Anodal Transcranial Direct Current Stimulation: the Effects on Corticospinal Excitability and Motor Performance

A dissertation by

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Dedicated to

My Loving Parents

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Abstract

Transcranial direct current stimulation (tDCS) is a noninvasive neuromodulatory technique extensively used as a method in neuroscience research and in treatment of various neurological and psychiatric disorders. Application of anode over the target cortical area is called anodal tDCS (a-tDCS), increases corticospinal excitability (CSE). Although a-tDCS is a promising technique for brain modulation, optimal parameters of stimulation are still not entirely set. The broad aim of this thesis was to provide optimal parameters of a-tDCS for enhancement of CSE and to establish a-tDCS protocol for induction of larger CSE changes and motor performance improvement, which lasts longer.

Prior to the main studies, a systematic review and meta-analysis was conducted to verify the effects of a-tDCS with different stimulation parameters on CSE and motor performance in both healthy individuals and subjects with stroke. From the findings of the meta-analysis, it was concluded that longer application of a-tDCS and larger current densities are associated with longer and larger lasting effects. The trend of changes was in favour of motor performance improvement in both healthy individuals and patients with stroke.

Healthy right-handed participants were recruited for all studies. Sample size was calculated based on the power and effect size (Appendix 1) of a pilot data analysis. Surface electrodes recorded electromyography activity of extensor carpi radialis (ECR) (Studies 3-5 and 7) and first dorsal interossei (FDI) muscles (Study 6) at rest. Single-pulse transcranial magnetic stimulation (TMS) was used to assess primary motor cortex

(M1) excitability changes by recording peak-to-peak amplitude of motor evoked potentials (MEPs) of the target muscle(s).

The first two experimental studies were conducted to test the intra- and inter-session reliability of the elicited MEPs (Study 2) and to fine-tune the set-up for application of TMS as an assessment tool and a-tDCS as the intervention (Study 3). Once, the set-up has been developed and tested, 4 other studies were conducted.

Study 4 investigated the optimal current density for application of a-tDCS using four different current intensities (0.3, 0.7, 1.4 and 2 mA) with a constant electrode size of 6×4 cm^2. The findings showed that the smallest current intensity (0. 3 mA) produces significantly larger CSE changes than the next two higher current densities (0.7 and 1.4 mA) with considerably less total charge to the cortical area.

In study 5 the role of active electrode sizes on the induced M1 CSE changes was assessed. It was found that reducing stimulation electrode size (12 cm^2) to one third of the conventional one (35cm^2) increases the efficacy of a-tDCS for induction of larger M1 CSE. This increase could be due to spatially more focused stimulation.

Study 6 investigated the optimal within-session repetition rate of a-tDCS applications (once, twice or three 10 minutes) and interval (5 or 25 minutes) between these stimulations for induction of longest lasting effects on M1 CSE and motor performance. The results showed that compared to a single 10 minutes stimulation, both twice and three times repetition of a-tDCS, induced excitability enhancements which lasted up to 24 hours. It should be noted that, significant improvement was only seen in motor performance following three times repetition with 25 minutes inter stimulus intervals.

Study 7 investigated the effects of a novel noninvasive neuromodulatory paradigm named transcranial pulsed current stimulation (tPCS) on M1 CSE. Anodal-tPCS (a-tPCS) with short (a-tPCS$_{SIPI}$) and long inter-pulse interval (a-tPCS$_{LIPI}$) was compared to a-tDCS and sham a-tPCS$_{SIPI}$. a-tPCS$_{SIPI}$, but not a-tPCS$_{LIPI}$ and sham a-tPCS$_{SIPI}$ induced larger excitability changes in the human cortex compared to the conventional a-tDCS. Furthermore, a-tPCS$_{SIPI}$ induced larger CSE changes compared to a-tPCS$_{LIPI}$. This suggests the importance of IPI in induction of CSE changes.

This thesis demonstrated optimal parameters for a-tDCS application on healthy individuals. Establishing optimal parameters of stimulation is of particular importance to increase the a-tDCS lasting effects.

List of publications

Peer-reviewed papers published during candidature

- **Bastani A.** & Jaberzadeh S. (2012). Does anodal transcranial direct current stimulation enhance excitability of the motor cortex and motor function in healthy individuals and subjects with stroke: A systematic review and meta-analysis. Clinical Neurophysiology. 123 (4): 644–657.

- Jaberzadeh S., **Bastani A.** Kidgell D. (2012). Does the Longer Application of Anodal-transcranial Direct Current Stimulation Increase Corticomotor Excitability Further? A Pilot Study. Basic and Clinical Neuroscience. 3(4): 28-35.

- **Bastani A.** & Jaberzadeh S. (2012). Higher Number of TMS-Elicited MEP from a combined Hotspot Improves Intra- and Inter-Session Reliability of the Upper Limb Muscles in Healthy Individuals. PLosONE. 7(10): e47582. Doi: 10.1371/journal.pone.0047582.

- **Bastani A.** & Jaberzadeh S. (2013). Differential modulation of corticospinal excitability by different current densities of anodal transcranial direct current stimulation. PLosONE. 8(8): e72254. Doi:10.1371/journal.pone.0072254.

- **Bastani A.** & Jaberzadeh S. (2013). a-tDCS differential modulation of corticospinal excitability: the effects of electrode size. Brain Stimulation. 6 (6): 932- 937.

- Jaberzadeh S., **Bastani A.** Zoghi M. (2013). Anodal transcranial pulsed current stimulation: A novel technique to enhance corticospinal excitability. Clinical Neurophysiology. 125 (2): 344-351.

- **Bastani A.**, Jaberzadeh S. (2014). Within session repeated a-tDCS: the effects of repetition rate and inter-stimulus interval on corticospinal excitability. Clinical Neurophysiology. DOI: 10.1016/j.clinph.2014.01.010.

Conference presentations and posters during candidature

Oral Presentations

- **Bastani A.**, Jaberzadeh S. (2010) Effects of anodal transcranial direct current stimulation over the human motor cortex on corticomotor excitability and motor function: a systematic review and meta-analysis. Higher degree research (HDR) student festival- Hosted by School of Primary Health Care Research Group - Notting Hill.

- **Bastani A.**, Jaberzadeh S. (2011) The effect of anodal transcranial direct current stimulation on human brain excitability. Three minute thesis competition, Melbourne, Australia.

- **Bastani A.**, Jaberzadeh S. (2012) Anodal transcranial direct current stimulation: the effects of electrode size on corticomotor excitability in healthy individuals. ISEK 2012, Brisbane, Australia.

- Jaberzadeh S., **Bastani A.,** Zoghi M**.** (2012) Anodal transcranial pulsed current stimulation: a novel technique to enhance corticomotor excitability in healthy individuals. ISEK 2012, Brisbane, Australia.

- **Bastani A.**, Jaberzadeh S. (2012) A study of a-tDCS current density and electrode size on brain excitability. The international Basic and Clinical Neuroscience congress, Tehran, Iran.

- **Bastani A.**, Jaberzadeh S. (2012) a-tDCS and corticospinal excitability: optimal dosage to induce longer lasting effects. Higher degree research (HDR) seminar. Hosted by School of Primary Health Care Research Group - Notting Hill. Melbourne, Australia.

- Vaseghi B., Jaberzadeh S., **Bastani A.** (2012) Anodal transcranial direct current stimulation (a-tDCS) technique for chronic pain: a systematic review and meta-analysis. The international Basic and Clinical Neuroscience congress 2012, Tehran, Iran.

- **Bastani A.**, Jaberzadeh S. (2013) Effects of a-tDCS on corticospinal excitability and motor performance: single versus repeated applications at different intervals. 11th Motor Control & Human Skill Conference, Melbourne, Australia.

- **Bastani A.**, Jaberzadeh S. (2013) Anodal transcranial direct current stimulation: Optimal parameters for induction of corticospinal excitability. Australasian Brain Stimulation meeting, Melbourne, Australia.

Poster Presentations

- **Bastani A.**, Jaberzadeh S. (2011) Anodal transcranial direct current stimulation enhances excitability of the motor cortex and motor function: a systematic review and meta- analysis. The 31th annual meeting of the Australian neuroscience society- Skycity convention centre, Auckland, New Zealand. Poster presentation.

- **Bastani A.**, Jaberzadeh S. (2011) Anodal transcranial direct current stimulation enhances excitability of the motor cortex: a systematic review and meta-analysis of stimulus parameters. The 31th annual meeting of the Australian neuroscience society- Skycity convention centre, Auckland, New Zealand. Poster presentation.

- Jaberzadeh S., **Bastani A.** (2011) Does longer application of anodal transcranial current stimulation coincides with larger excitability changes in human primary motor cortex? The 31th annual meeting of the Australian neuroscience society- Skycity convention centre, Auckland, New Zealand. Poster presentation.

- Jaberzadeh S., **Bastani A**. Dawson K. (2011) Does the longer application of anodal- transcranial direct current stimulation coincide with larger excitability changes in the human primary motor cortex? 10th Motor Control & Human Skill Conference, Perth, Australia. Poster presentation.

- **Bastani A.**, Jaberzadeh S. (2012) The number of TMS-elicited MEPs affects intra and inter-session reliability of the upper limb muscles in healthy individuals. ISEK 2012, Brisbane, Australia. Poster presentation.

- Jaberzadeh S., **Bastani A.** (2012) Does current intensity affects the size of anodal-tDCS induced corticomotor excitability in healthy individuals? ISEK 2012, Brisbane, Australia. Poster presentation.

- Vaseghi B., Jaberzadeh S., Zoghi M., **Bastani A**. (2012) Pain management by anodal transcranial direct current stimulation (a-tDCS): a systematic review and meta-analysis. Higher Degree Research (HDR) seminar. Hosted by School of Primary Health Care Research Group - Notting Hill. Melbourne, Australia. Poster presentation.

- **Bastani A.**, Jaberzadeh S., Vaseghi B. (2013) Optimal repetition rate and break duration for application of anodal-transcranial direct current stimulation. The 33th annual meeting of the Australian neuroscience society. Exhibition convention centre, Melbourne, Australia. Poster presentation.

- Jaberzadeh S., Vaseghi B., Zoghi M., **Bastani A.** (2013) The effects of Cathodal Transcranial direct current stimulation on modifying pain intensity processing: A systematic review and meta-analysis. Australian Neuroscience Society Inc. 33rd Annual meeting.

- Vaseghi B., Jaberzadeh S., **Bastani A.** (2013) The effects of anodal transcranial direct current stimulation on pain threshold and pain level: A systematic review and meta-analysis. Australian Neuroscience Society Inc. 33rd Annual meeting.

General Declaration

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

'I hereby declare that this thesis 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 6 original papers published in peer reviewed journals and 1 unpublished publications. The core theme of the thesis is Anodal transcranial direct current stimulation: the effects on corticospinal excitability 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 Faculty of Medicine, Nursing and Health Sciences under the supervision of Dr. Shapour Jaberzadeh.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapter 2, Chapter 3, Chapter 4, Chapter 5, Chapter 6, Chapter 7 and Chapter 8 my contribution to the work involved the following:

Project design (in consultation with my supervisor); review of appropriate literature; securing ethics approval; recruitment of participants; data collection; conducting data analysis and writing of papers.

Thesis chapter	Publication title	Publication status	Nature and extent of candidate's contribution
2	Does anodal transcranial direct current stimulation enhance excitability of the motor cortex and motor function in healthy individuals and subjects with stroke: A systematic review and meta-analysis	Published	80% Project design; review of appropriate literature; securing ethics approval; recruitment of participants; data collection; conducting data analysis and writing of papers (*)
3	A higher number of TMS-elicited MEP from a combined hotspot improves intra- and inter-session reliability of the upper limb muscles in healthy individuals	Published	80%*
4	Does the longer application of anodal-transcranial direct current stimulation increase corticomotor excitability further? A pilot study	Published	80%*
5	Differential modulation of corticospinal excitability by different current densities of anodal transcranial direct current stimulation	Published	80%*
6	a-tDCS differential modulation of corticospinal excitability: the effects of electrode size	Published	80%*
7	Within-session repeated a-tDCS: the effects of repetition rate and inter-stimulus interval on corticospinal excitability	Published	80%*
8	Anodal transcranial pulsed current stimulation: A novel technique to enhance corticospinal excitability	Published	80%*

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Signed: 

Date: 30.09.2013

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Andisheh Bastani

List of Abbreviations

Abbreviations	Definition
ADM	Abductor Digiti Minimi Muscle
Ag/Agcl	Silver Silver Chloride
AMED	Allied Health And Complementary Medicine Database
AMI	Australian Medical Index
ANOVA	Analysis of Variance
APB	Abductor Pollicis Brevis Muscle
a-tDCS	Anodal Transcranial Direct Current Stimulation
BBT	Box and Block Test
c-tDCS	Cathodal Transcranial Direct Current Stimulation
CENTRAL	Cochrane Central Register of Controlled Trials
CI	Confidence Interval
CINAHL	Cumulative Index to Nursing And Allied Health
cm	Centimeter
CM	Cortico Motoneuronal
CNS	Central Nervous System
CSE	Corticospinal Excitability
CT	Computerized Tomography
CV	Coefficient of Variation

Cz	Vertex
DC	Direct Current
D&B	Down and Black Quality Assessment Scale
EBM reviews	Evidence Based Medicine Reviews
ECR	Extensor Carpi Radialis
EEG	Electroencephalography
EMBASE	Excerpta Medica Database
EMG	Electromyography
FCR	Flexor Carpi Radialis
FDI	First Dorsal Interosseous
fMRI	Functional Magnetic Resolution Imaging
GABA	Gamma Aminobutyric Acid
GPT	Grooved Pegboard Test
Hz	Hertz
ICC	Intra-Class Correlation
JTT	Jebsen Taylor Test
LSD	Least Significant Difference
LTP	Long Term Potentiation
LTD	Long Term Depression
M1	Primary Motor Cortex

MEG	Magnetoencephalography
MEP	Motor Evoked Potential
MeSH	Medical Subject Headings
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
MUHREC	Monash University Human Research Ethics Committee
μV	Microvolt
MVC	Maximum Volountary Contraction
NIBS	Non Invasive Brain Stimulation
NMDA	N-Methyl-D-Aspertic Acid
OP	Opponeus Pollicis
PEDro	Physiotherapy Evidence Database
PET	Positron Emission Tomography
PMT	Photon Migration Tomography
PPT	Purdue Pegboard Test
RCTs	Randomized Control Trials
RMT	Resting Motor Threshold
rTMS	Repeated Transcranial Magnetic Stimulation
SD	Standard Deviation

SE	Standard Error
SEM	Standard Error of Measurment
SMD	Standard Mean Difference
SPECT	Single Photon Emission Computed Tomography
SPSS	Statistical Package for Social Sciences
T	Tesla
TA	Tibialis Anterior
tACS	Transcranial Alternating Direct Current Stimullation
tDCS	Transcranial Direct Current Stimulation
tES	Transcranial Electric Stimulation
TMS	Transcranial Magnetic Stimulation
tPCS	Transcranial Pulsed Current Stimulation
tRNS	Transcranial Random Noise Stimulation
W	Watt
WMD	Weighted Mean Difference

Thesis Outline

This thesis will present the results of a body of work (Figure 1) investigating optimal parameters of a-tDCS for the induction of CSE and motor performance. In addition, the tDCS effect on CSE will be compared to that of a new tES paradigm, named transcranial pulsed current stimulation (tPCS).

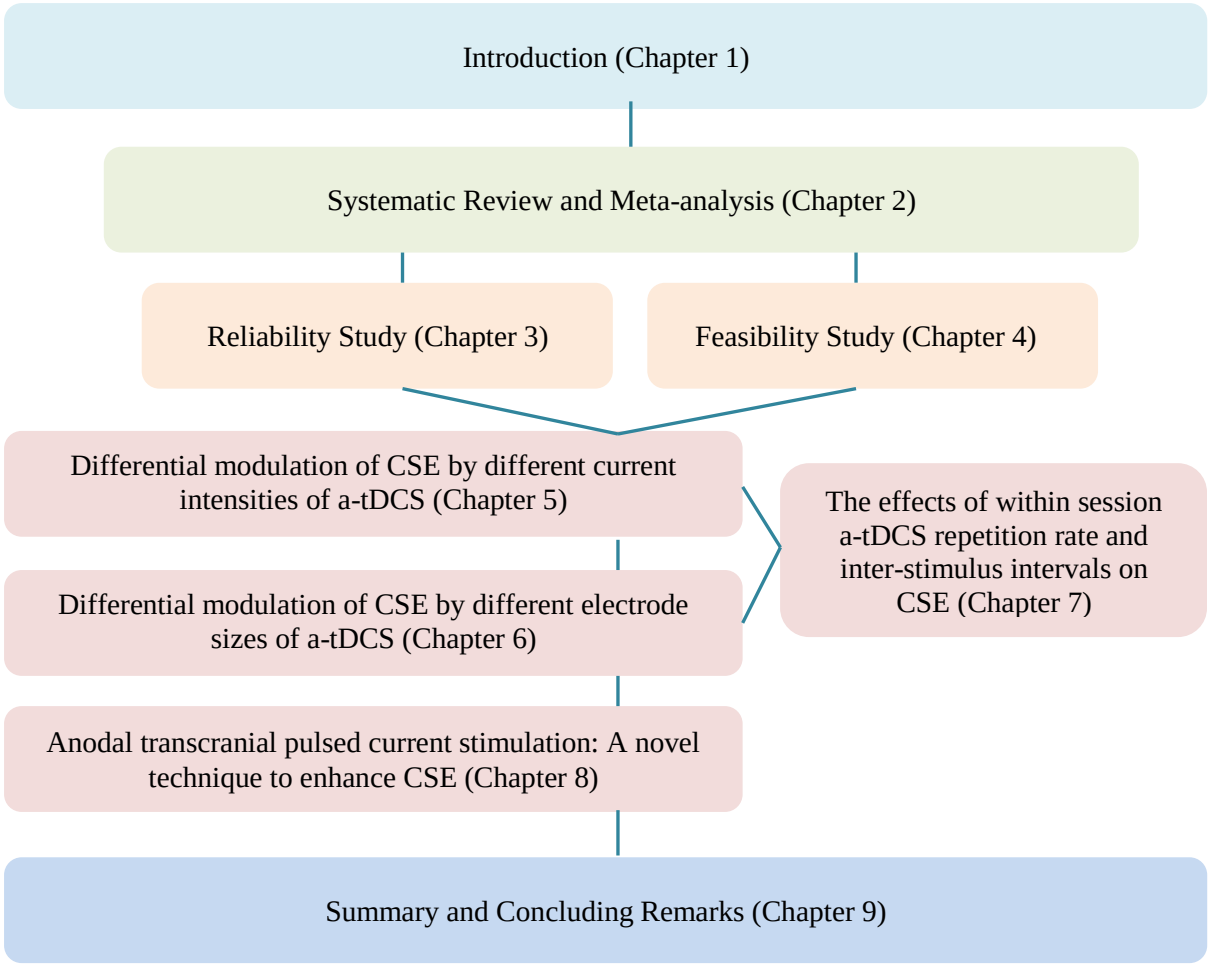

Figure 1: Thesis structure

Chapter 1 provides an introduction to the topic and background information on the neuroplasticity and physiology of the cerebral cortex, M1 and corticospinal tract, in order to anchor the framework of the research field that this thesis is related to. Also, the introduction presents the concept of the NIBS method, neurostimulatory and neuromodulatory techniques, safety issues, and tools for assessment of CSE and motor performance related to the work in this thesis.

Chapter 2 presents a systematic review and meta-analysis of current literature on the effects of a-tDCS when compared to sham/no stimulation on the CSE of M1 and motor performance in healthy individuals and subjects with stroke. Also, the effects of a-tDCS parameters on CSE in both healthy individuals and subjects with stroke are determined.

Chapter 3 outlines intra rater reliability of the assessor and the protocol for elicitation of TMS-induced MEPs. This study aims to compare the intra- and inter-session reliability of peak-to-peak amplitude and latency of different blocks (5, 10 and 15 MEPs per block) of simultaneous elicitation of MEP from the extensor carpi radialis (ECR) and first dorsal interossei (FDI) muscles at rest.

Study 3 (Chapter 4) is a pilot work to fine-tune the set-up for application of TMS as an assessment tool and a-tDCS as a therapeutic technique. All necessary changes are added to the data acquisition system. Also, this pilot study is carried out to collect data for sample size calculation using effect size index (Appendix 1).

Chapter 5 compares the effects of a-tDCS with four different current densities on the size of CSE in healthy individuals.

Similarly, Chapter 6 investigates the differential effects of a-tDCS with three different electrode sizes on the size of CSE in healthy individuals.

Chapter 7 examines how the number of a-tDCS repetitions and the intervals between the stimulations affect the size and extent of CSE and motor performance changes.

Chapter 8 evaluates a new neuromodulatory paradigm to increase CSE. It was decided to interrupt direct current and break it into a number of unidirectional pulses, named transcranial pulsed current stimulation (tPCS). The primary aim is to compare the effects of a-tPCS with sham a-tPCS and conventional a-tDCS on the enhancement of CSE in healthy individuals. The secondary aim is to compare the effects of shorter and longer inter-pulse intervals of a-tPCS.

The final Chapter (Chapter 9) summarises the findings and provides conclusions for different studies in this thesis.

Chapter: 1 General Introduction

Non-invasive brain stimulation (NIBS) represents a number of new breakthrough approaches for treatment of a range of motor, somatosensory, visual and cognitive disorders focused on using magnetic or electrical energy to improve brain function (Nitsche et al. 2008). These techniques are also used for research in healthy individuals and in people with psychological (Gershon et al. 2003; Kincses et al. 2004; Fregni et al. 2005a; George et al. 2007) or neurological (Byrnes et al. 2001; Uy et al. 2003; Boggio et al. 2007; Bolognini et al. 2009; Benninger et al. 2010) problems.

While currently available medications and/or physiotherapeutic techniques are effective for many patients, unfortunately a substantial number of them do not always respond fully to conventional treatments. For example, side effects of conventional psychiatric medications may limit the effectiveness of conventional treatments (Arana 2000). On the other hand, when the person is medication intolerant, or their problem is resistance to medication, their condition can become chronic. In such instances, the use of brain stimulation treatments may allow treatment of a condition that otherwise could not be treated. This can happen if the brain stimulation treatments replace medications, or allow the use of lower doses of medications or medications that are more tolerable to the patient but less effective by themselves. Furthermore, despite significant advances in the development of motor training protocols, motor recovery following neurological disorders, such as stroke, is often incomplete (Nakayama et al. 1994; Hendricks et al. 2002; Schaechter 2004; Marshall et al. 2007) and approximately two thirds of patients are left with long term disabilities (Strokefoundation 2013). In this scenario, enhancing

cortical connectivity prior to (Nitsche et al. 2003a; Boggio et al. 2006b) or during motor training (Reis et al. 2009; Stagg et al. 2011) may lead to greater clinical outcomes than could be achieved with traditional therapies alone.

Recent NIBS approaches are now being used to prime the effects of other therapeutic techniques (Dobkin 2003), as stand-alone techniques in areas such as pain treatment (Rosen et al. 2009; O'Connell et al. 2010) and in the treatment of some psychological (George et al. 2007) and neurological disorders (Fregni & Pascual-Leone 2007; Schulz et al. 2013). Priming is achieved by enhancing the sensitivity of the brain to therapy using techniques that increase or decrease the excitability of the cortex (Schabrun & Chipchase 2012). In this context, NIBS appears to be a promising option. A number of NIBS techniques have been developed and are now being tested for their ability to prime the brain in conditions such as stroke (Boggio et al. 2007; Takeuchi et al. 2008), psychological disorders (Novakovic et al. 2011; Vallar & Bolognini 2011), Parkinson's disease (Fregni et al. 2006b), chronic pain (Fregni et al. 2006a; Fregni et al. 2006c; Boggio et al. 2009a) and dystonia (Schabrun et al. 2009). These techniques are non-invasive, painless and induce changes in corticospinal excitability (CSE) that outlast the period of stimulation and have no or few side effects (Rossi et al. 2009). These characteristics make NIBS techniques attractive for use in different clinical settings.

NIBS induced alternations in the excitability of the cortex are considered to be a key component for functional gains (Karni et al. 1998; Kolb & Whishaw 1998). Over the last decade there has been increasing evidence of links between NIBS induced CSE enhancement , skill learning (Hummel et al. 2005; Hummel & Cohen 2005; Fregni et al. 2005b; Boggio et al. 2006a; Hunter et al. 2009; Reis et al. 2009; Matsuo et al. 2011) and

motor performance (Nitsche et al. 2003a; Hummel et al. 2010). A growing number of research indicates that improvements in function coincides with enhanced 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 patients with different pathological conditions (Uy et al. 2003; Hummel & Cohen 2005; Kim et al. 2006).

A primary goal of neuroscientists in this area of research is to develop NIBS protocols to prime the effects of other treatments (Wagner et al. 2007). NIBS paradigms aimed at modifying CSE include repetitive transcranial magnetic stimulation (rTMS) and transcranial electric stimulation (tES) (Pascual-Leone et al. 1994b; Paulus 2011). Despite rTMS being a neurostimulatory technique, tES is an umbrella term for the description of a number of neuromodulatory techniques such as transcranial direct current stimulation (tDCS), transcranial alternating current stimulation (tACS) and transcranial random noise stimulation (tRNS) (Paulus 2011). The most utilized tES techniques is tDCS, which is the application of a low-amplitude direct current which can modulate CSE in a polarity-dependent manner (Nitsche & Paulus 2000).

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

The studies introduced in this thesis are motivated by the need for the development of non-medical adjunct therapies to prime the effects of current therapies. Current standard tDCS protocols (Nitsche et al. 2008; Paulus 2011) advocate the utilisation of large electrodes (i.e. $5\times7cm^2$). tDCS focality is limited by using these electrodes and tDCS might not only stimulate the intended, but also the adjacent functional areas of the cortex, which may have a negative impact on the desired effects. On the other hand, current intensity delivery has varied between 1-2 mA in most published studies. These higher intensities make it impossible to use smaller electrodes and at the same time keep the current density at a safe level. Therefore the studies in this thesis were designed in a way that would solve this problem.

Another strategy to improve tDCS effects on the size of excitability changes and the length of its lasting effects is to increase the stimulation duration. However, due to homeostatic mechanisms this strategy only works to a limited extent (Monte-Silva et al. 2013). The alternative approach for this problem is to use within-session multiple application of tDCS. Another important issue is whether the neurophysiological finding can be translated into clinical effects: for instance, whether an increase in CSE induced by tDCS coincided with increased motor performance.

As such, the primary aim of this thesis is to determine optimal parameters of tDCS for enhancement of CSE. The secondary aim is to investigate the lasting effect of within-session repeated tDCS on the CSE and motor performance improvements. These studies are detailed in Chapters 3 - 8.

To address these aims, a number of studies were designed and carried out on healthy participants. To establish a framework for understanding the results of these studies, a brief review of the anatomical/physiological characteristics of the central nervous system (CNS), comprising the human motor system and a description of NIBS induced neuroplastic changes, are provided in the next section.

Neuroplasticity in the CNS

Neuroplasticity refers to the intrinsic ability of the nervous system (Pascual-Leone et al. 2005) to develop, react or adjust, throughout the course of its life, to internal and external environmental changes (Trojan & Pokorny 1999), both under physiological and pathological conditions. Neuroplasticity has replaced the formerly-held viewpoint that the brain is a physiologically static organ.

For a long time, the consensus among neuroscientists was that as we aged, the connections in the brain remained fixed. This belief has been challenged by findings revealing the dynamism of the nervous system which indicate that many aspects of the brain remain plastic even into adulthood (Rakic 2002; Pascual-Leone et al. 2005; Pascual-Leone et al. 2011). The brain reorganises itself by forming new connections between neurons, by changing the internal structure of existing synapses (cellular modification), and by increasing rates of neuronal survival when an injury occurs (Karmarkar & Dan 2006). The effects of neuroplasticity can lead to either positive or negative changes during development (evolutionary neuroplasticity), after short-term or transient exposure to a stimulus (reactive neuroplasticity), after long-term, continuous or repeated exposure

(adaptive neuroplasticity), and during functional or structural recovery of damaged neuronal circuits (reparation neuroplasticity) (Trojan & Pokorny 1999).

The above mentioned three types of neuroplasticity are categorized as physiological mechanisms (Trojan & Pokorny 1999). In physiological neuroplasticity, an increase or decrease in synaptic plasticity involves several molecular regulating processes. Two recognized processes that have been studied extensively across various species are long-term potentiation (LTP) and long-term depression (LTD) (Malenka & Bear 2004). These are conditions which may bring about an increase of transmitter release, change in the density of postsynaptic receptors, and change in the number of presynaptic invaginations (Schuster et al. 1990).

Pathological neuroplastic changes are based on different mechanisms, such as 'unmasking' of synapses or pathways that may ordinarily be inactive; 'denervation hypersensitivity', in which the target of a partially lesioned projection produces a great number of receptors to bind to a reduced number of available neurotransmitter molecules; and 'compensatory collateral sprouting', wherein the injured distal components of axons that are spared by a lesion sprout to occupy adjacent synapses vacated by lesioned neighbouring axons (Hamori 1990; Hallett 2001).

Cerebral Cortex Organization

The cerebral cortex, referred to as the ‘gray matter’ of the brain, is the outermost sheet of neural tissue of the cerebrum. It is typically 3 - 4 mm thick (Edelman & Mountcastle 1978; Taylor 1999), covering the gyri and sulci and containing most of the somas of the cerebral neurons. It encompasses about two-thirds of the brain mass and lies over and around most of the structures of the brain. It is the most highly developed part of the human brain and most of the actual information processing in the brain takes place in the cerebral cortex. It is divided into frontal, temporal, parietal and occipital lobes that contain functionally distinguished locales such as motor, somatosensory, and visual areas and a multitude of their subdivisions (Figure **2**). Although there are small inter-individual variations, each cortical area has its typical location in terms of the sulci and gyri.

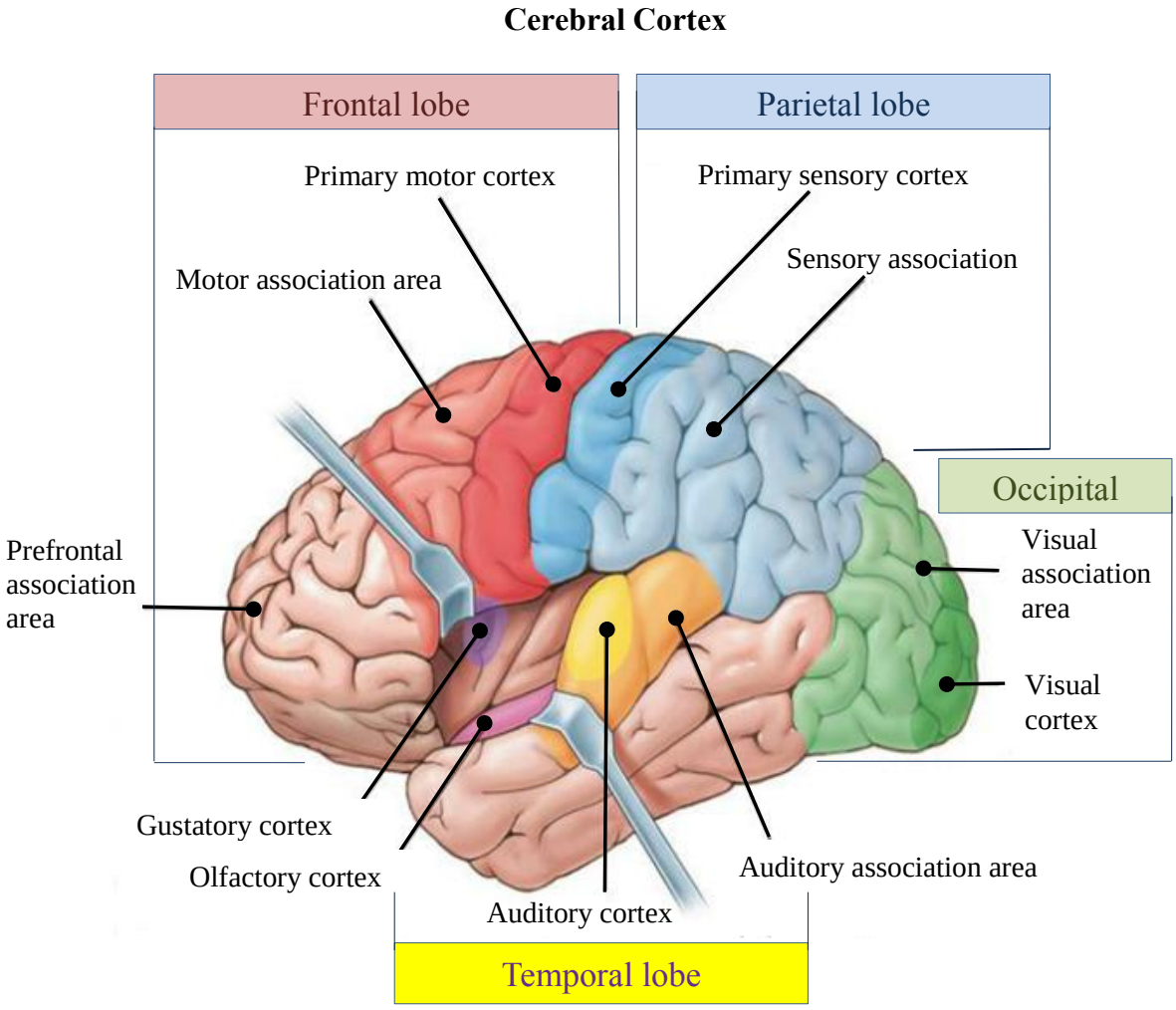

Figure 2 The cerebral cortex is divided into frontal, temporal, parietal and occipital lobes that contain sensory areas of perception, motor areas that direct movement and association areas that integrate information. Adapted from (Silverthorn et al. 2007).

In general the cerebral cortex consists of six layers (I-VI) of histologically and functionally distinct cells (Figure 3). Neurons in the cerebral cortex are distributed in horizontal layers and vertical columns (Garey 1994). The relative thickness of each layer varies with the function of the region of the cortex (Dinse et al. 2013).

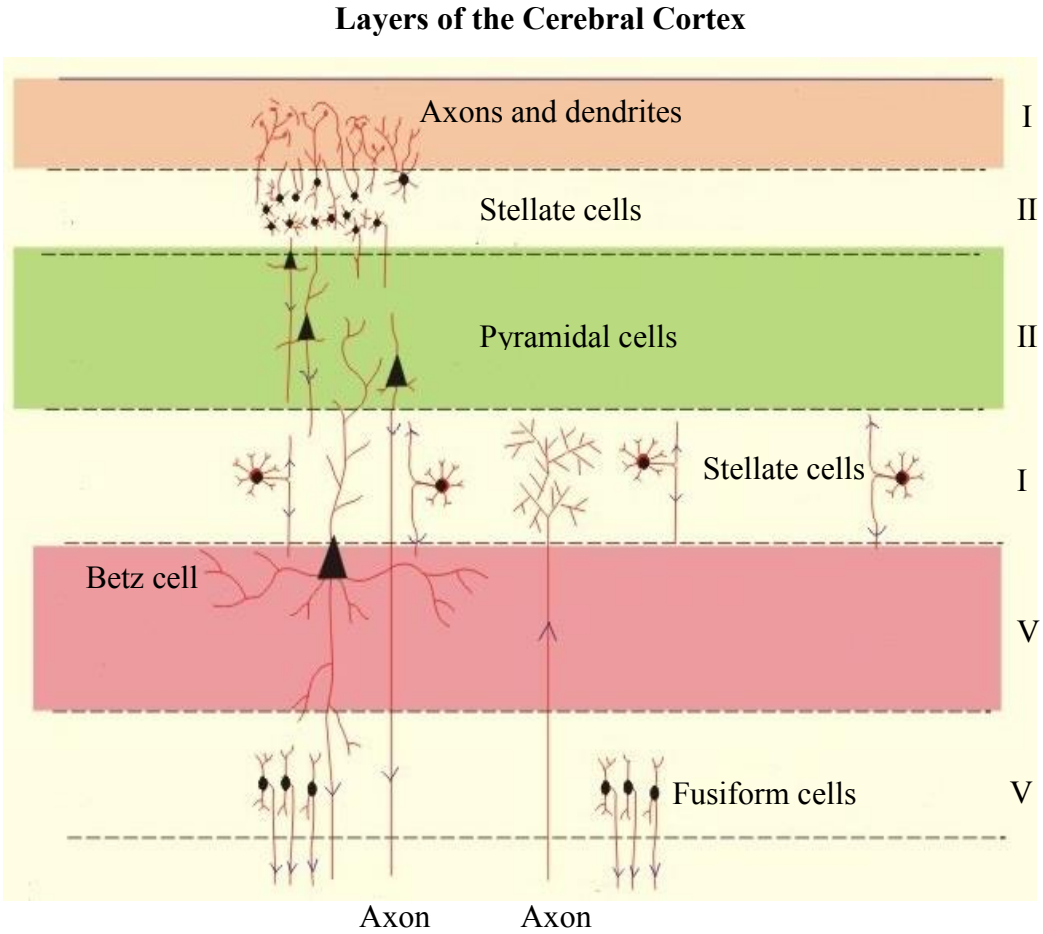

Figure 3 The cerebral cortex is commonly divided into 6 layers, showing the locations of the Pyramidal and Stellate neurons, and the axons and dendrites in the outermost layer. Adapted from (Paulev 1999).

Horizontal organization

The layers of the cerebral cortex are numbered with Roman numerals from superficial to deep. Layer I is the molecular layer, which contains very few neurons; layer II the external granular layer; layer III the external pyramidal layer; layer IV the internal granular layer; layer V the internal pyramidal layer; and layer VI the multiform, or fusiform layer. Each cortical layer contains different neuronal shapes, sizes and densities as well as different organizations of nerve fibres.

The layers of the cerebral cortex can also be divided functionally into three parts. The supra-granular layers consist of layers I to III. The supra-granular layers are the primary origin and termination of intracortical connections, which are either associational (i.e., with other areas of the same hemisphere), or commissural (i.e., with connections to the opposite hemisphere, primarily through the corpus callosum). The supra-granular portion of the cortex is highly developed in humans and permits communication between a portion of the cortex and other regions. The internal granular layer, layer IV, receives thalamocortical connections, especially from the specific thalamic nuclei. This is most prominent in the primary sensory cortices. The infragranular layers, layers V and VI, primarily connect the cerebral cortex with subcortical regions. These layers are most developed in motor cortical areas. Layer V gives rise to all of the principal cortical efferent projections to basal ganglia, brain stem and spinal cord. Layer VI, the multiform or fusiform layer, projects primarily to the thalamus.

There are several identifiable cell types in the cerebral cortex, particularly pyramidal or projection neurons and non-pyramidal neurons or interneurons (Brodal 1969). Pyramidal neurons are oriented, on average, perpendicular to the cortical surface, while

interneurons do not have a preferred orientation. These cells form complex neuronal networks in which the information processing takes place.

Pyramidal cells are found in layers II-VI but are most prevalent in layers III and V (Porter & Lemon 1993). Dendrites of pyramidal cells extend both horizontally and vertically into all layers of the cortex, forming extensive networks in layers II-IV. These intrinsic connections between dendritic spines presumably allow the flexible synaptic organization of the motor cortex. These cells can be extremely large in layer V of the motor cortex, giving rise to most corticobulbar and corticospinal fibres. The largest of these neurons are called 'Betz cells', sometimes reaching 100 μm in diameter. These cells are pyramidal in shape, with an apical dendrite that extends all the way to layer I of the cortex (Rothwell 1991). Betz cells send their axons down to the spinal cord where in humans they synapse directly with anterior horn cells, which in turn synapse directly with their target muscles.

There are also several basal dendrites projecting laterally from the base of these neurons. Dendrites of cortical neurons have many spines that are sites of synapses with other neurons (Figure 4).

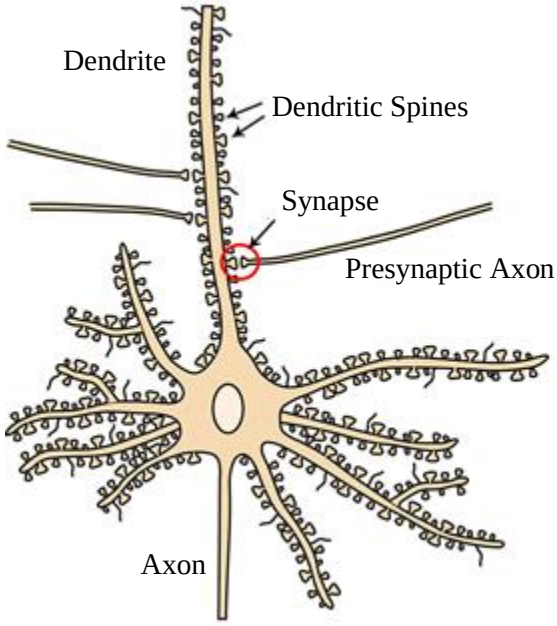

Figure 4 A cortical neuron, dendrites, and dendritic spines. Adapted from (Smrt & Zhao 2010).

The thin axon that arises from the base of the pyramidal cell has collaterals and a long process that leave the cortex. This is the process that connects with other brain regions by extending through the white matter deep to the cortex. Pyramidal cells use excitatory amino acid glutamate as the primary neurotransmitter (Cotman & Monaghan 1988).

Stellate cells, or granular cells, which act as interneurons within the motor cortex (Rothwell 1991) constitute approximately 25% of the neurons in the motor cortex, and are located in layers II-VI, but most prominent in layer IV. Their dendritic trees are organised radially and axons are almost exclusively intrinsic to the cortex (remain in the cortex). The most prevalent stellate cells in the motor cortex are basket cells, which

make inhibitory synaptic contact with pyramidal neurons, using the neurotransmitter gamma-aminobutyric acid (GABA) (Jones 1983; Meyer 1987).

Columnar organizations

In addition to the distribution of neurons in layers, groups of cells work together in vertical units called cortical columns (Edelman & Mountcastle 1978; Horton & Adams 2005). The basic unit of the mature cerebral cortex is the minicolumn, a narrow chain of neurons extending vertically across the cellular layers II–VI, perpendicular to the pia matter (Edelman & Mountcastle 1978). Cortical columns are formed by many minicolumns bound together by short-range horizontal connections. This columnar organization is characterised by extensive synaptic communication between neurons, the majority of which is inhibitory (Jones 1981).

The layer V neurons are clustered into groups distributed intermittently in the horizontal dimension. In the human motor cortex, pyramidal and non-pyramidal cells are clustered into columnar aggregates ~300 μm wide, separated by 100 μm cell-sparse zones (Meyer 1987). Forty percent of neurons in such clusters project to a single motoneuron pool in the spinal cord; the remainder project to the motoneuron pools of muscle groups active in similar movements. The recurrent axon collaterals of pyramidal cells project vertically into a 300 – 500 μm zone that extends through the cellular layers. This provides a strong excitatory drive to adjacent neurons and, via inhibitory interneurons, a columnar surround inhibition (Keller 1993) for the sharpening of motor commands.

Each cortical column is a discrete complex processing unit that communicates with the adjacent columns and other regions of the cortex through extensive horizontal connections (Edelman & Mountcastle 1978). Stimulation of a given motor column may activate a single muscle. More commonly stimulation of a column activates several muscles to produce a coordinated movement.

Cortical Motor Areas

Roland and Zilles proposed a definition of cortical motor areas as being those areas with projections to spinal motor neurons (Roland & Zilles 1996). The motor cortex comprises the premotor cortex, supplementary motor area and the primary motor cortex (M1) (Figure 5). The premotor cortex is involved in initiating and planning voluntary movement. The supplementary motor area is involved in programming complex sequences of movements and coordinating bilateral movements. The M1 generates commands for specific muscles or muscle groups, which are communicated via the corticospinal tract. The M1 also appears to be heavily involved in various aspects of motor skill learning (Sanes & Donoghue 2000) and changes in motor representations (Pascual-Leone et al. 1994a). Discharge properties of cortical neurones (Classen et al. 1998) can be seen during motor skill learning and motor performance in humans.

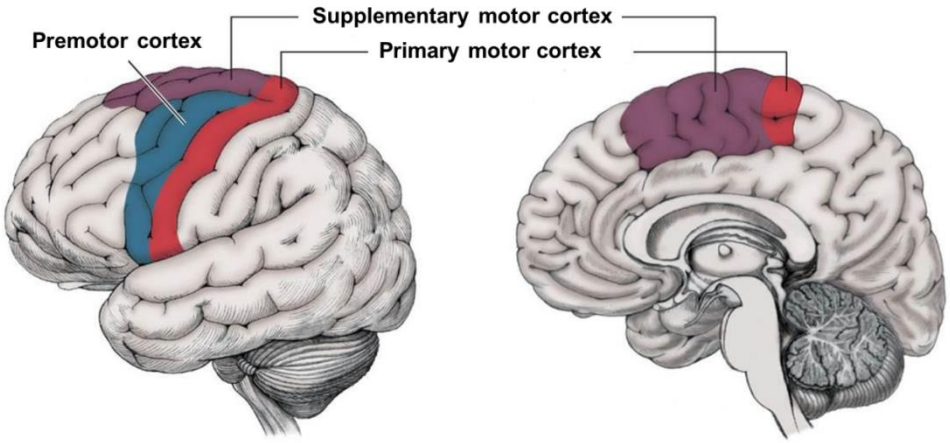

Figure 5 The motor cortex includes the premotor cortex, supplementary motor area and the M1 – the primary motor cortex. Adapted from (Kalat 2011).

Neural circuits responsible for motor performance

The neural circuits responsible for the control of movement can be divided into four distinct but highly interactive subsystems (Purves et al. 2008), each of which makes a unique contribution to motor control (Figure 6). These subsystems include local spinal cord and brainstem circuits, descending modulatory pathways, the cerebellum, and the basal ganglia. All commands for motor performance are ultimately conveyed to the muscles of interest by the activity of the lower motor neurons in initiating movement. The descending modulatory pathways consist of the upper motor neurons. The upper motor pathways that arise in the cortex are essential for the initiation of voluntary movements and for complex spatiotemporal sequences of skilled movements. In particular, descending projections from the M1 are essential for planning, initiating, and directing sequences of movements involving the limbs. The M1 generates commands for the target muscle(s) which are communicated through direct output on the spinal cord

via the corticospinal tract and brainstem circuits. It also receives direct input from basal ganglia and from the cerebellum (Purves et al. 2008).

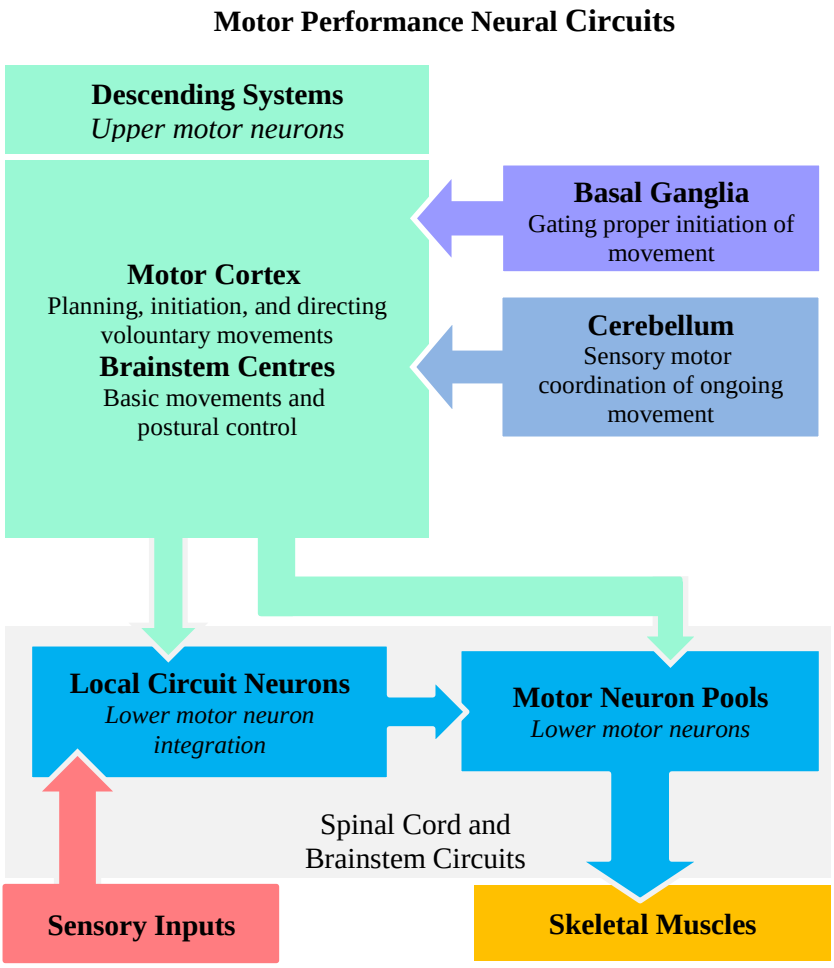

Figure 6 The overall organization of neural structures involved in the control of motor performance is a well-organised system. Adapted from (Purves et al. 2008).

Literature indicates a strong relationship between enhancement of motor performance and M1 excitability in humans (Georgopoulos et al. 1992; Ashe 1997). The tDCS

technique used in this thesis aims to enhance excitability of the M. This could be used as a stand-alone therapeutic technique or as an adjunct therapy to prime the effects of other therapeutic interventions. The M1 will be explained in more detail in the following section.

The primary motor cortex or M1

The primary motor cortex also known as the M1; is located in the precentral gyrus area of the frontal lobe of the cerebral cortex and extends onto the medial cortical surface within the longitudinal fissure (Garey 1994). The M1 is characterised by the lack of granule cells in layer IV and distinguished from the premotor cortex by the presence of Betz cells in layer V (Meyer 1987). The organization of the M1 in the cortex involves a small, distorted, discontinuous map of the body (Homunculus) (Figure 7), with larger areas devoted to body regions characterized by fine or complex movements and smaller areas to body regions characterized by gross movements involving few muscles. Hand, face, intraoral and, to some extent, foot muscles are particularly well represented on M1 (Geyer et al. 1996).

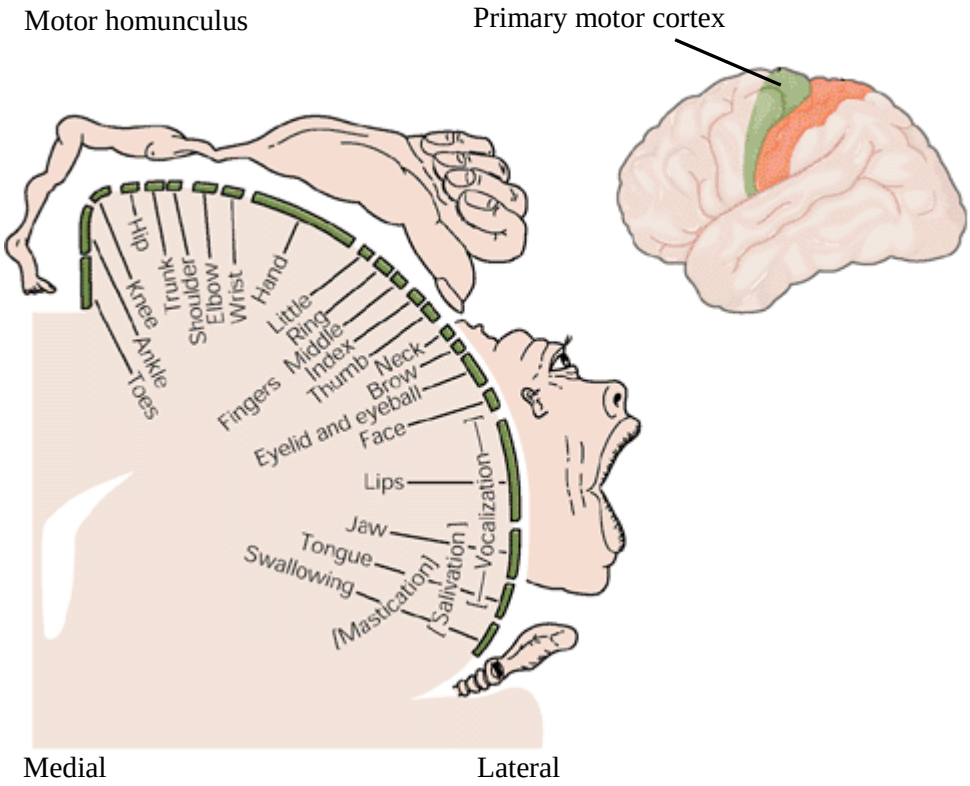

Figure 7 The homunculus of the M1 and the primary somatosensory motor cortex. Note that each hemisphere receives information from the opposite side of the body. Adapted from (Penfield & Rasmussen 1950).

Betz cells and other layer V pyramidal cells give rise to excitatory cortical and spinal projections and have numerous local collateral branches (Ghosh & Porter 1988), with horizontal connection systems within the M1 extending over 1cm (Huntley & Jones 1991; Hess & Donoghue 1994).

The strength of these excitatory glutamatergic horizontal pathways (Hess et al. 1994) is possibly influenced by GABA-ergic inhibitory interneurons (Hess & Donoghue 1994;

Donoghue 1995; Hess et al. 1996). There is increasing evidence that these extensive horizontal connections provide a basis for cortical plasticity.

In addition to extensive horizontal local cortico-cortical connections, the M1 receives afferent sensory input pertaining to the activity of muscles via the thalamus and primary somatosensory cortex (Ghosh et al. 1987). Additional afferent inputs come from the premotor cortices, cingulate motor area and area 5 of the parietal cortex (Muakkassa & Strick 1979; Ghosh et al. 1987; Tokuno & Tanji 1993) in a roughly somatotopic arrangement. In addition, there are transcallosal afferents from the contralateral M1 (Sloper & Powell 1979), and sparse transcallosal inputs from the contralateral premotor areas (Rouiller et al. 1994). These connections, derived from macaque data, are assumed to be present in humans. The output projections of the M1 layer V pyramidal cells consist predominantly of direct, prominent connections to the spinal cord via the corticospinal tract. In the next section the anatomy of the corticospinal tract is briefly reviewed.

The corticospinal tract

The pyramidal tract is one of the most important motor tracts in the human body. By definition, the majority of the fibres that comprise the tract (up to 60%) originate in layer V of the M1 and the adjacent premotor cortex, while the remaining fibres arise from the primary somatosensory cortex and the parietal cortex (Nathan et al. 1990). These fibres are known as the corticospinal tract (Figure 8). 70-90% of these fibres decussate and continue on as the lateral corticospinal tract, to synapse in the ventral horn of the spinal

cord with the motor neurons that innervate limb and trunk muscles. The remaining 10-30% of these uncrossed fibres descend in the ventral columns of the spinal cord as the ventral corticospinal tract, and terminate in the thoracic spinal cord, their function being to innervate trunk muscles (Nathan et al. 1990).

