Transcranial direct current stimulation: The effects on excitability and pain perception in healthy adults

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"It is only when we become conscious of our part in life, however modest, that we shall be happy. Only then, we will be able to live in peace and die in peace, for only this lends meaning to life…"

Wind, Sand, and Stars
Antoine de Saint-Exupery

I would like to dedicate this thesis, however modest, to my beloved parents.

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Publications in peer-reviewed journals

1. **Vaseghi B.**, Zoghi M., and Jaberzadeh S., Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study. Clin Neurophysiol, 2014. 125 (9): 1847-58.
2. **Vaseghi B.**, Jaberzadeh S., and Zoghi M., Inter-pulse Interval Affects the Size of Single-pulse TMS-induced Motor Evoked Potentials: a Reliability Study. Basic and Clinical Neuroscience, 2015. 6 (1): 44-51.
3. **Vaseghi B.**, Zoghi M., and Jaberzadeh S., How does anodal transcranial direct current stimulation of the pain neuromatrix affect brain excitability and pain perception? A randomised, double-blind, sham-control study. PLoS One, 2015. 10(3): e0118340.
4. **Vaseghi B.**, Zoghi M., and Jaberzadeh S., A meta-analysis of site-specific effects of cathodal transcranial direct current stimulation on sensory perception and pain. PLoS One, 2015, doi: 10.1371/journal.pone.0123873.
5. **Vaseghi B**., Zoghi M., and Jaberzadeh S., Differential effects of cathodal-tDCS of prefrontal, motor, and somatosensory cortices on cortical excitability and pain perception: a double-blind randomised sham-controlled study. European Journal of Neuroscience, 2015.

Paper accepted awaiting publication

1. Vaseghi B., Zoghi M., and Jaberzadeh S., The effects of anodal-tDCS on corticospinal excitability: conventional versus unihemispheric concurrent dual-site stimulation. Frontiers in Human Neuroscience, 2015.

Paper under peer review

1. Vaseghi B., Zoghi M., and Jaberzadeh S., Unihemispheric concurrent dual-site cathodal transcranial direct current stimulation: the effects on size and duration of corticospinal excitability alteration in healthy adults. (Submitted to Brain Stimulation Journal)
2. Vaseghi B., Zoghi M., and Jaberzadeh S., Unihemispheric dual-site stimulation of the cortical areas of pain neuromatrix induces distinct sensory and pain threshold alteration. (Submitted to Brain Stimulation Journal)

Oral presentation

1. **Vaseghi B**., Jaberzadeh S., Zoghi M., Inter-pulse interval affects the size of single-pulse TMS-induced motor evoked potentials: a reliability study, Australian Brain Stimulation Meeting, Melbourne, Australia, July 25-26 2013.
2. **Vaseghi B.**, Jaberzadeh S., Bastani A., 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, November 7-9 2012.
3. Jaberzadeh S., Bastani A., **Vaseghi B.**, Aminitehrani M., Hashemirad F., Zoghi M., Kouzani A., Fitzgerald P.B., The effects of mobile phone radiation on primary motor cortex excitability: a pilot study.

Poster presentation

1. **Vaseghi B.**, Jaberzadeh S., Zoghi M., Inter-pulse interval affects the size of single-pulse TMS-induced motor evoked potentials, 11th Motor Control and Human Skill Conference, Melbourne, Australia, November 27-29 2013.
2. Jaberzadeh S., **Vaseghi B.**, Zoghi M., Bastani A., 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, 3-6 February 2013
3. **Vaseghi B.**, Jaberzadeh S., Bastani A., 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, 3-6 February 2013
4. **Vaseghi B.**, Jaberzadeh S.,Zoghi M., Bastani A., Pain management by anodal transcranial direct current stimulation (a-tDCS): a systematic review and meta-analysis. Higher Degree Research (HDR) student festival, Monday-26 November 2012
5. **Vaseghi B.**, Zoghi M., Moseley GL., Jaberzadeh S., An investigation of MEP size and reliability: the effects of single pulse inter stimulus interval, 11th Motor Control and Human Skill Conference, Melbourne, Australia, November 2013.
6. Jaberzadeh S., **Vaseghi B.**, Zoghi M., Unihemispheric dual-channel a-tDCS of pain neuromatrix: a novel technique for modulation of corticospinal excitability, 1ST International Brain Stimulation Conference, 2-4 March, Singapore 2015.
7. Zoghi M., **Vaseghi B.**, Bastani A., Jaberzadeh S., Galea M., Do Hormonal changes during menstrual cycle affect excitability? A pilot study, 1ST International Brain Stimulation Conference, 2-4 March, Singapore 2015.
8. Vaseghi B., Zoghi M., Jaberzadeh S., Unihemispheric dual-site cathodal-tDCS: a novel neuromodulatory technique to enhance corticospinal excitability of primary motor cortex, INS 12th world congress, Montreal, Canada, 6-11 June 2015.

Thesis abstract

Painful stimuli are processed in a network called the pain neuromatrix (PNM) which comprises both the cortical and subcortical areas of the brain. The primary motor cortex (M1), primary sensory cortex (S1), and dorsolateral prefrontal cortex (DLPFC) are the cortical sites of the PNM. Literature indicates that modulatory changes occur in the excitability of these cortical sites during pain processing. These changes coincide with behavioral modulations such as sensory (STh) and pain (PTh) thresholds, and pain level (PL) changes. Transcranial direct current stimulation (tDCS) of cortical sites provides supportive evidence for the modulation of these cortical and behavioral changes. tDCS is a non-invasive neuromodulatory technique with a polarity dependent manner. The application of an anode over the target cortical sites (a-tDCS) increases corticospinal excitability (CSE), and the application of a cathode over the target (c-tDCS) decreases the CSE. Although there is an upward trend using a- and c-tDCS over M1, S1, and DLPFC for increasing STh and/or PTh, there is no consensus on the superiority of different tDCS modes and stimulation sites on the aforementioned effects. Therefore, the broad aim of the present study was to investigate the effects of tDCS modes and stimulation sites on CSE and STh/PTh modulation.

Prior to the experimental studies, two systematic review and meta-analyses (Studies 1-2) were conducted to verify the effect of a-tDCS and c-tDCS on different cortical sites of the PNM on STh and PTh in healthy individuals and patients with chronic pain. These reviews confirmed that these stimulation effects are site specific in both healthy and patient groups following a- and c-tDCS.

A reliability study was then conducted to test the intra- and intersession reliability of elicited MEPs and to fine-tune the set-up for application of TMS as an assessment tool (Study 3). The reliability study was a necessary step before conduction of the other experimental studies in this thesis.

In Studies 4 and 5, we investigated how single-site a-tDCS and c-tDCS of functionally connected

cortical sites of the PNM affect the level of M1 and S1 excitability. The result of Study 4 showed that a-tDCS of M1 and DLPFC are the best cortical sites for induction of greater CSE. This site specificity was not found for STh/PTh changes and a-tDCS of these three cortical sites increased STh/PTh in healthy adults. The results of Study 5 showed that c-tDCS of M1, S1, and DLPFC reduce M1 and S1 excitability, while it had opposite effects on STh/PTh. In fact, no site specificity was found following c-tDCS of these cortical sites in healthy adults.

Studies 6 and 7 compared the effects of single-site (conventional) tDCS and a novel tDCS technique termed unihemispheric concurrent dual-site tDCS ($tDCS_{UHCDS}$) on M1 CSE, short-interval intracortical inhibition (SICI), and intracortical facilitation (ICF). In this technique two unihemispheric functionally connected sites of the PNM were concurrently stimulated to intensify tDCS-induced CSE changes. Study 6 indicated that a-$tDCS_{UHCDS}$ of M1-DLPFC induces larger M1 CSE with day-long lasting effects, compared to M1 a-tDCS. This increase was mainly associated with an ICF increase. Study 7 showed that the application of c-$tDCS_{UHCDS}$ on cortical sites of the PNM not only failed to induce inhibitory effects, but even induced excitatory changes in some experimental conditions. These changes were associated with an ICF increase and a SICI decrease. Overall, in these two studies, we concluded that $tDCS_{UHCDS}$ is a more effective technique for induction of CSE changes compared to single-site tDCS.

In Study 8, this novel technique is used to explore the effect of both a- and c-$tDCS_{UHCDS}$ of cortical sites of the PNM on STh/PTh. The results in this concluding chapter revealed that, compared to single-site tDCS and c-$tDCS_{UHCDS}$, a-$tDCS_{UHCDS}$ of M1-DLPFC is the most efficient technique to enhance STh and PTh with day-long lasting effects.

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 2 unpublished (under review) publications. The core theme is Transcranial Direct Current Stimulation: The effects on M1 cortico spinal excitability (CSE), and sensory/pain perception in healthy adults. 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 and Dr. Maryam Zoghi.

The inclusion of co-authors reflects 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, chapter 8, and Chapter 9 my contribution to the work involved the following:

Project design (in consultation with my supervisors); 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	Number and extend of candidate's contribution
2	Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study	Published	80%*
3	A meta-analysis of site-specific effects of cathodal transcranial direct current stimulation on sensory perception and pain.	Published	80% *
4	Inter-pulse Interval Affects the Size of Single-pulse TMS-induced Motor Evoked Potentials: a Reliability Study.	Published	80% *
5	How does anodal transcranial direct current stimulation of the pain neuromatrix affect brain excitability and pain perception? A randomised, double-blind, sham-control study.	Published	80% *
6	Differential effects of cathodal-tDCS of prefrontal, motor, and somatosensory cortices on cortical excitability and pain perception: a double-blind randomised sham-controlled study	Published	80% *
7	The effects of anodal-tDCS on corticospinal excitability: conventional versus unihemispheric concurrent dual-site stimulation.	Accepted	80% *
8	Unihemispheric concurrent dual-site cathodal transcranial direct current stimulation: the effects on size and duration of corticospinal excitability alteration in healthy adults	Under review	80% *
9	Sensory and pain threshold enhancement in healthy adults by unihemispheric concurrent dual-site transcranial direct current stimulation	Under review	80% *

(*) Project design; review of appropriate literature; securing ethics approval; recruitment of participants; data collection; conducting data analysis and writing of papers

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

Candidate's name: Bita Vaseghi Signed: Date: 15-Sep-2015

Main supervisor's name: Dr. Shapour Jaberzadeh Signed: Date: 15-Sep-2015

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Bita Vaseghi

Abbreviations

Abbreviations	Definition
a-tDCS	Anodal Transcranial Direct Current Stimulation
a-tDCS$_{UHCDS}$	Unihemispheric Concurrent Dual-site Anodal Transcranial Direct Current Stimulation
Ag/Aghcl	Silver/Silver Chloride
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
c-tDCS	Cathodal transcranial direct current stimulation
c-tDCS$_{UHCDS}$	Unihemispheric Concurrent Dual-site Cathodal Transcranial Direct Current
CBF	Cerebral blood flow
CENTRAL	Cochrane Central Register of Controlled Trialls
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

D&B	Down and Black quality assessment scale
DC	Direct Current
DLPFC	Dorsolateral Prefrontal Cortex
FDI	First Dorsal Interossei
fMRI	Functional Magnetic Resolution Imaging
GABA	*gamma*-Aminobutyric acid
Hz	Hertz
ICC	Intra-class Correlation Coefficient
ICF	Intracortical Facilitation
IPI	Inter-pulse Interval
ISI	Inter-stimulus Interval
LICI	Long-interval Intracortical Inhibition
LTD	Long Term Depression
LTP	Long Term Potentiation
M1	Primary Motor Cortex
mA	Milliampere
MEG	Magnetoencephalography
MEP	Motor Evoked Potential
MeSH	Medical Subject Heading
MN	Median Nerve
MRC	Medical Research Council

MRI	Magnetic resonance Imaging
MUHREC	Monash University Human Research Ethics Committee
MVC	Maximum Voluntary Contraction
NIBS	Non-invasive Brain stimulation
NMDA	*N*-Methyl-D-aspartic acid
PCTs	Randomized Control Trials
PEDro	Physiotherapy Evidence Database
PET	Positron Emission Tomography
PL	Pain Level
PMT	Photon Migration Tomography
PNM	Pain Neuromatrix
PTh	Pain Threshold
RMT	Resting Motor Threshold
rTMS	Repeated Transcranial Magnetic Stimulation
S1	Primary Sensory Cortex
SD	Standard Deviation
SE	Standard Error
SEM	Standard Error of Measurement
sEMG	Surface electromyography
SICF	Short-interval Intracortical Facilitation
SICI	Short-interval Intra Cortical Inhibition

SMD	Standard Mean Difference
SPECT	Single Photon Emission Computed Tomography
SPSS	Statistical Package for Social Sciences
SSEP	Somatosensory Evoked Potential
STh	Sensory Threshold
T	Tesla
tACS	Transcranial Alternative Direct Current Stimulation
tDCS	Transcranial Direct Current Stimulation
tES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
tRNS	Transcranial Random Noise Stimulation
W	Watt
μV	Microvolt

Thesis Outline

The present thesis will present the results of a body of work (Figure 1) investigating the effect of tDCS over cortical sites of the pain neuromatrix (PNM) on the excitability level of primary motor (M1) and sensory (S1) cortices and sensory (STh)/pain (PTh) thresholds in healthy adults. In addition, the effect of a new tDCS paradigm named unihemispheric concurrent dual-site tDCS ($tDCS_{UHCDS}$) on CSE and STh/PTh will be compared to that of a conventional paradigm. To investigate the mechanisms behind the alteration induced by $tDCS_{UHCDS}$, SICI and ICF changes are assessed by paired-pulse TMS.

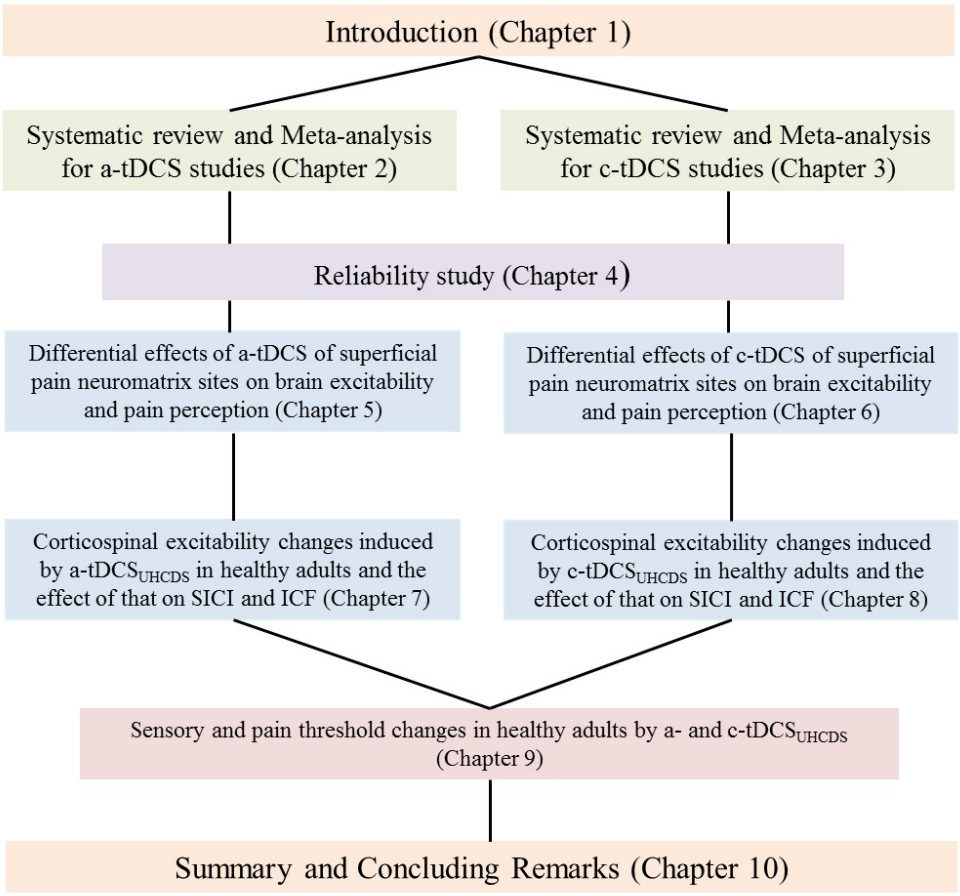

Figure 1: Thesis structure

Chapter 1 provides an introduction to the topic and background information on the neuroplasticity and physiology of the cerebral cortex, cortical sites of PNM, and pain-induced neuroplasticity, in order to anchor the framework of the research field that this thesis is related to. Also, the introduction presents the concept of NIBS methods, neurostimulatory and neuromodulatory techniques, safety issues, and tools for assessment of M1/S1 excitability and STh/PTh, which are the main concepts in this thesis.

Chapters 2-3 present two systematic reviews and meta-analyses of the current literature on the effects of a-tDCS (Chapter 2) and c-tDCS (Chapter 3) of cortical sites of the PNM (M1, S1, and DLPFC) on STh and PTh in healthy individuals and pain level (PL) in patients with chronic pain, compared to no stimulation and sham control.

Chapter 4 outlines inter- and intra-session reliability of the assessor and the optimal TMS protocol for elicitation of MEPs. This study aims to compare the intra- and inter-session reliability of peak-to-peak amplitudes of MEPs with short (4 sec) and long (10 sec) inter-pulse intervals (IPIs) recorded from the first dorsal interossei (FDI) muscle at rest.

Chapters 5-6 investigate the effect of a-tDCS (Chapter 5) and c-tDCS (Chapter 6) of functionally connected cortical sites of the PNM on the M1/S1 excitability in healthy adults. The STh and PTh alterations following the application of a- and c-tDCS are also evaluated.

Chapters 7-8 evaluate the effect of a new neuromodulatory tDCS paradigm named unihemispheric concurrent dual-site tDCS ($tDCS_{UHCDS}$) on CSE. The primary aim of Chapter 7 is to compare the effect of a-$tDCS_{UHCDS}$ of two cortical interconnected sites of M1 with a conventional paradigm. In chapter 8, the effect of c-$tDCS_{UHCDS}$ is compared with the conventional c-tDCS paradigm. The secondary aim in both studies was to compare the level of changes in intracortical facilitation and

inhibition following $tDCS_{UHCDS}$ with a conventional paradigm.

Chapter 9 examines how a- and c-$tDCS_{UHCDS}$ of these cortical sites of PNM affect STh and PTh. The primary aim of this study is to compare the effect of $tDCS_{UHCDS}$ and conventional tDCS to find the most efficient paradigm for STh and PTh enhancement.

The final chapter (Chapter 10) summarizes the findings and provides conclusions for different studies in this thesis.

Chapter 1: Introduction

General introduction

Non-invasive brain stimulation (NIBS) represents a number of new breakthrough approaches for the modulation of cortical sites of the pain neuromatrix (PNM) focused on using magnetic or electrical energy to modulate pain-induced neuroplasticity (Luedtke et al. 2012, O'Connell et al. 2011). These techniques are used for research in healthy individuals and patients with different pathologies including painful conditions. While currently available medications and/or physiotherapeutic techniques are effective for many patients, unfortunately a substantial number of patients do not always respond fully to these interventions. 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 the problem is resistant to medication, side effects of medication can become chronic, lasting for long periods of time in some cases (Apkarian et al. 2005, Medeiros et al. 2012). In such situations, pharmacological treatments or other methods of pain management like spinal cord stimulators, implantable drug delivery systems, and surgery, may not be effective enough to manage these conditions. In addition, based on the results of an epidemiological study conducted by the University of Sydney, the cost of chronic pain management in 2007 was $34.3 billion in Australia, or $10,847 for each affected case (Australia 2010).

Regarding the physical, psychological, and economic effects of pain on the quality of life of patients suffering from chronic pain, finding an efficient and safe technique to non-invasively reduce pain-induced neuroplasticity is an urgent need. Furthermore, despite significant advances in the development of pain treatment protocols, controlling the pain following some neuropathic chronic pain disorders, such as Fibromyalgia or Multiple sclerosis is often incomplete (Fagerlund et al. 2015, Fregni et al. 2006b, Mori et al. 2010). In this scenario,

modulating the functionally connected cortical sites of PNM by NIBS may lead to greater clinical outcomes than could be achieved with traditional therapies.

Recent NIBS approaches have begun to build on methods to prime the effects of other therapeutic techniques (Dobkin 2003) or to be used as a stand-alone technique in pain treatment (O'Connell et al. 2010, Rosen et al. 2009) and treatment of some psychological (George et al. 2007) and neurological (Fregni and Pascual-Leone 2007, Schulz et al. 2013) disorders. Priming could be achieved by enhancing the sensitivity of the brain to therapy using techniques that modulate the excitability of the cortex (Schabrun and Chipchase 2012). In this context, NIBS appears to be a promising option (Fregni and Pascual-Leone 2007). A number of NIBS techniques have been developed and are now being tested for their ability to prime the brain in conditions such as chronic pain (Boggio et al. 2009a, Fregni et al. 2006a, Fregni et al. 2006c) 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. 2009a). These characteristics make NIBS techniques attractive for use in different clinical settings.

NIBS induced alternations in the excitability of the cortex is considered to be a key component for pain modulation. Over the last decade there has been increasing evidence of links between NIBS induced CSE modulations for pain treatment (Huntley and Jones 1991, Mendonca et al. 2011, Nitsche et al. 2003a, Riberto et al. 2011). A growing body of research indicates that improvement in cortical outputs (CSE enhancement) coincides with an increase in sensory (STh) and pain (PTh) thresholds as behavioral outputs of pain in healthy individuals (Bachmann et al. 2010, Grundmann et al. 2011).

A primary goal of neuroscientists in this area of research is to develop NIBS protocols to prime the effects of other pain management methods such as pharmacological and surgical treatments (Nitsche and Paulus 2011, Price 2000, Wagner et al. 2007b). NIBS paradigms have been

developed to modulate CSE by different methods such as repetitive transcranial magnetic stimulation (rTMS) and transcranial electric stimulation (tES) (Pascual-Leone et al. 1994, Paulus 2011). In addition to rTMS, which is a neurostimulatory technique, tES is an umbrella term to describe 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). tDCS is the most common technique in which a low-amplitude direct current is applied on the target area of the brain to modulate CSE in a polarity-dependent manner (Nitsche and Paulus 2000a).

In current standard tDCS protocols, the primary motor cortex (M1) has been widely stimulated to affect cortical and behavioral outputs of pain (Bachmann et al. 2010, Luedtke et al. 2012). However, existing literature indicates that utilizing the electrodes over the other cortical sites of PNM (i.e., the primary sensory cortex (S1) and the dorsolateral prefrontal cortex (DLPFC)) resulted in increasing the level of STh/PTh in healthy individuals or decreasing the level of pain in patients with chronic pain (Grundmann et al. 2011, Naylor et al. 2014). As a result, there is no consensus on the optimal stimulation site for the most efficient pain management. In addition, both a- and c-tDCS have been used in pain-related studies. Crucially the optimal parameters of tDCS – such as site and mode of stimulation – need to be taken into consideration in both the realm of research and in its future clinical applications. Optimization of tDCS parameters can have a profound impact on its efficacy for M1 CSE enhancement and pain perception improvement in the near future in both healthy patients and patients with chronic pain.

The studies introduced in this thesis are motivated by the need for development of non-medical adjunct therapies to enhance CSE and STh/PTh. In the current tDCS protocols, the effects of tDCS on functionally connected sites of PNM are unknown. Therefore, it is hard to introduce best stimulation sites and tDCS modes to maximally enhance M1 CSE with longer lasting effects and to significantly increase STh and PTh. The studies in this thesis were designed

partly to address these issues. A probable strategy to improve tDCS effects on CSE enhancement and STh/PTh increase is concurrent stimulation of more than one cortical site of PNM in the same hemisphere. This technique is completely novel but may boost the immediate and longer lasting effects of tDCS. Another important issue is finding the probable mechanisms behind the efficacy of concurrent dual-site tDCS. In addition, it is important to explore whether the neurophysiological findings can be translated into clinical effects – for instance, whether a tDCS-induced CSE enhancement can be coincided with STh/PTh increase in healthy individuals or PL decrease in patients with chronic pain. As such, the primary aim of this thesis is to determine the optimal stimulation site and tDCS mode to increase the level of M1 CSE, STh, and PTh more than the current tDCS approaches do in healthy individuals. The secondary aim is to investigate the probable effects of these optimal parameters on intracortical inhibition (ICI) and facilitation (ICF) in healthy adults. These studies are detailed in Chapters 2- 9.

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 is given of the anatomical/physiological characteristics of the areas of the central nervous system (CNS) involved in sensory and pain processing.

The Cerebral Cortex

The cerebral cortex is the outer layer of the brain that covers the gray matter over the hemispheres. Typically it covers the gyri and sulci with a thickness of 3-4 mm (Edelman and Mountcastle 1978, Taylor 1999) and contains 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 areas such as motor, somatosensory, and visual areas, as well as a multitude of their subdivisions. Although

there are small inter-individual variations, each cortical area has its typical location in terms of the sulci and gyri.

Horizontal organization

In general, the cerebral cortex consists of six layers (I-VI) of histologically and functionally distinct cells. Neurons in the cerebral cortex are distributed in horizontal layers and vertical columns (Garey 1994) depending on the function of the regions of the cortex. The relative thickness of each layer may be varied (Dinse et al. 2013). The layers are numbered with Roman numerals from superficial to deep. Layer I is the molecular layer, which contains the apical dendrites of pyramidal cells. The distal branches of axons located in the thalamus project to the cortex. Layers II and III are the external granular layers, which contain small and medium pyramidal and stellate cells respectively. Layer IV is the inner granular layer. This layer receives the afferents from the thalamic relay nuclei. Layer V is the internal pyramidal layer, which contains large pyramidal cells projecting to the corpus striatum, brain stem, and spinal cord. Layer VI is the multiform, or fusiform layer, which contains modified pyramidal cells projecting to the thalamus (Figure 1).

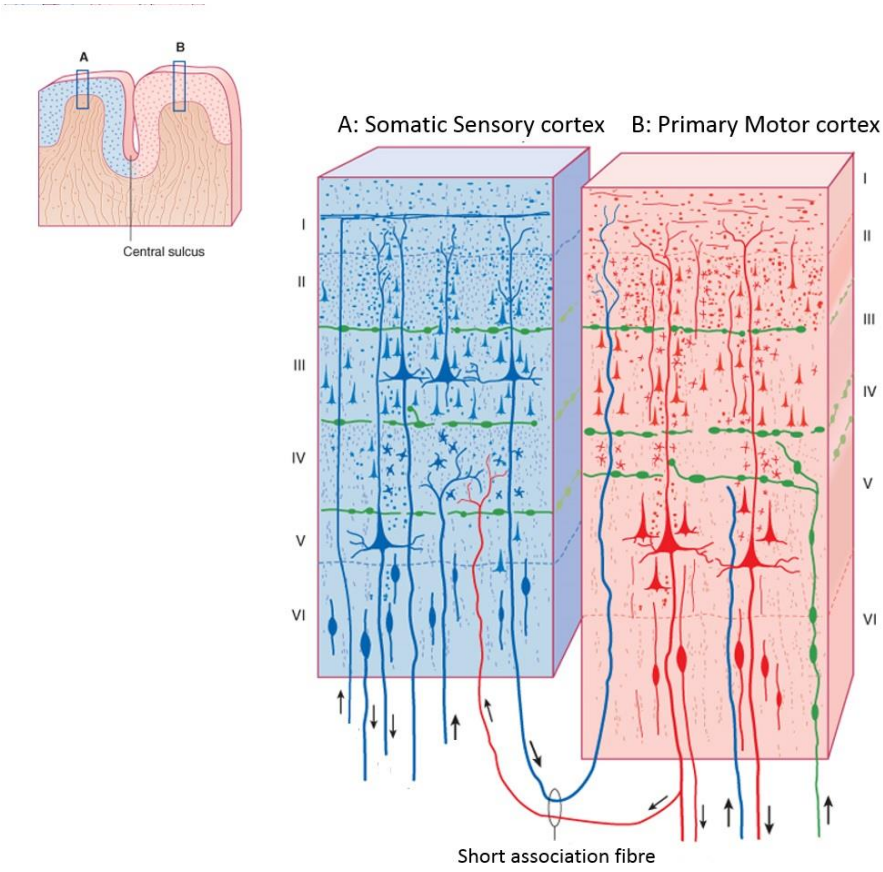

Figure 2: The cerebral isocortex. (A) Somatic sensory cortex. Cortical laminas I-VI are numbered on the left. (B) Primary motor cortex. Cortical laminas I-VI are numbered on the right. Short associated fibres show the fibres which are passing to the motor or sensory cortex. This figure is adapted from Clinical Neuroanatomy and Neuroscience (Mtui et al. 2011).

Different areas of the brain are functionally and anatomically connected to each other by the horizontal layers. Layers I to III 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). These layers permit communication between one portion of the cortex and other regions. Layer IV receives thalamocortical connections, especially from specific thalamic nuclei. This is most prominent in the primary sensory cortices. Layers V and VI primarily connect the cerebral cortex with subcortical regions. These layers are most developed in motor cortical areas. Layer V goes to the principal cortical efferent projections in basal ganglia, the brain stem and the spinal cord. Layer VI, which is the multiform or fusiform layer, projects to

the thalamus (Ab Aziz and Ahmad 2006, Brodal 1981).

The pyramidal cells, named ‘Betz cells’, can be extremely large in layer V of the motor cortex. The Betz cells in the motor cortex come from most corticobulbar and corticospinal fibres (Porter and Lemon 1993). 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.

Pyramidal cells use excitatory amino acid glutamate as their primary neurotransmitter (Cotman and Monaghan 1988). Stellate cells or granular cells, which act as interneurons within the motor cortex (DeFelipe and Fariñas 1992) constitute approximately 25% of the neurons in the motor cortex, and are located in layers II-VI, but most prominent in layer IV. The most prevalent satellite cells in the motor cortex are basket cells, which make inhibitory synaptic contacts with pyramidal neurons, using the neurotransmitter gamma- aminobutyric acid (GABA) (Jones 1983a, Meyer 1987).

Columnar organization

In addition to the distribution of neurons in layers, groups of cells work together in vertical units called cortical columns (Edelman and Mountcastle 1978, Horton and Adams 2005). In the mature human cortex, a narrow chain of neurons, called a minicolumn, is the basic unit. Cortical columns are formed by many minicolumns bound together by short-range horizontal connections and vertically extended across the cellular layers II–VI, perpendicular to the pia matter (Edelman and Mountcastle 1978). This columnar organization is characterized by extensive synaptic communication between neurons, the majority of which is inhibitory (Jones 1983b). The recurrent axon collaterals of pyramidal cells project vertically, which provides a strong excitatory drive to adjacent neurons. These axons are connected to a columnar surround inhibition, via inhibitory interneurons, for the sharpening of motor commands (Keller 1993). It has been shown that 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 and Mountcastle 1978).

In Chapters 7 and 8, the function of inhibitory (GABA) and facilatory (glutamergic) mechanisms are evaluated in the motor cortex to investigate the possible mechanisms behind the efficacy of a novel tDCS technique, which is introduced in this thesis for the first time. As a result, to better understand the function of inhibitory and excitatory interneurons in the brain, the next section provides a brief review of the neurochemistry of glutamergic and GABAergic mechanisms.

Gamma-aminobutyric acid (GABA) in the central nervous system

Bazemore described the function of GABA in 1956 for the first time (Bazemore et al. 1956). A number neurochemical and electrophysiological investigations have confirmed the inhibitory effects of GABAergic mechanisms in CNS (Basile 2002, Kubota et al. 2003, Szabo et al. 2014, Vicario-Abejon et al. 2000). There are many examples of GABAergic projection neurons such as the Purkinje cell of the cerebellar cortex.

There are three types of GABA receptors termed $GABA_A$, $GABA_B$, and $GABA_C$ (Kahsai et al. 2012). The activation of GABA receptors increases the permeability of chloride (CL^-) and bicarbonate (HCO_3) ions (Momiyama and Koga 2001). Although pharmacologically, electrophysiologically, and biometrically $GABA_A$ and $GABA_B$ are different, both of them induce inhibition (Momiyama 2002). Electrophysiological studies have indicated that $GABA_A$ mediates the membrane conductance increase by hyperpolarization of the membrane and increasing the firing threshold (Homanics et al. 1997). Hence, inhibition of action potential production reduces in neurons, which leads to neuronal inhibition. The reduction of membrane resistance is accompanied with GABA-dependent facilitation of CL^- ion influx through a receptor-associated channel. As a result, the level of intracellular CL^- increases, and this can activate the Ca^{2+} entry via voltage-gated channels (Momiyama 2002).

$GABA_A$ receptors

$GABA_A$ receptors can be found in the majority of GABAergic synapses (Schofield et al. 1987). To date, sixteen $GABA_A$ receptor subunits (α1-6, β1-4, γ1-4, δ, ε) have been noted (DeLorey and Olsen 1992). Based on the type of subunit bindings, different ion channels may be opened and consequently the permeability to CL^- is increased, which leads to the influx of CL^- and membrane hyperpolarization (de Azeredo et al. 2010). There are two sites of GABA-recognition in a GABA receptor. As a result, increasing the level of GABA concentration results in the induction of doubly liganded receptors and consequently the average time for opening the ion channels increases (Bormann 1988, Macdonald and Olsen 1994).

It has been shown that the ionic permeability increase in $GABA_A$ receptors is transient in the continuing presence of an agonist (Cash and Subbarao 1987). This is called desensitization. The underlying mechanism is not clear yet but the mediation of opening of the CL^- channels is the base of the desensitization (Cash and Subbarao 1987).

$GABA_B$ receptors

There are seven transmembrane segments for $GABA_B$ receptors. These segments are coupled through G-proteins to K^+ or Ca^{2+} channels. Activation of these receptors results in a K^+ increase or Ca^{2+}conductance decrease, which mediates slow synaptic inhibition (Curtis et al. 1974, Emson 2007). To date, three subunits have been cloned and are termed $GABA_B$ R1a, $GABA_B$ R1b, and GABAB R2 (Kaupmann et al. 1997, Nicoll 2004).

$GABA_B$ receptors are located on neurons and glia and they are able to induce both presynaptic and postsynaptic inhibition by inhibiting the presynaptic Ca^{2+} entry and consequently neurotransmitter release (Emson 2007). In addition, $GABA_B$ receptors are indirectly coupled to K^+ channels. By activating the K^+ channels, the level of Ca^{2+} conductance decreases, which leads to hyperpolarization and inhibition of cyclic Adenosine Monophosphate (cAMP) production mediated by G-proteins (Hill 1985). $GABA_B$ receptors are mainly found in the

cerebral cortex, the thalamus, the superior colliculus, the cerebellum, and in the dorsal horn of the spinal cord. The $GABA_B$ receptor concentration in these cortical sites has inhibitory effects on the pre- and postsynaptic neurons (Figure 2).

$GABA_C$ receptors

An analogue of GABA, cis-aminocrotonic acid (CACA), is able to bind to a GABA receptor, which is different from either $GABA_A$ or $GABA_B$ receptors (Drew et al. 1984, Johnston et al. 1975). $GABA_C$ receptors have three subunits (ρ1-3) (Johnston 1996) which are coupled to the CL^- selective ion channel. These receptors are activated by GABA and CACA and blocked by Picrotoxin.

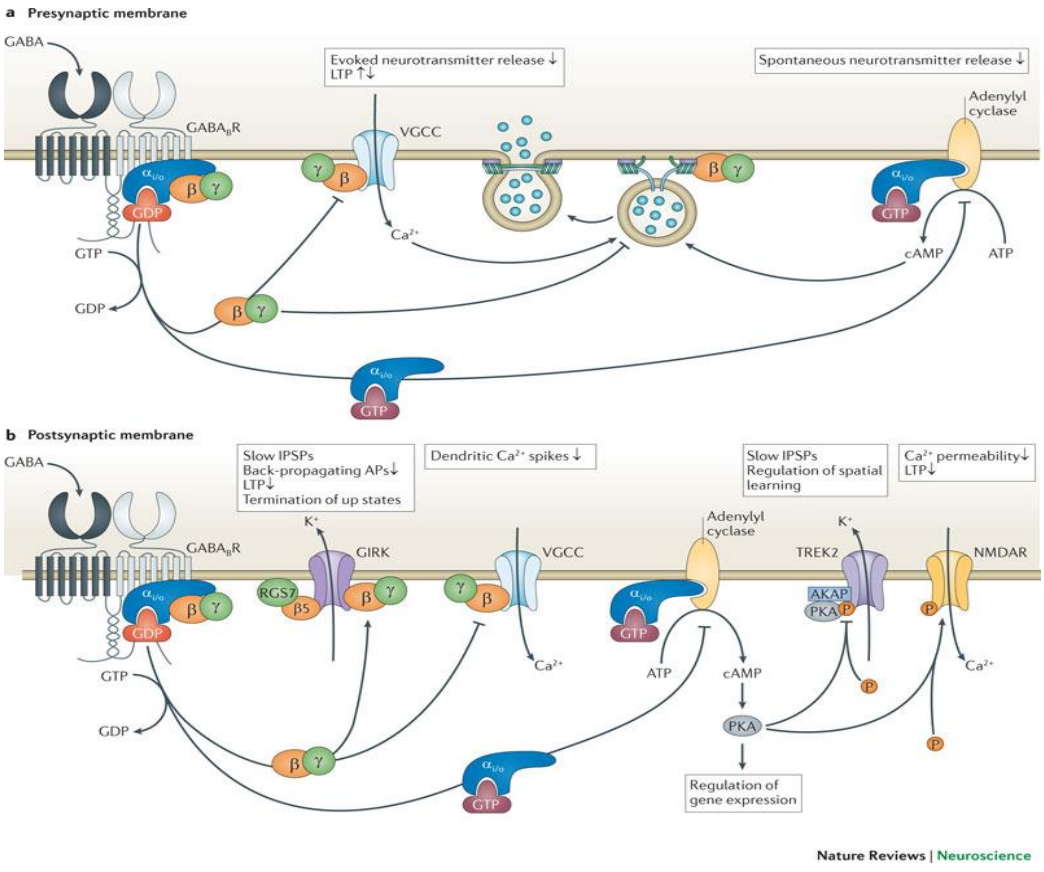

Figure 3: Physiological roles of $GABA_B$ receptor. Adapted by the Nature Review Journal (Gassmann and Bettler 2012) .In presynaptic neural membranes, GABAB receptors (GABABRs) activate G proteins to decrease the level of cyclic adenosine monophosphate (cAMP). At the axon terminals (A in Figure 3), cAMP prevents vesicle fusion, which results in less or no neurotransmitter release. G-beta-gamma (Gβγ) inhibits the voltage-gated Ca2+ channels

(VGCCs). Therefore, less Ca2+ dependent neurotransmitter will be released. Gβγ also directly binds to a protein named SNARE, which is the complex required for vesicle fusion. Therefore, less neurotransmitter is released. All these factors lead to less long-term potentiation (LTP) and the initiation of a initiating long-term depression (LTD) process.In postsynaptic compartments (B in figure 3), Gβγ opens dendritic G protein-activation to rectify potassium channels (GIRKs). GIRKs inhibit the excitability of neurons by the shunting of excitatory currents, the generation of slow inhibitory postsynaptic potentials (IPSPs), and the inhibition of back-propagating action potentials (APs). GIRKs accelerate the activation and deactivation kinetics of the GABABR-mediated K+-current response. Inhibition of VGCCs prevents dendritic Ca2+ spikes. GABABR-mediated inhibition of adenylyl cyclase reduces protein kinase A (PKA) activity, thereby alleviating an A-kinase anchoring protein (AKAP)-dependent and tonic inhibition of TREK2 channels. The Reduction of PKA activity by GABABRs inhibits the Ca2+ permeability of NMDA-type glutamate receptors (NMDARs) without affecting the overall synaptic currents through NMDARs. GABABR-mediated down regulation of PKA activity also influences gene expression (Gassmann and Bettler, 2012).

Glutamic acid (Glutamate) in the central nervous system

Glutamate (Glutamic acid) is the main excitatory neurotransmitter located in the neuronal cells, especially in the brain (Aoyama and Nakaki 2013, Castro-Alamancos and Borrell 1993). Glutamergic receptors are responsible for the glutamate-mediated postsynaptic excitation. There are two types of glutamergic receptors including metabotric (mGluRs) and ionotropic (iGluRs) glutamate receptors. Both receptors are involved in postsynaptic plasticity but the speed and duration of induced-changes are different (Honoré et al. 1982, Zhang et al. 2013). Increasing or decreasing the number of glutamate receptors in the membrane of postsynaptic cells may induce long-term potentiation (LTP) or long-term depression (LTD) (Anggono and Huganir 2012, Bassani et al. 2013, Henley and Wilkinson 2013, Song and Huganir 2002).

