	0

20. *Were the main outcome measures used accurate (valid and reliable)?*

For studies where the outcome measures are clearly described, the question should be answered yes. For studies which refer to other work or that demonstrates the outcome measures are accurate, the question should be answered as yes.

yes	1
no	0
unable to determine	0

Internal validity - confounding (selection bias)

21. *Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?*
For example, patients for all comparison groups should be selected from the same hospital. The question should be answered unable to determine for cohort and case-control studies where there is no information concerning the source of patients included in the study.

yes	1
no	0
unable to determine	0

22. *Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?*
For a study which does not specify the time period over which patients were recruited, the question should be answered as unable to determine.

yes	1
no	0
unable to determine	0

23. *Were study subjects randomised to intervention groups?*
Studies which state that subjects wererandomised should be answered yes except where method of randomisation would not ensure random allocation. For example alternate allocation would score no because it is predictable.

yes	1
no	0
unable to determine	0

24. *Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?*
All non-randomised studies should be answered no. If assignment was concealed from patients but not from staff, it should be answered no.

yes	1
no	0
unable to determine	0

25. *Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?*
This question should be answered no for trials if: the main conclusions of the study were based on analyses of treatment rather than intention to treat; the distribution of known confounders in the different treatment groups was not described; or the distribution of known confounders differed between the treatment groups but was not taken into account in the analyses. In non-randomised studies if the effect of the main confounders was not investigated or confounding was demonstrated but no adjustment was made in the final analyses the question should be answered as no.

yes	1
no	0
unable to determine	0

26. *Were losses of patients to follow-up taken into account?*
If the numbers of patients lost to follow-up are not reported, the question should be answered as unable to determine. If the proportion lost to follow-up was too small to affect the main findings, the question should be answered yes.

yes	1
no	0
unable to determine	0

Power

27. *Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?*
Sample sizes have been calculated to detect a difference of x% and y%.

	Size of *smallest* intervention group	
A	$<n_1$	0
B	n_1–n_2	1
C	n_3–n_4	2
D	n_5–n_6	3
E	n_7–n_8	4
F	n_9+	5

Appendix 12. Web Plot digitizer

Plot or Graph Digitizer is a Java program, which is used to digitize scanned plots of many types of functional data. Often data is found presented is represented in reports and references as functional X-Y type scatter, linear, semi-log, or log-log plot. To use this data, it must somehow be digitized.