The descending corticospinal tract terminates at the spinal cord, synapsing either on interneurons, or directly on motorneurons (Brinkman & Kuypers 1973). The corticospinal tract has assumed the pre-eminent role in the control of human muscles (Porter & Lemon 1993). In the current study, transcranial magnetic stimulation (TMS) induced responses are generated by the stimulation of Betz cells in the cerebral cortex, the propagation of action potentials through this pathway, and the induction of muscle responses in the target muscles through spinal nerves.

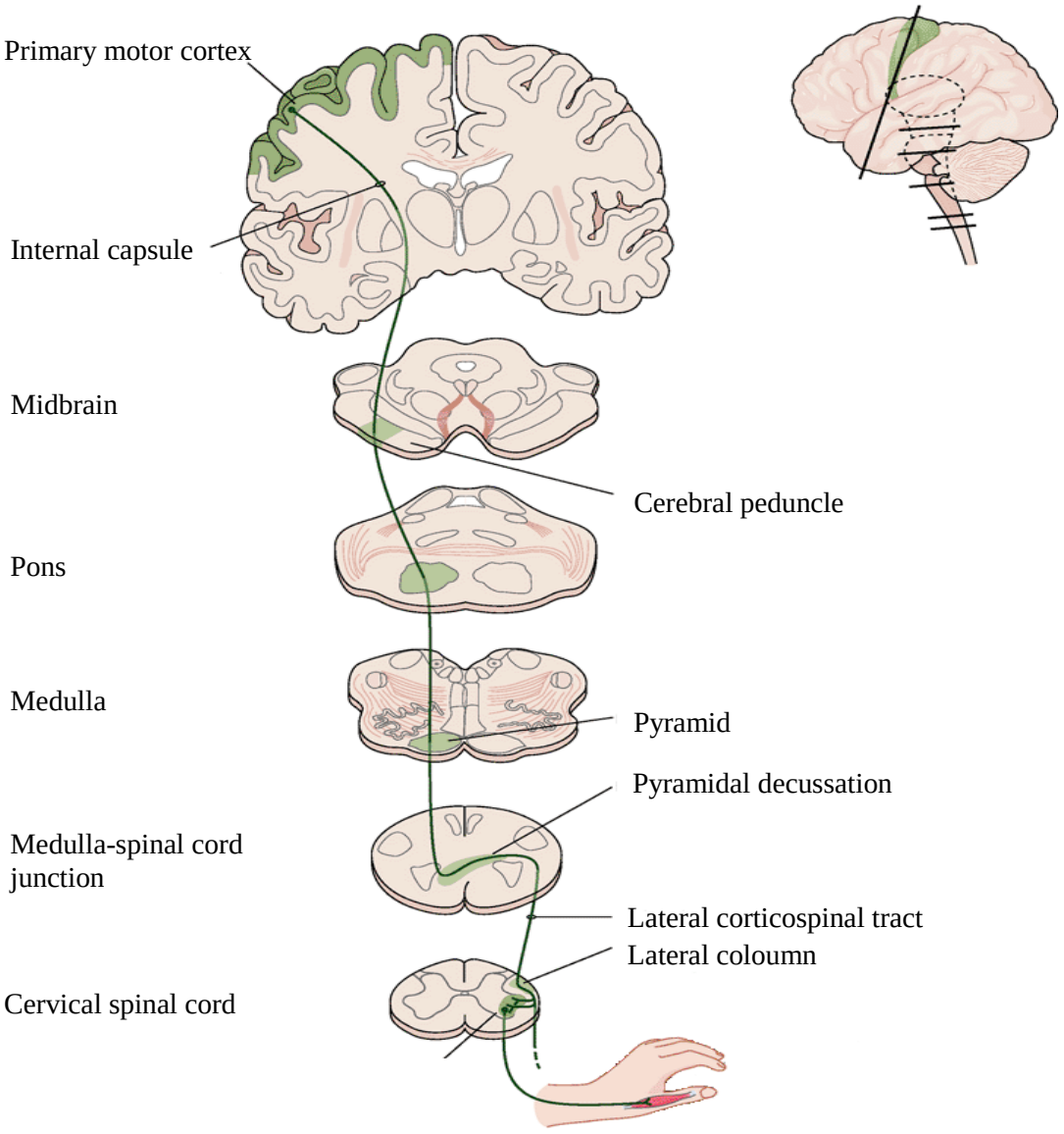

Figure 8 The coronal view of the descending lateral corticospinal pathway shows this tract arising from the M1, passing through the medullary pyramids, and terminating at the spinal cord to be directed to the target muscle. A majority of corticospinal fibres cross to the contralateral side in the medulla-spinal cord junction (pyramidal decussation) and descend as the lateral corticospinal tract. The remaining fibres descend ipsilaterally as the anterior corticospinal tract (not shown here). Adapted from http://www.ib.cnea.gov.ar.The following section outlines different NIBS techniques, provides an overview of the techniques, and then describes the intervention and assessment technique used in the present thesis.

NIBS techniques

NIBS can involve either or both neurostimulatory and neuromodulatory techniques.

Neurostimulatory techniques

One method of NIBS is TMS, a non-invasive tool for stimulating the human brain by means of rapidly changing magnetic fields (Wassermann et al. 2008). The stimulating effect is achieved by induction of brief cortical currents, which depolarize the cell membranes of both cortical excitatory pyramidal cells and inhibitory interneurons. If the depolarization exceeds a threshold level, the neuron will discharge.

TMS can be used as an assessment or as a therapeutic technique. rTMS is a therapeutic tool which is defined as application of regularly repeated single TMS pulses at certain high or low frequencies (Rossi et al. 2009). High-frequency rTMS (more than 1Hz) increases CSE (Pascual-Leone et al. 1994b; Rossi et al. 2009), whereas low-frequency rTMS (1 Hz and lower) can transiently suppress CSE (Chen et al. 1997; Hsiao & Weh-Hau Lin 2001).

In all of the studies presented in this thesis; TMS (single-pulse paradigm) is used as an assessment tool, which will be further discussed later.

Neuromodulatory techniques

Despite the above neurostimulatory techniques, tES covers a group of NIBS techniques which does not stimulate cortical neurons. These techniques most likely target neuronal signalling by manipulating ion channels or by shifting electrical gradients, which

influences the electrical balance of ions inside and outside of the neuronal membranes, thus modulating the membrane potential. tES is an umbrella term used for: tDCS, a low-amplitude direct current which modulates CSE in a polarity-dependent manner; tRNS, an oscillatory current which is no longer sensitive to the direction of current flow (Chaieb et al. 2009); and tACS, a sinusoidally applied current which allows manipulation of ongoing and intrinsic cortical oscillations with externally applied electrical frequencies, which is expected to synchronise active cortical rhythms (Paulus 2011). A variant of tACS is slow oscillatory tDCS (so-tDCS) where the stimulation is monophasic due to a DC offset added to the sinusoidal current (Antal et al. 2008; Bergmann et al. 2009; Groppa et al. 2010). The most utilised tES technique is tDCS.

tDCS is the intervention of interest in the present thesis. Compared with other NIBS techniques, tDCS is portable, painless, inexpensive and therefore feasible for home use. In addition, the feasibility of inducing long-lasting excitability modulations makes this technique a potentially valuable tool for induction of CSE. tDCS is the most studied NIBS technique and has the potential to be used as an adjunct or stand-alone intervention for psychological or neurophysiological disorders. tDCS will be described in more detail in the next section.

tDCS

tDCS is a safe and painless technique of brain modulation that has been increasingly investigated in healthy individuals and as a clinical tool for neuropsychiatric and neurological conditions. Direct current was first introduced by Galvani's (1791) and Volta's (1792) experiments on animal and human electricity (Piccolino 1997). This

initiated the clinical application of direct current stimulation in 1804, when Aldini successfully treated melancholic patients with this technique. The discovery of electroconvulsive therapy by Bini and Cerletti in the 1930s, however, led to an abrupt loss of interest in the technique of tDCS. In the 1950s and 1960s this method had a brief reprise and its effects were primarily investigated in animals (Creutzfeldt et al. 1962; Bindman et al. 1964; Purpura & McMurtry 1965). During that time it could already be shown that tDCS is able to affect brain functions via modulation of CSE (Albert 1966a; Albert 1966b). Most of the effects and mechanisms of DC stimulation, as explored in these animal studies, seem to be similar to those found to account for the tDCS effects in humans (Nitsche et al. 2009).

tDCS is delivered by a constant current stimulator to the cortex through a pair of saline-soaked surface sponge electrodes. It induces focal and prolonged- yet reversible- shifts of CSE (Priori et al. 1998; Nitsche & Paulus 2000; Nitsche & Paulus 2001; Priori 2003). Membrane potential changes induced by chemical neurotransmission, either pre- or postsynaptically may play an important role in tDCS effects (Liebetanz et al. 2002). The direct current enters the brain through the anode (positive charged electrode), then travels through the brain tissues, and finally exits through the cathode (negative charged electrode) (Figure 9) (George 2009). During application of tDCS, some of the injected current is shunted through the scalp. The actual amount of shunted current is dependent on the electrode dimensions, position and the proximity of the anode and the cathode. Increasing the distance between the electrodes over the scalp increases the relative amount of current entering the brain, than ‘shunted’ across the scalp (Miranda et al. 2006). Using smaller electrodes could consequently increase the distance between the

electrodes (Nathan et al. 1993). It also affects the portion of the injected current that reaches the brain or which is shunted through the scalp (Datta et al. 2009).

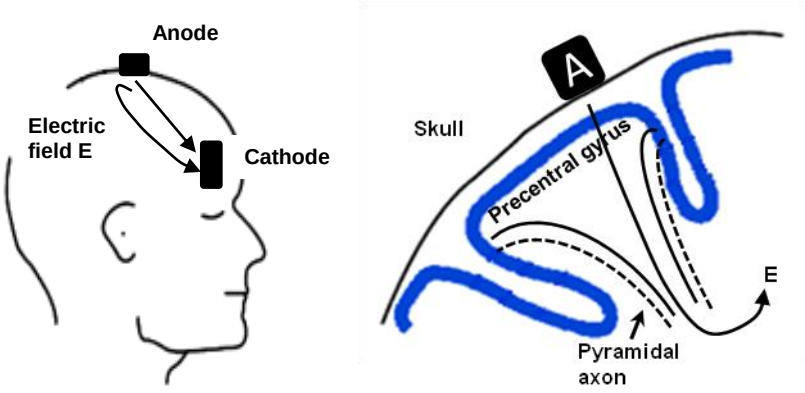

Figure 9 tDCS current flows from the anode to the cathode, creating an electric field (E) in the same direction. The line labelled E is parallel and radial to the scalp (shown schematically here). This figure is adapted from (Hallett & Chokroverty 2005).

Electrode montage is critical for achieving expected effects. The 'active' electrode is always placed over the targeted cortical region (i.e. M1) (Nitsche & Paulus 2001; Nitsche et al. 2003a; Hummel & Cohen 2005; Boggio et al. 2007; Boggio et al. 2009b). The 'reference' or 'indifferent' electrode is most often placed over the contralateral supraorbital ridge (Nitsche et al. 2003a; Hummel & Cohen 2005; Iyer et al. 2005; Floel et al. 2008). This is the most utilized montage for application of tDCS, therefore it was used for the application of tDCS in the studies presented in this thesis.

In addition to the motor cortex, other regions including the prefrontal cortex (Antal et al. 2004; Kincses et al. 2004; Fregni et al. 2005a), the occipital cortex (Matsunaga et al.

2004; Antal et al. 2006; Boggio et al. 2009b), Broca's area (Monti et al. 2008), and Wernicke's area (Floel et al. 2008; Sparing et al. 2008) have also been targeted.

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. On the other hand, anodal tDCS (a-tDCS), application of the positive charged electrode (anode) over the target area of stimulation, results in cortical depolarization, indicating increased CSE (Nitsche & Paulus 2000; Nitsche & Paulus 2001) (Figure 10).

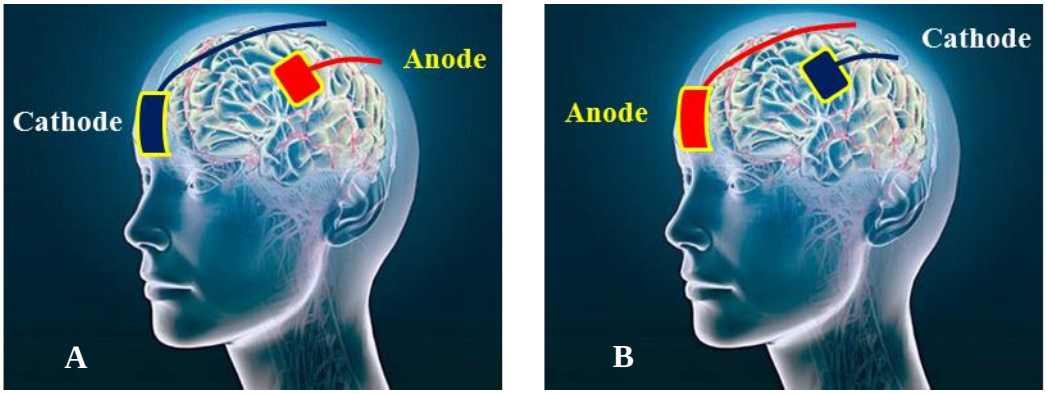

Figure 10 The placement of electrodes for a-tDCS (A) and c-tDCS (B) of the M1. For a-tDCS the anode is placed over the M1 of the target muscle, and the cathode is placed over the contralateral supraorbital ridge. For c-tDCS the placement of electrodes is reversed.

Once the electrodes are placed and fixed correctly, stimulation can be started. The current intensity as well as the duration of stimulation can be set in the tDCS device (Sparing & Mottaghy 2008). Many devices have a built-in capability that allows the current to be 'ramped up' or increased slowly until the necessary current is reached. This decreases the sudden stimulation effects felt by the person at the beginning of the tDCS application. The current will then continue unchanged during the set treatment time and finally will be automatically shut off.

tDCS safety

Due to widespread use of tDCS in neuroscience research on both healthy individuals and on patients with pathological conditions, its safety is of central importance. tDCS is a very safe method (Nitsche et al. 2003b). A precise experimental design is also critical in achieving the desired safety issues. Previous animal studies are the basis for safety conclusions made by tDCS researchers (Yuen et al. 1981; Agnew & McCreery 1987; McCreery et al. 1990).

Safety of brain stimulation depends on the amplitude of applied current, the size of the electrodes and the duration of the stimulation (Priori et al. 1998; Nitsche & Paulus 2000; Nitsche & Paulus 2001; Nitsche et al. 2003b; Iyer et al. 2005). To determine the safety limits of tDCS, current density and total charge of the applied current have to be considered (Agnew & McCreery 1987; Nitsche et al. 2003b). The following formulas are used to calculate the current density and total charge:

$$Current\ density\ (\frac{mA}{cm^2}) = \frac{stimulus\ intensity\ (mA)}{electrode\ size\ (cm^2)}$$

$$Total\ charge\ (\frac{C}{cm^2}) = \frac{stimulus\ intensity\ (mA)}{electrode\ size\ (cm^2)} \times total\ stimulus\ duration\ (s)$$

or

$$Total\ charge\ (\frac{C}{cm^2}) = \frac{stimulation\ strength\ (mA)}{electrode\ size\ (cm^2)} \times pulse\ duration\ \times number\ of\ pulses\ (s)$$

The recommended safety guideline was determined by McCreery et al. (1990) and Yuen et al. (1981) as 25 mA/cm^2 for current density and 216 C/cm^2 for total charge (Yuen et al. 1981; McCreery et al. 1990). Furthermore, research has been done to determine relevant parameters for safe application of tDCS in humans. Studies of tDCS safety shows 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 cm^2 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 individulas and patients with stroke Gandiga et al. (2006) have shown that tDCS (1mA current intensity; 25 cm^2 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 cm^2 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 a two-year period – 70.6% noticed a mild tingling under the electrodes, 35.3% felt fatigue following treatment, and 30.4% felt itching under the electrodes. Additionally, headache (11.8%), nausea (2.9%), and insomnia (0.98%) were reported. The authors concluded that tDCS is still safe to use when safety guidelines are followed (Poreisz et al. 2007). However, it was recently reported that the use of 2 mA caused skin lesions in five patients following 2 weeks (5 days per week) of 20 minute tDCS administrations. These potential side effects should be communicated with participants during the administration of tDCS at an intensity of 2 mA (Palm et al. 2008), and other long applications of tDCS even when using smaller intensities.

Furthermore, literature suggests that tDCS under safe protocols does not cause heating effects under the electrodes (Nitsche & Paulus 2000), does not increase the serum neurone-specific enolase level (Nitsche & Paulus 2001; Nitsche et al. 2003b) and does not result 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, was observed (Nitsche et al. 2003b; Nitsche et al. 2004).

In conclusion, in following the tDCS safety guidelines safety was ensured by all the stimulation parameters used in the current thesis. The induced a-tDCS total charge varied between 7.5 to 50 C/cm^2 which is far below the reported safety limit (216 C/cm^2) (Yuen et al. 1981). All participants tolerated the applied currents very well and there was no interruption of experimental procedures due to the side- or adverse-effects of the applied currents in all of the presented studies in this thesis.

Tools for assessment of cortical organization

Neuroscience research methods have developed dramatically in recent decades. The availability of noninvasive neuroimaging and electrophysiological techniques allows us to study cortical reorganization in the intact human brain. Single- and multi- neuron recording, EEG, computerised tomography (CT), positron emission tomography (PET), single photon emission computed tomography (SPECT), photon migration tomography (PMT), magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI), electromagnetically based magnetoencephalography (MEG) and TMS are examples of methods which enable researchers to identify the normal or abnormal functions of different brain regions.

Each approach investigates the human brain from a different perspective and complements the other techniques. CT produces anatomical images with x-rays. Also introduced in the 1970s, MRI provides anatomical images of the brain with tissue contrast superior to CT. PMT, also called near-infrared spectroscopy or optical imaging, is a new method for measuring cortical activity by registering the scattering of near-infrared light from the brain tissue. MEG, EEG, PET, SPECT and fMRI are based on electrophysiological, hemodynamic or metabolic changes that occur during task performance and therefore depend on cooperation of the subject. PET and fMRI have high spatial resolution but very limited temporal resolution (Baudewig et al. 2001; Lang et al. 2005): therefore, they can neither provide detailed information on the timing of task-related activation during a specific motor act, nor show the relative importance of each cortical area for different components of task performance. MEG and EEG have

excellent temporal resolution, but it can be difficult to identify the neurons responsible for the signal.

To determine which brain areas show changes in activity in relation to movements, the mentioned techniques with high spatial resolution are certainly powerful, but they provide limited information about the contribution of these areas to the control of the movement or to the nature of the neuronal activity. Also, these methods lack temporal resolution and cannot alone prove that an area is essential for a particular function. On the other hand, TMS presents the advantage of a precise timing and relatively good localization. With the TMS technique an external stimulus is used to elicit motor responses for evaluation of M1 excitability. TMS, unlike other techniques, can also be frequently repeated.

At the present, TMS is the only available technique that can be used as a direct neuronal activation tool to evaluate the activity of the corticospinal tract, and intracortical interneurons, in relation to different movements in humans and their functional connectivities. TMS can be used to measure various parameters in the motor cortex, allowing us to evaluate different aspects of corticospinal excitability (Hallett 2000). During the past two decades, the availability of this approach has triggered lots of clinical and physiological studies. This assessment technique was utilized as a gold standard measurement tool for assessment of CSE.

TMS is the core assessment technique for evaluation of CSE changes in the studies listed in this thesis, and will be explained in more detail in the following section. It was

also used to locate the M1 of the target muscle for the placement of active electrode during tDCS application (Nitsche et al. 2008).

TMS

In 1985, Barker introduced the technique of TMS as a painless and non-invasive method of magnetic stimulation of the human motor cortex (Barker 1985). This technique has been used extensively in human motor control research since that time (See Petersen et al. 2003 for review).

A magnetic stimulator consists of a capacitor and an inductor (the stimulating coil), and the operating principles are based on Faraday's Law. In the 19th century, Michael Faraday demonstrated that a current was induced in a secondary circuit when it was brought in close proximity to the primary circuit in which a time-varying current was flowing. A changing electrical field produces a changing magnetic field that, consistent with Faraday's Law, causes current to flow in nearby conducting material. With TMS, electrical charge is stored in capacitors. When the machine is discharged, a brief pulse of current of up to 5000 ampere passes through the copper stimulating coil (Jalinous & Chris 2006). Discharge of this stored energy produces a time-varying electrical field and in turn produces a transient magnetic field that causes current to flow in a nearby secondary conducting material, such as the brain.

The scalp tissues and skull present little impedance to a magnetic field of rapidly changing intensity. This magnetic field passes unimpeded through the scalp and tissue. The ability of the current induced in the brain to excite nerve cells depends upon its time-course, magnitude and direction. The intensity of the magnetic field is represented

by influx lines around the coil and is measured in Tesla (T), it declines rapidly with distance. The direction of current flow in the coil is opposite to the direction of the induced current in the nervous tissue (Figure 11).

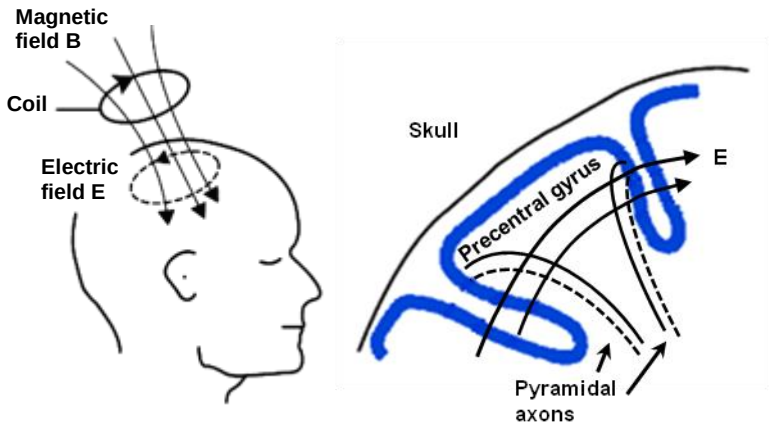

Figure 11 In TMS, current in the coil generates a magnetic field B that induces an electric field E. The lines of B go through the coil; the lines of E form closed circles. The above drawing schematically illustrates a lateral view of the precentral gyrus. Two pyramidal axons are shown with a typical orientation of the cranium. The intracranial electric field E is parallel to the scalp, and causes an electric pulse to travel down the pyramidal axons. This figure is adapted from (Hallett & Chokroverty 2005).

TMS has been used for many different purposes including studying CSE changes and brain mapping (Cohen et al. 1998). TMS methodology has been widely used in clinical studies, demonstrating excitability alterations in various diseases, including Parkinson's disease (Valls-Sole et al. 1994; Morgante et al. 2006), dystonia (Sohn & Hallett 2004; Quartarone et al. 2005), Huntington's disease (Lorenzano et al. 2006), Tourette's syndrome (Berardelli et al. 2003), and essential tremor (Cantello 2002; Modugno et al. 2002).

TMS-induced evoked responses are called motor evoked potentials (MEPs), which can be measured by electromyography (EMG). Fibres that run parallel to the cortical surface are preferentially excited by focal TMS with anteroposterior orientation of the coil. TMS

results in activation of the corticospinal tract and the induced MEPs can be recorded from the target muscle by EMG (Figure 12).

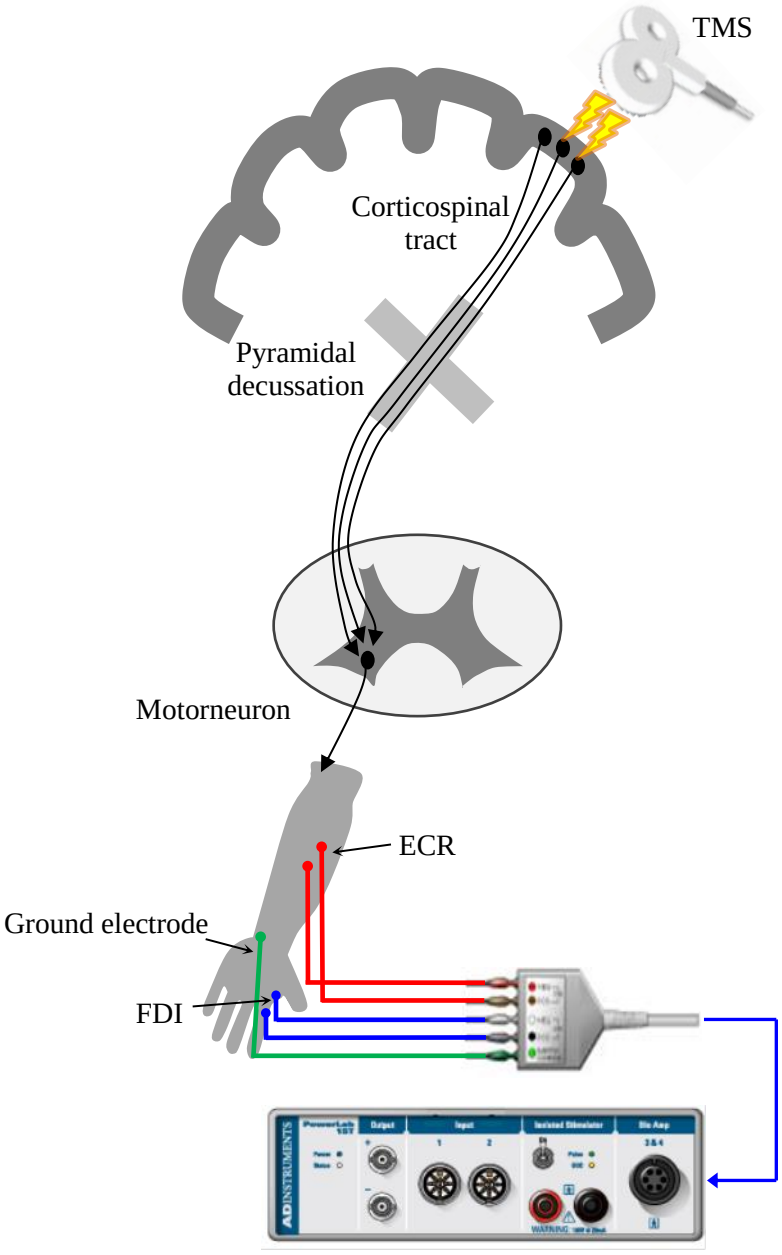

Figure 12 MEPs generated by TMS are recorded from the right extensor carpi radialis (ECR) in studies 2 to 5 and 7, and the right first dorsal interossei (FDI) in studies 2 and 6.

MEP recording

The subject should be seated comfortably during MEP recording, with easy access available to the subject's head for stimulation of the target area(s). The area of stimulation, contralateral to the target muscle, is first determined through rough measurement of the scalp using the conventions of the EEG 10/20 system to approximate a representative spot over the M1 that would allow measurement of the largest MEP responses. Then the exact location of this spot is determined by scanning of nearby areas for maximum MEP response in the desired target muscle. This brain region is called the ‘hotspot’. After localizing the hot spot, the coil's position is marked with a permanent marker on the scalp to guide the experimenter during the remainder 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. 1994). Small alterations in the orientation of the TMS coil on the scalp can alter the efficacy of stimulation and result in excitation of different populations of cortical neurons (Amassian et al. 1992).

For MEP studies presented in this thesis, the magnetic stimulator was connected to a standard EMG machine to synchronize the recording with the TMS pulse. When a TMS pulse was delivered over the M1, an MEP was observed in EMG recordings from the muscle controlled by that segment of the motor cortex. Surface EMG was recorded from the target muscle using bipolar Ag/AgCl disposable surface electrodes. To ensure good surface contact and to reduce skin resistance, a standard skin preparation procedure of cleaning and abrading was performed for each electrode site (Gilmore & Meyers 1983;

Schwartz 2003; Robertson et al. 2006). The location of the target muscle was determined based on anatomical landmarks (Perotto & Delagi 2005) and also observation of muscle contraction in the testing position (Kendall et al. 2010). Location of stimulation over M1 was reliably assured by neurophysiological confirmation that the coil was centred over the hotspot that produced the maximal amplitude of MEP in the desired target muscle. The accuracy of the EMG electrode placement was verified by asking the subject to maximally contract the muscle(s) of interest while the investigator monitored online EMG activity. A ground electrode was placed ipsilaterally on the styloid process of the ulnar bone (Basmajian & De Luca 1985; Oh 2003). The electrodes were secured by hypoallergenic tape (Micropore, USA). All raw EMG signals were band pass filtered, amplified and collected for offline analysis on commercially-available PC software.

In all studies, the stimulus intensity was set at suprathreshold level (1.2 times resting threshold) and we expected to induce MEP in all traces. The amplitude and latency of MEPs are the most important features of induced responses and will be explained in the next sections.

MEP amplitude

MEP amplitude provides an immediate quantitative measure of the degree of CSE changes (Wassermann et al. 2008). In addition to inter-individual differences it provides great inter-trial variability even in the same subject (Kiers et al. 1993). Several technical factors influence MEP amplitude. These include coil positioning (Wassermann et al. 2008), direction of induced electrical field (Wassermann et al. 2008), movements of the

coil (Gugino et al. 2001), and TMS inter-pulse interval (Vaseghi et al. 2013). In addition, a number of physiological factors may also influence the size of MEPs: the number of recruited motor neurons in the spinal cord (Keenan et al. 2006), the number of motor neurons discharging more than once to the stimulus (Magistris et al. 1998; Z'graggen et al. 2005), the synchronization of the TMS-induced motor neuron discharges (Wassermann et al. 2008), the level of background muscle activity (Hess et al. 1986), limb position (Labruna et al. 2011), and state of arousal (Labruna et al. 2011). Even with all conditions stable, however, there remains a considerable between-trial variability that is essentially random (Kiers et al. 1993). Facilitation manoeuvres such as voluntary contraction of the target muscle increase MEP amplitude. Even low background contraction may significantly increase MEP amplitude (Darling et al. 2006). This is particularly helpful in the lower extremities and trunk muscles where MEPs are sometimes difficult to obtain even at maximal stimulator output.

In the studies presented in this thesis, peak-to-peak MEP amplitude (Figure 13) is detected and measured automatically (Figure 14) using a custom designed macro in Powerlab 8/30 software. The setup is shown in Appendix 8.

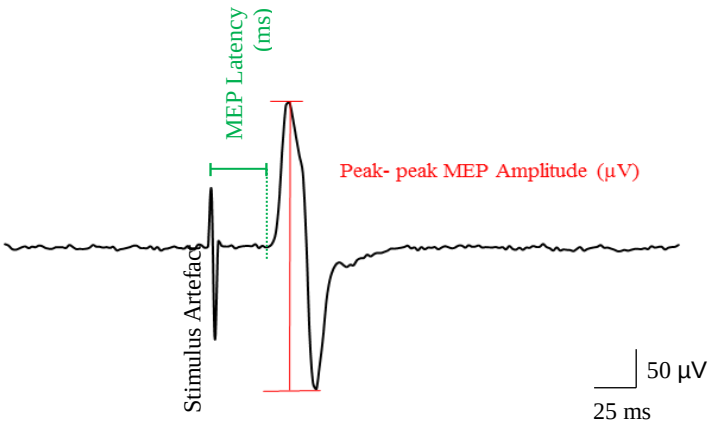

Figure 13 Recorded MEP from the extensor carpi radialis muscle. MEP amplitude is measured from peak-to-peak. MEP latency is measured from the TMS stimulus artefact to the onset of the recorded MEP from the target muscle.

Recording MEPs using LabChart

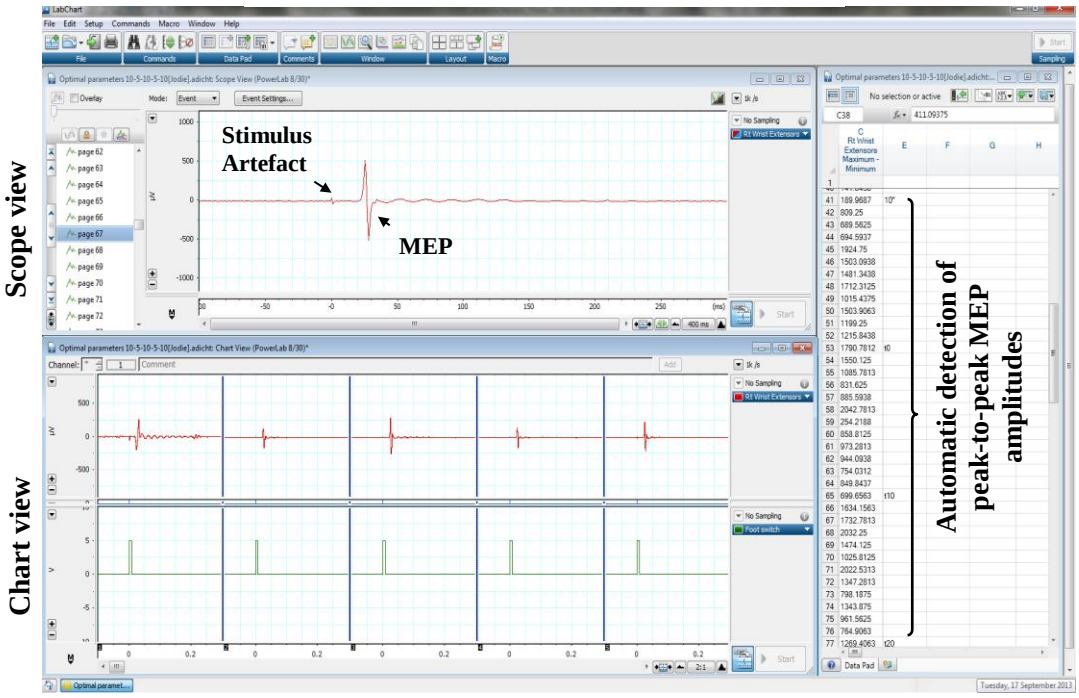

Figure 14 The automatic detection of peak-to-peak MEP amplitudes using Labchart software from the Adinstrument Company.

MEP latency

The latency of the MEP is the time between the cortical stimulation and the onset of an evoked response in the target muscle. The latency of the MEPs is generally regarded as its most reliable characteristic (Rothwell 1997) (Figure 13).

Motor threshold

MEP threshold is defined as the lowest stimulus intensity of TMS that gives a recordable MEP in a target muscle. The motor threshold is used as a reference to set the stimulation intensity during subsequent MEP recordings and may serve as a physiological measure for CSE. Motor threshold can be measured with the muscle of interest at rest and is referred to as rest motor threshold (RMT) or when the muscle is in a low level of sustained contraction referred to as active motor threshold. In all studies included in this thesis, RMT has been recorded, which is explained in more detail in the next section.

Resting Motor Threshold

RMT reflects the global excitability of the motor pathway, including large pyramidal cells, cortical excitatory and inhibitory interneurons, and spinal motorneurons (Ziemann 2004).

RMT has been classically defined as the amount of TMS machine output (intensity) necessary to produce an MEP that exceeds a defined peak-to-peak amplitude (usually 50 μV) 50% of the time in a finite number of trials. Accurate estimation of RMT is of

utmost importance in both research and clinical studies as it is the unit most commonly used for TMS application (Wassermann 2002). Inaccurate estimation of RMT can lead to overstimulation of a subject's cortex, which can increase the probability of TMS-induced seizures (Pascual-Leone et al. 1993; Wassermann 1998; Pascual-Leone et al. 1999). However, timesaving estimation of RMT is also important for the sake of subject comfort, as well as expedience in the laboratory or treatment room.

Guidelines for assessment of RMT by Rothwell et al. (1998) suggest that the TMS operator starts with a suprathreshold TMS intensity and decreases in steps of 2% or 5% of machine output until a level is reached below which reliable responses disappear (where the definition of 'reliable response' is based on the stimulus strength at which successful MEPs are observed 50% of the time in 10 to 20 consecutive stimuli) (Rothwell et al. 1998). An interstimulus interval of > 3 seconds has been recommended for determination of MEP threshold to prevent any facilitatory or inhibitory influence on the subsequent stimulation (Rothwell et al. 1999).

In this thesis the procedure recommended by Rothwell and colleagues (1998) has been followed. The only modification was that the RMT for each subject was determined by increasing/ decreasing the stimulus intensity in gradations of 1% or 2%.

RMT is usually lower for upper extremity muscles compared to lower extremity muscles. This has been associated with stronger corticospinal projections to these muscles (Chen et al. 1998). In addition, RMT is generally lower for distal rather than proximal muscles in both upper and lower limbs. In upper extremity muscles, lowest threshold values are reported for intrinsic hand muscles and finger extensors. This is

consistent with the larger cortical representations of these muscles (Rossini et al. 1994; Mills & Nithi 1997). Also, lower thresholds have been reported for the representation of the muscle(s) in the dominant hemisphere (Macdonell et al. 1991; Triggs et al. 1994).

The size of the RMT could be affected by a number of factors including the consumption of caffeine (in coffee and energy drinks), sleep deprivation and sedative medicines. Other factors that have been shown to influence RMT are sodium-channel blockers (Ziemann et al. 1996), sitting or standing vs. lying supine (Ackermann et al. 1991), neck rotations (Alagona et al. 2001), mental activity, and closing and opening of the eyes (Rossini & Rossi 1998). In addition, any slight contraction of the target muscle decreases MEP threshold, and it is therefore important to assure complete muscle relaxation when determining the RMT.

For all MEP measurements in Chapters 3 - 8, the TMS intensity is set at 120% of each individual's RMT. Twelve stimuli (Studies 3 and 6) or fifteen (Studies 2, 4, 5 and 7) were delivered in order to assess CSE with a frequency of 0.2 Hz (one TMS stimulus every 5 seconds). The stimulus intensity was kept constant throughout the study session for each subject.

TMS coil types

There are a number of different types of TMS coils on the market. These coils are designed for different purposes. Depending on the stimulation target, coil designs with various degrees of focality and field penetration depth could be desirable. The shape and

size of the TMS coils determines the output (peak magnetic field) of the coil and its stimulating characteristics (strength, depth of penetration and size of stimulated tissue) (Terao & Ugawa 2002). The most commonly used coil shape in TMS studies are the circular, figure-of-eight and double cone coils. Generally, circular and double cone coils allow direct stimulation of deeper brain regions and are less focal, while figure-of-eight coils allow focal stimulation of superficial cortical regions. Compared to circular coils which stimulate a large number of cortical columns, figure-of-eight coils are less stimulating. The physical characteristics of these TMS coils are described in Table 1.

Table 1: The physical characteristics, maximum calculated outputs, and advantages and disadvantages of the coils used with the Magstim 200^2. Adapted from (Jalinous & Chris 2006).

	Circular 50 mm	Circular 70 mm	Circular 90 mm	Figure-of-8 25 mm	Figure-of-8 50 mm	Figure-of-8 70 mm	Double cone
Inside diameter (mm)	25	40	66	18 (×2)	34 (×2)	56 (×2)	96 (×2)
Outside diameter (mm)	77	94	123	42 (×2)	74 (×2)	87 (×2)	125 (×2)
Peak magnitude field strength (T)	3.6	2.6	2	4	N/A	2.2	1.4
Peak magnitude electric field	600	530	530	660	N/A	660	N/A
Number of discharges at 100%	65	63	145	40	78	60	584
Advantage	The activation of neurons that lie deep and about 1.5 to 2.0 cm below the scalp surface			Focal stimulation			Stimulation of deep parts of the brain
Disadvantage	Non-focal stimulation			The activation of more superficial cortical areas			Non-focal stimulation

In all studies presented in this thesis, a 70 figure-of-eight magnetic coil mm new generation model was used (Explained in more detail in the next section).

Figure-of-eight coils

A figure-of-eight or butterfly coil is composed of two adjacent windings or round coils put together side by side on the same plane, each passing current in the opposite direction (Figure 15). The coils are made smaller than stand-alone round coils to improve 'focality' of the induced current (Ueno et al. 1988). The standard 70 mm figure-of-eight (70 mm in diameter each circle) has a peak magnetic field power of 2.2 T (Jalinous & Chris 2006). Such a coil induces two current loops that are superimposed at the junction of the two loops, where a maximum in the magnitude of the induced electric field is formed. Due to this configuration in the figure-of-eight coil the current flows in two opposite directions generating two electric fields. The figure-of-eight coil elicits more focal stimulation with maximal activation occurring beneath the intersection site, but produces a weaker and less penetrating magnetic field than the circular coil (Epstein et al. 1990).

The figure-of-eight coil allows the isolated stimulation of one hemisphere. Furthermore, by moving the coil along the central gyrus it is possible to activate muscle groups separately within a limb.

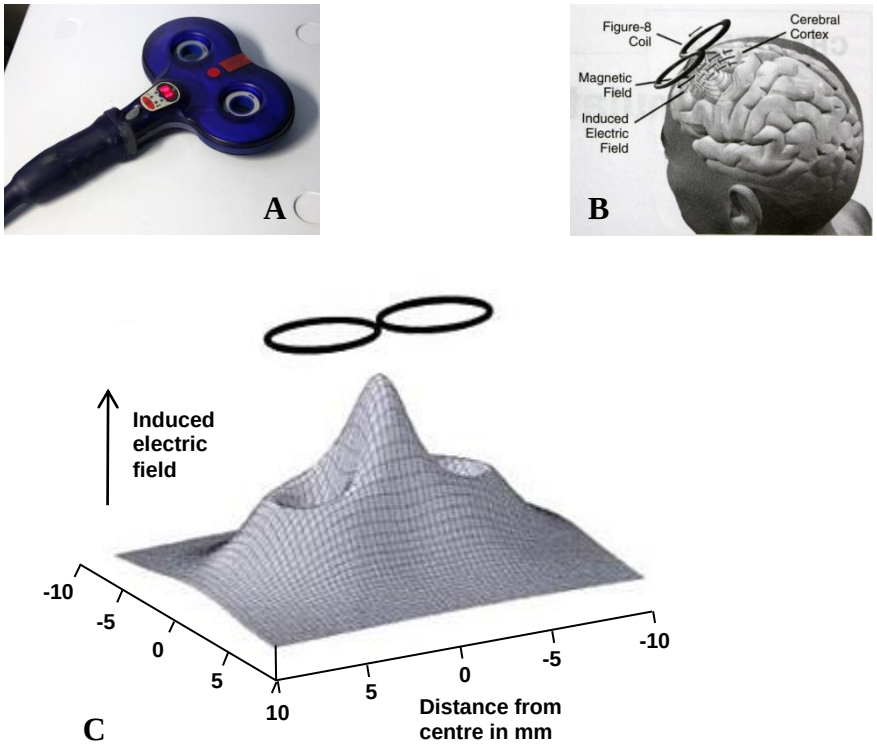

Figure 15 A) The figure-of-eight magnetic coil. B) The magnetic and electric field produced by a figure-of-eight coil. The two narrow black arrows show the current directions in the two side loops, which will be added together at the coil junction. Adapted from The Oxford Handbook of Transcranial Stimulation (Wassermann et al. 2008). C) The strength of the electric field induced below a figure of eight coil. Adapted from (Hallett & Chokroverty 2005).

Types of stimulators

TMS can be applied by one stimulus at a time (called 'single-pulse' TMS) or in pairs of stimuli separated by a variable interval ('paired-pulse' TMS). Single or paired-pulses of TMS are used to obtain and assess electrophysiological information about the

excitability of the motor cortex. Paired-pulse TMS is a valuable tool for investigating the intracortical inhibitory and facilitatory mechanisms within the motor cortex (Kujirai et al. 1993; Bütefisch et al. 2003) but it is not relevant to this thesis. Single-pulse TMS, however, is introduced in the following section.

Single- pulse

Single pulsed TMS is the most widely used stimulator paradigm. It has good temporal resolution and refers to TMS techniques with an inter-stimulus interval that is longer than five seconds. A number of different and valuable neurophysiological measures of CSE can be derived from single-pulse TMS. These include motor threshold, MEP size, representational motor mappings, input-output curves (MEP amplitude versus TMS intensity), and silent period duration. A single pulse TMS may be monophasic, biphasic or polyphasic. Each of these has its own properties and so may be useful in particular circumstances.

In all of the presented studies, single pulse magnetic stimuli were delivered using a Magstim 200^2 stimulator (Magstim, UK) (Figure 16). Magstim 200^2 produces a monophasic pulse with no current reversal. Monophasic discharge currents reduce heat dissipation in the coil, discharge click noise, stimulus artefact and increase stimulus accuracy in comparison to biphasic stimulators. In addition, the stable and well defined monophasic pulse allows for a better understanding of the mechanisms involved in magnetic nerve stimulation, particularly when used for cortical stimulation.

Figure 16 A) Participants position sitting upright in an adjustable podiatry chair. B) Magstim 2002 and a figure-of-eight 70 mm stimulating coil. This machine is widely used in research and neurology departments throughout the world to evoke motor responses from healthy individuals or patients undergoing a clinical neurological examination.

Given the potential for variability in the parameters of the MEP responses elicited by TMS and the growth in research applications for TMS, certain factors should be reported and/or controlled in single or paired pulse TMS studies. Recently, Chipchase et al. (2012) developed a checklist for these factors (Chipchase et al. 2012). The use of this checklist is essential in ensuring research findings are correctly interpreted. The recommendations in this checklist [once it was published online in 2012] are considered in studies presented in this thesis (Table 2).

Table 2: Checklist for reported and/or controlled factors in single or paired pulse TMS studies (Chipchase et al. 2012).

Were the following participant factors	Reported?	Controlled?
Age of subjects	☐	☐
Gender of subjects	☐	N/A
Handedness of subjects	☐	☐
Subjects prescribed medication	☐	☐
Use of CNS active drugs (e.g. anti-convulsants)	☐	☐
Presence of neurological/ psychiatric disorders	☐	☐
When studying healthy subjects		
Any medical conditions	☐	☐
History of specific repetitive motor activity	☐	☐
Were the following methodological factors		
Position and contact of EMG electrodes	☐	☐
Amount of relaxation/contraction of target muscles	☐	☐
Prior motor activity of the muscle to be tested	☐	☐
Level of relaxation of muscles other than those being tested	N/A	☐
Coil type (size and geometry)	☐	☐
Coil orientation	☐	☐
Direction of induced current in the brain	☐	☐
Coil location and stability (with or without a neuronavigation system)	☐	☐
Type of stimulator used (e.g. brand)	☐	☐
Stimulation intensity	☐	☐
Pulse shape (monophasic or biphasic)	☐	☐
Determination of optimal hotspot	☐	☐
The time between MEP trials	☐	☐
Time between days of testing	☐	☐
Subject attention (level of arousal) during testing	☐	☐
Method for determining threshold (active/resting)	☐	☐
Number of MEP measures made	☐	☐
Paired pulse only: Intensity of test pulse	☐	☐
Paired pulse only: Intensity of conditioning pulse	☐	☐
Paired pulse only: Inter-stimulus interval	☐	☐
Were the following analytical factors		
Method for determining MEP size during analysis	☐	☐
Size of unconditioned MEP	☐	☐

Safety of TMS

TMS is believed to only cause a transient change in neural activity without long-lasting effects (Bridgets & Delaney 1989; Bridgers 1991; Pascual-Leone et al. 1992; Chokroverty et al. 1995; Yamada et al. 1995). However, the possibility of unforeseen risks in the long term has not been excluded (Wassermann et al. 2008). TMS safety is a function of stimulation rate (Wassermann 1998). As the stimulation frequency of TMS increases, the risk of unwanted side effects increases. In many studies, single-pulse TMS (< 1 Hz) in healthy adults appears to pose no significant health risk. Prospective studies designed to systematically evaluate health effects have found no changes in heart rate, serum prolactine, blood pressure, cerebral blood flow, EEG, memory or cognition (Cohen & Hallett 1987; Bridgers 1991; Ferbert et al. 1991). The most commonly reported side effect of TMS is headache (5%) (Daskalakis et al. 2002). Subjects may experience some discomfort under the coil due to muscle contraction and stimulation of nerves on the scalp. If a subject develops headache, it is usually easily managed with standard analgesics.

A study in which three monkeys received 7000 stimuli each at maximum intensity over thirty days demonstrated no short- or long-term deficits in higher cerebral function, nor any other adverse effects (Yamada et al. 1995). In normal healthy subjects, prolonged high intensity rTMS with rates of 10-25 Hz can produce partial seizures with or without secondary generalization (Xiao-Ming & Ju-Ming 2011). A study of rTMS in healthy young participants indicates that exposure of healthy men to 12960 magnetic pulses a day for up to 3 days in 1 week failed to produce seizure or any other significant side effects (Anderson et al. 2006). A literature review on the safety and tolerability of rTMS

indicates that even by using rTMS in epileptic patients the risk of seizure is very small (0.35%) (Bae et al. 2007).

Certain conditions increase the risks associated with TMS (Appendix 13). The TMS Adult Safety Questionnaire was developed to alert investigators to factors in prospective subjects that may predispose them to adverse events during TMS (Wassermann 1998).

Furthermore, if the guidelines for safe application of TMS as the assessment technique are followed there will be no risks to participants. If not followed closely, risks are still minimal. Some potential risks are categorised and described below:

1. Effect of magnetic field on the biological tissue

The magnetic field generated can reach a peak of 2.2 T for a duration of less than 1 millisecond. This value is less than the level of 2.5 T used during MRI. In addition, while MRI typically exposes large portions of the human body to constant magnetic fields, TMS only exposes a small area of the body to intermittent transient magnetic fields. Therefore, no direct harmful effects on human tissue have been reported or expected due to the short duration as well as the relatively low level of magnetic stimulation.

2. Heat production

The total power dissipated at a constant TMS stimulus rate of 1 per second and 100% intensity is less than 10-3 Watts (W). Normal functioning of the body and brain produces 13W of energy in the adult human brain, and hence TMS heating effects are not considered to be harmful (Wassermann 1998).

3. Effects on immune system

Lateralized effects of single-pulse TMS on T lymphocyte subsets have been reported. The increases, which appear to be consistent across individuals, resolve within 48 hours. Comparable changes in lymphocyte subpopulations can occur with mild stress, normal circadian cycle, and the menstrual cycle. Therefore, the effects of TMS on immune system changes are not considered to be dangerous or harmful (Amassian et al. 1994; Sontag & Kalka 2007).

4. Seizures

There exists a very small possibility that seizures may be induced through the use of TMS, even though in the last 20 years of TMS usage, there have been no reported cases of accidental induction of seizures using single-pulse TMS in healthy individuals with no cortical lesions or abnormalities (Hallett 2000). Nevertheless, seizures have been produced by single-pulse TMS in several patients with large cerebral infarcts or other structural lesions (Kandler 1990; Fauth et al. 1992). There has also been the possibility that a minor degree of risk is involved in the use of TMS in people with epilepsy (Hufnagel et al. 1990; Düzel et al. 1996).

In all studies in this thesis the latest TMS and tDCS safety guidelines have been considered. A modified version of the TMS safety questionnaire (Appendix 14) was completed prior to all the experiments of the present studies to screen and exclude subjects for whom TMS was contraindicated.

Tools for the measurement of motor performance

To examine the effect of a-tDCS on the motor performance improvements, different assessment instruments are used. The most popular ones are: the Jebsen-Taylor Hand Function Test, designed to provide a short, objective test of hand functions commonly used in the activities of daily living (Jebsen et al. 1969); the Box and Block test, designed to assess unilateral gross manual dexterity (Mathiowetz et al. 1985); the Purdue pegboard test (PPT), which measures the gross movements of hands, fingers and arms, and the fine fingertip dexterity necessary in assembly tasks (Tiffin 1968) (detail in the next section); and the Grooved pegboard test (GPT), a variation of the PPT.

The GPT consists of key-shaped pegs that must be rotated to match the groove in the corresponding hole in a horizontal board (Tremblay et al. 2003). One advantage of the GPT is that there is no variation according to handedness; that is, left-handers complete the task in the same amount of time as right-handers (Ruff & Parker 1993).

In study 6 (Chapter 7), PPT is used for motor performance assessment before and after a single, or a number of a-tDCS applications. A full description of PPT is presented in the next section.

The Purdue pegboard test (PPT)

The PPT (Lafayette Instrument Company) is utilized as a reliable instrument for the assessment of manual dexterity and motor performance (Tiffin & Asher 1948). Several studies have shown the reliability of the PPT. The correlation coefficient for one-trial

administration of the test have ranged from adequate (0.60-0.79) (Tiffin & Asher 1948; Bass & Stucki 1951; Tiffin 1968; Reddon et al. 1988; Desrosiers et al. 1995) to excellent (> 0.80) (Desrosiers et al. 1995). A summary of validity studies of the PPT have shown that the obtained validity ranged from 0.07 to 0.76. (Tiffin & Asher 1948).

The PPT consists of a wood console with a shallow cup to contain the pegs on the top of the console and 50 holes (two parallel columns of 25 holes). Participants are seated directly in front of the pegboard and are instructed to pick up small pegs from the cup with the thumb and index finger and place them in holes. The aim is either to place as many pegs as possible in 30 seconds (Reddon et al. 1988) or to place 25 pegs on one side of the column in top-down order as fast as possible with the target hand. Participants are allowed to practice prior to the test in order to familiarize themselves with the PPT, and to control for potential learning effects. Motor performance improvement involves either an increase in the number of pegs placed within 30 seconds, or completing the task faster. In Chapter 7 of this thesis, measurement is done by timing how long it takes for each participant to complete the task. The participants are asked to place 25 pegs on the right hand side column in top-down order as fast as they can with their right hand. The time for completion of the task is considered as the outcome measure for evaluation of motor performance.

Declaration for Chapter 2

In the case of Chapter 2, contribution to the work involved the following:

Nature of contribution	**Extent of contribution (%)**
Identification and review of the relevant literature, data analysis, interpretation of the results and writing of the manuscript.	80 %

The following co-authors contributed to the work.

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Shapour Jaberzadeh	Guidance in the framing of the manuscript, review and provision of feedback on manuscript drafts	20 %

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	**Date** 30.09.2013
Signature	**Shapour Jaberzadeh** [redacted]	**Date** 24.09.2013

Preamble to Chapter 2

Chapter 2 provides a systematic review and meta-analysis to verify whether previous TMS studies support the view that a-tDCS increase CSE and motor performance in both healthy individuals and subjects with stroke. Also, the effects of a-tDCS parameters on CSE in both healthy individuals and subjects with stroke are determined.

Chapter 2: Does anodal transcranial direct current stimulation enhance excitability of the motor cortex and motor function in healthy individuals and subjects with stroke: A systematic review and meta-analysis

The format of this chapter is consistent with the Journal of *Clinical Neurophysiology*.

Supplementary tables for this chapter are provided in Appendices 2-7.

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Invited Review

Does anodal transcranial direct current stimulation enhance excitability of the motor cortex and motor function in healthy individuals and subjects with stroke: A systematic review and meta-analysis

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HIGHLIGHTS

- Anodal tDCS (a-tDCS) increases corticomotor excitability in both healthy individuals and subjects with stroke.
- A-tDCS could be used to produce changes in favor of functional improvement in both healthy individuals and subjects with stroke.
- Longer applications of a-tDCS or higher current densities under the active electrode induces larger corticomotor excitability in healthy individuals.