There are three subgroups of iGluRs including N-methyl-D-aspartate (NMDA), Kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Furukawa et al. 2005). iGluRs are liganded-gated nonselective channels which can be activated by binding the glutamates to pass K^+, Na^+, and sometimes Ca^{2+} (Fuchigami et al. 2015). As a result, the activation of iGluRs resultes in a postsynaptic depolarizing current. Following the action potential induced by voltage-gated channels located in presynaptic neurons, the glutamate vesicles are released in synapses (Fuchigami et al. 2015). AMPA and Kainate receptors respond to glutamates by opening Na^+ channels and initiating an action potential in postsynaptic neurons (Perkinton et al. 1999). In addition, NMDA receptors have an internal voltage-

dependent site to bind with Mg^{2+} ions to block the receptor. The outward current flow releases the glutamate to bind with NMDA receptors. Binding the glutamate and NMDA removes the Mg^{2+} which leads to opening the NMDA receptors and increasing the permeability of the membrane to Ca^{2+} (Paoletti and Neyton 2007, Song and Huganir 2002). The flow of Ca^{2+} may lead to the induction of more action potentials and AMPA receptors activation which leads to modifying the strength of the synaptic connection. Prolongation of Ca^{2+} may regulate the gene expression (Perkinton et al. 1999) (Figure 3).

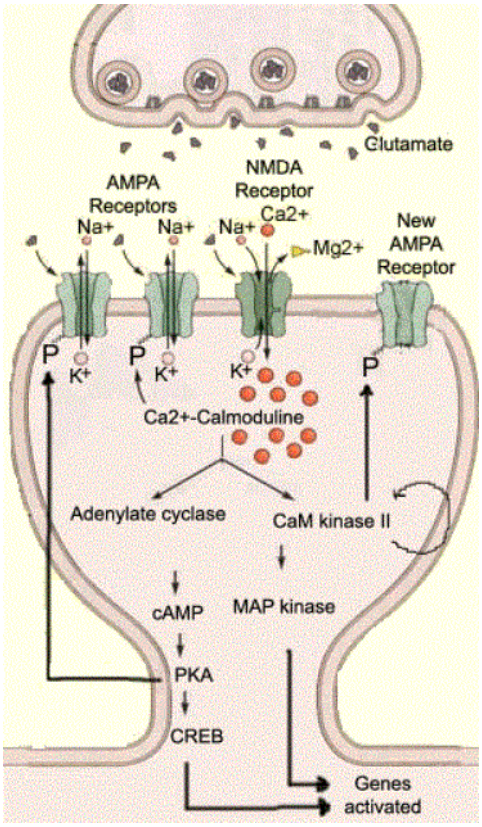

Figure 4: Excitatory effects of glutamergic mechanisms in the formation of long-term potentiation (LTP). The figure is adapted from http://thebrain.mcgill.ca/flash/a/a_07/a_07_m/a_07_m_tra/a_07_m_tra.html.

In all experimental studies presented in this thesis, the cortical sites of PNM are stimulated. As

a result, the following section briefly summarises the function of cortical and subcortical sites of PNM and the functional connectivities between these sites.

The pain neuromatrix (PNM)

The processing of painful stimuli is a multidimensional phenomenon mediated by a network of neurons in the brain called the 'Pain neuromatrix' (Derbyshire 2014, Iannetti and Mouraux 2010). Collaboration between vast areas of the brain including cortical and subcortical areas is needed to process not only sensory but also the affective, motoric and cognitive elements of painful stimuli (Bushnell and Duncan 1989, Wiech et al. 2008). In early functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) scan studies, it was demonstrated that the thalamus, the anterior cingulate cortex (ACC), and the S1 and the secondary sensory cortex (S2) are activated to process painful heat stimuli (Jones et al. 1991, Talbot et al. 1991). Since then, many fMRI and PET scan studies during different experimental and clinical pain conditions have been conducted to define all active brain regions during pain processing. The current PNM expanded to cortical and subcortical regions. The cortical sites are the S1, S2, prefrontal (Hsieh et al. 1995, Kwan et al. 2000, May et al. 1998, Petrovic et al. 2000), M1 and supplementary motor areas. The subcortical sites are the thalamus, the ACC, the midbrain regions of pre-aqueductal grey matter (PAG), the lenticular complex (Apkarian et al. 2005, Iannetti and Mouraux 2010), the insula (Davis et al. 1998, Hsieh et al. 1996), the inferioparietal, and the anterior cingulate cortices (Apkarian et al. 2005, Iannetti and Mouraux 2010) (Figure 4).

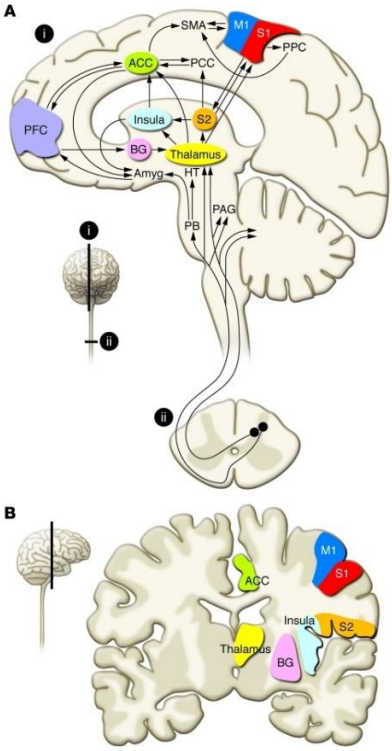

Figure 5: A schematic representation of ascending pain pathways and the PNM. The color-coded regions superimposed on an anatomical MRI (coronal slice) (B) are shown. S1: primary sensory cortex; S2: secondary sensory cortex; ACC: anterior cingulate cortex; PFC: dorsolateral prefrontal cortex, M1: primary sensory cortex. BG: basal ganglia; HT: hypothalamus; and Amyg: amygdala. Adapted from the European Journal of Pain. This figure adapted from (Apkarian et al. 2005).

Cortical sites of the pain neuromatrix

In all studies presented in this thesis, the effects of tDCS over S1, M1, and DLPFC on cortical sites and behavioural outcomes are evaluated. The selection of these sites was based on the fact that they are superficial sites of this matrix and it is possible to directly induce modulatory changes by tDCS. As a result, the next section provides a brief summary of the function of these cortical sites (Figure 5).

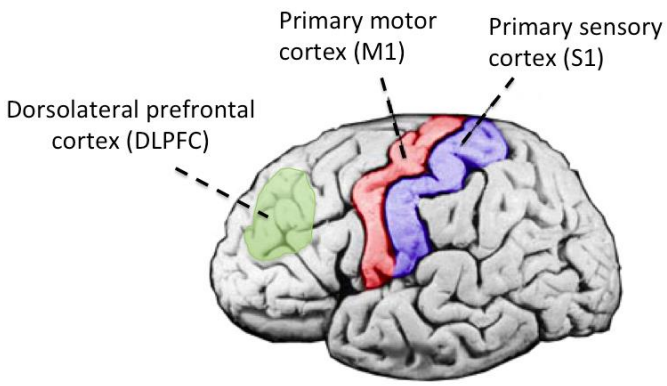

Figure 6: Cortical sites of the pain neuromatrix

The Primary Motor Cortex or M1

The primary motor cortex (M1) is located in the pre-central gyrus area of the frontal lobe of the cerebral cortex and extends onto the medial cortical surface within the longitudinal fissure (Rademacher et al. 1993). The organization of M1 in the cerebral cortex is like that of a small, distorted, discontinuous map of the body (*homunculus*) (Figure 6), 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 the M1 (Geyer et al. 1996).

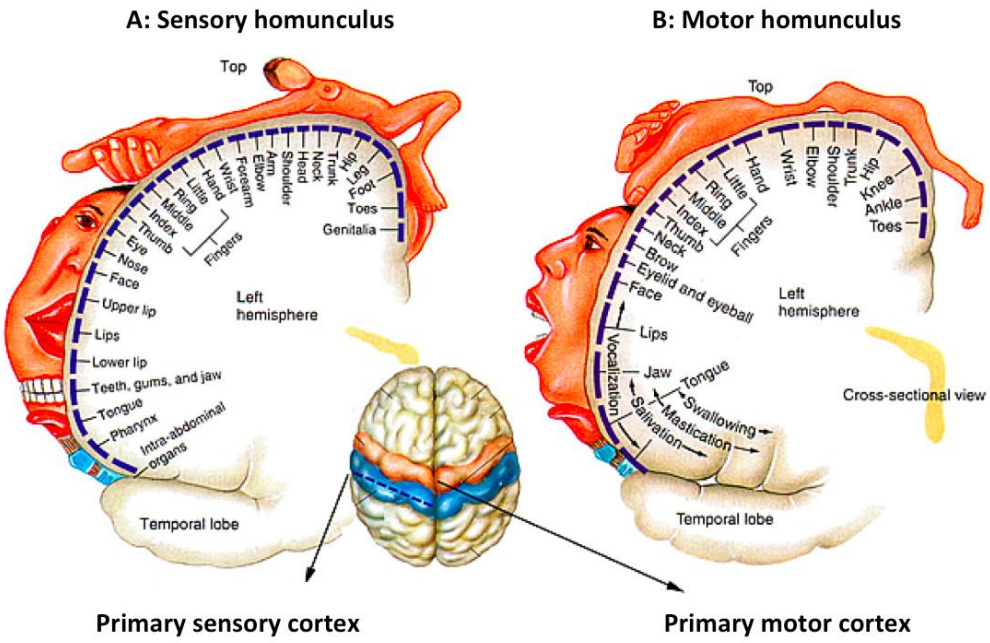

Figure 7: The homunculus of the M1 and the S1 (Squire et al. 2012)

The strength of excitatory glutamergic horizontal pathways (Hess et al. 1994) is possibly influenced by GABAergic inhibitory interneurons (Donoghue 1995, Hess et al. 1996, Hess et al. 1994). 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 the S1 (Ghosh and Porter 1988). Additional afferent inputs come from the premotor cortices, the cingulate motor area and the parietal cortex (Ghosh et al. 1987, Muakkassa and Strick 1979, Tokuno and Tanji 1993) in a roughly somatotopic arrangement. In addition, there are transcallosal afferents from the contralateral M1 (Sloper and Powell 1979), and sparse transcallosal inputs from the contralateral premotor areas (Rouiller et al. 1994).

Based on the result of some animal studies, there are many dopamine D_2 receptors in M1

neurons, which play an important role in pain control (Viisanen et al. 2012, Viisanen and Pertovaara 2010). Dopamine D_2 receptors modulate striatal and spinal dopamine D_2 receptors resulting in increasing the inhibitory effect of dopaminergic mechanisms and activation of sensorimotor gating of nociceptive information (Hagelberg et al. 2004).

The Primary Sensory Cortex or S1

The primary sensory cortex is also known as the S1, and is located in the lateral post central gyrus in the parietal lobe of the cerebral cortex. It was initially defined as the Brodmann Area 3 (Geyer et al. 1999) (Figure 5). Like the M1, the S1 contains a map of sensory areas in an inverted fashion from toe (at the top of the cerebral hemisphere) to the mouth (at the bottom), which is called the sensory homunculus (Figure 6). The devoted areas of each part of body in the homunculus refers to the relative density of receptors in that part of body. Each hemisphere receives the somatic senses from the contralateral side of the body.

The function of the S1 can be categorized into three types:

A. Exteroceptive functions include the sensations of touch, temperature, and pain, which can be divided into three modalities

1. Mechanoreception: All non-painful mechanical stimuli are received by mechanoreceptors including Pacinian corpuscles, Meissner's corpuscles, Merkel's discs, and Ruffini endings.

2. Thermoreception: All heat and cold stimuli are received by thermoreceptors.

3. Nociception: the sensations of burning and/or sharp pain are received by C and γ fibres.

B. Proprioceptive function includes the kinesthetic senses of position and movement. The sensory inputs from muscles, tendons, and joints are received by proprioceptors to provide information related to the position of joints and the direction, force, and speed of movements.

Muscle spindles and the Golgi Tendon (neurotendinous spindles) are responsible for providing proprioceptive information.

C. **Interoceptive functions** provide information about the internal organs.

All myelinated and unmyelinated axons enter the sensory information lateral fibres and terminate in layers I and IIa of the dorsal horn and layers V, VI, and X of the intermediate horn of the spinal cord (Desmedt 1987). The nociceptive, thermal and non-discriminatory touch signals are transmitted by the anterolateral system and the vibration, fine touch, two-point discrimination, and proprioception signals are conveyed by the posterior column medial lemniscus pathway.

The Dorsolateral Prefrontal Cortex or DLPFC

The DLPFC is located in the middle frontal gyrus of the frontal lobe (approximately areas 9 and 46 of Brodmann) (Hoshi 2006) (Figure 5). The DLPFC is heavily connected to the orbitofrontal cortex and many primary and secondary areas of the cortex including the M1 and S1 (Apkarian et al. 2005, Iannetti and Mouraux 2010, Petrican and Schimmack 2008). It is also connected to subcortical areas of the brain such as the dorsal caudate nucleus of the basal ganglia, thalamus, and hippocampus (MacDonald et al. 2000, Nagel et al. 2008, Staphorsius et al. 2015). The executive control of information processing, behavioral expression (mood and emotional judgment), the maintenance of information (working memory), inhibition of irrelevant stimuli, the evaluation and selection of the best response to stimuli (decision making), and attention to stimuli are responsibilities of the DLPFC (MacDonald et al. 2000). According to Mayberg (1997), there are two streams for the regulation and processing of the received sensory and painful stimuli (Mayberg 1997):

1. The dorsal cortical stream: It is composed of the DLPFC, the dorsal part of the ACC, the dorsomedial part of the prefrontal cortex, and the dorsal anterolateral prefrontal cortex. This

stream regulates behavior and experience as well as executive functions, attention, and the planning of movements (Davidson and Irwin 1999).

2. The Ventral cortical stream: This stream includes the DLPFC, the subgenual cingulate gyrus, the ventrolateral cortex, the orbitofrontal cortex, the amygdala, the anterior insula, the ventral striatum, the medial thalamus, and the hippocampus. The ventral cortical stream is mostly activated in pain or painful stimuli processing and provides behavioural responses to sensory and/or behavioural stimuli.

Subcortical areas of the pain neuromatrix

Sensory and pain information is relayed through a number of subcortical areas including the reticular formation (Almeida et al. 2004, Sessle 2000), the thalamus (Ab Aziz and Ahmad 2006) and the ACC, the amygdala, and the insula (Valet et al. 2004). The following points should be noted:

- The **reticular formation** consists of many small networks with different functions located in the brain stem. The reticular formation is involved in the sleep-awake cycle, motor-control, pain management, cardiovascular control, and habituation. It is also the origin of the descending analgesic pathway (Andy 1986)

- The **thalamus** is one of the most important structures receiving projections from different sensory pathways and affects the nociceptive stimuli before interpreting the information in the S1. The thalamus is involved in both the medial and lateral pain systems (Willis and Westlund 1997).

  The nociceptive neurons from the ventrobasal complex of the thalamus project to the S1 and other parts of lateral pain system which are involved in the sensory discrimination aspect of pain management (Herrero et al. 2002). The interalaminar thalamic nuclei project to the S1 and

the limbic system, which are involved in the effective-motivation aspect of pain management. There are also many projections from the ACC, which is responsible for the motivational aspect of pain management (Petrovic et al. 2000). Points to note are:

- The **ACC** is located around the corpus callosum and integrates pain or painful stimuli information. Due to the connectivity of the CC and the DLPFC, M1, amygdala, hypothalamus and anterior part of insula, the ACC is also involved in the perception of the suffering and emotional responses (Nakata et al. 2014).

- The **amygdalae** are two almond-shaped groups of nuclei located in the temporal lobe and considered as part of limbic system. It is connected to the thalamus, hypothalamus, reticular formation, and the trigeminal/facial nerves. Amygdalae are involved in motivation and emotional behaviour (Gallagher and Chiba 1996).

Functional connectivities between cortical and subcortical sites of PNM

Both cortical and subcortical sites of the PNM make two distinct but highly interacted subsystems – the lateral and medial pain systems (Figure 7). All sites of these two systems are functionally and/or anatomically connected to cover sensory discrimination and affective-motivation aspects of painful stimuli processing. To note:

- The lateral pain system: The S1, S2, thalamus, and the posterior part of the insula are collectively called the lateral pain system. This system is responsible for sensory discrimination of painful stimuli (Chen et al. 2013, Willis and Westlund 1997).

- The medial pain system: The DLPFC, M1, ACC, the anterior part of the insula, the amygdala, and the periaqueductal gray matter (PAG) comprise the medial pain system (Apkarian et al. 2005). The medial pain system is involved in the affective-motivation processing of painful

stimuli (Craig et al. 1994, Kulkarni et al. 2005, Lang et al. 2004, O'Connell et al. 2011, Vaseghi et al. 2014).

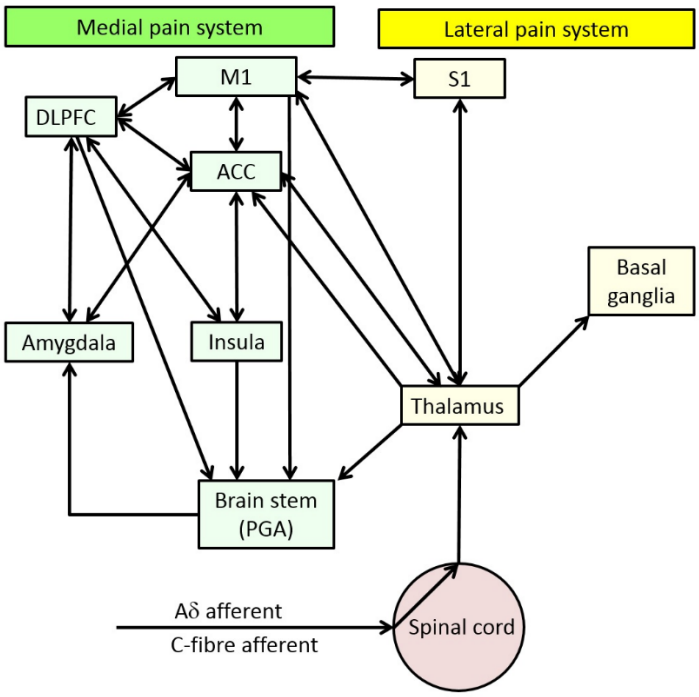

Figure 8: A schematic diagram of the medial and lateral pain systems of the 'pain neuromatrix', and their functional connectivities.

In Chapters 2-3, 5-6, and 9, the STh and PTh levels are measured to evaluate the site-specific effects of tDCS over the cortical sites of the PNM, and to measure the efficacy of a novel tDCS technique. Hence, the following section outlines the physiology of sensory and pain perception to clarify how STh and PTh levels may reflect the neuroplasticity induced by tDCS.

The physiology of sensory and painful stimuli perception

Unmyelinated C fibres and tiny myelinated Aδ fibres detect the sensory and painful stimuli induced by various modalities. The transient receptor potential-generated channels or TRP channels and purinergic channels convert the physiochemical properties of stimuli to electrical activity. To induce action potential, the electrical activities are amplified by sodium channels and sent to Lamina I and II of the dorsal horn of the spinal cord via glutamergic synapses. After

integration and assessment of the stimuli in the dorsal horn of the spinal cord, the integrated output pass through one of the ascending pathways. (This process will be explained in the following sections). Pain is perceived and analyzed in different sites of the PNM for sensory-discrimination, affective-motivation, and cognition aspects. The integrated results send to related parts of the PNM including the M1, DLPFC, and reticular formation, for a proper response.

The different forms and levels of plasticity induced by painful stimuli

Painful stimuli can induce temporal or permanent neuroplastic changes in the PNM. The following section provides a brief summary of painful stimuli-induced neuroplasticity.

Due to receiving intensive and/or prolonged painful stimuli, the efficacy of synapses increases in somatosensory neurons in the dorsal horn of the spinal cord and the S1 by changing the number of neurotransmitter receptors located on the pre and post synaptic nerves, and changing the quality of neurotransmitters in the synapses (synaptic level of neuroplasticy) (Woolf and Salter 2000, Woolf and Thompson 1991). These synaptic changes are the molecular basis for the concept of central sensitization (Willis and Westlund 1997). Central sensitization heightens synaptic transmission, which results in PTh reduction and increased pain sensitivity.

NMDA receptors are known as triggers and effectors in central sensitization. The NMDA receptor channels are blocked in a voltage-dependent manner by a magnesium (Mg^{2+}) ion sitting in the receptor pore (Qian and Johnson 2006) (cellular level of neuroplasticity). Substance P causes a long-lasting membrane depolarization (Henry 1976) and contributes to the temporal summation of C- fibre–evoked synaptic potentials (Dougherty and Willis 1991, Xu et al. 1992) as well as to intracellular signaling. Sustained release of glutamate and substance P by painful stimuli depolarizes the neuron membrane and forces Mg^{2+} to leave the NMDA receptor pore, whereupon glutamate binding to the receptor generates an inward current (Qian and Johnson 2006). This process is the major mechanism for rapidly boosting

synaptic strength and allows entry of Ca^{2+} into the neurons. Prolonged painful stimuli increase the level of Ca^{2+} (Molecular level of neuroplasticity) (Kuner 2010), which contributes to the central sensitization maintenance and the STh/PTh decrease in the painful condition. Increasing the level of excitability in the membranes and facilitation of the synapses results in excessive integrated sensory inputs analyzed by sensory-discrimination, affective-motivation, and the cognition centers of the PNM. As a result, the co-activation of both cortical and subcortical sites of the PNM mediates at network level (Network Level of Neuroplasticity) (Woolf and Salter 2000, Woolf and Thompson 1991). A long-lasting increase in synaptic strength and Ca^{2+} levels in the cortex induces long-term potentiation (LTP) **(Ji et al. 2003).** There is tremendous potential for plasticity at network-level processing of painful stimuli inputs. Depending upon how the networks affected by pain stimulate the output of different regions, PNM can change. These outputs are STh/PTh, PL, the level of movement phobia, and motor impairment.

tDCS is a NIBS technique to modulate the pain-induced neuroplastic changes such as cortical excitability and STh/PTh decrease. As a result, the next section introduces one of the NIBS techniques, tDCS, and related behavioral and cortical assessment techniques used in the present thesis.

NIBS techniques

NIBS can be defined as neurostimulatory and neuromodulatory techniques. Transcranial magnetic stimulation (TMS) and repeated TMS (rTMS) are two neurostimulatory non-invasive tools for stimulating the human brain. TMS rapidly changes the magnetic fields to induce 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 (Wassermann et al. 2008). As well, TMS can be used as an assessment or therapeutic technique. rTMS regularly induces repeated TMS pulses at certain high or low frequencies

(Rossi et al. 2009b).

Despite the above neurostimulatory techniques, transcranial electrical stimulation (tES) covers the neuromodulatory group of NIBS techniques. Manipulating ion channels and shifting electrical gradients are the most significant changes induced by tES, which influences the electrical balance of ions inside and outside the neuronal membranes. tES is an umbrella term used for tDCS, transcranial random noise stimulation (tRNS), and transcranial alternative current stimulation (tACS).

tDCS is the intervention of interest in the present thesis. This technique is simple, 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 the induction of CSE and STh/PTh changes. 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. Beside the numerus studies indicating the positive effects of tDCS, it is worth noting that negative findings are less likely to be published. The results of a systematic review and meta-analysis indicated that tDCS has no effect on pain reduction in patients with chronic pain (O'connell et al. 2011). However there are many methodological, neurophysiological reasons behind this result, which is extensively discussed in Chapter 2. In addition, there some interindividual factors which may affect the responses to tDCS (Weithoff et al. 2014). These interindividual factors are differences in cranial and brain anatomy, the level of motor cognition, neurotransmitters and receptor sensitivity, and the functional organization of local inhibitory and excitatory circuits within the cortex. tDCS will be described in more detail in the next section.

Transcranial direct current stimulation (tDCS)

tDCS is a safe and painless technique for 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). 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 comeback and its effects were primarily investigated in animals (Bindman et al. 1964, Creutzfeldt et al. 1962, Purpura and McMurtry 1965). During that time it could already be shown that tDCS is able to affect brain functions (Albert 1966a, Albert 1966). Most 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 neuroplastic changes (Nitsche and Paulus 2000a, Nitsche and Paulus 2001a, Priori 2003, Priori et al. 1998). 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 active electrode (anode or cathode), then travels through the brain tissues, and finally exits through the reference electrode. During application of tDCS, some of the injected current is shunted through the scalp, which 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 'shunts' across the scalp (Miranda et al. 2006). Consequently, using smaller electrodes can increase the distance between the electrodes (Nathan et al. 1993). It also affects the fraction of the injected current that reaches the brain or shunts through the scalp (Datta et al. 2009).

Electrode montage is critical for achieving expected effects. Conventionally, the 'active' electrode is always placed over the targeted cortical region (i.e., the M1) (Boggio et al. 2007, Boggio et al. 2009b, Hummel and Cohen 2005, Nitsche and Paulus 2001a, Nitsche et al.

2003b). The ‘reference’ or ‘indifferent’ electrode is most often placed over the contralateral supraorbital ridge (Floel et al. 2008, Hummel and Cohen 2005, Iyer et al. 2005, Nitsche et al. 2003b) (Figure 8). 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.

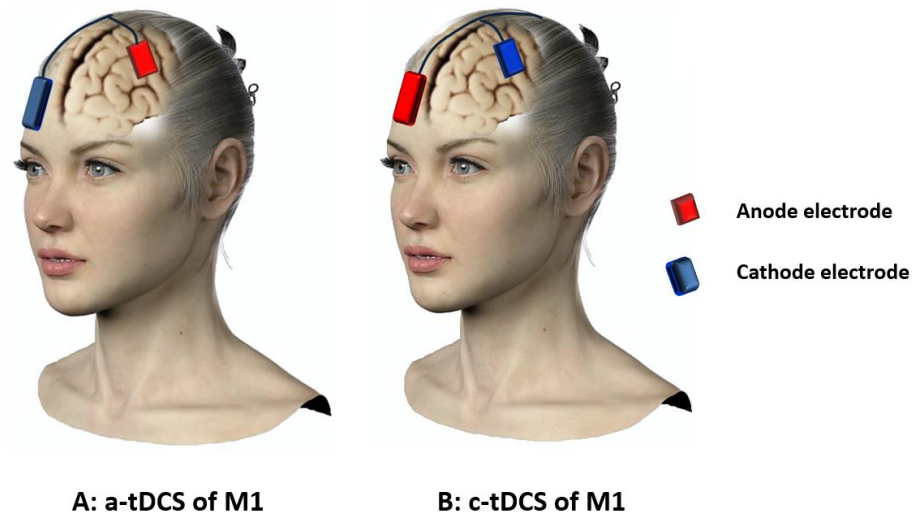

Figure 9: tDCS configuration

Depending on the stimulation polarity, tDCS increases or decreases neuronal excitability in the stimulated area (Priori et al. 1998, Rowny and Lisanby 2008, Wagner et al. 2007a). Cathodal tDCS (c-tDCS) application of the negatively charged electrode (cathode) over the target area of stimulation, leads to hyperpolarization of cortical neurons, inducing 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, inducing increased CSE (Nitsche and Paulus 2000b, 2001b).

Once the electrodes are placed and fixed with two perpendicular straps (Figure 9), the stimulation can be started. In the tDCS device the current intensity as well as the duration of stimulation can be set (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. Then, the current will continue unchanged during the set treatment time and finally will be automatically shut off.

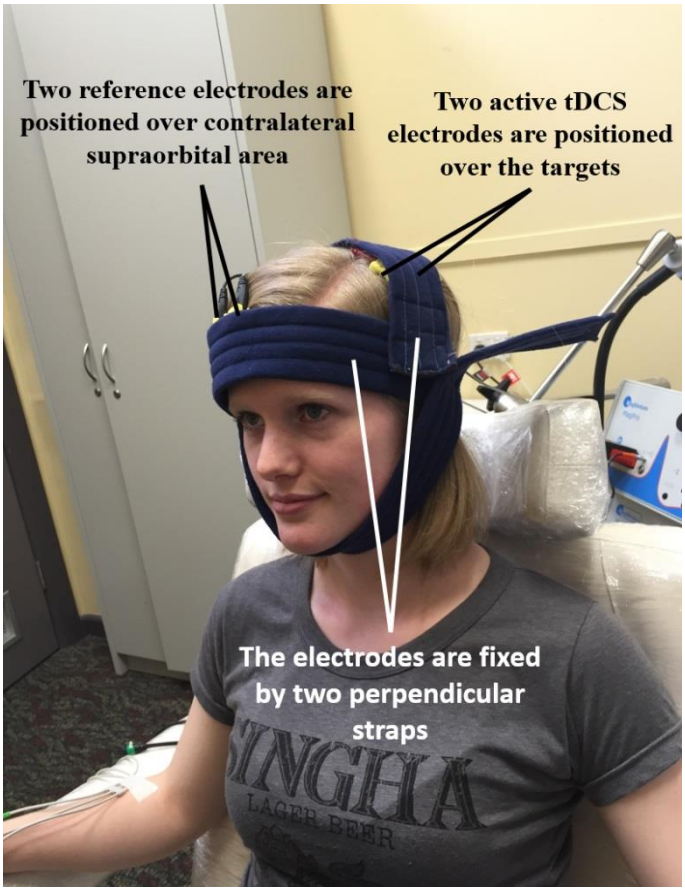

Figure 10: Two custom-designed perpendicular straps fix the active and reference electrodes.

tDCS safety

Due to the 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 and its safety is ensured by the following safety protocol introduced by Nitsche et al., (2003) (Nitsche et al. 2003b). A precise experimental design is critical in achieving the desired safety issues. Previous animal studies are the basis for safety conclusions made by tDCS

researchers (Agnew and McCreery 1987, McCreery et al. 1990, Yuen et al. 1981).

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

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

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

The recommended safety guideline was determined by McCreery et al. (1990) and Yuen et al. (1981) as less than 25 mA/cm^2 for current density and 216 C/cm^2 for total charge in adult humans (McCreery et al. 1990, Yuen et al. 1981). Furthermore, there has been some work done to determine harmful effects of tDCS (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 min of treatment (Iyer et al. 2005). Also, Gandiga et al. (2006), who studied both healthy individulas and patients with stroke, found that tDCS (1mA current intensity; 25 cm^2 electrode size) elicited a minimal discomfort 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: 1) 70.6% noticed a mild tingling under the electrodes; 2) 35.3% felt fatigue following treatment; and 3) 30.4% felt itching under the electrodes. Additionally, headache (11.8%), nausea (2.9%), and insomnia (0.98%) were reported, but, however, 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, which lead the authors to suggest that researchers should inform participants of this potential side effect during the administration of tDCS at an intensity of 2 mA (Palm et al., 2008) or long applications of tDCS even though using smaller intensities.

Furthermore, it is shown that tDCS under safe protocols does not cause heating effects under the electrodes (Nitsche and Paulus 2000a), does not increase serum neurone-specific enolase levels (Nitsche et al. 2003b, Nitsche and Paulus 2001a) 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 edema, necrosis or alterations of the blood–brain barrier or cerebral tissue, nor any sign of cell death, was observed (Nitsche et al. 2004, Nitsche et al. 2003b).

The parameters used in Chapters 5 to 9 are selected based on the tDCS safety guidelines to ensure that the tDCS parameters are safe for participants. In summary, in all experimental studies (Chapters 5-9), the current density of 0.1 mA/ cm^2 (current intensity of 0.3 mA) was applied for 20 min. As a result, the total charge was 120 C/cm^2 which is far below the reported safety limit (216 C/cm^2) (Yuen et al. 1981). All participants (Studies 4-8) 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.

Outcome measures

The effects of tDCS on cortical sites of the PNM on M1 CSE and STh/PTh changes are

evaluated in the present thesis. Therefore there are two types of outcome measures in this project: first, outcome measures for assessment of cortical changes such as M1 (Chapters 5-8) and S1 (Chapters5-6) excitability changes and the level of short-interval intracortical inhibition (SICI) and ICF (Chapters 7-8); second, outcome measures for assessment of behavioral changes such as STh/PTh (Chapters 5-6, and 9). The assessment tools to evaluate changes in these outcome measures will be described in more detail in the following sections.

Over the past decades, neuroscience research methods have developed dramatically. The availability of noninvasive neuroimaging and electrophysiological techniques allows us to study cortical reorganization in the intact human brain. Single- and multi-neuron recordings, EEG, somatosensory evoked potential (SEP), computerized tomography (CT), positron emission tomography (PET), single photon emission computed tomography (SPECT), photon migration tomography (PMT), MRI, fMRI, 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 (Baudewig et al. 2001, Lang et al. 2005). In this thesis, TMS is used as an assessment tool to elicit motor evoked responses for evaluation of M1 CSE modulations. Compared to the majority of the other techniques, which are mainly imaging techniques, TMS provides in vivo assessment of cortical changes.

TMS is the core assessment technique for the evaluation of CSE, ICI, and ICF changes in the studies listed in this thesis (Hallett 2000) and will be explained in more detail in the following section. It was also used to locate the M1 of the target muscle for application of an active electrode during tDCS application (Nitsche et al. 2008).

Transcranial magnetic stimulation (TMS) for assessment of M1 CSE

The application of TMS was introduced as a painless and noninvasive technique to stimulate

the human motor cortex by Barker and his colleagues in 1985 (Barker et al. 1985). Since that time, TMS has been extensively used to assess M1 CSE and the integrity of the corticospinal tract (Petersen et al. 2003).

The pyramidal tract, which encompasses both the corticospinal and corticobulbar tracts, is one of the most important motor tracts in the human body. The majority of the fibres in the tract (up to 60%) originate in layer V of the M1 and the adjacent premotor cortex, while the remaining fibres arise from the S1 and parietal cortex (Nathan et al. 1990). These fibres are collectively known as the corticospinal tract (Figure 10). 70-90% of these fibres decussate and continue on as the lateral corticospinal tract, to synapse with the motor neurons in the ventral horn of the spinal cord that innervate limb and trunk muscles. The remaining 10-30% are uncrossed fibres which descend in the ventral columns of the spinal cord as the ventral corticospinal tract and terminate in the thoracic spinal cord to innervate trunk muscles (Figure 10). TMS induced evoked responses are generated by the stimulation of Betz cells in the M1. They propagate action potentials through this pathway, and the induction of muscle responses in the target muscles through spinal nerves.

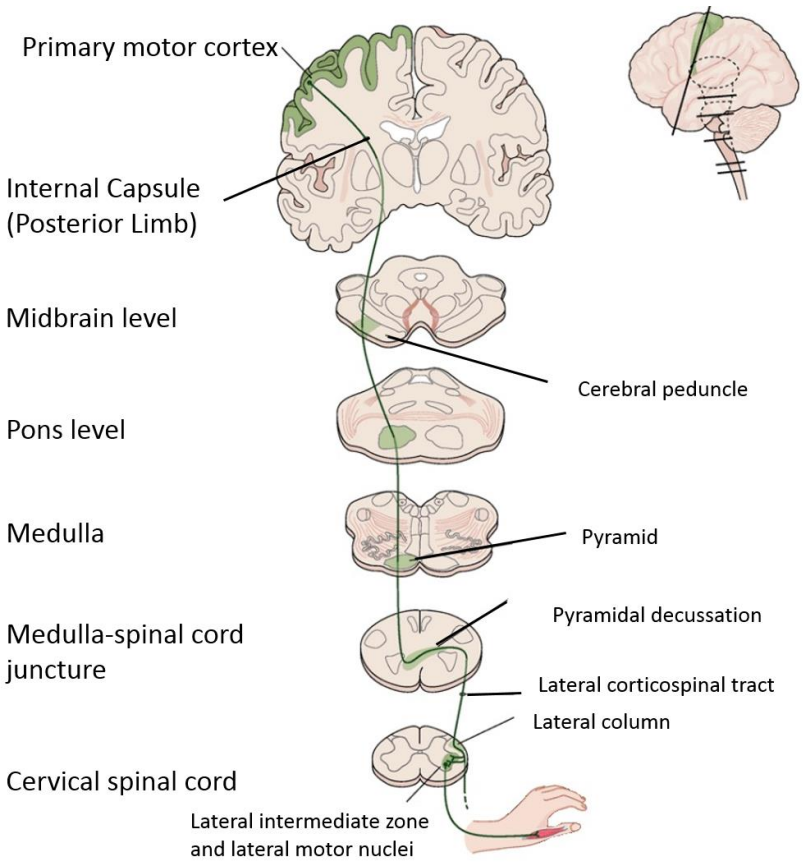

Figure 11: The corticospinal tract (Squire et al. 2012)

A magnetic stimulator induces a magnetic pulse, which can be applied on the skull by a magnetic coil. Based on Faraday's law, an electrical current can be induced in a secondary circuit when it is brought into close proximity to the primary magnetic circuit in which a time-varying current is flowing. By discharging the magnetic circuit induced by the magnetic stimulator, a brief pulse of current of up to 5000 ampere (A) passes through the stimulating coil (Jalinous and Chris 2006). This transient magnetic field is able to induce electrical current to flow in a nearby secondary conducting material, such as the brain. Depending on the time-course of the induced electrical current, the nerve cells in the stimulated area may be excited. The intensity of the magnetic field is represented by influx lines around the coil and is measured in Tesla (T). This intensity 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).

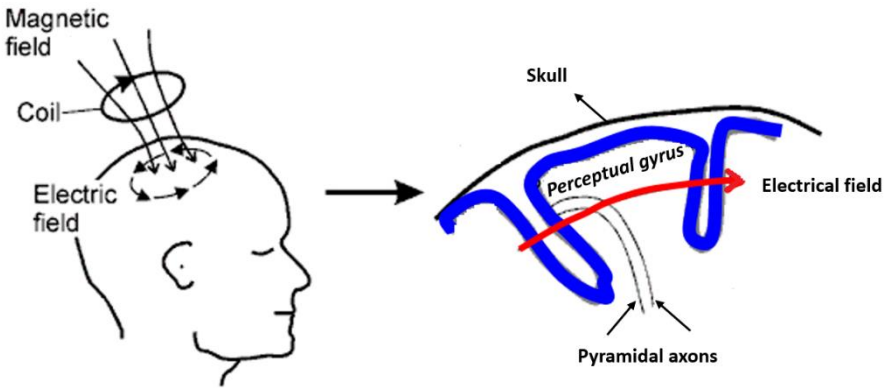

Figure 12: In the TMS, current in the coil generates a magnetic field that induces an electrical field. The above drawing schematically illustrates a lateral view of the percentral gyrus. Two pyramidal axons are shown with a typical orientation of the cranium. The intracranial electric field is parallel to the scalp, and causes an electric pulse to travel down the pyramidal axons. The figure is adapted from (Hallett and Chokroverty 2005).

TMS-induced evoked responses are called motor evoked potentials (MEPs), which can be measured by electromyography (EMG) from the target muscle.

TMS has been used for therapeutic and diagnostic (assessment) purposes (Rossini and Rossi 2007). As an assessment tool, TMS has been used to evaluate the CSE, the timing of cortical processes, cortico-cortical connectivities, and the inhibitory and facilitatory interaction of cortical processes by single- or paired-pulse TMS (Anand and Hotson 2002, Chen et al. 2004, Di Lazzaro et al. 2004, Pascual-Leone et al. 2000, Sanger et al. 2001).

In the present thesis, both single and paired-pulse TMS are used to assess the level of CSE, ICI, and ICF. As a result, these two TMS paradigms are describe in more detail in the following sections.

Single-pulse TMS

Single-pulse TMS are the most widely used TMS paradigm with a good temporal resolution. A number of different and valuable neurophysiologic measures of MEP can be derived from single-pulse TMS. These include MEP threshold, MEP size, representational motor mappings, input-output curves, and assessment of the silent period.

To minimize a large number of confounding variables which may affect elicited MEPs, the guideline checklist developed by Chipchase et al. (2012) was followed in all experiments in this thesis (Chipchase et al. 2012).

Paired-pulse TMS

A paired-pulse technique was originally introduced by Kujirai et al. (1993) in which a subthreshold conditioning stimulus is applied prior to a suprathreshold test stimulus (Kujirai et al. 1993). Depending on the length of the inter-stimulus interval (ISI) between conditioning and the test stimuli, paired-pulse TMS can be used for assessment of ICI or ICF (Figure 12). In fact, inhibitory or excitatory intracortical connections to the pyramidal tract neurons can be stimulated by different ISIs in paired-pulse TMS.