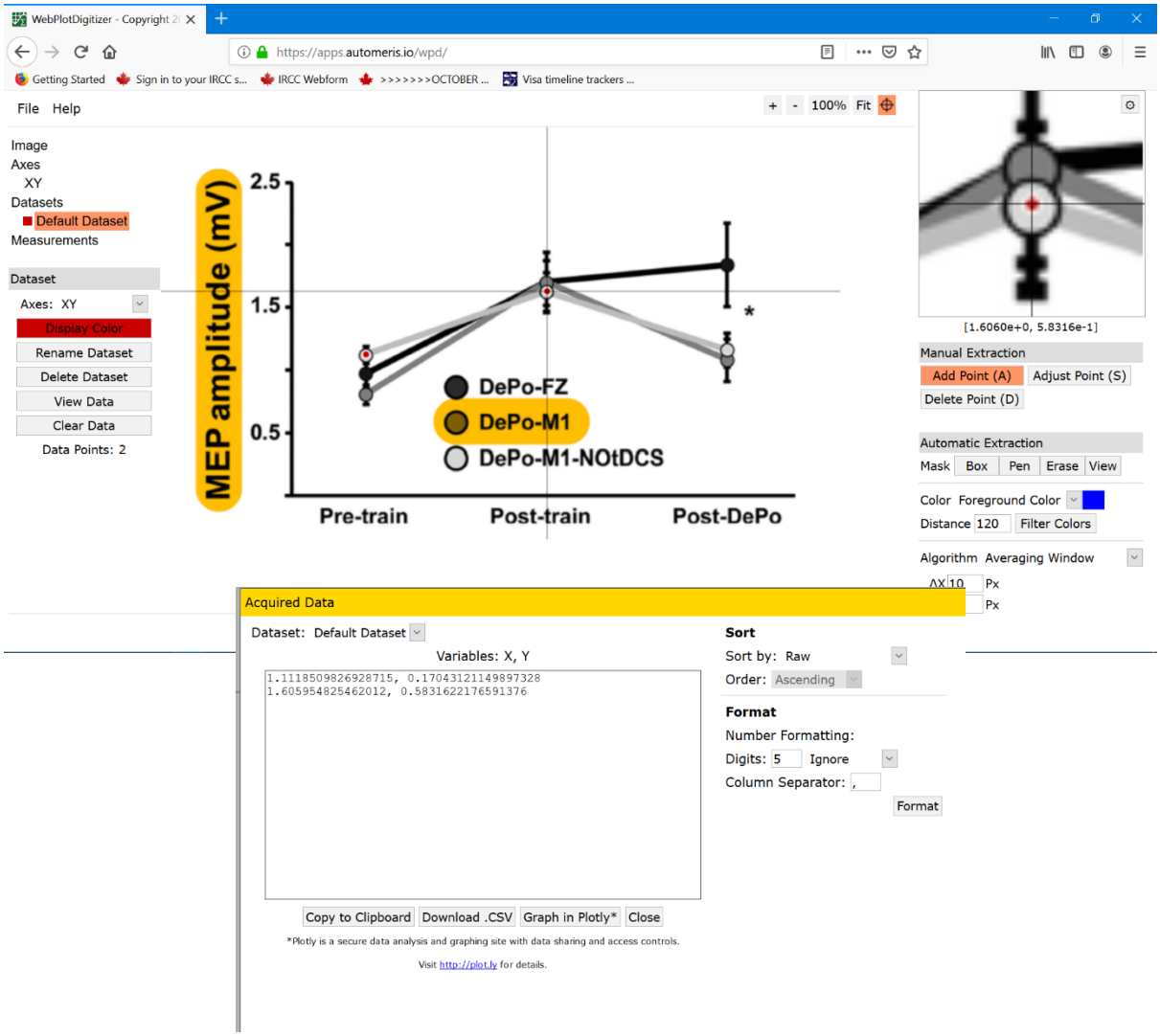
This program will allow you to take a scanned image of a plot (in JPEG or Bitmap) and quickly digitize values off the plot just by clicking the mouse on each data point after calibration. Any 3 non-collinear points can be used for calibration and calibration points **do not** need to be on the axes. Data can be export to ASCII, MS Excel, or MS Word files and used where ever you need them. Besides digitizing points off of data plots, this program can be used to digitize other types of scanned data (such as scaled drawings or orthographic photos).

Usage Notes

Quick Instructions: To use this program, first scan a plot with your favorite scanning system, then save the plot as Bitmap or JPEG format file. Run Plot Digitizer, open the scanned image file from the "Open image file" command in the "File" menu. Then calibrate the plot by clicking on the calibration option or from the "Tool" menu and then digitize the points.

Hint: If you want to digitize plots from published technical reports that are available electronically in PDF format, you can copy the image with the

Snapshot tool and paste and save in a graphics program, such as "Print" and then you can use that file with Plot Digitizer.



An illustration of data extraction from a graph- Using web Plot Digitizer.

Appendix 13. Participant recruitment advertisement

Appendix 14. Case report

Significant Reduction in the Size of Motor-Evoked Potentials After Transient Paraesthesia During Transcranial Magnetic Stimulation Measurements in a Young Healthy Male Adult

To the Editor:

WHAT HAPPENED/PROBLEM?

The incident happened during a single-pulse transcranial magnetic stimulation (TMS) testing session on a neurologically intact, right-handed, 20-year-old man. During the testing session, the participant was sitting comfortable in a fully adjustable treatment chair (MagVenture Co, Farum, Denmark) while his both arms/forearms were rested on pillows. The hotspot for recording motor-evoked potentials (MEPs) from the first dorsal interosseous (FDI) representation on the left primary motor cortex (M1) was identified by using a 70-mm figure-of-eight coil connected to a MagPro magnetic stimulator (MagVenture Co). The coil was held tangentially to the skull with an angle of 45 degrees laterally to the midline and the handle was pointing backward. Twenty-five TMS-induced MEPs with the size of 1 mV (peak-to-peak amplitude) were recorded with the intensity of 36% of maximal stimulator output (MSO). After recording the baseline MEPs, he received 28 minutes of anodal transcranial direct stimulation over the hotspot of FDI while he was keeping the previously mentioned position.