ABSTRACT

The primary aim of this review is to evaluate the effects of anodal transcranial direct current stimulation (a-tDCS) on corticomotor excitability and motor function in healthy individuals and subjects with stroke. The secondary aim is to find a-tDCS optimal parameters for its maximal effects. Electronic databases were searched for studies into the effect of a-tDCS when compared to no stimulation. Studies which met the inclusion criteria were assessed and methodological quality was examined using PEDro and Downs and Black (D&B) assessment tools. Data from seven studies revealed increase in corticomotor excitability with a small but significant effect size (0.31 [0.14, 0.48], $p = 0.0003$) in healthy subjects and data from two studies in subjects with stroke indicated significant results with moderate effect size (0.59 [0.24, 0.93], $p = 0.001$) in favor of a-tDCS. Likewise, studies examining motor function demonstrated a small and non-significant effect (0.39 [−0.17, 0.94], $p = 0.17$) in subjects with stroke and a large but non-significant effect (0.92 [−1.02, 2.87], $p = 0.35$) in healthy subjects in favor of improvement in motor function. The results also indicate that efficacy of a-tDCS is dependent on current density and duration of application. A-tDCS increases corticomotor excitability in both healthy individuals and subjects with stroke. The results also show a trend in favor of motor function improvement following a-tDCS. A-tDCS is a non-invasive, cheap and easy-to-apply modality which could be used as a stand-alone technique or as an adds-on technique to enhance corticomotor excitability and the efficacy of motor training approaches. However, the small sample size of the included studies reduces the strength of the presented evidences and any conclusion in this regard should be considered cautiously.

Contents

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1. Introduction

Several non-invasive strategies aimed at modifying corticomotor excitability have emerged in recent years. They include transcranial magnetic stimulation (TMS), repetitive transcranial magnetic stimulation (rTMS) (Pascual-Leone et al., 1994) and transcranial direct current stimulation (tDCS) (Nitsche and Paulus, 2000). Compared to the other two approaches, tDCS has a number of advantages. First of all, it is a non-invasive and painless corticomotor modulatory technique with no or minimal side effects and it can be applied by an inexpensive battery-operated device which is very simple to operate (Jeffery et al., 2007; Bolognini et al., 2009), even by patients.

tDCS involves application of very low-amplitude direct currents (2 mA or less) via surface scalp electrodes (Webster et al., 2006). This produces a sub-sensory level of electrical stimulation which remains imperceptible by most people during its application. In a small percentage of patients it may cause minimal discomfort with a mild tingling sensation, which usually disappears after a few seconds (Nitsche et al., 2003a,b). The applied current modifies the transmembrane neuronal potential and thus influences the level of excitability (Priori et al., 1998; Nitsche et al., 2008). Depending on the polarity of active electrodes over the primary motor cortex (M1) contralateral to the target muscles, tDCS can increase or decrease corticomotor excitability (Nitsche et al., 2003b, 2008). Cathodal tDCS leads to hyperpolarization (Nitsche and Paulus, 2000, 2001) and reduces the size of the TMS-induced motor evoked potentials (MEPs), indicating decreased motor cortex excitability. On the other hand anodal tDCS (a-tDCS) results in corticomotor depolarization and increases the size of MEPs, indicating an increased motor cortex excitability (Nitsche and Paulus, 2000, 2001). A-tDCS can be used as a stand-alone therapeutic intervention or can be used as an adds-on technique to prime the effects of other training methods (Hummel and Cohen, 2006; Hesse et al., 2007).

The literature indicates that any improvement in corticomotor excitability coincides with functional improvement (Traversa et al., 1997; Liepert et al., 1998; Hesse et al., 2007; Celnik et al., 2009; Nowak et al., 2010). In this regard, purposeful modulation of corticomotor excitability with a-tDCS can be used as a therapeutic technique for promotion of functional performance (Nowak et al., 2010).

TMS is a non-invasive brain stimulation technique which provides a quantitative measure of corticomotor excitability (Paulus et al., 2003). TMS uses electromagnetic induction to induce weak electric currents using a rapidly changing magnetic field. These tiny currents cause stimulation of corticospinal neurons in specific parts of M1 and produces muscle responses (MEPs) in contralateral target muscles (Chiappa, 1997). Calculation of peak-to-peak amplitudes from recorded MEPs, provide an objective outcome measure for assessment of corticomotor excitability (Wassermann et al., 2008). In this context, any corticomotor changes following application of a-tDCS could be detected by measurement of the size of these MEPs (Wassermann et al., 2008).

1.1. tDCS characteristics

tDCS involves application of very weak direct currents to M1 for a period of time. The extent which a-tDCS can directly modify corticomotor excitability or prime the effects of motor training, depends on the current density and duration of its application (Purpura and McMurtry, 1965; Nitsche and Paulus, 2000, 2001; Nitsche et al., 2008). Likewise, the safety of tDCS application is also related to these two parameters (Nitsche et al., 2003a). McCreery et al. (1990) suggested that the appropriate parameter for safety limits of tDCS should be the current density, which is

Table 1
Inclusion and exclusion criteria for identified studies.

	Inclusion	Exclusion
Participants	– Studies in which individuals were over 18 years of age – Either healthy or suffering from stroke as a primary diagnosis (no limits were applied to the type (infract/hemorrhage), anatomical location or stage (acute, subacute or chronic) of stroke)	– Studies involving individuals suffering from other type of neurologically diseases (i.e. from MS, Parkinson, brain, spinal cord injury and spinal tumor) – Non-human subjects
Intervention	– Studies that involve a-tDCS as intervention of interest	– Studies in which there was a combination of interventions or other kind of facilitatory techniques, (i.e. electrical stimulations, somatosensory stimulations, imagery, mirror, strength and isometric muscle activity, constraint induced therapy, active or passive movements task attention, sensory block or anesthesia)
Comparison	– Studies in which the comparison of interest is "no treatment"/ sham treatment – Placebo control	– Other control group
Outcomes	– Studies in which the outcome measures of interest were the MEP amplitude as measured by single pulse TMS and outcome measures of physical performance such as time taken to finish a functional task or number of objects moved during a certain time	– Other type of evaluation of MEPs (measured by rTMS, fMRI, PET, paired TMS)
Trial design	– Randomized control trials, controlled clinical trials, and pre–post trials	– Review articles – Case report – Selective review
Data reported	– Data that enables analysis and estimation of the effects of a-tDCS on characteristics of MEPs or physical performance must be reported	
Type of publications	– Published in a peer-reviewed journal, regardless of the year of publication – As the services for translation do not exist, only English publications will be considered	– Non English articles

determined by the stimulation strength (A) per area of stimulating electrode size (cm^2). They stated that the current densities below 25 mA/cm^2 are safe without inducing any brain tissue damage. In previous a-tDCS studies, the maximum applied current densities were 0.066 mA/cm^2 which is a thousand-fold smaller than the current density limit proposed by McCreery et al. (1990). The current density is independent of stimulation duration, therefore Nitsche et al. (2003a) point out that "duration of stimulation is also an important additional factor in causing tissue damage" and total charge should be considered in tDCS stimulation studies (Nitsche et al., 2003a). The induced total charge reflects the effects of stimulation duration and current density. Until now, no single study has compared the effects of different current densities or total charges with the effect of duration time of stimulation induced by a-tDCS.

In this review the studies addressing the effects of a-tDCS on corticomotor excitability and/or motor function in healthy individuals and subjects with stroke were systematically reviewed. The primary aim of this review was to synthesize and analyze the results of investigations into the effects of a-tDCS when compared to sham/no stimulation on the corticomotor excitability of M1 in healthy individuals and subjects with stroke and on the physical performance in subjects with stroke.

Likewise, the optimal parameters for more effective a-tDCS are not clear. Therefore, the secondary aim of this systematic review was to determine the effect of stimulation parameters on corticomotor excitability in both healthy individuals and subjects with stroke using a-tDCS.

We hypothesized that a-tDCS increases M1 excitability in healthy individuals and subjects following stroke and enhances the effects of motor training approaches in subjects with stroke. In addition, we also hypothesized that longer application of a-tDCS duration or larger current densities under active electrode within the safety limit, induces more effective changes in corticomotor excitation.

2. Methods

2.1. Literature search

To locate eligible articles, a literature search was performed using the following databases: PubMed, Physiotherapy Evidence Database (PEDro), AMED, CINAHL, EMBASE, CENTRAL (Cochrane Central Register of Controlled Trials), Scopus, PROQuest, SPORTDiscuss, AMI (Australian Medical Index), OvidMedline, EBM Reviews, Cochrane, Meditext and Psychoinfo, from their inception to October 2010.

Our key search terms were: 'transcranial direct current stimulation', 'tDCS', 'cranial direct current stimulation' and 'direct current stimulation', 'transcranial magnetic stimulation' or 'magnetic stimulation' and 'cortical plasticity' or 'cortical reorganization' or 'cortical excitability' or 'corticomotor excitability' and 'functional performance' or ' motor function' or 'motor skill', 'motor performance' and any associated variation. These terms were used in various combinations to find relevant studies. In addition to searching the database, the reference lists of all retrieved papers were searched for any related publications unidentified by the initial search strategy. Two reviewers (AB and SJ) independently screened the title and abstract of papers identified in the initial search strategy against the inclusion criteria (Table 1) and potentially relevant studies were retrieved for evaluation of the full texts. Differences of opinion between reviewers were resolved by consensus.

2.2. Selection criteria

Papers were included if they met the inclusion and exclusion criteria listed in Table 1: (1) application of a-tDCS in healthy individuals or subjects with stroke over 18 years of age; (2) MEPs amplitude used as outcome measure of interest measured by μv, mv or percentage scales; (3) assessment of motor function following application of a-tDCS intervention; (4) studies published as

Table 2
Study characteristics and outcome measures.

Included studies	Trial design	Subject characteristics	Muscle	Outcome measure	How this outcome is measured	↑ or ↓ MEP amplitude	↑ or ↓ Motor Function
Antal et al. (2007)	Pre–post test	Healthy subjects	FDI[a]	MEP amplitude	TMS	↑	–
Boros et al. (2008)	Pre–post test	Healthy subjects	ADM[b]	MEP amplitude	TMS	↑	–
Edwards et al. (2009)	Pre–post test	Subjects with stroke	FCR[c]	MEP amplitude	TMS	↑	–
Furubayashi et al. (2008)	Pre–post test	Healthy subjects	FDI	MEP amplitude	TMS	↑	–
Jeffery et al. (2007)	Pre–post test	Healthy subjects	TA[d]	MEP amplitude	TMS	↑	–
Nitsche and Paulus (2000)	Pre–post test	Healthy subjects	ADM	MEP amplitude	TMS	↑	–
Uy and Ridding (2003)	Pre–post test	Healthy subjects	FDI	MEP amplitude	TMS	↑	–
Nitsche et al. (2005)	Pre–post test	Healthy subjects	ADM	MEP amplitude	TMS	↑	–
Hummel et al. (2005)	Double–blind crossover	Subjects with stroke	FDI	MEP amplitude Function test	TMS,JTT	↑	↑
Boggio et al. (2007)	Double binded-sham controlled	Subjects with stroke	–	Function test	JTT	–	↑
Fregni et al. (2005)	Double binded-sham controlled	Subjects with stroke	–	Function test	JTT	–	↑
Kim et al. (2009)	Single-blinded, sham-controlled	Subjects with stroke	–	Function test	BBT	–	↑
Boggio et al. (2006a)	Double binded-sham controlled	Healthy subjects		Function test	JTT	–	↑
Hummel et al. (2009)	Double binded-sham controlled	Healthy subjects		Function test	JTT	–	↑

[a] FDI: first dorsal interossei.
[b] ADM: abductor digiti minimi.
[c] FCR: flexor carpi radialis.
[d] TA: tibialis anterior.

peer-reviews in journals or books; (5) pre–post and controlled clinical study designs. Included papers were limited to humans and full-text English language publications. Full-text versions were analyzed and studies in which there was a combination of therapeutic interventions with a-tDCS, (such as: electrical stimulations, active or passive movement, imagery, mirror, strength and isometric muscle activity, constraint-induced therapy, task attention, sensory block or anesthesia), or paired associative stimulation, or which did not measure corticomotor excitability with TMS, were excluded. With papers presenting as ambiguous, such as when the abstracts did not violate any exclusion criteria, or when there was insufficient detail in the abstract to make an informed decision, the full text of the papers were reviewed.

2.3. Outcome measures

We included prospective studies that evaluated the effects of a-tDCS on MEPs amplitude and/or motor function as primary and secondary outcome measures. TMS-induced MEPs amplitude is the most commonly used outcome measure for assessment of corticomotor excitability (Wassermann et al., 2008). The peak-to-peak amplitude of induced MEPs of target muscles determines the level of corticomotor excitability.

Motor performance improvement is usually determined by the time taken to finish a task or the number of moving items in a specific time duration (Mathiowetz et al., 1985). In this review motor function improvements in included studies have been assessed with the Jebsen Taylor test of hand function (JTT) which is a valid and reliable hand function test regarding activities of daily living (Jebsen et al., 1969; Hackel et al., 1992) and includes a range of seven subset fine motor, weighted and non-weighted hand function activities. The other hand dexterity test was Box and Block test (BBT), a reliable measure of upper extremity function and involves repeatedly moving blocks from one side of a box to another during a specified time (Mathiowetz et al., 1985).

2.4. Quality assessment

A quality assessment was conducted for each included study by using the Physiotherapy Evidence Database (PEDro scale) (Moseley et al., 2002; Maher et al., 2003) to assess the methodological quality of included articles. The PEDro scale includes 11 specific criteria, graded on a "yes"/"no" scale in which the first item relates to external validity and the other 10 items assess the internal validity of a clinical trial. The first criterion does not count toward the overall score that the paper receives for the quality of its study design. The PEDro scale is marked out of 10, the higher the PEDro score, the higher the assumed "quality" of the trial as assessed by the following cut-points defined by Foley et al.: 9–10, excellent; 6–8, good; 4–5, fair and below 4, poor (Foley et al., 2002). The process was also repeated using the Downs and Black tool (D&B) (Downs and Black, 1998). The reason behind using this scale is simply because most of these studies are non-randomized trials (Saunders et al., 2003). The original D&B tool ranged from 0 to 32 (Downs and Black, 1998). The D&B tool contains 27 questions, of which 25 are graded on 0 or 1 ("yes"/"no" or "not determined"), and one item in the reporting subscale which is scored 0 to 2 and a single item on power, which is scored 0–5. Eng et al. (2007) modified the item about the power of study, from a scale of 0 to 5 to a scale of 0 to 1 (Eng et al., 2007). Thus, their modified version ranged from 0 to 28, with a higher score indicating higher methodological quality.

2.5. Data extraction

The following data relevant to the aims of this study were extracted: (1) study design; (2) characteristics of subjects (Table 2); (3) outcome measures (Table 2) and a-tDCS parameters (Table 3); and (4) mean ± standard deviation (SD) of immediately post intervention MEPs amplitude and motor function outcomes. Since for the purpose of this study the data could be extracted regardless

Table 3
A- tDCS parameters.

Included studies	Size of active electrode (cm^2)	Intensity (mA)	Current density (mA/cm^2)	Time (min)
Antal et al. (2007)	35	1	0.029	10
Boros et al. (2008)	33	1	0.03	13
Edwards et al. (2009)	35	1	0.029	20
Furubayashi et al. (2008)	15	1	0.066	10
Jeffery et al. (2007)	35	2	0.057	10
Nitsche and Paulus (2000)	35	1	0.029	5
Uy (2003)	25	1	0.04	10
Nitsche (2005)	35	1	0.029	13
Hummel et al. (2005)	25	1	0.04	20
Boggio et al. (2007)	35	1	0.029	20
Fregni et al. (2005)	35	1	0.029	20
Kim et al. (2009)	25	1	0.04	20
Boggio et al. (2006a)	35	1	0.029	20
Hummel et al. (2009)	25	1	0.04	20

Table 4A
Extracted outcome measures.

Included studies	Outcome measure	Experimental group			Control group		
		Mean	SD	Total (n)	Mean	SD	Total (n)
Antal et al. (2007)	MEP amplitude	1.13	0.13	12	1	0.13	12
Boros et al. (2008)	MEP amplitude	1.41	0.62	8	0.87	0.08	8
Edwards et al. (2009)	MEP amplitude	1.36	0.42	6	0.77	0.18	6
Furubayashi et al. (2008)	MEP amplitude	1.28	0.72	8	0.3	0.11	8
Jeffery et al. (2007)	MEP amplitude	1.06	0.16	8	0.97	0.16	8
Nitsche and Paulus (2000)	MEP amplitude	1.34	0.24	19	1	0.05	19
Uy and Ridding (2003)	MEP amplitude	2.46	1.42	10	1.93	1.23	10
Nitsche et al. (2005)	MEP amplitude	2.07	0.65	12	1.59	0.79	12
Hummel et al. (2005)	MEP amplitude	1.96	1.17	6	1.42	0.78	6
Hummel et al. (2005)	Function test	39.72	5.24	6	43.57	5.75	6
Boggio et al. (2007)	Function test	49.4	12.4	4	54	16.2	4
Fregni et al. (2005)	Function test	59.33	16.54	6	63.8	18.22	6
Kim et al. (2009)	Function test	43.3	20.19	10	35.8	18.58	10
Boggio et al. (2006a) $_{DS}$	Function test	24.46	1.75	5	24.26	2.5	5
Boggio et al. (2006a) $_{NDS}$	Function test	24.66	3.33	5	27.22	3.63	5
Hummel et al. (2009)	Function test	28	4.07	10	29.89	4.48	10

Table 4B
Calculated effect size of included studies.

Included studies	Sample size	SMD	95% CI
Antal et al. (2007)	12	0.13	(0.03, 0.23)
Boros et al. (2008)	8	0.54	(0.11, 0.97)
Edwards et al. (2009)	6	0.59	(0.22, 0.96)
Furubayashi et al. (2008)	8	0.98	(0.48, 1.48)
Jeffery et al. (2007)	8	0.09	(−0.07, 0.25)
Nitsche and Paulus (2000)	19	0.34	(0.23, 0.45)
Uy and Ridding (2003)	10	0.53	(−0.63, 1.69)
Nitsche et al. (2005)	12	0.48	(−0.10, 1.06)
Hummel et al. (2005) $_{MEPs}$	6	0.54	(−0.59, 1.67)
Boggio et al. (2007)	4	0.28	(−1.12, 1.68)
Fregni et al. (2005)	6	0.24	(−0.90, 1.37)
Hummel et al. (2005) $_{Function}$	10	0.65	(−0.53, 1.82)
Kim et al. (2009)	10	0.37	(−0.52, 1.26)
Boggio et al. (2006a) $_{DS}$	5	−0.20	(−2.87, 2.47)
Boggio et al. (2006a) $_{NDS}$	5	2.56	(−1.76, 6.88)
Hummel et al. (2009)	10	1.89	(−1.86, 5.64)

of the subject's cortical dominancy, the findings of Boggio et al. study (2006a) were stated as two individual studies of Boggio et al. (2006a) dominant side (DS) and non-dominant side (NDS).

2.6. Data analysis

The effect of a-tDCS on corticomotor excitability and/or motor function was estimated by using effect size index. Effect size is also referred to as standard mean difference (SMD) which provides the measurement of differences in mean outcome after the intervention (Slade and Keating, 2007). Once completed, relevant data were grouped and entered into the effect size calculator using REVMAN 5 software (Cochrane Collaboration, 2008). In meta-analysis for continuous data measurements, the number of participants in each of the two groups, their mean response and the standard deviation of their responses are required (Table 4A). REVMAN calculates statistical significance of the difference between means, 95% confidence intervals (CIs) for the mean difference and uses Hedges' adjusted g, which is very similar to Cohen's d, but includes an adjustment for small sample bias (Deeks and Higgins, 2010) of randomized control trials (Table 4B).

For included studies of pre–post designs, we assigned pre-test (baseline) data, as control in the effect size calculator (Rubin, 2009). When the SD was not reported, it was estimated using the formula $SD = SE\sqrt{n}$ (n = number of subjects in each group) (Higgins and Green, 2008). In instances where there was uncertainty regarding the information and results presented by the authors and when data were not accessible from figures and graphs, an e-mail was sent to the corresponding author(s) to request for mean ± SD of desired outcome measures. Where mean ± SD values were not provided for baseline/control and post-intervention parameters as numerical data, it was pooled out from the graphs with Plot Digitizer software.

A Java-based Plot Digitizer program (Joseph, 2010) was used to digitize scanned plots of functional data. Plot Digitizer is a useful program for extracting data found presented in papers and references as linear, logarithmic axis scales and scatter plots. After

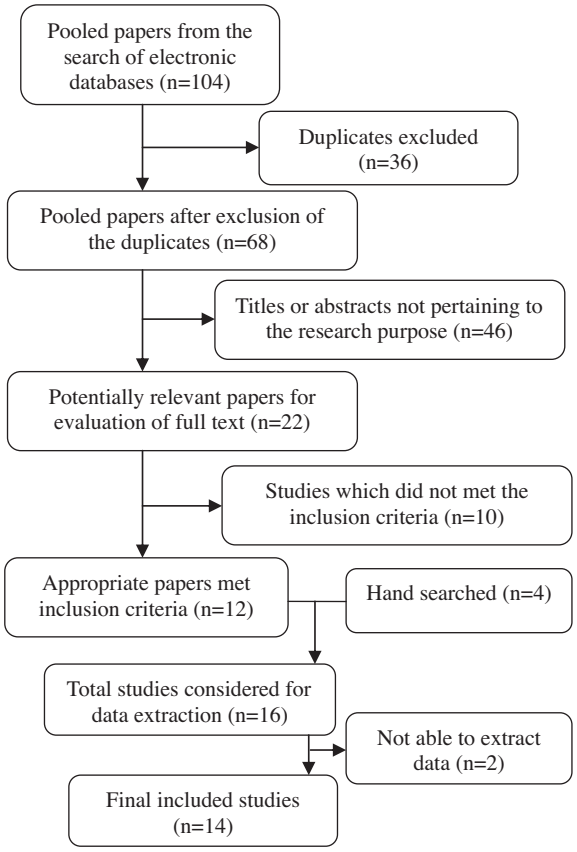

Fig. 1. QUORUM flow chart of studies through the review.

Table 5
Quality assessment of included studies.

Included studies	PEDro (1999)	D&B (Downs and Black, 1998)
Antal et al. (2007)	–	16
Boros et al. (2008)	–	16
Edwards et al. (2009)	–	15
Furubayashi et al. (2008)	–	15
Jeffery et al. (2007)	–	17
Nitsche and Paulus (2000)	–	14
Uy and Ridding (2003)	–	14
Nitsche et al. (2005)	–	15
Hummel et al. (2005)	7	–
Boggio et al. (2007)	7	–
Fregni et al. (2005)	8	–
Kim et al. (2009)	6	–
Boggio et al. (2006a)	8	–
Hummel et al. (2009)	8	–
Mean	(7.3) Good	(15.25) Medium

calibration of the image; we can extract the data values by merely clicking on the data points.

For included studies, the effect sizes were pooled into a meta-analysis. Each study is represented by a block (square) at the point estimate of intervention effect with a horizontal line extending either side of the block. The area of the block indicates the weight assigned to that study in the meta-analysis while the horizontal line shows the CIs. The CIs represents the range of intervention effects compatible with the study's result. The larger size of the block indicates studies with larger weight (usually those with narrower CIs), which dominate the calculation of the pooled result in favor of experiment or control group. Effect size calculation was adjusted so that positive pooled estimate for MEPs amplitude was in favor of increase in corticomotor excitability and negative pooled estimates were in favor of decrease in corticomotor excitability. For motor function performance, the explanation of effect size as being in favor of experiment or control was dependent upon the type of motor function test. When improvement in some motor function scales was shown with positive effect size, while others with negative effect size, the mean values from one set of studies was multiplied by −1 to ensure that all the scales point in the same direction (Higgins and Green, 2008). Data for comparable trials were pooled in the meta-analysis using a fixed or random effect model. The meta-analysis was performed using fixed effect analysis where no heterogeneity was calculated. However, if statistical heterogeneity was detected between trials, data was pooled in the meta-analysis using a random effects model. Statistical heterogeneity is typically assessed and considered likely if $p < 0.1$ for chi-squared testing. Heterogeneity can also be quantified using the I^2 statistic (Higgins et al., 2003). Heterogeneity is considered substantial if the I^2 statistic is found to be greater than 50%. Weighted mean difference (WMD) or SMD was used as the effect measure for MEPs amplitude and motor function assessments with inverse variance being the statistical method of choice. The WMD was employed when outcome measures in all trials were measured on the same scale and the SMD was used to estimate effect size when different outcome measures were used in different trials to measure a comparable outcome. The SMD calculation in RevMan 5 software is given by:

$$N_i = n_{1i} + n_{2i} \quad s_i = \sqrt{\frac{(n_{1i}-1)sd_{1i}^2 + (n_{2i}-1)sd_{2i}^2}{N_i - 2}}$$

$$MD_i = m_{1i} - m_{2i} \quad SMD_i = \frac{m_{1i} - m_{2i}}{s_i}\left(1 - \frac{3}{4N_i - 9}\right)$$

and the WMD calculation is given by:

$$MD_i = m_{1i} - m_{2i}$$

The value of a pooled effect size; thus, gives us the opportunity to clarify the degree of improvement or no improvement in our outcome measures of interest after the intervention. As we know, effect size values are between 0 and 1. If you consider a continuous spectrum line with 0 and 1 at each end, the effect size point on this line, represents the size of intervention effectiveness, with the value closer to 1 is bigger in effect size, in favor of experiment or control group. According to Cohen (1988), an effect size of 0.2 indicates small effect, 0.5 a moderate effect and 0.8 and more large intervention effects (Cohen, 1988). The other scale introduced by Higgins and Green (2008) (Cochrane Handbook of systematic

Table 6
Demographic and stroke characteristics of participants.

Included studies	Sample size	Cause of stroke		Mean time duration after stroke	Mean muscle strength (MRC scale)
		Infarction (n)	Hemorrhage (n)		
Boggio et al. (2007)	4	4	0	40.9 months	4.3
Fregni et al. (2005)	6	6	0	27.1 months	4.18
Hummel and Cohen (2005)	6	6	0	44.3 months	4.8
Kim et al. (2009)	10	8	2	6.4 weeks	$\geqslant 3$ but < 5

review of intervention) is slightly different and considers values less than 0.40 as small, between 0.40 and 0.70 as moderate, and values more than 0.70 as large (Higgins and Green, 2008).

3. Results

3.1. Identification and selection of studies

After removal of the duplicates, the literature search resulted in the identification of 68 studies, of which only 12 were considered appropriate for inclusion in this review for MEPs amplitude and/or motor function outcome measures. Four further studies were identified through hand-searching of reference lists (Fig. 1). Two papers were excluded because no data could be provided either from corresponding authors or graphs, bringing the total number of studies to 14 (Fig. 1).

3.2. Method of quality assessment

The quality PEDro score of included clinical studies ranged between 6 and 8 (with a mean method score of 7.3), which indicates good quality score for controlled clinical trials. Similarly, the 27-item D&B quality checklist provided a medium quality score of included pre–post studies with a mean method score of 15.25. Table 5 shows the calculated quality score of included studies with PEDro and the D&B assessment tool.

3.3. Participants in included studies

In total 77 healthy individuals and 12 subjects with stroke received a-tDCS for MEPs amplitude outcome measurements. For motor function outcome measurements, a total of 20 healthy individuals and 26 subjects with stroke received a-tDCS intervention across the six included studies. Stroke duration ranged from 6.4 weeks to 44.3 months. The majority of included subjects had suffered an ischemic stroke (n = 24 infarction, and n = hemorrhage) (Table 6). All studies examined the effect of a-tDCS intervention in hand muscles. For subjects with stroke the mean muscle strength ranged between 4.18 and 4.8 MRC (Medical Research Council scale for grading muscle strength) in three studies (Fregni et al., 2005; Hummel et al., 2005; Boggio et al., 2007) and $\geqslant$3 but <5 for all paretic finger flexors and extensors in one of the included studies (Kim et al., 2009).

3.4. Electric field orientation

A key parameter during a-tDCS application is the orientation of the electric field which is determined by position and polarity of the electrodes. The anode is defined as the positively charged electrode, whereas the cathode is the negatively charged one. Current flow is from cathode to anode electrode and all tDCS protocols should specify electrode orientation and placements as accurately as possible. Different current flow directions impose different corticomotor effects (Priori et al., 1998; Nitsche and Paulus, 2000). The orientation and placement of electrodes were similar in all included studies (anodal application of tDCS), with the anode on the affected M1 (subjects with stroke) or dominant/non dominant side M1 (healthy individuals), and the cathode on the contralateral supraorbital area. This orientation of electrodes produces an anterior–posterior directed current flow.

3.5. Current density

The most important parameters for tDCS application are current intensity and the size of the active electrode. Out of 12 included studies, 10 used an intensity of 1 mA (Nitsche and Paulus, 2000; Uy and Ridding, 2003; Hummel et al., 2005; Nitsche et al., 2005; Fregni et al., 2005; Antal et al., 2007; Boggio et al., 2007; Furubayashi et al., 2008; Edwards et al., 2009; Kim et al., 2009) while two studies provided an intensity of 0.1 (Boros et al., 2008) and 2 mA (Jeffery et al., 2007).

To increase consistency in reporting of a-tDCS application parameters in included studies, the concept of current density, which is the product of both stimulation intensity and the electrode size, is used. The amount of electric current passing through a cross-sectional area (perpendicular to the direction of current) of an electrode is termed current density, which is commonly expressed in amperes per square centimeter. Size of current density dictates the extent of modulatory effects of a-tDCS on corticomotor excitability and also plays a significant role in safety considerations (McCreery et al., 1990). The highest current density was applied through an electrode size of 15 cm^2 with an intensity of 1 mA (Furubayashi et al., 2008), whereas the lowest current densities were yielded with a 35 cm^2 active electrode size and 1 mA intensity. Current density delivered has varied between 0.029 and 0.066 mA/cm^2 in included studies (Table 3).

3.6. Duration of a-tDCS application

In all included studies on subjects with stroke, the duration of a-tDCS application was 20 min (Fregni et al., 2005; Hummel et al., 2005; Boggio et al., 2007; Edwards et al., 2009; Kim et al., 2009) compared to a range of 5–20 min for the studies on healthy individuals (Nitsche and Paulus, 2000; Uy and Ridding, 2003; Nitsche et al., 2005; Boggio et al., 2006a; Antal et al., 2007; Jeffery et al., 2007; Boros et al., 2008; Furubayashi et al., 2008; Hummel et al., 2009) (Table 3).

3.7. Pooled data analysis

3.7.1. Effects on MEPs amplitude

Fig. 2A summarizes the pooled data (size of MEPs) extracted from seven studies on healthy individuals (Nitsche and Paulus, 2000; Uy and Ridding, 2003; Nitsche et al., 2005; Antal et al., 2007; Jeffery et al., 2007; Boros et al., 2008; Furubayashi et al., 2008). We found a small significant effect size of SMD: 0.31 (95% CI: 0.14, 0.48) from the random effects model (p = 0.0003). As shown in Fig. 2B. the pooled analysis of two trials in subjects with stroke (Hummel et al., 2005; Edwards et al., 2009) indicated that the effect of SMD is significant and moderate: 0.59 (95% CI: 0.24, 0.93) (p = 0.001) in favor of a-tDCS.

3.7.2. Effects on motor function

Four studies examined the effect of a-tDCS on motor function in subjects with hemiparesis (Fregni et al., 2005; Hummel et al., 2005; Boggio et al., 2007; Kim et al., 2009). In three studies (Fregni et al., 2005; Hummel et al., 2005; Boggio et al., 2007), JTT was used to measure hand motor function. The other study (Kim et al., 2009) measured hand function improvement by BBT (Mathiowetz et al., 1985).

The pooled SMD was 0.39 (95% CI: −0.17, 0.94) (p = 0.17) for the fixed effects model in favor of improvement in hand function after a-tDCS (see Fig. 3A).

As shown in Fig. 3B, the pooled analysis of three trials in healthy subjects (Boggio et al., 2006a; Hummel et al., 2009) indicated a large non significant effect size of: 0.92 (95% CI: 0.24, 0.93) for the fixed effects model in favor of a-tDCS (see Fig. 3B).

3.7.3. The effects of tDCS parameters

Pooled analysis of two trials with 13 min of a-tDCS (Nitsche et al., 2005; Boros et al., 2008), indicate that the effects were

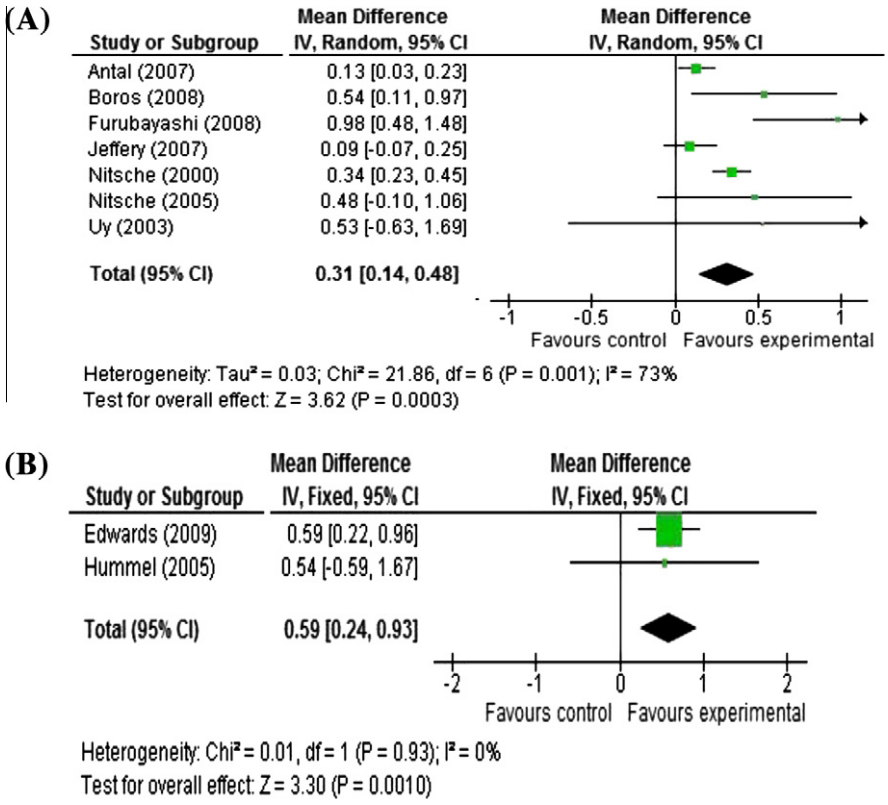

Fig. 2. Forest plot for MEPs amplitude in healthy individuals (A) and subjects with stroke (B). ■ = effect size for one trial; horizontal line = 95% confidence interval; ♦ = pooled effect size for all trials. CI: confidence interval, IV: inverse variance.

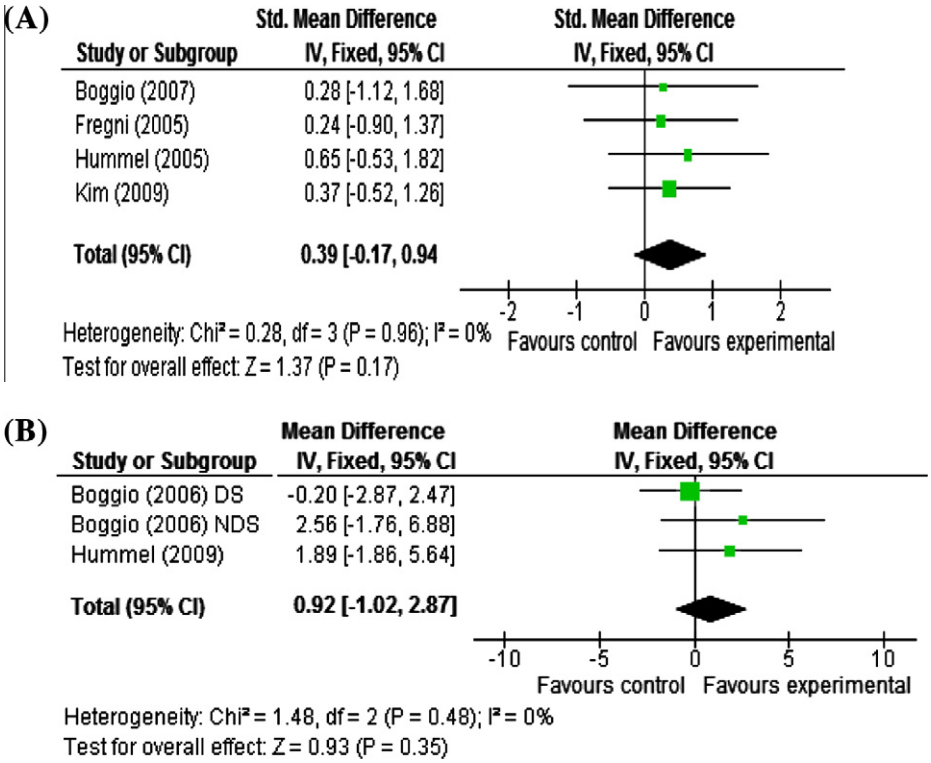

Fig. 3. Forest plot for the effect of a-tDCS on function in subjects with stroke (A) and healthy individuals (B). ■ = Effect size for one trial; horizontal line = 95% confidence interval; ♦ = pooled effect size for all trials. CI: confidence interval, IV: inverse variance. Note: The SMD for Boggio et al. (2007), Fregni et al. (2005) and Hummel et al. (2005) was multiplied by (−1) to ensure that all the scales point in the same direction.

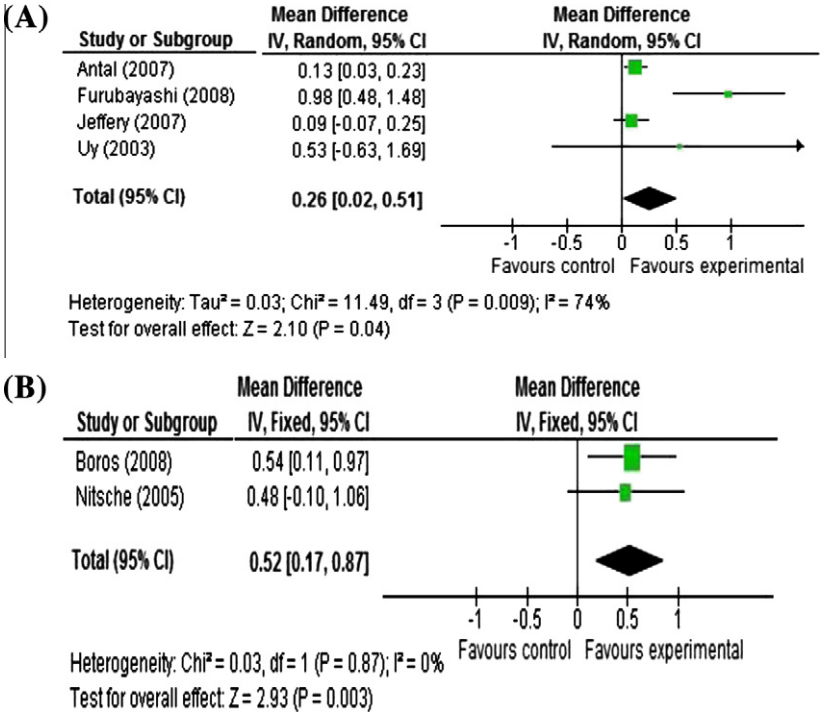

Fig. 4. Forest plots for MEPs amplitude after 10 min (A) and 13 min (B) of a-tDCS ■ = effect size for one trial; horizontal line = 95% confidence interval; ♦ = pooled effect size for all trials. CI: confidence interval, IV: inverse variance.

significant and moderate in size, (SMD: 0.52; 95% CI: 0.17, 0.87), compared to four included studies with 10 min of application (Uy and Ridding, 2003; Antal et al., 2007; Jeffery et al., 2007; Furubayashi et al., 2008), with small and significant effect (SMD: 0.26; 95% CI: 0.02, 0.51) both in favor of a-tDCS stimulation (Fig. 4A and B).

As shown in Fig. 5A and B, pooled analysis of MEPs amplitude in studies with constant current density above 0.029 mA/cm^2 (Uy and Ridding, 2003; Jeffery et al., 2007; Boros et al., 2008; Furubayashi et al., 2008), indicated that the effects were moderate and significant (pooled SMD: 0.50; 95% CI: 0.02, 0.97) compared to constant

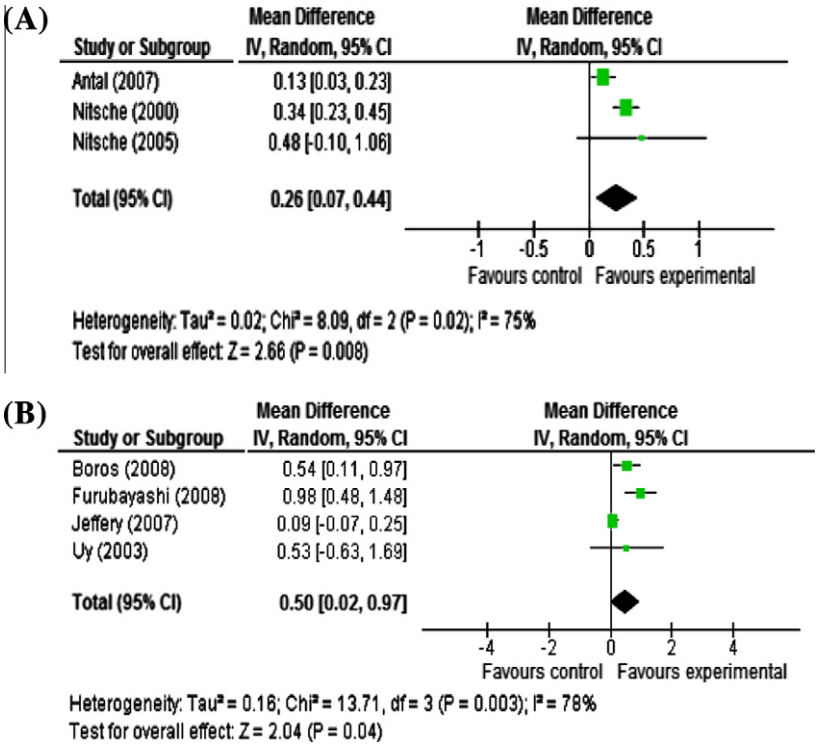

Fig. 5. Forest plots for MEPs amplitude after a-tDCS with current density below 0.029 mA/cm^2 (A) and above 0.029 mA/cm^2 (B) ■ = effect size for one trial; horizontal line = 95% confidence interval; ♦ = pooled effect size for all trials. CI: confidence interval, IV: inverse variance.

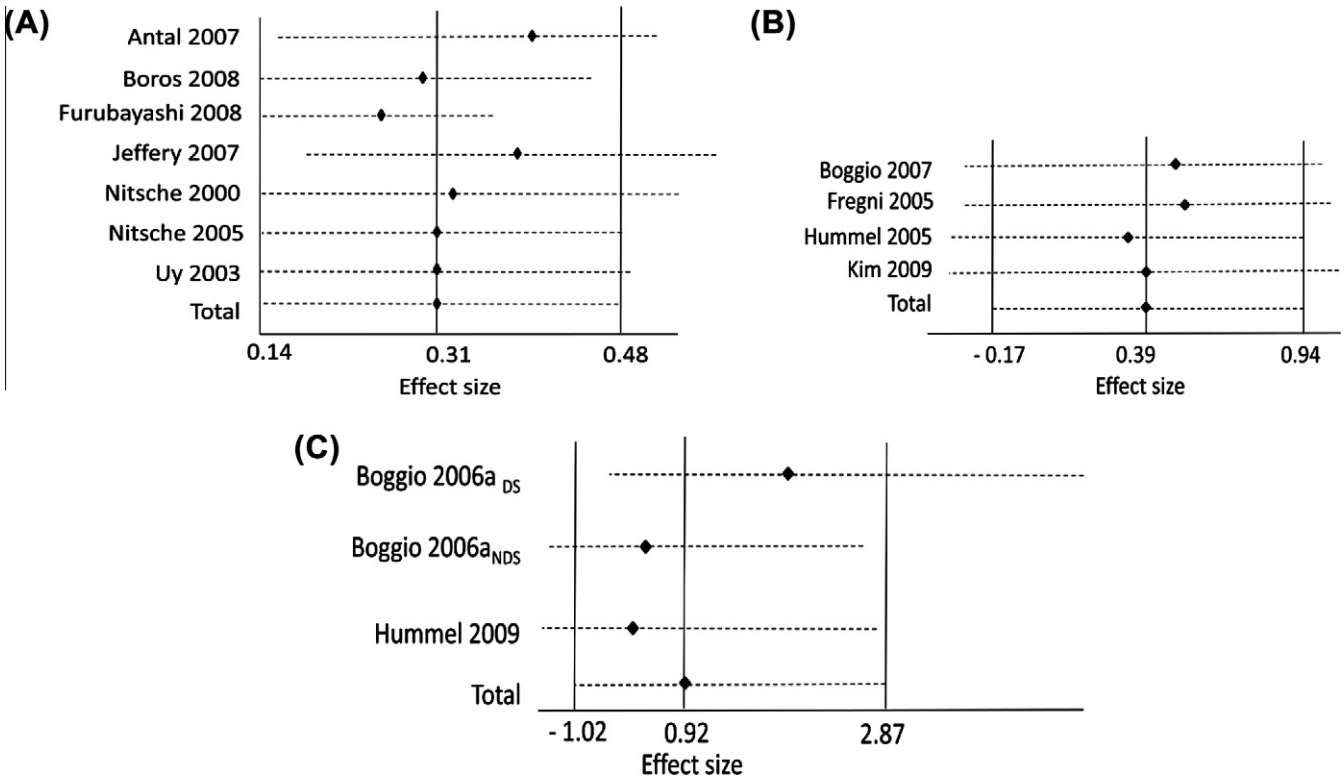

Fig. 6. Assessment of the individual influence of each corticomotor excitability study in healthy individuals (A) and each motor function study in subjects with stroke (B) and healthy individuals (C) after a-tDCS. Effect sizes are Cohen's d (SMD) and error bars represent the 95% confidence interval. The left, middle and right vertical lines are indicator for the minimum, mean and maximum value of total effect size, respectively.

current density below 0.029 mA/cm^2 (Nitsche and Paulus, 2000; Nitsche et al., 2005; Antal et al., 2007) with small significant effects (pooled SMD: 0.26; 95% CI: 0.07, 0.44) in favor of a-tDCS stimulation experiment.

3.8. Impact of each tDCS study on overall results

3.8.1. A-tDCS and corticomotor excitability

The impact of individual studies on the overall meta-analysis estimates were evaluated (Fig. 6A). Interestingly, the total result does not change if we exclude any one single study at a time for MEPs outcome measures in healthy individuals. The pooled data would slightly increases if the studies of Antal et al. (2007) (increases to 0.40), Jeffery et al. (2007) (increases to 0.39) and Nitsche and Paulus (2000) (increases to 0.33) were excluded. The pooled data slightly decrease if the studies of Furubayashi et al. (2008) (decreases to 0.24) or Boros et al. (2008) (decreases to 0.29) were excluded. Remarkably, the pooled data would not change if the studies of Nitsche et al. (2005) or Uy and Ridding (2003) were excluded. Therefore the overall finding of a positive effect of a-tDCS on increased corticomotor excitability as compared to baseline would remain significant even after the exclusion of any single study. Unfortunately, the impact of individual studies can only be examined with at least three included studies, so the results could not be investigated for MEPs amplitude outcome measures (Hummel et al., 2005; Edwards et al., 2009) in two studies of subjects with stroke.

3.8.2. A-tDCS and motor function

Likewise, the impact of individual studies on the meta-analysis estimates, omitting one study, was evaluated for functional improvement outcome (Fig. 6B and C). For the meta-analysis of subjects with stroke, the pooled data would slightly increase if the studies of Fregni et al. (2005) (increases to 0.43) and Boggio et al. (2007) (increases to 0.41) were excluded. The pooled data slightly decrease if the study of Hummel et al. (2005) (decreases to 0.31) was excluded. Remarkably, the pooled data would not change if the study of Kim et al. (2009) was excluded with no change in the significance of pooled effects (Fig. 6B). Acceptably, for the meta-analysis of healthy individuals, the size of pooled data would change if the study of Boggio et al. (2006a) dominant side was excluded, because the study had a small effect in favor of control (Fig. 6C).

4. Discussion

In this study we used meta-analysis to investigate the effects of a-tDCS on corticomotor excitability and motor function enhancement in both healthy individuals and subjects with stroke. According to Cochrane handbook for systematic reviews of interventions "Potential advantages of meta-analyses include an increase in power, an improvement in precision, the ability to answer questions not posed by individual studies, and the opportunity to settle controversies arising from conflicting claims" (Higgins and Green, 2008).

The pooled results facilitate healthcare decision-making by patients and the general public, clinicians and administrators. The value of a pooled effect size; thus, gives us the opportunity to clarify the degree of improvement or no improvement in our outcome measures of interest after the intervention.

4.1. A-tDCS and brain corticomotor excitability

The results of this meta-analysis support our hypothesis that a-tDCS can be effective in increasing corticomotor excitability in healthy individuals (Nitsche and Paulus, 2000; Uy and Ridding,

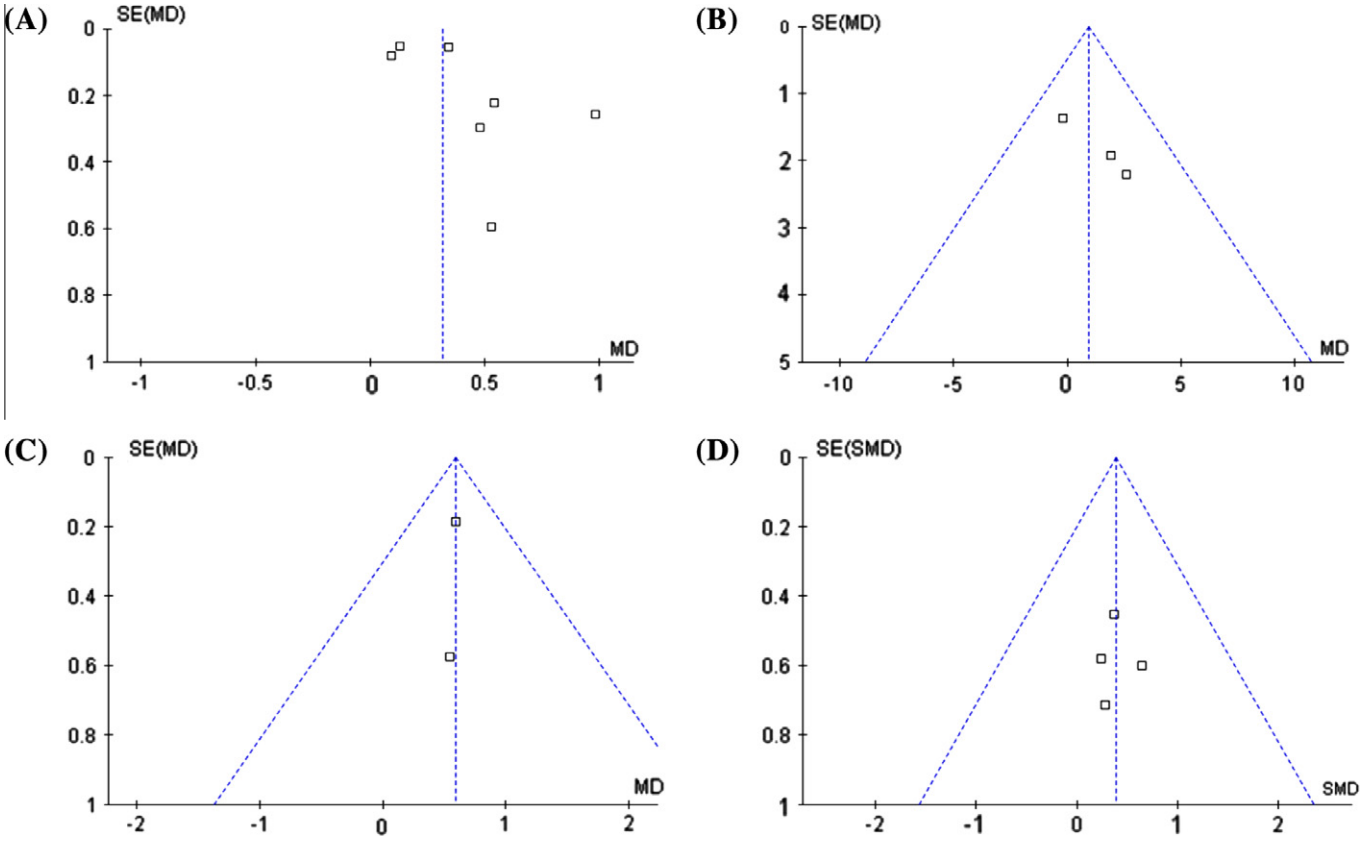

Fig. 7. Funnel plots representative of publishing bias: (A) MEPs amplitude in healthy individuals; (B) functional improvement after a-tDCS in healthy individuals; (C) MEPs amplitude in subjects with stroke and (D) functional improvement after a-tDCS in subjects with stroke.

2003; Nitsche et al., 2005; Antal et al., 2007; Jeffery et al., 2007; Boros et al., 2008; Furubayashi et al., 2008) and most likely subjects with stroke (Hummel et al., 2005; Edwards et al., 2009). Furthermore we demonstrated the evidence against significant heterogeneity and that the results remain robust after excluding each single study in healthy individuals.

Neuroplasticity is defined as the capacity of the brain to develop new neuronal/synaptic interconnections and thereby develop and adapt new functions and roles or to reorganize to compensate for changes (Stuss et al., 1999). The changes in cortical plasticity and CM cell excitability can be enhanced after a-tDCS and the facilitation of the M1 is specific for CM cells controlling the muscles engaged in motor performance.

4.2. A-tDCS and enhancement of motor function

A small number of studies have examined the effects of a-tDCS on the motor function of subjects with stroke (Fregni et al., 2005; Hummel et al., 2005; Boggio et al., 2007; Kim et al., 2009) and healthy subjects (Boggio et al., 2006a; Hummel et al., 2009). One common characteristic for all included stroke studies is that subjects had mild to moderate motor deficits and they had recovered to the point of being able to perform required motor function tests. The results of the meta-analysis on these studies supports that a-tDCS of the affected motor cortex might enhances motor function of the contralateral target muscles, which was demonstrated by a non-significant small effect size (Fig. 3A). Despite the non significant results of this meta-analysis, the trend of changes was in favor of motor function improvement. Indeed, most of the included studies in this meta-analysis was carried out on subjects with chronic stroke. The likelihood of any improvement in this chronic stage is low; therefore, any positive changes in the motor function of these subjects might represent a functional benefit for them. Moreover, the results of the meta-analysis on the studies with healthy subjects demonstrate a non-significant large effect size which supports the possible enhancement of the motor function of the target muscles by a-tDCS (Fig. 3B). This indicates that more studies with larger sample sizes in both healthy individuals and subjects with stroke are required to increase statistical power and bring the *p* value in significant range and increase the likelihood of using this technique in clinical settings.