In this paradigm, subthreshold conditioning stimulus intensity is applied as 80% of the RMT, followed by a second suprathreshold test stimulus. In this thesis the test stimulus is adjusted to achieve a baseline single-pulse TMS-induced MEP of around 1 mV (Batsikadze et al. 2013).

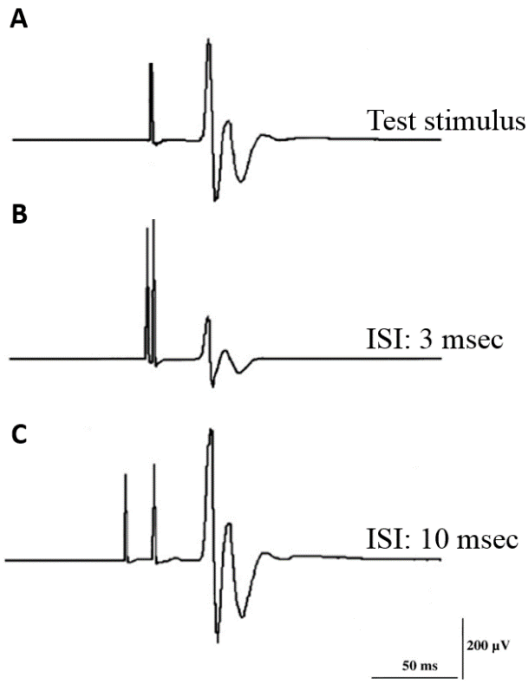

Figure 13: Recorded MEPs from relaxed right first dorsal interosseous (FDI) muscle. Representative single-pulse induced MEP using test stimulus (A), and when this stimulus conditioned by a subthreshold conditioning stimulus of 3 msec (B), and 10 msec (C) earlier. The inter-stimulus intervals (ISIs) were 3 and 10 msec to measure short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) respectively. Paired-pulse TMS was delivered by a MagPro R30 (MagOption) stimulator (MagVenture, Denmark).

MEP recording

For MEP recording, the participants were seated comfortably in a fully adjustable treatment chair (MagVenture, Denmark) with head and arm rests, and with easy access to the their head for stimulation of target area(s) (Figure 13).

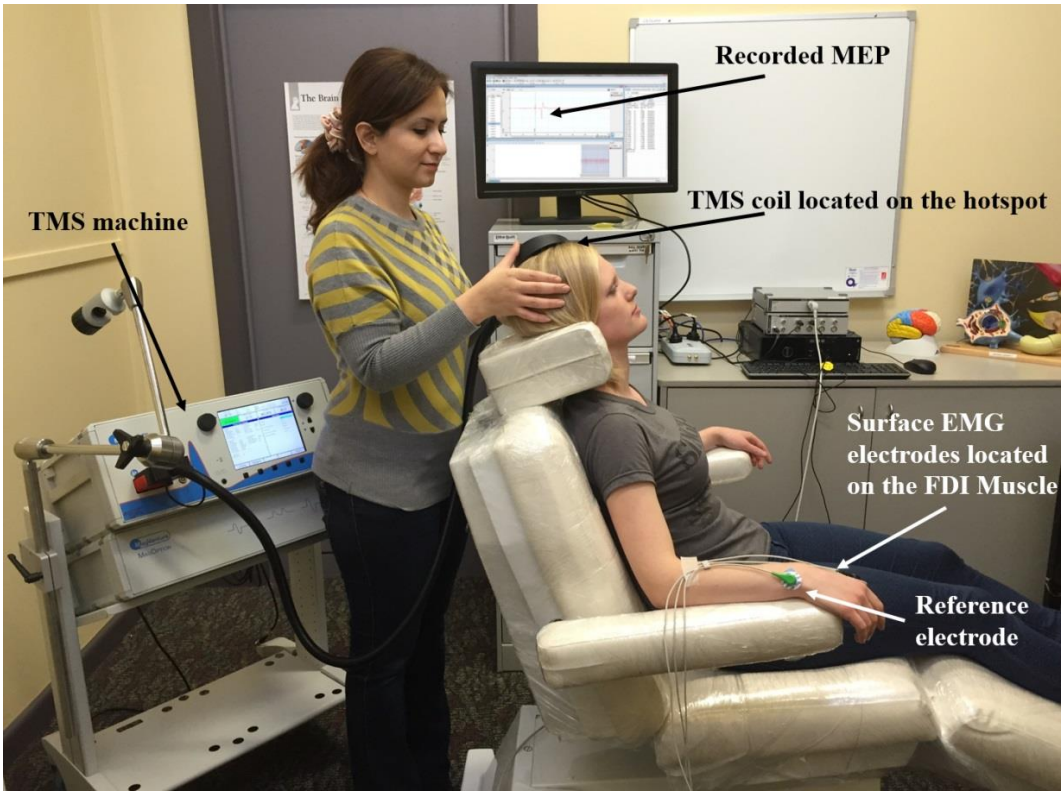

Figure 14: The participants sit upright in an adjustable podiatry chair. A TMS coil is positioned on the hotspot for the first dorsal interossei (FDI) muscle. When a TMS pulse is delivered over the M1, surface EMG is recorded from the FDI muscle using bipolar Ag/AgCl disposable surface electrodes.

The stimulation site, i.e. M1, contralateral to the target muscle, is first determined by using the international EEG 10/20 system. Then to find the optimal site for recording the MEP responses, the coil is moved around the M1 until the largest motor MEPs can be recorded from the target muscle. This area is called the 'hotspot' for the target muscle (Neggers et al. 2004). After localizing the hot spot, the coil's position is marked on the scalp to guide the experimenter during the rest of the testing.

The orientation of the coil is set at an angle of 45° to the midline and tangential to the scalp, such that the induced current flows in a posterior-anterior direction in the brain (Brasil-Neto et al. 1992, Rossini et al. 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). Surface EMG is recorded by bipolar Ag/AgCl disposable self-adhesive and pre-gelled surface electrodes (Figure 14).

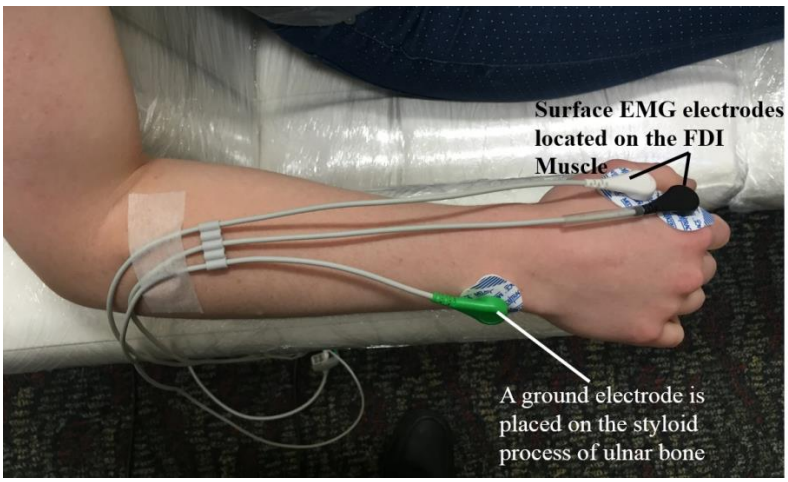

Figure 15: Surface electromyography (EMG) electrodes are positioned over FDI muscles with a 2 cm inter electrode space. The reference electrode is placed over the ulnar bone.

To ensure good surface contact and to reduce skin resistance, a standard skin preparation procedure of cleaning and abrading will be performed for each electrode site (Gilmore and Meyers 1983, Robertson et al. 2006, Schwartz 2003). The location of the surface electrodes on the target muscle (right FDI muscle) is determined based on anatomical landmarks (Perotto and Delagi 2005) and also observation of muscle contraction in the testing position (Kendall et al. 2010). The accuracy of EMG electrode placement is verified by asking the subject to maximally contract this muscle while the investigator monitors online EMG activity. A ground electrode is placed ipsilaterally on the styloid process of ulnar bone (Basmajian and De Luca 1985, Oh 2003). The electrodes are secured by hypoallergenic tape (Micropore, USA). All raw EMG signals are band pass filtered (10-500 Hz), amplified (x1000) and collected for offline analysis (The PowerLab 8/30, ADinstrument, Australia) on a PC running commercially available software (Figure 15).

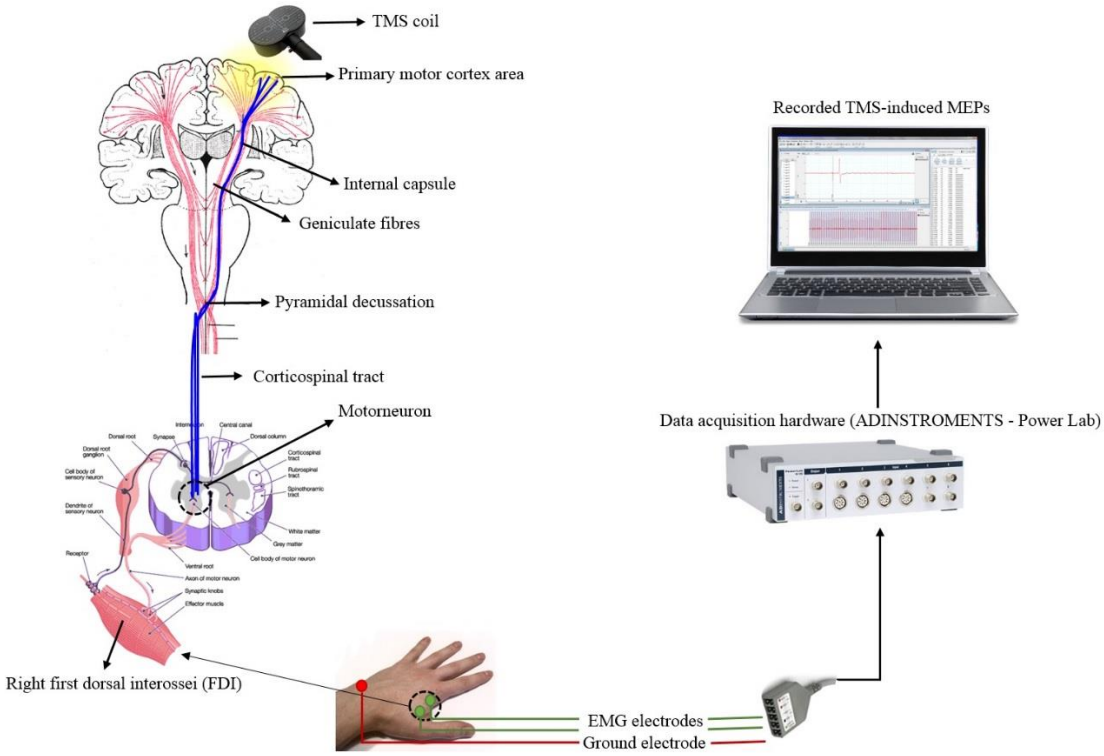

Figure 16: MEPs generated by TMS are recorded from the target muscle, right first dorsal interossei (FDI) in this figure.

MEP threshold

MEP threshold can be measured with the muscle of interest at rest and is referred to as the resting motor threshold (RMT), or when the muscle is in a low level of sustained contraction referred to as the active motor threshold. In all experimental studies included in this thesis (Chapters 5-9), RMT is recorded, which is explained in more detail in the next section. RMT has been classically defined as the TMS machine output (intensity) necessary to produce a MEP that exceeds defined peak-to-peak amplitude (usually 50 µV) 50% of the time in a finite number of trials (Neggers et al. 2004). The guideline for assessment of RMT by Rothwell et al.

(1998) suggests 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 by which five MEPs in a series of 10 with minimum amplitude of 50-100 μV are elicited in the relaxed muscle) (Rothwell et al. 1998). RMT reflects the global excitability of the motor pathway, including large pyramidal cells, cortical excitatory and inhibitory interneurons and spinal motor neurons (Ziemann 2004b).

Accurate estimation of RMT is very important in both research and clinical studies as it is the value most commonly used for estimation of the therapeutic dosage or the size of test stimulus in TMS applications (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, Pascual-Leone et al. 1998, Wassermann 1998b). In addition to this, accurate measurement of RMT is also important for the sake of subject comfort, and expedience in the laboratory or treatment room.

It has been indicated that NMDA antagonists, including Ketamine, reduce RMT whilst the block of voltage-gated sodium channels enhances RMT. Furthermore, it was shown that GABA has no effect on RMT (Ziemann et al. 1996b). A number of environmental factors could affect the size of RMT such as consuming caffeine (coffee, energy drinks), sleep deprivation, and sedative medicines. Other factors that have been shown to influence RMT are sodium-channel blockers (Ziemann et al. 1996a) posture (lower when sitting or standing vs. lying supine) (Ackermann et al. 1991), neck rotations (Alagona et al. 2001), mental activity and closing and opening of eyes (Rossini and 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.

MEP amplitude

The peak-to-peak amplitude of the MEP provides an immediate quantitative measure for the degree of CSE (Wassermann et al. 2008). In addition to inter-individual differences there is great inter-trial variability even in the same subject (Kiers et al. 1993). Several technical factors influence MEP amplitude. They 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 the 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 maneuvers, such as voluntary contraction of the target muscle, increases MEP amplitude. Even low background contractions 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.

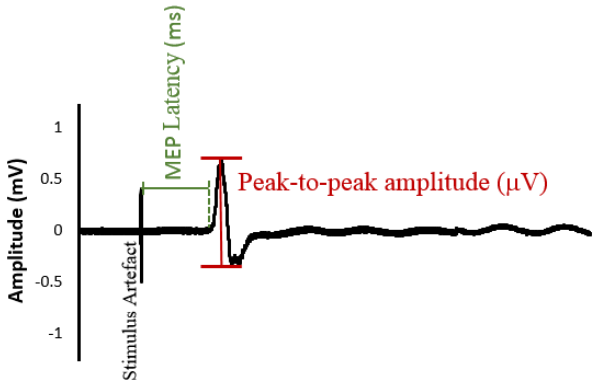

Figure 17: Recorded MEP from the first dorsal interossei (FDI) muscle. MEP amplitude is measured from peak-to-peak. Latency is measured from the TMS stimulus artefact to the onset of the recorded MEP from the target muscle (Rothwell 1997).

In the studies presented in this thesis, peak-to-peak MEP amplitude of the elicited MEPs (Figure 17), was measured automatically using a custom designed macro in Powerlab 8/30 software. The peak-to-peak values for each MEP were listed in a spreadsheet (Figure 17C). The setup is shown in the Appendices.

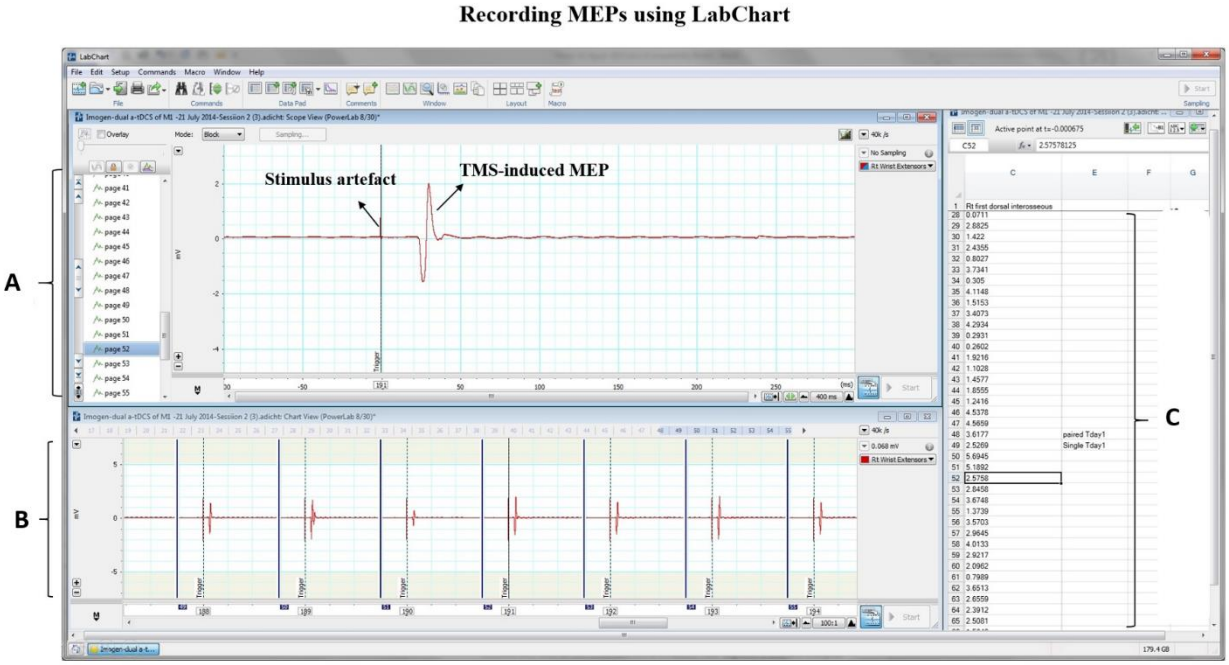

Figure 18: The Automatic detection of peak-to-peak MEP amplitudes using LabChart software from the Adinstrument Company. (A) Scope View provides an additional way of displaying and analysing the capabilities of a digital storage oscilloscope using PoweLab. In Scope View, each sweep is recorded and represented in a single page, creating a list of recorded MEPs that can be averaged and overlaid for analysis. (B) Chart View is the main window in which data can be dynamically viewed. (C) The peak-to-peak MEP amplitudes are automatically detected and recorded, using a custom designed macro with PowerLab 8/30 software.

There are a multitude of measures and protocols in the paired-pulse TMS paradigm including SICI, long interval intracortical inhibition (LICI), ICF, and short-interval intracortical facilitation (SICF) to measure ICI and ICF (Figure 18). Such measures and protocols are used in

different mechanism-base TMS studies to evaluate the function of GABAergic and/or Glutamergic mechanisms. In Chapters 7-8 of the present thesis, the paired-pulse paradigm is used to evaluate the mechanisms behind the efficacy of a novel tDCS technique on CSE enhancement, named unihemispheric concurrent dual-site tDCS ($tDCS_{UHCDS}$). In these chapters, SICI and ICF are measured, which will be described in more detail in the next section.

Short interval intracortical inhibition (SICI)

To measure SICI, a subthreshold conditioning stimulus is delivered 1 to 5 ms prior to a suprathreshold test stimulus (Kujirai et al. 1993) (Figure 18A). Compared to single-pulse induced MEPs, the recorded MEP is suppressed at a cortical level (Kujirai et al. 1993). Since it was shown that magnetic conditioning stimulus has no effect on electrical test pulses, which activates the axon of the corticospinal tract, it was concluded that the inhibition observed in SICI is not due to the refractoriness of the corticospinal tract (Kujirai et al. 1993). SICI has been associated with the activity of $GABA_A$ receptors (Cohen et al. 1998, Di Lazzaro et al. 2000).

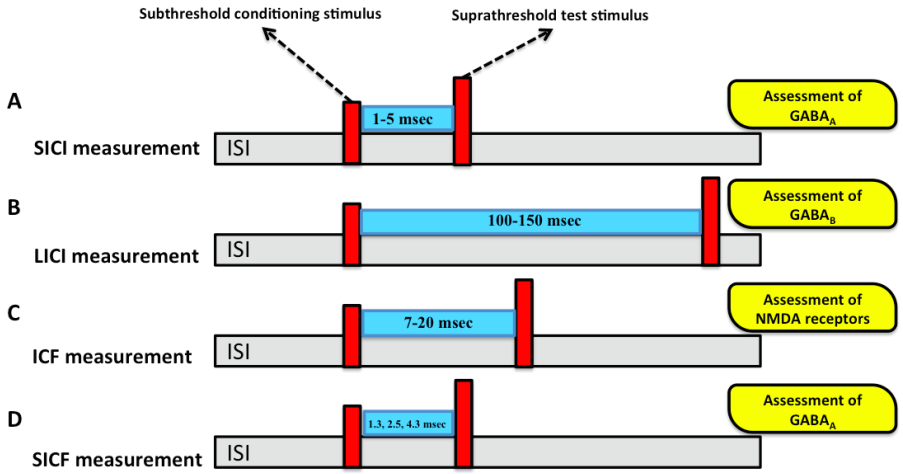

Figure 19: TMS paired-pulse protocols for testing short intracortical inhibitors (SICI) (A), long intracortical inhibitors (LICI) (B), intracortical facilitation (ICF) (C), and short intracortical facilitation (SICF) (D). The blue blocks show the inter-stimulus interval (ISI).

Intracortical facilitation (ICF)

In the paired-pulse paradigm, if the subthreshold conditioning stimulus is delivered 7 to 20 msec prior to the suprathreshold test stimulus, the responses will be facilitated, compared to the single-pulse paradigm (Kujirai et al. 1993) (Figure 18C). ICF is an index for the function of excitatory circuits in the motor cortex (Ziemann et al. 1996b). Based on pharmacological studies, NMDA antagonists like Lorazepam decrease ICF (Ziemann et al. 1996b). In contrast $GABA_B$ agonists like Baclofen enhance the levels of ICF (Ziemann 2004a). Regarding the ICF reduction by NMDA antagonists, it is suggested that glutamate plays an important role in ICF enhancement (Ziemann et al. 1998).

The literature indicates that ISIs of 3 and 10 msec are the most efficient protocols to measure the function of cortical inhibition (Batsikadze et al. 2013, Ni et al. 2011, Oliveri et al. 2000, Udupa et al. 2010) and facilitation (Du et al. 2014, Kothari et al. 2014, Sato et al. 2013) circuits respectively. As a result, in Chapters 7 and 8, paired-pulse magnetic stimuli were delivered by a MagPro R30 (MagOption) stimulator (MagVenture, Denmark) to measure SICI (with an ISI of 3 msec) and ICF (with an ISI of 10 msec).

The safety of TMS

TMS is believed to cause only a transient change in neural activity (Bridgers 1991, Bridgets and Delaney 1989, Chokroverty et al. 1995, Pascual-Leone et al. 1992, 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 1998b). As the stimulation frequency of TMS increases, the risk of unwanted side effects increases. In many studies, single-pulse (<1 Hz) TMS 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 (Bridgers 1991, Cohen and Hallett 1987, 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 or 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 and 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 seizures or any other significant side effects (Anderson et al. 2006). A literature review on the safety and tolerability of rTMS (Bae et al. 2007) indicates that even by using rTMS in epileptic patients, the risk of seizure in these patients is very small (0.35%).

Certain conditions increase the risks associated with TMS (Appendix 12). 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 1998b).

Furthermore, if the guidelines for safe application of TMS as the assessment technique are followed, there will be no risks to participants. If they are not followed, there may be some potential risks as described below:

1. The effect of magnetic fields on biological tissues.

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 an MRI. In addition, while an 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 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 1998a).

3. Effects on the 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, and comparable changes in lymphocyte subpopulations can occur with mild stress, a normal circadian cycle and the menstrual cycle. Therefore, the effects of TMS on the immune system are not considered to be dangerous or harmful (Amassian et al. 1994, Sontag and 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 (Fauth et al. 1992, Kandler 1990). There has also been the proposition that a minor degree of risk is involved in the use of TMS in people with epilepsy (Düzel et al. 1996, Hufnagel et al. 1990).

   In all studies in this thesis, we have considered the latest TMS and tDCS safety guidelines (Appendixes 10-11). A modified version of TMS safety questionnaire (Appendix 13) was

completed prior to all the experiments of the present studies to screen and exclude subjects for whom TMS was contraindicated.

Somatosensory evoked potential for assessment of S1 excitability

Sensory Evoked Potential Responses (SEP) is a noninvasive technique which could be used to assess excitability of the S1. The S1 is responsible for sensory discrimination of painful stimuli or pain. SEP recording involves elicitation and recording of the cortical waves in response to electrical signals (sensory and painful stimuli). They are a series of positive and negative deflections that reflect the sequential activation of neural structures along the somatosensory pathways. In fact, these responses are the result of the synchronous response (action potential and synaptic potentials) of a series of neurons activated in response to sensory signals (Aminoff and Eisen 1998).

The recorded signals are low-amplitude (1-20 μV) (Freye 2005). These signals are mixed with other electrical potentials including the activity of spontaneous nervous system like rhythmic cardiac activity, which generates undesirable noise. As a result, improving signals and decreasing the signal to noise ratio is required in order to have a clear response. Averaging and amplifying the signals are the best ways to have reliable data (Desmet et al. 1987). The remaining signals represent the potential signals evoked by sensory stimulus. This technique was developed by Dawson in 1954.

The SEP involves a series of sinusoidal waves, whose peaks are characterized by specific latency (in msec) from the stimulation (Cruccu et al. 2008, Freye 2005). Morphology of peaks and inter-peak latencies are other important SEP parameters (Figure 19). The amplitude of SEP waves represents the number of neurons/fibres and their synchronization when fired (Freye 2005).

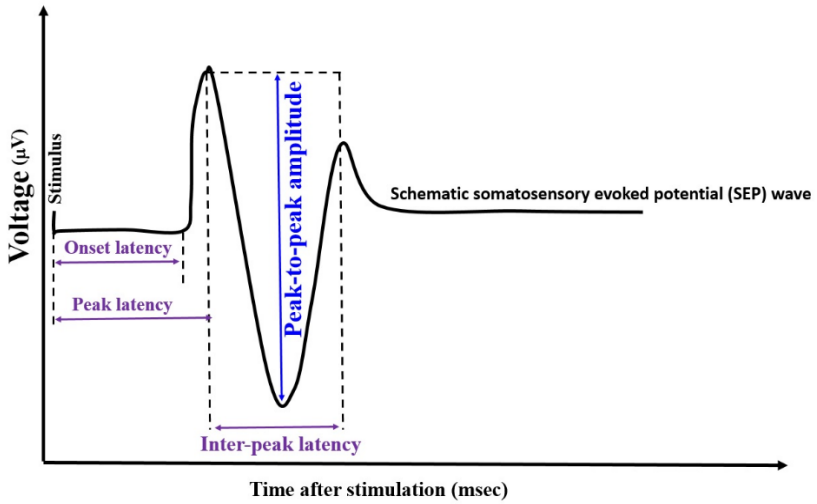

Figure 20: SEP components are characterized by amplitude, latency, onset latency, and inter-peak latency. Adapted from Banoub 2003 (Banoub et al. 2003).

Animal studies have demonstrated that SEPs are primarily mediated by dorsal column-medial leminscal tract (Cohen et al. 1981, Cruccu et al. 2008, Cusick et al. 1979). Other somatosensory tracts like the spinothalamic tract can affect the components of SEPs (Aminoff and Eisen 1998, Toleikis 2005). Finally, all sensory inputs terminated to S1 and can be recorded from scalp.

In clinical studies, electrical or mechanical stimulation on the distal part of a peripheral nerve is applied and SEPs are elicited from related areas in the S1 (Aminoff and Eisen 1998). Tactile stimulation, as a mechanical stimulation, can been applied using a pneumatic stimulator on a finger to record SEPs (Wienbruch et al. 2006). Alternatively, transcutaneous electrical stimulation of a peripheral nerves, usually the median or ulnar nerves, at the wrist level for upper extremity monitoring, can be used for elicitation of SEPs (Berger and Blum 2007, Shellhaas et al. 2011). Based on the American Clinical Neurophysiology Society's (2011) recommendations, it is suggested that monophasic rectangular pulses with a duration of 100-300 μs and a frequency of 3-5 Hz are used. The intensity of electrical stimulation should be set so as to induce visible muscle twitching in related muscles. For example, a muscle twitch should be observed in abductors of the thumb by stimulation of the median nerve (Aminoff and Eisen 1998, Berger and Blum 2007, Cruccu et al. 2008). An earth electrode should be

positioned between the stimulation and recording sites (Shellhaas et al. 2011). SEP waves are composed of both low and high frequencies resulting in noisy SEPs. As a result, depending on the setting and aims of studies, either low or high frequencies can be filtered. The band pass filters of 5-30Hz and 2000-4000Hz for the low- and high-pass filters are the most common filtering protocol (Berger and Blum 2007, Freye 2005, Shellhaas et al. 2011).

To record SEP waves, electrical stimulation is applied to the selected nerve ending and the responses are recorded by two standard EEG electrodes (impedance: < 5kΩ) which are placed over the scalp (Cruccu et al. 2008, Freye 2005). High-resolution scalp recordings can be performed using a cap with electrodes located according to the International 10-20 System (Lascano et al. 2009, van de Wassenberg et al. 2008). The latency between the peripheral nerve stimulation to elicited SSEPs from the scalp is about 40 msec for the upper extremity. This latency can be prolonged by neurological problems such as hypothermia or anaesthesia. To be sure that the recoded signals are not artefacts, it is recommended each recording be replicated (Cruccu et al. 2008, Shellhaas et al. 2011).

The reference electrode should be theoretically placed over an isoelectrical (zero potential) part of the body. As the heart and nerves produce electrical potentials in the whole body (Geselowitz 1998), the ideal location of the reference electrode is still under debate (Desmedt 1987). Therefore, in some studies the reference electrode is positioned on a non-cephalic part of the body (Cruccu et al. 2008) while other studies use the cephalic reference (Geselowitz 1998, Murray et al. 2008). As the cephalic reference electrode is the most common configuration, in the studies in this thesis (Chapters 4 and 5), the reference electrode was placed over the mid-frontal area (Fz) (Fukuda 2006, Matsunaga et al. 2004) in which the net source of potential is zero (Murray et al. 2008).

SEP components

The SEP components are determined according to polarity (positive (P) and negative (N)

components) and the average post-stimulus latency from a sample from a normal adult population (Berger and Blum 2007, Shellhaas et al. 2011). For instance, P20 means that the peak is positive and appears 20ms after the stimulus. Conversely, N25 is a negative peak which appears 25 ms after the stimulus (Desmedt 1987).

SEP waves can also distinguish between near-field and far-field electrical potentials according to the distance from their source with the recording electrodes.

- Near-field potentials refer to long-latency cortical components of neurons located in the grey matter of cortical areas, within 3-4 cm from the recording electrodes.

- Far-field potentials refer to short-latency components of subcortical or peripheral neurons located in the white matter corresponding to SEPs whose generators are subcortical or peripheral (Banoub et al. 2003, Berger and Blum 2007, Freye 2005, Gilmore 1989). The characteristics of short-latency peaks produced after stimulation of the median nerve are N9, P9, N11, P11, N13, P13, P14, N18, N20, P20, P22, P25, P27, N30, P30, N35, P45 (Desmedt 1987).

  N9 and P9 are the first peaks of near-field potentials recorded from Erb's point and provide information related to the brachial plexus (Desmedt 1987). Therefore, both N9 and P9 show the function of peripheral nerves.

  N11 and P11 are recorded from the lower cervical spine and reflect the function of primary somesthesic neurons near the dorsal root entry zone in the spinal cord (Desmedt 1987).

  N13 and P13 are also recorded over the lower cervical spine and evaluate the function of the postsynaptic activity of neurons in the posterior horn of the lower cervical spinal cord and in ascending afferents in the cuneate tract (Berger and Blum 2007, Desmedt 1987, Shellhaas et al. 2011).

P14 generated far-field potential recorded referentially from the scalp and having a widespread distribution. P14 is the result of activity in the dorsal column nuclei or the caudal medial lemniscus (Shellhaas et al. 2011).

N18 subcortically generated far-field potential was recorded referentially from the ipsilateral scalp electrodes. N18 reflects the postsynaptic activity of the brain stem and the thalamus (Shellhaas et al. 2011).

N20 is a near-field potential derived from the contra-lateral parietal cortex and it is the first cortical negativity, reflecting activation in the contralateral S1 from thalamocortical radiations projecting from the VPL (Berger and Blum 2007, Desmedt 1987, Shellhaas et al. 2011).

P22 is generated by frontal nerves located near the central sulcus (Desmedt 1987).

N20, P20, P25, N30, P30 and N35 are the result of the activity of neurons located in the hand area of the contralateral S1 and in the cortical association areas (Cracco and Cracco 1976) (Figure 20)

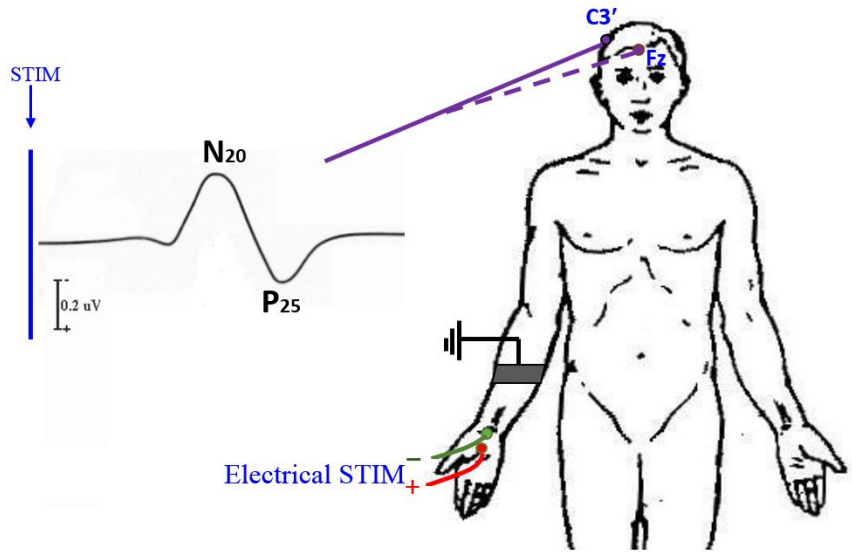

Figure 21: The normal Somatosensory Evoked Potential (SEP) following electrical stimulation of the right Median nerve at the wrist level. The intensity of stimulation was fixed at the motor threshold.

Factors altering the sensory evoked responses

Many factors can affect the latency, amplitude, morphology and topographical distribution of SEPs (Banoub et al. 2003, Freye 2005). Besides the pathologic factors, the responses can be different by changing the stimulation parameters including:

- **Type of stimulation**: Compared to mechanical stimulations, electrical stimulations induce bigger amplitude responses (Aminoff and Eisen 1998).

- **Intensity of stimulus**: Increasing the intensity of stimulations leads to amplitude enhancement, while the latency of SEPs recorded following median nerve stimulation cannot be affected by changing the intensity of stimulation (Aminoff and Eisen 1998).

- **Frequency of stimulus**: Increasing the frequency of the stimulus can decrease the amplitude of responses but has no effect on the latency (Araki et al. 1999).

- **Setting of filtering:** The effect of filtration on SEP responses has been explained previously.

Chapters 5-6 detail the recording of SEPs from the S1 following electrical stimulation of the right median nerve at the wrist level at 2Hz with a pulse width of 0.2ms. The intensity of stimulation was fixed at the motor threshold. The electrical potentials were recorded in epochs from 0 to 200ms after the stimulus. A total of 500 stimulus-related epochs were recorded. Peak-to-peak amplitudes of N20-P25 responses generated in the S1 is measured.

Equally important, some characteristics of participants can also affect the SEP responses (Freye 2005):

- **Age**: it has been shown that aging induces a gradual increase in both the amplitude and the latency of responses in healthy people (Aminoff and Eisen 1998, Shagass and Schwartz 1965,

Zumsteg and Wieser 2002). The investigation indicated that the conduction velocity at the median nerve decreases by about 0.16 m/s/year, which is equal to increasing the latencies. In young human adults, peripheral conduction is 71.1±4.0 m/s, whereas it decreases to 61.2±5.9m/s in healthy octogenarians (Desmedt 1987). In fact, aging also affects the spatial distribution of SEPs (Zumsteg and Wieser 2002).

- M**aturation**: The result of SEP responses illustrated that the nervous system in infants is still maturing (Berger and Blum 2007, Gilmore 1989). As a result, the inter-peak latency after stimulation of the median nerve decreases rapidly from age 0 to 2 years, then slower from 2 to 6 years old, and reaches adult values at 8 years old.

- **Gender:** As females are usually shorter than males, the latency of the median nerve in females is 1ms shorter than males (Banoub et al. 2003).

- **Height**: The length of the somesthesic pathways increases with the patient's height and consequently the latency increases as well in adults.

- C**utaneous and core temperatures**: Temperature directly affects the conduction velocity of peripheral nerves (a 5% velocity increase per 1°C temperature increase) and causes minor changes in the central pathways. In the case of increasing hypothermia, central conduction time and latency lengthen, and some components tend to disappear (Banoub et al. 2003). Conversely, hyperthermia results in a decrease in conduction time and latency, whereas the amplitude is not affected (Banoub et al. 2003). As a result, recordings should be performed at a constant temperature (21-23°C) with the cutaneous temperature at 34°C and the central body temperature at 37°C.

- C**onsciousness Level**: The amplitude, latency and waveform of some components such as N20 are subject to change during the different stages of sleep and vigilance (Niedermeyer and da Silva 2005).

- **Attention**: Some early cortical components recorded before 50 msec could be modified by attention-related processes (Niedermeyer and da Silva 2005).

- B**lood pressure**: Lower blood pressure can result in a slight reduction in the amplitude of responses (Banoub et al. 2003, Long).

- A**naesthesia administration**: Anaesthesia can generate diverse cortical SEP components, while subcortical SEPs show no change (Banoub et al. 2003, Berger and Blum 2007).

In Chapters 5 and 6, the amplitudes of N20-P25 were used as an index of S1 excitability in healthy young adults.

Tools for assessment of behavioural changes

STh and PTh are two behavioral outcome measures assessed in the current thesis. The following sections provide an overview of STh and PTh assessment tools.

Tools for sensory and pain threshold assessment

Based on the International Association for the Study of Pain (IASP), STh is defined as the minimum intensity required for a stimulus to be perceived for the first time. PTh is defined as the minimum intensity required for a stimulus to be perceived as a painful stimulus for the first time. STh and PTh are self-reported experiences (Gracely et al. 1988). The excitability of peripheral receptors, afferent nerves, sensory tracts including the anterolateral tract and the posterior column-medial lemniscus tract, and the S1 (as the sensory-discrimination center) (Adrendt-Nielsen et al. 1990) can affect the level of STh and PTh. Arousal (Chapman 2002), psychosocial (Price 2000), or cultural factors (Turk and Okifuji 1999) may also affect STh and PTh.

There are many methods and instruments used to quantify the level of STh and PTh. Quantitative sensory testing (QST) is one of the most reliable methods for assessing STh (the detection threshold for innocuous stimuli), PTh, and sensations evoked by suprathreshold stimuli (Cornelissen et al. 2014, Lindblom and Verrillo 1979). There are two types of QST including Static and dynamic.

- Static QST measures the threshold determination (including pain detection, tolerance and threshold) and stimulus intensity rating (including the visual analogue scale or VAS). As one point from a complex pain processing system is involved in this static type of measurement, dynamic QST measurement is suggested (Arendt-Nielsen and Graven-Nielsen 2011).

- Dynamic QST measures the central temporal and spatial integration of stimuli and controls the descending pathways of sensory and pain processing. This type of QST is still being evolved.

QST is a semi-subjective method, which is able to quantify sensory and painful stimuli. The stimulus target fibres and the central pathways, which can be assessed by QST, are summarized in Table 1.

Table 1: An overview of stimulus, target fibres, and sensory pathways can be assessed in QST.

Stimulus	QST	Target sensory fibre	Sensory pathway
Cold	Thermal testing QSTs	Aδ	spinothalamic
Heat	Thermal testing QSTs	C	spinothalamic
Light touch	Calibrated monofilament	Aβ	Lemniscal
Vibration	Vibrometer	Aβ	Lemniscal
Pinprick	Calibrated Pin	Aδ, C	spinothalamic
Pressure	Algometer	Aδ, C	spinothalamic

Types of modalities and pain measurement parameters included in QST are summarized in Table 2.

Table 2: Stimulus modalities and pain measurement parameters in QST.

Stimulus Modalities	Pain Measurement Parameters
Electrical	Pain Threshold
Contact Thermal (heat, cold)	Pain Threshold/Tolerance
Immersion Thermal (heat, cold)	Suprathreshold Scaling (e.g.VAS, NRS)
Mechanical (Pressure, Touch, Vibration)	Pain Threshold/Tolerance, Temporal Summation
Thermal, Ischemic	Conditioned Pain Modulation
Chemical (e.g. capsaicin, hypertonic saline, glutamate)	Cerebral Responses (e.g. EEG, fMRI, PET) Muscle Reflexes

Sensory and pain stimuli are received by different peripheral receptors (Figure 21), transferred by anterolateral and posterior column-medial lemniscus tracts, and finally received by the S1. Anterolateral tract neurons located in the dorsal root carry sensory signals by Aδ fibres (in the spinothalamic tract) or C fibres (in the spinoreticular tract) from the peripheral to the dorsal

horn of the spinal cord. From there, their axons usually decussate and ascend by direct (in the spinothalamic tract) or indirect pathways (in the spinoreticular tract) and synapse with reticular formation. Finally the axons ipsilaterally send sensory information to the striatum, the S1, S2, ACC, and DLPFC (Figure 22).