By the end of the anodal transcranial direct stimulation intervention, the participant complained of local paraesthesia in his right forearm, followed by tingling sensation and numbness in his ring and little fingers. He requested to move his arm, and after a couple of minutes of moving his arm, he felt alright again and sat in the same position. At this point, the postintervention measurement was started, which included measuring the resting motor threshold for his right FDI muscle and the test intensity to produce 1-mV MEPs in this muscle again. Resting motor threshold was measured first which was similar to the preintervention value; however, the assessor was not able to record 1-mV MEPs from this muscle even by increasing the test intensity as high as 50% to 55% of the MSO. At this point, the assessor asked the participant about his feeling in his right arm, and he reported that the tingling sensation and numbness in his ring and little fingers came back a few minutes earlier.

HOW WE SOLVED THE PROBLEM?

The participant was asked to move his right arm again until he had normal sensation in his arm and fingers again. Then he sat down and rested his arm directly on the armrest of the treatment chair without using a pillow this time. The assessor managed to complete the postintervention session by recording 25-mV MEPs from right FDI with the test intensity of 39% of the MSO. It should be noted that the participant did not report any tingling or numbness sensation in his right arm until the end of the testing session.

WHY IT HAPPENED?

The target muscle in this test was the FDI muscle, which is innervated by the deep motor branch of ulnar nerve at the wrist. The ulnar nerve is the extension of the medial cord of the brachial plexus. It is a mixed nerve that provides the sensation to the ulnar side of the palm, the fourth and fifth fingers and power to some intrinsic hand muscles including FDI.[1] In this case, the long-term pressure over the nerve caused by static arm position led to transient ulnar nerve palsy, which in turn led to reduction of the MEPs' size from FDI muscle.

HOW THE ISSUE IS RESOLVED?

Releasing the pressure over the right ulnar nerve by voluntary movements of the right arm and hand returned the normal function to the nerve and resumed the size of MEPs to their normal range.

HOW SUCH AN INCIDENT MAY AFFECT THE RESULTS OF TMS STUDIES IN PRE-POST DESIGNS?

This kind of incident can affect the size of elicited MEPs in any stage of data recording. Usually, it does not happen at the beginning of the study because it requires prolonged pressure on peripheral nerves. In acute cases like the case herein, that the participant had a complete numbness in his fingers, it would be easy to be identified and fixed promptly. However, if mild pressure on peripheral nerves remains unnoticed, it may directly affect the size of the recorded MEPs. Therefore, it can act as a confounding variable that should be carefully controlled by TMS assessors in any TMS study.

RECOMMENDATIONS

To avoid the effect of long-term positional pressure on the peripheral nerves innervating the target muscle/s and reducing its adverse effects on the size of elicited MEPs, the following recommendations are required:

- Advice the participants that they should inform the assessor as soon as they start losing their normal sensations in the assessment area, for example, slight tingling sensation, so the adjustment can be made straight away.
- Participants should be careful monitored to assure normal sensation in the assessment area during long TMS testing session.
- Allocate a few minutes to let the participants to move around for releasing the pressure on different parts of their body, especially during long testing sessions.

Maryam Hassanzahraee, MSc
Non-invasive Brain Stimulation and Neuroplasticity Laboratory
Department of Physiotherapy
School of Primary and Allied Health Care
Faculty of Medicine, Nursing and Health Science
Monash University
Melbourne, Australia
maryam.hassanzahraee@monash.edu

Maryam Zoghi, PhD
Department of Rehabilitation, Nutrition and Sport
School of Allied Health
La Trobe University
Melbourne, Australia

Shapour Jaberzadeh, PhD
Non-invasive Brain Stimulation and Neuroplasticity Laboratory
Department of Physiotherapy
School of Primary and Allied Health Care
Faculty of Medicine, Nursing and Health Science
Monash University
Melbourne, Australia

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