It is important to note that the interpretation of the effect size is different for each individual subject, each individual studies and also based on individual variables (age, gender, baseline functional level, and even lesion site and severity of condition in subjects with stroke). Therefore, the effect size should be interpret as a relative percentage of functional gain and improvement progress compared to baseline values in both healthy individuals and subjects with stroke.

A number of studies using TMS have illustrated that any improvement in motor performance is coincided with increase in corticomotor excitability (Pascual-Leone et al., 1995; Muellbacher et al., 2001; Ziemann et al., 2001; Garry et al., 2004; Boggio et al., 2006b). In a study done by Ziemann et al. (2001) a remarkable increase in MEPs size of biceps muscle was reported after a training session of repeated voluntary elbow flexion movements. Their results accord with those of Pascual-Leone et al. (1995) which showed improvement in performance after a five-finger piano exercise, associated with enhancement of cortical motor outputs to the muscles involved in the task. Furthermore, Muellbacher et al. (2001) described a linear correlation between the increased MEP amplitude size and the increased pinch force and acceleration of the muscle directly involved in a ballistic finger movement task. Consistent with the above findings, Garry et al. (2004) showed

corticomotor excitability enhancement of hand muscles used in purdue pegboard task performance, with no change seen in muscles unrelated to the task.

These findings confirm the strong evidence for rapid practice-induced changes in M1 excitability and reflect the early stages of memory consolidation in M1 following training (Donoghue and Sanes, 1994; Sanes and Donoghue, 2000; Muellbacher et al., 2002). Recovery following damage to M1 is enhanced by various training programs (Feeney et al., 1982; Wolf et al., 1989; Bütefisch et al., 1995; Nudo et al., 1996). Previous studies suggest that motor recovery following stroke or motor relearning of the paretic limb can be maximized by a-tDCS (Nitsche et al., 2003c; Butefisch et al., 2004). A-tDCS not only increases corticomotor excitability, but may also promote the recovery of the affected limbs following stroke (Nowak et al., 2010), which perhaps is caused by the enhancement of motor learning (Schwenkreis et al., 2005).

4.3. A-tDCS parameters

As a therapeutic technique, optimization of a-tDCS parameters may have a profound impact on its efficacy for enhancement of brain excitability and motor performance. Likewise, in this systematic review we explored the dependence of the efficacy of a-tDCS on treatment time and current density.

4.3.1. Treatment time

The meta-analysis of included studies with constant duration of a-tDCS for 10 or 13 min revealed significant medium effects for longer stimulation duration compared to small effects for shorter stimulation periods (see Fig. 4A and B). Larger effects have been attained with longer durations in a single session of stimulation within a-tDCS safety limits. The reason for this is that the larger amount of total charge under the stimulating electrode induces more changes in the neuronal membrane potential.

4.3.2. Current density

The current review provides evidence for correlation between the level of M1 excitability and current density.

Pooled analysis of studies with constant current density above 0.029 mA/cm^2 compared to current densities below 0.029 mA/cm^2 indicated significant moderate effects for larger current densities, and significant small effects for smaller current densities under the active electrode. Since larger current densities will increase the penetration depth of the magnetic field and therefore will increase cutaneous pain sensation and may affect different primary sensory neurons, we should ensure the efficiency and safety of these stimulation parameters during clinical use (Nitsche et al., 2003a), especially when prolongation of the effects of a-tDCS for an extended time course is desired.

4.4. Publication bias

To investigate the presence of publication bias in our systematic review, four funnel plots were generated. As it is shown in Fig. 7, the funnel plot is not symmetrical for the effects of a-tDCS on corticomotor excitability in healthy subjects (Fig. 7A), although this impression is mainly caused by one small study on the right side of the plot. This may be due to publication bias, however there are other factors leading to an asymmetrical plot. The other possible explanations could be the quality of included studies, non-published studies with a negative or either those showing no difference results, and also studies that may have been performed in a particular population, which often result in exaggerated or overestimated true effect sizes. In the absence of the bias the plot approximately resemble a symmetrical funnel. This is illustrated in Fig. 7A, C and D for the effects of a-tDCS on corticomotor excitability in subject with stroke and motor function improvement in both healthy individuals and subjects with stroke.

4.5. Limitations of the study

Studies of a-tDCS have shown promising results so far, but the following limitations should be considered when discussing the results. First, database searching was limited to English language papers, which possibly has decreased the number of pooled papers in this area. On the other hand, the small sample size in some included studies is associated with the larger effect size that can affect the overall result and statistical significance. Additionally, all included studies looking for the effect of a-tDCS on MEPs amplitude and functional improvement in both healthy individuals and subjects with stroke are a pre–post study designs, without a control group.

4.6. Areas for future research

Regarding the increase of corticomotor excitability in healthy individuals after a-tDCS, any changes in motor learning relevant to the stimulated target muscles should be addressed in future studies. Given the limited number of clinical trials that have assessed the efficacy of a-tDCS on motor function, the studies conducted in subjects with stroke and healthy individuals thus far have been limited to a single session of approximately 20 min. It might be likely that longer sessions or multiple applications could result in benefits of significant greater magnitude in motor function improvements.

Only one study (Hummel et al., 2005) looked at the effects of a-tDCS on both motor function and corticomotor changes. Therefore, further studies are needed to find out the extent of corticomotor changes in compatible with the improvements in motor function. Moreover, it will be important to determine the extent to which tDCS influence different types of motor tasks commonly used in neuro-rehabilitation trials of fine distal hand movements or those involving more proximal functions and functional impairment levels. Further investigations for the effectiveness of a-tDCS on motor training of the target muscle in subjects with stroke should consider the dominancy of the stroke hemisphere, the type of stroke, the role of the lesion site and timing after stroke (Koski et al., 2004).

Bringing together the outcome of this systematic review, a potential driver of cortical plastic changes can be suggested by a combination of a-tDCS application and motor functional therapies. This combination might have more clinical gains than administering of a-tDCS or functional treatments alone. Future studies should explore various aspects likely relevant for the efficacy of combination therapies and the optimal parameters of stimulation.

5. Conclusion

This meta-analysis shows that a-tDCS can produce statistically significant effects on corticomotor excitability enhancement in healthy individuals and subjects with stroke. However, the small sample size in studies on subjects with stroke reduces the strength of the presented evidences and any conclusion in this regard should be considered cautiously. Although the results of this a-tDCS meta-analysis did not alter by excluding any single study in healthy individuals, the effect size of interest was small. In this context a-tDCS could be a potential option as a stand-alone technique or as an add-on technique to improve motor function and corticomotor excitability. For a-tDCS, there was a small, non significant effect size in subjects with stroke and a non significant but large effect size in healthy subjects on motor function improvements. In addition, longer application of a-tDCS or larger current densities under active electrodes induces more effective changes

in corticomotor excitation. More studies are needed in this area to define the optimal parameters and their effectiveness on after-effects duration. Therefore, our findings inspire more work in this field before transferring a-tDCS to the clinics.

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Declaration for Chapter 3

In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
Review of literature, Project design, ethics application and approval, participant recruitment, data collection, data analysis, interpretation of the results and writing of the manuscript.	80 %

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Shapour Jaberzadeh	Supervisory input on study design, Guidance in the framing of the manuscript, discussion of findings, review and provision of feedback on manuscript drafts	20 %

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	**Date** 30.09.2013
Signature	**Shapour Jaberzadeh**[redacted]	**Date** 24.09.2013

Preamble to Chapter 3

Any application of tDCS involves measurement of changes before and after intervention. Therefore, in order to make sure that the changes following interventions are not due to systematic errors and methodological inconsistencies, a reliability study is conducted. Chapter 3 examines the intra and inter-reliability of peak-to-peak amplitude and latency of MEPs recorded from ECR and FDI muscles at rest.

Chapter 3: The number of TMS elicited MEP affects intra and inter-session reliability of the upper limb muscles in healthy individuals

The format of this chapter is consistent with the Journal of *PLOS ONE*.

The setup system used in this study, Ethics approval, TMS safety and Edinburg handedness questionnaires and consent form are provided in Appendices 8-9 and 12-15.

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A Higher Number of TMS-Elicited MEP from a Combined Hotspot Improves Intra- and Inter-Session Reliability of the Upper Limb Muscles in Healthy Individuals

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Abstract

We aimed to determine, using transcranial magnetic stimulation (TMS), the number of elicited motor evoked potentials (MEPs) that induces the highest intra- and inter-sessions reliability for the extensor carpi radialis (ECR) and first dorsal interosseus (FDI) muscles. Twelve healthy subjects participated in this study on two separate days. Single pulse magnetic stimuli were triggered with Magstim 200^2 to obtain MEPs from the muscles of interest, with the subjects in a relaxed position. Reliability of MEP responses was investigated in three blocks of 5, 10 and 15 trials. The intra- and inter-session reliability of the MEPs' amplitudes and latencies were assessed using intraclass correlation coefficients (ICCs). Repeated measures ANOVA and paired t-tests revealed no significant time effect in the MEP amplitude and latency measurements ($P>0.05$). The ICCs indicated high intra-session reliability in the MEPs' amplitudes for the ECR and FDI muscles (0.77 to 0.99, 0.90 to 0.99, respectively) and latency (0.80 to 1.00, 0.75 to 0.97, respectively). The MEPs' amplitudes also had high inter-session reliability (0.84 to 0.97, 0.88 to 0.93, respectively), as did their latency (0.80 to 0.90, 0.75 to 0.97, respectively). Highest intra- and inter-session reliability was achieved for blocks of 10 and 15 trials. Our data suggest that intra- and inter-session comparisons should be performed using at least 10 MEPs in "combined hotspot" stimulation technique to ensure highest reliability.

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Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive, safe and painless technique for assessment of brain corticospinal excitability in both healthy individuals and patients with neurological conditions [1–4]. One of the major advantages of TMS is the ability of the magnetic pulses to pass unchanged through the scalp to induce an electric current in underlying conductive brain tissues [1,5,6]. When applied over the primary motor cortex (M1) of a target muscle, TMS depolarizes nerve cells descending corticospinal pathways to contralateral muscle(s) of interest and elicits a motor response called "motor evoked potential" (MEP). This response can be recorded using surface electromyography (EMG) electrodes placed over the target muscle(s) [7–9].

TMS-induced MEPs have been used as a reliable outcome measure of corticospinal excitability changes *in a range of research protocols* [10–12]. Two important characteristics of recorded MEPs are amplitude and latency, which provide valuable information about corticospinal pathways. MEP amplitude is an indicator of M1 corticospinal excitability [13]: larger amplitudes indicate higher excitability and smaller amplitudes indicate lower excitability [13]. On the other hand, variation in MEP latency indicates change in the central and peripheral conduction time required for transmission of induced action potential from the M1 to the target muscle(s) [9].

A significant aspect of any clinical or experimental assessment tool is its test-retest reliability [7,8,14,15]. Reliability refers to 'the consistency of measurements' [16]: it tests the stability of scores over time and involves the degree to which repeated measurements provide similar results [17]. A reliable measurement of MEPs guarantees stable amplitude size and latency in different testings over time [14,18]. Reliability assures that any changes observed in the repeated measure designs and/or pre- and post-therapeutic interventions are genuine and are due to physiological changes rather than errors arising from methodological variabilities [19].

Previous studies suggested a relationship between the number of recorded MEPs and the level of reliability [14,15,20]. Studies using a mean of 5 recorded MEPs resulted in good to high reliability in amplitude measures compared to studies involving one to four MEPs per block; for example, Christie et al's recordings of two, three or four MEPs per block resulted in poor reliability [14,20]. Recent intra- and inter-session reliability studies of MEPs also suggest that recorded MEPs are more reliable when larger numbers of trials are recorded and averaged for analysis [14,15,20]. Doeltgen et al. reported that an average of 10 MEPs provides high reliability in inter-session measurements [15]. The number of MEPs required to produce reliable measurement may vary in different settings and be specific to the study design, number of examinees, assessment and reliability measurement methods or techniques, and recorded muscle(s) of interest.

Despite the widespread use of TMS in recent years, few studies have focused on the test-retest reliability of resting MEPs in upper limb muscles [14,20,21]. Two studies, showed moderate reliability in MEP amplitude for the abductor digiti minimi (ADM) and first dorsal interosseus (FDI) in healthy individuals [14,20]. In contrast, Livingstone et al. (2008) reported less consistency in the resting MEP amplitude for the abductor pollicis bravis (APB), FDI and ADM muscles. Nevertheless, Livingstone et al's [21] MEP amplitude coefficients were lower than those reported by Kamen [20] for the biceps muscle, prompting the hypothesis that the reliability of MEP amplitude may be muscle specific [20,22]. In addition, Kamen [20] reliably measured MEP amplitude during simultaneous M1 stimulation of the biceps and FDI muscles (0.95 and 0.081, respectively). On the other hand, Livingston and Ingersoll [21] demonstrated high reliability for MEP latency obtained from APB, FDI and ADM muscles [21].

In TMS studies several researchers found a single hotspot for a given muscle and then analyzed MEPs simultaneously evoked from that site but in other muscles for which the TMS parameters were not optimized [7,15,20,21,23]. This is not a flawless approach and fails to show a complete picture of cortical changes in all targeted muscles. To address this issue, it might be better to use a "combined hotspot" with overlap M1s for all muscles of interest.

To our knowledge, while investigations of the intra- and inter-session MEP reliability of multiple upper limb muscles exist [7,15,20,21,23], no researchers have assessed the reliability of MEPs recorded from a "combined hotspot", which could be useful for the studies in which MEPs of two or more muscles are simultaneously elicited.

The purpose of the current study was to compare the intra- and inter-session reliability of peak-to-peak amplitude and latency of different blocks of simultaneous elicitation of MEPs from the combined hotspot for ECR and FDI muscles at rest. We hypothesized that MEPs elicited from a combined hotspot, with optimized parameters for all target muscles, are reliable. Due to the stochastic nature and trial-to-trial variability of the TMS-elicited MEPs in all muscles and the fact that averaging may reduce this variability, we also hypothesized that there is a direct relationship between the number of MEPs in each block and reliability, and that the intraclass correlation coefficients (ICCs) of MEPs amplitude and latency are not muscle specific.

Materials and Methods

2.1. Subjects

Twelve healthy volunteers (six women, six men) with a mean age of 30.3±6.8 (yrs) (range 21 to 47 yrs) a mean weight of 74.5±10.4 (kg) and a mean height of 171.4±7.8 (cm) were tested in two sessions separated by at least 48 hours. All were consistent right-handers according to the 10-item version of the Edinburgh Handedness Inventory (mean laterality index = 100) [24]. Prior to the experiments, all participants completed the Adult Safety Screening Questionnaire [25] to determine their suitability for TMS. Participants were informed about the experimental procedures and gave written informed consent according to the declaration of Helsinki. All experimental procedures were approved by the Monash University Human Research Ethics Committee. Each subject was tested at the same time of the day to avoid diurnal variation.

2.2. Electromyography (EMG) recording

Participants were seated in an adjustable podiatry chair, with the right forearm pronated and the wrist joint in neutral position on the arm rest. To ensure good surface contact and reduce skin resistance, a standard skin preparation procedure of cleaning and abrading was performed for each site of electrode placement [26–28]. MEPs were recorded from the right ECR and FDI muscles at rest, using pre-gelled self-adhesive bipolar Ag/AgCl disposable surfaces electrodes with an inter-electrode distance of 3 cm for the ECR and 2 cm for the FDI muscle (measured from the centres of the electrodes). The locations of ECR and FDI muscles were determined based on anatomical landmarks [29] and observations of muscle contraction in the testing position (wrist extension and radial deviation for ECR, and index finger abduction for FDI muscle) [30]. The accuracy of EMG electrode placement was verified by asking the subject to maximally contract the muscles of interest while the investigator monitored online EMG activity. The ground electrode was placed ipsilaterally on the styloid process of the ulnar bone [31]. Then, the electrodes were secured with tape. All raw EMG signals were band-pass filtered (10–500 Hz), amplified (×1000) and sampled at 1000 Hz and collected on a PC running commercially-available software (LabChart^{TM} software, ADInstruments, Australia) via a laboratory analogue-digital interface (The PowerLab 8/30, ADInstruments, Australia) for later off-line analysis.

2.3. Measurement of corticospinal excitability by TMS

Participants were seated upright and comfortable with head and neck supported by a head rest. Single pulse magnetic stimuli were delivered using a Magstim 200^2 (Magstim, UK) stimulator with a flat 70 mm figure-of-eight magnetic coil. Using the international 10–20 system, the vertex (C_z) point was measured and marked to be used as a reference [28]. The magnetic coil was placed over the left hemisphere (cortex), contralateral to the target muscles. The coil was set at an angle 45° to the midline and tangential to the scalp, such that the induced current flowed in a posterior-anterior direction. To determine the optimal site of stimulation (hotspot), the coil was moved around the M1 of the target muscles to trigger the M1 overlapped area for both the ECR and FDI muscles that gave the largest MEP response. This overlapped M1 area was called the "combined hotspot".

The surface area of representation and the coordinates of the combined hotspot for the FDI and ECR muscles were found and marked based on the size of the MEP amplitude. As illustrated by Devanne et al. [32], the optimal spot for stimulation of the FDI muscle is more anteriorly and laterally located relative to the vertex than that for the ECR muscle. Figure 1 explains the concept of the combined hotspot.

After localizing the optimal stimulation site, the coil position was marked on the scalp to ensure consistency in the placement of the coil for the remainder of the testing. The full hotspot identification procedure was performed in each session. Resting motor threshold (RMT) was defined as the minimal stimulus intensity that evoked 5 MEPs in a series of 10 tests with amplitude of at least 50 μV [*4,6,9,33*] from the combined hotspot of both ECR and FDI muscles. Hence, the same RMT was used for both muscles. The RMT for each subject was determined by increasing and decreasing stimulus intensity in 1–2% intervals until MEPs of appropriate size were elicited. For all further MEP measurement, the TMS intensity was set at 120% of each individual's RMT. Fifteen stimuli were elicited to assess corticospinal excitability at each time point. The stimulus intensity remained constant throughout the study session for each subject.

2.4. Procedures

All individuals participated in two experimental sessions. The protocol in session 1 enabled us to study the reliability of MEP

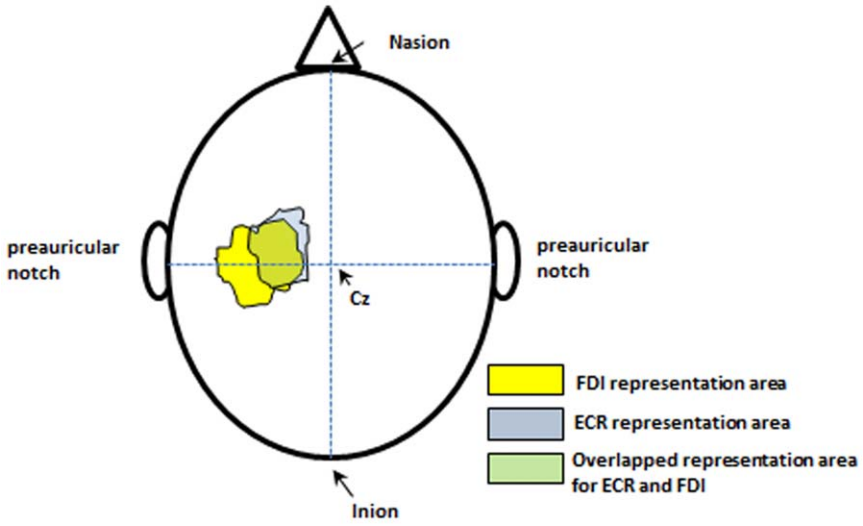

Figure 1. Contours plot of the ECR and FDI M1 representations. The overlap between the two representations is shown in green. Cz: The position of vertex. Adapted with modification from Devanne et al. (2006) study [32].
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amplitude and latency within a session (intra-session reliability). The corticospinal excitability of the ECR and FDI muscles was assessed at three consecutive time points (T1, T2 and T3) separated by intervals of 20 minutes. The EMG electrodes were left in place and the TMS coil was removed while the subjects rested for 20 minutes, with no hand or wrist movements allowed.

The second session of testing was held at least 48 hours after the first one. This session was shorter and only involved recording of MEPs at one time point (T1). Comparison of these data with the T1 from session 1 enabled us to study the inter-session reliability of MEPs' size and latency for the ECR and FDI muscles.

2.5. Data management and statistical analysis

Twelve subjects were required for a true ρ 0 of 0.7 against an alternative ρ1 of 0.9, based on a 95% significant level and a power of 80% ($\beta = 0.20$) for three time points [34].

The peak-to-peak amplitudes and latencies of elicited MEPs were measured for the ECR and FDI muscles. The MEPs' amplitudes were measured from the positive to the negative peak of the signals and MEPs latency was calculated from the stimulus artifact indicator to the first deflection of the signal. To assess the intra- and inter-session reliability of recorded MEPs, the averaged MEPs at each time point (T1, T2 and T3) were calculated in separate blocks of the first 5 (Block 1), first 10 (Block 2) and all 15 responses (Block 3).

Two-way repeated measure analysis of variance (ANOVA) and paired t-tests were used to detect systematic bias between the repeated measurements within or between days, respectively. This test shows the degree of agreement between the measurements and assesses the closeness of the repeated measures [35]. The correlation between the measurements was assessed using the ICC [8], which is the most appropriate reliability outcome measure for measurements on a continuous scale.

ICCs were calculated for blocks of the first 5, 10 and all 15 elicited MEPs in order to identify the number of trials which produced the greatest intra-session reliability. The same protocol was applied to calculate the inter-session reliability (between sessions 1 and 2) for MEPs' amplitudes and latencies. The ICCs, based on a two-way single measure mixed effects model ($ICC_{(3,1)}$), were calculated for averaged MEPs in each block for both inter- and intra-session reliability. The reliability coefficient ranges from 0 to 1, with values closer to 1 representing stronger reliability. Although the interpretation of ICCs is subjective, Portney and Watkins (2009) [35], suggested that coefficients below 0.50 represent poor reliability, from 0.50 to 0.75 correspond to moderate reliability, and values above 0.75 signal high reliability.

Unlike the ICC, which is a relative measure of reliability, standard error of measurement (SEM) was calculated which provides an absolute index of reliability [36]. The SEM quantifies the precision of individual scores on a test (within-subject reliability) and indicates to what extent the values observed at different time points vary from the 'true' value of that excitability parameter for a given subject [37]. The interpretation of SEM focuses on the assessment of reliability for individual subjects [38], and SEM determines the effect of measurement error on the test score of an individual examinee. SEM is estimated as follows:

$$SEM = SD\sqrt{1 - ICC}$$

where SD is the standard deviation of the scores from all subjects and ICC is the reliability coefficient [19,37,39]. The larger the SEM, the lower the reliability of the test and the less precision there is in the measurements taken and scores obtained.

All data are presented as mean6SD, the level of statistical significance was set at 5%, and all analyses were conducted using SPSS for Windows Version 19.

Results

All participants completed both sessions of data collection. The mean interval between sessions of measurements was 52.7±4.6 hours.

3.1. Intra-session reliability

MEPs amplitude and latency. The averaged RMT and consequent stimulus intensity for both muscles were 45% (45.2±10.4) and 54% (54.3±12.5) of the stimulator output, respectively. A representative single subject's data (Figure 2a and b) showed minimal changes for the mean amplitude of the MEPs for ECR and FDI muscles at all three time points. Indeed, repetition of the measurements by the same examiner every 20 minutes after the first test revealed no significant differences in the group mean values of any of the measurements recorded (Tables 1

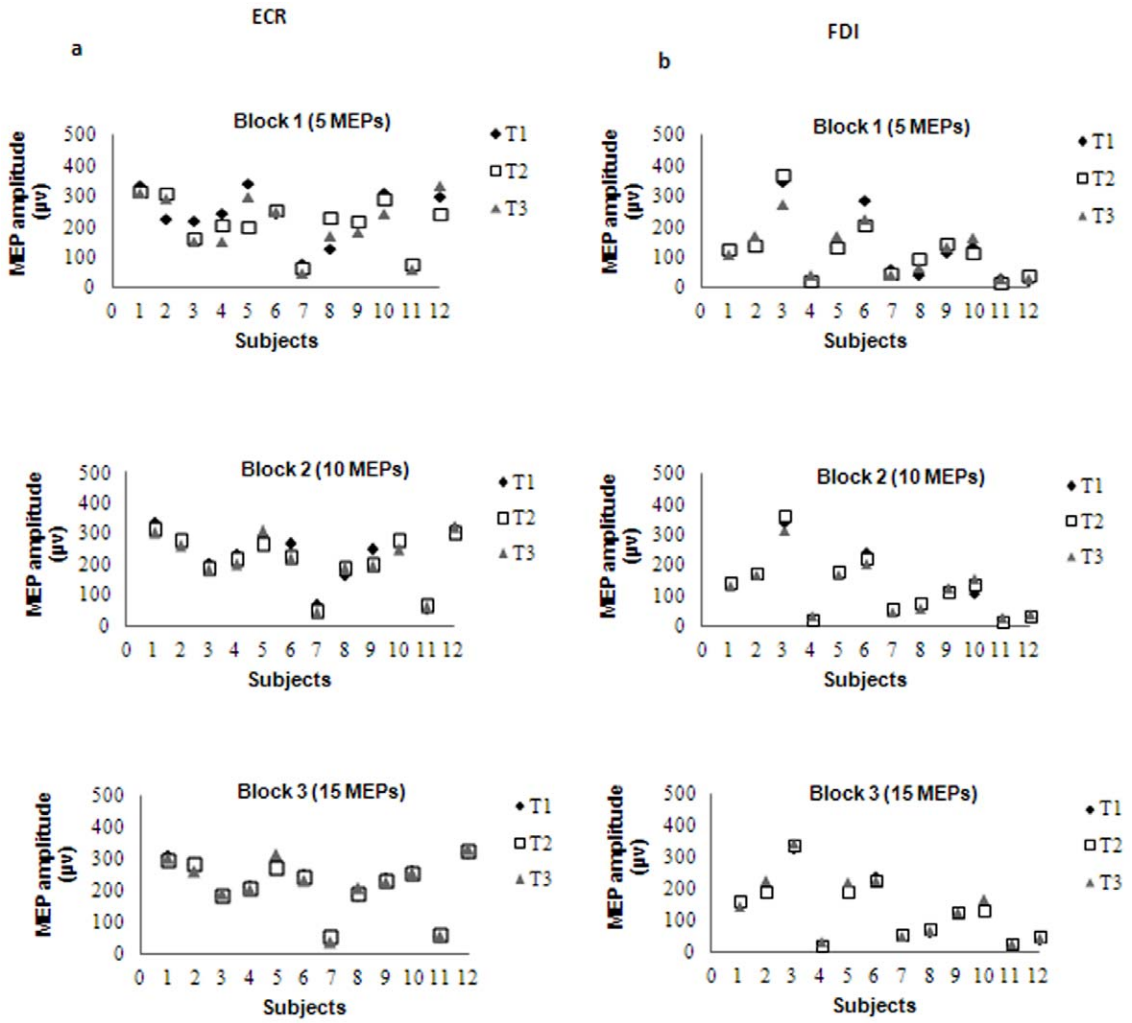

Figure 2. Comparison of MEPs amplitude in 12 subjects within a session. a) ECR, and b) FDI muscles with 5, 10 and 15 MEPs per block in three time points.
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and 2). Repeated measures ANOVA revealed no significant time effect in any of the measurements for ECR muscle and FDI muscles (Tables 1 and 2).

ICCs ranged from 0.77 for block 1 (5 MEPs) to 0.99 for block 3 (15 MEPs). MEP amplitudes showed high reliability within a session for both ECR and FDI muscles (Table 3). As expected,

Table 1. Mean, standard deviation and level of agreement of MEPs amplitude for three blocks of trials recorded from ECR and FDI muscles.

						ANOVA		Paired T-test	
		Mean amplitude (μv)±SD				(Intra session)		(Intra session)	
Muscle	Blocks	T1- session 1	T2- session 1	T3- session 1	T1- session 2	F (2,22)	P-value	T (11)	P-value
ECR	Block 1	229.51±95.63	215.92±80.27	208.99±95.98	221.07±78.95	0.97	0.39	0.59	0.56
	Block 2	228.34±90.64	215.94±85.06	212.23±88.89	217.13±83.71	0.73	0.44	1.49	0.16
	Block 3	224.0±88.04	220.91±85.45	220.81±0.92	215.8±83.66	0.59	0.55	1.70	0.11
FDI	Block 1	121.42±102.96	122.98±96.67	121.97±82.13	129.98±98.89	0.227	0.877	−0.60	0.56
	Block 2	124.70±95.76	132.17±100.17	124.99±85.94	132.74±99.40	0.571	0.638	−0.78	0.45
	Block 3	131.12±96.14	135.12±95.02	141.99±100.86	133.64±101.33	0.678	0.571	−0.24	0.81

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Table 2. Mean, standard deviation and level of agreement of MEPs latency for three blocks of trials recorded from ECR and FDI muscles.

						ANOVA		Paired T-test	
		Mean latency (ms)±SD				(Intra session)		(Intra session)	
Muscle	Blocks	T1- session 1	T2- session 1	T3- session 1	T1- session 2	F (2,22)	P-value	T (11)	P-value
ECR	Block 1	16.66±1.07	16.50±1.24	16.58±1.24	16.41±0.99	0.47	0.62	1.39	0.19
	Block 2	16.66±1.30	16.58±0.99	16.58±1.37	16.75±0.96	0.18	0.83	−0.32	0.75
	Block 3	16.66±1.30	16.66±1.30	16.66±1.30	16.75±1.05	0.314	0.815	−0.56	0.58
FDI	Block 1	22.66±1.15	22.66±1.23	22.58±1.44	22.83±0.93	0.401	0.753	−0.69	0.50
	Block 2	22.41±1.08	22.91±1.24	22.58±1.16	22.75±1.21	1.486	0.236	−1.44	0.16
	Block 3	22.41±1.31	22.75±1.42	22.50±1.31	22.91±1.24	0.647	0.59	−2.17	0.05

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higher ICCs were achieved for blocks of 10 and 15 MEPs in all comparisons.

The mean and ICC results for MEP latency of the ECR and FDI muscles are shown in Tables 2 and 4, respectively. MEP latency showed high stability over the three replicates within a session for both the ECR (ICCs = 0.80 to 1.00) and FDI (ICCs = 0.75 to 0.97) muscles. As expected, slightly higher ICCs were achieved for blocks of 10 and 15 trials in all comparisons.

3.2. Inter-session reliability

MEP amplitude and latency. The averaged RMTs and consequent stimulus intensities for both muscles were 46% (46±10.8) and 55% (55.2±13.0) of stimulator output, respectively. A representative single subject's data showed minimal changes in mean MEP amplitude for the ECR and FDI muscles (Figure 3a and b). Moreover, repetition of the measurements by the same examiner in two different sessions held at least 48 hours apart did not reveal any significant differences in the group mean MEP amplitude and latency values (Tables 1 and 2). Paired t-tests comparing the means of all variables between the two sessions showed no statistically significant differences for the ECR and FDI muscles (Tables 1 and 2). ICCs for MEP amplitudes ranged from 0.84 for block 1 (5 MEPs) to 0.97 for block 2 (10 MEPs) for the ECR muscle and 0.88 for block 1 (5 MEPs) to 0.93 for block 2 (10 MEPs) for the FDI muscle. Marginally higher ICCs were achieved for block 3 (15 MEPs) for the ECR muscle, with no change in the ICCs of the FDI muscle for blocks of 10 and 15 trials (Table 3).

ICC values for MEP latency ranged from 0.82 to 0.90 for block 1 and 2 (5 and 10 MEPs, respectively) for ECR and 0.75 to 0.80 for block 1 and 2 (5 and 10 MEPs, respectively) for the FDI muscle (Table 4). As expected, slightly higher ICCs were achieved for block 3 (15 MEPs) in all comparisons. The ICCs were higher in all three blocks for the ECR muscle compared to the FDI muscle.

Discussion

In this study we assessed the intra- and inter-session reliability of the amplitude and latency of different blocks of simultaneous TMS-elicited MEPs from the ECR and FDI muscles. Correlations between individuals and sessions were determined using ICCs [8]. Systematic bias was evaluated by measuring the level of agreement using ANOVA or a paired t-test where appropriate. The reliability established in this study is also intra-rater reliability, because all data were collected by the same rater.

4.1. Intra-session reliability

The agreement and high values of ICCs between pre- and post-MEP measurements observed in both ECR and FDI muscles indicate high within-session reliability. These intra-session reliability results are in agreement with those of Christie et al. [14], who reported an ICC of 0.97 for the reliability of MEP amplitude derived from the ADM muscle. Furthermore, MEP latencies ranged from 16.4±0.9 ms for the ECR to 22.9±1.2 ms for the FDI muscles, results in agreement with MEP latency data reported

Table 3. Comparison of between MEPs correlation of the recorded MEPs amplitude from ECR and FDI muscles.

		Intra session reliability					Inter session reliability	
		ICCs					Inter session reliabilityICCs	
Muscle	Blocks	T1- T2	T1- T3	T2-T3	T1-T2-T3	SEM	T1	SEM
ECR	Block 1	0.77	0.90	0.82	0.83	12.70	0.84	8.93
	Block 2	0.97	0.96	0.97	0.97	2.62	0.95	3.37
	Block 3	**0.99**	0.98	0.98	0.98	1.15	**0.97**	1.62
FDI	Block 1	0.94	0.93	0.90	0.93	5.29	0.88	7.87
	Block 2	**0.99**	0.97	0.97	0.98	1.52	**0.93**	3.67
	Block 3	**0.99**	0.98	0.98	0.98	1.24	**0.93**	3.60

Largest ICCs values of each comparison are in bold. ECR: extensor carpi radialis; FDI: first dorsal interosseus; ICCs: inter class correlations; SEM: standard error of measurement.

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Table 4. Comparison of between MEPs correlation of the recorded MEPs latency from ECR and FDI muscles.

		Intra session reliability					Inter session reliability	
		ICCs					ICCs	
Muscle	Blocks	T1- T2	T1- T3	T2-T3	T1-T2-T3	SEM	T1	SEM
ECR	Block 1	0.80	0.90	0.91	0.87	0.10	0.82	0.1
	Block 2	0.83	0.97	0.87	0.90	0.07	0.89	0.13
	Block 3	**1.00**	**1.00**	**1.00**	**1.00**	-	**0.90**	0.05
FDI	Block 1	0.75	0.86	0.87	0.83	0.14	0.75	2.18
	Block 2	0.76	0.94	0.88	0.89	0.13	0.77	2.06
	Block 3	0.88	**0.97**	0.89	0.91	0.05	**0.80**	1.97

Largest ICCs values of each comparison are in bold. ECR: extensor carpi radialis; FDI: first dorsal interosseus; ICCs: inter class correlations; SEM: standard error of measurement.

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by Ravnborg and Dahl [40] and Wu et al. [41]. As expected, motor evoked latencies demonstrated an absolute intra-session consistency for the FDI muscle and very high reliability for the ECR muscle. This can be explained by the careful positioning of EMG surface electrodes within the session and the consistency in the alignment and position of the TMS magnetic coil on the combined M1.

The results indicate a direct relationship between the number of recorded MEPs in each block of stimulation and the level of reliability, supporting the hypothesis of our study. We established high reliability in this session for 5, 10 and 15 MEPs per block, indicating that even an average of 5 MEP amplitudes is enough to establish high within-session reliability, in agreement with Christie et al. [14]. This result also supports Kamen's [20] findings of good

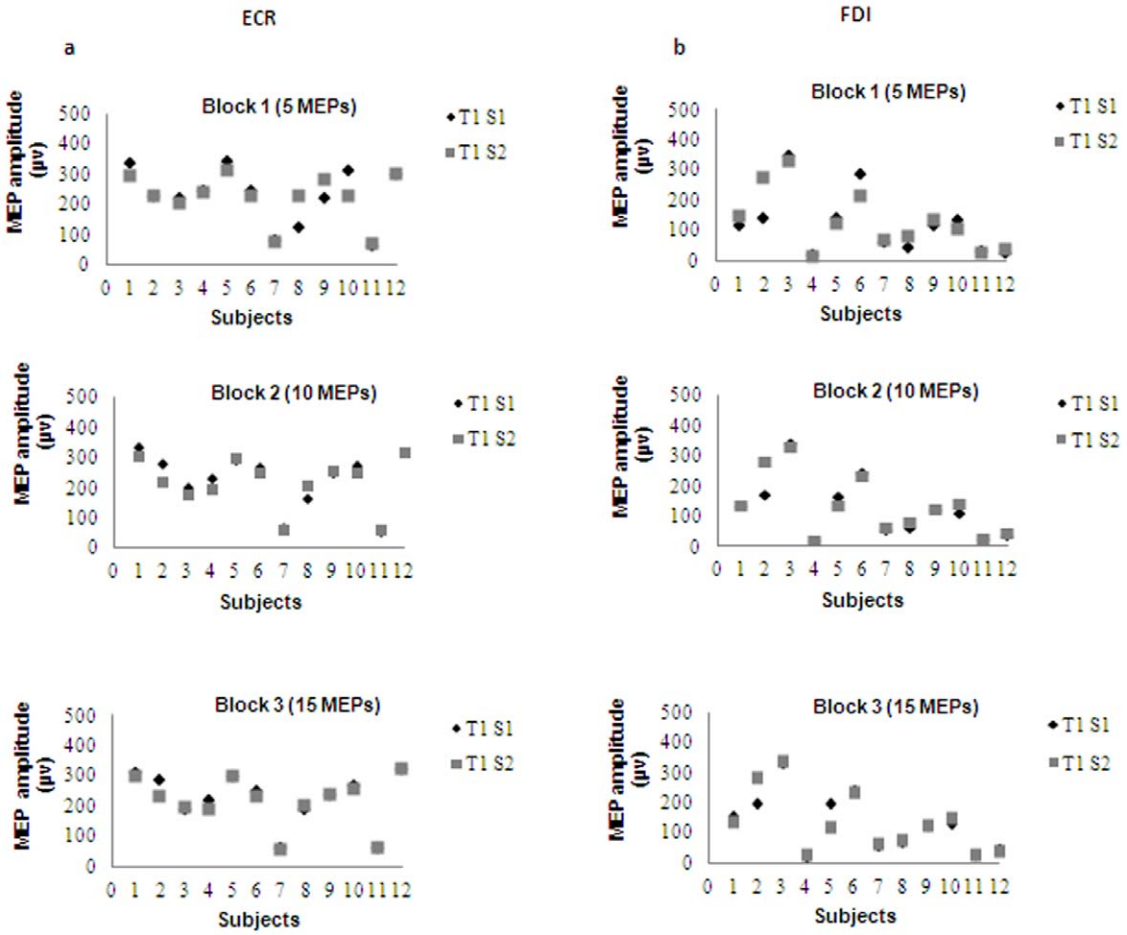

Figure 3. Comparison of MEPs amplitude in 12 subjects between two sessions. a) ECR and b) FDI muscle with 5, 10 and 15 MEPs per block in three time points.

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to high reliability of MEP amplitude in the FDI and biceps muscles in healthy individuals.

4.2. Inter-session reliability

The agreement and also high and consistent ICCs indicate high inter-session reliability of MEP measurement in both ECR and FDI muscles. The ICCs of all three blocks in the present experiment are larger than those reported by Kamen [20] for the FDI muscle (0.60–0.81) and Christie et al. [14] for the ADM muscle (0.65–0.83). Although no previous reliability studies focused on forearm muscles, our ICCs for the ECR muscle were comparable with Kamen's findings [20] for the biceps muscle (0.95–0.99) for blocks of 10 and 15 MEPs. These values are higher than those reported by Livingston and Ingersoll [21], who found small (0.28) to moderate (0.72) ICCs for the FDI, APB and ADM muscles. Our results indicate that MEP amplitude remains constant in healthy subjects, even with a 48 hour interval between testing sessions.

MEP latency is sensitive to electrode positioning [42], particularly given that electrode placement over forearm muscles is inevitably more variable than in intrinsic hand muscles. Therefore, the high reliability of MEP latency found in this study suggests the consistent positioning of EMG electrodes across the two sessions. Although the reliability of MEP latency has not been previously investigated for forearm muscles, our results are in keeping with those of Livingston and Ingersoll [21], who showed that the MEP latency of distal hand muscles remained stable, with an ICC of 0.87 across different sessions.

In this study, the combined hotspot was more toward the periphery for the FDI muscle. Therefore, one potential explanation for the small MEPs recorded in the FDI muscle is that the MEP size might be smaller in the periphery of the cortical representation compared to that at the hotspot. However, it is interesting to see that the reliability remained high despite this small MEP size. In agreement with previous studies, reliability measures reached high values when 5 trials were included in the present analysis, with a slight increase in reliability when 10 or all 15 trials were considered. As the highest reliability was achieved by increasing the number of MEPs per block, we recommend the use of at least 10 MEP trials when the research includes multiple independent sessions of data collection and simultaneous M1 stimulations.

The high reliabilities demonstrated by high ICCs for MEP amplitude and latency in our study are in agreement with data reported for the upper limb muscles by some authors, regardless of whether they had used ICCs [7,43], ANOVA or coefficient of variation (CV) [44,45] for the statistical analysis. The ICC values recorded in the present study showed an overall reliability of over 0.75 in both the intra- and inter-session assessments.

The shape, size and orientation of the coil are main factors that determine the size of stimulated area as well as the direction of the induced current flow [46]. Moreover, a factor that could theoretically affect MEP amplitudes' reliability is the use of a neuronavigation system in eliciting MEPs. However, two recent studies found no decrease in the variability [47] and no further improve in reliability [48] of MEPs with TMS navigated systems. We used a conventional TMS assessment technique without a navigation system, but our results were in agreement with previous studies demonstrating high reliability in TMS mapping parameters with smaller numbers of MEPs, both with [49] and without [14] the use of a neuronavigation system.

The results support our hypothesis that TMS-elicited MEPs are not muscle specific. High reliability in both ECR and FDI muscles confirms data reported by Lefebvre et al. [50] demonstrating that TMS reliability is not muscle specific. However, Kamen [20] produced contradictory findings indicating that reliability varies according to the muscle of investigation, and that higher reliability in the biceps muscle could be a function of its location or M1 size in comparison to distal hand muscles.

It is important to note that SEM values were lower in blocks 2 and 3 (10 and 15 MEPs, respectively) than in block 1 (5 MEPs) for both the ECR and FDI muscles. In addition, SEM was similar in blocks 2 and 3 for the FDI muscle. Overall, the SEM became smaller as the number of MEPs per block increased from 5 to 15. As the observed values lie within the SEM from the true score, this shows the significance of increasing the number of recorded MEPs to bring the observed values closer to the true scores.

Based on the data presented here, TMS-elicited MEPs can be reproduced with a high degree of consistency to simultaneously assess the corticospinal pathways from both ECR and FDI muscles when performed in a controlled laboratory environment. Our findings are also useful for interpreting individual intervention effects in TMS-related studies where any changes in MEP responses can be considered as an intervention effect. TMS is frequently used in investigations such as brain mapping or recruitment curves, and can involve 250 or more stimulations. Our results indicate acceptable reliability with 5 MEPs per block, enabling researchers to avoid unnecessary stimulations to the brain. However, to increase the reliability of inherently variable and sensitive measurements, more MEPs per block should be recorded.

One limitation of our study is that we studied only healthy young participants, so findings cannot be extrapolated to older and/or unwell subjects. This study was also limited in that it only evaluated one intensity (120% RMT), so we are unable to expand our findings to higher or lower intensities, although previous studies have shown that stimulation by higher intensities provides higher reliability [49].

The results of our study only indicate intra-rater reliability. An obvious further direction is to perform similar study by testing the inter-rater reliability for multi-center studies.

Author Contributions

Conceived and designed the experiments: AB SJ. Performed the experiments: AB. Analyzed the data: AB. Contributed reagents/materials/analysis tools: AB SJ.

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Chapter 3 Postscript

Using a figure-of-eight magnetic coil, Study 2 showed a high intra- and inter-session reliability of elicited MEPs from the ECR and FDI combined hotspot. This point is always located within the overlap area of individual representations of muscles in M1. This is a necessary approach in studies where assessment of multiple muscles is required. One of the shortfalls of this technique is the fact that the size of elicited MEPs is smaller compared to the ones recorded from a single muscle hotspot. Therefore, as the primary aim of this thesis is to determine optimal parameters of a-tDCS for enhancement of CSE, this could be a confounding variable which may affect the results. Thus, the further studies in this thesis are conducted using single hot spots.

Declaration for Chapter 4

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
Review of literature, Project design, ethics application and approval, participant recruitment, data collection, data analysis, interpretation of the results and writing of the manuscript.	80 %

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Shapour Jaberzadeh	Supervisory input on study design, Guidance in the framing of the manuscript, discussion of findings, review and provision of feedback on manuscript drafts	15 %
Dawson Kidgell	Review and provision of feedback on final manuscript draft	5 %

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	**Date** 30.09.2013
Signatures	**Shapour Jaberzadeh** [redacted]	**Date** 24.09.2013
	Dawson Kidgell [redacted]	**Date** 20.09.2013

Preamble to Chapter 4

Chapter 4 is a feasibility study, planned to fine-tune the setup for application of TMS as an assessment tool and a-tDCS as the neuromodulatory technique. Technically, all necessary changes have been made, such as addition of a foot switch for hands-free triggering of TMS, setting of the Lab chart software (LabChartTM software, ADInstruments, Australia) in a way to facilitate recording of the TMS induced MEPs single handedly and addition of a macro to the data acquisition system (PowerLab 8/30, ADInstruments, Australia) and its dedicated software for automatic measurement of the TMS induced MEPs peak-to-peak amplitude.

This study also compares the effects of stimulation duration on the size of CSE enhancements.

Chapter 4: Does the longer application of anodal-transcranial direct current stimulation increase corticomotor excitability further? A pilot study

The format of this chapter is consistent with the Journal of *Basic and Clinical Neuroscience*.

The setup system used in this study, Ethics approval, TMS safety and Edinburg handedness questionnaires and consent form are provided in Appendices 8, 10 and 12-15.

Does the Longer Application of Anodal-transcranial Direct Current Stimulation Increase Corticomotor Excitability Further? A Pilot Study

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ABSTRACT

Introduction: Anodal transcranial direct current stimulation (a-tDCS) of the primary motor cortex (M1) has been shown to be effective in increasing corticomotor excitability.

Methods: We investigated whether longer applications of a-tDCS coincide with greater increases in corticomotor excitability compared to shorter application of a-tDCS. Ten right-handed healthy participants received one session of a-tDCS (1mA current) with shorter (10 min) and longer (10+10 min) stimulation durations applied to the left M1 of extensor carpi radialis muscle (ECR). Corticomotor excitability following application of a-tDCS was assessed at rest with transcranial magnetic stimulation (TMS) elicited motor evoked potentials (MEP) and compared with baseline data for each participant.

Results: MEP amplitudes were increased following 10 min of a-tDCS by 67% ($p = 0.001$) with a further increase (32%) after the second 10 min of a-tDCS ($p = 0.005$). MEP amplitudes remained elevated at 15 min post stimulation compared to baseline values by 65% ($p = 0.02$).

Discussion: The results demonstrate that longer application of a-tDCS within the recommended safety limits, increases corticomotor excitability with after effects of up to 15 minutes post stimulation.

1. Introduction

Transcranial direct current stimulation (tDCS) is a simple, safe, non-invasive neuromodulatory technique that uses low intensity direct current (DC) delivered directly to the area of interest over the cerebral cortex via two surface electrodes (Nitsche & Paulus, 2000; Nitsche et al., 2003a; Nitsche et al., 2003b). When these electrodes are placed in the regions of interest, the applied current induces very weak intracortical current flow (Nitsche & Paulus, 2001). Depending on the polarity of active electrodes over the primary motor cortex (M1) contralateral to target muscles, tDCS can either increase or decrease corticomotor excitability (Nitsche et al., 2003a; Nitsche et al., 2008). Application of the positive charged electrode (anode) over M1 (anodal tDCS, a-tDCS) induces intracortical current flow which

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results in cortical depolarization and increases the size of MEPs in the target muscles of the specific area being stimulated, indicating increased corticomotor excitability (Nitsche & Paulus, 2000, 2001). On the other hand, application of the negative charged electrode (cathode) over M1 (cathodal tDCS, c-tDCS) leads to hyperpolarization and reduces the size of the transcranial magnetic stimulation (TMS) induced motor evoked potentials (MEPs), indicating decreased corticomotor excitability.

The extent of modulatory effects induced by a-tDCS, depends on the current density and duration of its application (Purpura & McMurtry, 1965a; Nitsche & Paulus, 2000; Nitsche & Paulus, 2001; Nitsche et al., 2008). For example, a series of studies have examined the effects of different durations of a-tDCS on corticomotor excitability indicating a linear relationship between the duration of application and the increase in corticomotor excitability (Nitsche & Paulus, 2000, 2001; Furubayashi et al., 2008). Nitsche and Paulus (2000) reported that when comparing shorter and longer application of a-tDCS (1, 2, 3, 4 and 5 min) there was a linear relationship between the duration of a-tDCS and the increase in corticomotor excitability (Nitsche & Paulus, 2000). In addition, a large number of studies have shown that a-tDCS increases corticomotor excitability that lasts beyond the stimulation period (Purpura & McMurtry, 1965; Nitsche & Paulus, 2000, 2001; Nitsche et al., 2005; Boros et al., 2008; Furubayashi et al., 2008; Nitsche et al., 2008; Utz et al., 2010; Fricke et al., 2011).

The safety of tDCS as a neuromodulatory technique is determined by both the current density which is established by the amplitude (A) per surface area of the stimulating electrode (cm^2), and the duration of stimulation (Nitsche et al., 2003b). Experimental data has shown that current densities below 25 mA/cm^2 are safe and have no detrimental effects on the underlying cerebral tissue (McCreery et al., 1990). In addition, the current density is independent of stimulation duration; therefore identifying the optimal duration of stimulation is important for the safe application of tDCS (Nitsche et al., 2003a).

There are several cross-sectional studies that have used a-tDCS to induce corticomotor excitability; however, no studies to date have used an application duration of more than 13 min in healthy individuals (Nitsche et al., 2005; Boros et al., 2008). Therefore the primary aim of the current study was to compare the effects of shorter (10 min) and longer (10+10 min) durations of a-tDCS on the excitability of M1 for the right extensor carpi radialis muscle (ECR) and to investigate if longer (10+10 min) durations of a-tDCS could be tolerated or not. We hypothesized that longer application (10+10 min) of a-tDCS would induce larger increases in corticomotor excitability compared to shorter application (10 min) and that the application would be well tolerated by participants.

2. Methods

2.1. Participants

Ten healthy volunteers (four males, six females), aged between 20-51 years, (mean age 35.8 ± 8.9 years) participated in this study (Table 1). Participants were recruited from Monash University students or staff. All participants were consistent right-handers according to the 10-item version of the Edinburgh Handedness Inventory (mean laterality index =100) (Oldfield, 1971). Prior to the experiment, all participants completed the Adult Safety Screening Questionnaire to determine their suitability for TMS and tDCS application (Keel et al., 2001). Volunteers with a family history of epilepsy or any other neurological/psychiatric disorders and

Table 1. Subject baseline demographic and clinical characteristics.

Subject characteristics	Statistics	
Healthy	Number	10
• Heavy Smokers		0
• Sleep deprivation		0
• Taking excessive caffeine		0
• Taking excessive energy drinks		0
• Taking any medications affecting nervous system		0
• Extraneous exercise of wrist extensor muscles prior to testing session		0
Age (years)	Range	20.51
	Mean	35.8
	SD	8.9
Sex	Male (N, %)	4 (40%)
	Female (N, %)	6 (60%)
Weight (kg)	Range	55-90
	Mean	69.4
	SD	13.12
Height (cm)	Range	158-191
	Mean	169.6
	SD	10.6
BMI	Range	21-28
	Mean	24
	SD	1.9

those with metallic implants/implanted electrical devices or pacemakers were excluded. Participants were informed about the experimental procedures and gave their written informed consent according to the declaration of Helsinki. All experimental procedures were approved by the Human Research Ethics Committee of the University.

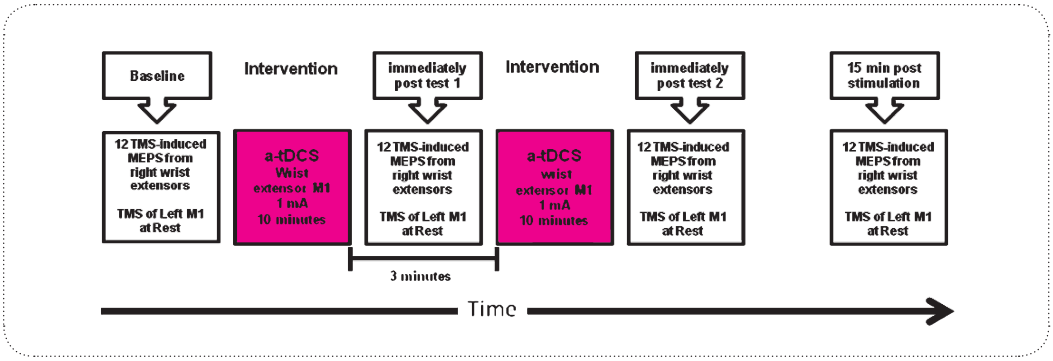


Figure 1. Corticomotor excitability was assessed before and after 10 minutes of a-tDCS and also immediately and 15 minutes following the second 10 minutes of a-tDCS application.

2.2. Experimental Design

Figure 1 illustrates the one-way within-subjects experimental design used in this study. All recruited individuals participated in one experimental session. Corticomotor excitability of their ECR M1 was measured (using TMS) before the application of a-tDCS (baseline value) and at three time points following a-tDCS, including; immediately post 10 min (post-test 1), immediately post 10+10 min (post-test 2) and 15 min post a-tDCS (follow up).

2.3. Electromyographic (EMG) Recording

Participants were seated in a chair with their forearm pronated and resting on the armrest of a purpose-built chair (Figure 2). MEPs were recorded from the right ECR muscle using Ag/AgCl disposable surface electrodes with an inter-electrode distance of 2 cm. The ground electrode was placed over the styloid process of ipsilateral ulnar bone (Oh, 2003). In order to ensure good surface contact and reduce skin resistance, a standard skin preparation procedure of cleaning and abrad-

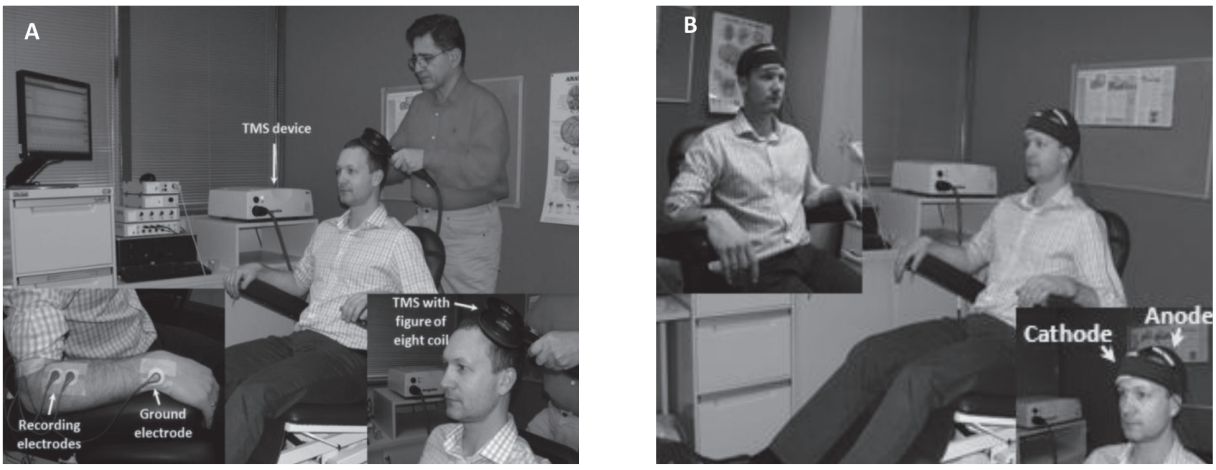


Figure 2. Participants were seated in a podiatry chair with their forearm pronated and resting on the armrest of the chair. A) TMS application with a figure of eight magnetic coil placed at 45° angle to the midline and tangential to the scalp for eliciting MEPs over the left M1. EMG was recorded from ECR muscle. Recording and ground electrodes were secured with tape.