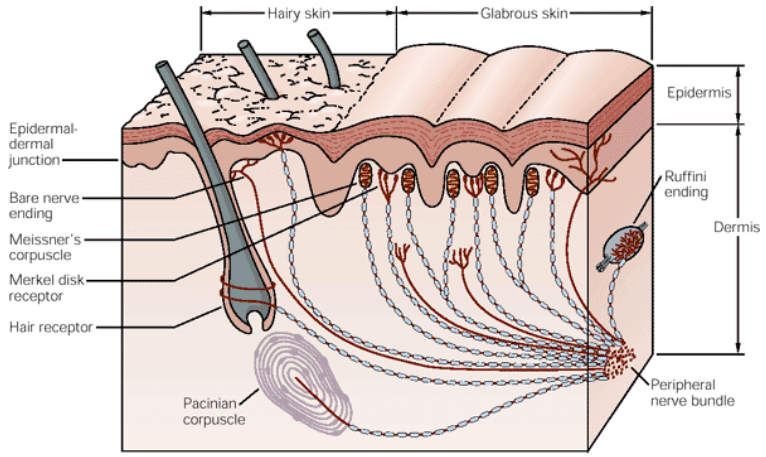

Figure 22: The location and morphology of mechanoreceptors in hairy and hairless (glabrous) skin of the human hand. The receptors of glabrous skin are Meissner's corpuscles, located in the dermal papillae; Merkel disk receptors, located between the dermal papillae; and bare nerve endings. The receptors of the hairy skin are hair receptors, Merkel's receptors, and bare nerve endings. Subcutaneous receptors, beneath both glabrous and hairy skin, include Pacinian corpuscles and Ruffini endings. Nerve fibres that terminate in the superficial layers of the skin are branched at their distal terminals, innervating several nearby receptor organs; nerve fibres in the subcutaneous layer innervate only a single receptor organ. The structure of the receptor organ determines its physiological function. The figure is adapted from www.ib.cnea.gov.ar.

In the posterior column-medial lemniscus tract, Aß and Aδ fibres send sensory and/or painful stimuli from the peripheral to the dorsal horn of the spinal cord. The axons of neurons in the dorsal horn ascend ipsilaterally through the dorsal column and decussate in the lower medulla (medial lemniscus). The medial lemniscal axons ascend and provide information to the ventral posterior lateral nucleus of the thalamus. The information from the halamus goes to the S1. Due to the decussation in the sensory tracts, the sensory and/or painful stimuli from one side are analyzed in the contralateral S1.

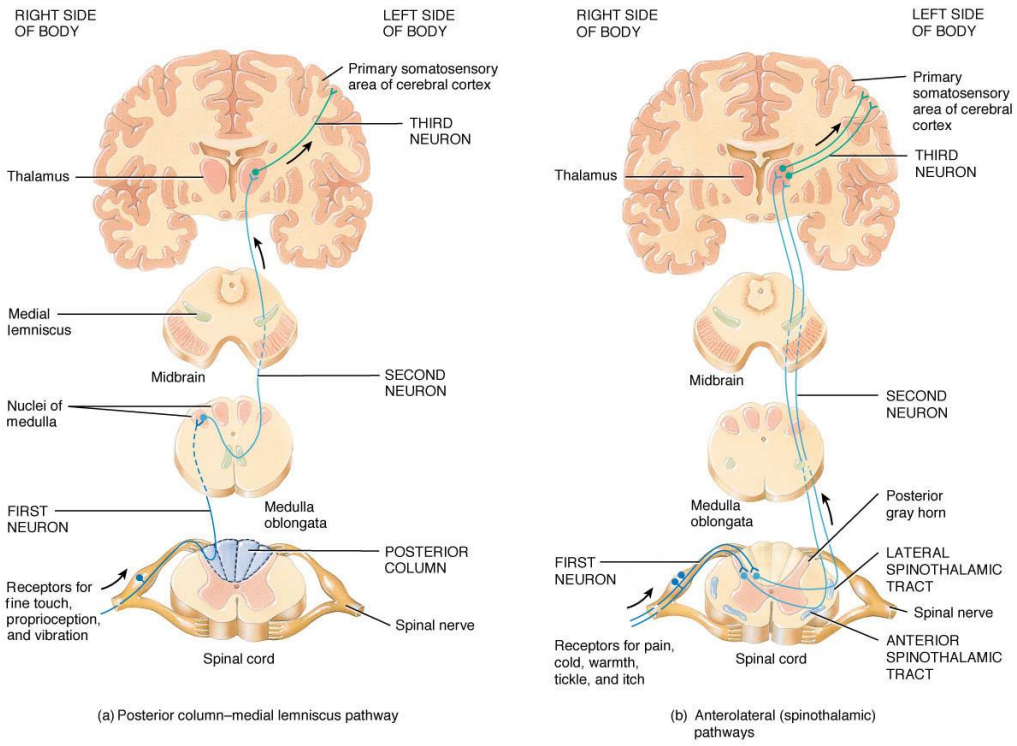

Figure 23: The lateral and posterior column pathway (Squire et al. 2012)

Depending on the type of stimuli, QST can assess both small and large fibre dysfunction. Touch and vibration measure the function of large myelinated Aα and Aβ sensory fibres. Thermal stimulation devices are used to evaluate the pathology of small myelinated and unmyelinated nerve fibres; they can be used to assess heat and cold sensations, as well as thermal pain thresholds. Pressure-specified sensory devices (PSSDs) assess large myelinated sensory nerve function by quantifying the thresholds of pressure detected with light, and static and moving touch. Finally, current perception threshold testing involves the quantification of the sensory threshold to transcutaneous electrical stimulation. In current threshold testing, typically 3 different frequencies are tested: 5 Hz, designed to assess C fibres; 250 Hz, designed to assess Aδ fibres; and 2,000 Hz, designed to assess Aβ fibres. Results are compared with those of a reference population.

In Chapters 5-6 and 9, STh and PTh are evaluated by electrical stimulation. The electrical stimuli was applied by a pen electrode (model: 2762CC, Chattanooga, USA) to the right median nerve (pulse duration: 200μ s) at wrist level. In these chapters, the PTh to pressure (PpTh) is also measured, using a pressure algometer (model: FDX 50, Wagner, USA; capacity: 50Å~ 0.05Ibf, accuracy: ±0.3% of full scale) on the belly of FDI muscle with a flat circular metal probe dressed in a plastic cover (Figure 23). Force was displayed digitally in increments of 0.1N. More details are described in Chapters 5-6 and 9.

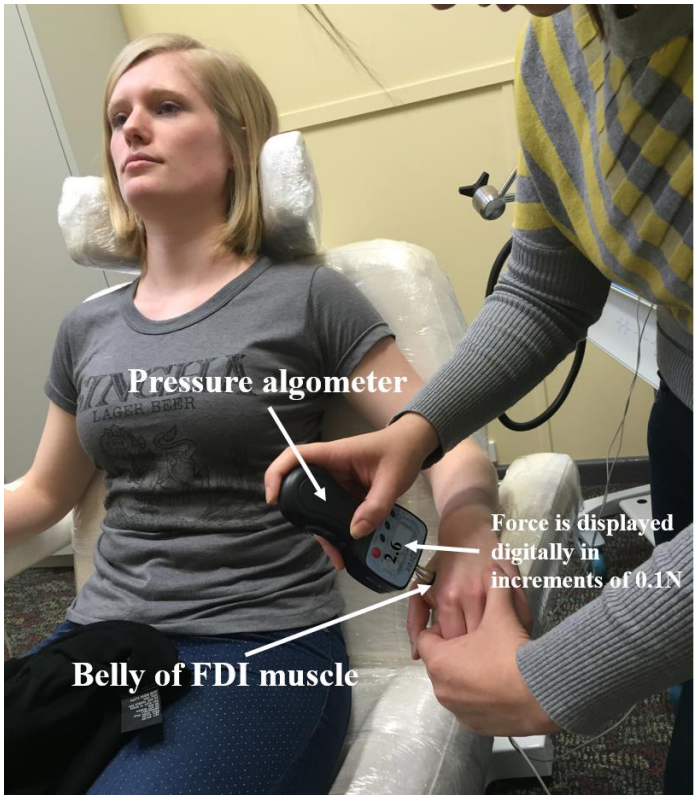

Figure 24: Pressure pain threshold (PpTh) measurement using pressure algometer on first dorsal interossei (FDI) muscle

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 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 framing of the manuscript, review and provision of feedback on manuscript drafts	15 %
Maryam Zoghi	Guidance in framing of the manuscript, review and provision of feedback on manuscript drafts	5 %

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

Candidate's Signature: [redacted] Date: 15-Sep-2015

Signature: [redacted] Date: 15-Sep-2015

Signature: [redacted] Date: 15-Sep-2015

Preamble to Chapter 2

Chapter 2 provides a systematic review and meta-analysis to verify whether previous tDCS studies support the view that a-tDCS increases STh/PTh in healthy individuals and PL in patients with chronic pain.

Chapter 2: Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study

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

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Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study

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HIGHLIGHTS

- Anodal tDCS (a-tDCS) of the primary motor cortex increases sensory and pain threshold in healthy individuals.
- a-tDCS of the primary sensory cortex increases pain threshold significantly.
- a-tDCS of both primary motor cortex and dorsolateral prefrontal cortex decreases pain level in patients with chronic pain.

ABSTRACT

Objective: The primary aim of this systematic review was to evaluate the effects of anodal transcranial direct current stimulation (a-tDCS) on sensory (STh) and pain thresholds (PTh) in healthy individuals and pain levels (PL) in patients with chronic pain.
Methods: Electronic databases were searched for a-tDCS studies. Methodological quality was examined using the PEDro and Downs and Black (D&B) assessment tools.
Results: a-tDCS of the primary motor cortex (M1) increases both STh ($P < 0.005$, with the effect size of 22.19%) and PTh ($P < 0.001$, effect size of 19.28%). In addition, STh was increased by a-tDCS of the primary sensory cortex (S1) ($P < 0.05$ with an effect size of 4.34). Likewise, PL decreased significantly in the patient group following application of a-tDCS to both the M1 and dorsolateral prefrontal cortex (DLPFC). The average decrease in visual analogue score was 14.9% and 19.3% after applying a-tDCS on the M1 and DLPFC. Moreover, meta-analysis showed that in all subgroups (except a-tDCS of S1) active a-tDCS and sham stimulation produced significant differences.
Conclusions: This review provides evidence for the effectiveness of a-tDCS in increasing STh/PTh in healthy group and decreasing PL in patients. However, due to small sample sizes in the included studies, our results should be interpreted cautiously. Given the level of blinding did not considered in inclusion criteria, the result of current study should be interpreted with caution.
Significance: Site of stimulation should have a differential effect over pain relief.

1. Introduction

Sensory and emotional processing of pain involves parallel brain structures (Rainville, 2002; Porro, 2003). Lateral thalamic nuclei and the somatosensory cortex (S1) are thought to subserve sensory-discriminative aspects of pain such as threshold, quality, location, and judgement of its intensity, whereas medial thalamic nuclei, the prefrontal cortex and the limbic system are considered to subserve the affective-emotional dimension of pain. The overlap between these areas and emotion-processing regions of the brain could explain the human subjective qualities of pain (Bornhovd et al., 2002; Porro, 2003; Wager et al., 2004).

Brain mapping studies have reasonably consistently identified the brain areas that are active when someone is in pain (Laurent et al., 2000; Peyron et al., 2000). These areas are mostly multimodal and respond to salient non-noxious stimuli as well as noxious stimuli (Mouraux et al., 2011). Brain areas that are

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involved in pain processing signals and are also superficial to the skull are the primary sensory cortex (S1), primary motor cortex (M1), and dorsolateral prefrontal cortex (DLPFC) (Antal et al., 2010).

S1, with its topographical organization, was long presumed to be a key location of pain-related brain activity. However, the evidence behind this notion is not compelling. Some studies clearly show S1 activity is related to pain intensity (Antal et al., 2008; Grundmann et al., 2011) and others show no such relation (Kanda et al., 2000; Peyron et al., 2000; Bingel et al., 2003; Porro, 2003). Some researchers have predicted that S1 activity will most closely relate to pain when the pain is felt in the skin (Simoes and Hari, 1999; Timmermann et al., 2001).

M1 activation can affect pain reduction not only because of neural connections existed between S1 and M1, but also because of functional relationship between M1 and thalamus (Coghill et al., 1999), and activation of thalamus leads to activation of other pain-related structures such as anterior cingulate, and periaqueductal grey areas which have major role in pain management (Tsubokawa et al., 1993; Fomberstein et al., 2013). A vast literature shows that the motor output of M1 changes with pain (Moseley and Brugger, 2009). This includes reduced amplitude and velocity of movement (Lund et al., 1991), altered muscle coordination (Hodges and Moseley, 2003), decreased motor unit discharge rate (Farina et al., 2004; Hodges et al., 2008) and decreased maximal voluntary contraction force (Graven-Nielsen et al., 2002). The mechanisms behind the involvement of M1 are largely unknown but we know that M1 activity has a clear link with the pain network, which makes it an intuitively sensible target of interventions to reduce pain (Apkarian et al., 2004; Baliki et al., 2012).

DLPFC is one of the areas of the brain most commonly activated during pain, regardless of where the pain is felt (Apkarian et al., 2005). Changes in connectivity between the DLPFC and deeper pain-related areas (Baliki et al., 2012) and reduction in grey matter density and DLPFC volume (Apkarian et al., 2004) have been implicated in chronic pain for an alternative result (Scarpazza et al., 2013) and for a compelling argument for disregarding brain volume studies altogether. DLPFC activation does seem to be related to cognitive and attentional processing of noxious stimuli (Peyron et al., 1999; Bornhovd et al., 2002) and probably has a role in modulating pain expectation (Sawamoto et al., 2000) and pain-induced anxiety (Ploghaus et al., 1999).

Non-invasive brain stimulation strategies aimed at modifying corticospinal excitability for different purposes have emerged in recent years. In recent pain studies, transcranial magnetic stimulation (TMS) (Leon-Sarmiento et al., 2013), repetitive transcranial magnetic stimulation (rTMS) (Lefaucheur et al., 2006; Hosomi et al., 2013; Jette et al., 2013; Perocheau et al., 2013) and transcranial direct current stimulation (tDCS) (Flor et al., 1997; Riberto et al., 2011) have been used to modulate pain. tDCS is a common method of modulating the cortical activity of superficial pain-relevant areas; it has been used to treat a variety of clinical conditions, and is a painless technique with minimal side effects (Jeffery et al., 2007; Bolognini et al., 2009). tDCS delivers low direct currents via scalp electrodes to the cerebral cortex that result in the modulation of cortical excitability . A part of this current is shunted through the scalp and the rest flows into the cerebral cortex (Miranda et al., 2006; Nitsche et al., 2008). tDCS is usually applied through two surface electrodes, one serving as an anode and the other as a cathode. Anodal tDCS (a-tDCS, involving the application of an anode over the target area) typically has an excitatory effect on the underlying cerebral cortex by depolarizing neurons, while cathodal tDCS (c-tDCS, involving the application of a cathode over the target area) decreases cortical excitability by inducing hyperpolarization (Nitsche and Paulus, 2000). The proposed mechanism behind immediate effects of tDCS is polarity-dependent shifts of the resting membrane potential and consequent alteration of corticospinal excitability at the stimulation site. The idea is that this alteration leads to facilitation or inhibition of the superficial structures and of deeper and more remote brain areas related to pain modulation (Willis and Westlund, 1997; Petrovic et al., 2000; Casey et al., 2001; Lorenz et al., 2003; Lang et al., 2005). Furthermore, long-lasting effects of tDCS depend on *N*-methyl-D-aspartate (NMDA) receptor-efficacy changes (Liebetanz et al., 2002). Involvement of NMDA receptors induces neuroplasticity in which transformation of synaptic strength takes place by Long-term potentiation and depression (LTP & LTD) mechanisms (Islam et al., 1995; Nitsche and Paulus, 2001; Liebetanz et al., 2002).

S1, M1 and DLPFC are relatively superficial brain areas that contribute to the neural substrate of pain. Pain can be operationalized into key variables, for example sensory threshold (STh), pain threshold (PTh), and pain level (PL) (Fernandez and Turk, 1992; Bornhovd et al., 2002; Giesecke et al., 2005) although these variables are not closely correlated (Wolff, 1964). Some tDCS studies have reported that excitatory effects of a-tDCS may increase the function of superficial areas of pain neuromatrix led to pain management by increasing the level of STh/PTh (Antal et al., 2008; Csifcsak et al., 2009) and decreasing the level of PL (Fregni et al., 2006a,b; Roizenblatt et al., 2007; Antal et al., 2010).

There is now a large literature concerning tDCS for pain relief. Recently, systematic reviews of all tDCS pain-related studies have concluded that insufficient evidence exists to make firm conclusions (O'Connell et al., 2011; Luedtke et al., 2012), a problem compounded by the recent questioning of the assumption that the most commonly used intensity of tDCS can be easily blinded (O'Connell et al., 2012; Russo et al., 2013). These studies raise a very important question: what is the evidence for the effectiveness of a-tDCS in modulating pain according to the site of stimulation? According to the common understanding that S1, M1 and DLPFC make independent contributions to pain, the site of stimulation should have a differential effect over pain relief.

As a result, based on the existed studies, we investigated the site-specific effects of a-tDCS on STh/PTh in healthy individuals and PL in patients with chronic pain. We hypothesized that:

1. STh is modulated immediately after application of a-tDCS over S1 and M1 in healthy individuals.
2. PTh is modulated immediately after application of a-tDCS over S1 and M1 in healthy individuals.
3. PL is modulated immediately after application of a-tDCS over S1 and M1in patients with chronic pain.
4. Application of sham stimulation to different areas of the brain has no effect on STh/PTh in healthy individuals, nor on PL in patients with chronic pain.

2. Methods

2.1. Inclusion criteria

We included studies that recruited participants over the age of 18 years who were healthy or had experienced chronic pain for more than three months (Smith et al., 2001; Latremoliere and Woolf, 2009). All types of study designs, parallel or cross-over, were included regardless of blinding. Studies that utilised a-tDCS on the S1, M1, or DLPFC in healthy subjects or patients experiencing chronic pain were included if:

(1) The subjects were over 18 years of age.
(2) The outcome measure was VAS in the patient group or STh/PTh in the healthy group.
(3) Sham tDCS or active control was applied (Table 1).

Given the fact that M1, S1, and DLPFC are the only superficial areas of pain neuromatrix which are accessible to stimulate by tDCS, we included studies investigated the effects of a-tDCS on these areas in both healthy (Table 2), and patient group with chronic pain regardless of their pathology (Table 3). All modalities that evoked a sensory or painful sensation were included (i.e., laser, heat, cold, and mechanical stimuli). Moreover, in patient group, chronic pain is specified as a refractory pain which is resistant to medical intervention or drug management more than three month (Smith et al., 2001; Latremoliere and Woolf, 2009). We included studies that placed electrodes over M1, DLPFC, or S1 regions.

2.2. Exclusion criteria

Studies were excluded if:

(1) They did not involve brain stimulation.
(2) The duration of symptoms for patient groups was unclear.
(3) The study used surgical brain stimulators, rTMS, TMS, or electrical stimulation with pulse currents (Table 1).
(4) The studies used c-tDCS or other forms of non-invasive brain stimulations (TMS, rTMS, or cranial electrical stimulation); indirect forms of stimulation (caloric vestibular stimulation or occipital nerve stimulation) or invasive forms of brain stimulation involving the use of electrodes implanted within the brain.

2.3. Outcome measures

The outcome measures for STh and PTh were percentage changes in stimulus intensities at which participants reported the onset of sensation (STh) or pain (PTh). For PL in patient groups, we pooled studies that used a visual analogue scale (VAS).

Because the included trials involved post-intervention assessments at varying periods, these were partitioned into short-term and long-term outcomes. 'Short-term' was arbitrarily defined as less than one hour after intervention. If a trial had multiple assessments during that period, the assessment performed closest to the intervention was used. 'Long-term' was defined as greater than one

Table 1
Inclusion and exclusion criteria for identified studies.

	Inclusion	Exclusion
Participants	18 or more years of age Either healthy or suffering from chronic pain (musculoskeletal, neural, or central pain syndrome), anatomical location	Suffering from other type of diseases (e.g., depression or other mental disorders, cancerous pain) With primary symptoms other than pain (e.g., depression or schizophrenia) Non-human subjects
Intervention	a-tDCS and sham stimulation	
Comparison	"no treatment"/sham treatment Before and after a-tDCS	Other control group
Outcomes	NAS measured by QST[1] and LEP[2] amplitude in healthy individuals and VAS in patients with chronic pain	Other type of evaluation of sensory perception, PTh[3], and PL[4] (measured by rTMS[5], fMRI[6], PET[7], and Paired TMS)
Trial design	Randomized control trial, controlled clinical trials, and pre-post trials	Review articles Selective review
Data reported	Data that enable analysis and estimation of the effects of a-tDCS and sham stimulation on STh, PTh, and PL	
Type of publications	Peer-reviewed journal articles, regardless of the year of publication English language	Non-English articles

[1] Quantitative sensory testing.
[2] Laser evoked potential.
[3] Pain threshold.
[4] Pain level.
[5] Repeated transcranial magneticstimulation.
[6] Functional magnetic resonance imaging.
[7] Positron emission tomography.

Table 2
Study characteristics and outcome measure in healthy group.

Included studies	Trial design	No. participants	Stimulation method	Outcome measure	Intervention	Stimulated area
Boggio et al. (2009)	Double blinded – Sham controlled	20	E.S	VAS	a-tDCS	V1[1],M1[2],DLPFC[3]
Hansen et al. (2011)	Pre-post test	19	E.S[4]	VAS, PREP[5],BR[6]	a-tDCS, c-tDCS	M1
Bachmann et al. (2010)	Single blinded – Crossover	8	QST	VAS	a-tDCS, c-tDCS	M1
Grundmann et.al. (2011)	Pre-post test	12	QST	VAS	a-tDCS, c-tDCS	S1[7]
Reidler et al. (2012)	Double blinded – Sham controlled	15	QST	VAS,	a-tDCS	M1
Jurgens et al. (2012)	Pre-post test	17	QST	VAS	a-tDCS, c-tDCs	M1
Antal et al. (2008)	Pre-post test	10	LASER	VAS, LEP[8]	a-tDCS, c-tDCS	S1
Csifcsak et al. (2009)	Pre-post test	10	LASER	VAS, LEP	a-tDCS, c-tDCS	M1
Ragert et al. (2008)	Double blinded – Sham controlled	10	Tactile discrimination	VAS	a-tDCS	S1
Rogalewski et al. (2004)	Single blinded – Sham controlled	13	Tactile perception	VAS	a-tDCS, c-tDCS	S1

[1] Visual cortex.
[2] Primary motor cortex.
[3] Dorsolateral prefrontal cortex.
[4] Electrical stimulation.
[5] Pain related evoked potential.
[6] Blink reflex.
[7] Somatosensory cortex.
[8] Laser evoked potential.

Table 3
Study characteristics and outcome measure in patient group.

Included studies	Trial design	No. participants	Patients	Stimulation area	Intervention	Outcome measure
Riberto et al. (2011)	Double blinded – Sham control	23	Fibromyalgia	M1[1]	a-tDCS	VAS, SF-36
Valle et al. (2009)	Double blinded – Sham control	41	Fibromyalgia	M1, DLPFC[2]	a-tDCS	VAS, FIQ[3]
Fregni et al. (2006a,b)	Double blinded – Sham control	32	Fibromyalgia	M1, DLPFC	a-tDCS	VAS,FIQ, Mood
Roizenblatt et al. (2007)	Double blinded – Sham control	32	Fibromyalgia	M1, DLPFC	a-tDCS	VAS, RAM, REM
Mendonca et al. (2011)	Double blinded – Sham control	30	Fibromyalgia	M1, SO[4]	a-tDCS c-tDCS	VAS
Mori et al. (2010)	Double blinded – Sham control	19	Multiple sclerosis	M1	a-tDCS	VAS, MC Gill
Antal et al. (2010)	Double blinded – Crossover	12	Trigeminal neuralgia Post stroke pain Syndrome Back pain Fibromyalgia	M1	a-tDCS	VAS, MEP[5]
Fregni et al. (2006a,b)	Double blinded – Sham control	17	Spinal cord injury	M1	a-tDCS	VAS, PGA[6]
Fenton et al. (2009)	Double blinded – Sham control	7	Chronic pelvic pain	M1	a-tDCS	VAS
Soler et al. (2010)	Double blinded – Sham control	39	Spinal cord injury	M1	a-tDCS	VAS
Boggio et al. (2009)	Double blinded – Crossover	8	Chronic neurogenic pain	M1	a-tDCS	VAS
Antal et al. (2011)	Double blinded – Sham control	26	Chronic migraine	V1[7]	c-tDCS	VAS
Dasilva et al. (2012)	Double blinded – Sham control	13	Chronic migraine	M1	a-tDCS	VAS
Antal et al. (2011)	Double blinded – Sham control	1	Refractory orofacial pain	M1	a-tDCS c-tDCS	VAS

[1] Primary motor cortex.
[2] Dorsolateral prefrontal cortex.
[3] Fibromyalgia impact questionnaire.
[4] Supra-orbital area.
[5] Motor evoked potential.
[6] Patient general assessment.
[7] Visual motor cortex.

hour after intervention; long-term outcomes were not included in meta-analyses.

2.4. Methods for identifying studies

We searched for relevant studies published in English. To locate eligible articles, a literature search was performed using PubMed, Physiotherapy Evidence Databases (PEDro), CINAHL, CENTRAL (Cochrane Central Register of Controlled Trials), Scopus, PROQuest, SPorTDiscuss, AMI (Australian Medical Index), Ovid Medline, EBM Review, Cochrane, Meditex and PsycINFO, from their inception to July 2012. All reference lists of retrieved papers were searched to identify additional relevant articles unidentified by initial search strategy. The key search terms were: 'transcranial direct current stimulation', 'tDCS', 'sensory perception', 'pain', 'pain perception', 'pain tolerance', 'sensory threshold', 'pain threshold', 'sensory stimulation' and 'pain trigger'.

2.5. Selection of the included studies

Two reviewers (B.V. and S.J.) independently screened the titles and abstracts of papers identified in the initial search strategy against the inclusion criteria. If the information in the title and the abstract was insufficient to make a decision, the reviewers assessed the full paper to include or exclude the study. All included studies were then double-checked by a full-text appraisal. If the reviewers disagreed, resolution was attempted by discussion. If resolution was not achieved, the third reviewer (M.Z.) was consulted.

2.6. Risk of bias and quality assessment

To assess the methodological quality of included studies, we assessed risk of bias using the Cochrane 'Risk of bias. assessment tool outlined in Chapter 8 of the *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.2 (Higgins and Green, 2011). Fig. 1 is a methodological quality graph for all included studies.

Further quality assessment was conducted for each included study by using the Physiotherapy Evidence Database (PEDro scale) (Moseley et al., 2002; Maher et al., 2003). The PEDro scale includes 10 criteria for internal validity; studies are awarded a point for each criterion met. The PEDro cut-points are 9–10, excellent; 6–8, good; 4–5, fair and below 4, poor (de Morton, 2009). Because some of the studies we identified were not randomised controlled trials, the process was repeated using the Down and Black tool (D&B) (Downs and Black, 1998). The D&B contains 27 questions, of which 25 are graded 0 or 1 ("yes", "no" or "not determined"), one is scored 0–2 and one, on power, is scored 0–5. Eng et al. (2007) modified scoring for the final item on power to 0 or 1 (Eng et al., 2007) (Table 4).

2.7. Outcome measures

Our primary outcome measures were the STh and PTh of healthy individuals and PL in patients who suffered from chronic pain. STh is usually measured by quantitative sensory testing (QST) using mechanical, vibration or thermal methods (Chong and Cros, 2004). A subject's STh is classically defined as the level of stimulus intensity necessary for sensation to be just detectable. PTh is defined as the level of stimulus intensity at which pain is detected. PL in patients with chronic pain showed the average pain that they experience during a day, usually measured by VAS (Bolton and Wilkinson, 1998).

2.8. Subgroup analysis and intervention of heterogeneity

We assessed heterogeneity using the Chi^2 test and I^2 statistic. Also, the effect of a-tDCS and sham stimulation on STh, PTh, and PL were measured in the M1 and S1 in healthy participants and in the M1 and DLPFC in patients with chronic pain.

2.9. Data extraction

The following data relevant to the aims of this study were extracted: study design; characteristics of subjects (Table 2) outcome

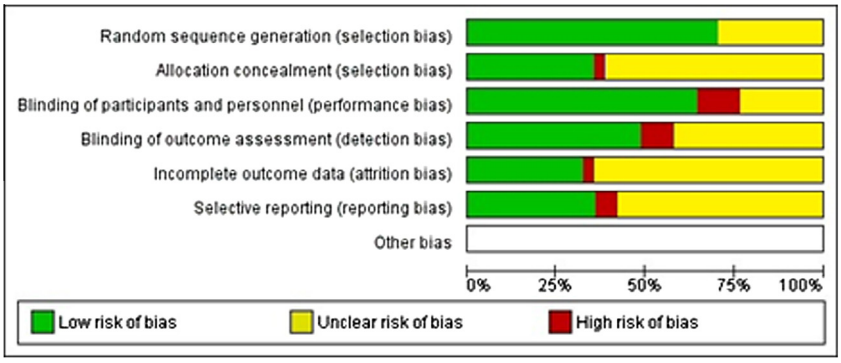

Fig. 1. Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all included studies. The evaluation has six domains: (1) Adequate sequence generation, (2) Allocation sequence concealment, (3) Blinding, (4) Incomplete outcome data, (5) Selective outcome reporting, (6) Other source of bias.

Table 4
Quality assessment of included studies in healthy (A) and patient (B) groups.

Included studies	Type of study	PEDro (1999)	D&B Downs and Black, (1998)
A: Healthy group			
Csifcsak et al. (2009)	Pre-post test	–	17
Grundmann et.al. (2011)	Pre-post test	–	16
Antal et al. (2008)	Pre-post test	–	18
Hansen et al. (2011)	Pre-post test	–	17
Boggio et al. (2008)	Randomized control trial	7	–
Bachmann et al. (2010)	Randomized control trial	7	–
Reidler et al. (2012)	Randomized control trial	8	–
Rogalewski et al. (2004)	Randomized control trial	7	–
Ragert et al. (2008)	Randomized control trial	8	–
B: Patient group			
Riberto et al. (2011)	Randomized control trial	8	–
Valle et al. (2009)	Randomized control trial	7	–
Antal et al. (2011)	Randomized control trial	5	–
Antal et al. (2010)	Randomized control trial	8	–
Fregni et al. (2006a,b)	Randomized control trial	8	–
Mendonca et al. (2011)	Randomized control trial	7	–
Mori et al. (2010)	Randomized control trial	8	–
Fregni et al. (2006a,b)	Randomized control trial	8	–
Fenton et al. (2009)	Randomized control trial	6	–
Soler et al. (2010)	Randomized control trial	8	–
Boggio et al. (2009)	Randomized control trial	8	–
Dasilva et al. (2012)	Randomized control trial	7	–

measures (Table 2) and a-tDCS parameters in both healthy and patient groups (Table 5); and percentages of VAS changes immediately post-intervention compared to baseline (pre-test) and sham values (Tables 6 and 7) (Chong and Cros, 2004). 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, 2011). When there was uncertainty regarding the information and results and when data were not accessible from figures and graphs, we contacted the corresponding author(s) and requested the mean ± SD of desired outcome measures. Where mean ± SD values were not provided for baseline/control and post-intervention parameters as numerical data, they were pooled out from the graphs with Plot Digitizer software (Joseph, 2011).

A Java-based Plot Digitizer program (Higgins and Green, 2011) was used to digitize scanned plots of functional data. Data were entered into the effect size calculator using REVMAN 5.1 software (Cochrane Collaboration, 2008) (Bax et al., 2007). 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). Extracted data were entered into the meta-analysis using the generic inverse-variance method as suggested in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins and Green, 2011).

We pooled results using RevMan 5 software (version 5.1). We used a random effects model to conduct separate meta-analyses for different forms of stimulation (a-tDCS and sham). Where more than one data point was available for short-term outcomes, we used the first post-stimulation measure. Two forest plots were generated for each outcome measure. In the first one, the percentage changes in STh, PTh and PL after applying a-tDCS compared to baseline values were assessed. In the second one, the percentage changes in STh, PTh and PL after a-tDCS were compared to the percentage changes after effects of sham stimulation.

3. Results

3.1. Identification and selection of studies

The search strategy identified 283 studies, including 221 duplicates. Screening by title and abstract identified 49 studies as potentially eligible for the review. 31 studies which did not meet inclusion criteria were excluded. Seven studies were identified from hand-searching of the reference lists of included studies, of which one were not retrievable in abstract or full manuscript form. Two papers were excluded because no data could be provided

Table 5
tDCS parameters in both healthy (A) and patient (B) groups.

Included studies	Type of tDCS	Size of electrode (cm^2)	Intensity (mA)	Current density (mA/cm^2)	Time of stimulation (min)	Electrode position
A: Healthy group						
Boggio et al. (2008)	a-tDCS	35	2	0.057	5	C3, F3, Oz
Bachmann et al. (2010)	a-tDCS c-tDCS	35	1	0.029	15	C3
Grundmann et al. (2011)	a-tDCS c-tDCS	35	1	0.029	15	C3
Reidler et al. (2012)	a-tDCS	35	2	0.057	20	C3
Antal et al. (2008)	a-tDCS c-tDCS	35	1	0.029	15	2 cm posterior to ADM[1] hot spot
Csifcsak et al. (2009)	a-tDCS c-tDCS	35	1	0.029	10	C3
Ragert et al. (2008)	a-tDCS	25	1	0.04	20	2 cm posterior to C3
Rogalewski et al. (2004)	a-tDCS c-tDCS	35	1	0.029	7	C4
Hansen et al. (2011)	a-tDCS c-tDCS	16	1	0.063	20	Cz, 1 cm above the supraorbital nerve
Boggio et al. (2009)	a-tDCS	35	2	0.057	5	C3, F3, Oz
B: Patient group						
Riberto et al. (2011)	a-tDCS	35	2	0.057	20	C3
Valle et al. (2009)	a-tDCS	35	2	0.057	20	C3, F3
Fregni et al. (2006a,b)	a-tDCS	35	2	0.057	20	C3 or C4
Roizenblatt et al. (2007)	a-tDCS	35	2	0.057	20	C3
Mendonca et al. (2011)	a-tDCS c-tDCS	16, 80	2	0.125,0.0125	20	C3
Mori et al. (2010)	a-tDCS	35	2	0.057	20	C3 or C4
Antal et al. (2010)	a-tDCS	16	1	0.063	20	C3
Fregni et al. (2006a,b)	a-tDCS	35	2	0.057	20	C3 or C4
Fenton et al. (2009)	a-tDCS	35	1	0.029	20	C3 or C4
Soler et al. (2010)	a-tDCS	35	2	0.057	20	C3 or C4
Boggio et al. (2009)	a-tDCS	35	2	0.057	30	C3 or C4
Dasilva et al. (2012)	a-tDCS	35	2	0.057	20	C3
Antal et al. (2011)	a-tDCS	35	2	0.057	20	C3

[1] Abductor digiti minimi.

either from corresponding authors or graphs, bringing the final number of studies to 22 (Fig. 2).

3.2. Risk of bias and quality assessment

No study was judged to have a low risk of bias across all criteria. Fig. 1 summarises the risk of bias assessment results. All trials had unclear or inadequate bias control in one or more of the domains for the assessment of risk of bias. Based on the results, allocation of blinding was the major potential source of bias in this meta-analysis. As well, outcome assessment of sensation and pain was not blinded in 50% of the studies, representing a high risk of bias. However, PEDro scores ranged between 5 and 8 in patient studies (with a mean score of 7.3/11) and between 7 and 8 in healthy volunteer studies (with a mean method score of 7.4/11), which indicate good quality controlled clinical trials. Similarly, the 27-item D&B quality checklist provided a medium-quality mean method score of 17/27 for studies involving healthy participants. Table 4 show the PEDro and D&B scores of the included studies.

3.3. Participants in included studies

In total across the included studies, 146 healthy individuals and 276 patients with chronic pain received a-tDCS and sham for VAS measurement. All studies examined the effect of a-tDCS intervention in one or more of the M1, S1 or DLPFC. In the patients with chronic pain, the average VAS was more than 5.

There was no study in healthy group in which DLPFC was stimulated to measure STh/PTh. Also, the effect of a-tDCS of S1 on PL has not been investigated yet. As a result, we investigated the effects of a-tDCS on two site of stimulation, S1 and M1, in healthy group and two, M1 and DLPFC, in patient group.

3.4. Pooled data analysis

For all studies, the standard error (SE) was calculated from the 95% confidence interval of the standardised mean difference and entered into the meta-analysis using the generic inverse variance method. Pre-post a-tDCS studies and active-sham studies were evaluated to assess whether a-tDCS can change STh, PTh, and PL and whether studies using a sham group as a control produced results significantly different from those of pre-post studies. The percentage changes before and after applying a-tDCS and sham a-tDCS were calculated and pooled in meta-analysis.

3.4.1. Effects of a-tDCS on STh in healthy participants

Fig. 3A summarizes the pooled data (percentage of changes) extracted from seven studies on healthy individuals (Antal et al., 2008; Ragert et al., 2008; Csifcsak et al., 2009; Grundmann et al., 2011). As shown, the percentage STh changes were significant for stimulation of M1 with a mean effect size of 19.25%, while a-tDCS of S1 produced no significant mean STh change ($P = 0.09$). The overall analysis indicated that a-tDCS can change STh significantly ($P < 0.001$) with an effect size of 13.34%

Forest plot and meta-analysis results indicated an overall positive mean effect of a-tDCS on STh of 11.22 (Fig. 3B). The subgroup results demonstrated while sham and active a-tDCS generate significantly different STh changes in the M1 subgroup, with a mean effect size of 16.54%, this is not the case in the S1 subgroup.

3.4.2. Effects of a-tDCS on PTh in healthy participants

Seven studies examined the effect of a-tDCS on PTh in healthy individuals (Antal and Paulus, 2008; Csifcsak et al., 2009; Grundmann et al., 2011; Reidler et al., 2012). The stimulation site in two studies was the S1 (Antal et al., 2008; Grundmann et al.,

Table 6
Percentage STh (A) and PTh (B) changes in healthy participants after applying active a-tDCS and sham tDCS.

Papers	Stimulus	Stimulation area	Percentage of changes after-before active a-tDCS (mean ± SE)	Percentage of changes after-before sham a-tDCS (mean ± SE)
A: STh changes				
Boggio et al. (2008)	Electrical stimulation	M1	35.6 ± 7.11	2.65 ± 2.89
Bachmann et al. (2010)	Cold detection Warm detection Mechanical detection Vibration	M1	12.33 ± 6.01 12.33 ± 6.01 10.0 ± 8.0 27.66 ± 3.89	3.33 ± 1.1 4.1 ± 2.76 6.87 ± 3.96 1.93 ± 0.96
Antal et al. (2008)	Heat perception produced by LASER	S1	-10.12 ± 2.51	14.0 ± 10.76
Ragert et al. (2012)	Tactile perception	S1	37.42 ± 6.36	
Grundmann et al. (2011)	Cold detection Warm detection Mechanical detection Vibration	S1	22.33 ± 6.45 15.33 ± 4.33 2.33 ± 2.0 5.66 ± 4.61	2.66 ± 1.76 0.66 ± 1.03 2.0 ± 1.83 1.98 ± 2.65
Csifcsak et al. (2009)	Heat perception produced by LASER	M1	20.55 ± 19.51	2.89 ± 1.67
Rogalewski et al. (2004)	Tactile perception	S1	7.08 ± 1.96	3.43 ± 2.96
B: PTh changes				
Boggio et al. (2008)	Electrical stimulation	M1	17.2 ± 2.57	1.36 ± 1.2
Hansen et al. (2011)	Electrical stimulation	M1	28.37 ± 2.26	2.67 ± 1.06
Bachmann et al. (2010)	Cold pain Heat pain Mechanical pain	M1	8.66 ± 5.19 6.03 ± 4.13 4.03 ± 4.13	4.54 ± 0.3 3.38 ± 0.1 3.82 ± 0.33
Reidler et al. (2012)	Cold pain Mechanical pain	M1	56 ± 1.98 42.66 ± 3.7	36.0 ± 7.33 27.87 ± 5.77
Grundmann et al. (2011)	Cold pain Heat pain Mechanical pain	S1	8.66 ± 3.65 6.33 ± 2.4 4.36 ± 3.08	0.4 ± 0.2 0.5 ± 0.29 0.27 ± 0.29
Csifcsak et al. (2009)	Heat perception produced by LASER	M1	12.97 ± 4.71	0.10 ± 0.11
Antal et al. (2008)	Heat perception produced by LASER	S1	−0.94 ± 1.53	2.84 ± 5.7

2011) and in the five remaining studies tDCS was applied to the M1 (Fig. 4).