B) a-tDCS application with the anode electrode placed over the M1 for ECR and the cathode electrode was placed over the contralateral supra orbital area. The electrodes were fixed in place by two custom-designed straps.

ing was performed for each site of electrode placement (Gilmore & Meyers, 1983; Schwartz, 2003). All EMG signals (MEPs) were sampled at 2048 Hz and collected on a PC running commercially-available software PowerLab (ADinstruments, Australia) via a laboratory analogue-digital interface (PowerLab 8/30, ADinstrument, Australia) for later off-line analysis. EMG signals were filtered and amplified (1000×) with bandpass filtering between 20 Hz and 500 Hz and digitized at 1 kHz for 200 ms.

2.4. Measurement of Corticomotor Excitability by TMS

MEPs were evoked by TMS of the contralateral motor area controlling the right ECR using a Magstim 200² (Magstim company limited, UK), with a 70 mm wide figure of 8 magnetic coil. The size of evoked MEPs was considered as a dependent variable to assess changes in corticomotor excitability of M1 in the dominant side prior and following the application of a-tDCS. The optimal stimulation site (hotspot) for evoking MEPs from ECR was determined and marked to ensure accurate positioning of the coil between trials. The orientation of the coil was set at a 45° angle to the midline and tangential to the scalp, so that the induced current flowed in a posterior-anterior direction. Resting motor threshold (RMT) was determined by applying TMS at the optimal M1 site for evoking responses in ECR muscle at rest. RMT was defined as the minimal stimulus intensity that evoked 5 MEPs in a series of 10 with an amplitude of at least 50 µV (Rothwell et al., 1999). Following this, the test intensity was set at 120% of RMT. Twelve stimuli were given to elicit MEPs for the assessment of corticomotor excitability at each time point (see Figure 1).

2.5. Anodal-tDCS of the Primary Motor Cortex

A-tDCS was delivered by an Intelect® Advanced Therapy System (Chattanooga, USA) through a pair of saline-soaked surface sponge electrodes (42 cm^2). The active electrode (anode) was fixed with two straps over the left M1 for the right ECR as identified by TMS, and the indifferent electrode was placed over the right contralateral supra orbital area. The stimulation intensity was set to 1 mA and a-tDCS was applied continuously for 10 min which was repeated following TMS assessment of corticomotor excitability. Therefore, overall, each participant received 20 min (10+10 min) of a-tDCS with a time interval of 3 min between two stimulation periods.

2.6. Data Management and Statistical Analyses

In determining the optimal site, all MEPs collected (n = 12) with 200-millisecond recordings for each condition were displayed and averaged online for visual inspection, and then stored off-line for further analysis.

Figure 3 displays the resting state of muscle prior to stimulation, the stimulus artifact and a typical MEP response. MEP latency was calculated from the stimulus artifact to the first deflection of MEP and the size of MEP amplitude was measured from the maximum peak to the minimum peak of the recorded MEP. Mean and SE of MEP peak-to-peak amplitude (µV) from TMS

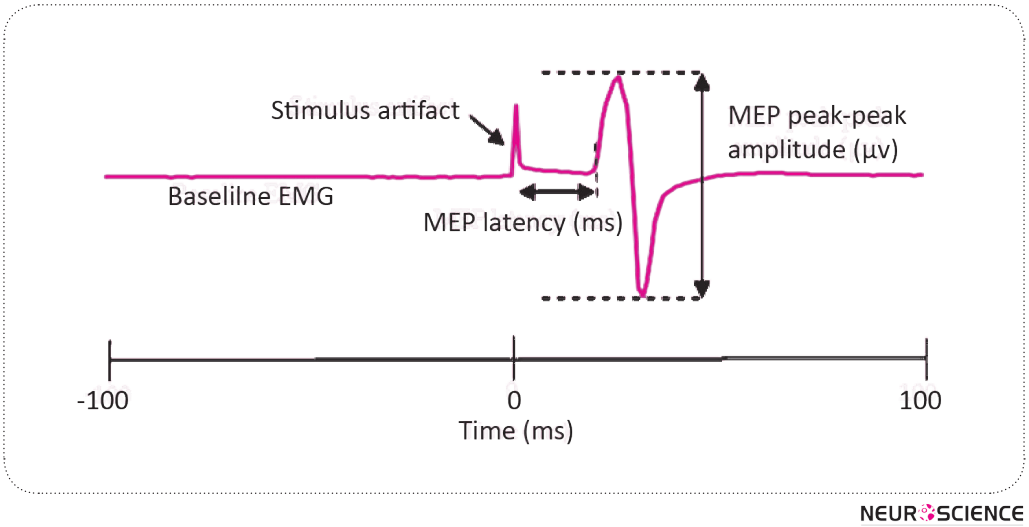


Figure 3. Typical MEP response recorded from resting ECR, showing, baseline EMG, stimulus artefact, latency (ms) and peak-to-peak amplitude (µV).

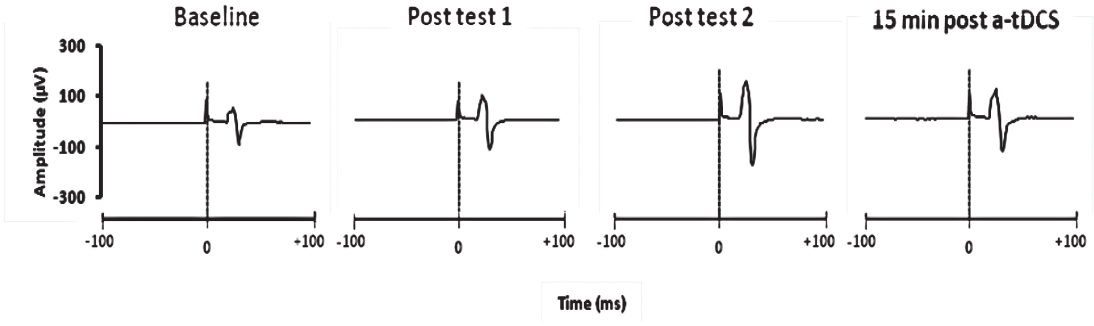
measurements at rest were calculated for the time points of baseline, immediately post-test 1, immediately post-test 2 and follow up.

A one-way within-subjects ANOVA was conducted to compare the effects of short and long durations of a-tDCS on corticomotor excitability at four different time points. Significance was set at $p \leq 0.05$, all results are displayed as means ± SE and statistical analysis was performed using SPSS software version 19.

3. Results

All participants tolerated the intervention used in this study and all finished the experiments. No side effects other than a mild tingling or itchiness were reported.

The ANOVA indicated that corticomotor excitability increased significantly over time ($F_{3,27} = 20.32$, $p = 0.000$, $\eta 2 = 0.69$). Furthermore, a series of pairwise comparisons revealed that the average MEP amplitude confidence level immediately following 10 min of a-tDCS (M = 220.58 μV, SE = 22.77, 95% CI [169.09, 272.06], p = 0.001), 10+10 min a-tDCS (M = 292.63 μV, SE = 31.99, 95% CI [220.31, 364.95], p = 0.005)



NEUROSCIENCE

Figure 4. Averaged MEP signal across 12 trials obtained from one participants right ECR muscle before (baseline), post-test 1, post-test 2 and 15 min post a-tDCS. There was a 79% increase in MEP amplitude immediately following 10 min a-tDCS, 138% increase immediately post 10+10 min a-tDCS and a 85% increase 15 min post a-tDCS, respectively.

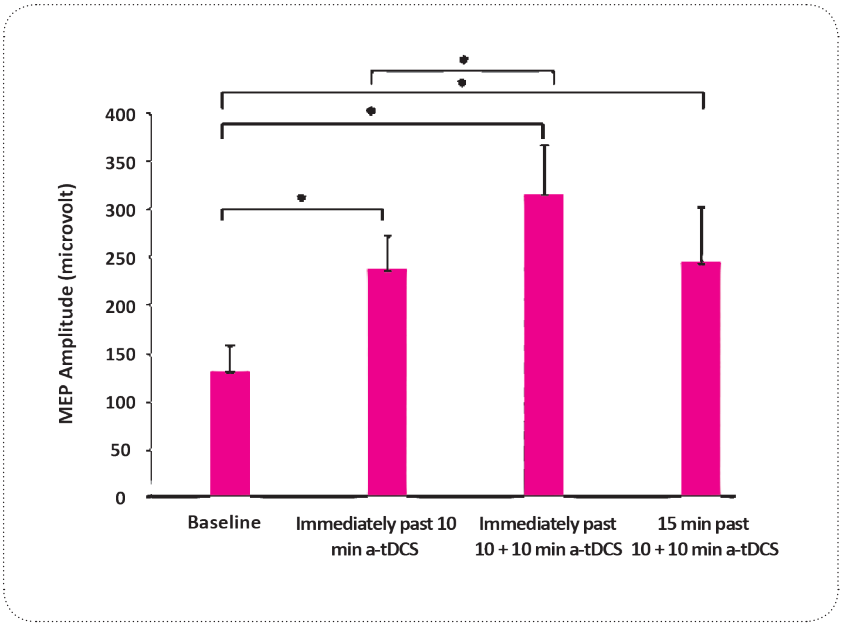


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Figure 5. Time course effect of a-tDCS applied over the left M1 on the amplitude of evoked MEPs from the left ECR motor area (mean and SE). Asterisks indicate significant differences between MEP amplitudes after a-tDCS stimulation and baseline.

and 15 min following 10+10 min a-tDCS (M = 218.04 μV, SE = 37.59, 95% CI [133.05, 303.02], p = 0.02) was significantly higher than the average MEP amplitude confidence level obtained at baseline (M = 131.93 μV, SE = 16.35, 95% CI [94.96, 168.90] Figure 4 & 5). Also, the MEP amplitude of ECR showed significant differences between post test 1 and following post test 2 (p = 0.03). Figure 5 indicates that there were no significant differences between 15 min follow up and both of the previous measurements (p > 0.05).

4. Discussion

The purpose of this study was to explore the effects of short duration and long duration of a-tDCS on modulating corticomotor excitability. Both short duration (10 min) and long duration (10+10 min) increased corticomotor excitability by 67% and 122% respectively. Further, there were significant after-effects of a-tDCS application, with corticomotor excitability still elevated 15 min after tDCS stimulation. This suggests several important findings. Foremost, corticomotor excitability was facilitated following a-tDCS with both short and long stimulation periods. Second, long duration a-tDCS elicited further facilitation in corticomotor excitability compared to short duration, showing that duration of stimulation is important for the therapeutic use of a-tDCS. In addition, the application of 10+10 min of a-tDCS using 6×7 cm (42 cm^2) electrodes was safe and well tolerated by all participants.

It was hypothesized that short duration (10 min) a-tDCS would facilitate corticomotor excitability and that an additional 10 min would elicit further increases compared to just 10 min of a-tDCS. In the present study we demonstrated a significant increase (67%) in the TMS-evoked MEPs following 10 min a-tDCS when compared to baseline. This finding is in agreement with several other studies that have used stimulation periods of between 5, 7 and 9 min (Nitsche & Paulus, 2000, 2001; Uy & Ridding, 2003; Lang et al., 2004; Nitsche et al., 2005; Fricke et al., 2011). Furthermore, the present finding following short duration of a-tDCS is also in agreement with Lang et al. (2004), Antal et al. (2007) and Furubayashi et al. (2008) who also demonstrated a single session of a-tDCS for 10 min increased corticomotor excitability.

The novel aspect of the current study was the application of an additional 10 min a-tDCS. We hypothesized that longer application (10+10 min) of a-tDCS would induce a larger increase in corticomotor excitability compared to a single 10 min stimulation period. The results are consistent with previous studies that have shown facilitated corticomotor excitability following 13 min of a-tDCS when compared to shorter applications (Nitsche et al., 2005; Boros et al., 2008), showing that longer applications of a-tDCS modulates corticomotor excitability to a greater extent compared to shorter applications.

Although the mechanism of a-tDCS remains largely unknown, the increases in MEP amplitudes observed in the current study are likely to be related to the effects of the direct currents inducing membrane polarization. These effects have been demonstrated in M1 by plasticity-inducing protocols (Nitsche & Paulus, 2000). As such it's conceivable that the increases in corticomotor excitability shown in the current study may have occurred due to mechanism associated with long-term potentiation. For example, anodal stimulation has been shown to result in neuronal membrane depolarization at the cellular level with increases in intracellular ca^{2+} levels that induce increase in corticomotor excitability (Nitsche et al., 2004). The induction of longer stimulation may have resulted in greater shifts in the resting membrane potential, thus modulating enhanced synaptic efficacy (Nitsche & Paulus, 2000).

Longer a-tDCS stimulation has been shown to trigger a membrane potential change that leads to N-methyl-D-aspartate (NMDA) receptor activation and/or more ca^{2+} influx into neurons (Liebetanz et al., 2002). It is well understood that long-lasting NMDA-receptor dependent cortical excitability and subsequent action potential activity shifts, are involved in neuroplastic modification, such as activity-dependant synaptic plasticity. The larger increase in corticomotor excitability following the longer application of a-tDCS in the present study is most likely due to increased neuronal membrane excitability and/or NMDA receptor efficacy (Liebetanz et al., 2002). Either membrane potential or synaptic mechanisms (increased presynaptic release of excitatory transmitters or an increased postsynaptic ca^{2+} influx) (Bennett et al., 2000) or both; may explain the larger increase in corticomotor excitability following longer application of a-tDCS. Therefore, we suggest that this longer application of a-tDCS allows time for other processes to develop, involving physiological factors associated with synaptic plasticity; that replaces the smaller size in corticomotor excitability following shorter stimulations.

The present study has also shown significant after-effects of increased corticomotor excitability following a-tDCS. This finding is consistent with a number of studies that have demonstrated enhanced corticomotor

excitability following the application of 1 mA a-tDCS (Nitsche & Paulus, 2001; Hummel & Cohen, 2006; Antal et al., 2007; Boros et al., 2008; Furubayashi et al., 2008). The after effects lasted at least 15 min post stimulation and the amplitude of the TMS evoked MEPs began to decrease nearly 15 min after the offset of a-tDCS, even though it remained higher than the baseline value. Experimental data has previously shown that shorter duration of stimulation of 5 and 7 min, results in after effects that are maintained for no longer than 5 min, and the application of a-tDCS for 9, 11 and 13 min results in elevated MEP amplitudes up to 30, 45 and 90 min, respectively (Nitsche & Paulus, 2001).

It is unlikely that membrane potential change is the only mechanism responsible for modulating the after-effects on increased corticomotor excitability produced by a-tDCS. Lasting effects beyond the stimulation must be explained by other mechanisms, such as adrenergic mechanisms which have been found to be involved in the stabilization of after effects (Nitsche et al., 2004; Nitsche et al., 2005) and must conform to the above speculated mechanism involved in longer a-tDCS application. Although this is a potential mechanism of action, the exact mechanism of action of a-tDCS still remains unclear and these concepts are purely hypothetical at present.

5. Conclusion

In conclusion, we have shown that it is possible to induce greater levels of corticomotor excitability following longer periods of a-tDCS application compared to shorter periods (i.e. 10 min), with these affects remaining elevated at least 15 min after the end of stimulation. Further experiments should explore the presumed physiological mechanisms more directly. In addition, further research is needed using a larger sample size and long-term follow-ups. The results of this study can be useful for increasing corticomotor excitability by repeating a-tDCS application within a session compared to longer applications of a-tDCS which may produce opposite effects (Monte Silva et al., 2011).

Glossary

a-tDCS: Anodal transcranial direct current stimulation

c-tDCS: Cathodal transcranial direct current stimulation

TMS: Transcranial magnetic stimulation

ECR: Extensor carpi radialis

EMG: Electromyography

MEP: Motor evoked potential

M1: Primary motor cortex

NMDA: N-methyl-D-aspartate

RMT: Resting motor threshold

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Declaration for Chapter 5

In the case of Chapter 5, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
Review of literature, Project design, ethics application and approval, participant recruitment, data collection, data analysis, interpretation of the results and writing of the manuscript.	80 %

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Shapour Jaberzadeh	Supervisory input on study design, Guidance in the framing of the manuscript, discussion of findings, review and provision of feedback on manuscript drafts	20 %

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	**Date** 30.09.2013
Signature	**Shapour Jaberzadeh**[redacted]	**Date** 24.09.2013

Preamble to Chapter 5

Despite initial success following the application of existing a-tDCS parameters, involving current intensities of 1-2 mA, the optimal intensity for induction of largest CSE changes are not reported yet. In particular, it is vital to systematically measure the effects of a range of common a-tDCS current intensities on CSE changes. Therefore, the focus of Chapter 5 is to study the effects of four different current intensities of a-tDCS on the size of CSE. Our data set in the following chapter allows addressing the optimal current intensity for application of a-tDCS.

Chapter 5: Differential modulation of corticospinal excitability by different current densities of anodal transcranial direct current stimulation

The format of this chapter is consistent with the Journal of *PLOS ONE*.

The setup system used in this study, Ethics approval, TMS safety and Edinburg handedness questionnaires and consent form are provided in Appendices 8, 10 and 12-15.

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Differential Modulation of Corticospinal Excitability by Different Current Densities of Anodal Transcranial Direct Current Stimulation

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Abstract

Background: Novel non-invasive brain stimulation techniques such as transcranial direct current stimulation (tDCS) have been developed in recent years. TDCS-induced corticospinal excitability changes depend on two important factors current intensity and stimulation duration. Despite clinical success with existing tDCS parameters, optimal protocols are still not entirely set.

Objective/hypothesis: The current study aimed to investigate the effects of four different anodal tDCS (a-tDCS) current densities on corticospinal excitability.

Methods: Four current intensities of 0.3, 0.7, 1.4 and 2 mA resulting in current densities (CDs) of 0.013, 0.029, 0.058 and 0.083 mA/cm^2 were applied on twelve right-handed (mean age 34.5±10.32 yrs) healthy individuals in different sessions at least 48 hours apart. a-tDCS was applied continuously for 10 minute, with constant active and reference electrode sizes of 24 and 35 cm^2 respectively. The corticospinal excitability of the extensor carpi radialis muscle (ECR) was measured before and immediately after the intervention and at 10, 20 and 30 minutes thereafter.

Results: Post hoc comparisons showed significant differences in corticospinal excitability changes for CDs of 0.013 mA/cm^2 and 0.029 mA/cm^2 ($P = 0.003$). There were no significant differences between excitability changes for the 0.013 mA/cm^2 and 0.058 mA/cm^2 ($P = 0.080$) or 0.013 mA/cm^2 and 0.083 mA/cm^2 ($P = 0.484$) conditions.

Conclusion: This study found that a-tDCS with a current density of 0.013 mA/cm^2 induces significantly larger corticospinal excitability changes than CDs of 0.029 mA/cm^2. The implication is that might help to avoid applying unwanted amount of current to the cortical areas.

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Introduction

As part of a growing understanding of neuroplasticity, novel non-invasive brain stimulation techniques have been developed in recent years. Brain stimulation paradigms aimed at modifying corticospinal excitability include repetitive transcranial magnetic stimulation (rTMS) and transcranial electric stimulation (tES) [1,2].

Despite the rTMS which is a neurostimulatory technique, tES is an umbrella term for description of a number of neuromodulatory techniques such as transcranial alternating current stimulation, transcranial random noise stimulation and transcranial direct current stimulation (tDCS) [2]. The most utilised techniques of tES is tDCS, application of a low-amplitude direct current which can modulate corticospinal excitability in a polarity-dependent manner [3] with several advantages. It is a painless technique with no or minimal side effects and it can be applied by an inexpensive direct current stimulator which is very simple to operate [3]. tDCS involves application of very low-amplitude direct currents (2 mA or less) via surface scalp electrodes to modify neuronal transmembrane potential and influence the level of excitability [4,5]. Depending on the polarity of the active electrode over the primary motor cortex (M1), contralateral to the target muscles, tDCS can increase or decrease corticospinal excitability [5,6]. Cathodal tDCS (c-tDCS) involves application of the negatively charged electrode (cathode) over M1, which leads to hyperpolarization [3,6] of cortical neurons and reduces the size of the TMS-induced motor evoked potentials (MEPs), indicating decreased corticospinal excitability. On the other hand, anodal tDCS (a-tDCS) involves the application of the positive charged electrode (anode) over M1, which results in cortical depolarization and increases the size of TMS-induced MEPs, indicating increased corticospinal excitability [3,6]. These changes in corticospinal excitability can lead to improved motor performances [7–9]; thus tDCS can be used as a stand-alone therapeutic intervention or as an add-on technique to prime the effects of other training methods [10,11].

tDCS can also be used for induction of cortical changes to provide information about the functioning of the human brain [5].

The extent of a-tDCS-induced corticospinal excitability changes depend on the current intensity/density, the electric current per electrode surface area [3], duration of current application [3,5,6,12] and the electrode's surface area [13]. As reported in a recent systematic review [14], a-tDCS with higher current densities (CDs) induce larger corticospinal excitability changes. Nitsche and Paulus (2000) compared five current intensities between 0.2 and 1 mA (CDs between 0.006 to 0.029 mA/cm^2). They found that a stimulus intensity of at least 0.6 mA (electrode size 35 cm^2; CD: 0.017 mA/cm^2) is required to induce a significant increase in MEP amplitude [3].

Although the general impression is that tDCS is a safe, well-tolerated technique with no evidence of serious adverse effects [15,16], recipients may experience mild and transient sensory side effects such as itching, tingling and burning sensations [17]. There is a direct link between current intensity and these side effects, therefore to minimise these side effects lower intensities should be used [17,18]. This is important, because new protocols designed to extend the duration of lasting effects recommend longer and/or multiple tDCS application sessions [2].

Despite clinical success following the application of existing tDCS parameters, involving current intensities of 1–2 mA and electrode sizes of 25–35 cm^2 [12], stimulation parameters are yet to be optimised; more research is required to fulfil this needs. In particular, it is vital to systematically measure the effects of a range of common a-tDCS CDs on corticospinal excitability changes. Therefore, the aim of the present study was to compare the effects of a range of CDs on a-tDCS induced corticospinal excitability in healthy individuals. The second aim of this study was to assess the tolerability of a-tDCS during stimulation. We hypothesized that there is a direct relationship between the CD under the active electrode and the magnitude of induced corticospinal excitability change in M1. We also hypothesized that there is a direct relationship between CD and the level of side effects.

Materials and Methods

Subjects

We conducted 48 experiments on twelve healthy volunteers (seven women, five men) recruited from Monash University students/staff with a mean age of 34.5±10.3 years (age range 20–51 years), a mean weight of 68.6±11.0 kg and a mean height of 168.9±15.5 cm. All were right-handers as determined by the Edinburgh Handedness Inventory (10 item version, mean laterality quotient = 87.9±19.5) [19]. All participants completed the Adult Safety Screening Questionnaire to determine suitability for TMS [20]. Participants were informed about the experimental procedures and gave their written informed consent according to the declaration of Helsinki. All experimental procedures were approved by the Monash University Human Research Ethics Committee.

a-tDCS of the Motor Cortex

a-tDCS was delivered by an Intelect® Advanced Therapy System (Chattanooga, USA) through a pair of saline-soaked surface sponge electrodes. The anode was placed over the left M1 for the right extensor carpi radialis muscle (ECR) as identified by TMS. The cathode was placed over the right contralateral supraorbital area [3]. The electrodes were fixed with two horizontal and perpendicular straps.

Each subject was tested at the same time of the day to avoid diurnal variations. A-tDCS was applied continuously for 10 minute for all stimulation protocols using active and reference electrodes of 24 and 35 cm^2 respectively. A larger electrode was used for the cathode electrode to decrease the CD and reduce side effects under the indifferent electrode with more focused density under the anode [13]. The only differences between the four stimulation protocols were different current intensities (0.3, 0.7, 1.4 and 2 mA) resulting in four different CDs (D1–D4) under the active electrode (D1 = 0.013, D2 = 0.029, D3 = 0.058 and D4 = 0.083 mA/cm^2).

Monitoring of Corticospinal Excitability

Participants were seated upright in an adjustable podiatry chair, with the forearm pronated and the wrist joint in neutral position resting on the armrest.

Single-pulse magnetic stimuli were delivered using a Magstim 200^2 (Magstim Company Limited, Whiteland, Wales, UK) stimulator with a flat 70 mm figure-of-eight standard magnetic coil (peak magnitude field, 2.2 T). The vertex (C_z) point was measured and marked to be used as a reference [21]. The magnetic coil was placed over the left hemisphere (cortex), contralateral to the target muscle. The orientation of the coil was set at an angle 45° to the midline and tangential to the scalp such that the induced current flowed in a posterior-anterior direction in the brain. The area of stimulation (hotspot) was determined through the measurement of the scalp using the convention of the EEG 10/20 system to find a spot over the ECR muscle M1 that would allow measurement of the largest MEP responses.

After localizing the hot spot, the coil's position was marked on the scalp to be used for remainder of the testing for the target muscle to ensure consistency in the placement of the coil. Resting motor threshold (RMT) was defined as the minimal stimulus intensity that evoked five MEPs in a series of 10 with an amplitude of at least 50 μV [22–24]. The resting thresholds for the ECR muscle were determined by incrementing and decrementing stimulus intensity in 1–2% intervals until MEPs of at least 50 μV were elicited [3]. For all further MEP measurements, the test TMS intensity was set at 120% of each individual's RMT. Fifteen stimuli were elicited to assess corticospinal excitability at each time point. The stimulus intensity remained constant throughout the study session for each subject.

Surface EMG was recorded from the right ECR muscle using bipolar Ag/AgCl disposable surface electrodes with an inter-electrode distance of 3 cm (measured from the centres of the electrodes). To ensure good surface contact and reduce skin resistance, a standard skin preparation procedure of cleaning and abrading was performed for each electrode site [21,25,26]. The location of ECR was determined based on anatomical landmarks [27] and also observation of muscle response in the testing position (wrist extension and radial deviation) [28]. The accuracy of EMG electrode placement was verified by asking the subject to contract the muscle(s) of interest while the investigator monitored online EMG activity. A ground electrode was placed ipsilaterally on the styloid process of the ulnar bone [29,30]. The electrodes were secured by hypoallergenic tape (Micropore, USA). All raw EMG signals were band pass filtered (10–1000 Hz), amplified (×1000) and sampled at 2000 Hz and collected on a PC running commercially-available software (Chart™ software, ADinstrument, Australia) via a laboratory analogue-digital interface (The PowerLab 8/30, ADinstrument, Australia). Peak-peak MEP amplitude was detected and measured automatically using a custom-designed macro in Powerlab 8/30 software after each magnetic stimulus.

Assessment of a-tDCS Tolerability

a-tDCS side effects were assessed by monitoring the presence of itching, tingling, burning sensation and any other discomfort, including headache; these are the sensory complaints most commonly reported during application of tDCS [5,31]. Tolerability and sensory changes were monitored based on participants' reports under the active and/or reference electrodes at the beginning, in the middle and at the end of a-tDCS application, using numeric analogue scales (NAS) (eg, 0 = no tingling to 10 = worst tingling imaginable).

Experimental Procedures

The study was conducted in a within-subject, randomised, counter-balanced cross-over design, illustrated in Figure 1. All recruited individuals participated in four experimental sessions at least 48 hours apart to avoid interference or carry-over effects of a-tDCS. Subjects were blinded to a-tDCS conditions. The order in which the experimental sessions were conducted was randomized between participants. Corticospinal excitability was measured before, immediately after (T0) and three more times at 10-minute intervals (T10, T20 and T30) after the cessation of a-tDCS.

Data Management and Statistical Analysis

Peak–peak amplitudes of 15 MEPs were calculated and averaged automatically for each time point before and after interventions. Post-intervention values were then normalized to the baseline value [32].

Differences in MEP amplitudes in the ECR muscle for four different a-tDCS CDs and at each of time points were analysed with a two-way repeated measure analysis of variance (ANOVA). The first within - subject independent factor was CD (four levels). The second independent factor was time points (four levels). Mauchly's test was used to assess the validity of the sphericity assumption for repeated measures ANOVA; it requires that the variances for each set of difference scores be equal. Greenhouse-Geisser corrected significance values were used when sphericity was lacking [33]. In case of significant main effects, post hoc comparisons were performed using the least significant difference adjustment for multiple comparisons. Baseline MEP amplitudes and RMT of the respective a-tDCS conditions were tested using one-way ANOVA to see whether they were identical in all conditions. Furthermore, using one way ANOVA, we examined whether our results were associated with an order effect. We considered the results of all statistical analyses significant at $P<0.05$. All results are expressed as the mean ± standard error of mean (SEM). Statistical analyses were performed using SPSS software version 20.

Results

Effects of Different CDs on Corticospinal Excitability

One-way repeated measure ANOVA showed that baseline a-tDCS MEP amplitudes ($P=0.12$) and RMT were identical between all conditions ($P=0.28$). Also, there was no significant order effect ($F(3, 33)=2.07$, $P=0.12$). Mauchly's test of sphericity indicated that this assumption was met for CD ($W=0.387$, $df=5$, $P=0.102$), so no corrections were applied to the F-ratio computations. The assumption of sphericity was violated for time ($W=0.318$, $df=5$, $P=0.05$) and CD × time interaction ($W=0.000$, $df=44$, $P<0.001$), so Greenhouse-Geisser correction was employed for the F-ratio computations.

The results of the two-way repeated measures ANOVA showed significant main effects of time ($F(1.75,19.31)=94.05$; $P<0.001$, $\eta_p^2=0.56$). Post hoc comparisons showed that there was significant difference between T0–T10 (Mean = 9.16, SE = 4.07) ($P=0.046$), T0–T20 (Mean = 21.10, SE = 6.37) ($P=0.007$), T0–

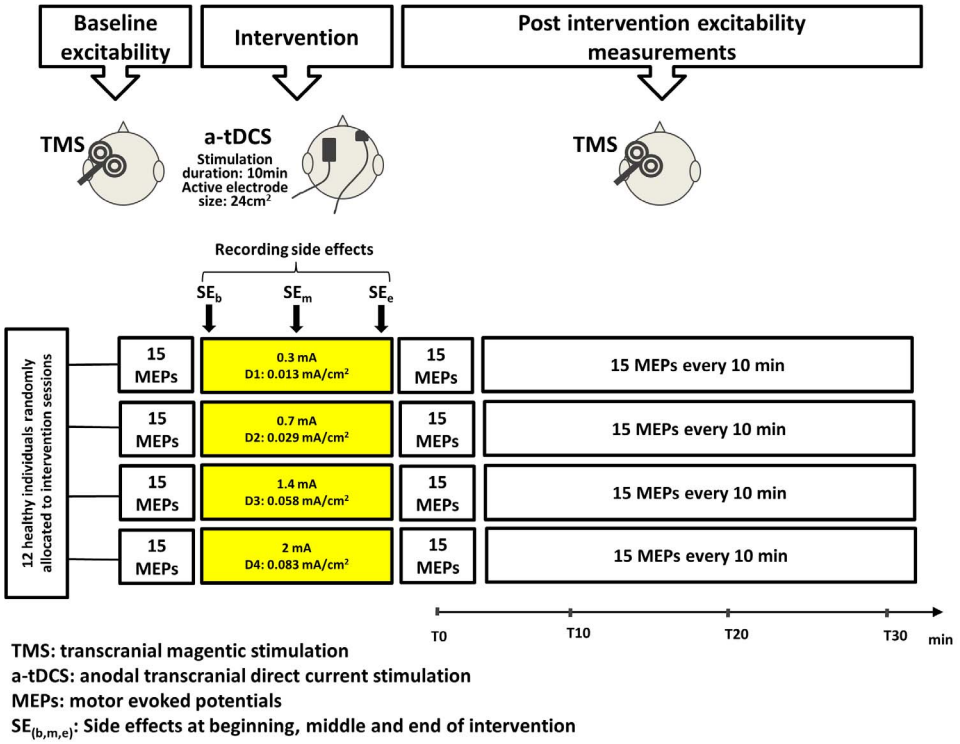

Figure 1. Experimental design. Comparison of the effects of different CDs (D1–D4) on corticospinal excitability.
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T30 (Mean = 27.80, SE = 5.76) ($P = 0.001$), T10–T20 (Mean = 11.94, SE = 3.58) ($P = 0.007$) and T10–T30 (Mean = 18.67, SE = 3.80) ($P < 0.001$). Post-hoc comparisons also indicated that there was no significant difference in the scores of T20 and T30 ($P = 0.063$).

We observed no significant changes between different time points of a-tDCS within each D1, D3 and D4 CD conditions ($P > 0.05$). However, in the D2 condition, we found significant differences between the amplitudes of ECR MEPs 20 and 30 minutes after the end of stimulation ($P < 0.05$) (Figure 2). Also the result of post hoc comparisons showed significant differences between D1–D2, D2–D4 and D3–D4 ($P < 0.05$) in all time points of T0, T10, T20 and T30 (Figure 3).

Two-way repeated measures ANOVA showed significant main effects of a-tDCS different CDs ($F(3,33) = 6.121$; $P < 0.05$, $\eta_p^2 = 0.36$). Post-hoc comparisons indicated that there was a significant difference in the scores of D1 and D2 (Mean = 50.94, SE = 17.34) ($P < 0.05$). Pairwise comparison indicated that there was a significant difference in the scores of D2–D4 (Mean = 69.04, SE = 18.05) ($P = 0.003$) and in the scores of D3–D4 (Mean = 51.38, SE = 16.97) ($P = 0.012$). Post hoc comparisons also showed that there was no significant difference between D1–D3 ($P = 0.080$), D1–D4 ($P = 0.484$) and D2–D3 ($P = 0.076$) (Figure 3).

As displayed in Figure 2, a-tDCS resulted in significant excitability enhancement lasting for 30 minutes after the end of stimulation in all conditions ($P < 0.005$). Finally, The results of the two-way repeated measures ANOVA showed no significant interaction of CD × time ($F(3.17,34.90) = 0.18$; $P = 0.91$, $\eta_p^2 = 0.01$). This means that the effect of CD on corticospinal excitability is not dependent on the levels of the time (T0–T30).

a-tDCS Side Effects and Tolerability

Participants described their experiences under the electrodes at the beginning, in the middle and at the end of the intervention. The only sensations related to the cathode electrode were a mild redness under the cathode electrode (reported by two participants). In contrast, most participants reported tingling, itching and/or burning under the anode electrode (Table 1). Overall, the findings support the tolerability of direct current stimulation using CDs of D1 and D2 compared to D3 and D4. D3 and D4 produced more unpleasant feelings under the anode and the D4 caused one participant to terminate the experiment (Note that the CDs used in

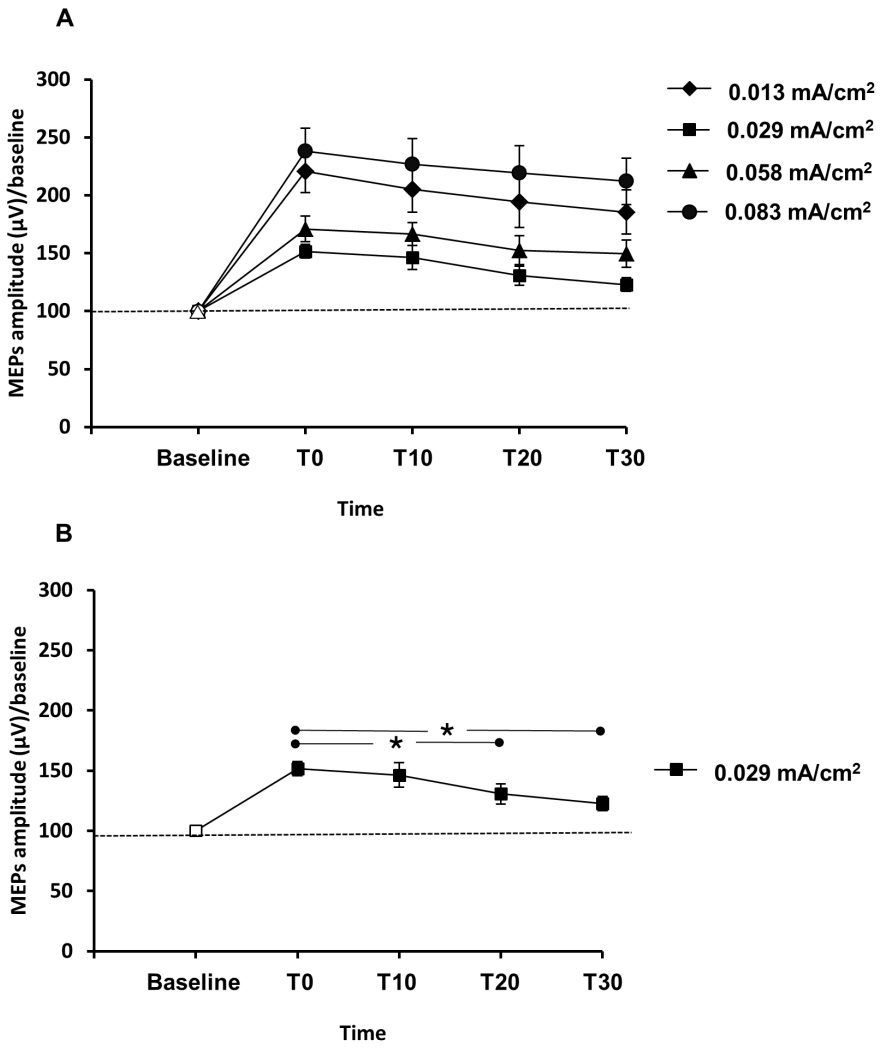

Figure 2. The effects of different CDs on the MEPs size over the 30 minutes. Filled symbols indicate significant deviation of the post-a-tDCS MEP amplitudes compared to baseline (A, B). The asterisks mark significant differences between time points during the 30 minutes after cessation of a-tDCS (B). The only significant differences were seen within D2 condition. Error bars represent SEM.
doi:10.1371/journal.pone.0072254.g002

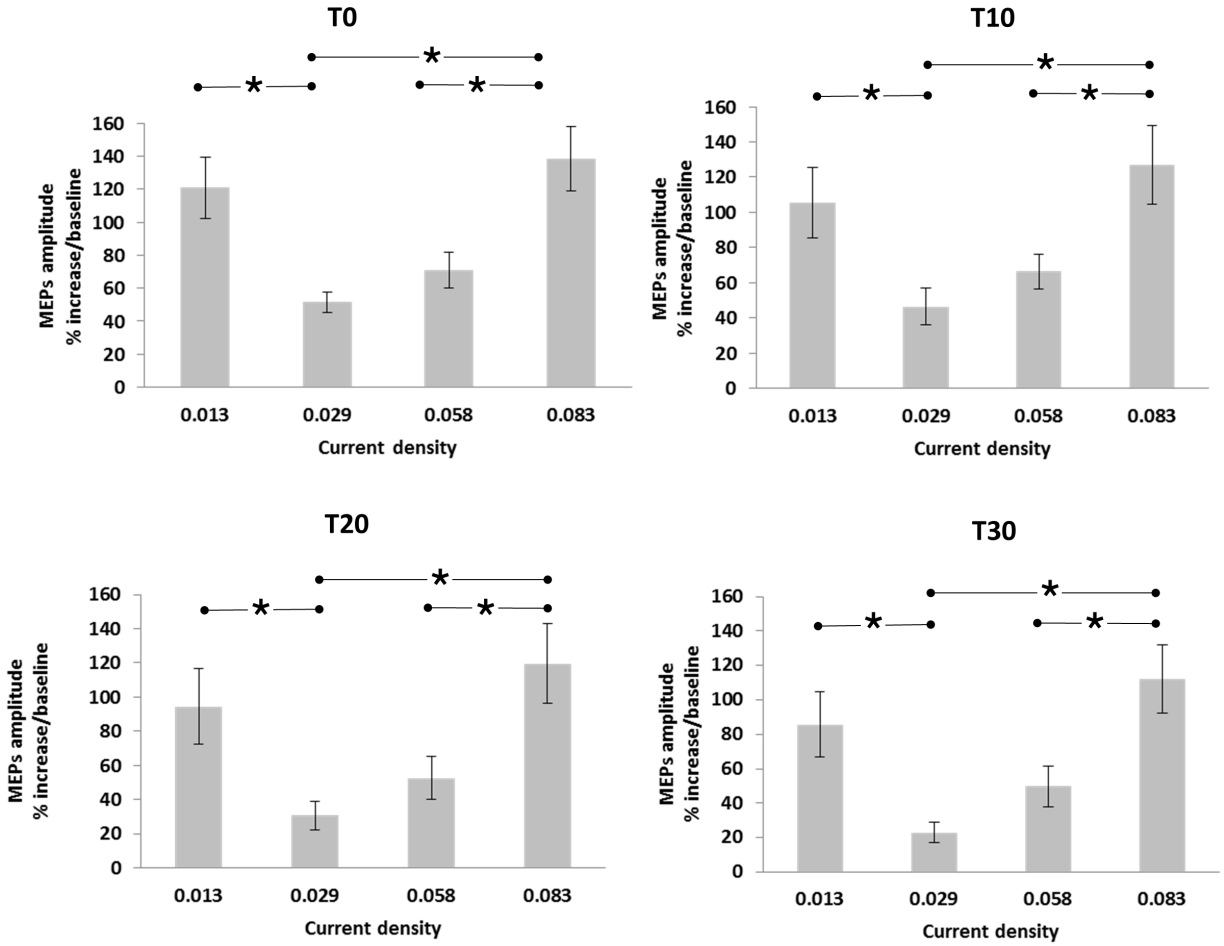

Figure 3. Percentage increase in corticospinal excitability after the intervention. The asterisks mark significant differences between ECR muscle MEP amplitudes after the end of a-tDCS in all time points of T0, T10, T20 and T30. Error bars represent SEM.
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this study were specifically selected to allow safe stimulation). There were no adverse effects related to application of a-tDCS during the follow-up period.

Discussion

Effects of Different CDs on Corticospinal Excitability

The present study was designed to determine the effects of four different CDs on corticospinal excitability in healthy individuals and generated several important findings. First, different CDs induce different corticospinal excitability changes. Second, there was a direct relationship between the density of the three largest applied currents (D2, D3 and D4) and the size of the excitability changes produced. Third, in apparent contradiction to the dose-response relationship implied by the previous finding, the lowest density (D1) induced more corticospinal changes than two higher applied intensities (D2 and D3). Fourth, a-tDCS applied to the M1 increased corticospinal excitability for at least 30 minutes after the stimulation period.

Table 1. Sensations under the anode reported by participants.

Current Density	No sensation	Tingling sensation			Itching sensation			Burning sensation			Not tolerated
		Beginning	Middle	End	Beginning	Middle	End	Beginning	Middle	End	
D1	83.3% (10)	16.6% (2)	–	–	–	–	8.3% (1)	–	–	–	–
D2	50% (6)	50% (6)	25% (3)	8.3% (1)	–	25.0% (3)	50.0% (6)	–	–	–	–
D3	8.3% (1)	50% (6)	33.3% (4)	33.3% (4)	46.6% (5)	46.6% (5)	58.3% (7)	–	8.3% (1)	16.6% (2)	–
D4	8.3% (1)	66.6% (8)	66.6% (8)	58.3% (7)	50% (6)	66.6% (8)	66.6% (8)	16.6% (2)	25% (3)	25% (3)	8.3% (1)

The values are showed as percentage followed by number of subjects in parentheses.
doi:10.1371/journal.pone.0072254.t001

We hypothesized that there is a direct relationship between the size of CDs under the active electrode and the size of induced corticospinal excitability changes in M1. The findings in the current study only support this hypothesis in part. The hypothesized direct relationship was only observed in the three largest CDs (D2, D3 and D4), but supports Nitsche and Paulus' (2000), finding of a direct relationship between current intensities/densities of 0.2 to 1 mA (CD = 0.006 to 0.029) and corticospinal excitability changes [3]. The finding that the smallest CD produced significantly larger corticospinal changes than the next two higher CDs has not been previously reported. The finding appears to be new. However, some possible differences between the presented study and the Nitsche and Paulus (2000) study can be explained.

The findings in current study are not in line with the findings of Nitsche and Paulus (2000). Contrary to the finding in current study which indicates that the smallest CD (0.3 mA) produced significantly larger corticospinal changes than the next two higher CDs, they found that for a-tDCS, a minimal stimulus intensity of 0.6 mA (0.017 mA/cm^2) is necessary to enhance corticospinal excitability. This discrepancy could be easily described by following differences between these two studies. First, the stimulation duration in Nitsche and Paulus (2000) study was considerably shorter than that of the current study. The stimulation time in Nitsche and Paulus (2000) study was 5 minutes compared to 10 minutes in the current study. The minimal stimulus of 0.6 may be right for 5 minutes of stimulation but that threshold should be less for longer applications. Second, Nitsche and Paulus (2000) used an electrode size of 35 cm^2 compared to 24 cm^2 in the current study. According to a recent study by our group [34], the electrode size has an important role on the size of induced corticospinal excitability. The electrode size of 24 cm^2 used in our study may also contribute to the discrepancy in results with Nitsche and Paulus (2000) study.

The mechanisms underlying these changes are not clear, but it is proposed that they are caused by alterations in the function of the membrane ion channels, leading to neuroplasticity [5]. The way that a single session of a-tDCS behaves could be due to short term potentiation (STP) [35] and/or early long term potentiation (e-LTP) [36]. e-LTP depends on activation of calcium-dependent kinases, which controls the trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and activation of N-methyl-D-aspartate (NMDA – a subtype of glutamate receptor) [37–41]. Excitatory synaptic changes in the brain are predominantly mediated by the neurotransmitter glutamate [42,43], while inhibitory transmission is mediated mainly by the neurotransmitter gamma-amino butyric acid (GABA) [42,44]. The level of excitation in the brain is kept in check through inhibitory control exerted by GABA neurons [45]. One pharmacological study showed an abolition of the intracortical effects of anodal tDCS after administration of lorazepam as a GABA agonist [46]. Also, in a recent animal study it was shown that any increase in NMDA activity coincides with an increase in the level of GABA secretion [47]. The mechanism behind this activation of GABA receptors could be that the Ca^{2+} influx through the NMDA receptors affects the adjacent inhibitory presynaptic sites and leads directly to release of GABA [48]. In addition to this, the activation of gated Ca^{2+} channels on the synaptic membrane may play a role [49,50]. Thus, any manipulation that influences the magnitude or dynamics of Ca^{2+} increases within dendritic spines may profoundly influence the form of the resulting synaptic plasticity.

Surprisingly, we found that the smallest CD (D1) induced larger corticospinal changes than the two consecutive higher CDs of D2 and D3. This finding has no precedent in the literature; it indicates that a different mechanism may be involved in induction of corticospinal excitability changes at lower current intensities/densities. Lower density of a-tDCS may induce more corticospinal changes than higher densities due to the relative activity of facilitatory and inhibitory mechanisms. Previous animal studies have reported that GABA activation is voltage dependent [51,52]. An increase in MEP amplitude with D1 may be due to the fact that at this low density the GABA and NMDA receptors are inactive and the excitatory changes are driven by activation of voltage-gated Ca^{2+} channels which normally have lower thresholds than NMDA or AMPA receptors. Apparently, this low direct current stimulation at 0.3 mA (0.013 mA/cm2), considered a weak form of a-tDCS, is sufficient to activate Ca^{2+} channels and raise intracellular Ca^{2+} concentrations. This may lead into cortical neuron depolarization that shifts the resting membrane potential more toward positive values and closer to the threshold level, a state called 'excitation'.

In the current study, lasting effects of a-tDCS (increased corticospinal excitability) were measured up to 30 minutes after the end of stimulation, consistent with previous investigations [6,53–56]. These observations suggest that the modulatory response of M1 pyramidal cells to a-tDCS might be dependent on the CD and subsequent degree of activated receptors.

a-tDCS Side Effects and Tolerability

Our findings confirm that the smallest CD (0.013 mA/cm^2) has the lowest side effects under the active electrode, thereby supporting our second hypothesis. The application of a-tDCS to the ECR M1 area was associated with a tingling sensation in 40.8% of the tests in all CD conditions, however; 25.0% of recipients of D3 and 66.7% of the participants who received D4 found the stimulation procedure mildly unpleasant.

Limitations

Our findings must be interpreted in the context of several limitations. First, our study involved only 12 non-randomly-selected participants, which limits the generalizability of the results. The data were obtained from a healthy population, so we cannot extrapolate the findings to patient populations. The effects of the stimulation were only assessed up to 30 minutes after delivery; longer assessment of lasting effects is recommended to evaluate their length. Another limiting factor is that the examiner was not blinded to the stimulation conditions.

Suggestions for Future Studies

A further study involving current intensities between 0.3–0.8 mA is suggested to investigate the turning point of the excitability changes. Furthermore, to underpin the mechanisms of action of lower CD, it is recommended that a study of motor cortex excitability be undertaken, by measuring silent period, intracortical inhibition, and facilitation, to indirectly assess the role of GABAa, GABAb and glutamergic receptors.

In addition, the effects of different CDs and their tolerability should be studied in patients with neurological problems, different age groups and genders. Additional pharmacological experiments using receptor agonists/antagonists are needed to prove that if a-tDCS with lower CD has different mechanisms compared with larger CD.

Conclusion

Our findings can be employed to develop a-tDCS protocols optimized for clinical application. The smallest CD used in this study (0.013 mA/cm^2) could be a promising parameter for the

modulation of corticospinal excitability with less total charge to the cortical area. In addition to its efficiency in inducing corticospinal excitability, it was much better tolerated than larger CDs and could be safely used in protocols with multi sessions of a-tDCS applications. Our results suggest that a deeper understanding of the mechanisms underlying a-tDCS-induced excitability is required.

Author Contributions

Conceived and designed the experiments: AB SJ. Performed the experiments: AB. Analyzed the data: AB. Contributed reagents/materials/analysis tools: AB. Wrote the paper: AB.

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Declaration for Chapter 6

In the case of Chapter 6, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Review of literature, Project design, ethics application and approval, participant recruitment, data collection, data analysis, interpretation of the results and writing of the manuscript.	80 %

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Shapour Jaberzadeh	Supervisory input on study design, Guidance in the framing of the manuscript, discussion of findings, review and provision of feedback on manuscript drafts	20 %

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	**Date** 30.09.2013
Main Supervisor's Signature	**Shapour Jaberzadeh**[redacted]	**Date** 24.09.2013

Preamble to Chapter 6

In the previous chapter, the effect of different current intensities on CSE enhancement is investigated. Electrode size is another important factor contributing to the final output of a-tDCS on CSE changes. Electrode size determines spatial focality of the applied current. In Chapter 6, the effects of active electrode size on the size of induced CSE change are examined.

Chapter 6: a-tDCS differential modulation of corticospinal excitability: the effects of electrode size

The format of this chapter is consistent with the Journal of *Brain Stimulation*.

The setup system used in this study, Ethics approval, TMS safety and Edinburg handedness questionnaires and consent form are provided in Appendices 8, 10 and 12-15.

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Original Articles

a-tDCS Differential Modulation of Corticospinal Excitability: The Effects of Electrode Size

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ABSTRACT

Background: Novel noninvasive brain stimulation techniques such as transcranial direct current stimulation (tDCS) have been developed in recent years. tDCS-induced corticospinal excitability changes depend on two important factors: current density and electrodes size. Despite clinical success with existing tDCS parameters; optimal protocols are still not entirely set.
Objective: The current study aimed to investigate the effects of anodal tDCS (a-tDCS) with three electrode sizes on corticospinal excitability.
Methods: a-tDCS was applied with three active electrode sizes of 12, 24 and 35 cm^2 with a constant current density of 0.029 mA/cm^2 on twelve right handed healthy individuals (mean age: 34.5 ± 10.32 years) in different sessions at least 48 h apart. a-tDCS was applied continuously for 10 min, with a constant reference electrode size of 35 cm^2. The corticospinal excitability of extensor carpi radialis muscle (ECR) was measured before and immediately after the intervention and at 10, 20 and 30 min thereafter.
Results: We found that smaller electrode may produce more focal current density and could lead to more effective and localized neural modulation than the larger ones. Post hoc comparisons showed that active electrode of 12 cm^2 size induces the biggest increase in the corticospinal excitability compared to bigger electrode sizes, 24 cm^2 ($P = 0.002$) and 35 cm^2 ($P = 0.000$). There was no significant difference between two larger electrode sizes (24 cm^2 and 35 cm^2) ($P = 0.177$). a-tDCS resulted in significant excitability enhancement lasting for 30 min after the end of stimulation in the 12 and 24 cm^2 electrode size conditions ($P < 0.005$). However, in 35 cm^2 electrode size condition, the MEP amplitudes of the ECR did not differ significantly from baseline value in 20 and 30 min post stimulation ($P > 0.005$).
Conclusion: Reducing stimulation electrode size to one third of the conventional one results in spatially more focused stimulation and increases the efficacy of a-tDCS for induction of larger corticospinal excitability. This may be due to the fact that larger electrodes stimulate nearby cortical functional areas which can have inhibitory effects on primary motor cortex.

Introduction

Transcranial direct current stimulation (tDCS) is a noninvasive brain modulation technique intensively used as therapeutic technique in treatment of various neurological and psychiatric disorders [1–4]. It is also extensively used as a method in neuroscience research [2,5–13]. tDCS utilizes low amplitude direct currents applied via scalp electrodes to modulate the level of the corticospinal excitability [14]. The direction of changes depends on the polarity of the active electrode. Application of anode over the target brain area is called anodal tDCS (a-tDCS), depolarizes the resting membrane potential and causes increased excitability. On the other hand, application of cathode over the brain target area is termed cathodal tDCS (c-tDCS), hyperpolarize the resting membrane potential and causes decreased excitability [15]. In the most commonly used configuration for a-tDCS, anode is placed over the region of interest, e.g., the motor cortex, and the other is placed over an indifferent area, e.g., above the contralateral supraorbital ridge.

Although tDCS is a promising tool for brain modulation, there are still many factors which should be addressed before its extensive use as a therapeutic technique. These factors are current density (the electric current per electrode surface area) [14], duration of application [12,14,16,17], number of treatments, length of interval between treatments and the electrodes surface area [11]. Electrode size is one of the important factor contributing to the final output of stimulation. Electrode size determines spatial focality of the applied current. tDCS is considered to be poorly focused using large rectangular-pad electrode configurations [18]. The spatial focality of induced cortical electric field increases by reducing electrode sizes [18]. Compared to smaller electrodes, the larger ones

Conflict of interest: The authors declare that they have no conflict of interest.

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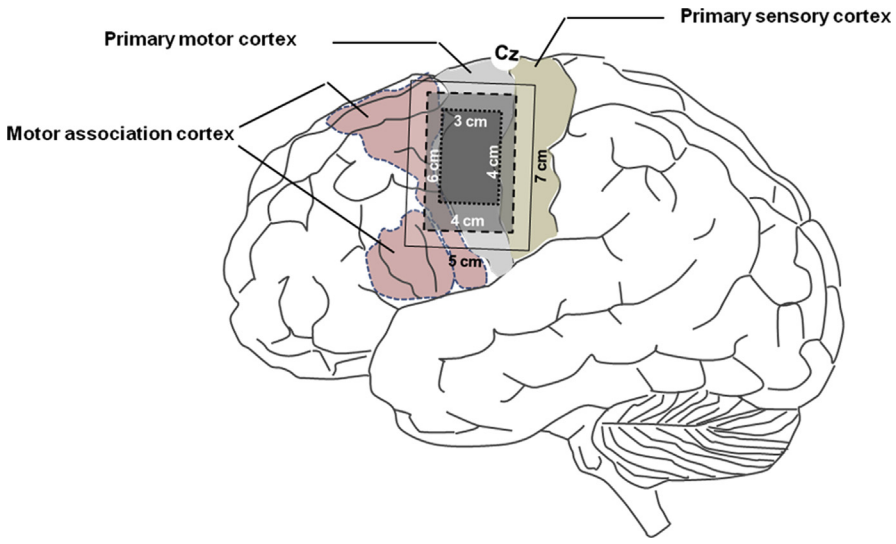

Figure 1. Schematic illustration of the area covered under the electrodes (7 × 5 cm = 35 cm^2, 6 × 4 cm = 24 cm^2 and 4 × 3 cm = 12 cm^2) over M1 of the ECR muscle. The 24 and 35 cm^2 electrodes are likely to cover the nearby functional areas.

can affect a larger cortical area which in turn may activate adjacent functional neuronal areas [18]. To the best of our knowledge only one study has formally examined the focality of a-tDCS [18]. In this study, the effects of a-tDCS were assessed by manipulating the size of conventional pad electrodes. They found that a-tDCS, with a 3.5 cm^2 anode placed over the abductor digiti minimi representation over M1, did not modulate the excitability of the neighboring representation of the first dorsal interosseus (FDI) muscle, which lay just outside of the physical boundary of the anode. In addition, based on computer modeling, tDCS delivered with relatively large electrodes resulted in diffuse electrical activation in regions under and between electrodes [15,19].

Recently, in order to increase efficiency and focality of tDCS, various types of electrodes, such as ring and concentric electrodes or montages employing one or more small electrodes, have been modeled [20–22] and tested against the conventional pad-type electrodes [23]. For example, tDCS was applied with several anodes and one cathode ring electrode placed according to the positions of EEG electrodes [23] or using one anode and several cathode ring electrodes [19,21,24] established some improved focality in a target region. This comes close to achieving unifocal stimulation, but using one electrode over the target region and several connected electrodes of the same size distributed around the perimeter of the head [25] could have some adverse effects on non-target areas [23].

Amount of the injected current shunted through the scalp, during tDCS, is dependent on the electrode dimensions, position and the proximity of the anode and the cathode. Increasing the distance between the electrodes over the scalp, increases the relative amount of current entering the brain than "shunted" across the scalp [26]. Using smaller electrodes could consequently increase the distance between the electrodes [27].

There is no attempt to systematically evaluate the effects of a-tDCS with different electrode sizes. Only one study [18] looked at the effects of electrode sizes on the corticospinal excitability. The present study was designed to assess the effects of different rectangular electrode sizes during application of a-tDCS on the corticospinal excitability of M1. We hypothesized that smaller active electrodes induces larger corticospinal changes. The basis for this hypothesis is the focality of active electrodes which increases by using smaller electrodes and avoids stimulation of nearby functional cortical areas with inhibitory effects on corticospinal excitability.

Materials and methods

Subjects

Twelve healthy volunteers (seven women, five men) recruited from Monash University students/staff with a mean age of 34.5 ± 10.3 years (age range 20–51 years), a mean weight of 68.6 ± 1 kg and a mean height of 168.9 ± 15.5 cm took part in 3 stimulation sessions. All were right-handers determined by the Edinburgh Handedness Inventory (10 item version, mean laterality quotient = 87.9 ± 19.5) [28]. All participants completed the Adult Safety Screening Questionnaire to determine suitability for TMS [29]. Participants were informed about the experimental procedures and gave their written informed consent according to the declaration of Helsinki. All experimental procedures were approved by the Monash University Human Research Ethics Committee.

a-tDCS of the motor cortex

a-tDCS was delivered by an Intelect® Advanced Therapy System (Chattanooga, USA) through a pair of saline-soaked surface sponge electrodes. The anode was placed over the left M1 for the right extensor carpi radialis muscle (ECR) as identified by TMS. The cathode was placed over the right contralateral supraorbital area [14]. The electrodes were fixed with two straps. Each subject was tested at the same time of the day to avoid any diurnal variations. a-tDCS was applied continuously for 10 min for all stimulation protocols using a constant reference electrode (35 cm^2) and three active electrode sizes (12, 24 and 35 cm^2) (Fig. 1). The current density under the anode was kept constant at 0.029 mA/cm^2 for all three conditions because this parameter determines the efficacy of a-tDCS [14]. In doing so, however, current intensities of 0.3, 0.7 and 1 mA were selected according to three electrode sizes of 12, 24 and 35 cm^2, respectively.

Assessment of corticospinal excitability

Participants were seated upright in an adjustable podiatry chair, with the forearm pronated and the wrist joint in neutral position resting on the armrest. Single pulse magnetic stimuli were delivered using a Magstim 200^2 (Magstim Company Limited, Whiteland, Wales, UK) stimulator with a flat 70 mm

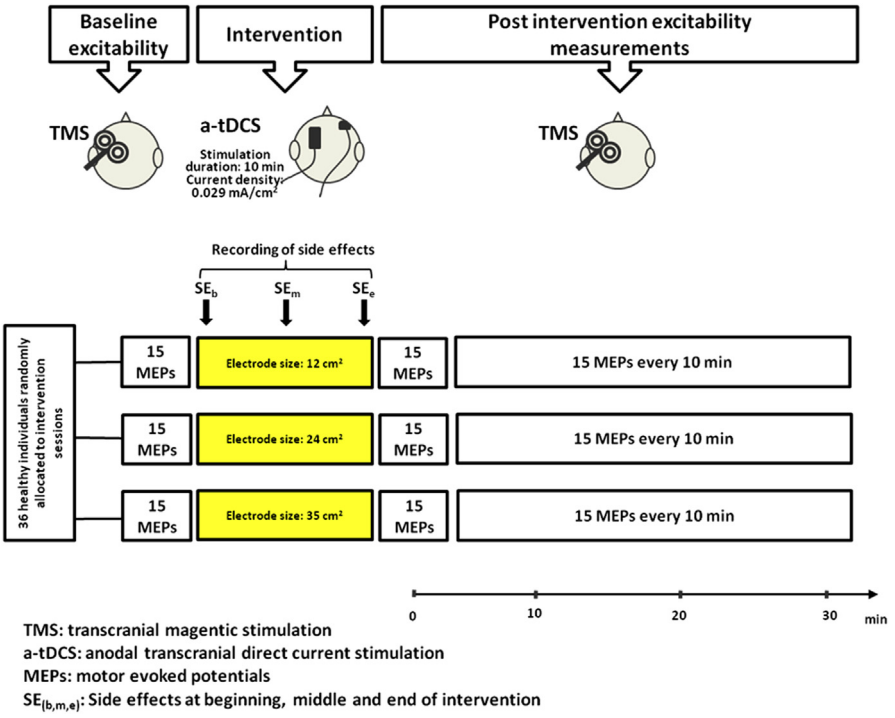

Figure 2. Experimental design for our comparison of the effects of different electrode sizes on corticospinal excitability.

figure-of-eight standard magnetic coil (peak magnitude field, 2.2 T). The vertex (C_z) point was measured and marked to be used as a reference [30]. The magnetic coil was placed over the left hemisphere (cortex), contralateral to the target muscle. The orientation of the coil was set at an angle 45° to the midline and tangential to the scalp, such that the induced current flowed in a posterior–anterior direction in the brain. The area of stimulation (hotspot) was determined through the measurement of the scalp using the convention of the EEG 10/20 system to find a spot over the ECR muscle M1 that would allow measurement of the largest MEP responses.

After localizing the hot spot, the coil's position was marked with a permanent marker on the scalp to be used for remainder of the testing for the target muscle to ensure consistency in the placement of the coil. Resting motor threshold (RMT) was defined as the minimal stimulus intensity that evoked five MEPs in a series of 10 with an amplitude of at least 50 μV [14,31–33].

The resting thresholds for the ECR muscle were determined by incrementing and decrementing stimulus intensity in 1–2% intervals until MEPs of at least 50 μV were elicited [14]. For all further MEP measurements, the TMS intensity was set at 120% of each individual's RMT. Fifteen stimuli were elicited to assess corticospinal excitability at each time point with a frequency of 0.2 Hz (one TMS stimulus every 5 s). The stimulus intensity remained constant throughout the study session for each subject.

Surface EMG was recorded from the right ECR muscle using bipolar Ag/AgCl disposable surface electrodes with an inter-electrode distance of 3 cm (measured from the center of the electrodes). To ensure good surface contact and reduce skin resistance, a standard skin preparation procedure of cleaning and abrading was performed for each electrode site [30,34,35]. The location of the ECR was determined based on anatomical landmarks [36] and also observation of muscle contraction in the testing position (wrist extension and radial deviation) [37]. The accuracy of EMG electrode placement was verified by asking the subject to maximally contract the muscles of interest while the investigator monitored online EMG activity. A ground electrode was placed ipsilaterally on the styloid process of ulnar bone [38,39]. The electrodes were secured by hypoallergenic tape (Micropore, USA). All raw EMG signals were band pass filtered (10–1000 Hz), amplified (×1000) and sampled at 2000 Hz and collected on a PC running commercially available software (Chart™ software, ADinstrument, Australia) via a laboratory analogue-digital interface (The PowerLab 8/30, ADinstrument, Australia). Peak–peak MEP amplitude was detected and measured automatically using a custom designed macro in Powerlab 8/30 software after each magnetic stimulus.

The intra and inter sessions reliability of the technique for assessment of corticospinal excitability is already established in a recent study on 12 healthy individuals [40].

Assessment of a-tDCS tolerability

a-tDCS side effects were assessed by monitoring the presence of itching, tingling, burning sensation and any other discomfort including headache; these are the sensory complaints most commonly reported during application of tDCS [16,41]. Tolerability and sensory changes were monitored during the first, the middle and the last 2 min of application based on participants' reports under the active and/or reference electrodes.

Experimental procedures

The study was conducted in a within-subject, randomized, counter-balanced design (Fig. 2). All recruited individuals, participated in three experimental sessions at least 72 h apart to avoid interference or carry-over effects of a-tDCS. Subjects were blinded to a-tDCS conditions. The order in which the experimental sessions were conducted was randomized between participants. The corticospinal excitability was measured before, immediately after (T0) and three more times at 10 min intervals (T10, T20 and T30) after the cessation of a-tDCS.

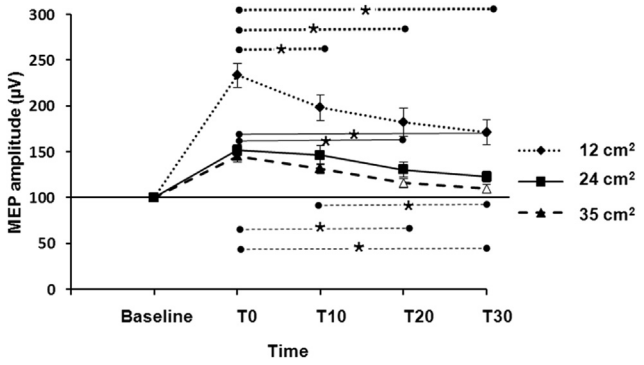

Figure 3. The effects of different electrode sizes on the lasting effects of a-tDCS and slope of decrease for MEPs amplitude over time. Filled symbols indicate significant deviation of the post-a-tDCS MEP amplitudes relative to baseline; the asterisks mark significant differences between MEP amplitudes for the ECR muscle during the 30 min after cessation of a-tDCS. Error bars represent SEM.

Data management and statistical analysis

Peak–peak amplitudes of 15 MEPs were calculated and averaged automatically for each time point before and after interventions. Post-intervention values were then normalized to the baseline value [42].

Differences in MEP amplitudes in the ECR muscle for three different a-tDCS electrode sizes and at each of five time points were analyzed with a two-way repeated measure analysis of variance (ANOVA). The first within-subject independent factor was electrode size (three levels). The second independent factor was time points (five levels). Mauchly's test was used to assess the validity of the sphericity assumption for repeated measures ANOVA; it requires that the variances for each set of difference scores be equal. Greenhouse–Geisser corrected significance values were used when sphericity was lacking [43]. In case of significant main effects, post hoc comparisons were performed using the Least Significant Difference adjustment for multiple comparisons. In order to rule out carry-over effects, baseline MEP amplitudes of the respective a-tDCS conditions were tested using one way ANOVA to see whether they were identical in all conditions. Furthermore, we examined whether our results were associated with an order effect. In addition, a homogeneity test was performed to compare the slopes of decrease in MEP amplitudes up to 30 min after the end of stimulation. We considered the results of all statistical analyses at $P < 0.05$. All results are expressed as the mean ± standard error of measurement (SEM). Statistical analyses were performed using SPSS software version 20.

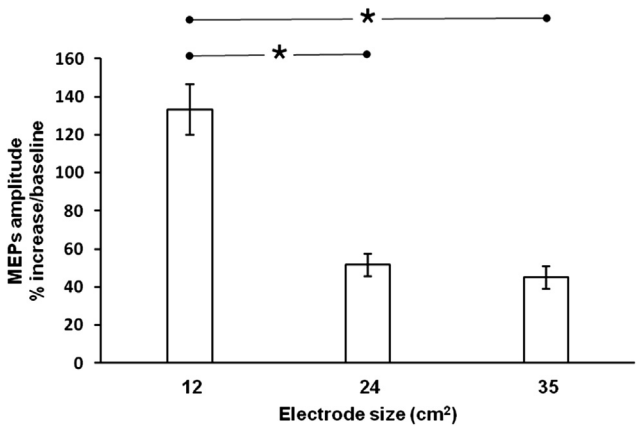

Figure 4. Percentage increase in the corticospinal excitability immediately after the intervention. The asterisks mark significant differences between ECR muscle MEP amplitudes after the end of a-tDCS. Error bars represent SEM.

Results

Effects of different electrode sizes on corticospinal excitability

Mauchly's test of sphericity indicated that this assumption was met for electrode size ($W = 0.700$, df = 2, $P = 0.168$), so no corrections were applied to the *F*-ratio computations.

The assumption of sphericity was violated for time ($W = 0.064$, df = 9, $P = 0.002$), so Greenhouse–Geisser correction was employed for the *F*-ratio computations. The results of the repeated measures ANOVA showed significant main effects of time (F (2.38, 26.17) = 72.61; $P < 0.001$, $\eta_p^2 = 0.87$). There were significant changes between different time points of a-tDCS in all three different conditions of electrode sizes ($P < 0.05$) (Fig. 3). Baseline a-tDCS MEPs amplitude was identical for all electrode sizes ($P > 0.05$) and there was no significant order effect ($F(2, 22) = 3.11$, $P = 0.65$). Two-way repeated measures ANOVA showed significant main effects of a-tDCS different electrode sizes (F (2, 22) = 19.46; $P < 0.001$, $\eta_p^2 = 0.64$). Post hoc comparisons indicated that there was significant difference in the scores of 12 cm^2 and 24 cm^2 electrode sizes (Mean = 46.69, SE = 11.62) ($P = 0.002$) (Fig. 4). A paired sample *t*-tests indicated that there was a significant difference in the scores of 12 cm^2 and 35 cm^2 electrode sizes (Mean = 56.55, SE = 9.96) ($P < 0.001$) (Fig. 4). Post hoc comparisons also showed that there was no significant difference between 24 cm^2 and 35 cm^2 electrode sizes (Mean = 9.86, SE = 6.83) ($P = 0.177$) (Fig. 4).

Mauchly's test of sphericity was not met for the slope of changes ($W = 0.497$, df = 2, $P = 0.03$), so Greenhouse–Geisser correction was considered for the *F*-ratio computations. We observed no significant differences ($F(1.33, 14.63) = 3.57$; $P = 0.069$) in the slope of decrease for MEP amplitudes for different electrode sizes over follow-up time (Fig. 3).

As displayed in Fig. 3, a-tDCS resulted in significant excitability enhancement lasting for 30 min after the end of stimulation in the 12 and 24 cm^2 electrode size conditions ($P < 0.005$). However, in 35 cm^2 electrode size condition, the MEP amplitudes of the ECR did not differ significantly in 20 and 30 min time points from baseline value after the end of stimulation ($P > 0.005$). Also, the assumption of sphericity was violated for the interaction of time and electrode size ($W = 0.000$, df = 35, $P = 0.001$), so Greenhouse–Geisser correction was employed for the *F*-ratio computations. ANOVA showed a significant interaction between time course × a-tDCS active electrode sizes (F (3.56, 39.20) = 9.576; $P < 0.001$, $\eta_p^2 = 0.47$). A significant interaction of the time by electrode size means that the pattern between time points in each stimulation protocols (different electrode sizes) is different.

a-tDCS side effects

Participants described their experiences under the electrodes at the beginning, in the middle and at the end of the interventions. The only sensations related to the cathode were a mild redness (reported by two participants). In contrast, most participants reported tingling, itching and/or burning under the anode (Table 1). Overall, the findings support the tolerability of direct current stimulation using a current density of 0.029 mA/cm^2 and all participants reported similar sensations under anode. There were no adverse effects related to application of a-tDCS during the follow-up period.

Table 1
Sensation under the anode reported by participants.

Electrode size	No sensation	Tingling sensation			Itching sensation			Burning sensation			Not tolerated
		Beginning	Middle	End	Beginning	Middle	End	Beginning	Middle	End	
12 cm^2	33.3% 3 (4)	25% (3)	25% (3)	–	–	25% (3)	46.66% (5)	–	–	–	–
24 cm^2	50% (6)	50% (6)	8.3% (1)	–	–	25% (3)	50% (6)	–	–	–	–
35 cm^2	50% (6)	25% (3)	25% (3)	–	–	33.3% (4)	50% (6)	–	–	–	–

The values are presented as percentage followed by number of subjects in parentheses.

Discussion

Effects of different electrode sizes on corticospinal excitability

The results in current study indicate electrode size dependency of excitability changes following a-tDCS. This finding supports the experimental hypothesis. Smaller active electrodes induce larger corticospinal changes. Reduction in electrode size while keeping current density constant, increases the spatial focality of stimulated area [18]. This highlights the efficiency of a-tDCS in increasing excitability by focusing the direct current for the muscle representation under the active electrode. a-tDCS resulted in largest excitability changes in the 12 cm^2 electrode size condition. As revealed by a mapping study on the ECR muscle [44], we assume that 12 cm^2 electrode size, only covers the M1 representational area of the ECR muscle; however, in 24 cm^2 and 35 cm^2 a-tDCS electrode size conditions, both M1 and adjacent functional areas are covered by the electrode surfaces.

The results of this study are in contrast to earlier findings of Nitsche et al [18]. The smallest electrode (12 cm^2) used in the present study was about 3.5 times larger than their electrode size of 3.5 cm^2 and the representation area of the ECR muscle was almost covered by the electrode. However, in Nitsche et al study the targeted muscle (FDI) representation field was outside the area covered by the electrode [18].

Our findings showed that there was no significant difference between the size of increase in corticospinal excitability in 24 cm^2 and 35 cm^2 electrode size conditions. One reason could be that the relatively large stimulation electrodes covers not only the area of interest, but also adjacent cortical functional areas, and this does not allow a selective stimulation of targeted cortical area. On the other hand, based on the modeling and imaging studies the current concentrates at the edge of the electrodes [45]; therefore, using smaller electrodes will maintain the edge of the electrode closer to the target area of stimulation. Indeed, by using smaller electrodes we can avoid undesired inhibitory effects from nearby cortical areas connected functionally to M1. Intra-hemispheric cortico-cortical connections (functional connectivity model) provide a number of tDCS strategies which could be used to promote M1 excitability [46,47]. The concept of functional connectivity is viewed as central for understanding the organized behavior of anatomic regions in the brain during their activity. This organization is thought to be based on the interaction between different and differently specialized cortical sites. For example, motor association cortex has inhibitory effects on M1 [48] while premotor cortex facilitates M1 by reducing short-interval intracortical inhibition [49].

To overcome these limitations, it would be desirable to precisely control stimulation area to reduce unnecessary exposure of other cortical areas by increasing the focality of DC stimulation with smaller electrode sizes (Fig. 1).

Another relevant aspect is the inter-electrode distance as it has a significant effect on the current distribution. Closely spaced electrodes produce more superficial stimulation [50]. It also affects the fraction of the injected current that reaches the brain or shunted through the scalp [21]. However, in this study the electrode montage and position were kept constant, but using smaller electrode sizes might reduce the percentage of shunted current due to the further proximity of the edges of the anode and the cathode and increase the amount of injected current passed through the scalp [26].

With all electrode sizes, a-tDCS resulted in significant excitability increase lasting up to 30 min.

Limitations

The present findings must be interpreted in the context of a number of potential limitations. One limitation of the study was the small sample size that limits generalizability of the results. The data were obtained from a healthy population with no neurological background; therefore the results might not be extrapolated to subjects with stroke or other neurological conditions. Another limitation of this study was that only a young group of participants were examined. So the data could not be generalized to elder age groups. Another limitation of this study was that we did not explored gender differences.

Suggestions for future studies

However, these results were encouraging in increasing the corticospinal excitability lasting for half an hour, but longer follow-ups are needed for monitoring the differences in lasting effects. Also, future studies should be focused on motor performance assessments in both healthy and neurological patients. A future study could assess age and gender effects.

Conclusion

Our findings might help to develop a-tDCS electrode sizes optimized for clinical application. The smallest electrode used in this study could be a promising size to modulate the corticospinal excitability. Reducing stimulation electrode size to one third of the standard one resulted in spatially more focused stimulation and increased the efficacy of a-tDCS. The results indicate that focality at the target area can be drastically improved over the conventional approach of using large electrodes.

We believe that our methodology and the presented results should help to elucidate the induced stimulation effect on the brain via smaller tDCS electrode sizes and should be useful for further investigation of more effective tDCS. Our results suggest that a deeper knowledge of the mechanisms underlying a-tDCS-induced excitability is required.

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Declaration for Chapter 7

In the case of Chapter 7, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution (%)**
Review of literature, Project design, ethics application and approval, participant recruitment, data collection, data analysis, interpretation of the results and writing of the manuscript.	80 %

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Shapour Jaberzadeh	Supervisory input on study design, Guidance in the framing of the manuscript, discussion of findings, review and provision of feedback on manuscript drafts	20 %

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	**Date** 30.09.2013
Signature	**Shapour Jaberzadeh**[redacted]	**Date** 24.09.2013

Preamble to Chapter 7

a-tDCS have relatively short lasting effects compared to the duration needed for any clinically relevant functional improvement. As indicated in Chapter 2 and 4, the longer applications of a-tDCS induce larger increases in CSE. However, recent literature indicates that due to homeostatic effects, longer applications are not the key for prolongation of the lasting effects. Therefore, instead of longer applications we introduced a within session repeated applications of a-tDCS for this purpose. However, the question remains open regarding the optimal within-session repeated rate and interval for application of these a-tDCS repetitions. This question is addressed in detail in Chapter 7.

Chapter 7: Within-session repeated a-tDCS: the effects of repetition rate and inter-stimulus intervals on corticospinal excitability and motor performance

The format of this chapter is consistent with the Journal of *Clinical Neurophysiology*.

The setup system used in this study, Ethics approval, TMS safety and Edinburg handedness questionnaires, consent form and Purdue pegboard test instruction are provided in Appendices 8 and 10-16.

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Within-session repeated a-tDCS: The effects of repetition rate and inter-stimulus interval on corticospinal excitability and motor performance

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HIGHLIGHTS

- Longer anodal transcranial direct current stimulation (a-tDCS) applications are not the key for prolongation of the effects.
- a-tDCS lasting effects might be prolonged by repetition of shorter applications.
- Repeated a-tDCS with longer intervals increase the lasting effect of a-tDCS.

ABSTRACT

Objective: This study investigated the effect of rate and stimulation interval of anodal transcranial direct current stimulation (a-tDCS) on CSE and motor performance.
Methods: Twelve healthy individuals participated in this study. CSE was assessed before and after five experimental conditions of one, two or three applications of 10 min of a-tDCS with an interval of 5 or 25 min. a-tDCS was applied with a constant current density of 0.016 mA/cm^2. Purdue pegboard-test was selected for motor performance assessment.
Results: Compared to single 10 min stimulation, the magnitude of the within-session repeated a-tDCS induced excitability was enhanced significantly after the second stimulation was performed with an interval of 25 min, but not 5 min. However, by increasing the number of a-tDCS to three repetitions the CSE was significantly increased and lasted for 2 h with both 5 and 25 min intervals. Furthermore, CSE enhancement remained significant for up to 24 h for within session a-tDCS repetitions with 25 min intervals. Likewise, significant improvement was seen in motor performance following three times repetition with 25 min inter-stimulus intervals.
Conclusions: The results suggest that within session repeated a-tDCS with longer intervals within the lasting effects of the previous stimulations are preferable for increasing induced excitability changes with longer lasting effects. Significance: It is of particular importance to increase the a-tDCS lasting effects to consolidate the neuroplastic CSE changes.

1. Introduction

Non-invasive induction of neuroplastic changes by transcranial direct current stimulation have been used increasingly in recent years (Nitsche and Paulus, 2000, 2001). It is of particular importance for inducing corticospinal excitability (CSE) effects which continue after stimulation. Anodal transcranial direct current stimulation (a-tDCS) increases CSE by depolarization of cortical neurons. Nitsche and Paulus (2000, 2001) reported a direct relationship between duration of a-tDCS application and duration of its after effects. Based on the current literature, it is hypothesized that longer applications of a-tDCS are associated with longer lasting effects (Bastani and Jaberzadeh, 2012a; Nitsche and Paulus, 2000, 2001; Ohn et al., 2008).

However, this hypothesis was challenged by Monte-Silva et al. (2013). They concluded that the observed direct relationship between the duration of a-tDCS application and the extent of

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lasting effects does not exist in longer applications of a-tDCS. One likely mechanism for explaining this observation is neuronal counter-regulation, which prevents over-excitation of the involved neurons (Monte-Silva et al., 2013). This finding highlights the challenge by Monte-Silva et al. (2013) that increasing the length of a-tDCS application is not the best strategy to increase the length of its lasting effect.

An alternative approach for prolongation of the lasting effects of a-tDCS might be the repetition of shorter a-tDCS applications, compared to a single long a-tDCS application (Fricke et al., 2010; Monte-Silva et al., 2013). In a recent study Monte-Silva et al. (2013) showed that repeated 13 min of a-tDCS application induces day-long excitability enhancements of the primary motor cortex (M1). Their observed extension of the lasting effects was dependent on the duration of the interval between a-tDCS applications. They repeated a-tDCS applications twice and found that the CSE would enhance if the second stimulation was applied during the lasting effects of the first one with an interval of 3 or 20 min. The results of the Monte-Silva et al. (2013) study failed to show significant changes during the first 2 h post-intervention, though they showed significant changes in M1 excitability later that day (same evening) and next morning. A confounding variable which was not controlled by the authors of this study was the number of applied TMS pulses over post stimulation period. The effects of within-session repeated cathodal transcranial direct current stimulation (c-tDCS) were also explored (Monte-Silva et al., 2010). Similarly, in that study conduction of the second stimulation protocol during the lasting effects of the first one enhanced efficacy of cathodal stimulation for 1 h, whereas a break duration of hours diminished the effects of c-tDCS. However, doubling the stimulation duration of c-tDCS without a break did not convert the lasting effects, but resulted in a prolongation of the effects (Monte-Silva et al., 2010).

An understanding of the interaction between CSE modulations and motor performance is critical for clinical approaches (Hummel et al., 2010; Nitsche et al., 2003). Research with both animals and humans has shown that modulation in the M1 neural representation area can be induced not only in response to motor training (Karni et al., 1998; Kolb and Whishaw, 1998), but also with the application of a-tDCS. On the other hand, enhancement of CSE can be reflected in increased performance in both healthy individuals and patients with neurological problems, as a result of modulation and reorganization of the M1 (Poldrack, 2000). The relationship between any increases in CSE induced by repeated application of a-tDCS and possible improvement in motor performance has not been investigated in prior researches. A direct relationship seems to be likely since increased CSE may facilitate motor performance improvements.

The current study aims to investigate how the number of a-tDCS repetitions (1 to 3) and the intervals between the stimulations (5 and 25 min) affect the size and extent of CSE and motor performance changes.

First, we hypothesized that within-session repeated a-tDCS induces larger CSE changes compared to a single application of a-tDCS. Second, we hypothesized that within-session repeated a-tDCS with longer, compared to shorter intervals, increase the duration of effects of a-tDCS on motor evoked potentials (MEPs) amplitude. It was also hypothesized that within-session repeated a-tDCS with longer intervals induces a greater increase in motor performance, and that this increase endures longer.

2. Materials and methods

2.1. Subjects

study 1 comprised five experimental conditions. In study 1 the participants were twelve healthy volunteers (ten women, two men) with no neurological or psychiatric disorders, recruited from Monash University (students and staff) with a mean age of 21.8 ± 1.4 years (age range 18–33 years), a mean weight of 62.4 ± 3.1 kg, and a mean height of 168.6 ± 2.9 cm. They were right-handers as determined by the Edinburgh Handedness Inventory (10 item version, mean laterality quotient = 78.71 ± 7.05) (Oldfield, 1971).

In study 2, with four experimental conditions, the participants (all women) were six of the twelve subjects of study 1 which were randomly selected. They presented with a mean age of 23.16 ± 2.54 years (age range 19–33 years), a mean weight of 59.16 ± 3.61 kg and a mean height of 165.83 ± 2.63 cm.

All participants completed the Adult Safety Screening Questionnaire to determine suitability for transcranial magnetic stimulation (TMS) (Keel et al., 2001). Participants were informed about the experimental procedures and gave their written informed consent according to the declaration of Helsinki. All experimental procedures were approved by the Monash University Human Research Ethics Committee.

2.2. a-tDCS of the motor cortex

In both studies, a-tDCS was delivered by an Intelect® Advanced Therapy System (Chattanooga, USA) via a pair of saline-soaked surface sponge electrodes. The anode was placed over the left M1 for the right first dorsal interossei muscle (FDI) as identified by TMS. The cathode was located contralaterally over the right supraorbital area (Nitsche and Paulus, 2000). The electrodes were fixed with two horizontal and perpendicular straps. Each subject was tested at the same time of day to avoid diurnal variations. a-tDCS was applied using a constant current density of 0.016 mA/cm^2. In a recent study of the same group, these parameters of current intensity and electrode size had the largest efficacy on CSE enhancement (Bastani and Jaberzadeh, 2013a,b).

2.3. Assessment of CSE

Participants were seated upright in an adjustable podiatry chair, with the forearm pronated and the wrist joint in neutral position resting on the armrest. TMS-elicited MEPs were recorded to measure CSE changes of the motor cortex representation area of the right FDI.

Single-pulse magnetic stimuli were delivered using a Magstim 200^2 stimulator (Magstim Company Limited, Whiteland, Wales, UK) with a flat 70 mm figure-of-eight standard magnetic coil (peak magnitude field, 2.2 T). The vertex (C_z) point was measured and marked to be used as a reference (Schwartz, 2003). The magnetic coil was placed over the left hemisphere (cortex), contralateral to the target muscle. The orientation of the coil was set at an angle 45° to the midline and tangential to the scalp so that the induced current flowed in a posterior-anterior direction in the brain. The area of stimulation (hotspot) was applied over the M1 of FDI muscle that would allow measurement of the largest MEPs responses. This was targeted by guiding the coil to the position C3 according to the international EEG 10–20 system (Herwig et al., 2003).

After localizing the hot spot, the coil's position was marked on the scalp to be used throughout the remainder of the testing for the target muscle to ensure consistency in the placement of the coil. Resting motor threshold (RMT) was defined as the minimal stimulus intensity that evoked five MEPs in a series of 10 with an amplitude of at least 50 μV (Hallett, 1996; Nitsche and Paulus, 2000; Rossini et al., 1994; Wassermann et al., 2008). The resting thresholds for the FDI muscle were determined by incrementing and decrementing stimulus intensity in 1–2% intervals until MEPs of at least 50 μV were elicited (Nitsche and Paulus, 2000; Rossini et al., 1994). For all further MEP measurements, the test TMS

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intensity was set at 120% of each individual's RMT. Twelve stimuli were elicited to assess CSE at each time point (Bastani and Jaberzadeh, 2012b) with a frequency of 0.2 Hz (one TMS stimulus every 5 s). The stimulus intensity remained constant throughout the study session for each subject.

Surface EMG was recorded from the right FDI muscle using bipolar Ag/AgCl disposable surface electrodes with an inter-electrode distance of 2 cm (measured from the center of the electrodes). To ensure good surface contact and reduce skin resistance, a standard skin preparation procedure of cleaning and abrading was performed for each electrode site (Gilmore and Meyers, 1983; Robertson et al., 2006; Schwartz, 2003). The location of FDI muscle was determined based on anatomical landmarks (Perotto and Delagi, 2005) and also observation of muscle response in the testing position (index finger abduction) (Kendall et al., 2010). The accuracy of EMG electrode placement was verified by asking the subject to contract the muscle while the investigator monitored online EMG activity. A ground electrode was placed ipsilaterally on the styloid process of the ulnar bone (Basmajian and De Luca, 1985; Oh, 2003). The electrodes were secured by hypoallergenic tape (Micropore, USA). All raw EMG signals were band pass filtered (10–1000 Hz), amplified (×1000) and sampled at 2000 Hz and collected on PC compatible commercially-available software (Chart™ software, ADinstrument, Australia) via a laboratory analog–digital interface (The PowerLab 8/30, ADinstrument, Australia). Peak-to-peak MEPs amplitude was detected and measured automatically using custom-designed macro in PowerLab 8/30 software after each magnetic stimulus.

2.4. Measurement of motor performance

The Purdue Pegboard Test (PPT, Lafayette Instrument Company) was utilized as a reliable and valid instrument for assessment of manual dexterity and motor performance (Tiffin and Asher, 1948). The PPT consists of a wood console with a shallow cup, to contain the pegs on the top of the console, and 50 holes (two parallel columns of 25 holes). The participants were seated directly in front of the pegboard and instructed to place 25 pegs on the right hand side column in top-down order as fast as they could with their right hand. The time for completion of the task was considered as the outcome measure for evaluation of motor performance. Participants were allowed to practice during three trials in order to be familiarized and stabilize their motor performance before the test. After the familiarization time, the pre a-tDCS PPT performance time was recorded and considered as the baseline value. Post-intervention tests were performed immediately and at different time points up to two hours after the end of a-tDCS applications (Fig. 1). For each experimental session, participants performed the PPT task twice for baseline evaluation and post-intervention (Hollak et al., 2013).

2.5. Measurement of a-tDCS side effects

All participants completed a questionnaire at different time points during the stimulation sessions. Participants rated the unpleasantness of any scalp sensations under the active and/or indifferent electrodes at the beginning (first 2 min), in the middle (minutes 4–6) and at the end (last 2 min) of a-tDCS application, using numeric analog scales (NAS) (e.g., 0 = no tingling to 10 = worst tingling imaginable). The questionnaire contained rating scales for the presence and severity of side effects such as itching, tingling, a burning sensation under the electrodes (George and Aston-Jones, 2009; Nitsche et al., 2008) and all other discomforts, including headache and pain during and after a-tDCS applications.

3. Experimental procedures

3.1. Study 1

Study 1 (with five experimental conditions) was conducted using a within-subject, randomized design (Fig. 1). The experimental sessions were separated by at least 72 h to avoid interference or carry-over effects of a-tDCS. Subjects were blinded to the stimulation parameters and potential effects of a-tDCS; but could not be blinded to the rate and stimulus interval of experimental conditions. We applied consecutive a-tDCS with two time intervals of 5 min, when the lasting effect of earlier a-tDCS application is near its maximum value, and 25 min, when the CSE is still above the baseline value but closer to it (Nitsche and Paulus, 2001). Experimental conditions in this study were:

(a) Single 10 min of a-tDCS (10).
(b) Two 10 min of a-tDCS with an interval of 5 min (10-5-10).
(c) Two 10 min of a-tDCS with an interval of 25 min (10-5-10).
(d) Three 10 min of a-tDCS with an interval of 5 min (10-5-10-5-10).
(e) Three 10 min of a-tDCS with an interval of 25 min (10–25-10–25-10).

Immediately after the final a-tDCS application (T0), 12 MEPs and PPT performance times were recorded every 10 min for half an hour, then every 15 min up to 1 h, and afterward every 30 min up to 2 h after the cessation of a-tDCS in all experimental conditions.

MEPs were also recorded at one or two more additional time points for within session repeated conditions: after the first 10 min a-tDCS for 10-5-10 and 10-25-10 conditions and after the first and second 10 min a-tDCS for 10-5-10-5-10 and 10-25-10-25-10 conditions, to track the possible changes during the repetitions.

3.2. Study 2

This study explored longer lasting effects of within-session repeated a-tDCS experimental conditions. Since we did not expect longer lasting effects induced by the single 10 min of a-tDCS, here this condition was not included in this study. The procedures were identical to that of the first study, with the exception that CSE and motor performance were only evaluated before and at 6 and 24 h post-intervention (Fig. 1). Participants' were asked not to do the tasks involving fine motor movements between the measurements. Indeed, in this study we skipped the multiple measurements immediately after intervention and up to 2 h post-intervention to avoid the possibility of TMS induced excitability changes produced by mass TMS testing during this period.

4. Data management and statistical analysis

For both studies the normal distribution of data for MEPs amplitude and PPT performance were assessed using the Shapiro–Wilk test. These tests assumed normal distribution.

4.1. The effects of within-session repeated a-tDCS on CSE

4.1.1. Study 1

Peak-to-peak amplitude of 12 MEPs were calculated and averaged automatically for each time point covering baseline and post stimulation values. Post-intervention values were then normalized intra-individually to baseline value (Antal et al., 2008). Baseline MEPs amplitude and RMT of the respective experimental

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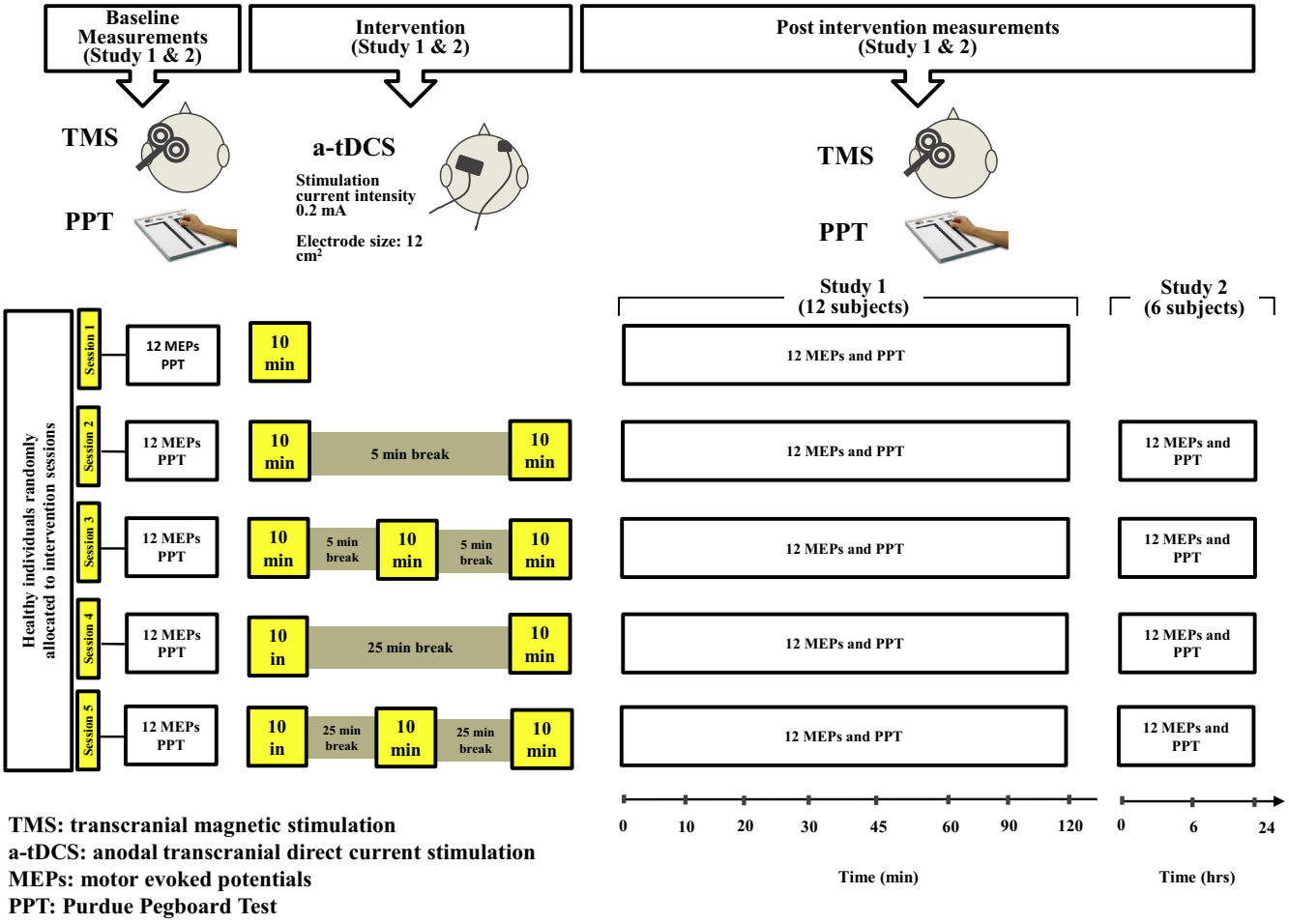

Fig. 1. Experimental set-up for study 1 and 2.

conditions were also tested using one-way repeated measure analysis of variance (ANOVA) to see whether the baseline MEPs and RMT were identical in all experimental conditions.

Differences in MEPs amplitude in the FDI muscle (as dependent factor) for the five different experimental conditions and for each time point (as within – subject independent factor) up to 2 h after the end of a-tDCS application were analyzed with a two-way repeated measure ANOVA. In addition, Mauchly's test was used to test the assumption of sphericity for repeated measures factor ANOVA. Greenhouse-Geisser corrected significance values were used when sphericity was lacking (Meyers et al., 2005). In case of significant main effects, post hoc comparisons were performed using the least significant difference (LSD) with adjustment for multiple comparisons. This tested whether the MEPs amplitude after a-tDCS differed significantly between the within-session repeated a-tDCS experimental conditions, whether the MEPs amplitude after within-session repeated a-tDCS differed significantly from the pre a-tDCS amplitudes, and whether the MEPs amplitude of the within-session repeated a-tDCS experimental conditions differed from the single 10 min a-tDCS experimental condition.

4.1.2. Study 2

Peak-to-peak amplitude of 12 MEPs were calculated and averaged automatically for three time points covering baseline, T6 and T24.

The analysis of differences in MEPs amplitude in the FDI muscle (as dependent factor) for four different within-session repeated a-tDCS experimental conditions and at two time points of T6 and T24 (as within – subject independent factors) after the end of a-tDCS application was conducted with a two-way repeated measure ANOVA. The remaining procedures were identical to that described for study 1.

4.2. The effects of within-session repeated a-tDCS on motor performance

4.2.1. Study 1

Baseline PPT performance time of the respective experimental conditions was tested using one-way repeated measure ANOVA to see whether the baseline values were identical in all conditions.

As with MEPs amplitude, differences in PPT performance time for five different experimental conditions and for each time point up to 2 h after the end of a-tDCS application were analyzed with a two-way repeated measure ANOVA. Mauchly's sphericity test was used to validate an assumption of repeated measures factor ANOVA. Greenhouse-Geisser corrected significance values were used when sphericity was lacking (Meyers et al., 2005). If the analysis was significant, post hoc testing was performed and corrected for multiple comparisons (LSD) to evaluate comparisons of post-intervention to baseline values for all experimental conditions. For the PPT performance time, we averaged the value of two runs performed at baseline and post-intervention at each time points.

4.2.2. Study 2

Differences in PPT performance time for four different experimental conditions and two time points of T6 and T24 after the

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end of a-tDCS application were analyzed with a two-way repeated measure ANOVA. The remaining procedures were identical to that described for study 1.

We considered the results of all statistical analyses significant at $P < 0.05$. All results are expressed as the mean ± standard error of mean (SE). Statistical analyses were performed using SPSS software version 20.

5. Results

5.1. Within-session repeated a-tDCS side effects

All participants tolerated the applied currents in different experimental conditions very well and there was no interruption of experimental procedures due to the side- or adverse-effects of the applied currents in both studies. The scalp sensations most commonly reported by participants were itchiness, tingling, and burning. Table 1 summarizes the numeric value means ± SE for reported side effects under the anode and cathode during within-session repeated a-tDCS applications in study 1. The mean values of tingling, itching and burning sensations are all low, indicating the mild nature of these side effects. There were no side effects reported by participants after the end of experimental sessions. Also, there were no reports of pain and headache during or after within-session repeated a-tDCS applications. Therefore these findings support the tolerability of a-tDCS using the presented parameters.

5.2. The effects of within-session repeated a-tDCS on CSE

5.2.1. Study 1

One-way repeated measure ANOVA showed that baseline MEPs amplitude ($F(2.61,28.80) = 0.97$, $P = 0.43$, partial $\eta^2 = 0.08$) and RMT ($F(4,44) = 1.48$, $P = 0.22$, partial $\eta^2 = 0.12$) were identical for all experimental conditions.

Mauchly's test of sphericity indicated that this assumption was not met for a-tDCS experimental conditions ($W = 0.161$, $df = 9$, $P = 0.049$), so Greenhouse-Geisser correction was employed for the F-ratio computations. Also, the assumption of sphericity was violated for time ($W = 0.000$, $df = 35$, $P < 0.001$), therefore Greenhouse-Geisser correction was employed for the F-ratio computations.

The results of the two-way repeated measures ANOVA showed significant main effects of experimental condition ($F(2.18,23.99) = 5.08$; $P = 0.013$, $\eta_p^2 = 0.793$) and time ($F(2.25,24.82) = 15.43$; $P < 0.001$, $\eta_p^2 = 0.58$). The results of the two-way repeated measures ANOVA showed non-significant main effects of time × experimental condition ($F(4.31,47.48) = 1.33$; $P = 0.271$, $\eta_p^2 = 0.10$).

As shown by the post hoc tests, the single session of a-tDCS for 10 min resulted in an average excitability enhancement of about 57% compared to baseline. Although 10-5-10 experimental condition induced an average increase of 83.75% in MEPs amplitude, post hoc tests showed no significant differences to that of single 10 min stimulation ($P = 0.42$) (Fig. 2A). However, the trend of

Table 1
Sensations reported by participants during the experimental conditions under the anode and cathode in study 1. Values are shown as averaged participants NAS score from 0 to 10 followed by % of participants reported the sensation. (–) Indicates that no sensation were reported.

Experimental condition	Time (min)	Sensations under anode NAS score (% participants)			Sensations under cathode NAS score (% participants)		
		Tingling	Itching	Burning	Tingling	Itching	Burning
10	1–2	1.6 (25)	1.7 (33.3)	2 (16.6)	1 (16.6)	2 (8.3)	2 (8.3)
	4–6	2.5 (16.6)	2.1 (50)	2.3 (25)	1.3 (33.3)	3 (8.3)	4 (8.3)
	8–10	1.5 (16.6)	2.8 (50)	2.6 (25)	1 (33.3)	2.5 (16.6)	2.5 (16.6)
10.5.10	1–2	1 (8.3)	1.25 (33.3)	3 (8.3)	1 (8.3)	–	–
	4–6	–	1.8 (41.6)	3 (8.3)	–	2 (8.3)	1 (8.3)
	8–10	2 (8.3)	2.6 (25)	3 (8.3)	–	2 (8.3)	1.5 (25)
	1–2	2 (16.6)	2 (50)	2 (16.6)	2 (25)	1.66 (25)	2 (16.6)
	4–6	1.5 (33.3)	2.28 (58.3)	2.5 (16.6)	1 (8.3)	3 (8.3)	1.5 (25)
	8–10	2 (16.6)	1.5 (58.3)	2 (16.6)	1 (8.3)	3 (8.3)	2 (16.6)
10.5.10.5.10	1–2	2 (8.3)	2.6 (41.6)	1 (16.6)	1 (8.3)	3 (8.3)	–
	4–6	1 (8.3)	1.85 (58.3)	1.5 (16.6)	1 (16.6)	2.5 (16.6)	–
	8–10	1 (16.6)	1.62 (66.6)	1 (16.6)	1 (16.6)	2 (16.6)	1 (8.3)
	1–2	1 (16.6)	2 (41.6)	1 (16.6)	–	1 (8.3)	1 (8.3)
	4–6	1.5 (16.6)	2.14 (58.3)	1 (16.6)	1 (16.6)	1.5 (16.6)	2 (8.3)
	8–10	1.5 (16.6)	2 (58.3)	1 (16.6)	2 (16.6)	2 (8.3)	2 (8.3)
	1–2	1 (8.3)	1.85 (58.3)	1.5 (16.6)	–	2 (8.3)	1 (16.6)
	4–6	1 (16.6)	1.62 (66.6)	1 (16.6)	–	2 (16.6)	1 (16.6)
	8–10	1 (16.6)	1.83 (50)	1 (8.3)	2 (16.6)	3 (8.3)	1 (8.3)
10.25.10	1–2	2 (16.6)	2 (25)	1 (16.6)	–	1.33 (25)	1 (8.3)
	4–6	1.6 (25)	2.14 (58.3)	2 (16.6)	1.33 (25)	2 (16.6)	1.5 (16.6)
	8–10	1.6 (25)	2.57 (58.3)	1.5 (16.6)	2 (16.6)	3.5 (16.6)	2 (16.6)
	1–2	1 (25)	2 (33.3)	1.5 (16.6)	1 (8.3)	3 (8.3)	1.5 (16.6)
	4–6	1 (25)	2 (33.3)	2 (16.6)	1 (8.3)	3.5 (16.6)	2 (16.6)
	8–10	1.3 (25)	2.16 (50)	2.5 (16.6)	1.3 (8.3)	3.5 (16.6)	1.5 (16.6)
10.25.10.25.10	1–2	2 (16.6)	2.2 (41.6)	2 (16.6)	2 (8.3)	–	1 (16.6)
	4–6	–	3.2 (41.6)	2.5 (16.6)	1.5 (16.6)	1.6 (25)	1 (8.3)
	8–10	–	3 (33.3)	1.5 (16.6)	–	3 (16.6)	1 (8.3)
	1–2	–	1.83 (50)	2 (25)	1 (16.6)	2 (16.6)	–
	4–6	–	2.2 (41.6)	1.5 (16.6)	1 (8.3)	1.5 (16.6)	–
	8–10	2 (8.3)	2 (41.6)	1.66 (25)	1 (8.3)	2 (16.6)	–
	1–2	1.5 (16.6)	1.8 (41.6)	1.66 (25)	1 (16.6)	1.6 (16.6)	1 (16.6)
	4–6	1 (8.3)	1.83 (50)	2.5 (16.6)	1 (8.3)	3 (16.6)	1 (16.6)
	8–10	1 (8.3)	1.83 (50)	2 (25)	1 (8.3)	3 (16.6)	1 (16.6)

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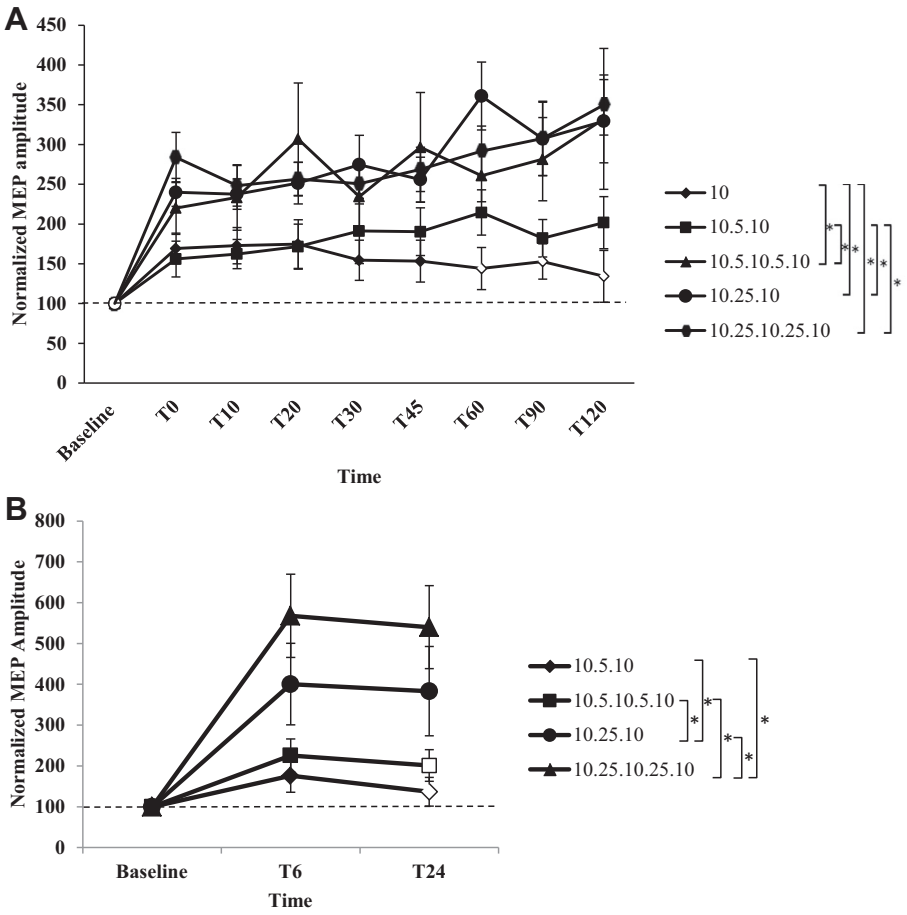

Fig. 2. (A) The effects of different a-tDCS experimental conditions on CSE over time. (B) The effects of different within-session repeated a-tDCS experimental conditions on CSE at two time points of 6 and 24 h after the end of the last stimulation. The mean values are normalized to baseline. Filled symbols indicate significant deviation of the post MEPs amplitude to the normalized value (horizontal dotted line). Error bars represent SE.

lasting excitatory effects in 10-25-10 experimental condition was similar to that of 10-5-10, but a significant difference was seen between these two experimental conditions ($P = 0.001$) in which 10-25-10 induced larger (182.12%) CSE (Fig. 2A).