As shown in Fig. 4A, a pooled analysis of eight trials of a-tDCS on the M1 in healthy subjects indicated a significant increase in PTh with a mean effect size of 22.19%. Furthermore, a-tDCS of the S1 significantly increased PTh by a mean of 4.34. The overall effect of a-tDCS on PTh was significant ($P = 0.007$) with the effect size of 16.42% [95% CI: 4.48 to 28.37].

Meta-analysis showed that a-tDCS of both M1 ($P = 0.003$, [95% CI: 22.19 (7.63, 36.76)]) and S1 ($P = 0.02$, [95% CI: 4.34 (0.78, 7.90)]) increased PTh significantly. Although there was no significant difference between sham and active a-tDCS of the S1, analysis of all studies applying a-tDCS onto both the M1 and S1 indicated a positive effect of a-tDCS on PTh ($P = 0.001$, [95% CI: 9.45% (3.70, 15.20)]) (Fig. 4B).

3.4.3. The effect of a-tDCS on PL in patients with chronic pain

Adequate data were available from 13 studies (Fregni et al., 2006a,b; Roizenblatt et al., 2007; Boggio et al., 2009; Fenton et al., 2009; Valle et al., 2009; Antal and Paulus, 2011; Mendonca et al., 2011; Riberto et al., 2011; Dasilva et al., 2012) that investigated the effects of a-tDCS on both the M1 and DLPFC in patients with chronic pain. As can be seen in Fig. 5, pooled analysis of twelve trials on M1 and two studies on DLPFC showed significant effects in both subgroups. The average score of PL decrease was 14.6% for a-tDCS of the M1 and 19.3 for a-tDCS of the DLPFC.

In studies that compared the effects of a-tDCS and sham stimulation, pain reduced significantly after applying a-tDCS to the M1, with a mean effect size of 9.59%, and after a-tDCS of the DLPFC, with a mean effect size of 15.79% (Fig. 5B).

4. Discussion

We aimed to determine the evidence for the effectiveness of a-tDCS in increasing STh/PTh and reducing PL according to the site of stimulation. We conducted meta-analyses of studies of STh and PTh after a-tDCS in healthy volunteers and PL in patients with chronic pain. Our results show that a-tDCS of M1 increased both STh and PTh in healthy individuals. Furthermore, a-tDCS of both M1 and DLPFC led to significant PL reduction. Similar results were found following comparison of active a-tDCS and sham stimulation (except in a-tDCS of the S1, in which no significant difference was observed).

4.1. The effects of a-tDCS on STh in healthy individuals

We hypothesized that STh is modulated immediately after application of a-tDCS over S1 and M1 in healthy individuals. This hypothesis was supported by the results of our meta-analysis in the M1 but not in the S1 subgroup. Of seven studies which involved the S1, two reported a-tDCS of the S1 decreased STh (Rogalewski et al., 2004; Antal and Paulus, 2008), and one study concluded that a-tDCS of the S1 had no effect on STh in healthy individuals (Wager et al., 2004). The other four studies found significant increases in STh (Fig. 3). After a-tDCS of the M1, STh increased significantly as compared to baseline condition and sham stimulation, while after a-tDCS of the S1 a non-significant increase in STh was observed. Surprisingly, we found no significant difference in STh between sham stimulation and a-tDCS of S1.

Our findings support those of Enomoto and colleagues, who suggested that rTMS of the S1/S2 does not alter STh (Enomoto et al., 2001). In addition, some tDCS studies showed that a-tDCS

Table 7
Percentage PL changes in patients with chronic pain after applying active a-tDCS and sham tDCS.

Papers	Patients	Stimulation area	Percentages of changes after-before active a-tDCS (mean ± SD)	Percentages of changes after-before sham a-tDCS (mean ± SD)
Antal et al. (2010)	Trigeminal neuralgia Post stroke pain syndrome Fibromyalgia	M1	3.33 ± 1.38	4.8 ± 0.91
Antal et al. (2011)	Refractory orofacial pain	M1	8.12 ± 1.22	2.69 ± 0.93
Boggio et al. (2009)	Chronic Neurogenic pain	M1	16.7 ± 0.72	5.6 ± 0.54
Dasilva et al. (2012)	Chronic Migraine	M1	14.29 ± 2.53	8.23 ± 1.94
Fenton et al. (2009)	Chr**onic pelvic pain	M1	20.31 ± 2.76	10.23 ± 1.99
Fregni et al. (2006a,b)	Fibromyalgia	M1	23.06 ± 3.15	12.17 ± 2.06
Fregni et al. (2006a,b)	Spinal cord injury	M1 DLPFC	25.83 ± 3.89 29.43 ± 3.96	14.34 ± 2.79 14.57 ± 3.16
Mendonca et al. (2011)	Fibromyalgia	M1	14.74 ± 2.67	6.38 ± 0.95
Mori et al. 2010	MS	M1	17.48 ± 3.58	8.94 ± 2.05
Riberto et al. (2011)	Fibromyalgia	M1	18.95 ± 3.56	6.95 ± 1.57
Roizenblatt et al. (2007)	Fibromyalgia	M1 DLPFC	20.03 ± 4.23 32.84 ± 6.97	9.63 ± 2.18 17.83 ± 5.97
Soler et al. (2010)	Spinal cord injury	M1	19.39 ± 3.03	3.94 ± 0.92
Valle et al. (2009)	Fibromyalgia	M1 DLPFC	23.71 ± 2.52 25.81 ± 3.86	13.52 ± 3.01 12.66 ± 2.94

of the S1 might have no effect on sensation (Rogalewski et al., 2004); therefore, any observed effects in STh after a-tDCS of the S1 are probably due to some other mechanism (Dieckhofer et al., 2006; Antal and Paulus, 2008; Ragert et al., 2008). Moreover, other studies reported that rTMS of the somatosensory cortex increased cold perception but not warmth perception (Summers et al., 2004; Oliviero et al., 2005).Sensation of warmth is transmitted via unmyelinated C-fibers and sensation of non-painful cold by small myelinated Aδ fibers (Fuller and Guiloff, 1989), so pooling data for different sensations (cold, warm, vibration, mechanical, etc.) might have altered our results, but the small number of studies made it impossible for us to do so.

4.2. The effect of a-tDCS on PTh in healthy individuals

We hypothesised that PTh is modulated immediately after application of a-tDCS over S1 and M1 in healthy individuals. After a-tDCS of the M1 and S1, significantly increased PTh was observed when compared with the baseline conditions, supporting our hypothesis. Additionally, our comparison of the after-effects of active a-tDCS and sham stimulation of the M1 demonstrated a significant difference in PTh.

These results seem consistent with some PET scan studies that showed that any changes in the resting membrane potential of nerve fibres caused regional cerebral blood flow increase in various structures such as the thalamus, anterior insula, and upper brainstem (Peyron et al., 1995; Garcia-Larrea et al., 1999; Grundmann et al., 2011). Considering the functional connections of motor cortex and deep structures related to pain and sensory processing, a-tDCS may act indirectly on these deep structures to increase PTh (Peyron et al., 1995; Grundmann et al., 2011). The mechanisms behind the efficacy of a-tDCS for PTh remain unclear. Based on the fact that a-tDCS increases corticospinal excitability, it is possible that excitation of neurons under the active electrode may led to the PTh increase. Other hypothesised mechanisms include long-term potentiation theory (Gamboa et al., 2010) and gating theory (Ziemann and Siebner, 2008), both of which highlight the multiple structures of the central nervous system involved in pain processing (Luedtke et al., 2012; Mylius et al., 2012). Stimulation of M1 is thought to modulate the sensory–discriminative aspects of pain (Porro, 2003). Resting membrane potential of axons might be modulated by a-tDCS, thus tDCS can be explained as mediated primarily by action on M1, but possibly also by action on S1 (Bornhovd et al., 2002; Antal et al., 2008; Mylius et al., 2012). Relevant to this is the finding that rTMS applied at high frequency over M1 improves sensory discrimination as well as providing some pain relief (Passard et al., 2007; Lefaucheur et al., 2012). Also, a-tDCS may impact on intracortical motor circuitry, as suggested by rTMS-induced changes in cortical excitability parameters.

4.3. The effect of a-tDCS on PL in patient group

We hypothesized that PL is modulated immediately after application of a-tDCS over S1 and M1in patients with chronic pain. Our results support this hypothesis, and the results of two systematic reviews (O'Connell et al., 2011; Luedtke et al., 2012) concluded that a-tDCS is an effective method for reducing pain. Our analysis of the effect of a-tDCS site on PL in patients with chronic pain found that stimulation of both M1 and DLPFC reduces pain in patients with chronic pain. The other interesting finding is, that compared to stimulation of M1, the effect size after applying a-tDCS on DLPFC is bigger. Significant differences in the PL of patients after applying a-tDCS and sham stimulation indicate the efficacy of a-tDCS in pain reduction.

The high level of risk of bias and heterogeneity in the studies we included suggests that more studies with larger sample sizes are required to draw firm conclusions about the effects of a-tDCS, especially of the DLPFC.

In the current study we included different diseases and pathologies (fibromyalgia, spinal cord syndrome, multiple sclerosis, and migraine) in the review and the meta-analysis, which led to substantial heterogeneity. There might be merit in analysing according to condition if sufficient data exist, but there is not an obvious biological reason to predict differential responses.

Although the exact mechanism behind the efficacy of a-tDCS is not clear yet, most of the included studies concluded that the probable mechanism was the changes of resting membrane potential in neurons directly under the active electrode and indirectly in other parts of the pain neuromatrix (like periaqueductal grey, insula, and thalamus) through functional interconnections (Lang et al., 2005). In addition, it has been proposed that tDCS of the M1 can activate descending inhibitory M1-thalamic projections that modulate chronic pain (Fregni et al., 2007). Several rTMS and tDCS studies have shown that stimulation of the DLPFC is associated with improvement of depression (Nitsche et al., 2009, 2012), and thus might have mechanisms of action similar to antidepressants, which are also capable of inducing analgesic effects.

4.4. Quality of evidence

Some of the included studies did not clearly report assessor blinding. This could explain the reduced heterogeneity of meta-analyses and the pooled effect size. A recent epidemiological study provided empirical evidence that incomplete blinding in controlled trials that measure subjective outcomes exaggerates the observed effect size by 25% (Wood et al., 2008). This may be the case here, because 2 mA was used in 11 of the 13 included studies of patients with chronic pain and in three of 10 studies of healthy individuals (Table 5). Recently, O'Connell et al. (2012) reported that proper blinding is not possible in a study that uses a current intensity of 2 mA. Though this conclusion was challenged by Russo et al. (2013) and Palm et al. (2013), the implication is that the overall quality of the evidence for the effects of a-tDCS on STh/PTh assessment in healthy individuals and PL assessment in patients with chronic pain should be considered cautiously. Results should be replicated using a current intensity for which blinding is universally accepted as possible.

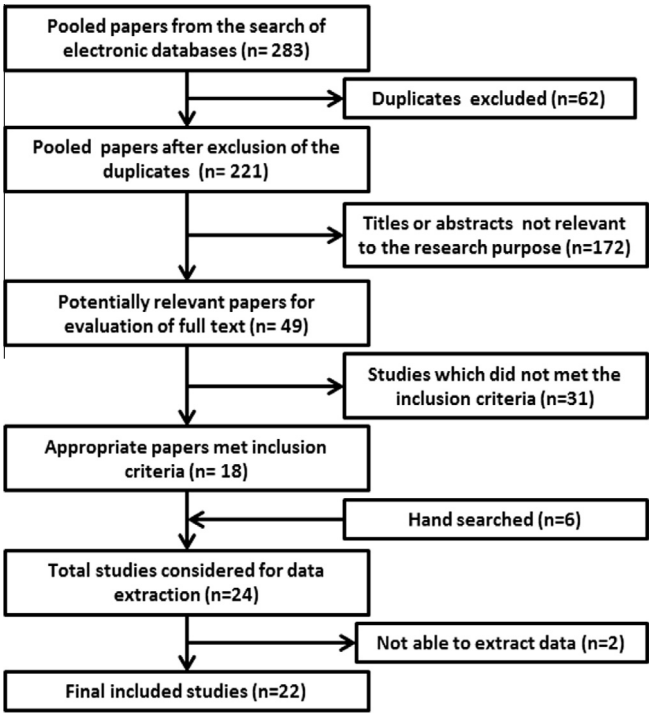

Fig. 2. QUORUM flowchart of studies included in the review.

4.5. Limitations

According to the *Cochrane Handbook for Systematic Reviews of Interventions*, "potential advantages of meta-analysis include an increase in power, an improvement in precision, the ability to answer question not posted by individual studies, and the opportunity to settle controversies arising from conflicting claims". That is, establishing clear protocols and inclusion and exclusion criteria can minimise the bias that the reviewer brings to the study. However, we cannot limit the bias that is within the literature itself. There is no doubt that negative findings are less likely to be published and most available studies are fundamentally flawed insofar as they do not include a control group or they do not verify participant blinding (O'Connell et al., 2012).

Other limitations of our study exist. The literature was limited to English-language articles, and most studies used small samples that inflated effect sizes and therefore might affect pooled results. Finally, studies of the effects of tDCS on STh and PTh used different types of sensation modalities and methods. As a result, because of small number of studies investigated the effects of a-tDCS on STh and PTh, we were not able to study the effect of a-tDCS on each stimulation method separately.

It is worth noting that the present study limited to immediate after-effects of a-tDCS not long-lasting effects. Due to limited number of included studies and mismatched measurement time-points, it was impossible to evaluate long-lasting after-effects of a-tDCS based on the site of stimulation.

4.6. Areas for future research

The results of our review indicate that a-tDCS of the M1 increases PTh in healthy individuals, and that a-tDCS of both the M1 and the DLPFC reduces PL of patients with chronic pain. An obvious future direction is to perform similar studies by testing the effects of cathodal tDCS (c-tDCS). The studies conducted in healthy subjects and patients with chronic pain thus far have been limited to a single a-tDCS session of approximately 20 min. It is

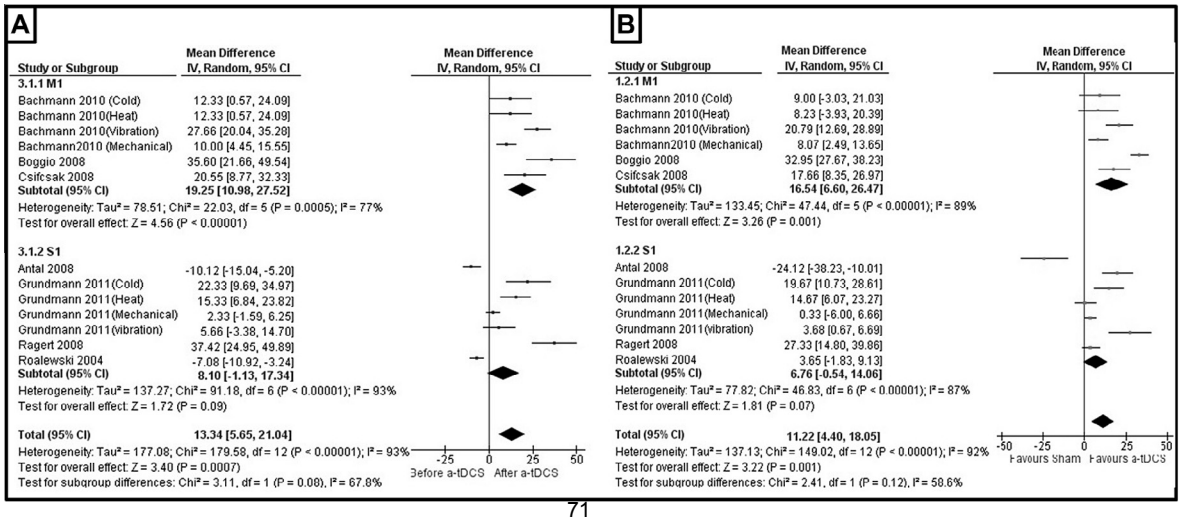

Fig. 3. Forest plot of comparison; (A) after-effects of a-tDCS compared to baseline value, (B) after-effects of a-tDCS compared to sham stimulation. Outcome: percentages of self-reported STh changes, subgroup analysis: M1 and S1.

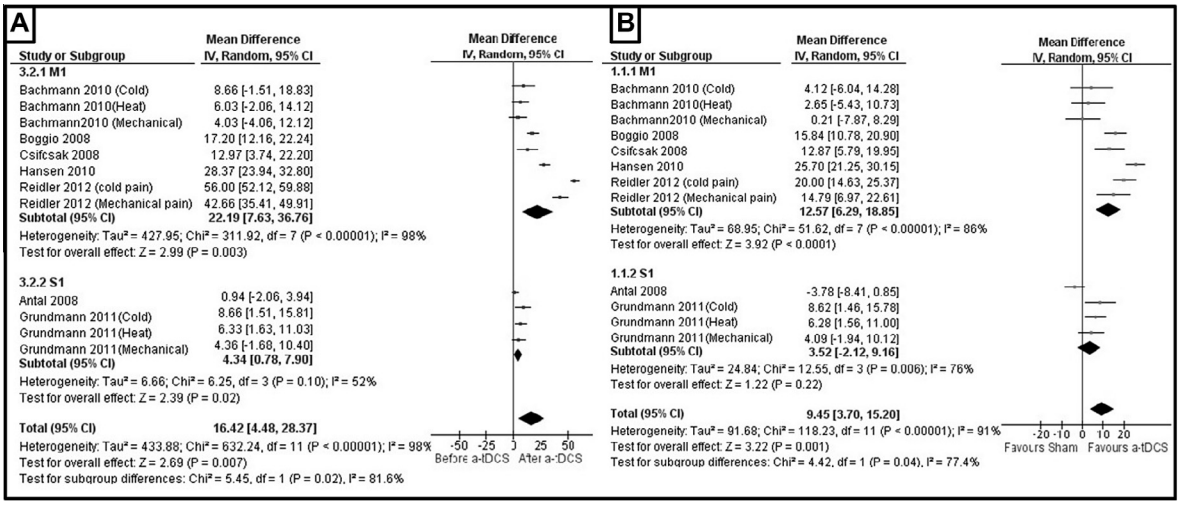

Fig. 4. Forest plot of comparison; (A) after-effects of a-tDCS compared to baseline value, (B) after-effects of a-tDCS compared to sham stimulation. Outcome: percentages of self-reported PTh changes, subgroup analysis: M1 and S1.

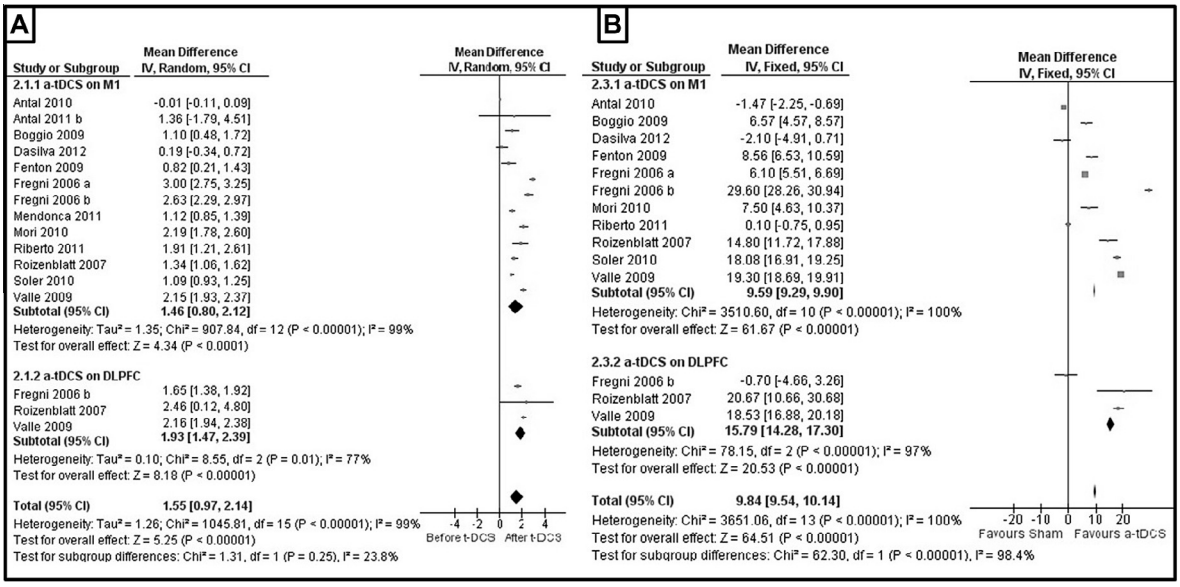

Fig. 5. Forest plot of comparison; (A) after-effects of a-tDCS compared to baseline value, (B) after-effects of a-tDCS compared to sham stimulation. Outcome: percentages of self-reported PL changes, subgroup analysis: M1 and DLPFC.

possible that longer application time or multiple applications could significantly increase pain thresholds or reduce pain levels (Nitsche et al., 2005; Furubayashi et al., 2008). Moreover, no study to date has optimized parameters regarding the analgesic effects of a-tDCS on both healthy and pain patient groups. Based on the fact that c-tDCS can suppress M1 excitability for up to 60 min after stimulation (Nitsche and Paulus, 2000; Rainville, 2002; Zaehle et al., 2011; Di Lazzaro et al., 2012), research focusing on the analgesic effects of c-tDCS could develop a more efficient method for pain treatment. Finally, since there are complicated relationships between different parts of the brain related to pain processing, subsequent research could aim to find the best stimulation sites and develop an efficient tDCS protocol to reduce pain.

Regarding the importance of therapeutic effects of tDCS in pain treatment, further investigation is required to evaluate the long-term effects of a-tDCS on STh/PTh in healthy individuals and PL in patients with chronic pain.

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References

Antal A, Brepohl N, Poreisz C, Boros K, Csifcsak G, Paulus W. Transcranial direct current stimulation over somatosensory cortex decreases experimentally induced acute pain perception. Clin J Pain 2008;24:56–63.

Antal A, Paulus W. Transcranial direct current stimulation and visual perception. Perception 2008;37:367–74.

Antal A, Paulus W. A case of refractory orofacial pain treated by transcranial direct current stimulation applied over hand motor area in combination with NMDA agonist drug intake. Brain Stimul 2011;4:117–21.

Antal A, Polania R, SchmidtSamoa C, Dechent P, Paulus W. Transcranial direct current stimulation over the primary motor cortex during fMRI. Neuroimage 2011;55(2):590–6. http://dx.doi.org/10.1016/j.neuroimage.2010.11.085.
Antal A, Terney D, Kuhnl S, Paulus W. Anodal transcranial direct current stimulation of the motor cortex ameliorates chronic pain and reduces short intracortical inhibition. J Pain Symptom Manage 2010;39:890–903.
Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain 2005;9:463–84.
Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, Gitelman DR. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. J Neurosci 2004;24:10410–5.
Bachmann CG, Muschinsky S, Nitsche MA, Rolke R, Magerl W, Treede RD, Paulus W. Transcranial direct current stimulation of the motor cortex induces distinct changes in thermal and mechanical sensory percepts. Clin Neurophysiol 2010;121(12):2083–9. http://dx.doi.org/10.1016/j.clinph.2010.05.005.
Baliki MN, Petre B, Torbey S, Herrmann KM, Huang L, Schnitzer TJ, Fields HL, Apkarian AV. Corticostriatal functional connectivity predicts transition to chronic back pain. Nat Neurosci 2012;15:1117–9.
Bax L, Yu LM, Ikeda N, Moons KG. A systematic comparison of software dedicated to meta-analysis of causal studies. BMC Med Res Methodol 2007;7(40):2007.
Bingel U, Quante M, Knab R, Bromm B, Weiller C, Buchel C. Single trial fMRI reveals significant contralateral bias in responses to laser pain within thalamus and somatosensory cortices. Neuroimage 2003;18:740–8.
Boggio PS, Zaghi S, Fregni F. Modulation of emotions associated with images of human pain using anodal transcranial direct current stimulation (tDCS). Neuropsychologia 2009;47:212–7.
Bolognini N, Pascual-Leone A, Fregni F. Using non-invasive brain stimulation to augment motor training-induced plasticity. J Neuroeng Rehabil 2009;6. 1743-0003.
Bolton JE, Wilkinson RC. Responsiveness of pain scales: a comparison of three pain intensity measures in chiropractic patients. J Manipulative Physiol Ther 1998;21:1–7.
Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C, Buchel C. Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. Brain 2002;125:1326–36.
Casey KL, Morrow TJ, Lorenz J, Minoshima S. Temporal and spatial dynamics of human forebrain activity during heat pain: analysis by positron emission tomography. J Neurophysiol 2001;85:951–9.
Chong PS, Cros DP. Technology literature review: quantitative sensory testing. Muscle Nerve 2004;29:734–47.
Coghill RC, Sang CN, Maisog JM, Iadarola MJ. Pain intensity processing within the human brain: a bilateral, distributed mechanism. J Neurophysiol 1999;82:1934–43.
Csifcsak G, Antal A, Hillers F, Levold M, Bachmann CG, Happe S, Nitsche MA, Ellrich J, Paulus W. Modulatory effects of transcranial direct current stimulation on laser-evoked potentials. Pain Med 2009;10:122–32.
Dasilva AF, Mendonca ME, Zaghi S, Lopes M, Dossantos MF, Spierings EL, Bajwa Z, Datta A, Bikson M, Fregni F. TDCS-induced analgesia and electrical fields in pain-related neural networks in chronic migraine. Headache 2012;52:1283–95.
de Morton NA. The PEDro scale is a valid measure of the methodological quality of clinical trials: a demographic study. Aust J Physiother 2009;55:129–33.
Deeks JJ, Higgins JP.Statistical algorithms in Review Manager 5. Statistical Methods Group of The Cochrane Collaboration 2010.
Di Lazzaro V, Manganelli F, Dileone M, Notturno F, Esposito M, Capasso M, Dubbioso R, Pace M, Ranieri F, Minicuci G, Santoro L, Uncini A. The effects of prolonged cathodal direct current stimulation on the excitatory and inhibitory circuits of the ipsilateral and contralateral motor cortex. J Neural Transm 2012;119:1499–506.
Dieckhofer A, Waberski TD, Nitsche M, Paulus W, Buchner H, Gobbele R. Transcranial direct current stimulation applied over the somatosensory cortex - differential effect on low and high frequency SEPs. Clin Neurophysiol 2006;117:2221–7.
Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health 1998;52: 377–84.
Eng JJ, Teasell R, Miller WC, Wolfe DL, Townson AF, Aubut JA, Abramson C, Hsieh JT, Connolly S, Konnyu K. Spinal Cord Injury Rehabilitation Evidence: methods of the SCIRE Systematic Review. Top Spinal Cord Inj Rehabil 2007;13:1–10.
Enomoto H, Ugawa Y, Hanajima R, Yuasa K, Mochizuki H, Terao Y, Shiio Y, Furubayashi T, Iwata NK, Kanazawa I. Decreased sensory cortical excitability after 1 Hz rTMS over the ipsilateral primary motor cortex. Clin Neurophysiol 2001;112:2154–8.
Farina D, Arendt-Nielsen L, Merletti R, Graven-Nielsen T. Effect of experimental muscle pain on motor unit firing rate and conduction velocity. J Neurophysiol 2004;91:1250–9.
Fenton BW, Palmieri PA, Boggio P, Fanning J, Fregni F. A preliminary study of transcranial direct current stimulation for the treatment of refractory chronic pelvic pain. Brain Stimul 2009;2:103–7.
Fernandez E, Turk DC. Sensory and affective components of pain: separation and synthesis. Psychol Bull 1992;112:205–17.
Flor H, Braun C, Elbert T, Birbaumer N. Extensive reorganization of primary somatosensory cortex in chronic back pain patients. Neurosci Lett 1997;224:5–8.
Fomberstein K, Qadri S, Ramani R. Functional MRI and pain. Curr Opin Anaesthesiol 2013;29:29.
Fregni F, Boggio PS, Lima MC, Ferreira MJ, Wagner T, Rigonatti SP, Castro AW, Souza DR, Riberto M, Freedman SD, Nitsche MA, Pascual-Leone A. A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. Pain 2006a;122:197–209.
Fregni F, Freedman S, Pascual-Leone A. Recent advances in the treatment of chronic pain with non-invasive brain stimulation techniques. Lancet Neurol 2007;6:188–91.
Fregni F, Gimenes R, Valle AC, Ferreira MJ, Rocha RR, Natalle L, Bravo R, Rigonatti SP, Freedman SD, Nitsche MA, Pascual-Leone A, Boggio PS. A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. Arthritis Rheum 2006b;54:3988–98.
Fuller GN, Guiloff RJ. The conduction velocities of peripheral nerve fibres conveying sensations of warming and cooling. J Neurol Neurosurg Psychiatry. 1989;52(6):810–2.
Furubayashi T, Terao Y, Arai N, Okabe S, Mochizuki H, Hanajima R, Hamada M, Yugeta A, Inomata-Terada S, Ugawa Y. Short and long duration transcranial direct current stimulation (tDCS) over the human hand motor area. Exp Brain Res 2008;185:279–86.
Gamboa OL, Antal A, Moliadze V, Paulus W. Simply longer is not better: reversal of theta burst after-effect with prolonged stimulation. Exp Brain Res 2010;204:181–7.
Garcia-Larrea L, Peyron R, Mertens P, Gregoire MC, Lavenne F, Le Bars D, Convers P, Mauguiere F, Sindou M, Laurent B. Electrical stimulation of motor cortex for pain control: a combined PET-scan and electrophysiological study. Pain 1999;83:259–73.
Giesecke T, Gracely RH, Williams DA, Geisser ME, Petzke FW, Clauw DJ. The relationship between depression, clinical pain, and experimental pain in a chronic pain cohort. Arthritis Rheum 2005;52:1577–84.
Graven-Nielsen T, Lund H, Arendt-Nielsen L, Danneskiold-Samsoe B, Bliddal H. Inhibition of maximal voluntary contraction force by experimental muscle pain: a centrally mediated mechanism. Muscle Nerve 2002;26:708–12.
Grundmann L, Rolke R, Nitsche MA, Pavlakovic G, Happe S, Treede RD, Paulus W, Bachmann CG. Effects of transcranial direct current stimulation of the primary sensory cortex on somatosensory perception. Brain Stimul 2011;4: 253–60.
Hansen N, Obermann M, Poitz F, Holle D. Diener HC., Antal A., Paulus W., Katsarava Z., Modulation of human trigeminal and extracranial nociceptive processing by transcranial direct current stimulation of the motor cortex. Cephalalgia. 2011;31(6):661–70. http://dx.doi.org/10.1177/0333102410390394.
Higgins, Green S. Cochrane Handbook for Systematic Reviews of Interventions, Cochrane Collaboration 2011.
Hodges PW, Ervilha UF, Graven-Nielsen T. Changes in motor unit firing rate in synergist muscles cannot explain the maintenance of force during constant force painful contractions. J Pain 2008;9:1169–74.
Hodges PW, Moseley GL. Pain and motor control of the lumbopelvic region: effect and possible mechanisms. J Electromyogr Kinesiol 2003;13:361–70.
Hosomi K, Shimokawa T, Ikoma K, Nakamura Y, Sugiyama K, Ugawa Y, Uozumi T, Yamamoto T, Saitoh Y. Daily repetitive transcranial magnetic stimulation of primary motor cortex for neuropathic pain: a randomized, multicenter, double-blind, crossover, sham-controlled trial. Pain 2013;154:1065–72.
Islam N, Aftabuddin M, Moriwaki A, Hattori Y, Hori Y. Increase in the calcium level following anodal polarization in the rat brain. Brain Res 1995;684:206–8.
Jeffery DT, Norton JA, Roy FD, Gorassini MA. Effects of transcranial direct current stimulation on the excitability of the leg motor cortex. Exp Brain Res 2007;182:281–7.
Jette F, Cote I, Meziane HB, Mercier C. Effect of single-session repetitive transcranial magnetic stimulation applied over the hand versus leg motor area on pain after spinal cord injury. Neurorehabil Neural Repair 2013;27:636–43.
Joseph A. (2011). ‘‘Plot Digitizer 2.5.1 ‘‘, from http://plotdigitizer.sourceforge.net/.
Jurgens TP, Schulte A, Klein T. May A, Transcranial direct current stimulation does neither modulate results of a quantitative sensory testing protocol nor ratings of suprathreshold heat stimuli in healthy volunteers. Eur. J. Pain. 2012;16(9):1251–63. http://dx.doi.org/10.1002/j.1532-2149.2012.00135.x.
Kanda M, Nagamine T, Ikeda A, Ohara S, Kunieda T, Fujiwara N, Yazawa S, Sawamoto N, Matsumoto R, Taki W, Shibasaki H. Primary somatosensory cortex is actively involved in pain processing in human. Brain Res 2000;853:282–9.
Lang N, Siebner HR, Ward NS, Lee L, Nitsche MA, Paulus W, Rothwell JC, Lemon RN, Frackowiak RS. How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? Eur J Neurosci 2005;22:495–504.
Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain 2009;10:895–926.
Laurent B, Peyron R. Garcia Larrea L, Mauguiere F. Positron emission tomography to study central pain integration. Rev Neurol 2000;156:341–51.
Lefaucheur JP, Ayache SS, Sorel M, Farhat WH, Zouari HG, Ciampi de Andrade D, Ahdab R, Menard-Lefaucheur I, Brugieres P, Goujon C. Analgesic effects of repetitive transcranial magnetic stimulation of the motor cortex in neuropathic pain: influence of theta burst stimulation priming. Eur J Pain 2012;16:1403–13.
Lefaucheur JP, Drouot X, Menard-Lefaucheur I, Keravel Y, Nguyen JP. Motor cortex rTMS restores defective intracortical inhibition in chronic neuropathic pain. Neurology 2006;67:1568–74.
Leon-Sarmiento FE, Granadillo E, Bayona EA. Present and future of the transcranial magnetic stimulation. Invest Clin 2013;54:74–89.
Liebetanz D, Nitsche MA, Tergau F, Paulus W. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain 2002;125:2238–47.

Lorenz J, Minoshima S, Casey KL. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain 2003;126:1079–91.
Luedtke K, Rushton A, Wright C, Geiss B, Juergens TP, May A. Transcranial direct current stimulation for the reduction of clinical and experimentally induced pain: a systematic review and meta-analysis. Clin J Pain 2012;28:452–61.
Lund JP, Donga R, Widmer CG, Stohler CS. The pain-adaptation model: a discussion of the relationship between chronic musculoskeletal pain and motor activity. Can J Physiol Pharmacol 1991;69:683–94.
Maher CG, Sherrington C, Herbert RD, Moseley AM, Elkins M. Reliability of the PEDro scale for rating quality of randomized controlled trials. Phys Ther 2003;83:713–21.
Mendonca ME, Santana MB, Baptista AF, Datta A, Bikson M, Fregni F, Araujo CP. Transcranial DC stimulation in fibromyalgia: optimized cortical target supported by high-resolution computational models. J Pain 2011;12:610–7.
Miranda PC, Lomarev M, Hallett M. Modeling the current distribution during transcranial direct current stimulation. Clin Neurophysiol 2006;117:1623–9.
Mori F, Codeca C, Kusayanagi H, Monteleone F, Buttari F, Fiore S, Bernardi G, Koch G. Centonze D. Effects of anodal transcranial direct current stimulation on chronic neuropathic pain in patients with multiple sclerosis, J Pain 2010;11(5):436–42. http://dx.doi.org/10.1016/j.jpain.2009.08.011.
Moseley AM, Herbert RD, Sherrington C, Maher CG. Evidence for physiotherapy practice: a survey of the Physiotherapy Evidence Database (PEDro). Aust J Physiother 2002;48:43–9.
Moseley GL, Brugger P. Interdependence of movement and anatomy persists when amputees learn a physiologically impossible movement of their phantom limb. Proc Natl Acad Sci USA 2009;106:18798–802.
Mouraux A, Diukova A, Lee MC, Wise RG, Iannetti GD. A multisensory investigation of the functional significance of the "pain matrix". Neuroimage 2011;54:2237–49.
Mylius V, Borckardt JJ, Lefaucheur JP. Noninvasive cortical modulation of experimental pain. Pain 2012;153:1350–63.
Nitsche MA, Boggio PS, Fregni F, Pascual-Leone A. Treatment of depression with transcranial direct current stimulation (tDCS): a review. Exp Neurol 2009;219:14–9.
Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F, Pascual-Leone A. Transcranial direct current stimulation: State of the art 2008. Brain Stimul 2008;1:206–23.
Nitsche MA, Koschack J, Pohlers H, Hullemann S, Paulus W, Happe S. Effects of frontal transcranial direct current stimulation on emotional state and processing in healthy humans. Front Psychiatry 2012;3:00058.
Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 2000;3:633–9.
Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 2001;57:1899–901.
Nitsche MA, Seeber A, Frommann K, Klein CC, Rochford C, Nitsche MS, Fricke K, Liebetanz D, Lang N, Antal A, Paulus W, Tergau F. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. J Physiol 2005;568:291–303.
O'Connell NE, Cossar J, Marston L, Wand BM, Bunce D, Moseley GL, De Souza LH. Rethinking clinical trials of transcranial direct current stimulation: participant and assessor blinding is inadequate at intensities of 2mA. PLoS One 2012;7:17.
O'Connell NE, Wand BM, Marston L, Spencer S, Desouza LH. Non-invasive brain stimulation techniques for chronic pain. A report of a Cochrane systematic review and meta-analysis. Eur J Phys Rehabil Med 2011;47:309–26.
Oliviero A, Esteban MR, de la Cruz FS, Cabredo LF, Di Lazzaro V. Short-lasting impairment of temperature perception by high frequency rTMS of the sensorimotor cortex. Clin Neurophysiol 2005;116:1072–6.
Palm U, Reisinger E, Keeser D, Kuo MF, Pogarell O, Leicht G, Mulert C, Nitsche MA, Padberg F. Evaluation of sham transcranial direct current stimulation for randomized, placebo-controlled clinical trials. Brain Stimul 2013;6:690–5.
Passard A, Attal N, Benadhira R, Brasseur L, Saba G, Sichere P, Perrot S, Januel D, Bouhassira D. Effects of unilateral repetitive transcranial magnetic stimulation of the motor cortex on chronic widespread pain in fibromyalgia. Brain 2007;130:2661–70.
Perocheau D, Laroche F, Perrot S. Relieving pain in rheumatology patients: Repetitive transcranial magnetic stimulation (rTMS), a developing approach. Joint Bone Spine 2013;17. 00116-00114.
Petrovic P, Petersson KM, Ghatan PH, Stone-Elander S, Ingvar M. Pain-related cerebral activation is altered by a distracting cognitive task. Pain 2000;85:19–30.
Peyron R, Garcia-Larrea L, Deiber MP, Cinotti L, Convers P, Sindou M, Mauguiere F, Laurent B. Electrical stimulation of precentral cortical area in the treatment of central pain: electrophysiological and PET study. Pain 1995;62:275–86.
Peyron R, Garcia-Larrea L, Gregoire MC, Costes N, Convers P, Lavenne F, Mauguiere F, Michel D, Laurent B. Haemodynamic brain responses to acute pain in humans: sensory and attentional networks. Brain 1999;122:1765–80.
Peyron R, Laurent B, Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol Clin 2000;30:263–88.
Ploghaus A, Tracey I, Gati JS, Clare S, Menon RS, Matthews PM, Rawlins JN. Dissociating pain from its anticipation in the human brain. Science 1999;284:1979–81.
Porro CA. Functional imaging and pain: behavior, perception, and modulation. Neuroscientist 2003;9:354–69.
Ragert P, Vandermeeren Y, Camus M, Cohen LG. Improvement of spatial tactile acuity by transcranial direct current stimulation. Clin Neurophysiol 2008;119:805–11.
Rainville P. Brain mechanisms of pain affect and pain modulation. Curr Opin Neurobiol 2002;12:195–204.
Reidler JS, Mendonca ME, Santana MB, Wang X, Lenkinski R, Motta AF, Marchand S, Latif L, Fregni F. Effects of motor cortex modulation and descending inhibitory systems on pain thresholds in healthy subjects. J Pain 2012;13:450–8.
Riberto M, Marcon Alfieri F, Monteiro de Benedetto Pacheco K, Dini Leite V, Nemoto Kaihami H, Fregni F, Rizzo Battistella L. Efficacy of transcranial direct current stimulation coupled with a multidisciplinary rehabilitation program for the treatment of fibromyalgia. Open Rheumatol J 2011;5:45–50.
Rogalewski A, Breitenstein C, Nitsche MA, Paulus W, Knecht S. Transcranial direct current stimulation disrupts tactile perception. Eur J Neurosci 2004;20:313–6.
Roizenblatt S, Fregni F, Gimenez R, Wetzel T, Rigonatti SP, Tufik S, Boggio PS, Valle AC. Site-specific effects of transcranial direct current stimulation on sleep and pain in fibromyalgia: a randomized, sham-controlled study. Pain Pract 2007;7:297–306.
Russo R, Wallace D, Fitzgerald PB, Cooper NR. Perception of comfort during active and sham transcranial direct current stimulation: a double blind study. Brain Stimul 2013;6:946–51.
Sawamoto N, Honda M, Okada T, Hanakawa T, Kanda M, Fukuyama H, Konishi J, Shibasaki H. Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/posterior insula: an event-related functional magnetic resonance imaging study. J Neurosci 2000;20:7438–45.
Scarpazza C, Sartori G, De Simone MS, Mechelli A. When the single matters more than the group: very high false positive rates in single case Voxel Based Morphometry. Neuroimage 2013;70:175–88.
Simoes C, Hari R. Relationship between responses to contra- and ipsilateral stimuli in the human second somatosensory cortex SII. Neuroimage 1999;10:408–16.
Smith BH, Elliott AM, Chambers WA, Smith WC, Hannaford PC, Penny K. The impact of chronic pain in the community. Fam Pract 2001;18:292–9.
Soler MD, Kumru H, Pelayo R, Vidal J, Tormos JM, Fregni F, Navarro X, Pascual-Leone A. Effectiveness of transcranial direct current stimulation and visual illusion on neuropathic pain in spinal cord injury. Brain 2010;133(9):2565–77. http://dx.doi.org/10.1093/brain/awq184.
Summers J, Johnson S, Pridmore S, Oberoi G. Changes to cold detection and pain thresholds following low and high frequency transcranial magnetic stimulation of the motor cortex. Neurosci Lett 2004;368:197–200.
Timmermann L, Ploner M, Haucke K, Schmitz F, Baltissen R, Schnitzler A. Differential coding of pain intensity in the human primary and secondary somatosensory cortex. J Neurophysiol 2001;86:1499–503.
Tsubokawa T, Katayama Y, Yamamoto T, Hirayama T, Koyama S. Chronic motor cortex stimulation in patients with thalamic pain. J Neurosurg 1993;78:393–401.
Valle A, Roizenblatt S, Botte S, Zaghi S, Riberto M, Tufik S, Boggio PS, Fregni F. Efficacy of anodal transcranial direct current stimulation (tDCS) for the treatment of fibromyalgia: results of a randomized, sham-controlled longitudinal clinical trial. J Pain Manag 2009;2:353–61.
Wager TD, Rilling JK, Smith EE, Sokolik A, Casey KL, Davidson RJ, Kosslyn SM, Rose RM, Cohen JD. Placebo-induced changes in FMRI in the anticipation and experience of pain. Science 2004;303:1162–7.
Willis WD, Westlund KN. Neuroanatomy of the pain system and of the pathways that modulate pain. J Clin Neurophysiol 1997;14:2–31.
Wolff BB. The Relationship of Experimental Pain Tolerance to Pain Threshold: A Critique of Gelfand's Paper. Can J Psychol 1964;18:249–53.
Wood L, Egger M, Gluud LL, Schulz KF, Juni P, Altman DG, Gluud C, Martin RM, Wood AJ, Sterne JA. Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study. Bmj 2008;336:601–5.
Zaehle T, Beretta M, Jancke L, Herrmann CS, Sandmann P. Excitability changes induced in the human auditory cortex by transcranial direct current stimulation: direct electrophysiological evidence. Exp Brain Res 2011;215:135–40.
Ziemann U, Siebner HR. Modifying motor learning through gating and homeostatic metaplasticity. Brain Stimul 2008;1:60–6.

Declaration for Chapter 3

In the case of Chapter 3, contribution to the work involved the following:

Nature of contribution	Extent of contribution (%)
Identification and review of the 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 framing of the manuscript, review and provision of feedback on manuscript drafts	15 %
Maryam Zoghi	Guidance in framing of the manuscript, review and provision of feedback on manuscript drafts	5 %

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

Candidate's Signature: [redacted] Date: 15-Sep-2015

Signature: [redacted] Date: 15-Sep-2015.

Signature: [redacted] Date: 15-Sep-2015

Preamble to Chapter 3

Chapter 3 provides a systematic review and meta-analysis to verify whether previous tDCS studies support the view that c-tDCS modifies STh/PTh in healthy individuals and PL in patients with chronic pain.

Chapter 3: A meta-analysis of site-specific effects of cathodal transcranial direct current stimulation on sensory perception and pain

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

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RESEARCH ARTICLE

A Meta-Analysis of Site-Specific Effects of Cathodal Transcranial Direct Current Stimulation on Sensory Perception and Pain

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Abstract

The primary aim of our meta-analysis was to evaluate the effects of cathodal transcranial direct current stimulation (c-tDCS) on sensory and pain thresholds (STh and PTh) in healthy individuals and pain level (PL) in patients with chronic pain. Electronic databases were searched for c-tDCS studies. Methodological quality was evaluated using the PEDro and Downs and Black (D&B) assessment tools. C-tDCS of the primary motor cortex (S1) increases both STh ($P<0.001$, effect size of 26.84%) and PTh ($P<0.001$, effect size of 11.62%). In addition, c-tDCS over M1 led to STh increase ($P<0.005$, effect size of 30.44%). Likewise, PL decreased significantly in the patient group following application of c-tDCS. The small number of studies precluded subgroup analysis. Nevertheless, meta-analysis showed that in all groups (except c-tDCS of S1) active c-tDCS and sham stimulation produced significant differences in STh/PTh in healthy and PL in patient group. This review provides evidence for the site-specific effectiveness of c-tDCS in increasing STh/PTh in healthy individuals and decreasing PL in patients with chronic pain. However, due to small sample sizes in the included studies, our results should be interpreted with caution. Given that the level of blinding was not considered in the inclusion criteria, the results of the current study should be interpreted with caution.

Introduction

Cathodal transcranial direct current stimulation (c-tDCS) is one of the non-invasive brain stimulation techniques which depends on the parameters of the applied current, may induce decreased or increased corticospinal excitability [1, 2]. The inhibitory effect of c-tDCS has been recently utilized for treatment of different clinical conditions including pain management [3–5]. To understand how c-tDCS modulates pain, it should be noted that a large distributed network of brain sites are activated during pain processing [6] which collectively is called pain neuromatrix [7, 8]. Some parts of the pain neuromatrix are superficial, including the primary sensory cortex (S1), primary motor cortex (M1), and dorsolateral prefrontal cortex (DLPFC).

Other areas of the pain neuromatrix such as the thalamus, insula, and anterior cingulate cortex, and pre-acuectal grey matter are deeper structures [9, 10].

Since S1, M1, and DLPFC make contributions to pain processing [11, 12], the site of stimulation should have a differential effect on pain relief. Some imaging studies have indicated that S1 is responsible for sensory discriminative component of pain, including stimulus localization, intensity and discrimination of pain quality [7, 10, 13–15]. Furthermore, functional connectivities between M1, ventro-lateral, and anterior thalamic nuclei affect medial thalamus, anterior cingulate cortex, and upper brainstem functions [16–18]. These connectivities are the means by which the central nervous system regulates the musculoskeletal system during painful conditions. Regardless of pain location, DLPFC affects cognition, attention, anticipation, and emotion aspects of pain during the pain processing [8, 15, 19–22]. There is also evidence that prefrontal cortex and the anterior cingulate cortex are activated during pain expectation [23] and pain-induced anxiety [24].

Pain can be operationalized into key variables including sensory threshold (STh) and pain threshold (PTh) in healthy individuals [15, 25, 26] and pain level (PL) in patients with chronic pain [27, 28]. Recent investigations have demonstrated that c-tDCS of superficial areas of pain neuromatrix induces excitability decrease [1] which results in STh/PTh increase [29, 30] and PL decrease [27]. In contrast, others report no effect on these behavioral variables [31]. These results raise a very important question: what is the evidence for the effectiveness of c-tDCS in modulating pain according to the site of stimulation? To date, no meta-analysis has drawn together the abundant evidence from the existing literature on the effects of c-tDCS over superficial areas of pain neuromatrix on STh/PTh and PL to reach a firm conclusion about the efficacy of c-tDCS in pain management. In the current study, we aimed to investigate the site-specific effects of c-tDCS on STh/PTh in healthy individuals and PL in patients with chronic pain.

Methodology

Inclusion criteria

English-language articles describing all types of study designs, including parallel or cross over studies, were included in the current study regardless of blinding. Studies that utilized c-tDCS on the S1, M1, or DLPFC in healthy individuals or patients experiencing chronic pain were recruited if the participants were over 18 years of age and either healthy or had experienced chronic pain for more than three months [6, 32], the outcome measures of interest were the visual analogue scale (VAS) in the patient group or STh/PTh in the healthy group, and sham tDCS or active control was applied (Table 1).

Given the fact that M1, S1, and DLPFC are the only superficial areas of pain neuromatrix which are accessible for stimulation by tDCS, we included studies investigated the effects of c-tDCS on these areas in both healthy subjects (Table 2) and patient groups with chronic pain regardless of their pathology (Table 3). All modalities that evoked a sensory or painful sensation were included (i.e., laser, heat, cold and mechanical stimuli). Chronic pain was specified as a refractory pain which is resistant to medical intervention or drug management for more than three months [6, 32]. We included studies that placed electrodes over M1, S1 or DLPFC regions.

Exclusion Criteria

Studies were excluded if they did not involve brain stimulation, the duration of symptoms for patient groups was not clear, the study used deep brain stimulation, transcranial magnetic stimulation, repetitive transcranial magnetic stimulation, or electrical stimulation with pulse

Table 1. Inclusion and exclusion criteria for identified studies.

	Inclusion	Exclusion
Participants	- Studies in which individuals were over 18 years of age	- Studies involving individuals suffering from other type of diseases (i.e., depression or other type of mental disorders, cancerous pain
	- Either healthy or suffering from chronic pain (no limits were applied to the type (musculoskeletal, neural, or central pain syndrome), anatomical location	- Studies on patients with primary symptoms other than pain (i.e., depression or schizophrenia)
Intervention	- Studies that involve c-tDCS and Sham as intervention of interest	
Comparison	- Studies in which the comparison of interest is "no treatment"/sham treatment	- Other control group
	- Before and after c-tDCS	
Outcomes	- Studies in which the outcome measure of interest were Numeric Analogue Scale (NAS) measured by Quantitative Sensory Testing (QST) method and LEP amplitude in healthy individuals and VAS in patients with chronic pain	- Other type of evaluation of sensory perception, pain threshold, and PL (Measured by rTMS, fMRI, PET, and Paired TMS)
Trial design	- Randomized control trial, controlled clinical trials, and pre-post trials	- Review articles
		- Selective review
Data reported	- Data that enable analysis and estimation of the effects of c-tDCS and Sham on STh, PTh, and PL must be reported	
Type of publications	- Published in a peer-reviewed journal, regardless of the year of publication	Non English articles
	- As the services for translation do not exist, only English publications will be considered	

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Table 2. Study characteristics and outcome measure in healthy individuals.

Included Studies	Trial design	No. Participants	Stimulation method	Outcome measure	Intervention	Stimulated area
Antal et al. 2008	Pre-Post test	10	LASER	NAS, LEP	a-tDCS, c-tDCS	S1
Bachmann et al. 2010	Single blinded, Crossover trail	8	QST	NAS	a-tDCS, c-tDCS	M1
Boggio et al. 2008	Double blinded, sham controlled	20	ES	NAS	a-tDCS	V1[1], M1[2], DLPFC[3]
Csfcsak et al. 2009	Pre-Post test	10	LASER	NAS, LEP	a-tDCS, c-tDCS	M1
Grundmann et al. 2010	Pre-Post test	12	QST	NAS	a-tDCS, c-tDCS	S1[6]
Hansen et al. 2010	Pre-Post test	19	ES	NAS, PREP[4], BR[5]	a-tDCS, c-tDCS	M1
Rogalewski et al. 2004	Single blinded, Sham controlled	13	Tactile perception	NAS	a-tDCS, c-tDCS	S1
Terney et al. 2008	Single blinded crossover trial	15	LASER	NAS, LEP	a-tDCS, c-tDCS	M1

1. Primary visual cortex
2. Primary motor cortex
3. Dorsolateral prefrontal cortex
4. Pain Related Evoked Potential
5. Blink Reflex
6. Somatosensory cortex

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Table 3. Study characteristics and outcome measure in patients with chronic pain.

Included Studies	Trial design	No. Participants	Patients	Stimulation area	Intervention	Outcome measure
Antal et al. 2011 a	Double blinded sham control	26	Chronic Migraine	V14	c-tDCS	VAS
Mendonca et al. 2011	Double blinded randomised control	30	Fibromyalgia	M1	a-tDCS, c-tDCS	VNS[1]

1. Visual Numeric Scale

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currents (Table 1). In addition, studies that used a-tDCS or indirect forms of stimulation (caloric vestibular stimulation or occipital nerve stimulation) were excluded.

Outcome measures

The outcome measures for STh and PTh were percentage changes in stimulus intensities at which participants reported the onset of sensation (STh) or onset of pain (PTh). For PL in the patient group, we pooled studies that used VAS (Tables 2 and 3).

Because the included trials involved post-intervention assessments at varying time points, they were partitioned into short-term and long-term outcomes. "Short-term" was arbitrarily defined as less than one hour after intervention. If a trial had multiple assessments during that period, the assessment performed closest to the intervention was used. "Long-term" was defined as greater than one hour after intervention; long-term outcomes were not included in the current meta-analyses.

Methods for identifying studies

To locate eligible articles, a broad search was performed on all English literatures through relevant databases including PubMed, Physiotherapy Evidence Databases (PEDro), CINAHL, Cochrane Central Register of Controlled Trials, Scopus, PROQuest, SPorTDiscuss, Australian Medical Index, Ovid Medline, EBM Review, Cochrane, Meditex and PsycINFO from their inception to December 2014. All reference lists of retrieved papers were searched to identify additional relevant articles missed in the initial search strategy. The key words were "transcranial direct current stimulation", "tDCS", "sensory perception", "pain", "pain perception", "pain tolerance", "sensory threshold", "pain threshold", "sensory stimulation" and "pain trigger".

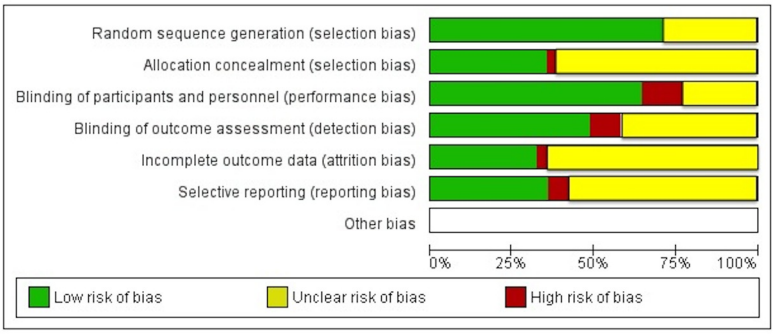

Fig 1. Risk of bias graph: Review authors' judgments about each risk of bias item presented as percentages across all 10 included studies.

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Selection of the included studies

Considering the inclusion criteria, both randomized and non-randomized trials were selected. Two independent raters (BV and SJ) reviewed the title and abstract of all selected papers. If the information in the title and abstract was insufficient to make a decision, the reviewers assessed the full paper to include or exclude the study. All included studies were then double-checked by a full-text appraisal. If the reviewers disagreed, resolution was attempted by discussion. If resolution was not achieved, the third reviewer (MZ) was consulted.

Risk of bias assessment

The risk of bias and methodological quality of included studies were evaluated by the assessed method defined in Chapter 8 of the *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.2* [33]. Fig 1 is a methodological quality graph for all included studies.

PEDro scale was used for further quality assessment [34, 35] in which there are 10 criteria for internal validity; studies are awarded a point for each criterion met. The PEDro cut-points are 9–10, excellent; 6–8, good; 4–5, fair and below 4, poor [36]. For non-randomized controlled trials, Down and Black tool (D&B) was used [37] (Table 4).

Outcome measures

Our primary outcome measures were the STh and PTh of healthy individuals and PL in patients who suffered from chronic pain. STh is usually measured by quantitative sensory testing using mechanical, vibration or thermal methods [38]. The STh is defined as the level of stimulus intensity at which sensation was detected for the first time. PTh is defined as the level of stimulus intensity at which pain is detected. PL in patients with chronic pain was defined as the average pain that they experience during a day, usually measured by the VAS [39].

Subgroup analysis and intervention of heterogeneity

The heterogeneity of included studies was evaluated by Chi^2 test and I^2 statistic. There are two subgroups in each meta-analysis assessing the effects of c-tDCS on STh and PTh in healthy individuals: (i) c-tDCS of S1, and (ii) c-tDCS of M1. Due to the limited included studies in patient group, the overall effect of c-tDCS on PL with no subgroup analysis was assessed in the patient group.

Table 4. Quality assessment of included studies.

	Included studies	PEDro (1999)	D & B (Downs and Black, 1998)
Healthy group	Bachmann et al. 2010	7	-
	Rogalewwski et al. 2004	7	-
	Terney et al. 2008	-	16
	Csfcsak et al. 2009	-	17
	Grundmann et al. 2010	-	16
	Henssen et al. 2010	-	17
	Antal et al. 2008	-	18
	Boggio et al. 2008	-	18
Patient group	Mendonca et al. 2011	7	-
	Antal et al. 2011	8	-

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Data extraction

The following data were extracted from the included studies: study design, characteristics of subjects, outcome measures; stimulated areas in healthy (Table 2) and patient group (Table 3). C-tDCS parameters, the position and size of active electrodes are also summarized in Table 5. We asked the corresponding author(s) to send us the mean ± SD of desired outcome measures. Where the requested data were not provided, mean ± SD values were extracted from tables or pooled from graphs using Plot Digitizer software [40]. In studies that did not report standard deviation (SD), we used the formula $SD = SE\sqrt{n}$ (n = number of subjects in each group) [33].

Plot Digitizer, a java program, was used to digitize data point off of scanned plots [33] was used to digitize scanned plots of functional data. Statistical significance of the difference between extracted data was calculated with 95% of confidence intervals (CIs) by RevMan software, version 5.1 (Cochrane Collaboration, 2008) [41]. RevMan is adjusted to calculate small sample bias [33]. Extracted data were entered into the meta-analysis using the generic inverse variance method as suggested in the *Cochrane Handbook for Systematic Reviews of Interventions* [33].We used a random effects model to conduct separate meta-analyses for different forms of stimulation (c-tDCS and sham). Where more than one data point was available for short term outcomes, we used the first post stimulation measure. Two forest plots were generated for each outcome measure. In the first one, the percentage changes in STh, PTh and PL after applying c-tDCS compared to baseline values were assessed. In the second one, the percentage changes in STh, PTh and PL after c-tDCS were compared to the percentage changes after effects of sham stimulation (Table 5). Based on the *Cochrane Handbook for Systematic Reviews of Interventions*: "Standard mean difference (SDM) is used to measure effect size when the trials all assess same outcomes, but measured in a variety of ways. As a result, the effect measure for STh/ PTh in healthy and PL in patient groups was assessed by SMD by which we had this opportunity to clarify the degree of improvement or no improvement in our outcome

Table 5. c-tDCS parameters in healthy individuals.

Included studies	Electrode size (cm^2)	Intensity (mA)	Current density (mA/cm^2)	Time (min)	Electrode Position
A: Healthy group					
Antal et al. 2008	35	1	0.029	15	2cm posterior to ADM[1] hot spot
Bachmann et al. 2010	35	1	0.029	15	C3
Boggio et al. 2008	35	2	0.057	5	C3, F3, Oz
Csfcsak et al. 2009	35	1	0.029	10	C3
Grundmann et al. 2010	35	1	0.029	15	C3
Hansen et al. 2010	16	1	0.063	20	Cz, 1 cm above the supraorbital nerve
Rogalewski et al. 2004	35	1	0.029	7	C4
Terney et al. 2008	35	1	0.029	15	ADM[1] hot spot
B: Patient group					
Antal et al. 2011 a					
Mendonca et al. 2011	16	1	0.063	20	C3
	16, 80	2	0.125, 0.0125	20	C3

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measures after the intervention. The SMD calculation in RevMan software is given by:

$$\text{SMD} = \frac{\text{Difference in mean outcome between groups}}{\text{Standard deviation of outcome among participants}}$$

Results

Identification and selection of studies

The search strategy identified 131 studies, including 62 duplicates. Screening by title and abstract, 17 studies, including 12 studies in healthy and 5 in patient group, were eligible to review in which three studies in the healthy and one study in the patient group were identified from manual searching the reference lists of included studies. Five studies, which did not meet inclusion criteria, were excluded. As requested data were not provided from corresponding authors and graphs or tables of two papers, they were excluded. Therefore, the final number of study is 10 (8 in healthy and 2 in patient group) (Fig 2).

Risk of bias and quality assessment

No study was judged to have a low risk of bias across all criteria. Fig 1 summarizes the risk of bias assessment results. All trials had unclear or inadequate bias control in one or more of the domains for the assessment of risk of bias. Lack of blinding of participants and personnel was the major potential source of bias in the current meta-analysis. Also, allocation concealment and completeness of outcome data were unclear in more than 50% of studies, representing a moderate risk of bias. However, the PEDro score was 7 in the healthy group (mean score of 7/

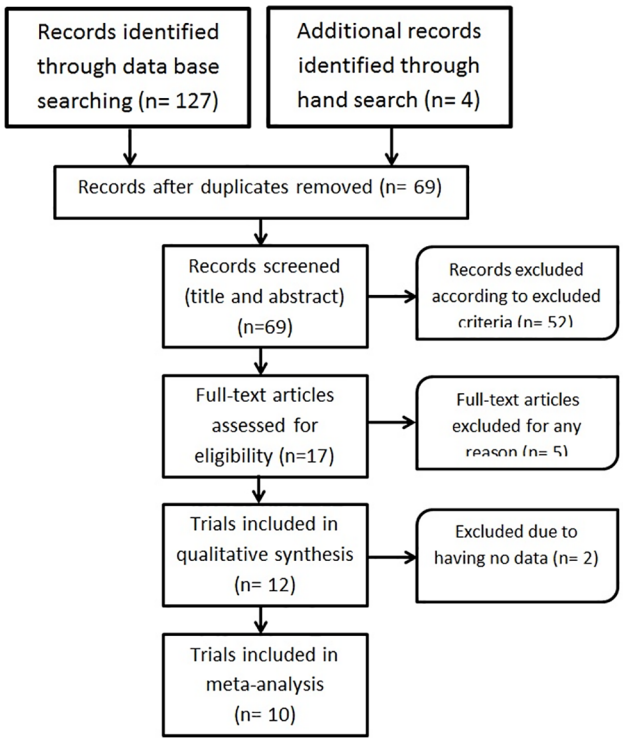

Fig 2. Flowchart study selection.

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11) and ranged between 7 and 8 in the patient group (mean score of 7.5/11), which is in the range of good quality controlled trials. Mean score of 12/27 in D&B quality checklist indicates that the mean quality checklist is medium in the healthy group. Table 3 shows the PEDro and D&B scores of the studies.

Participants in included studies

In total across the included studies, 107 healthy individuals and 56 patients with chronic pain received c-tDCS and sham for VAS measurement. All studies assessed the effect of c-tDCS in one or more of the M1, S1, or DLPFC. In patients with chronic pain, the average VAS score was more than 5.

No study in the healthy group involved stimulating the DLPFC to measure STh/PTh. Furthermore, no study could be identified on the effects of S1 c-tDCS on PL. Due to the patient group containing only two studies, it was impossible to evaluate the site-specific effects of c-tDCS in this group.

Pooled data analysis

For all studies the standard error (SE) was calculated from the 95% confidence interval of the standardized mean difference and entered into the meta-analysis using the generic inverse variance method. Pre-post c-tDCS studies and active/sham studies were evaluated to assess whether c-tDCS can change STh, PTh, and PL, and whether studies using a sham group as a control produced results significantly different from those of pre-post studies. The percentage changes before and after applying c-tDCS and sham were calculated and pooled in meta-analysis.

Effects of c-tDCS on STh in healthy participants

Fig 3A summarizes the pooled data (percentages of changes) extracted from six studies on healthy individuals [29–31, 42–44]. The pooled analysis of three studies (n = 81) in S1

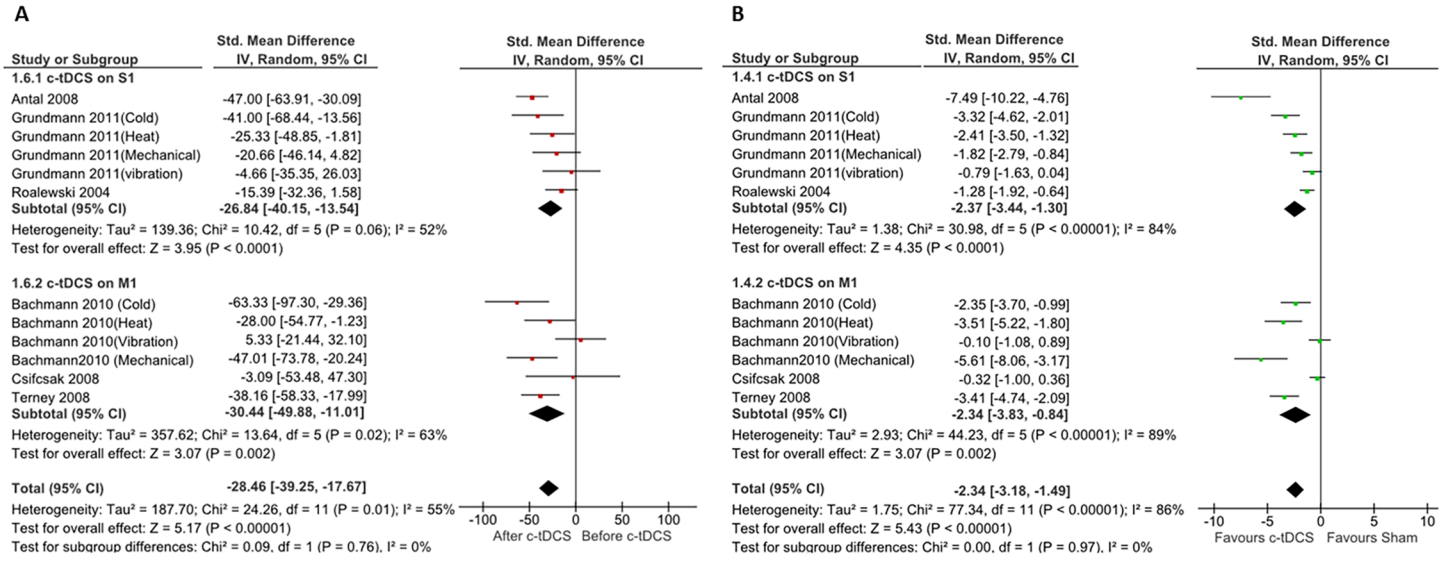

Fig 3. Forest plots of sensory threshold changes in healthy individuals. mparison of percentages of sensory threshold changes before and after c-tDCS (A), and comparison of after effects of sensory threshold changes between active and sham c-tDCS (B). Subgroup analysis: studies of M1 and S1 stimulation. ■ = the effect size for one trial; horizontal line = 95% of confidence interval; ◆ = pooled effect size for all trials. CI: confidence interval, IV: inverse variance.

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subgroup and three studies (n = 61) in M1 subgroup showed that c-tDCS of both S1 and M1 increased STh significantly. The heterogeneity of S1 stimulation was (I^2 = 84%) and the percentages of STh increase was 26.84% (P< 0.0001, 95% CI, (from 40.15% to 13.54%)). The heterogeneity for M1 stimulation was (I^2 = 69%), and the results suggest a significant benefit of c-tDCS on M1 ((28.25%, (95% CI 53.49%, 3.01%), P = 0.02)).

Overall, our meta-analysis of pooled data from all included studies indicates significant STh increase (P<0.00001) in healthy individuals with a main effect size of 27.30% (95% CI, (from 39.19 to 15.42%) (Fig 3A).

Fig 3B shows the result of comparison of sham and active c-tDCS. The results of meta-analysis of pooled studies demonstrated that there are significant STh changes in both S1 (pooled SMD: -2.37, (95% CI, (from -3.44 to -1.30)), P < 0.0001) and M1 (pooled SMD: -2.34, (95% CI, (from -3.34 to -1.49)), P = 0.002) subgroups. Forest plot and meta-analysis results also indicated a significant difference between sham and active c-tDCS (P<0.00001).

Effects of c-tDCS on PTh in healthy participants

Six studies assessed the effect of c-tDCS on PTh in healthy individuals [29, 31, 44–46]. Two studies (n = 46) stimulated S1 and four (n = 72) focused on M1 stimulation. The subgroup results demonstrated c-tDCS generated a significant PTh increase in the S1 subgroup, with a mean effect size of (11.62%, (95% CI, from 16.09% to 7.14%), P<0.00001) and heterogeneity of 0%; this was not the case in the M1 subgroup and there was no significant change in PTh after applying c-tDCS on M1 (Fig 4A). The analysis also indicated no significant overall effect on PTh (P = 0.08).

As can be seen in Fig 4B, meta-analysis showed that while there is a significant difference between sham and active c-tDCS of M1 (pooled SMD: -2.20, (95% CI, (from -3.12 to -1.28)), P < 0.00001), there is no significant difference between sham and active c-tDCS of S1 (pooled SMD: -1.32, (95% CI, (from -3.16 to 0.53), P = 0.16).

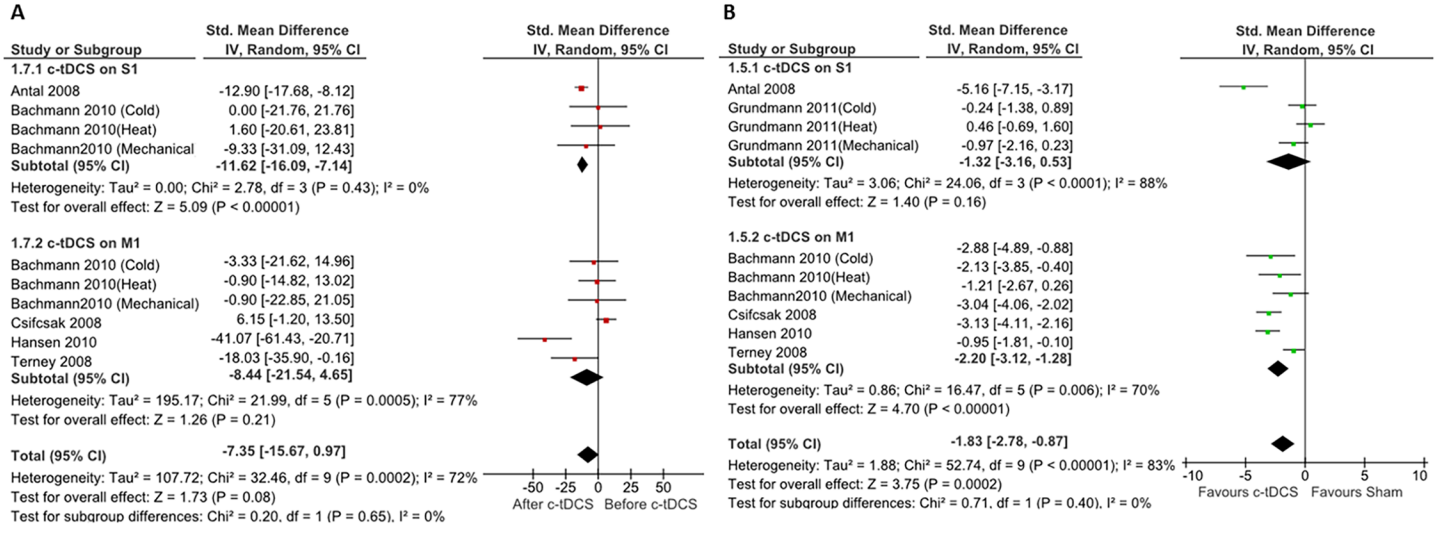

Fig 4. Forest plots of pain threshold changes in healthy individuals. Comparison of percentages of pain threshold changes before and after c-tDCS (A), and comparison of after effects of pain threshold changes between active and sham c-tDCS (B). Subgroup analysis: studies of M1 and S1 stimulation. ■ = the effect size for one trial; horizontal line = 95% of confidence interval; ◆ = pooled effect size for all trials. CI: confidence interval, IV: inverse variance.

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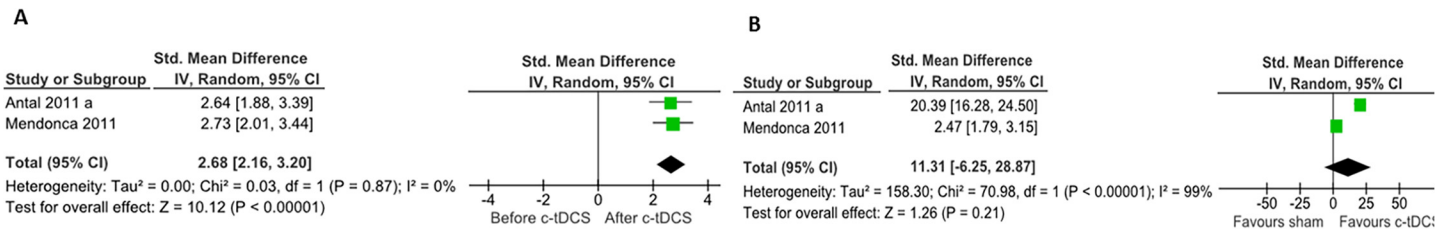

Fig 5. Forest plots of pain level changes in patients with chronic pain. Comparison of percentages of pain level changes before and after c-tDCS (A), and comparison of after effects of pain level changes between active and sham c-tDCS (B). Subgroup analysis: studies of M1 and dorsolateral prefrontal cortex (DLPFC) stimulation. ■ = the effect size for one trial; horizontal line = 95% of confidence interval; ◆ = pooled effect size for all trials. CI: confidence interval, IV: inverse variance.

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The effect of c-tDCS on PL in patients with chronic pain

We had insufficient data to investigate the effect of site of stimulation in the patient group, but we could investigate the overall effect of c-tDCS on PL in patients with chronic pain. Data were available from two studies (n = 56) [27, 28]. Regarding heterogeneity of included studies (I^2 = 0%), the evidence suggested that applying c-tDCS resulted in a significant decrease in PL in patients with chronic pain. The pooled effect was 2.68 (95% CI, (from 2.16 to 3.20)), P < 0.00001) (Fig 5A).

The comparison of active and sham c-tDCS indicates that a non-significant difference in PL after applying active and sham tDCS (pooled SMD: 11.31, (95% CI, (from -6.25 to 28.87)), P = 0.21) (Fig 5B).

The impact of individual studies on the overall results

The effect of each included study on the pooled effect size of overall analyses was examined in both healthy and patient groups.

C-tDCS and STh/PTh in healthy individuals

Based on the result of sensitivity analysis, the total effect size of meta-analysis evaluating after effects of S1 c-tDCS on STh did not change if any one single study was excluded although the pooled data was slightly decreased by excluding the study of Antal et al. 2008 (P = 0.001) and Grundmann et al. 2011 (cold and heat: P = 0.001, and mechanical: P = 0.0005). Exclusion of Grundmann (vibration) and Roalewski et al. (2004) data had no effect on the overall effect size (Fig 6A). The effect size of STh after c-tDCS of M1 was slightly decreased when Terney et al. (2008) (P = 0.03) and Bachmann et al. (2011) studies (cold and heat: P = 0.01, and mechanical: P = 0.02) were excluded. Conversely, the pooled dada increased after excluding that part of Bachmann's study evaluating the effect of M1 c-TDCS on vibration (Fig 6B).

The sensitivity analysis showed that excluding Antal et al. (2008) study and Bachmann et al. (2010) (mechanical) investigating the after effects of S1 c-tDCS on PTh in healthy individuals decreased the overall effect size to 0.68 and 0.05 respectively. Excluding other studies had no effect on pooled effect (Fig 6C). In addition, the pooled data did not changed by excluding each study evaluating the M1 c-tDCS on PTh (Fig 6D).

Likewise, the impact of individual studies on the meta-analyses comparing active and sham c-tDCS on STh and PTh were evaluated (Fig 7A–7D). For meta-analysis on STh, the pooled effect size would decrease to 0.0002 if the studies of Grundmann et al. (2011) on cold, heat, or mechanical sensation omitted. Exclusion of other including studies had no effect on the overall pooled effect (Fig 7A). The results also indicated that the overall effect size of meta-analysis compared the effects of M1 c-tDCS and sham on PTh did not change after exclusion of each

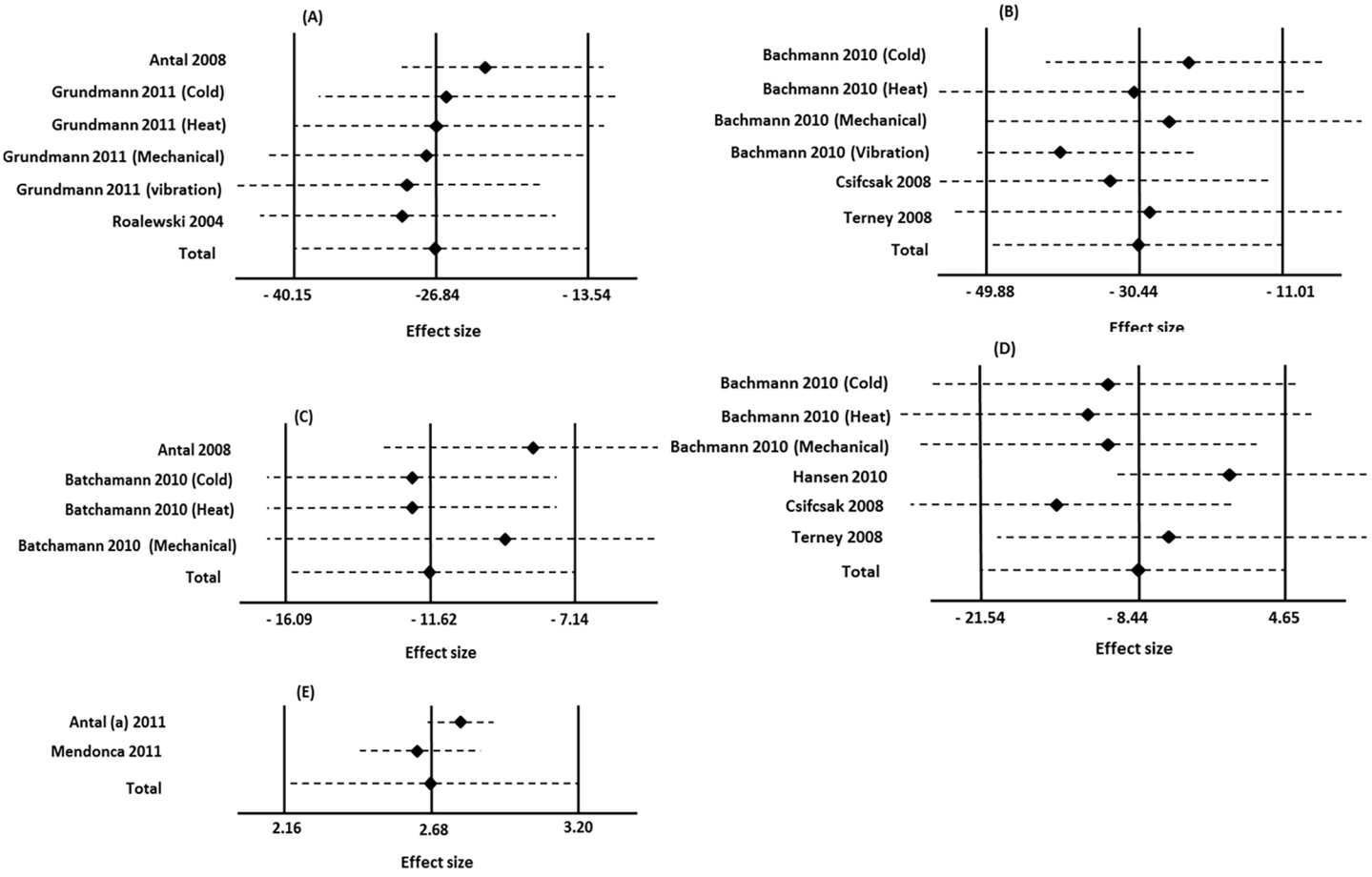

Fig 6. Assessment of the individual influence of included studies evaluating the after effects of c-tDCS on outcome measures. The Impact of single studies on overall effect size in studies evaluating the effect of c-tDCS of S1 (A) and M1 (B) on sensory threshold, c-tDCS of S1 (C) and M1 (D) on pain threshold in healthy individuals, and pain level (E) in patients with chronic pain were evaluated. The 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.