Post-hoc comparison showed significant differences between 10 and 10-5-10-5-10 experimental conditions ($P = 0.042$). Nevertheless, there were significant differences between single 10 min a-tDCS application with both 10-25-10 and 10-25-10-25-10 experimental conditions ($P = 0.001$; $P = 0.001$, respectively). The MEPs amplitude was significantly enlarged with three times repetition (182.12%) and also with two repetitions (182.09%) with a 25 min interval (Fig. 2A).

Furthermore, post hoc comparisons showed no significant differences between 10-5-10-5-10 and both 10-25-10-25-10 ($P = 0.81$) and 10-25-10 ($P = 0.78$) experimental conditions, with an average increase of 170.72% in CSE. Pairwise comparisons also showed a significant difference between 10-5-10 and 10-25-10-25-10 experimental conditions ($P = 0.004$) (Fig. 2A).

Compared to the single 10 min a-tDCS with 45 min of lasting effects, all repeated a-tDCS experimental conditions with 5 or 25 min intervals prolonged the excitatory lasting effects at least to 2 h (Fig. 2A).

5.2.1.1. MEPs size after first 10 min of a-tDCS. Mauchly's test of sphericity indicated that this assumption was met ($W = 0.303$, $df = 9$, $P = 0.267$). The results of the one-way repeated measures ANOVA showed non-significant main effects of condition ($F(4,44) = 0.557$; $P = 0.70$, partial $\eta^2 = 0.048$). This indicates that 10 min a-tDCS induced identical size of increase in MEPs between all five conditions.

5.2.1.2. Tests of between condition MEPs size after the second 10 min of a-tDCS. Mauchly's test of sphericity indicated that this assumption was violated for time ($W = 0.000$, $df = 44$, $P < 0.001$), therefore Greenhouse-Geisser correction was employed for the *F*-ratio computations. The results of the one-way repeated measures ANOVA showed significant main effects of time ($F(2.62,28.85) = 4.63$; $P = 0.012$, partial $\eta^2 = 0.276$). Post- hoc tests using LSD correction revealed that MEPs size were significantly increased after second 10 min of stimulation in both 10–25-10 ($P < 0.001$) and 10-25-10-25-10 conditions ($P < 0.001$). There were no significant increases in MEPs amplitude after second a-tDCS repetitions in the 10-5-10 ($P = 0.94$) and 10-5-10-5-10 ($P = 0.74$) conditions.

Furthermore, post hoc comparisons showed significant differences between the second and third ($P = 0.043$) and first and third ($P = 0.001$) block of a-tDCS application in the 10-25-10-25-10 condition. However, there were no significant differences between the second and third ($P = 0.135$) and first and third ($P = 0.083$) block of a-tDCS application in the 10-5-10-5-10 condition.

Post-hoc comparison showed no significant differences between the second block of stimulation of 10-5-10 and 10-5-10-5-10 experimental conditions ($P = 0.597$). Nevertheless, there were no significant differences between the second block of stimulation of 10-25-10 and 10-25-10-25-10 experimental conditions ($P = 0.85$). However, post hoc comparisons showed significant differences between the second block of stimulation of both

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10-25-10 and 10-25-10-025-10 to that of 10-5-10 and/or 10-5-10-5-10 experimental conditions ($P > 0.05$).

5.2.2. Study 2

One-way repeated measure ANOVA showed that baseline MEPs amplitude ($F(3,15) = 0.66$, $P = 0.58$, partial $\eta^2 = 0.11$) and RMT ($F(3,15) = 0.48$, $P = 0.70$, partial $\eta^2 = 0.08$) were identical for all experimental conditions.

Mauchly's test of sphericity indicated that this assumption was met for within-session repeated a-tDCS experimental conditions ($W = 0.073$, $df = 5$, $P = 0.092$) and time ($W = 0.895$, $df = 2$, $P = 0.801$), therefore no corrections were applied to the F-ratio computations.

The results of the two-way repeated measures ANOVA showed significant main effects of experimental conditions ($F(3,15) = 39$; $P < 0.001$, $\eta_p^2 = 0.89$) and time ($F(2,10) = 113.34$; $P < 0.001$, $\eta_p^2 = 0.96$). The results of the two-way repeated measures ANOVA showed significant main effects of time × experimental condition ($F(6,30) = 34.27$; $P < 0.001$, $\eta_p^2 = 0.87$).

Post-hoc comparisons showed non-significant effects between 10-5-10 and 10-5-10-5-10 experimental conditions ($P = 0.078$). Results of the post hoc tests showed a significant difference between a-tDCS experimental conditions comparing 10-5-10 to 10-25-10 ($P = 0.005$), 10-5-10 to 10-25-10-25-10 ($P < 0.001$), 10-5-10-5-10 to 10-25-10 ($P = 0.004$) and 10-5-10-5-10 to 10-25-10-25-10 ($P = 0.001$). The results showed a large increase in the size of MEPs in both 10-25-10 and 10-25-10-25-10 experimental conditions; but post hoc comparisons showed a significant difference between 10-25-10 and 10-25-10-25-10 experimental conditions ($P = 0.011$) with an average increase of 291.78% and 454.04% in CSE at the two time points of T6 and T24 respectively (Fig. 2B).

5.3. The effects of within-session repeated a-tDCS on motor performance

5.3.1. Study 1

One-way repeated measure ANOVA showed that baseline PPT performance time ($F(1.94,21.43) = 0.15$, $P = 0.85$, partial $\eta^2 = 0.01$) was identical for all experimental conditions.

Mauchly's test of sphericity indicated that this assumption was met for a-tDCS experimental conditions ($W = 0.342$, $df = 9$, $P = 0.35$), therefore no corrections were applied to the F-ratio computations. The assumption of sphericity was violated for time ($W = 0.000$, $df = 35$, $P = 0.006$), so Greenhouse-Geisser correction was employed for the F-ratio computations.

The results of the two-way repeated measures ANOVA showed significant main effect of time ($F(3.62,39.87) = 14.78$; $P < 0.001$, $\eta_p^2 = 0.573$) and non-significant main effects of experimental condition ($F(4,44) = 0.164$; $P = 0.956$, $\eta_p^2 = 0.015$) and experimental condition × time interaction ($F(6.56,72.19) = 1$; $P = 0.471$, $\eta_p^2 = 0.083$).

Post-hoc comparisons showed non-significant effects in all five experimental conditions ($P > 0.05$). Pairwise comparisons showed significant effects between baseline and all time bins of T0 to T120; T0 and all time bins of T20 to T120; T10 and T30, T45, T60, T120; and T20 and T60 ($P < 0.05$) (Fig. 3A).

5.3.2. Study 2

One-way repeated measure ANOVA showed that baseline PPT performance time ($F(3,15) = 1.83$, $P = 0.18$, partial $\eta^2 = 0.26$) was identical for all experimental conditions.

Mauchly's test of sphericity indicated that this assumption was met for a-tDCS experimental conditions ($W = 0.393$, $df = 5$, $P = 0.637$) and time ($W = 0.343$, $df = 2$, $P = 0.118$) so no corrections were applied to the F-ratio computations.

The results of the two-way repeated measures ANOVA showed non-significant main effects of time ($F(2,10) = 2.27$; $P = 0.153$, $\eta_p^2 = 0.313$) and experimental condition ($F(3,15) = 0.737$; $P = 0.546$, $\eta_p^2 = 0.128$) and significant main effects of experimental condition × time ($F(6,30) = 4.65$; $P = 0.002$, $\eta_p^2 = 0.482$).

Post-hoc comparisons showed non-significant effects in four experimental conditions ($P > 0.05$) and in time pairwise comparisons ($P > 0.05$). Compared to baseline, pairwise comparisons showed non-significant changes in motor performance values at T6 and T24 for all three experimental conditions of 10-5-10, 10-5-10-5-10 and 10-25-10. However, the improvement in motor performance lasted significantly for a day-long period in 10-25-10-25-10 experimental condition (Fig. 3B). A Pearson product-moment correlation coefficient was computed to assess the relationship between increases in CSE induced by repeated application of a-tDCS in the 10-25-10-25-10 condition and motor performance at two time points of T6 and T24. There was a non-significant negative correlation between the size of changes in CSE and motor performance improvements at both T6 ($r = -0.236$, $n = 6$, $P = 0.653$) and T24 ($r = -0.623$, $n = 6$, $P = 0.187$), showing that improvement in motor performance was not associated with increase in CSE. However,

6. Discussion

The results of the present study indicate that within-session repeated a-tDCS induces larger CSE changes compared to single 10 min of a-tDCS. Also, the findings show that within session repeated a-tDCS with longer intervals increase the duration of a-tDCS effects on CSE, thereby supporting the first and second hypothesis. However, the motor performance improvement following within session repeated a-tDCS with longer intervals only support the third hypothesis in part.

6.1. Comparing single versus within-session repeated a-tDCS effects on CSE

The results of the current study indicate that all within-session repeated a-tDCS experimental conditions increased CSE. In study 1 we found consistent results with the previous studies, that the experimental condition with single 10 min a-tDCS induced 45 min lasting effects in CSE (9 and 11 min of a-tDCS induced 30 and 45 min facilitation, respectively) (Monte-Silva et al., 2013; Nitsche and Paulus, 2000). However, compared to single 10 min stimulation, the magnitude of the within-session repeated a-tDCS induced excitability was only enhanced significantly if the second stimulation was performed after an interval of 25 min, but not 5 min. However, by increasing the number of a-tDCS to three repetitions the CSE was significantly increased and lasted for 2 h with both 5 and 25 min intervals.

6.2. Comparing within-session repeated a-tDCS effects on CSE

In this present study, the second or third within-session repetitions of a-tDCS were applied while the effects of previous stimulations were still present. Our results confirm the finding of Monte-Silva et al. (2013) who suggested that subsequent stimulations of within-session repeated a-tDCS should be within the lasting effects of the previous stimulations. Other studies have also found that the time interval between stimulation sessions is a critical factor in determining the outcomes of the second period of stimulation. For example, Monte-Silva et al. (2010) found that for c-tDCS, lasting inhibitory effects (measured to 2 h after the second stimulation period) were prolonged if the second stimulation was performed with short inter-stimulus intervals of 3 and 20 min.

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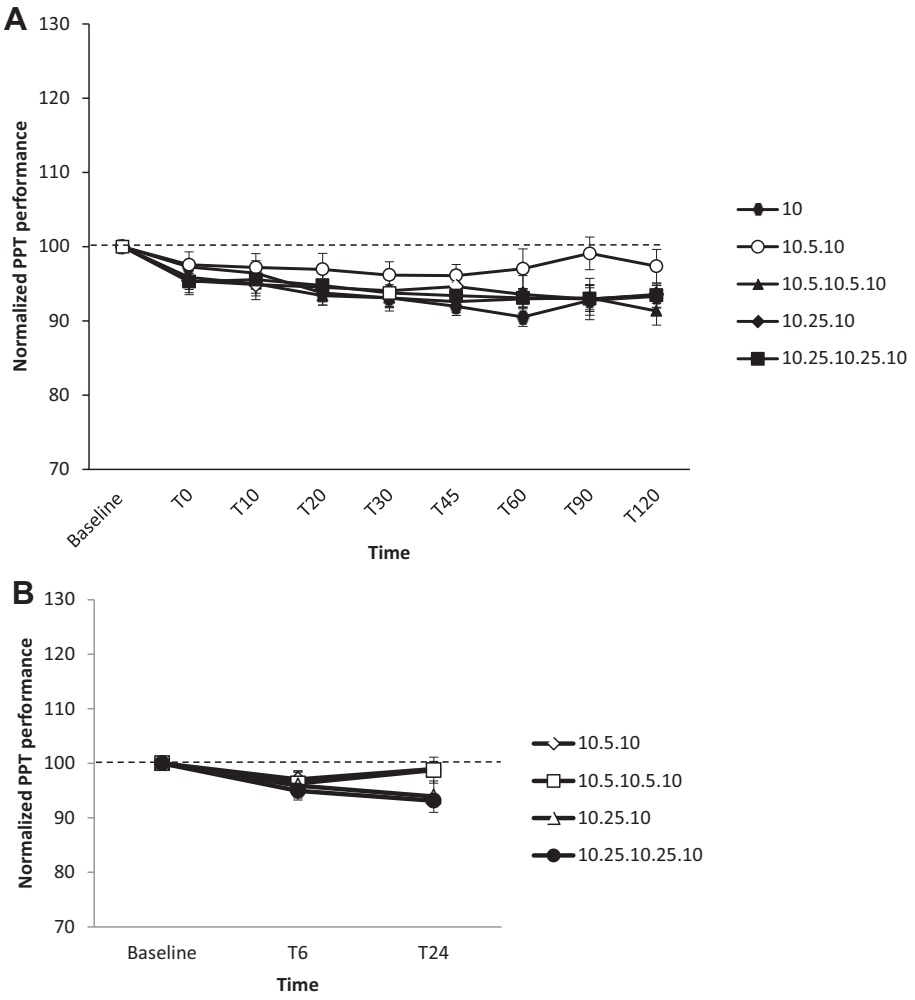

Fig. 3. (A) PPT performance time in study 1 and (B) study 2. Filled symbols indicate significant deviation of the post stimulation PPT score relative to baseline values. Error bars represent SE.

However, they found that when the second stimulation followed the first after 3 or 24 h, the c-tDCS induced inhibitory lasting effects were attenuated (Monte-Silva et al., 2010). For a-tDCS, Fricke et al. (2010) showed that excitatory effects could be enhanced, negated or unaffected as the inter-stimulation period was varied between 0 and 30 min. They compared the lasting effect of a single 5 min application of a-tDCS with the effects of a 5 min a-tDCS preceded by an identical 5 min conditioning stimulation administered 30, 20, 10, 3, 1 or 0 min beforehand. Five minutes a-tDCS increased CSE for about 5 min. Increasing the duration of a-tDCS to 10 min prolonged the duration of the effects. If two 5 min periods of a-tDCS were applied with a 30 min break between them, the effect of the second period of a-tDCS was identical to that of 5 min stimulation alone. If a second a-tDCS session followed the first one during an interval of 1, 3, 10 or 20 min the after effects of a-tDCS were suppressed or even reversed (Fricke et al., 2010).

As revealed by the results of the previous studies (Furubayashi et al., 2008, Lang et al., 2004; Nitsche and Paulus, 2000, 2001), a significant increase in MEPs amplitude could be induced initially after a-tDCS, which declines gradually over time. The Monte-Silva et al. (2013) used 13 min of a-tDCS in their study, and stated that this stimulation duration induces CSE enhancements of about 60 min duration. Then they applied the second a-tDCS after two inter-stimulus intervals of 3 and 20 min. Indeed, this group applied the second stimulation when the effect of the first treatment was at its highest level. In this current study, the subsequent stimulations were applied when the effect of the previous stimulation was at its higher (inter-stimulus intervals of 5 min) and lower (inter-stimulus intervals of 25 min). In addition, CSE has been measured between the different a-tDCS applications in all within session repeated conditions. Our results confirm the significant level of increase in CSE changes in the subsequent a-tDCS applications (second and third) only when longer inter-stimulus intervals were applied.

Although the subsequent a-tDCS application time and duration of the inter-stimulation intervals used by the Monte Silva group differs to that used in this research, Monte-Silva et al. (2013) do share some similarities in findings. The Monte-Silva et al. (2013) found that the excitability enhancement was larger for the experimental condition involved a longer interval (20 min), which supports the findings of this study. However, in the study by Monte Silva et al. (2013), two within-session repetition a-tDCS with an interval of 3 or 20 min showed enhanced efficacy in CSE. Yet these changes were non-significant for up to 90 min after stimulation and CSE was at baseline level 2 h post stimulation. Unlike the findings of Monte Silva et al. (2013), in the present study we found significant changes even during the first 2 h after the completion of stimulation. This discrepancy could be easily explained by comparing the differences between these two studies. First, they used an electrode size of 3 cm^2 compared to 12 cm^2 in the current study.

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According to a recent study by our group (Bastani and Jaberzadeh, 2013b), the electrode size has an important role in the size of induced CSE. Second, the current intensity of 0.2 mA used in our study may also contribute to the discrepancy in results in comparison with Monte Silva et al. (2013) study with an intensity of 1 mA. This current intensity was chosen based on the findings of a recent study comparing the effects of different intensities on CSE (Bastani and Jaberzadeh, 2013a). Furthermore, it is also possible that the slight reduction and non-significant MEPs size in Monte Silva et al. (2013) study might be caused by the homeostatic metaplasticity of CSE changes (Turrigiano et al., 1998). The homeostatic metaplasticity carries the risk of triggering an uncontrolled increase in synaptic effectiveness (Kuo et al., 2008). This can be potentially destabilizing and overpower other inputs in the system (Turrigiano, 2008; Turrigiano and Nelson, 2004). This could have triggered with doubled excitability enhancement, by not only a-tDCS but also a large number of magnetic pulses induced by TMS at each time point (25 MEPs recorded per time point). This can be explained by the concept of state-dependency effects of TMS (Silvanto and Pascual-Leone, 2008; Silvanto et al., 2008). This induction is dependent on the prior activity of the cortical network, neurons, or synapses, which helps to avoid "runaway" or uncontrolled excitation, and thus preserve the stability of the network (Monte-Silva et al., 2013). Delivering a large number of magnetic stimuli with short intervals between each time points is an influential concept; however, it has been a largely ignored issue in the TMS literature. Therefore, in our second study design, TMS induced excitability effects were avoided by only recording at two time points of T6 and T24, which also enabled us to follow up the CSE effects further.

Extending findings from study 1, the results of the second study confirm our hypothesis that compared to other experimental conditions; within-session repetition of a-tDCS with 25 min inter-stimulus intervals induced the largest CSE changes which also lasted longer. The results showed that if the second or third stimulations were performed with an inter-stimulus interval of 5 or 25 min, the combined lasting effects of the two or three a-tDCS were present for 6 or 24 h, respectively, after the end of stimulation. However, Monte Silva et al. (2013) found enhancement in CSE excitability lasting for 24 h with both 3 and 20 min inter-stimulus interval experimental conditions. Further experiments should be done to find out any possible differences in lasting effects of a-tDCS repetitions with longer intervals (i.e., 10-25-10 and 10-25-10-25-10).

6.3. The effects of within-session repeated a-tDCS on motor performance

In both studies we hypothesized that within-session repeated a-tDCS with 25 min inter-stimulus intervals would induce a greater and more enduring increase in motor performance. We expected that within-session repeated a-tDCS with longer intervals increases the possible changes in motor performance improvements regarding any increase in MEPs size (Hummel and Cohen, 2005). The findings in the current study only support our hypothesis in part. We found significant performance improvement over time in all a-tDCS experimental conditions in study 1 (except in the 10-5-10 experimental condition) but the results did not support our hypothesis. This shows that a one-to-one transferability of the presented results obtained on CSE to performance improvements is unlikely. The hypothesized possible improvements in motor performance were observed only in the 10-25-10-25-10 a-tDCS experimental condition in study 2. However, any correlation between the increase in CSE and motor performance improvements at T6 and T24 were not statistically supported. The lack of significance in this area may be due to the small sample size. Therefore, further studies with larger sample size are required.

A possible explanation for this might be that a within-session repeated a-tDCS was not sufficient to show any possible differences between a-tDCS experimental conditions in healthy individuals. Previous studies indicated that the efficacy of a-tDCS to improve motor function after stroke (Boggio et al., 2007; Kim et al., 2010) might be enhanced if repeated stimulation protocols, as proposed by the current study, were applied, instead of a daily single stimulation, which is currently applied most often in clinical studies. From this present study we suggest that plasticity induction with performance improvements will be more significant in patients, and follow different rules of consolidation of the effects, than plasticity induction in healthy individuals with intact and perfect motor performance. It could also be concluded that PPT is not the best method and sensitive enough to be used as an assessment tool for motor performance changes in healthy individuals.

6.4. Clinical implications

The results of this study should be taken into account in the development of treatment protocols using a-tDCS in patients with neurological or psychological problems. It is of particular importance to increase the a-tDCS lasting effects to consolidate the neuroplastic CSE changes, which hopefully could promote motor performance improvement and/or motor learning.

6.5. Limitations

Studies 1 and 2 had a number of limitations. First, the effects of the within session repeated a-tDCS experimental conditions were only assessed up to 24 h after delivery; which limits our knowledge regarding possible ongoing lasting effects. Second, the data were obtained from a healthy population with no neurological background; therefore the results might not be extrapolated to subjects with stroke or other neurological conditions. Third, we only evaluated the effects on young healthy subjects; older healthy individuals may respond differently to a-tDCS with the presented protocols. Fourth, the study should be conducted with larger number of participants. Also, we did not explore gender differences in study 1 and all volunteers who participated in study 2 were women. Finally, due to different treatment times, the blinding of the investigator was not feasible. Therefore, the administration, collection and data analysis for both a-tDCS experimental conditions and PPT performance time was directed by one investigator.

6.6. Suggestions for future studies

Future experiments should explore the physiological mechanisms of action to a larger extent and more directly. The current study investigated the lasting effects of a-tDCS for up to 24 h. Important research still has to be performed to explore longer follow-ups. Furthermore, to underpin the mechanisms of action it is recommended that measurements are made of silent periods, intracortical inhibition, and facilitation, in order to assess the function of γ-aminobutyric acid (GABAa and GABAb) and glutamatergic receptors. Future studies should also assess CSE and motor performance in neurological patients with motor disorders or psychiatric disorders, and of varying age and gender.

7. Conclusions

Our findings might help to develop a-tDCS within-session repetition rates and inter-stimulus intervals to optimum levels for therapeutical strategies in neurorehabilitation and clinical

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applications. Within-session repeated a-tDCS with longer intervals used in this study could be a promising protocol for modulation of the CSE. However, increasing the number of repetitions could also lead to longer lasting effects compared to the effects of a single a-tDCS, regardless of the inter-stimulus interval. In addition, increasing the number of within-session a-tDCS repetitions with longer intervals increases both the size and lasting effects of CSE. It might be speculated that within-session repeated a-tDCS is suited to improving motor performance in humans. Our results suggest that a deeper knowledge of the mechanisms underlying a-tDCS induced excitability is required.

Conflict of interest

The authors declare that they have no conflict of interest.

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Declaration for Chapter 8

In the case of Chapter 8, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Review of literature, Project design, ethics application and approval, participant recruitment, data collection, data analysis, interpretation of the results and writing of the manuscript.	80 %

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Shapour Jaberzadeh	Supervisory input on study design, Guidance in the framing of the manuscript, discussion of findings, review and provision of feedback on manuscript drafts	15 %
Maryam Zoghi	Discussion of findings, review and provision of feedback on manuscript drafts	5 %

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	**Date** 30.09.2013
Signatures	**Shapour Jaberzadeh** [redacted]	**Date** 24.09.2013
	Maryam Zoghi [redacted]	**Date** 19.09.2013

Preamble to Chapter 8

Chapter 8 investigates the effects of a novel noninvasive neuromodulatory paradigm on CSE. In this paradigm, named transcranial pulsed current stimulation (tPCS), direct current is interrupted and broke into a number of pulses to take advantage of two extra parameters of pulse duration and inter pulse interval. Chapter 8 provides detailed information on the theoretical aspects of this new paradigm and also summarises the effects of tPCS on the size of CSE changes, compared to conventional a-tDCS.

Chapter 8: Anodal transcranial pulsed current stimulation: A novel technique to enhance corticospinal excitability

The format of this chapter is consistent with the Journal of *Clinical Neurophysiology*.

The setup system used in this study, Ethics approval, TMS safety and Edinburg handedness questionnaires and consent form are provided in Appendices 8 and 12-15 and 17.

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Anodal transcranial pulsed current stimulation: A novel technique to enhance corticospinal excitability

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HIGHLIGHTS

- Transcranial pulsed current stimulation (tPCS) is a novel non-invasive neuromodulatory paradigm with less side effects compared to the conventional transcranial direct current stimulation (tDCS).
- Despite tDCS which modifies neuronal excitability by tonic depolarization of the resting membrane potential, tPCS modifies neuronal excitability by a combination of tonic and phasic effects.
- tPCS appears to be a promising tool for clinical neuroplasticity research as a new method of delivering transcranial stimulation for modulation of corticospinal excitability.

ABSTRACT

Objective: We aimed to compare the effects of anodal-transcranial pulsed current stimulation (a-tPCS) with conventional anodal transcranial direct current stimulation (a-tDCS) on corticospinal excitability (CSE) in healthy individuals.
Methods: CSE of the dominant primary motor cortex of the resting right extensor carpi radialis muscle was assessed before, immediately, 10, 20 and 30 min after application of four experimental conditions: (1) a-tDCS, (2) a-tPCS with short inter-pulse interval (a-tPCS$_{SIPI}$, 50 ms), (3) a-tPCS with long inter-pulse interval (a-tPCS$_{LIPI}$, 650 ms) and (4) sham a-tPCS. The total charges were kept constant in all experimental conditions except sham condition. The outcome measure in this study was motor evoked potentials.
Results: Only a-tDCS and a-tPCS$_{SIPI}$ ($P < 0.05$) induced significant increases in CSE, lasted for at least 30 min. Post-hoc tests indicated that this increase was larger in a-tPCS$_{SIPI}$ ($P < 0.05$). There were no significant changes following application of a-tPCS$_{LIPI}$ and sham a-tPCS. All participants tolerated the applied currents in all experimental conditions very well.
Conclusions: Compared to a-tDCS, a-tPCS$_{SIPI}$ is a better technique for enhancement of CSE. There were no sham effects for application of a-tPCS. However, unlike a-tDCS which modifies neuronal excitability by tonic depolarization of the resting membrane potential, a-tPCS modifies neuronal excitability by a combination of tonic and phasic effects.
Significance: a-tPCS could be considered as a promising neuromodulatory tool in basic neuroscience and as a therapeutic technique in neurorehabilitation.

1. Introduction

Non-invasive induction of neuroplastic changes by transcranial stimulation techniques have been increasingly used in recent years. Apart from transcranial magnetic stimulation (TMS) and repetitive transcranial magnetic stimulation, which are neurostimulatory techniques, transcranial direct current stimulation (tDCS) is a well-known neuromodulatory technique. This technique has been involved in a number of important discoveries in the field of human cortical function and has become a well-established method for enhancing brain function in healthy participants (Antal et al., 2007; Boggio et al., 2006; Boros et al., 2008; Uy and Ridding, 2003) and patients with neurological conditions (Boggio et al.,

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2007; Fregni et al., 2005; Hummel et al., 2005; Benninger et al., 2010). The direction of corticospinal excitability (CSE) changes depends on the polarity of the active electrode. The application of anode over the target brain area is called anodal tDCS (a-tDCS) and it depolarizes the resting membrane potential and causes increased excitability. On the other hand, the application of cathode over the brain target area is termed cathodal tDCS (c-tDCS) and it hyperpolarizes the resting membrane potential and causes decreased excitability (Nitsche and Paulus, 2000). A recent systematic review and meta-analysis of the efficacy of a-tDCS in healthy individuals and people with stroke indicated a-tDCS effectively enhances CSE and motor performance (Bastani and Jaberzadeh, 2012). This review indicates that the induced CSE changes in both healthy participants and patients with stroke depend on current intensity and its duration of application (Nitsche and Paulus, 2000; Nitsche et al., 2003b; Nitsche and Paulus, 2001). Another parameter which may also affect the outcome of stimulation, and which is the focus of the current study, is current type.

The use of tDCS involves the employment of direct current, which is an uninterrupted flow of charged particles in one direction (Fig. 1a). Polarity, referring to two oppositely charged poles, one positive (+) and the other negative (−), determines the direction in which the current flows. Indeed, polarity in the context of electric current means "*charge imbalance*". If direct current is applied to the body via skin-mounted electrodes, there will be a build-up of ions under the electrodes. Under the cathode, due to the excess of positive ions such as sodium ions and its combination with water, acidic reactions may happen. Under the anode, there will be a corresponding accumulation of negatively charged ions such as chloride ions (Cameron, 2012; Michlovitz et al., 2005). Combination of these ions with water may produce a basic (alkaline) reaction under the anode. These acidic and basic reactions are called electrochemical effects of direct current (Ledger, 1992). The body's response to changes in pH of the skin is to increase blood flow to the area in an attempt to restore normal pH. Blistering or chemical burns may occur if normal pH cannot be maintained. These chemical reactions could be a source of sensory side effects of tDCS such as burning sensation, itching and tingling.

Transcranial alternating current stimulation (tACS) is another neuromudulatory paradigm which has been introduced to directly modulate human cortical excitability (Antal et al., 2008; Paulus, 2011; Zaghi et al., 2010; Kanai et al., 2010; Pell et al., 2011; Jung and Ziemann, 2009). It employs a continuous flow of charged particles in alternating directions, and the direction of flow cycles back and forth over time. This is a balanced current because alternating biphasic pulses have equal electric charges, therefore the net direct current component (NDCC), the average value of the voltage or current over application time, is zero. Compared to tDCS, tACS allows manipulation of CSE not only based on intensity, but also based on the frequency of the applied current. Unlike tDCS which its excitatory or inhibitory effects are polarity dependent, tACS effects are determined by the frequency of the current (Kanai et al., 2010; Zaghi et al., 2010) and are not polarity dependent. In addition, sinusoidal tDCS (tSDCS) (Antal et al., 2008) or slow oscillatory tDCS (so-tDCS) (Bergmann et al., 2009; Groppa et al., 2010) are modified protocols where the alternative currents are added to a DC offset. In tSDCS or so-tDCS, anodal or cathodal stimulation is sinusoidally modified at a given frequency. The tSDCS has a given single low or high frequency. However, so-tDCS is applied with a slow frequency range (Bergmann et al., 2009; Groppa et al., 2010; Kirov et al., 2009). A recent study by Antal et al. (2008) did not find any significant effects in CSE after application of both anodal or cathodal tSDCS to M1 of hand muscle (Antal et al., 2008).

Moreover, one known side effect for alternative, sinusoidal or oscillatory types of current is a very slight flashing of light in eyes. These light flashes – a phenomena characterized by the experience of seeing light without light actually entering the eye – are also known as phosphenes, or retinal phosphenes (Lakhanpal et al., 2003). Phosphenes can be directly induced by mechanical, electrical, or magnetic stimulation of the retina or visual cortex as well as by random firing of cells in the visual system (Kanai et al., 2008). It has been reported that phosphenes result from the normal activity of the visual system after being stimulated by other stimuli rather than light.

The current study was designed to investigate the effects of a new neuromodulatory paradigm which uses transcranial pulsed current stimulation (tPCS). In this paradigm, the tDCS was interrupted by a typical modern electrical stimulator to take advantage of two extra parameters, "pulse duration (PD)" and "inter-pulse interval (IPI)", which may dramatically affect the size of CSE. In this new neuromodulatory paradigm, the current flows in unidirectional pulses separated by an IPI instead of a continuous flow of direct current in tDCS. Even though the physiological mechanisms underpinning these effects are not understood yet, but it was assumed that the new paradigm induces its effects not only by polarity-dependent modulation of the baseline activity of the motor cortex, but also through the on–off nature of pulses on voltage gated carrier proteins (Bennett, 2000; Malenka and Bear, 2004; Rioult-Pedotti et al., 2000) in the membranes of M1 neurons.

The extent of activation within the cortex during tPCS may be influenced by a number of variables, including the size of the electrodes and their positions over the head; intensity and frequency of the pulses; the intervals between the pulses; output waveforms

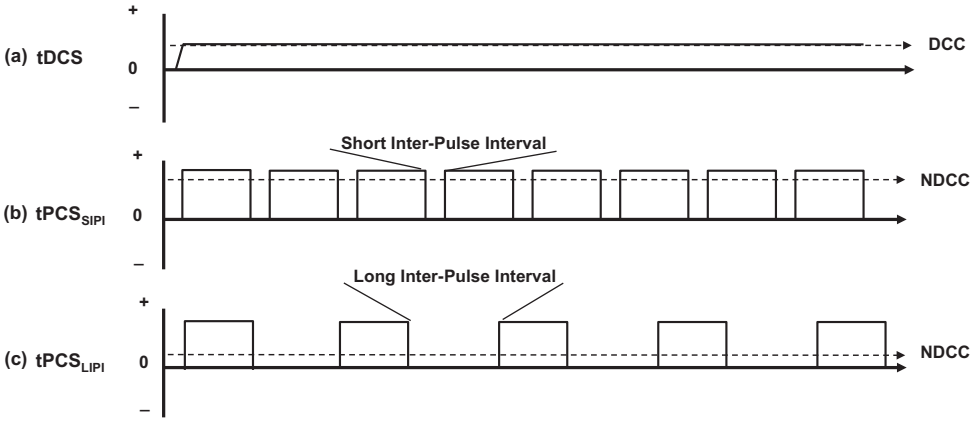


Fig. 1. (a) Transcranial direct current stimulation (tDCS), (b) tPCS$_{SIPI}$: transcranial pulsed current stimulation (short inter-pulse interval) and (c) tPCS$_{LIPI}$: transcranial pulsed current stimulation (long inter-pulse interval). DCC, direct current component; NDCC, net direct current component.

(monophasic vs. biphasic) and the anatomy of the region under stimulation.

tPCS could be applied with short inter-pulse intervals ($tPCS_{SIPI}$) or long inter-pulse intervals ($tPCS_{LIPI}$). Due to the unidirectional nature of tPCS it is an unbalanced current with some degrees of NDCC. The tPCS could also be modulated by keeping IPIs constant and changing pulse duration. Pulsatile currents could also be delivered in bi-directional form. This is a balanced type of current because biphasic pulses have equal electric charges; therefore NDCC in this type of current is zero. This type of current is not the focus of the present study.

Similar to tDCS, tPCS also involves the application of very low-amplitude currents via surface scalp electrodes. Therefore it is expected to be tolerated well by participants. However, due to the interrupted nature of tPCS, the presence of phosphene is expected.

The primary aim of the current project is to compare the effects of anodal tPCS (a-tPCS) with sham a-tPCS and conventional a-tDCS on the enhancement of CSE in healthy individuals. The secondary aim is to compare the effects of shorter and longer IPIs. Based on a pilot study on 3 healthy individuals, it is hypothesized that a-$tPCS_{SIPI}$ induces larger CSE changes compared to both a-tDCS and a-$tPCS_{LIPI}$. We also hypothesized that a-tDCS induces less CSE changes compared to a-$tPCS_{LIPI}$.

2. Methods

2.1. Subjects

2.1.1. Experimental group

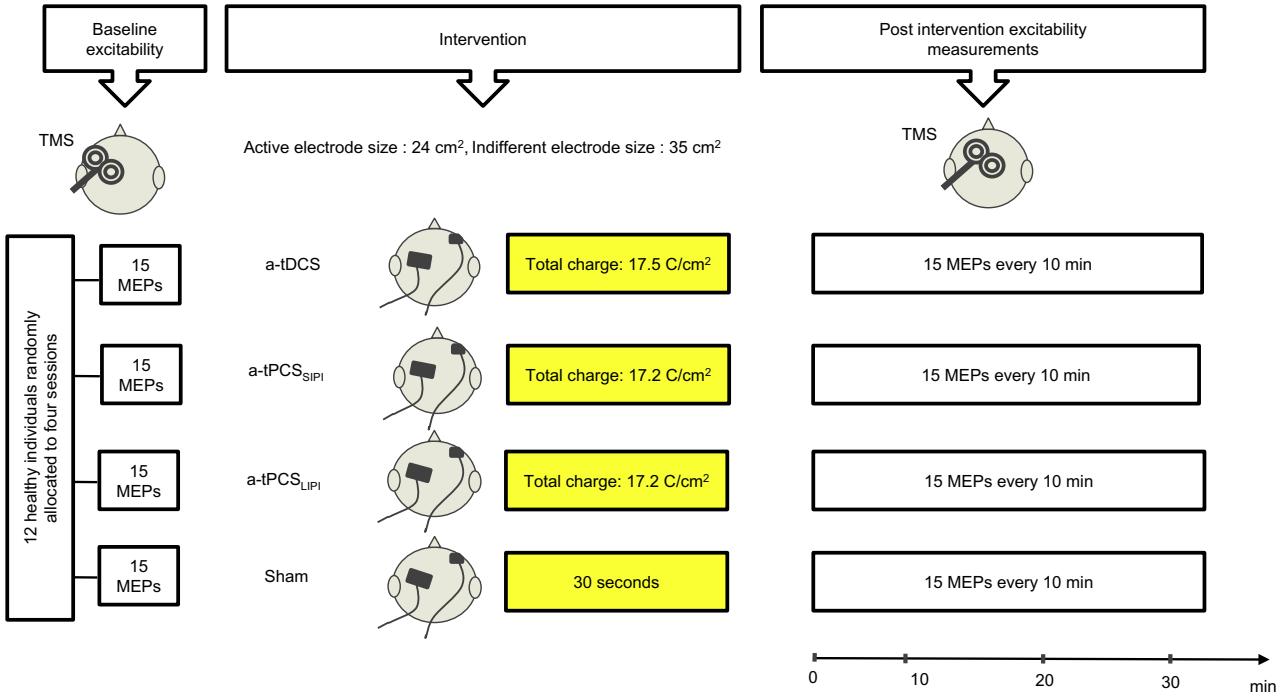
Twelve healthy volunteers participated in four testing conditions: a-tDCS, a-$tPCS_{SIPI}$, a-$tPCS_{LIPI}$ and sham a-$tPCS_{SIPI}$. The volunteers comprised 7 women and 5 men. Their age ranged from 20 to 51 years and the mean age was 32.5 ± 9.41. The mean weight of the volunteers was 67.41 ± 10.24 (kg) and their mean height was 169.95 ± 12.82 (cm). They were all right-handers according to the 10 item version of the Edinburgh Handedness Inventory (85.7 ± 6.1) (Oldfield, 1971).

In a separate control study, we examined the phosphene effect on CSE changes. This experiment was carried-out to rule out any excitatory effects of induced phosphene by a-tPCS. In this study everything was kept identical to original study (a-$tPCS_{SIPI}$) except the application of current over contralateral M1. MEPs were recorded from Rt ECR muscle. Six of the subjects (4 women, 2 men) who had reported phosphene took part in this experiment (mean age; 22.33 ± 2.87, mean weight: 68.66 ± 10.03 (kg) and mean height: 175.33 ± 17.70 (cm)).

Prior to the experiments, all participants completed the Adult Safety Screening Questionnaire (Keel et al., 2001) to determine their suitability for TMS. Participants were informed of the experimental procedures and gave their written informed consent according to the declaration of Helsinki. All experimental procedures were approved by the Ethics Committee of Monash University. Each subject was tested at the same time of the day to avoid diurnal variation.

2.2. Study design

Fig. 2 illustrates the placebo-controlled experimental set-up used in this study. The order in which the real and sham experimental conditions were conducted was randomized between participants. All recruited individuals completed their experimental conditions at least 48 h apart, to avoid interference or carry over effects of transcranial stimulations. Subjects were blinded to different stimulation conditions. During the



TMS: transcranial magentic stimulation
IPI: inter pulse interval
atDCS: anodal transcranial direct current stimulation
atPCS$_{SIPI}$: anodal transcranial pulsed current stimulation, Short IPI
atPCS$_{LIPI}$: anodal transcranial pulsed current stimulation, Long IPI
MEPs: motor evoked potentials

Fig. 2. Study design for the comparison of different current types on CSE.

experiments, subjects were at rest with no hand and wrist movements allowed. The CSE was measured at five consecutive time points: baseline, immediately after, and every 10 min up to 30 min after the end of stimulation (T0, T10, T20 and T30). The total charge under the active electrodes was kept constant in all real experimental conditions.

2.3. Application of a-tDCS

An Intelect® Advanced Therapy System (Chattanooga, USA) was used to deliver a-tDCS through a pair of saline-soaked surface sponge electrodes. The active electrode (anode, 24 cm^2) was placed over the left M1 for the right extensor carpi radialis (ECR) muscle as identified by TMS, and the indifferent electrode (cathode, 35 cm^2) was placed over the right contralateral supraorbital area (Nitsche and Paulus, 2000). The electrodes were fixed with two horizontal and perpendicular straps. a-tDCS was applied continuously for 10 min with a current intensity of 0.7 mA (total charge ~17 C/cm^2).

2.4. Application of a-tPCS

An Intelect® Advanced Therapy System (Chattanooga, USA) was used for delivery of a-tPCS through a pair of saline-soaked surface sponge electrodes. The anode (24 cm^2) was placed over the left M1 for the right ECR muscle as identified by TMS, and the cathode (35 cm^2) was placed over the right contralateral supraorbital area (Nitsche and Paulus, 2000). The impedance between the electrodes and the skin was kept below 10 kΩ. The waveform of the stimulation was unidirectional, pulsed and rectangular. The a-tPCS was delivered with the following parameters for a-$tPCS_{SIPI}$: current intensity: 1.5 mA, pulse duration: 500 ms, IPI: 50 ms and with a total duration of 5 min (total charge ~17 C/cm^2). For a-$tPCS_{LIPI}$ it was: current intensity: 1.5 mA, pulse duration: 500 ms, IPI: 650 ms and with t otal duration of 10 min (total charge ~17 C/cm^2).

2.5. Application of sham a-$tPCS_{SIPI}$

For sham stimulation, the electrodes were placed in the same positions as for real experimental conditions; however, the stimulator was turned off after 30 s of stimulation. Therefore, the subjects felt the initial sensations, but received no current for the rest of the stimulation period. This procedure allowed to blind subjects for the respective stimulation condition (Nitsche et al., 2003a).

2.6. Measurement of side effects

All the subjects completed a questionnaire during and after the experimental conditions. The questionnaire contained rating scales for the presence and severity of side effects such as itching, tingling, burning sensations under the electrodes (George and Aston-Jones, 2009; Nitsche et al., 2008) and other discomforts including eye flashing, headache and pain during and after a-tDCS, a-$tPCS_{SIPI}$ and a-$tPCS_{LIPI}$. All participants rated the unpleasantness of any scalp sensations using numeric analog scales (NAS) (e.g., 0 = no tingling to 10 = worst tingling imaginable).

2.7. Measurement of CSE by TMS

Participants were seated upright and comfortable while their head and neck was supported by a head rest. Single pulse magnetic stimuli were delivered using a Magstim 200^2 (Magstim Company Limited, UK) stimulator with a flat 70 mm figure-of-eight magnetic coil. Using an international 10–20 system, the vertex (C_z) point was measured and marked to be used as a reference (Schwartz, 2003). The magnetic coil was placed over the left hemisphere (cortex), contralateral to the target muscles. The orientation of the coil was set at an angle of 45° to the midline and tangential to the scalp, such that the induced current flowed in a posterior-anterior direction. To determine the optimal site of stimulation (hotspot), the coil was moved around the M1 of ECR muscle to trigger the area with the largest motor evoked potentials (MEPs) response.

After localizing the optimal stimulation site, the coil position was marked with a marker on the scalp to be used for the remainder of the testing for the target muscle to ensure consistency in the placement of the coil. The resting motor threshold (RMT) was defined as the minimal stimulus intensity that evoked 5 MEPs in a series of 10 with the amplitude of at least 50 μV (Hallett, 1996; Nitsche and Paulus, 2000; Rossini et al., 1994; Wassermann et al., 2008) from the hotspot of the ECR muscle. The RMT for each subject was determined by increasing and decreasing stimulus intensity in 1–2% intervals until MEPs of appropriate size were elicited. For all further MEP measurement, the TMS intensity was set at 120% (1.2 times) of each individual's RMT. Fifteen stimuli were elicited to assess CSE at each time point. The stimulus intensity remained constant throughout the experimental conditions for each subject.

2.8. Electromyography (EMG) recording

Participants were seated in an adjustable podiatry chair with their forearm pronated and the wrist joint in a neutral position, resting on the armrest of the chair. To ensure good surface contact and reduce skin resistance, a standard skin preparation procedure of cleaning and abrading was performed for each site of electrode placement (Gilmore and Meyers, 1983; Robertson et al., 2006; Schwartz, 2003). MEPs were recorded from the right ECR muscle at rest, using pre-gelled self-adhesive bipolar Ag/AgCl disposable surface electrodes with an inter-electrode distance of 3 cm, measured from the center of the electrodes. The location of the ECR muscle was determined based on anatomical landmarks (Perotto and Delagi, 2005) and also observation of muscle contraction in the testing position (wrist extended and radially deviated) (Kendall et al., 2010). The accuracy of EMG electrode placement was verified by asking the subject to maximally contract the ECR muscle while the investigator monitored online EMG activity. The ground electrode was placed ipsilaterally on the styloid process of the ulnar bone (Oh, 2003). Then, the electrodes were secured by tape. All raw EMG signals were band pass filtered (10–1000 Hz), amplified (×1000) and sampled at 2000 Hz, and were collected on PC running commercially-available software (Chart™ software, ADinstrument, Australia) via a laboratory analog–digital interface (The PowerLab 8/30, ADinstrument, Australia). Peak-to-peak MEPs amplitude was detected and measured automatically using a custom designed macro in Powerlab 8/30 software after each magnetic stimuli.

2.9. Data management and statistical analysis

In this study, 15 MEP amplitudes were calculated automatically before and after real and sham experimental conditions. MEP amplitudes were normalized to the baseline value.

Baseline MEP amplitudes and RMT of the respective conditions were tested using one-way repeated measure ANOVA to see whether the baseline MEPs or RMT were identical in all conditions. This test was also carried-out on the mean values from NAS test to analyses the sensation differences between different conditions.

A two-way repeated measure ANOVA was used to assess the effects of different current types of transcranial stimulations (a-tDCS, a-$tPCS_{SIPI}$, and a-$tPCS_{LIPI}$ and sham a-$tPCS_{SIPI}$) on MEP's amplitude over time. The first within-subject independent factor was differ-

ent conditions (four levels). The second independent factor was time points (four levels). Mauchly's sphericity test was used to validate an assumption of repeated measures factor ANOVA. Greenhouse–Geisser corrected significance values were used when sphericity was lacking. Post hoc comparisons were performed using the least significance difference (LSD) adjustment for multiple comparisons when appropriate. As an additional analysis, it was assessed whether sham and active stimulations were distinguishable. The subjects were asked to indicate if they thought the stimulation was either active or sham at the end of their participation in all testing conditions. Data were analyzed using Pearson's chi-square. The results were considered significant at the level of $P < 0.05$ for all statistical analyses. All results are expressed as the mean ± standard error of mean (SEM) and statistical analyses were performed using SPSS software version 20.

3. Results

3.1. Comparison of sensations in different conditions

All participants tolerated the applied currents in different conditions very well and there was no interruption of experimental procedures due to the adverse or side effects of the applied currents. Table 1 summarizes the numeric value means ± SEM for reported side-effects under the anode and cathode during a-tDCS, a-tPCS and sham a-tPCS$_{SIPI}$. The mean values of side effects are all low indicating their mild nature. Light flashing is a side effect of a-tPCS which was experienced by two thirds of the participants during a-tPCS$_{LIPI}$, a-tPCS$_{SIPI}$ and sham a-tPCS$_{SIPI}$ in a frequency dependent manner. There were no side effects reported by participants after the end of experimental sessions. Also, there were no reports of burning sensations, pain or headaches during or after a-tDCS or a-tPCS applications. The results of one-way ANOVA showed that sensations were differed significantly across the four conditions ($P < 0.001$) (Table 2). LSD Post-hoc comparisons are listed in Table 2.

3.2. Participants' awareness of active versus sham conditions

Table 3 provides the data on participants' awareness of the stimulation being used in testing conditions. Pearson's chi square was not significant (χ^2 (6df) = 1.95, $P = 0.92$), suggesting that participants could not accurately determine the type of stimulation received and they were successfully blinded. Overall, the percentage of participants' who correctly guessed the active condition was 25% (excluding 'cannot say' responders) and 81% (including 'cannot say' responders).

Table 1
Numeric sensation scores reported by participants during experimental conditions. Scores are reported as mean ± SEM.

	Sensation	Active electrode (Anode)	Indifferent electrode (Cathode)
a-tDCS	Itching	3.3 ± 0.29	2.41 ± 0.16
	Tingling	2.52 ± 0.23	1.89 ± 0.11
	Eye flashing	None	
a-tPCS$_{SIPI}$	Itching	1.08 ± 0.39	0.41 ± 0.28
	Tingling	0.99 ± 0.29	0.33 ± 0.14
	Eye flashing	2.68 ± 0.41	
a-tPCS$_{LIPI}$	Itching	1.41 ± 0.48	0.36 ± 0.17
	Tingling	0.62 ± 0.18	0.26 ± 0.08
	Eye flashing	2. 21 ± 0.37	
Sham a-tPCS$_{SIPI}$	Itching	0.75 ± 0.42	0
	Tingling	0.5 ± 0.33	0
	Eye flashing	0.53 ± 0.41	

Table 2
Comparison of side effects between tDCS, tDCS$_{SIPI}$, tPCS$_{LIPI}$ and sham conditions. The results were considered significant at the level of $P < 0.05$.

Sensation	Comparison between conditions	Anode		Cathode	
		F value (3,44)	*P* value	*F*-value (3,44)	*P* value
Itching					
	tDCS-tPCS$_{LIPI}$	28.07	$P < 0.001$	99.12	$P < 0.001$
	tDCS-tDCS$_{SIPI}$		$P < 0.001$		$P < 0.001$
	tDCS-Sham		$P < 0.001$		$P < 0.001$
	tDCS$_{SIPI}$- tPCS$_{LIPI}$		$P = 0.29$		$P = 0.72$
	tDCS$_{SIPI}$-Sham		$P = 0.29$		$P = 0.01$
	tPCS$_{LIPI}$-Sham		$P = 0.03$		$P = 0.02$
Tingling					
	tDCS-tPCS$_{LIPI}$	24.40	$P < 0.001$	95.89	$P < 0.001$
	tDCS-tDCS$_{SIPI}$		$P < 0.001$		$P < 0.001$
	tDCS-Sham		$P < 0.001$		$P < 0.001$
	tDCS$_{SIPI}$- tPCS$_{LIPI}$		$P = 0.18$		$P = 0.58$
	tDCS$_{SIPI}$-Sham		$P = 0.08$		$P = 0.01$
	tPCS$_{LIPI}$-Sham		$P = 0.64$		$P = 0.04$
Eye flashing					
	tDCS-tPCS$_{LIPI}$	11.30	$P < 0.001$	13.28	-
	tDCS-tDCS$_{SIPI}$		$P < 0.001$		-
	tDCS-Sham		$P = 0.34$		-
	tDCS$_{SIPI}$- tPCS$_{LIPI}$		$P = 0.38$		-
	tDCS$_{SIPI}$-Sham		$P < 0.001$		-
	tPCS$_{LIPI}$-Sham		$P = 0.004$		-

Table 3
Number of subject's who guessed the active or sham stimulation conditions.

		Actual testing conditions ($n = 12$)				
		a-tDCS	a-tPCS$_{SIPI}$	a-tPCS$_{LIPI}$	Sham	Total
Perceived stimulation	Active	2	4	3	3	12
	Sham	3	2	2	1	8
	Cannot say	7	6	7	8	28
	Total	12	12	12	12	48

3.3. Comparison of different conditions

One-way repeated measure ANOVA showed that baseline MEPs' amplitude ($F_{(3,33)} = 1.43$, $P = 0.25$, partial $\eta^2 = 0.11$) and RMT ($F_{(1,11)} = 1.26$, $P = 0.28$, partial $\eta^2 = 0.10$) were identical for all conditions.

A two-way repeated measures ANOVA was used to compare the effects of four different conditions on CSE. The assumption of sphericity had been met for time ($W = 0.60$, $df = 5$, $P = 0.43$). The assumption of sphericity had been violated for condition ($W = 0.28$, $df = 5$, $P = 0.031$) and condition × time interaction ($W = 0.000$, $df = 44$, $P = 0.002$). Therefore, Greenhouse–Geisser correction was considered for the *F*-ratio computations.

The results of the two-way repeated measures ANOVA showed significant main effects of time ($F_{(3,33)} = 5.95$, $P = 0.002$, partial $\eta^2 = 0.35$). Pairwise comparison showed no significant changes between different time points of T10 and T30 ($P = 0.32$), T10 and T20 ($P = 0.516$), T20 and T30 ($P = 0.502$), but significant changes between all other time points ($P < 0.001$).

Two-way repeated measures ANOVA showed significant main effects of condition ($F_{(3,33)} = 8.19$, $P < 0.001$, partial $\eta^2 = 0.42$). Post-hoc comparisons indicated that a-tPCS$_{SIPI}$ produced larger CSE compared to a-tPCS$_{LIPI}$. This increase was statistically significant (Mean = 69.01, SEM = 10.01) ($P < 0.001$). It also indicated that a-tDCS induced significant larger effects on CSE compared to a-tPCS$_{LIPI}$ (Mean = 30.59, SEM = 6.21) ($P < 0.001$). This change is also significantly larger after a-tPCS$_{SIPI}$ compared to a-tDCS

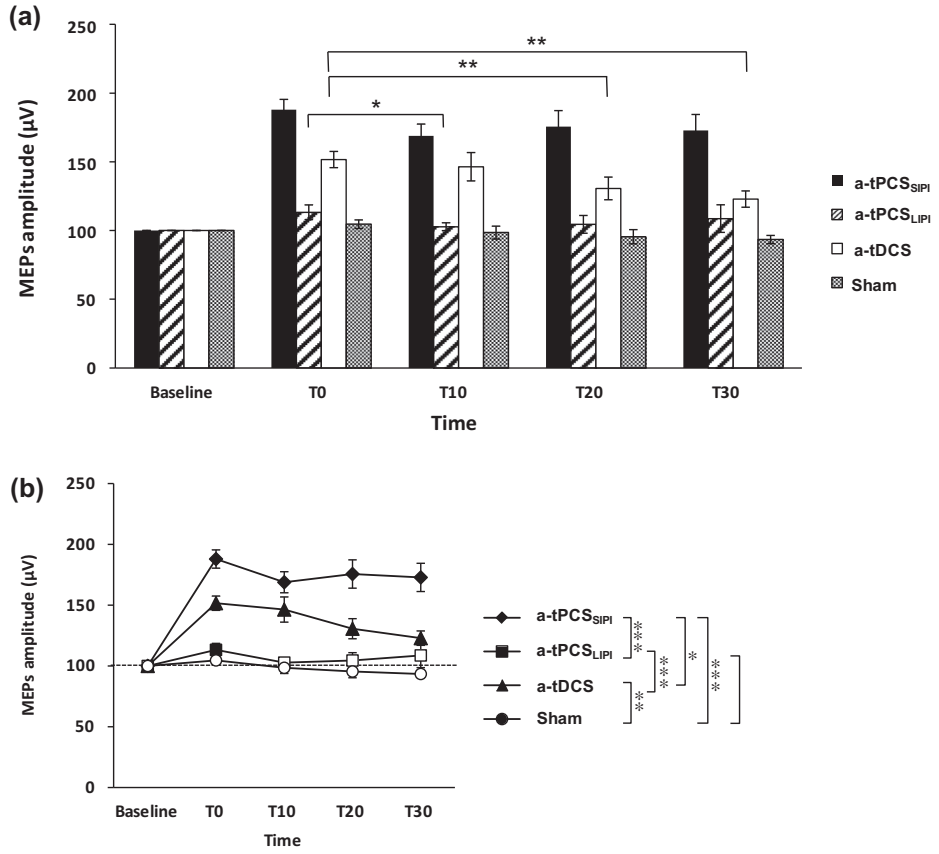


Fig. 3. The effects of different current types on the lasting effects and slope of decrease for MEPs' amplitude over time. (a) The asterisks mark significant differences between repeated post stimulation readings of MEP amplitudes within each condition and (b) Filled symbols indicate significant deviation of the post transcranial stimulation MEP amplitudes relative to the baseline; the asterisks mark significant differences between different testing conditions. Data are reported as mean ± SEM.