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single study. However, pooled effect size reached to 0.01 by omitting Bachmann et al. (2010) experiments (Fig 7B).

The result of sensitivity analysis focused on the effects of active and sham S1 (Fig 7C) and M1 (Fig 7D) c-tDCS on PTh in healthy individuals indicated that excluding each individual study had no effect on the overall pooled effect size.

C-tDCS and PL in Patients with chronic pain

The result of sensitivity analysis in overall effect size in meta-analysis evaluating the after effects of c-tDCS on PL in patient group illustrated that omitting each study had no effect on overall effect size (Fig 6E). In contrast, in meta-analysis comparing sham and active c-tDCS on PL, exclusion of Antal et al. (2011a) study would increase the pooled effect size to 0.002 (Fig 7E).

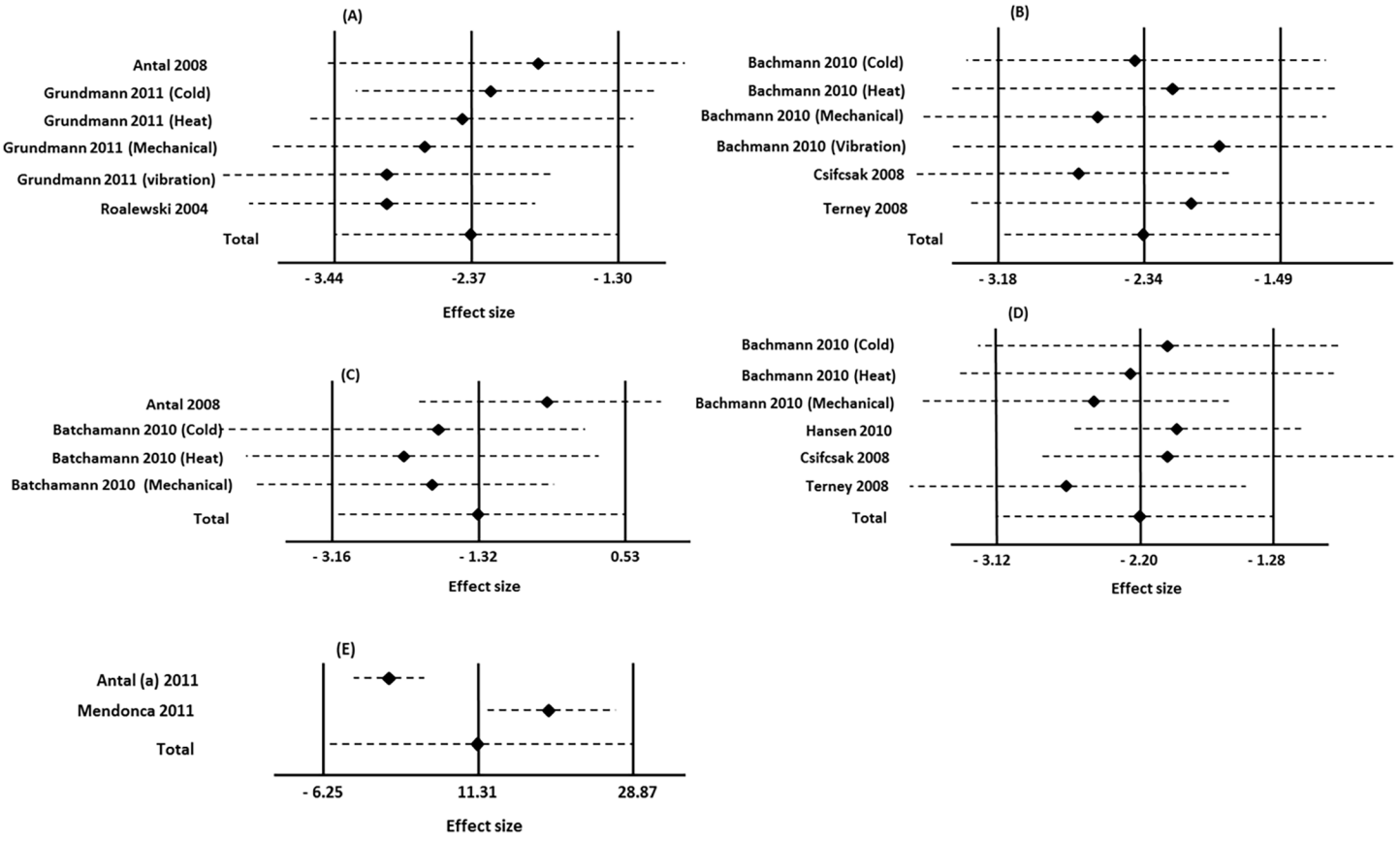

Fig 7. Assessment of the individual influence of included studies evaluating the effect of active and sham c-tDCS on outcome measures. The Impact of single studies on overall effect size in studies evaluating the effect of active and sham c-tDCS of S1 (A) and M1 (B) on sensory threshold, c-tDCS of S1 (C) and M1 (D) on pain threshold in healthy individuals, and pain level (E) in patients with chronic pain were evaluated. The 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.

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Discussion

Our meta-analysis involved eight studies of the effects of c-tDCS on STh and PTh in healthy individuals and two studies of the effects of c-tDCS on PL in patients with chronic pain. We aimed to evaluate the effectiveness of c-tDCS in increasing STh/PTh and PL according to the site of stimulation. The results of subgroup analyses in healthy individuals showed that, compared to baseline values, c-tDCS of S1 increased both STh and PTh in healthy individuals. Furthermore, c-tDCS of M1 led to significant STh but not PTh increase. Due to the scarcity of studies applying c-tDCS in patients with chronic pain, we could not conduct subgroup analysis in the patient group, but c-tDCS significantly decreased PL in the patient group overall. Similar results were found from comparison of active c-tDCS and sham stimulation, except for c-tDCS of S1, in which no significant difference was observed. More studies are needed to reach a firm conclusion in this regard.

The effects of c-tDCS on STh in healthy individuals

In spite of the small number of studies available, the results of our meta-analysis showed that application of c-tDCS on both M1 and S1 increases STh in healthy individuals. Of six studies,

which involved the S1, five reported c-tDCS of the S1 increased STh [30, 43, 45], and one study concluded that c-tDCS of S1 had no effect on STh [30] (Fig 3).

Four of six studies reported a significant increase in STh after c-tDCS of M1, and the remaining two failed to show such an increase. In addition, our comparison of the after effects of active c-tDCS and sham stimulation of the M1 and S1 subgroups demonstrated a significant increase in STh.

As can be seen in Fig 3, the heterogeneity of each subgroup and overall heterogeneity was moderate. We used sensitivity analysis [47] to assess the impact of excluding the studies with high risk of bias; the results demonstrate no changes in heterogeneity, which indicate insufficient data from which to draw a firm conclusion. Also, the different stimulation methods applied in the included studies to assess sensory threshold (laser, heat, cold, and electrical stimulation) could be another reason for the moderate heterogeneity.

Due to the fact that unmyelinated C-fibers transmit the sensation of warmth and small myelinated Aδ fibers transmit the sensation of non-painful cold [48], pooling data for different sensation (cold, warm, vibration, mechanical, etc.) might affect the results. The findings of Summers (2004) and Oliviviero (2005) suggested that repetitive transcranial magnetic stimulation of the somatosensory cortex increased cold perception but not warmth perception [49, 50]. Studies with larger sample sizes using the same methods of sensory threshold assessment will increase statistical power and decrease heterogeneity.

There are several basic mechanisms to explain the increased after effects of c-tDCS. First, prolonged constant electric field alters ionic concentration in stimulated area, which led to migration of transmembrane proteins and acid-base balance changes [51, 52]. Second, direct currents dissociate pure water to H^+ and OH^- [53] resulted in acid-base balance changes by inducing acidosis or alkalosis that in turn strongly affect membrane, receptor and cell function [54]. Because changes in intracellular pH and (Ca^{2+}) are tightly correlated [55], one possibility is that c-tDCS changes pH and Ca^{2+} concentration and increases STh

The effects of c-tDCS on PTh in healthy individuals

Meta-analysis of the included studies demonstrates conflicting results in two subgroups. Compared with the baseline conditions, c-tDCS of S1 significantly increased PTh, whilst c-tDCS of M1 had no effect on PTh. Additionally, our comparison of the immediate after effects of active c-tDCS and sham stimulation of M1 demonstrated a significant difference in PTh. We also found no significant difference in PTh between sham stimulation and c-tDCS of S1.

The included studies used a wide range of stimulation parameters and methods, which might explain the substantial heterogeneity.

The effect of c-tDCS on PL in patient group

The overall effect of c-tDCS was significant decreases in PL. Significant differences in PL of patients after application of c-tDCS and sham stimulation indicate the efficacy of c-tDCS in pain reduction. Due to the low number of c-tDCS studies in our patients group and its substantial heterogeneity, it was impossible to review site-specificity effects of c-tDCS on PL in different subgroups. The small number of included studies and participants, different pathology, site of stimulation, and stimulation parameters created substantial heterogeneity in the patient group data.

The results of our meta-analyses are in line with the conclusions from published systematic reviews [56–58], which allows us to conclude that c-tDCS can relieve pain in patients with chronic pain; however, more c-tDCS studies in chronic pain patients with different pathologies and sites of stimulation are recommended to improve the quality of the evidence.

The exact mechanisms underpinning the effects of c-tDCS on pain relief are not clear yet, but recent evidence categorized the effects of c-tDCS into two types: immediate after effects and long-lasting effects [59, 60]. The immediate after effects of c-tDCS can be explained by changes in the acid-base balance of neuron membranes [54, 61], excitability diminution [62], and consecutive reduction of NMDA receptor activity [62–64]. As a result, direct changes in membrane function, outside of synapses, change the activity of NMDA receptors indirectly and decrease the function of brain areas related to pain management [47, 59]. Based on the Kinkelin's study (2000) it can be concluded that the after effects of tDCS do not arise from NMDA synaptic involvement alone. Although NMDA receptors are present on peripheral axons [65], they have not been reported on axons in the CNS [60, 63, 64].

Quality of evidence

Assessor blinding status was not clearly reported which affect both homogeneity and pooled effect size. Regarding an epidemiological study, incomplete blinding in controlled trials may exaggerate the effect size by 25% [66]. Recently, O'Connell et al. (2012) reported that proper blinding is not possible in a study that uses a current intensity of 2 mA or greater. Though this conclusion was challenged by Russo et al. (2013) and Palm et al. (2013), the implication is that the overall quality of the evidence for the effects of c-tDCS on STh/PTh assessment in healthy individuals and PL assessment in patients with chronic pain is low and should be considered cautiously [67, 68]. Results should be replicated using a current intensity for which blinding is universally accepted as possible. Therefore, the effect size of our meta-analysis of STh and PTh measurements may be affected by incomplete blinding of included studies.

Potential bias in the review process

Substantial variation exists between the included studies of c-tDCS in both the healthy and patient groups. Studies varied in terms of the stimulation parameters used, gender and range of ages of participants, and the number of treatment sessions, all of which increased the heterogeneity of subgroups. This heterogeneity was reflected in the I^2 statistics for the overall c-tDCS meta-analyses. In addition, several studies in the healthy group used heat and others used cold stimuli for assessment of STh/PTh, and these different stimuli activate different pathways, so this can be considered as a source of methodological bias in final responses.

Publication bias

Six funnel plots were generated to examine the result of each meta-analysis for evidence of publication bias. Three plots show the bias of included studies comparing after effects of c-tDCS on STh (Fig 8A) and PTh (Fig 8B) in healthy individuals, and PL (Fig 8C) in patients with chronic pain. As can be seen, the funnel plots are asymmetrical. In addition, funnel plots for the meta-analyses comparing the effect of active and sham c-tDCS on STh (Fig 8D), PTh (Fig 8E), and PL (Fig 8F) are also asymmetrical. These asymmetrical plots indicate the possibility of publishing studies with significant positive results and being reluctant to publish studies with non-significant results. Regarding the level of heterogeneity of meta-analyses in the current study, the other possibility is the small number of included studies and participants which often result in exaggerated or overestimated true effect size.

Limitations of the study

The findings of current meta-analysis should be interpreted in the context of some limitations. First, the small sample sizes in some included studies were associated with larger effect sizes

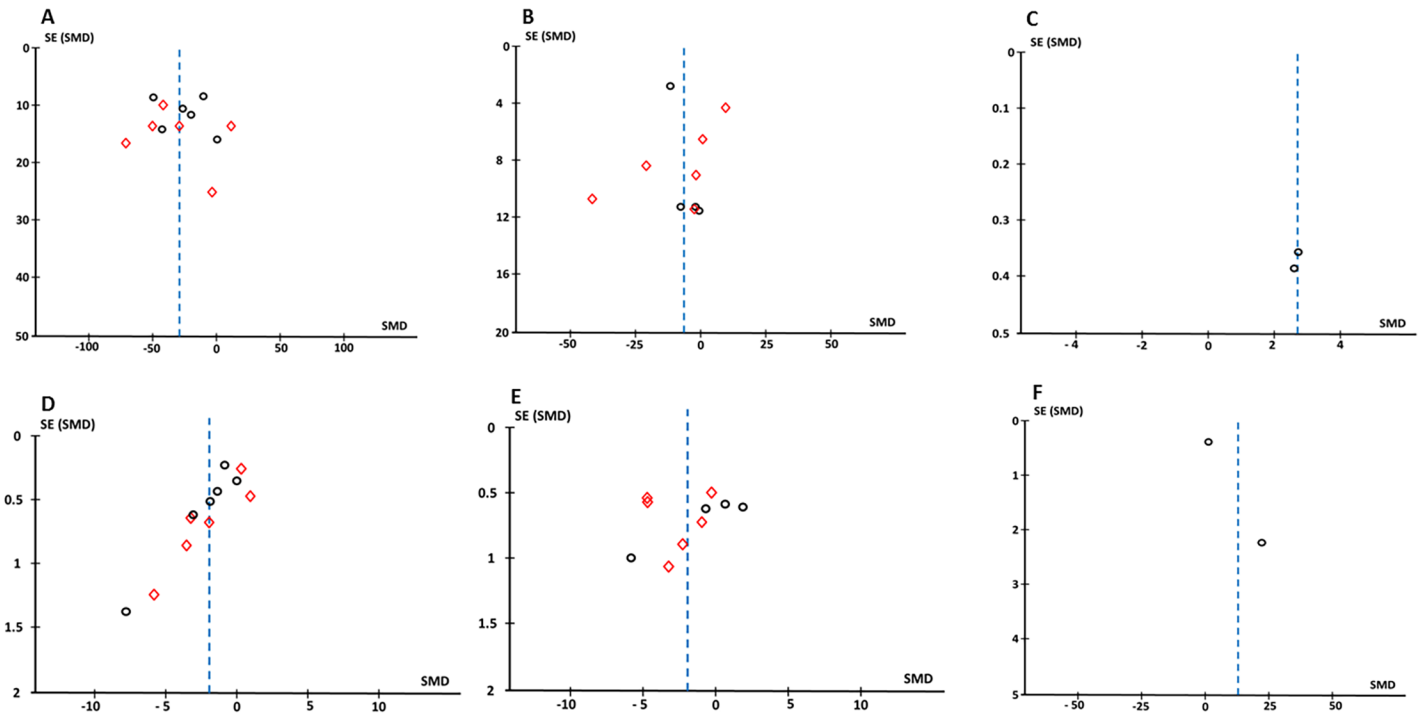

Fig 8. The funnel plots representative of publication bias. After effects of c-tDCS on sensory threshold (A), pain threshold (B), and pain level (C) in patient group. Also the publication bias in studies investigating sham effects of c-tDCS on sensory threshold (D), pain threshold (B), and pain level (E) in patient group are evaluated. In Figures A, B, D, and E, circles indicates S1 subgroup analysis and squares show M1 subgroup analyses.

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that might have affected the overall results and statistical significance. Second, Because of the scarcity of studies on the effect of c-tDCS on pain, it was impossible to analyses subgroups with fixed stimulation parameters. Finally, it is worth noting that our study considered only the immediate after effects of c-tDCS, not long-lasting effects. Due to the limited number of included studies and mismatched measurement time-points, it was impossible to evaluate long-lasting after effects of c-tDCS based on the site of stimulation.

Areas for future research

The results of our study concern the immediate after effects of a single c-tDCS session. It is possible that longer applications or multiple applications could significantly increase PTh or decrease PL [69, 70]. Given the small number of clinical trials that have assessed the efficacy of c-tDCS to reduce pain level in patients with chronic pain, investigation of the effects of c-tDCS in patients with different pathologies would be useful. C-tDCS has short-term analgesic effects [59] so can be used in acute cases; as a result, opportunities exist for more studies of c-tDCS during its use to reduce acute and chronic pain in health centers.

Supporting Information

S1 PRISMA Checklist. PRISMA Checklist.
(DOC)

Author Contributions

Conceived and designed the experiments: BV SJ MZ. Performed the experiments: BV SJ. Analyzed the data: BV. Contributed reagents/materials/analysis tools: BV. Wrote the paper: BV MZ SJ. Searched the databases: BV MZ SJ.

References

1. Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol. 2000; 527 Pt 3: 633–639. PMID: 10990547
2. Batsikadze G, Moliadze V, Paulus W, Kuo MF, Nitsche MA. Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. J Physiol. 2013; 591: 1987–2000. doi: 10.1113/jphysiol.2012.249730 PMID: 23339180
3. Fregni F, Boggio PS, Lima MC, Ferreira MJ, Wagner T, Rigonatti SP, et al. A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury. Pain. 2006; 122: 197–209. PMID: 16564618
4. Fregni F, Freedman S, Pascual-Leone A. Recent advances in the treatment of chronic pain with non-invasive brain stimulation techniques. Lancet Neurol. 2007; 6: 188–191. PMID: 17239806
5. Roizenblatt S, Fregni F, Gimenez R, Wetzel T, Rigonatti SP, Tufik S, et al. Site-specific effects of transcranial direct current stimulation on sleep and pain in fibromyalgia: a randomized, sham-controlled study. Pain Pract. 2007; 7: 297–306. PMID: 17986164
6. Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain. 2009; 10: 895–926. doi: 10.1016/j.jpain.2009.06.012 PMID: 19712899
7. Kulkarni B, Bentley DE, Elliott R, Youell P, Watson A, Derbyshire SW, et al. Attention to pain localization and unpleasantness discriminates the functions of the medial and lateral pain systems. Eur J Neurosci. 2005; 21: 3133–3142. PMID: 15978022
8. Peyron R, Laurent B, Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol Clin. 2000; 30: 263–288. PMID: 11126640
9. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain. 2005; 9: 463–484. PMID: 15979027
10. Bingel U, Lorenz J, Glauche V, Knab R, Glascher J, Weiller C, et al. Somatotopic organization of human somatosensory cortices for pain: a single trial fMRI study. Neuroimage. 2004; 23: 224–232. PMID: 15325369
11. Laurent B, Peyron R, Garcia Larrea L, Mauguiere F. [Positron emission tomography to study central pain integration]. Rev Neurol. 2000; 156: 341–351. PMID: 10795010
12. Mouraux A, Diukova A, Lee MC, Wise RG, Iannetti GD. A multisensory investigation of the functional significance of the "pain matrix". Neuroimage. 2011; 54: 2237–2249. doi: 10.1016/j.neuroimage.2010.09.084 PMID: 20932917
13. Ostrowsky K, Magnin M, Ryvlin P, Isnard J, Guenot M, Mauguiere F. Representation of pain and somatic sensation in the human insula: a study of responses to direct electrical cortical stimulation. Cereb Cortex. 2002; 12: 376–385. PMID: 11884353
14. Kanda M, Nagamine T, Ikeda A, Ohara S, Kunieda T, Fujiwara N, et al. Primary somatosensory cortex is actively involved in pain processing in human. Brain Res. 2000; 853: 282–289. PMID: 10640625
15. Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C, Buchel C. Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. Brain. 2002; 125: 1326–1336. PMID: 12023321
16. Garcia-Larrea L, Peyron R, Mertens P, Gregoire MC, Lavenne F, Le Bars D, et al. Electrical stimulation of motor cortex for pain control: a combined PET-scan and electrophysiological study. Pain. 1999; 83: 259–273. PMID: 10534598
17. Soler MD, Kumru H, Pelayo R, Vidal J, Tormos JM, Fregni F, et al. Effectiveness of transcranial direct current stimulation and visual illusion on neuropathic pain in spinal cord injury. Brain. 2010; 133: 2565–2577. doi: 10.1093/brain/awq184 PMID: 20685806
18. Reidler JS, Mendonca ME, Santana MB, Wang X, Lenkinski R, Motta AF, et al. Effects of motor cortex modulation and descending inhibitory systems on pain thresholds in healthy subjects. J Pain. 2012; 13: 450–458. doi: 10.1016/j.jpain.2012.01.005 PMID: 22515945
19. McCarthy G, Luby M, Gore J, Goldman-Rakic P. Infrequent events transiently activate human prefrontal and parietal cortex as measured by functional MRI. J Neurophysiol. 1997; 77: 1630–1634. PMID: 9084626

20. Bantick SJ, Wise RG, Ploghaus A, Clare S, Smith SM, Tracey I. Imaging how attention modulates pain in humans using functional MRI. Brain. 2002; 125: 310–319. PMID: 11844731

21. Iannetti GD, Mouraux A. From the neuromatrix to the pain matrix (and back). Exp Brain Res. 2010; 205: 1–12. doi: 10.1007/s00221-010-2340-1 PMID: 20607220

22. Hoshi E. Functional specialization within the dorsolateral prefrontal cortex: A review of anatomical and physiological studies of non-human primates. Neuroscience Research. 2006; 54: 73–84. PMID: 16310877

23. Sawamoto N, Honda M, Okada T, Hanakawa T, Kanda M, Fukuyama H, et al. Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/posterior insula: an event-related functional magnetic resonance imaging study. J Neurosci. 2000; 20: 7438–7445. PMID: 11007903

24. Ploghaus A, Tracey I, Gati JS, Clare S, Menon RS, Matthews PM, et al. Dissociating pain from its anticipation in the human brain. Science. 1999; 284: 1979–1981. PMID: 10373114

25. Fernandez-de-Las-Penas C, Madeleine P, Martinez-Perez A, Arendt-Nielsen L, Jimenez-Garcia R, Pareja JA. Pressure pain sensitivity topographical maps reveal bilateral hyperalgesia of the hands in patients with unilateral carpal tunnel syndrome. Arthritis Care Res (Hoboken). 2010; 62: 1055–1064. doi: 10.1002/acr.20189 PMID: 20235189

26. Giesecke T, Gracely RH, Williams DA, Geisser ME, Petzke FW, Clauw DJ. The relationship between depression, clinical pain, and experimental pain in a chronic pain cohort. Arthritis Rheum. 2005; 52: 1577–1584. PMID: 15880832

27. Mendonca ME, Santana MB, Baptista AF, Datta A, Bikson M, Fregni F, et al. Transcranial DC stimulation in fibromyalgia: optimized cortical target supported by high-resolution computational models. J Pain. 2011; 12: 610–617. doi: 10.1016/j.jpain.2010.12.015 PMID: 21497140

28. Antal A, Kriener N, Lang N, Boros K, Paulus W. Cathodal transcranial direct current stimulation of the visual cortex in the prophylactic treatment of migraine. Cephalalgia. 2011; 31: 820–828. doi: 10.1177/0333102411399349 PMID: 21398419

29. Bachmann CG, Muschinsky S, Nitsche MA, Rolke R, Magerl W, Treede RD, et al. Transcranial direct current stimulation of the motor cortex induces distinct changes in thermal and mechanical sensory percepts. Clin Neurophysiol. 2010; 121: 2083–2089. doi: 10.1016/j.clinph.2010.05.005 PMID: 20570558

30. Grundmann L, Rolke R, Nitsche MA, Pavlakovic G, Happe S, Treede RD, et al. Effects of transcranial direct current stimulation of the primary sensory cortex on somatosensory perception. Brain Stimul. 2011; 4: 253–260. doi: 10.1016/j.brs.2010.12.002 PMID: 22032740

31. Csifcsak G, Antal A, Hillers F, Levold M, Bachmann CG, Happe S, et al. Modulatory effects of transcranial direct current stimulation on laser-evoked potentials. Pain Med. 2009; 10: 122–132. doi: 10.1111/j.1526-4637.2008.00508.x PMID: 18823388

32. Smith BH, Elliott AM, Chambers WA, Smith WC, Hannaford PC, Penny K. The impact of chronic pain in the community. Fam Pract. 2001; 18: 292–299. PMID: 11356737

33. Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions. 2011, Cochrane Collaboration.

34. Moseley AM, Herbert RD, Sherrington C, Maher CG. Evidence for physiotherapy practice: a survey of the Physiotherapy Evidence Database (PEDro). Aust J Physiother. 2002; 48: 43–49. PMID: 11869164

35. Maher CG, Sherrington C, Herbert RD, Moseley AM, Elkins M. Reliability of the PEDro scale for rating quality of randomized controlled trials. Phys Ther. 2003; 83: 713–721. PMID: 12882612

36. de Morton NA. The PEDro scale is a valid measure of the methodological quality of clinical trials: a demographic study. Aust J Physiother. 2009; 55: 129–133. PMID: 19463084

37. Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health. 1998; 52: 377–384. PMID: 9764259

38. Chong PS, Cros DP. Technology literature review: quantitative sensory testing. Muscle Nerve. 2004; 29: 734–747. PMID: 15116380

39. Bolton JE, Wilkinson RC. Responsiveness of pain scales: a comparison of three pain intensity measures in chiropractic patients. J Manipulative Physiol Ther. 1998; 21: 1–7. PMID: 9467094

40. Joseph A. *Plot Digitizer 2.5.1* 2011; Available: http://plotdigitizer.sourceforge.net/.

41. Bax L, Yu LM, Ikeda N, Moons KG. A systematic comparison of software dedicated to meta-analysis of causal studies: BMC Med Res Methodol. 2007 Sep 10; 7:40.; 2007. PMID: 17845719

42. Antal A, Paulus W. Transcranial direct current stimulation and visual perception. Perception. 2008; 37: 367–374. PMID: 18491714

43. Rogalewski A, Breitenstein C, Nitsche MA, Paulus W, Knecht S. Transcranial direct current stimulation disrupts tactile perception. Eur J Neurosci. 2004; 20: 313–316. PMID: 15245504

44. Terney D, Bergmann I, Poreisz C, Chaieb L, Boros K, Nitsche MA, et al. Pergolide increases the efficacy of cathodal direct current stimulation to reduce the amplitude of laser-evoked potentials in humans. J Pain Symptom Manage. 2008; 36: 79–91. doi: 10.1016/j.jpainsymman.2007.08.014 PMID: 18358692

45. Antal A, Brepohl N, Poreisz C, Boros K, Csifcsak G, Paulus W. Transcranial direct current stimulation over somatosensory cortex decreases experimentally induced acute pain perception. Clin J Pain. 2008; 24: 56–63. doi: 10.1097/AJP.0b013e318157233b PMID: 18180638

46. Hansen N, Obermann M, Poitz F, Holle D, Diener HC, Antal A, et al. Modulation of human trigeminal and extracranial nociceptive processing by transcranial direct current stimulation of the motor cortex. Cephalalgia. 2011; 31: 661–670. doi: 10.1177/0333102410390394 PMID: 21233281

47. Khedr EM, Kotb H, Kamel NF, Ahmed MA, Sadek R, Rothwell JC. Longlasting antalgic effects of daily sessions of repetitive transcranial magnetic stimulation in central and peripheral neuropathic pain. J Neurol Neurosurg Psychiatry. 2005; 76: 833–838. PMID: 15897507

48. Fuller GN, Guiloff RJ. The conduction velocities of peripheral nerve fibres conveying sensations of warming and cooling: J Neurol Neurosurg Psychiatry. 1989 Jun; 52(6):810–2.; 1989. PMID: 2746283

49. Summers J, Johnson S, Pridmore S, Oberoi G. Changes to cold detection and pain thresholds following low and high frequency transcranial magnetic stimulation of the motor cortex. Neurosci Lett. 2004; 368: 197–200. PMID: 15351448

50. Oliviero A, Esteban MR, de la Cruz FS, Cabredo LF, Di Lazzaro V. Short-lasting impairment of temperature perception by high frequency rTMS of the sensorimotor cortex. Clin Neurophysiol. 2005; 116: 1072–1076. PMID: 15826847

51. Stollberg J, Fraser SE. Acetylcholine receptors and concanavalin A-binding sites on cultured Xenopus muscle cells: electrophoresis, diffusion, and aggregation. J Cell Biol. 1988; 107: 1397–1408. PMID: 3170634

52. Debanne D, Daoudal G, Sourdet V, Russier M. Brain plasticity and ion channels. J Physiol Paris. 2003; 97: 403–414. PMID: 15242652

53. Loeb GE, Gans C. Electromyography for experimentalists: University of Chicago Press; 1986.

54. Chesler M. Regulation and modulation of pH in the brain. Physiol Rev. 2003; 83: 1183–1221. PMID: 14506304

55. Islam N, Aftabuddin M, Moriwaki A, Hattori Y, Hori Y. Increase in the calcium level following anodal polarization in the rat brain. Brain Res. 1995; 684: 206–208. PMID: 7583224

56. O'Connell NE, Wand BM, Marston L, Spencer S, Desouza LH. Non-invasive brain stimulation techniques for chronic pain. A report of a Cochrane systematic review and meta-analysis. Eur J Phys Rehabil Med. 2011; 47: 309–326. PMID: 21494222

57. Luedtke K, Rushton A, Wright C, Geiss B, Juergens TP, May A. Transcranial direct current stimulation for the reduction of clinical and experimentally induced pain: a systematic review and meta-analysis. Clin J Pain. 2012; 28: 452–461. doi: 10.1097/AJP.0b013e31823853e3 PMID: 22569218

58. Vaseghi B, Zoghi M, Jaberzadeh S. Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study. Clin Neurophysiol. 2014.

59. Ardolino G, Bossi B, Barbieri S, Priori A. Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. J Physiol. 2005; 568: 653–663. PMID: 16037080

60. Nitsche MA, Jaussi W, Liebetanz D, Lang N, Tergau F, Paulus W. Consolidation of human motor cortical neuroplasticity by D-cycloserine. Neuropsychopharmacology. 2004; 29: 1573–1578. PMID: 15199378

61. Tang CM, Dichter M, Morad M. Modulation of the N-methyl-D-aspartate channel by extracellular H+. Proc Natl Acad Sci U S A. 1990; 87: 6445–6449. PMID: 1696732

62. Nitsche MA, Liebetanz D, Schlitterlau A, Henschke U, Fricke K, Frommann K, et al. GABAergic modulation of DC stimulation-induced motor cortex excitability shifts in humans. Eur J Neurosci. 2004; 19: 2720–2726. PMID: 15147306

63. Liebetanz D, Nitsche MA, Tergau F, Paulus W. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain. 2002; 125: 2238–2247. PMID: 12244081

64. Netzer R, Pflimlin P, Trube G. Dextromethorphan blocks N-methyl-D-aspartate-induced currents and voltage-operated inward currents in cultured cortical neurons. Eur J Pharmacol. 1993; 238: 209–216. PMID: 7691620

65. Kinkelin I, Brocker EB, Koltzenburg M, Carlton SM. Localization of ionotropic glutamate receptors in peripheral axons of human skin. Neurosci Lett. 2000; 283: 149–152. PMID: 10739897

66. Wood L, Egger M, Gluud LL, Schulz KF, Juni P, Altman DG, et al. Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study. Bmj. 2008; 336: 601–605. doi: 10.1136/bmj.39465.451748.AD PMID: 18316340

67. Russo R, Wallace D, Fitzgerald PB, Cooper NR. Perception of comfort during active and sham transcranial direct current stimulation: a double blind study. Brain Stimul. 2013; 6: 946–951. doi: 10.1016/j.brs.2013.05.009 PMID: 23835166

68. Palm U, Reisinger E, Keeser D, Kuo MF, Pogarell O, Leicht G, et al. Evaluation of sham transcranial direct current stimulation for randomized, placebo-controlled clinical trials. Brain Stimul. 2013; 6: 690–695. doi: 10.1016/j.brs.2013.01.005 PMID: 23415938

69. Nitsche MA, Seeber A, Frommann K, Klein CC, Rochford C, Nitsche MS, et al. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. J Physiol. 2005; 568: 291–303. PMID: 16002441

70. Furubayashi T, Terao Y, Arai N, Okabe S, Mochizuki H, Hanajima R, et al. Short and long duration transcranial direct current stimulation (tDCS) over the human hand motor area. Exp Brain Res. 2008; 185: 279–286. PMID: 17940759

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.

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	Supervisory input on study design, Guidance in the framing of the manuscript, Review and provision of feedback on final manuscript draft	5 %

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

Candidate's Signature: [redacted] Date: 15-Sep- 2015

Signature: [redacted] Date: 15-Sep-2015

Signature: [redacted] Date: 15-Sep-2015

Preamble to Chapter 4

Any application of tDCS involves measurement of changes before and after interventions. Therefore, in order to make sure that changes following interventions are not due to systematic errors and methodological inconsistencies, a reliability study is conducted. Chapter 4 examines the effects of inter-pulse intervals of TMS pulses on the size of MEPs and their effects on intra and inter-session reliabilities.

Chapter 4: Inter-Pulse interval affects the size of single-pulse TMS induced motor evoked potentials: a reliability study

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

The setup system used in this study and consent form is provided in Appendix.

Inter-pulse Interval Affects the Size of Single-pulse TMS-induced Motor Evoked Potentials: a Reliability Study

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ABSTRACT

Introduction: Measuring the size of motor evoked potentials (MEPs) induced by transcranial magnetic stimulation (TMS) is an investigational technique to show the level of corticospinal excitability; however, some of the fundamental methodological aspects of TMS (such as the effects of inter-pulse intervals (IPI) on MEP size) are not fully understood, this issue raises concerns about the reliability of MEPs, especially in pre-test post-test studies.

Methods: MEP size at short and long IPIs was assessed during two separate sessions. Inter- and intra-session reliability of MEP size also was assessed at both short and long IPIs.

Results: The results indicated that long IPIs induced larger MEPs ($p < 0.05$) across all time points. The intra-class correlation coefficient (ICC) indicated high intra- and inter-session reliability for short (0.87 to 0.96) and long (0.80 to 0.97) IPIs respectively. The amplitude of MEPs also had high intersession reliability for short (ICC = 0.87) and long (ICC = 0.80) IPIs.

Conclusion: This study provides evidence that the length of IPIs determines the size of MEPs. As a result, it is recommended to add the length of IPI to the international checklist of considerations for TMS application.

1. Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive, safe and painless technique for assessment of corticospinal excitability (CSE) in both healthy individuals and patients with neurological conditions. One of the major advantages of TMS is the ability of the magnetic pulses to pass unchanged through the scalp in order to induce an electric field within the conductive brain tissues (Wassermann 2002). When applied over the primary motor cortex (M1) of a target muscle, it induces a response known as the motor evoked potential (MEP). MEPs can be recorded using surface electromyography (EMG) electrodes placed over the muscle of interest (Wassermann 2002; Malcolm et al. 2006). Two characteristics of recorded MEPs are amplitude and latency; the amplitude provides valuable information about the excitability of corticospinal pathways. TMS-induced MEPs have been used as a reliable outcome measure of CSE changes in a range of research protocols (Nitsche and Paulus 2000a). Larger MEP amplitudes indicate higher CSE and smaller amplitudes indicate lower CSE (Nitsche and Paulus 2000a; Di Lazzaro et al., 2004).

While the latency of MEP is relatively stable, the size of these responses is highly changeable (Kiers et al., 1993). Many factors can affect MEP size. Technical factors include coil type (Fleming et al. 2012), placement (Ngomo et al., 2012), orientation (Thomson et al., 2013) and TMS intensity (Fisher et al., 2002). Physiological factors include muscle fatigue (Milanovic et al., 2013), background muscle activity (Ngomo et al., 2012), arousal, attention, emotional context, and afferent feedback of different parts of the brain (such as the supplementary motor area or dorsal premotor cortex) (Schmidt et al., 2009).

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Although TMS has been employed as an investigational technique for more than two decades, some of its fundamental methodological principles are not fully understood. For instance, TMS inter-pulse interval (IPI) may have profound effects on MEP size. Even though, work in our laboratory conducted over the past 5 years suggests induction of larger MEPs with longer IPIs. To the best of our knowledge, this relationship has not been reported in the literature up to date which may be associated with a net drop in haemoglobin levels following each stimulation, this may reduce the neural activation in stimulated area for about 8-10 seconds and may affect the size of MEPs (Thomson et al., 2012b).

An important aspect of any clinical or experimental assessment tool and method is its test-retest reliability (Schmidt et al. 2009). Reliability refers to the consistency of measurements; it tests the stability of scores over time and the degree to which repeated measurements provide similar results (de Vet et al. 2006). To be an effective assessment tool, the size of TMS-induced MEPs must be reliable. A reliable measurement of MEPs guarantees stable amplitude size over time in the absence of an intervention (Lexell and Downham 2005; Christie et al. 2007). If the IPIs of TMS pulses affect the size of MEPs, then we should avoid using different IPIs in pre-test post-test study designs. For example, if we use lower TMS IPIs (e.g., four seconds) during baseline measurements we must use identical IPIs for post intervention measurements. If we fail to do so, IPI length becomes a confounding variable and contaminates the intervention effects.

The primary aim of this study was to investigate the effects of shorter (four-second) and longer (10-second) IPIs on the size and reliability of the induced MEPs. We hypothesised that longer IPIs induce larger MEPs. We also hypothesised that longer IPIs induce more reliable MEPs.

2. Methods

Twelve healthy volunteers (six women and six men) with a mean age of 32.27 (SD=7.2 years) a mean weight of 70.9 (SD= 11.4 kg) and mean height of 173.8 (SD= 7.3 cm) were tested in two sessions separated by at least 48 hours. All participants were consistent right-handers according to the 10-item version of the Edinburgh Handedness Inventory (mean laterality index=100) (Oldfield 1971) with no neurological, psychological, or endocrinological problems. None were taking any medication. Prior to the experiments, all participants completed the Adult Safety Screening Questionnaire (Keel et al. 2001) to determine their safety for TMS application. Participants gave informed consent according to the declaration of Helsinki. Monash University's Human Research Ethics Committee approved the experimental procedure. Each subject was tested at the same time of the day to avoid diurnal variation.

EMG recording

Participants were seated upright in an adjustable podiatry chair with head and neck supported by a headrest and the right forearm on the armrest with the wrist joint in a pronated and neutral position. 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). MEPs were recorded from the first dorsal interosseous (FDI) muscle at rest, using pre-gelled self-adhesive bipolar Ag/AgCl disposable surface electrodes with an inter-electrode distance of 2 cm (measured from the centres of the electrodes). The location of the FDI muscle was determined based on anatomical landmarks and observations of muscle contraction in the testing position (index finger abduction) (Kendall et al. 1983). The accuracy of EMG electrode placement was verified by asking the subject to maximally contract the muscle while the investigator monitored online EMG activity. The ground electrode was placed ipsilaterally on the styloid process of the ulnar bone (Oh 2003) and 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, AD Instruments, Australia) via a laboratory analogue-digital interface (The PowerLab 8/30, ADInstruments, Australia) for later off-line analysis.