(Mean = 38.42, SEM = 12.65) ($P = 0.011$). Although the CSE was larger in a-tPCS$_{LIPI}$ compared to the sham group, there was no significant difference (Mean = 9.30, SEM = 5.61) ($P = 0.12$) (Fig. 3a and b). Finally ANOVA showed a non-significant interaction of condition × time course ($F_{(3.33,36.69)} = 1.47$, $P = 0.23$, partial $\eta^2 = 0.11$) (Fig. 3b).

A paired sample *t*-test with Bonferroni correction was used to compare mean MEPs in different time points within each condition (see Fig. 3a and b). All comparisons between different time points were statistically non-significant ($P > 0.01$), except those highlighted in Fig. 3a and b.

A test for homogeneity was carried out to find the slope of decrease in CSE in experimental conditions. Mauchly's test of sphericity was met for the slope of changes ($W = 0.930$, $df = 2$, $P = 0.697$). There were no significant differences ($F_{(3,\ 33)} = 1.53$; $P = 0.24$) in the slope of decrease for MEPs amplitude between different current types throughout the follow up period (Fig. 3b).

The lasting effects of changes in different conditions are presented in Fig. 3b, where a-tDCS and a-tPCS$_{SIPI}$ resulted in significant excitability enhancement lasting at least for 30 min ($P < 0.005$). However, for a-tDCS$_{LIPI}$ this change was only significant at T0 ($P = 0.03$). Following sham a-tPCS$_{SIPI}$, there were no significant changes in the MEPs amplitude in different time points ($P > 0.05$).

A One-way repeated measure ANOVA was used to compare the effects of phosphene on CSE. The assumption of sphericity had been met for time ($W = 0.007$, $df = 9$, $P = 0.08$). There were no significant changes ($F_{(4,20)} = 0.26$, $P = 0.90$, partial $\eta^2 = 0.05$) in the MEPs amplitude in different time points.

4. Discussion

4.1. Safety and side effects of a-tDCS and a-tPCSs

4.1.1. General observations

Overall, the findings of the current study supports the use of a-tPCS, with minimal or no side effects, in healthy individuals. The participants tolerated a-tPCS better than the conventional a-tDCS. No adverse effects such as seizure, headache and nausea were recorded or resulted in termination of experiments. In a systematic review, itching, tingling, headaches, burning sensations, and general discomfort were considered as the most often reported side effects of active tDCS vs. sham tDCS (Brunoni et al., 2011). The reported side effects in the present study are consistent with the ones reported in Brunoni's review and include itchiness and tingling. These skin sensations could occur due to electrochemical effects of direct currents under the electrodes (Palm et al., 2008; Durand et al., 2002; Dundas et al., 2007). These side effects were minimized during and after the application of a-tPCSs regardless of IPI parameter. In general, the tDCS-induced sensations were perceived more frequently and strongly than real tPCS or sham a-tPCS$_{SIPI}$ conditions for all of the sensations reported. Also, participants were unable to distinguish whether the stimulation was real or sham.

4.2. The experience of phosphene

During the application of a-tPCS, two thirds of the participants experienced phosphene. The rate of these flashings was correlated

to the frequency of pulses during the application of a-tPCS$_{SIPI}$ and a-tPCS$_{LIPI}$. The high sensitivity of the retina to electrical stimulation could be the reason for retinal phosphene. The closer the transcranial currents are applied to the retina, the more likely retinal stimulation occurs (Kanai et al., 2008). In this study, the electrode configuration was identical in all real and sham experimental conditions. During the a-tDCS application, the current was ramped up over a couple of seconds and remained constant throughout the session. This could be the reason why participants were not able to recognize any retinal phosphenes. However, during a-tPCS application, as a result of the on/off nature of the a-tPCS, the retinal phosphene was present during the stimulation and disappeared when the stimulation concluded. Overall it can be concluded that the induced phosphene by a-tPCS does not have any effect on CSE changes.

4.3. Comparison of different conditions

It was hypothesized that applying a-tPCS$_{SIPI}$ induces larger CSE changes compared to a-tDCS. The findings in this study support this hypothesis.

Current knowledge of the effects of tPCS on the modulation of CSE is limited, and the mechanisms behind the efficacy of tPCS for the induction of CSE are not yet understood. Several different mechanisms may account for understanding the results presented in this study. The effect of tDCS is due to its direct current component. However, unlike a-tDCS which modifies neuronal excitability by *tonic* depolarization of the resting membrane potential, a-tPCS modifies neuronal excitability by a combination of *tonic* and *phasic* effects.

The *tonic* effects of a-tPCS could be due to induced NDCC. In this case, neuronal excitability will be modified by tonic depolarization of the resting membrane potential (Medeiros et al., 2012). Neurons under the anode electrode will be 'excited' and their resting membrane potentials shift towards depolarization, and an increased rate of spontaneous neuronal firing could occur (Nitsche et al., 2005). On the other hand, neurons under the cathode electrode will be 'inhibited' and their resting membrane potentials shift towards hyperpolarization, and again reduced neuronal firing could occur (Bindman et al., 1964; Purpura and McMurtry, 1965).

The *phasic* effects of a-tPCS are caused by the on/off nature of pulsatile currents. The a-tPCS$_{SIPI}$ involves a succession of 500 ms rectangular pulses (on-phase) separated by 50 ms intervals (off-phase) (Fig. 1b). Therefore this effect may be attributed to the repeated opening and closure of Ca^{2+} or Na^{+} channels. During the on-phase, depolarization of nerve cell membranes occur for 500 ms, followed by 50 ms of IPI with no current delivery. These changes in depolarization of cell membranes happen over and over by the delivery of every single pulse. It seems that there is an accumulation effect for these tiny depolarizations which presents as larger induced CSE changes compared to a-tDCS.

It was also hypothesized that a-tPCS$_{LIPI}$ induces larger CSE changes compared to a-tDCS. The findings in this study did not support this hypothesis. Indeed, a-tDCS induced larger CSE changes than a-tPCS$_{LIPI}$. This indicates that the on–off nature of the a-tPCS is not the only factor for induction of further CSE changes. The other factors could be the ratio between pulse duration and the length of the IPI. The pulse width is easily eliminated because its length was kept identical in both a-tPCS conditions. Therefore it seems that the length of IPI plays a major role in the induction of CSE changes.

a-tPCS$_{LIPI}$ involves a flow of 500 ms rectangular pulses (on-phase) with 650 ms intervals (off-phase) (Fig. 1c). During on-phase, depolarization of nerve cell membranes happens for 500 ms, followed by 650 ms of IPI with no current delivery. These tiny changes in depolarizations of cell membranes happen over and over by delivery of every single pulse. It seems that the accumulation of these changes in depolarizations during the off-phase will not occur due to the long IPI. This could explain why a-tPCS$_{LIPI}$ failed to show added CSE changes compared to a-tPCS$_{SIPI}$.

The third hypothesis in this study was that a-tPCS$_{SIPI}$ induces larger CSE changes compared to a-tPCS$_{LIPI}$ when exposed to a constant total charge under the active electrode. This hypothesis has been supported by the findings. This provides extra support for the importance of IPI in induction of CSE changes, as the length of the interval between stimulation pulses may determine the size of facilitation or the direction of the stimulation effect (Jung and Ziemann, 2009). In the a-tPCS$_{LIPI}$ condition, cells return to their resting state due to the long IPI. However, in a-tPCS$_{SIPI}$ condition, subsequent pulses arrive after a very short IPI which causes a summation effect. As discussed earlier, another explanation for small changes in a-tPCS$_{LIPI}$ could be due to less tonic effect.

Although the current type used in current study is different to that of Bergmann et al. (2009) and Groppa et al. (2010), but the results can be also discussed in relation to excitability effects of so-tDCS which to some extent shares similar characteristics with tPCS. The rationale behind os-tDCS or tACS protocols is to interact with endogenous oscillatory cortical activity. In good agreement with current study, they concluded that anodal so-tDCS and a-tDCS can induce comparable effects on CSE when the total current charge is matched. However, the result of current study is in contrast to their findings that found no significant difference in the amount of CSE increase between a-tDCS and anodal so-tDCS. This discrepancy with the present study could be explained by different stimulation durations (2×20 min and 10 min vs. 5 min) and lower frequency (0.75 and 0.8 Hz vs. 1.8 Hz) that have been used by Bergmann et al. (2009) and Groppa et al. (2010), respectively.

4.4. Limitations of the study

The present findings must be interpreted in the context of a number of potential limitations. First, even though we showed that retinal phosphine on its own does not have any effect on CSE, but interaction of this sensory side effect with stimulation might have modulatory effects on CSE. Due to methodological limitations, this interaction was not tested in this study. Second, in the current study to keep electrode size and total charge identical, the stimulus intensity was variable in different conditions. Therefore the result of this study should be interpreted by consideration of the fact that stronger stimulation will result in larger electrical field strength in deeper areas, which might result in effects on different neuronal populations. Third, small sample size in the current study restricts generalizability of the results. Fourth, the data were obtained from a healthy population with no neurological history; therefore the results may not necessarily be extrapolated to subjects with stroke or other neurological conditions. Fifth, the lasting effects of the stimulation were only assessed up to 30 min. Even though, the length of the lasting effect was not the focus of this study, but we considered it as a limitation. Finally, the effects were evaluated on only young healthy subjects. Older healthy individuals may respond differently to a-tDCS or a-tPCS.

4.5. Suggestions for future research

The current study assessed the lasting effects of a-tPCS over a time duration of up to 30 min. Further study needs to be carried out to monitor the length of a-tPCS when applied over a longer duration. The effects of different characteristics of pulsatile currents such as the effects of pulse duration, length of IPI and frequency of pulsatile currents, are further areas which should be systematically studied to determine the optimal parameters in prolonging the effects of a-tPCS even further.

In addition, future studies should also assess motor performance in both healthy and neurological patients. Furthermore, to underpin the mechanisms of action in a-tPCS, it is recommended that a study of motor cortex excitability be undertaken, by measuring silent period, intracortical inhibition, and facilitation, to assess the function of GABAa, GABAb and glutamergic receptors.

Further studies combined with direct EEG measurements and technical developments such as improvements of electrode design will allow refining tPCS as a method of frequency dependent brain stimulation.

In conclusion, a-tPCS is a novel method of delivering transcranial stimulation for the modulation of M1 areas, and it appears to be a promising tool for clinical neuroplasticity research. It is a painless, selective, focal, noninvasive approach which induces reversible excitability changes in the human cortex. Therefore this study could provide valuable information for the development of new therapeutical strategies in neurorehabilitation.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgement

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Preamble to Chapter 9

Chapter 9 summarises the thesis findings and provides a list of limitations and suggestions for future research studies.

Chapter 9: Summary and Concluding Remarks

In this thesis, the intention is to bring together the optimal parameters of stimulation for a-tDCS in order for it to induce its largest and longest lasting effects on CSE and motor performance. Anodal transcranial direct current stimulation (a-tDCS) is the most investigated non-invasive neuromodulatory technique. In recent years a large body of research has been devoted to a-tDCS effects on corticospinal excitability (CSE). This research has raised new and interesting questions about optimal parameters of stimulation. Optimization of a-tDCS parameters can have a profound impact on its efficacy for enhancement of brain excitability and motor performance.

The primary aim of this thesis is to determine optimal parameters of a-tDCS for enhancement of CSE. The secondary aim is to establish an a-tDCS protocol for induction of larger and longer lasting CSE changes. To discuss these aims, and provide concluding remarks as to how they have been satisfied, I have divided the following section into four parts: a systematic review of the literature (Study 1), reliability and feasibility studies (Studies 2 and 3), the determination of optimal parameters for induction of larger M1 CSE changes (Studies 4-5 and 7), and the development of a protocol for induction of larger and more enduring CSE and motor performance changes (Study 6).

1. Systematic review of the literature

In *Chapter 2* a systematic review and meta-analysis is carried out to verify whether previous studies support the view that a-tDCS increases CSE and motor performance in healthy individuals and patients with stroke. From the findings of the meta-analysis it is concluded that longer applications of a-tDCS and larger current densities are associated with larger and longer lasting effects (Nitsche & Paulus 2000; Nitsche & Paulus 2001; Ohn et al. 2008; Bastani & Jaberzadeh 2012a).

The trend of changes is in favour of motor performance improvement in both healthy individuals and patients with stroke. Yet, as described in this chapter, there is not enough evidence to consider optimal parameters of stimulation to produce larger CSE changes with longer lasting effects. To determine these optimal parameters the first two studies are carried out to establish the intra-rater reliability of myself in my evaluation of the CSE changes using transcranial magnetic stimulation (TMS), and to assess feasibility and to fine-tune the a-tDCS technique used in this thesis.

2. Reliability and feasibility studies

Any application of tDCS involves measurement of changes before and after intervention. Therefore, in order to make sure that the changes following interventions are not due to systematic errors and methodological inconsistencies, a reliability study is conducted (*Chapter 3*). The reliability study is also used to determine the effects of the number of recorded motor evoked potentials (MEPs) on the intra- and inter-session reliability of the averaged MEP values. The higher reliability is achieved by increasing the number of MEPs from 5 to 10 or 15 per block of TMS (Bastani & Jaberzadeh 2012b). In conclusion, while even a block of 5 MEPs resulted in acceptable reliability in

both extensor carpi radialis (ECR) and first dorsal interossei (FDI) muscles (ICC > 0.77), recording 10 to 15 MEPs per block (ICC > 0.98) is recommended to increase the reliability of inherently variable and sensitive measurements.

Following the reliability study, a feasibility study is used to fine-tune the setup for application of TMS as an assessment tool, and a-tDCS as the neuromodulatory technique (*Chapter 4*). All necessary technical changes are checked, including the addition of a foot switch for hands-free triggering of TMS, setting of the Lab chart software (LabChartTM software, ADInstruments, Australia) in a way to facilitate recording of the TMS induced MEPs single-handedly, and the addition of a macro and its dedicated software to the data acquisition system (PowerLab 8/30, ADInstruments, Australia) for automatic measurement of the TMS induced MEPs peak-to-peak amplitude.

3. Determination of optimal parameters for induction of larger M1 CSE changes

Optimization of a-tDCS parameters can have a profound impact on its efficacy for enhancement of M1 CSE and possibly motor performance. Study 3 (*Chapter 4*) shows that it is possible to apply a-tDCS for up to 20 minutes without any adverse effects such as seizure, headache and nausea. Also, long duration a-tDCS (20 minutes) induces larger CSE changes compared to short duration a-tDCS (10 minutes) (Jaberzadeh et al. 2012c). The results are consistent with previous studies (Nitsche et al., 2005, Boros et al., 2008) showing that longer applications of a-tDCS modulates CSE to a greater extent compared to shorter applications. However, this hypothesis has recently been challenged by Monte Silva et al. (2013). They concluded that due to homeostatic neuronal counter-regulation, the observed direct relationship between the duration of a-tDCS application and the extent of lasting effects does not exist in applications longer than 26 minutes (Monte-

Silva et al. 2013). From these findings it is concluded that larger a-tDCS application time does not necessarily lead to bigger CSE changes, and new protocols such as within session multiple applications of a-tDCS could be developed to induce larger changes that endure longer (C*hapter 7*).

Another important parameter for application of a-tDCS is current density, which is largely neglected in a-tDCS experimental examinations. Current density comprises the two parameters of current intensity and active electrode size. *Chapter 5* addressed the optimal current intensity for application of a-tDCS by testing a reasonable range of different current intensities (0.3, 0.7, 1.4 and 2 mA) with a constant electrode size of 24 cm^2. The findings indicate a direct relationship between the size of current densities and the size of induced CSE changes in M1 in the three largest current densities (0.7, 1.4 and 2 mA). From these findings, the smallest current intensity (0.3 mA) indeed produces significantly larger CSE changes than the next two higher current densities (0.7 and 1.4 mA), and with less side effects (Bastani & Jaberzadeh 2013a). This finding may promote a smaller current intensity, and thus help avoid the application of unwanted amounts of current to the cortical areas.

In *Chapter 6*, the role of active electrode size on the a-tDCS induced M1 CSE changes is investigated. Reduction in electrode size, while keeping the current density constant, increases the spatial focality of the stimulated area (Nitsche et al. 2007). a-tDCS results in the largest excitability changes in the 12 cm^2 electrode size condition as opposed to the electrode conditions (Bastani & Jaberzadeh 2013b). This highlights the importance of focality of active electrodes during a-tDCS applications. Indeed, by using smaller electrodes we can avoid undesired inhibitory effects from nearby cortical areas

connected functionally to M1 (Biswal et al. 1995; Greicius et al. 2003). In conclusion, reducing stimulation electrode size to one third of the conventional size results in spatially more focused stimulation and increases the efficacy of a-tDCS for induction of larger CSE.

Chapter 8 investigated the effects of current type on M1 CSE changes. In this chapter a novel noninvasive neuromodulatory paradigm is introduced – anodal transcranial pulsed current stimulation (a-tPCS) – and its effect is compared to conventional a-tDCS. This new paradigm is designed to take advantage of two extra parameters, 'pulse duration' and 'inter-pulse interval (IPI)'. a-tPCS is applied with short (a-tPCS$_{SIPI}$) and long inter-pulse interval (a-tPCS$_{LIPI}$). To compare the effects of a-tPCS with a-tDCS the total charge is kept constant in all experimental conditions. As a result, a-tPCS$_{SIPI}$, but not a-tPCS$_{LIPI}$, induces excitability changes in the human cortex that are larger than those produced by the conventional a-tDCS in healthy individuals.

On the other hand, a-tPCS$_{SIPI}$ induces larger CSE changes compared to a-tPCS$_{LIPI}$. This difference in induced CSE size suggests the importance of IPI in induction of CSE changes (Jaberzadeh et al. 2013c). In addition, the participants tolerated a-tPCS better than the conventional a-tDCS. However, retinal phosphene is experienced by two thirds of the subjects during a-tPCS$_{LIPI}$, a-tPCS$_{SIPI}$ and sham a-tPCS$_{SIPI}$ in a frequency dependent manner, as a result of the on/off nature of the current. A further control study confirms that the retinal phosphene does not have any effect on CSE enhancement. In conclusion, compared to a-tDCS, a-tPCS$_{SIPI}$ is a better technique for enhancement of CSE.

4. Inducing longer lasting effects on CSE enhancement and motor performance improvement

a-tPCS$_{SIPI}$ is particularly important in inducing CSE effects, which continue after stimulation. While the longer application of a-tDCS increases the CSE effects (*Chapter 4*), there is an upper limit for sustaining the excitatory lasting effects from a-tDCS (Monte-Silva et al. 2013). Therefore, the most efficient stimulation protocols may turn out to be repetitive daily a-tDCS applications to extend the lasting effects for longer. *Chapter 7* has investigated the effects of within-session repeated application of a-tDCS on the size and duration of CSE changes. In study 6, where one aim was to induce an effect lasting 24 hours using the best parameters of stimulation obtained in *Chapters 5* and *6*, the number of 10-minute repetitions (1, 2 or 3) and the intervals between these 10 minute applications (5 and 25 minutes) were considered as independent variables.

The results show that all single and within-session repeated a-tDCS conditions increase CSE. Compared to single 10 minutes stimulation, the magnitude of the within-session multiple application of a-tDCS induced excitability is enhanced significantly only if the second stimulation is performed after an interval of 25 minutes, but not 5 minutes (Bastani & Jaberzadeh 2014). However, by increasing the number of a-tDCS to three repetitions, the CSE is significantly increased and lasts for 2 hours with both 5 and 25 minute intervals. The results also indicate that if the second or third stimulations are performed with an inter-stimulus interval of 5 or 25 minutes, the combined lasting effects of the two or three a-tDCSs are present for 6 or 24 hours respectively after the end of stimulation. In conclusion, we can benefit from within session multiple applications of a-tDCS for the promotion of longer lasting CSE changes. Based on this

protocol we may bypass the reversal of a-tDCS effects due to homeostatic counter-regulation which happens after continuous long applications. To increase the lasting effects, applying subsequent applications of a-tDCS is a key factor when the effect of the previous stimulation is at a lower size (inter-stimulus intervals of 25 minutes).

Likewise, motor performance is improved following a-tDCS application in all testing conditions. However, in contrast to the study hypotheses no significant differences are seen between the testing conditions within 2 hours after the end of stimulation. Therefore, a one-to-one transferability of the presented results obtained on CSE cannot be extrapolated to the motor performance improvement. The only exception is the a-tDCS condition with three times repetition and a 25 minute inter-stimulus interval. The daylong lasting motor performance improvement seen in this condition is complementary to the related increase in CSE.

Thesis Limitations

Limitations have been provided within each study presented in this thesis. To avoid repetition, only the limitations in the framework of multiple studies are presented here. The group under investigation in all of the studies in this thesis is comprised of healthy young individuals, so findings cannot be extrapolated to older patients or patients with pathological conditions. As well as this, even though participants from both sexes participated in all studies, the gender differences are not explored. Finally, all studies in the present thesis are single-blinded (participants were not aware of the type of stimulation).

Recommendations for future research

The studies in this thesis lend themselves to a number of directions which have not yet been pursued. Further study involving current intensities between 0.2 - 0.7 mA is suggested in order to determine the turning point in the size of induced CSE enhancements. Similarly the impact of different characteristics of pulsatile currents such as the effects of pulse duration, length of IPI and frequency of pulsatile currents, are further areas which could be systematically studied in more detail to determine the optimal parameters in prolongation of the effects of a-tPCS even further. Additionally the analysis can be deepened by exploring the mechanisms of action of a-tPCS and a recommended lower current intensity of a-tDCS. Recommendations include measurement of the silent period, intracortical inhibition, and facilitation, to indirectly assess the role of γ-aminobutyric acid (GABAa and GABAb) and glutamatergic receptors. Additional pharmacological experiments using receptor agonists/antagonists are needed to explore the presumed physiological mechanisms more directly.

Moreover, the effects of different a-tDCS and a-tPCS parameters of stimulation and their tolerability could be studied in different age and gender groups, and patients with neurological problems or psychiatric disorders.

Given the limited number of clinical trials that have assessed the efficacy of a-tDCS on motor performance, further studies using larger sample sizes and long-term follow-ups are needed to find out the extent of CSE changes and their compatibility with the improvements in motor performance, both in healthy individuals and patients with neurological problems, such as stroke. As well, a combination of a-tDCS application and

motor functional therapies may be a potential driver of cortical plastic changes, and this needs to be investigated further. It will also be important to determine the extent to which a-tDCS influences different types of motor tasks commonly used in neuro-rehabilitation trials of fine distal hand movements, and those involving more proximal functions and functional impairment levels.

Now there is hope that finding the optimal parameters and application dosage of a-tDCS might assist in the development of treatment protocols in patients with neurological or psychological problems. This thesis takes a small step in that direction.

APPENDICES

Appendix 1 Sample size calculation

Power analysis for the analysis of variance

This appendix describes statistical procedures for power analysis and estimation of sample size for studies using analysis of variance. These procedures are based on the work of Cohen (Cohen 1988).

SPSS reports the effect size index as eta squared (η^2) or it can be calculated as below:

For the analysis of variance (ANOVA) the effect size index, *f*, is defined by

$$f = \sqrt{\frac{SS_b}{SS_e}}$$

Where SSe is the error sum of squares from the ANOVA summary table. For a one-way ANOVA, SSb is the between-groups sum of squares. For a two-way ANOVA, SSb can represent either an individual main effect or the interaction effect; that is, a separate effect size index can be computed for each effect.

Power table for the ANOVA is arranged according to the degrees of freedom associated with each F-test (df_b) in a one-way ANOVA, this is the between-group effect. In a two-way ANOVA these effects will include each main effect and an interaction effect. The below table give power estimates for different values of the effect size index, *f*, at $df_b = 1$ to 6, 8 and 10 at $\alpha = 0.05$.

Sample size needed for the ANOVA for α = 0.05

	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 2 Supplementary Tables - Chapter 2

Key search terms and associated variations

Cortical excitability	Transcranial magnetic stimulation	Anodal transcranial direct current stimulation	Physical performance	Stroke
- Cortical excitability - Corticomotor excitability - Cortical excitation - Cortical plasticity - Cort$ excita$ - Evoked potentials - Motor cortex - Motor evoked potential$ - Primary motor area - Cortical activation - Premotor activation	-Transcranial magnetic stimulation -TMS -Magnetic stimulation	-Anodal transcranial direct current stimulation -Anodal tDCS -transcranial direct current stimulation -tDCS -transcranialstimul$	-Physical performance -Motor performance -Motor function -Function$ -performance -Motor skill -Motor activity -Practice -Physical function	-Stroke -Brain infarction -Neurologically impaired -Neurologic$ disorder$ -Hemiplegi$

Databases with similar Search Strategy in each category

Search Strategy Category	Databases
1	CINAHL, Ovid Medline
2	AMED, CENTRAL, EBM Reviews,AMI, Meditext, PROQuestPsychinfo, SPORTDiscus, EMBASE, Scopus,CHOCHRANE
3	PubMed,PEDro

PEDro quality assessment for included studies

	Eligibility criteria were specified	Subjects were randomly allocated to groups	Allocation was concealed	Similar baseline measurements	blinding of all subjects	blinding of all therapists	blinding of all assessors	>85% subjects available for assessment	All subjects for whom outcome measures were available received the treatment or data for at least one key outcome was analysed by intention to treat.	The results of statistical comparisons are reported for at least one key outcome	The study provides both point measures and measures of variability for at least one key outcome	**Quality Score**
Hummel et al (2005)	✓	×	×	✓	✓	×	✓	✓	✓	✓	✓	**7**
Boggio et al (2007)	✓	×	×	✓	✓	×	✓	✓	✓	✓	✓	**7**
Kim et al (2009)	✓	×	×	✓	✓	×	×	✓	✓	✓	✓	**6**
Fregni et al (2005)	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	**8**
Boggio et al (2006a)	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	**8**
Hummel et al (2009)	✓	✓	×	✓	✓	×	✓	✓	✓	✓	✓	**8**

D&B quality assessment for included studies

	Is the hypothesis/aim/objective of the study clearly described?	Are the main outcomes to be measured clearly described in the Introduction or Methods section?	Are the characteristics of the patients included in the study clearly described?	Are the interventions of interest clearly described?	Are the distributions of principal confounders in each group of subjects to be compared clearly described?	Are the main findings of the study clearly described?	Does the study provide estimates of the random variability in the data for the main outcomes?	Have all important adverse events that may be a consequence of the intervention been reported?	Have the characteristics of patients lost to follow-up been described?	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?	Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	Was an attempt made to blind study subjects to the intervention they have received ?	Was an attempt made to blind those measuring the main outcomes of the intervention?	If any of the results of the study were based on "data dredging", was this made clear?	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?	Were the statistical tests used to assess the main outcomes appropriate?	Was compliance with the intervention/s reliable?	Were the main outcome measures used accurate (valid and reliable)?	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?	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?	Were study subjects randomised to intervention groups?	Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	Was there adequate adjustment for confound ding in the analyses from which the main findings were drawn?	Were losses of patients to follow-up taken into account?	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%?	**Quality Score**
Nitsche and Paulus (2000)	1	1	1	1	0	1	1	0	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	1	0	**14**
Jeffery et al (2007)	1	1	1	1	0	1	1	0	1	1	0	0	0	1	0	1	1	1	1	1	0	1	1	0	0	1	0	**17**
Boros et al (2008)	1	1	1	1	0	1	1	0	1	1	0	0	0	1	0	1	1	1	1	1	0	1	0	0	0	1	0	**16**
Edwards et al (2009)	1	1	1	1	0	1	1	0	1	1	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	1	0	**15**
Antal et al (2007)	1	1	1	1	0	1	1	0	1	1	0	0	0	1	0	1	1	1	1	1	0	1	0	0	0	1	0	**16**
Furubayashi et al (2008)	1	1	1	1	0	1	1	0	1	1	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	1	0	**15**
Uy and Ridding (2003)	1	1	1	1	0	1	1	0	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	1	0	**14**
Nitsche et al (2005)	1	1	1	1	0	1	1	0	1	1	0	0	0	0	0	1	1	1	1	1	0	1	0	0	0	1	0	**15**

Appendix 3 Search strategy for systematic review and meta-analysis

Databeses were grouped together based on their search function characteristics.

Group 1: Databases where papers were listed under and searched by MeSH otherwise known as indexing terms, as well as by keywords. These databases had functions that allowed the MeSH and keywords used in the used in the search to be combined using an appropriate combining term (AND or OR). They also utilized truncation symbols (e.g.?,$) that can be applied to keywords (Ovid Medline, CINAHL).

Group 2: Databases where papers were searched by keywords only and had limitedability for combination of keyword searches with an AND or OR. Wherever possible,truncation symbols were applied. Allied Health and Complementary Medicine Database (AMED), CENTRAL, Evidence Based Medicine (EBM) Reviews, AMI, Meditext, PROQuest, Psychinfo, SPORTDiscus, EMBASE, Scopus, CHOCHRANE).

Group 3: Databases where papers were searched by keywords only and had no ability to apply truncation symbols. A single term, for example “cortical excitability” or “anodal transcranial direct current stimulation” was searched for separately (PEDro, PubMed).

Group 1: Medline

1. cortical excitability.mp. [mp= title, original title, mesh subject heading]
2. corticomotor excitability.mp. [mp= title, original title, mesh subject heading]
3. cortical excitation.mp. [mp= title, original title, mesh subject heading]
4. cort$ excit$. mp. [mp= title, original title, mesh subject heading]
5. cortical plasticity. mp. [mp= title, original title, mesh subject heading]
6. Evoked potentials.mp. [mp= title, original title, mesh subject heading]
7. Motor cortex.mp. [mp= title, original title, mesh subject heading]
8. Motor evoked potential$.mp. [mp= title, original title, mesh subject heading]
9. Primary motor area.mp. [mp= title, original title, mesh subject heading]
10. Primary motor cortex.mp. [mp= title, original title, mesh subject heading]
11. Cortical activation.mp. [mp= title, original title, mesh subject heading]
12. Premotor activation.mp. [mp= title, original title, mesh subject heading]
13. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11or 12
14. anodaltranscranial direct current stimulation$.mp. [mp= title, original title, mesh subject head
15. anodal tDCS .mp. [mp= title, original title, mesh subject heading]
16. transcranial stimulation$.mp. [mp= title, original title, mesh subject heading]
17. transcranial direct current stimulation.mp. [mp= title, original title, mesh subject heading]
18. tDCS.mp. [mp= title, original title, mesh subject heading]
19. 14 or 15 or 16 or17 or 18
20. TMS.mp. [mp= title, original title, mesh subject heading]
21. transcranial magnetic stimulation.mp. [mp= title, original title, mesh subject heading]
22. magnetic stimulation.mp. [mp= title, original title, mesh subject heading]
23. transcrani$ magnetic$ stimul$.mp. [mp= title, original title, mesh subject heading]
24 . 20 or 21 or 22 or 23
25. physical performance.mp. [mp= title, original title, mesh subject heading]
26. motor function.mp. [mp= title, original title, mesh subject heading]
27. physical function.mp. [mp= title, original title, mesh subject heading]
28. motor performance.mp. [mp= title, original title, mesh subject heading]
29. function$ recovery.mp. [[mp= title, original title, mesh subject heading]
30. motor skill.mp.[mp= title, original title, mesh subject heading]
31. Motor activity.mp. [mp= title, original title, mesh subject heading]
32. Practice.mp. [mp= title, original title, mesh subject heading]
33. Physical function.mp. [mp= title, original title, mesh subject heading]
34. 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33
35. Stroke.mp. [mp= title, original title, mesh subject heading]
36. Brain infarction.mp. [mp= title, original title, mesh subject heading]
37. Neurologically impaired .mp. [mp= title, original title, mesh subject heading]
38. Neurologic$ disorder$.mp. [mp= title, original title, mesh subject heading]
39. Hemiplegi$.mp. [mp=abstract, heading words, title]
40. 35 or 36 or 37 or 38 or 39
41. 13 and 19 and 24 and 40
42. 13 and 19 and 24 and 34 and 40
43. limit 41 to (English language and humans)
44. limit 42 to (English language and humans)

Group 2: EBM Reviews

1. cortical excitability.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
2. corticomotor excitability.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
3. cortical excitation.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
4. cort$ excit$. mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
5. cortical plasticity.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
6. Evoked potentials.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
7. Motor cortex.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
8. Motor evoked potential$.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
9. Primary motor area.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
10. Primary motor cortex.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
11. Cortical activation.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
12. Premotor activation.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
13. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11or 12
14. anodaltranscranial direct current stimulation$.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
15. anodal tDCS .mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
16. transcranial stimulation$.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
17. transcranial direct current stimulation.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
18. tDCS.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
19. 14 or 15 or 16 or17 or 18
20. TMS.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
21. transcranial magnetic stimulation.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
22. magnetic stimulation.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
23. transcrani$ magnetic$ stimul$.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
24 . 20 or 21 or 22 or 23
25. physical performance.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
26. motor function.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
27. physical function.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
28. motor performance.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
29. function$ recovery.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
30. motor skill.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
31. Motor activity.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
32. Practice.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
33. Physical function.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
34. 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33
35. Stroke.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
36. Brain infarction.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
37. Neurologically impaired .mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
38. Neurologic$ disorder$.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
39. Hemiplegi$.mp. [mp=ti, ot, ab, tx, kw, ct, sh, hw]
40. 35 or 36 or 37 or 38 or 39
41. 13 and 19 and 24 and 40
42. 13and 19 and 24 and 34 and 40
43. limit 41 to (English language and humans)
44. limit 42 to (English language and humans)

Group 3: PEDro
1. cortical excitability 2. corticomotor excitability 3. cortical excitation 4. cortical plasticity 5. Evoked potentials 6. Motor cortex 7. Motor evoked potentials 7. Primary motor area 9.Primary motor cortex 10. Cortical activation 11. Premotor activation 12. anodal transcranial direct current stimulations 13. anodal tDCS 14. transcranial stimulations 15.transcranial direct current stimulation 16.tDCS 16. TMS 18. transcranial magnetic stimulation 19. magnetic stimulation

Appendix 4 PEDro scale

Pedro criteria	Definition
1. Eligibility criteria were specified	This criterion is satisfied if the report describes the source of subjects and a list of criteria used to determine who was eligible to participate in the study
2. Subjects were randomly allocated to groups (in a crossover study, subjects were randomly allocated an order in which treatments were received)	A study is considered to have used random allocation if the report states that allocation was random. The precise method of randomisation need not be specified. Procedures such as coin-tossing and dice-rolling should be considered random. Quasi-randomisation allocation procedures such as allocation by hospital record number or birth date, or alternation, do not satisfy this criterion
3. Allocation was concealed	Concealed allocation means that the person who determined if a subject was eligible for inclusion in the trial was unaware, when this decision was made, of which group the subject would be allocated to. A point is awarded for this criteria, even if it is not stated that allocation was concealed, when the report states that allocation was by sealed opaque envelopes or that allocation involved contacting the holder of the allocation schedule who was "off-sit
4. The groups were similar at baseline regarding the most important prognostic indicators	At a minimum, in studies of therapeutic interventions, the report must describe at least one measure of the severity of the condition being treated and at least one (different) key outcome measure at baseline. The rater must be satisfied that the groups' outcomes would not be expected to differ, on the basis of baseline differences in prognostic variables alone, by a clinically significant amount. This criterion is satisfied even if only baseline data of study completers are presented.

5. There was blinding of all subjects 6. There was blinding of all therapists who administered the therapy 7. There was blinding of all assessors who measured at least one key outcome	*Blinding* means the person in question (subject, therapist or assessor) did not know which group the subject had been allocated to. In addition, subjects and therapists are only considered to be "blind" if it could be expected that they would have been unable to distinguish between the treatments applied to different groups. In trials in which key outcomes are self-reported (eg, visual analogue scale, pain diary), the assessor is considered to be blind if the subject was blind.
8. Measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups	This criterion is only satisfied if the report explicitly states both the number of subjects initially allocated to groups and the number of subjects from whom key outcome measures were obtained. In trials in which outcomes are measured at several points in time, a key outcome must have been measured in more than 85% of subjects at one of those points in time.
9. All subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analysed by "intention to treat"	An intention to treat analysis means that, where subjects did not receive treatment (or the control condition) as allocated, and where measures of outcomes were available, the analysis was performed as if subjects received the treatment (or control condition) they were allocated to. This criterion is satisfied, even if there is no mention of analysis by intention to treat, if the report explicitly states that all subjects received treatment or control conditions as allocated.

10. The results of between-group statistical comparisons are reported for at least one key outcome	A between-group statistical comparison involves statistical comparison of one group with another. Depending on the design of the study, this may involve comparison of two or more treatments, or comparison of treatment with a control condition. The analysis may be a simple comparison of outcomes measured after the treatment was administered, or a comparison of the change in one group with the change in another (when a factorial analysis of variance has been used to analyse the Data, the latter is often reported as a group × time interaction). The comparison may be in the form hypothesis testing (which provides a "p" value, describing the probability that the groups differed only by chance) or in the form of an estimate (for example, the mean or median difference, or a difference in proportions, or number needed to treat, or a relative risk or hazard ratio) and its confidence interval
11. The study provides both point measures and measures of variability for at least one key outcome	A point measure is a measure of the size of the treatment effect. The treatment effect may be described as a difference in group outcomes, or as the outcome in (each of) all groups. Measures of variability include standard deviations, standard errors, confidence intervals, interquartile ranges (or other quantile ranges), and ranges. Point measures and/or measures of variability may be provided graphically (for example, sds may be given as error bars in a figure) as long as it is clear what is being graphed (for example, as long as it is clear whether error bars represent sds or ses). Where outcomes are categorical, this criterion is considered to have been met if the number of subjects in each category is given for each group.

From PEDro (1999), http://www.pedro.org.au/scale_item.html

Appendix 5 Decision rules for the PEDro scale

Criteria	Decision Rule
All Criteria	Points are only awarded when a criterion is clearly satisfied. If on a literal reading of the trial report it is possible that a criterion was not satisfied, a point should not be awarded for that criterion.
Criterion 1	This criterion is satisfied if the report describes the source of subjects and a list of criteria used to determine who was eligible to participate in the study.
Criterion 2	A study is considered to have used random allocation if the report states that allocation was random. The precise method of randomisation need not be specified. Procedures such as coin-tossing and dice-rolling should be considered random. Quasi-randomised allocation procedures such as allocation by hospital record number or birth date, or alternation, do not satisfy this criterion.
Criterion 3	Concealed allocation means that the person who determined if a subject was eligible for inclusion in the trial was unaware, when this decision was made, of which group the subject would be allocated to. A point is awarded for this criteria, even if it is not stated that allocation was concealed, when the report states that allocation was by sealed opaque envelopes or that allocation involved contacting the holder of the allocation schedule who was "off-site".
Criterion 4	At a minimum, in studies of therapeutic interventions, the report must describe at least one measure of the severity of the condition being treated and at least one (different) key outcome measure at baseline. The rater must be satisfied that the groups' outcomes would not be expected to differ, on the basis of baseline differences in prognostic variables alone, by a clinically significant amount. This criterion is satisfied even if only baseline data of study completers are presented.
Criterion 4, 7-11	Key outcomes are those outcomes which provide the primary measure of the effectiveness (or lack of effectiveness) of the therapy. In most studies, more than one variable is used as an outcome measure.

Criterion 5-7	Blinding means the person in question (subject, therapist or assessor) did not know which group the subject had been allocated to. In addition, subjects and therapists are only considered to be "blind" if it could be expected that they would have been unable to distinguish between the treatments applied to different groups. In trials in which key outcomes are self-reported (eg, visual analogue scale, pain diary), the assessor is considered to be blind if the subject was blind.
Criterion 8	This criterion is only satisfied if the report explicitly states both the number of subjects initially allocated to groups and the number of subjects from whom key outcome measures were obtained. In trials in which outcomes are measured at several points in time, a key outcome must have been measured in more than 85% of subjects at one of those points in time.
Criterion 9	An intention to treat analysis means that, where subjects did not receive treatment (or the control condition) as allocated, and where measures of outcomes were available, the analysis was performed as if subjects received the treatment (or control condition) they were allocated to. This criterion is satisfied, even if there is no mention of analysis by intention to treat, if the report explicitly states that all subjects received treatment or control conditions as allocated.
Criterion 10	A between-group statistical comparison involves statistical comparison of one group with another. Depending on the design of the study, this may involve comparison of two or more treatments, or comparison of treatment with a control condition. The analysis may be a simple comparison of outcomes measured after the treatment was administered, or a comparison of the change in one group with the change in another (when a factorial analysis of variance has been used to analyse the data, the latter is often reported as a group x time interaction). The comparison may be in the form of hypothesis testing (which provides a "p" value, describing the probability that the groups differed only by chance) or in the form of an estimate (for example, the mean or median difference, or a difference in proportions, or number needed to treat, or a relative risk or hazard ratio) and its confidence interval.
Criterion 11	A point measure is a measure of the size of the treatment effect. The treatment effect may be described as a difference in group outcomes, or as the outcome in (each of) all groups. Measures of variability include standard deviations, standard errors, confidence intervals, inter-quartile ranges (or other quantile ranges), and ranges. Point measures and/or measures of variability may be provided graphically (for example, SDs may be given as error bars in a Figure) as long as it is clear what is being graphed (for example, as long as it is clear whether error bars represent SDs or SEs). Where outcomes are categorical, this criterion is considered to have been met if the number of subjects in each category is given for each group.

Appendix 6 D&B Quality Assessment scale

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	1
		Partially	0
		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 finding 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

Yes	1
No	0
Unable to determine	0

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.

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 nonparametric 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. *Internal validity - confounding (selection bias)*

Yes	1
No	0
Unable to determine	0

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)*
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 were randomised 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

Yes	1
No	0
Unable to determine	0

between the treatment groups but was not taken into account in the analyses. In nonrandomised 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.

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	*<n1*	*0*
B	*n1-n2*	*1*
C	*n3-n4*	*2*
D	*n5-n6*	*3*
E	*n7-n8*	*4*
F	*n8+*	*5*

Appendix 7 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 in reports and references as functional X-Y type scatter, linear, semi-log, or log-log plot. In order to use this data, it must somehow be digitized.

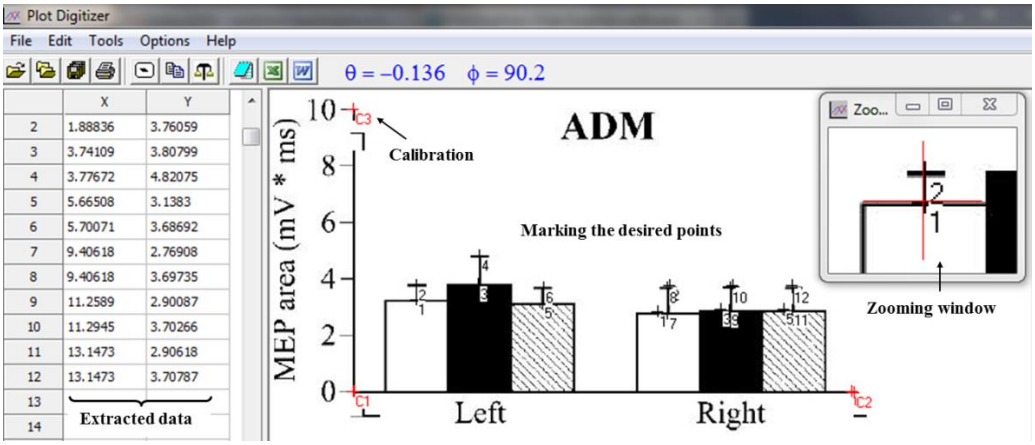
This program will allow you to take a scanned image of a plot (in JPEG or Bitmap) and quickly digitize values off the plot just by clicking the mouse on each data point after calibration. Any 3 non-collinear points can be used for calibration and calibration points **do not** need to be on the axes. Data can be export to an 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 "Tool" menu and then digitize the points.

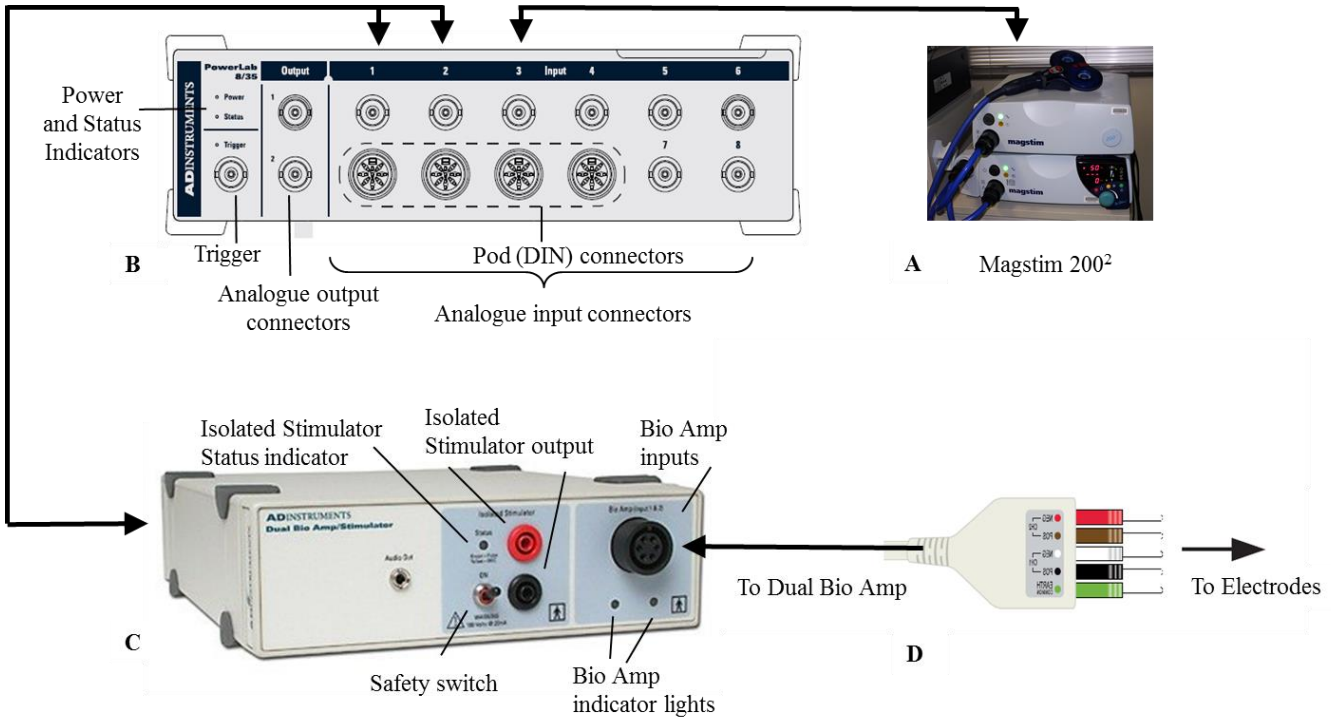
Hint: If you want to digitize plots from published technical reports that are available electronically in PDF format, you can copy the image with the

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 Plot Digitizer.

Appendix 8 The set up system used in the present thesis



A) Magstim 200^2 **B)** The powerlab 8/30 has three indicators at the left of the 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 pod (DIN) connectors for inputs 1-4, for recording external signals **C)** Dual Bioamp/stimulator **D**) Cables for recording EMG of the target muscle(s).

Appendix 9 Reliability study ethics approval

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

Date: **23 February 2011**

Project Number: **CF10/2590 - 2010001443**
Project Title: **Intra and inter-session reliability of muscle responses elicited by transcranial magnetic stimulation**
Chief Investigator: **Dr Shapour Jaberzadeh**
Approved: **From: 23 February 2011** **To: 23 February 2016**

Terms of approval

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, and a copy forwarded to MUHREC before any data collection can occur at the specified organisation. **Failure to provide permission letters to MUHREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research**.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the 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 University letterhead and the Monash University complaints clause must contain your project number.
6. **Amendments to the approved project (including changes in personnel):** Requires the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
8. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

[redacted]

Professor Ben Canny
Chair, MUHREC

cc: Ms Andisheh Bastani Jahromi

Appendix 10 a-tDCS study ethics approval

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

Date: **20 June 2011**

Project Number: **CF11/0741 - 2011000367**
Project Title: **Anodal transcranial direct current stimulation and enhancement of corticomotor excitability: the effects of duration and intensity**
Chief Investigator: **Dr Shapour Jaberzadeh**
Approved: **From: 20 June 2011 To 20 June 2016**

Terms of approval

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, and a copy forwarded to MUHREC before any data collection can occur at the specified organisation. **Failure to provide permission letters to MUHREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research**.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is the 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 University letterhead and the Monash University complaints clause must contain your project number.
6. **Amendments to the approved project (including changes in personnel):** Requires the submission of a Request for Amendment form to MUHREC and must not begin without written approval from MUHREC. Substantial variations may require a new application.
7. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
8. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. This is determined by the date of your letter of approval.
9. **Final report:** A Final Report should be provided at the conclusion of the project. MUHREC should be notified if the project is discontinued before the expected date of completion.
10. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by MUHREC at any time.
11. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

[redacted]

Professor Ben Canny
Chair, MUHREC

cc: Ms Andisheh Bastani Jahromi

Appendix 11 Ethics amendment approval

MUHREC Amendment CF11/0741 - 2011000367: Anodal transcranial direct current stimulation and enhancement of corticomotor excitability: the effects of duration and intensity

Dear Researchers

Thank you for submitting a Request for Amendment to the above named project.
This is to advise that the following amendments have been approved and the project can proceed according to your approval given on 20 June 2011:

Change to procedures:

1. To increase the length of a-tDCS application from 25 minutes (in the current project) to 30 minutes to compare the effects of continuous 30 min with multi session (10+10+10 minutes of a-tDCS application with 25 minutes intervals).
2. The addition of Purdue pegboard as a test for evaluation of functional performance. This is a test to see how quickly and accurate one can work with his hands. The test board consists of four cups across the top and two vertical columns of 25 small holes. The cups contain 25 pins each. In this test subjects are asked to take up one peg at a time with their right hand from these cups. Starting with the top hole, they place each peg in the right handed column. Performance requires participants to place 25 pins down the right column as fast as possible with their right hand.
3. To increase the length of post intervention follow-up measurements from 30 minutes up to 6 hours (See Fig 1).15 MEPs and the time for completion of Pegboard test will be recorded every 30 minutes up to two hours and then they will be recorded every 1 hour to compare the after effects of these single session/multi sessions applications of a-tDCS.

Thank you for keeping the Committee informed.

Professor Ben Canny
Chair, MUHREC
Human Ethics
Monash Research Office

Appendix 12 Edinburgh Handedness Questionnaire

Subject's Code:

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 absolutely forced to, put two checks (✓✓).
If you are indifferent, put one check 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 13 TMS safety Questionnaire

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:

☐ Has psychiatric or neurological illnesses (including brain injury, cranial surgery)?
☐ Has seizure, epilepsy, heat convulsion, head injury and has epilepsy and seizure in first degree relatives?
☐ Has any metal in the head (outside the mouth); any metallic particles in the eye, implanted cardiac pacemaker or any intracardiac lines?
☐ Has frequent or severe headaches, history of migraine?
☐ Has any implanted neurostimulators, surgical clips, medical pumps and any implanted electrical biomedical device (defibrillator, acoustic device)?
☐ If pregnant?
☐ Has taking any medications, excessive use of caffeine or energy drinks?
☐ Has sleep deprivations?
☐ Has unable to speak, read or write English

Status for study: ☐ INCLUDED ☐ EXCLUDED

Full name: ……..

Date: ……..

Contact details: ……

Telephone: …… **Email:** ……..

Address: ……

Appendix 14 Transcranial Magnetic Stimulation Adult Safety Screen

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 Electroencepalogram (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 it's 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 15 Consent Form

MONASH University
Medicine, Nursing and Health Sciences

Project Title: ……..

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 participate in two phases of testing**

☐ **I agree to take part in the following experimental procedures:**
a. TranscranialDirectCurrentStimulation (tDCS)
b. TranscranialMagneticbrain Stimulation (TMS)
c. Recording of muscle activity using surface electrodes

☐ **I understand that I can withdraw all records of my participation in study up till 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 penalised 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 a secure storage and accessible to the research team. I also understand that the data will be destroyed after a 5 year period.

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's name: .....

Signature: .....**Date:** .....

Researcher's name: .....

Signature: ..... **Date:** .....

Appendix 16 Purdue pegboard test instruction

When beginning the assessment for right handed participants, she/he is supposed to start off on the right side of the board.

- Sit comfortably in front of the board.

This is a test to see how quickly and accurate you can work with your hands. Before you start the test you would be told what to do and then you have an opportunity to practice. Be sure you understand exactly what to do.

- You are supposed to start off on the right side of the board.
- Take up one peg at a time with your right hand from the above cup. Starting with the top hole, place each peg in the right handed row. Now you can start a few pegs for practice. If during the test you drop a peg, do not start to pick it up and continue by picking another peg from the cup.

After the practice

- Now take out the practice pegs and put it in the cup with your left hand.

When I say begin, place 25 pegs as rapidly as you can in the right handed row, until I say stop. Start with the top hole.

Are you ready?

Begin

Stop

Appendix 17 a-tPCS study ethics approval

MUHREC Amendment CF12/2764 – 2012001504: Anodal-transcranial pulsed current stimulation: the effects of inter pulse interval, pulse duration and frequency on corticospinal excitability

Dear Researchers

Thank you for submitting a Request for Amendment to the above named project.

This is to advise that the following amendments have been approved and the project can proceed according to your approval given on 23 November 2012:

Changes to Title:

1. From 'Anodal-transcranial pulsed current stimulation: The effects of inter pulse interval, pulse duration and frequency on corticospinal excitability' to 'Transcranial pulsed current stimulation : an innovative neuromodulatory technique to boost capacity of our ever-changing brain further'

Changes to Personnel:

2. Addition of Ms Andishe Bastani and Ms Prue Morgan

Changes to Procedures

3. Addition of an extra group of participants, with chronic stroke, at least 6 months post-stroke.

Thank you for keeping the Committee informed.

Professor Ben Canny Chair, MUHREC
Human Ethics
Monash Research Office

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