Procedure

All individuals participated in two experimental sessions. The protocol in session 1 enabled us to study the within-session reliability of MEPs (intra-session reliability). The CSE of the FDI's representation in M1 was assessed before and after 20 minutes of no intervention. Follow-up assessments were carried out at four consecutive time points (T0, T20, T40, T60), 20 minutes apart. The EMG electrodes were left in place and the TMS coil was removed while the subjects rested between the pre and post measurements, with no hand or wrist movements allowed.

Each participant's second session of testing was occurred at least 48 hours after the first one. This session was shorter and involved recording of MEPs at a single time point (Tday 2). Comparison of these data with the T0 from ses-

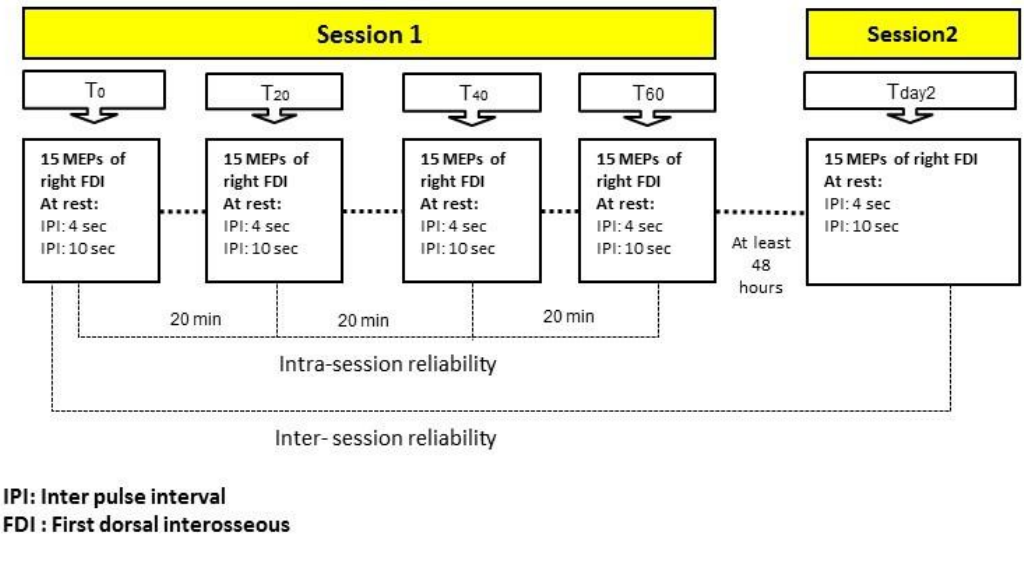

Figure 1. Experimental set-up; TMS was delivered on first dorsal interosseus (FDI) hotspot and 15 MEPs were recorded during a two-session experiments with at least 48 hours separation. Randomization of the short and long IPIs' order were applied at both sessions.

sion 1 enabled us to study the inter-session reliability of the MEP sizes (Fig.1). Randomization of the short and long IPIs' order were applied at both sessions.

CSE measurement by TMS

Single-pulse magnetic stimuli were delivered using a Magstim 2002 (Magstim, UK) stimulator with a flat 70 mm figure-of-eight magnetic coil. Using the international 10-20 system, the vertex (Cz) point was measured and marked for the use as a reference. The magnetic coil was placed over the left M1 area, contralateral to the target muscle. The coil was set at 45˚ to the midline and tangential to the scalp, such that the induced current flowed in a posterior-anterior direction (Rossini and Rossi 1998; Schmidt et al. 2009). To determine the optimal site of stimulation (hotspot), the coil was moved around the M1 of the target muscle to find the area with the largest MEP responses.

After localizing the optimal stimulation site, the coil position was marked on the scalp to ensure consistency in placement throughout the testing session. The full hotspot identification procedure was performed in each session. Resting motor threshold (RMT) was defined as the minimal stimulus intensity that evoked five MEPs in the series of 10 tests with an amplitude of at least 50 μV from the FDI hot spot (Devanne et al. 2006). The RMT for each subject was determined by increasing and decreasing stimulus intensity in 1-2% intervals until MEPs of appropriate size were elicited (Rothwell et al. 1999). Fifteen stimuli were delivered (Bastani and Jaberzadeh 2012) to assess CSE at each time point, with the stimulus intensity set at 120% of each individual's RMT. The stimulus intensity remained constant throughout the study session for each subject. The excitability of M1 related to the FDI muscle was tested with both short and long IPIs randomly in two separated blocks of 15 MEPs (Bastani and Jaberzadeh 2012). Short IPI was defined as a four-second rest between each pulse and long IPI was defined as a ten-second rest.

Data management and data analysis

The average of 15 MEPs at each time point (T0, T20, T40, T60, and Tday2) was calculated for both short and long IPIs. Data analysis was carried out in two phases. In phase A, a two-way repeated measure ANOVA was used to study the effects of IPI on the size of TMS evoked MEPs. The first within-subject independent factor was IPI (two levels). The second independent factor was time points (five 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 where performed when sphericity was lacking. Post hoc comparisons were performed using the least significance difference (Bonferoni) adjustment for multiple comparisons when appropriate. In phase B, the within- and between-session reliability of elicited MEP sizes for both IPIs were calculated using Intra Class Correlation (ICC) (Pourtney and Watkins 2000). To assess the

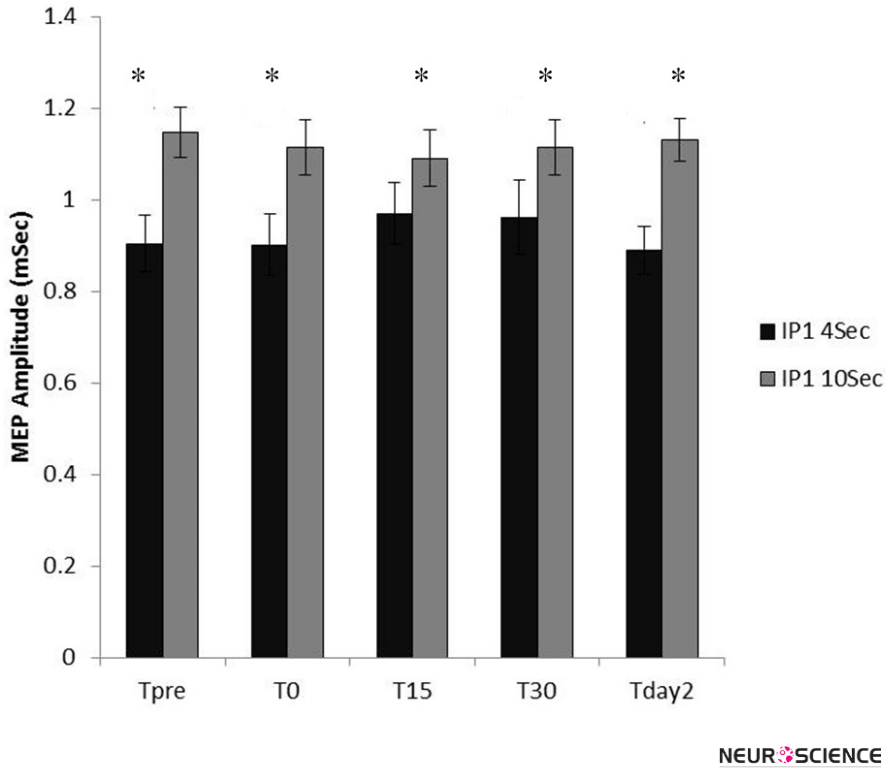

Figure 2. Comparison of short and Long IPIs in each time point of measurement. * indicates statistically significant (P< 0.05) and each column represents mean percentage change ± SEM.

agreement between the repeated measurements, a one-way ANOVA was carried out for each interval. The reliability coefficient ranges from 0 to 1, with values closer to 1 representing stronger reliability. Although the interpretation of ICCs is subjective, it has been suggested that coefficients below 0.50 represent poor reliability, those from 0.50 to 0.75 correspond to moderate reliability, and values above 0.75 indicate high reliability (Pourtney and Watkins 2000).

3. Results

3.1. Comparison of short and long IPIs

Long IPIs (10 seconds) yielded significantly greater mean MEP amplitude than short IPIs (four seconds) (Fig. 2). Table 1 depicts the MEP amplitudes; the differences between short and long IPIs were significant at all time-points.

3.2. Reliability of TMS-induced MEPs

Intra-session reliability

The RMT and consequent stimulus intensity (120% RMT) for the FDI muscle were 43.2% (SD=9.87) and 51.78% (SD= 12.38) of stimulator output respectively. MEP amplitude changed minimally: repetition of the measurements by the same examiner every 20 minutes after the first test revealed no significant differences in group mean values. Repeated measures ANOVA revealed no significant time effect on any of the MEP measurements. ICCs ranged from 0.80 to 0.91 for IPIs of four seconds and 0.79 to 0.96 for IPIs of 10 seconds. MEP amplitudes showed high within-session reliability for both four and 10-second IPIs (Table 1).

Inter-session reliability

The averaged RMTs and consequent stimulus intensities for short and long IPIs were 37% (SD= 8.94) and 44.4% (SD= 12.6) of stimulus output respectively. Comparing the mean MEP amplitude after applying long and short IPIs represented more consistency in MEP amplitudes after applying TMS with long IPI. Moreover, repetition of the measurements by the same examiner in two different

Table 1. Comparison of short and long IPIs at five measurement points by two-tailed, paired t-test

	T_0 (IPI: 4-10 Sec)	T_{20} (IPI: 4-10 Sec)	T_{40} (IPI: 4-10 Sec)	T_{60} (IPI: 4-10 Sec)	T_{day2} (IPI: 4-10 Sec)
P-value	0.000	0.000	0.03	0.000	0.003
T(11)	-5.58	-6.52	-2.48	-4.92	-3.78

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sessions held an average of 48 hours apart did not reveal any significant differences in mean MEP amplitude values. A paired T-test comparing the means of the size of MEPs between the two sessions showed no significant differences for the FDI muscle. According to the ICC, all MEP amplitude measures were highly reliable for both short and long IPIs. Despite the ICC values, the standard errors of measurement (SEM) values were relatively low, suggesting the measurements were precise (Table 2).

4. Discussion

Comparison of short and long IPIs

We hypothesised that an IPI of 10 seconds would induce larger MEPs than an IPI of four seconds. This hypothesis is strongly supported by the results of the present study. While no direct similar studies exist, some studies in literature support our finding. In a near infra-red spectroscopy (NIRS) study, the level of oxyhaemoglobin (HbO) decreased following each single-pulse TMS due to the contraction of vessels in the stimulated area, and a period of 8-10 sec was needed in order to return to original state (Thomson et al. 2012b). In a similar study, Thomson et al. (2011) showed that each TMS pulse stimulates smooth muscles in the walls of blood vessels and reduces blood flow for 8–10 seconds (Thomson et al., 2011). As a result, it can be concluded that TMS pulses can change the hemodynamic statute of stimulated areas (Thomson et al., 2012a).

The finding of the current study is supported by those of rTMS studies in which the reduction of HbO led to elicitation of smaller MEPs (Fitzgerald et al. 2006; Thomson et al. 2012b). Significant reduction in HbO, which was observed with 130% rTMS, suggested that normal hemodynamic homeostatic mechanisms might be disrupted by TMS pulses. HbO concentration began to increase about four seconds after onset of TMS pulses and finally returned to normal levels after 15 seconds (Mochizuki et al. 2006). It seems that vasoconstriction resulting from suprathreshold TMS disrupts constant perfusion in stimulated area (Mochizuki et al. 2006; Vernieri et al. 2009) Non-U S Gov't </work-type> <urls> </urls> </record> </Cite> </EndNote>. These findings show that neurons in the stimulated area need at least 10 seconds to return to optimal state with maximum delivery of oxygen for peak performance. This mechanism easily explains smaller MEP sizes with a shorter IPI: when using an IPI of four seconds, the stimulus applies while the blood circulation in the stimulated area is not in an optimal state. Therefore, due to vasoconstriction after each TMS stimulus (Rollnik et al. 2002; Speer et al. 2003), the ability of the hemodynamic system is diminished and neurons cannot mount a proper response.

Given that a direct relationship exists between regional cerebral blood flow (rCBF) and excitatory synaptic activity (Mochizuki et al. 2004), the other probable mechanism is that vasoconstriction following TMS stimuli decreases the level of excitatory activity in the stimulated area. This may lead to decreased MEP sizes. It is likely that larger IPIs provide enough time for rCBF and excitatory circuits to return to the normal levels associated with larger MEPs.

Table 2. Comparison of intra- and inter-session reliability of short and long IPIs by Inter Class Correlation (ICC) and Standard Error of Measurement (SEM)

IPI	Intra-session reliability							Inter-session reliability	
	ICCs						SEM (%)	ICCs	SEM (%)
	T_0-T_{20}	T_0-T_{40}	T_0-T_{60}	T_{20}-T_{40}	T_{20}-T_{60}	T_0-T_{20}-T_{40}-T_{60}		T_0-T_{day2}	
4 Sec	0.91	0.95	0.96	0.96	0.94	0.96	1.2	0.87	1.3
10 Sec	0.86	0.83	0.86	0.93	0.97	0.95	1.7	0.80	2.2

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Reliability of TMS-induced MEPs at larger IPIs

The shape, size, orientation of the TMS coil, and direction of the induced current flow may affect the size of MEPs. All these factors were similar in all measurement points (Hallett and Chokroverty 2005). Moreover, the other factor that could theoretically affect MEP amplitudes' reliability is the use of a neuro navigation system in eliciting MEPs. However, two recent studies found no decrease in the variability (Jung et al. 2010) and no further improve in reliability (Fleming et al. 2012) of MEPs with TMS navigated systems. In the current study, we used a conventional TMS assessment technique without a navigation system, and our results were in agreement with previous studies demonstrating high reliability in TMS mapping parameters with smaller numbers of MEPs, both with (Ngomo et al. 2012) and without (Christie et al. 2007) the use of a navigation system.

Intra-session reliability

The agreement and high value of ICCs for measurements of pre- and post-MEPs with both short and long IPIs in FDI muscles indicate high within-session reliability. Although the intra-session reliability of MEP size at different IPIs has not been investigated before, our findings of intra-session reliability are in agreement with studies reporting high levels of reliability of MEP amplitude derived from the abductor digiti minimi (ICC of 0.97) (Christie et al. 2007), erector carpi radialis (ICC of 0.93) and FDI (ICC of 0.97) (Bastani and Jaberzadeh 2012). We also hypothesised that longer IPIs have higher level of ICCs, but the results of current study did not support this hypothesis. Our result indicates that both short and long IPIs have high level of reliability. These results support other studies in which a high reliability of MEP amplitude was detected in upper arm muscles (Kamen 2004; Christie et al. 2007).

Inter-session reliability

Inter-session reliability of MEPs in FDI was high for both short and long IPIs. Although no previous researchers have investigated the effect of different IPIs on inter-session MEP reliability, the ICCs obtained in our experiment are larger than those reported by Kamen (Kamen 2004) for the FDI muscle (0.60–0.81) and Christie et al. (Christie et al. 2007) for the abductor digiti minimi (ADM) muscle (0.65–0.83). In addition, the range of ICCs in our study was similar to those reported by Bastani et al. (2012) with the same number of TMS stimuli (15 per time point) for the FDI (0.93–0.99) and Extensor carpi radialis (ECR) (0.97–0.99) muscles. Our results demonstrate that MEP amplitude remains constant with both short and long IPIs in healthy subjects, even with an average of 48 hours between testing sessions.

Our finding has substantial implications for TMS application. It is recommended to add IPI length to the international checklist of considerations for TMS application (Chipchase et al. 2012) as an MEP modulatory criterion. Reporting the IPI may be important because our results suggest that length of IPI is a strong confounding variable in TMS studies.

The size of TMS-induced MEPs has been used as an index of CSE in neurophysiological and neurological studies (Nitsche and Paulus 2000b). IPI length was not reported in the cited studies or in many other studies which used TMS in assessment of CSE; therefore, the results of these studies should be considered cautiously.

Limitations

It should be noted that, while some studies suggested that neuro-navigational systems provide more robust data compared to detection of hot spots by conventional method, others demonstrated that there is no significant differences between these two methods. Current study utilised the conventional method and therefore interpretation of data should be considered accordingly. Furthermore, since we studied a small group of healthy young participants, findings cannot be extrapolated to older and/or patient groups. We only evaluated one intensity (120% RMT) in a relaxed muscle, so our findings might not hold true for higher or lower intensities or active muscles. In addition, we used only the figure-of-eight magnetic coil to collect data; different results could be obtained using circular coil.

Suggestions for future research

Our work indicates that a 10 second IPI induces larger MEPs than a four-second interval with the same level of reliability. An obvious future research direction is to test a wider range of IPIs with different TMS stimulus intensities. It is also important to test the reported effects while the target muscles are active. Finally, given that rCBF changes with age and due to disease, the effects of different IPIs on elderly people and patients with different health conditions should be investigated.

To our knowledge this is the first study to investigate the effects of short and long IPIs on MEP size. The present study revealed that there is a positive relationship between the length of IPIs and the size of evoked MEPs.

The results also indicate high reliability in the size of MEPs under both short and long IPIs.

References

Bastani A, and Jaberzadeh S. (2012). 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. PLoS One, 7: e47582.

Chipchase L, Schabrun S, Cohen L, Hodges P, Ridding M, Rothwell J, Taylor J and Ziemann U. (2012). A checklist for assessing the methodological quality of studies using transcranial magnetic stimulation to study the motor system: an international consensus study. Clin Neurophysiol, 123: 1698-1704.

Christie A, Fling B, Crews RT, Mulwitz LA, and Kamen G. (2007). Reliability of motor-evoked potentials in the ADM muscle of older adults. J Neurosci Methods, 164: 320-324.

de Vet HCW, Terwee CB, Knol DL and Bouter LM. (2006). When to use agreement versus reliability measures. Journal of Clinical Epidemiology, 59: 1033-1039.

Devanne H, Cassim F, Ethier C, Brizzi L, Thevenon A and Capaday C. (2006). The comparable size and overlapping nature of upper limb distal and proximal muscle representations in the human motor cortex. Eur J Neurosci, 23: 2467-2476.

Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Mazzone P, Insola A, Tonali PA and Rothwell JC. (2004). The physiological basis of transcranial motor cortex stimulation in conscious humans. Clin Neurophysiol, 115: 255-266.

Fisher RJ, Nakamura Y, Bestmann S, Rothwell JC and Bostock H. (2002). Two phases of intracortical inhibition revealed by transcranial magnetic threshold tracking. Exp Brain Res, 143: 240-248.

Fitzgerald PB, Fountain S and Daskalakis ZJ. (2006). A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clin Neurophysiol, 117: 2584-2596.

Fleming MK, Sorinola IO, Newham DJ, Roberts-Lewis SF and Bergmann JH. (2012). The effect of coil type and navigation on the reliability of transcranial magnetic stimulation. IEEE Trans Neural Syst Rehabil Eng, 20: 617-625.

Gilmore KL and Meyers JE. (1983). Using surface electromyography in physiotherapy research. Aust J Physiother, 29: 3-9.

Hallett M and Chokroverty S. (2005). Magnetic stimulation in clinical neurophysiology, Elsevier Health Sciences.

Jung NH, Delvendahl I, Kuhnke NG, Hauschke D, Stolle S and Mall V. (2010). Navigated transcranial magnetic stimulation does not decrease the variability of motor-evoked potentials. Brain Stimul, 3: 87-94.

Kamen G. (2004). Reliability of motor-evoked potentials during resting and active contraction conditions. Med Sci Sports Exerc, 36: 1574-1579.

Keel JC, Smith MJ and Wassermann EM. (Apr. 2001). A safety screening questionnaire for transcranial magnetic stimulation. Clin Neurophysiol, 112(4): 720.

Kendall FP, McCreary EK and Kendall HO. (1983). Muscles, testing and function, Williams & Wilkins Baltimore, MD.

Kiers L, Cros D, Chiappa KH and Fang J. (1993). Variability of motor potentials evoked by transcranial magnetic stimulation. Electroencephalogr Clin Neurophysiol, 89: 415-423.

Lexell JE and Downham DY. (2005). How to assess the reliability of measurements in rehabilitation. Am J Phys Med Rehabil, 84: 719-723.

Malcolm MP, Triggs WJ, Light KE, Shechtman O, Khandekar G and Gonzalez Rothi LJ. (2006). Reliability of motor cortex transcranial magnetic stimulation in four muscle representations. Clin Neurophysiol, 117: 1037-1046.

Milanovic S, Filipovic SR, Radovanovic S, Blesic S, Ilic NV, Kostic VS and Ljubisavljevic MR. (2013). Changes in motor cortex excitability associated with muscle fatigue in patients with Parkinson's disease. Vojnosanit Pregl, 70: 298-303.

Mochizuki H, Terao Y, Okabe S, Furubayashi T, Arai N, Iwata NK, Hanajima R, Kamakura K, Motoyoshi K and Ugawa Y. (2004). Effects of motor cortical stimulation on the excitability of contralateral motor and sensory cortices. Experimental Brain Research, 158: 519-526.

Mochizuki H, Ugawa Y, Terao Y and Sakai KL. (2006). Cortical hemoglobin-concentration changes under the coil induced by single-pulse TMS in humans: a simultaneous recording with near-infrared spectroscopy. Exp Brain Res, 169: 302-310.

Ngomo S, Leonard G, Moffet H and Mercier C. (2012). Comparison of transcranial magnetic stimulation measures obtained at rest and under active conditions and their reliability. J Neurosci Methods, 205: 65-71.

Nitsche and Paulus. (2000a). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol, 3: 633-639.

Nitsche MA and Paulus W. (2000b). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol, 3: 633-639.

Oh SJ. (2003). Clinical electromyography: nerve conduction studies, Lippincott Williams & Wilkins.

Oldfield RC. (1971). The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia, 9: 97-113.

Pourtney L and Watkins M. (2000). Foundations of clinical research: application to practice. Connecticut: Appleton and Lange.

Rollnik JD, Dusterhoft A, Dauper J, Kossev A, Weissenborn K and Dengler R. (2002). Decrease of middle cerebral artery blood flow velocity after low-frequency repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex. Clin Neurophysiol, 113: 951-955.

Rossini PM and Rossi S. (1998). Clinical applications of motor evoked potentials. Electroencephalography and clinical neurophysiology, 106: 180-194.

Rothwell JC, Hallett M, Berardelli A, Eisen A, Rossini P and Paulus W. (1999). Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol Suppl, 52: 97-103.

Schmidt S, Cichy RM, Kraft A, Brocke J, Irlbacher K and Brandt SA. (2009). An initial transient-state and reliable measures of corticospinal excitability in TMS studies. Clin Neurophysiol, 120: 987-993.

Speer AM, Willis MW, Herscovitch P, Daube-Witherspoon M, Shelton JR, Benson BE, Post RM and Wassermann EM. (2003). Intensity-dependent regional cerebral blood flow during 1-Hz repetitive transcranial magnetic stimulation (rTMS) in healthy volunteers studied with H215O positron emission tomography: I. Effects of primary motor cortex rTMS. Biol Psychiatry, 54: 818-825.

Thomson RH, Cleve TJ, Bailey NW, Rogasch NC, Maller JJ, Daskalakis ZJ and Fitzgerald PB. (2013). Blood oxygenation changes modulated by coil orientation during prefrontal transcranial magnetic stimulation. Brain Stimul, 6: 576-581.

Thomson RH, Daskalakis ZJ and Fitzgerald PB. (2011). A near infra-red spectroscopy study of the effects of pre-frontal single and paired pulse transcranial magnetic stimulation. Clin Neurophysiol, 122: 378-382.

Thomson RH, Maller JJ, Daskalakis ZJ and Fitzgerald PB. (2012a). Blood oxygenation changes resulting from trains of low frequency transcranial magnetic stimulation. Cortex, 48: 487-491.

Thomson RH, Maller JJ, Daskalakis ZJ and Fitzgerald PB. (2012b). Blood oxygenation changes resulting from trains of low frequency transcranial magnetic stimulation. Cortex, 48: 487-491.

Vernieri F, Maggio P, Tibuzzi F, Filippi MM, Pasqualetti P, Melgari JM, Altamura C, Palazzo P, Di Giorgio M and Rossini PM. (2009). High frequency repetitive transcranial magnetic stimulation decreases cerebral vasomotor reactivity. Clin Neurophysiol, 120: 1188-1194.

Wassermann EM. (2002). Variation in the response to transcranial magnetic brain stimulation in the general population. Clin Neurophysiol, 113: 1165-1171.

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.

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	Supervisory input on study design, Guidance in the framing of the manuscript, Review and provision of feedback on final manuscript draft	5 %

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

Candidate's Signature:  Date: 15-Sep- 2015

Signature:  Date: 15-Sep-2015

Signature:  Date: 15-Sep-2015

Preamble to chapter 5

Based on the findings in the first systematic review and meta-analysis (Chapter 2), a-tDCS of the M1, S1, and DLPFC increases STh/PTh. In Chapter 5, the effects of a-tDCS of these cortical sites on M1/S1 excitability and STh/PTh are assessed. The current study is the first which collectively evaluates these effects. The data in Chapter 5 provides evidence for functional connectivities between these cortical sites.

Chapter 5: How does anodal transcranial direct current stimulation of the pain neuromatrix affect brain excitability and pain perception? A randomised, double-blind, sham-control study

The format of this chapter is consistent with the Journal of PlosOne.

The setup system used in this study, Ethics approval, TMS safety, Personal health history form, and Edinburg handedness questionnaires and consent form are provided in Appendices 7-13.

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RESEARCH ARTICLE

How Does Anodal Transcranial Direct Current Stimulation of the Pain Neuromatrix Affect Brain Excitability and Pain Perception? A Randomised, Double-Blind, Sham-Control Study

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Abstract

Background

Integration of information between multiple cortical regions of the pain neuromatrix is thought to underpin pain modulation. Although altered processing in the primary motor (M1) and sensory (S1) cortices is implicated in separate studies, the simultaneous changes in and the relationship between these regions are unknown yet. The primary aim was to assess the effects of anodal transcranial direct current stimulation (a-tDCS) over superficial regions of the pain neuromatrix on M1 and S1 excitability. The secondary aim was to investigate how M1 and S1 excitability changes affect sensory (STh) and pain thresholds (PTh).

Methods

Twelve healthy participants received 20 min a-tDCS under five different conditions including a-tDCS of M1, a-tDCS of S1, a-tDCS of DLPFC, sham a-tDCS, and no-tDCS. Excitability of dominant M1 and S1 were measured before, immediately, and 30 minutes after intervention respectively. Moreover, STh and PTh to peripheral electrical and mechanical stimulation were evaluated. All outcome measures were assessed at three time-points of measurement by a blind rater.

Results

A-tDCS of M1 and dorsolateral prefrontal cortex (DLPFC) significantly increased brain excitability in M1 ($p < 0.05$) for at least 30 min. Following application of a-tDCS over the S1, the amplitude of the N20-P25 component of SEPs increased immediately after the stimulation ($p < 0.05$), whilst M1 stimulation decreased it. Compared to baseline values, significant

STh and PTh increase was observed after a-tDCS of all three stimulated areas. Except in M1 stimulation, there was significant PTh difference between a-tDCS and sham tDCS.

Conclusion

a-tDCS of M1 is the best spots to enhance brain excitability than a-tDCS of S1 and DLPFC. Surprisingly, a-tDCS of M1 and S1 has diverse effects on S1 and M1 excitability. A-tDCS of M1, S1, and DLPFC increased STh and PTh levels. Given the placebo effects of a-tDCS of M1 in pain perception, our results should be interpreted with caution, particularly with respect to the behavioural aspects of pain modulation.

Trial Registration

Australian New Zealand Clinical Trials, ACTRN12614000817640, http://www.anzctr.org.au/.

Introduction

Pain is a multidimensional phenomenon with sensory-discriminative, affective-motivational, motor and autonomic components [1–5]. Primary (S1) and secondary (S2) somatosensory cortices, the thalamus, and posterior part of the insula collectively called lateral pain system which are responsible for sensory-discrimination of pain [6]. In contrast, the anterior cingulate cortex (ACC) and anterior part of the insula have been involved in affective-motivation processing of pain, which is referred to as the medial pain system [4, 7–9]. Cognitive aspects of pain is related to dorsolateral prefrontal cortex (DLPFC) [1]. Recent studies have shown that the motor cortex is also involved in pain modulation [10–16]. Some other areas of the brain including the preacuectal grey matter (PAG) system and nucleus cuneiformis also play a major role in modulation of pain [6]. Involvement of these areas of brain in pain processing occurs in a large distributed neural network called pain neuromatrix (PNM) [17]. Some parts of the PNM such as S1, M1, and DLPFC are superficial, and some others such as the thalamus, insula, and anterior cingulate cortex are deep structures [1, 4].

A growing body of evidence indicates co-activation of S1, M1 and DLPFC during pain processing [7, 18, 19], which may provide evidence for functional connectivity between these cortical sites. The connectivity between M1 and S1 has already been established by a number of studies. Matsunaga et al. showed that anodal transcranial direct current stimulation (a-tDCS) over the M1 can induce a long-lasting increase in the size of ipsilateral cortical components of sensory evoked potentials (SEPs) [20]. This connectivity was also studied by Schabrun et al. looking at the pain-induced changes in S1 and M1 excitability. They showed that the S1 excitability was reduced during and after pain, while M1 excitability was suppressed only after the resolved pain [21]. Furthermore, positron emission tomography (PET) studies also indicated mixed results which indicate that this relationship is not very straight forward. They showed that pain-induced S1 activity may coincide with increased [22, 23], decreased [24], or unchanged [25] M1 activity. Therefore, there is no consensus on direction of M1 activation in response to pain induced changes in S1. The relationship for DLPFC and M1 is more straightforward. Despite the direct effects of DLPFC on the frontal-parietal network and subgenual cortex [26], literature indicates that increased DLPFC activity coincides with increased M1 activity and modulation of the medial pain system [27, 28].

Recent investigations have also demonstrated that the excitability changes in superficial areas of PNM induces changes in some key variables operationalizing pain, including sensory threshold (STh) and pain threshold (PTh) [29–31]. It is reported that increasing the excitability of M1 and/or S1 results in different STh/PTh responses [32, 33]. Based on the results of a recent systematic review, there is a site-specific effect in STh/PTh modulation following an increase in the excitability of M1/S1 in healthy individuals and M1/DLPFC in patients with chronic pain [9]. Unlike M1 and S1, the role of DLPFC on STh/PTh has not been investigated. The closest study in this regard is a recently published systematic review by O'Connell et al. (2014) which indicated that excitability modulation following application of repeated transcranial magnetic stimulation (rTMS), tDCS, or cerebral electrotherapy stimulation (CES) over the DLPFC has no effect on the pain level in patients with chronic pain [34]. The above studies could be categorised in two groups: first, the induced changes in a single PNM site is followed by measurement of excitability and STh/PTh changes in another site. Second, pain induced temporal association between the changes in activity of PNM sites are studied. To the best of our knowledge, there is no study in the literature to collectively investigate the effects of changes in one of these three superficial sites of PNM on the other two sites.

A-tDCS is a powerful non-invasive neuromodulatory technique which could be used to study the functional connectivity [35–37]. Therefore, in the present study, the primary aim is to simultaneously measure the level of M1 and S1 excitability following a-tDCS of M1, S1, and DLPFC to investigate the functional connectivities between these sites in healthy individuals. The secondary aim was to investigate how M1 or S1 excitability modulation affects STh and PTh. We also aimed to investigate the placebo effects of a-tDCS on modulation of M1, S1 excitability and STh and PTh. Indeed, the results of this pilot study generate further hypotheses relating to complex mechanisms of different brain stimulation localisations on sensory/pain thresholds.

Materials and Methods

Study design

We conducted a single-center, doubled-blinded, randomized, sham-controlled crossover study to determine the site-specific effect of a single session of a-tDCS on M1 and S1 excitability and STh and PTh in healthy volunteers. This study conformed to the ethical standards of the Declaration of Helsinki and was approved by the institutional ethics committee at Monash University, Clayton, Australia (S1 Ethics). Considering the WHO definition, it was impossible to publish the current study as an "Original Article". As a result, it was registered as a clinical trial on the Australian New Zealand Clinical Trial (registry number: ACTRN12614000817640, http://www.anzctr.org.au/) after enrolment of all participants. The authors confirm that all ongoing and related trials for tDCS studies are registered.

Participants

Between October and December of 2013, we conducted 60 experiments on 12 healthy volunteers (four men and eight women, all Monash University students) with a mean age of 23.6±5.3 years (age range 20–34). All were right-handers as determined by the Edinburgh Handedness Inventory (10-item version, mean laterality quotient = 87.9±10.5) [38]. Eligibility criteria were: age between 18 and 35 years, no clinically significant or unstable medical, neuropsychiatric, or chronic pain disorder, no history of substance abuse or dependence, no use of central nervous system-effective medication, no history of brain surgery, tumour, or intracranial metal implantation. All participants were interviewed and examined by a physician prior to enrolment in

the study and provided written, informed consent. The protocol for this trial and CONSORT checklist are available; see S1 CONSORT Checklist and S1 Protocol.

Experimental procedures

The healthy participants received intervention with tDCS under each of five different conditions in a random order: a-tDCS of M1, a-tDCS of S1, a-tDCS of DLPFC, sham a-tDCS, and no-tDCS. The experimental sessions were separated by at least 72 hours to avoid interference or carry-over effects of tDCS, and completed at the same time of day (mornings or early afternoon) to avoid diurnal variation. The duration of tDCS application was 20 minutes in all experiments. MEPs, SEPs, STh, PTh to peripheral electrical stimulation, and PTh to pressure stimulation (P_pTh) were assessed before (T_{pre}), immediately after (T_0) and 30 minutes (T_{30}) after each intervention. Participants were blinded to the condition of tDCS (sham or active). The progress of the clinical trial through various phases (Enrollment, Allocation, Follow-up, and Analysis) is shown in Fig. 1. Two researchers (assessor of outcome measures and tDCS administrator) were involved in the current study; the assessor who measured SEP, MEP, STh, PTh, and P_pTh and took part in data analysis, was blinded to all experimental conditions. The tDCS administrator, who was responsible for delivering the tDCS was not blinded to the tDCS condition.

A-tDCS of superficial regions of PNM

Anodal tDCS was administered through an active saline-soaked surface sponge electrode (2×1.5cm) over the target area and a reference electrode (3×4cm over the right contralateral

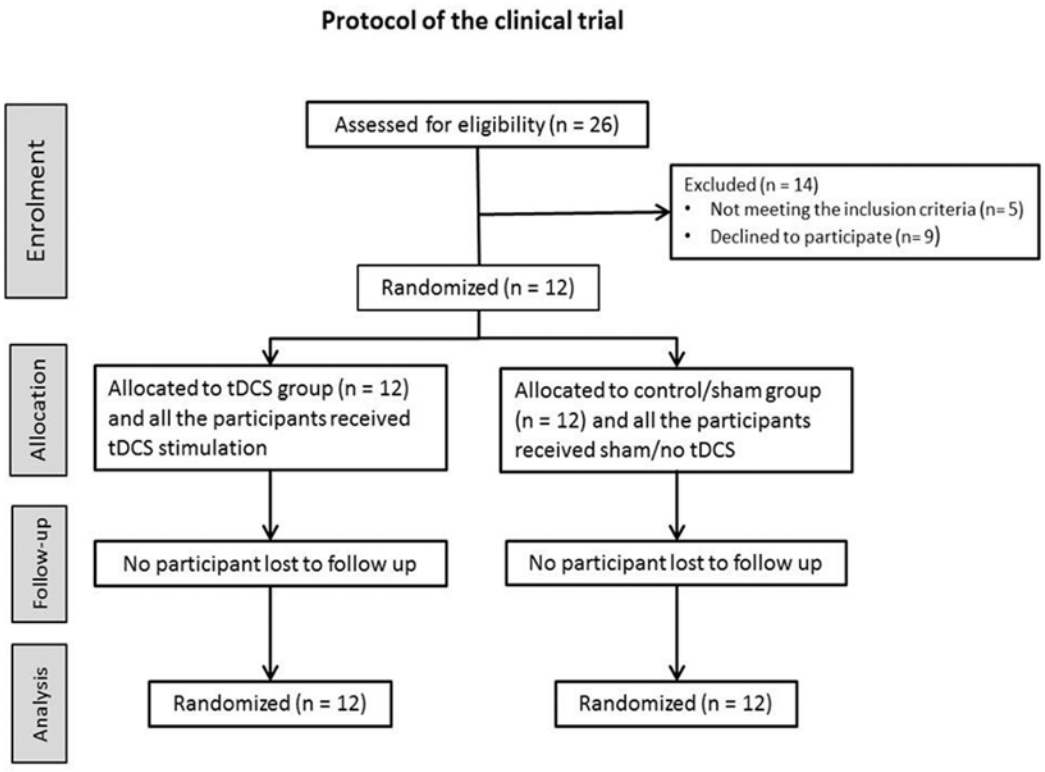

Fig 1. Flow diagram of the progress through the phases (Enrolment, Allocation, Follow-up, and Analysis) of the randomized clinical trial of transcranial direct current stimulation (tDCS) and sham/control groups.

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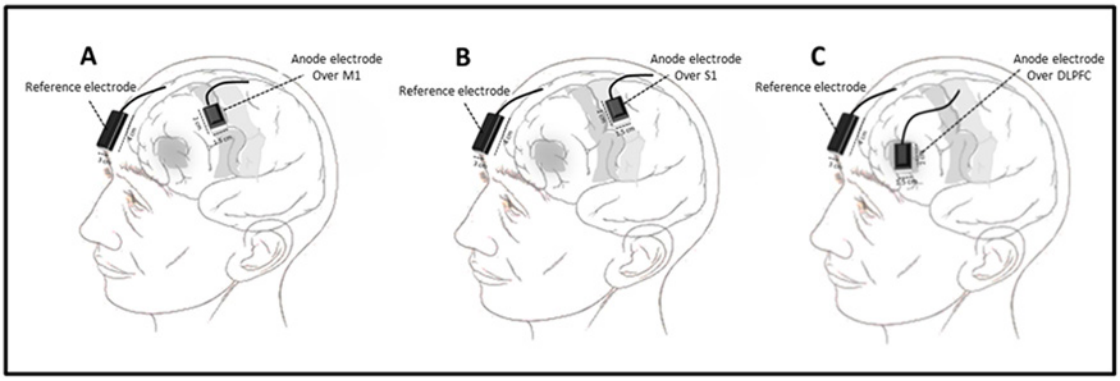

Fig 2. Schematic illustration of electrode montage. Stimulation of primary motor cortex (M1), primary sensory cortex (S1), and dorsolateral prefrontal cortex (DLPFC), the anode electrode was positioned on C3 (A), C′3 (B), and F3 (C) consecutively. The reference electrode was placed over the contralateral supraorbital area in all conditions.

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supraorbital area [35]. The larger size for the reference electrode decreases current density (CD) and reduces side effects under the indifferent electrode with more focused density under the anode [39]. The tDCS stimulator (Intelec Advanced Therapy System, Chattanooga, USA) was programmed to deliver 0.3mA direct current for 20 minutes, with 10 seconds of linear fade in and fade out. The electrodes were fixed with two horizontal and perpendicular straps.

Current intensity was set at 0.3mA which enabled us to considerably decrease the size of the electrodes [40]. In all experiments, the CD was kept at 0.1 mA/cm^2 which is in a safe range with limited side effects [35, 41, 42]. There is evidence for superiority of this intensity in induction of corticospinal excitability [41, 43–45]. The small size of the anode electrode authorized highly focused stimulation of M1 and S1 [46].

For anodal stimulation of M1 and S1, the anode electrode was placed over C3 and C′3 (2cm backward relative to C3) based on the 10–20 system. For anodal stimulation of DLPFC, the anode electrode was placed over F3 (Fig. 2). The reference electrode (cathode) was conventionally placed over the contralateral supraorbital area with an assumption of negligible or zero neuromodulatory effects on the subgenual cortex. We kept the size of this electrode four times larger than the active anode (the density was four times less) to minimize neuromodulatory effects under the cathode, but in reality, the subgenual cortex may be affected even by this very low density of currents under the cathode [47–49]. In the sham condition, the electrodes were placed in the same positions as for anodal M1 or S1 stimulation randomly, but the stimulator was turned off after 30 seconds of stimulation. For the no-tDCS session, participants were asked to sit in a podiatry chair during the 20-minute intervention time although no electrode was placed on PNM regions. All pre and post evaluations were identical to those in other conditions.

Measurement of side effects

To record side effects or adverse effects resulting from stimulation, all participants were asked to complete questionnaires both during and after all experimental conditions. The questionnaire contained rating scales for the presence and severity of side effects such as itching, tingling, burning sensations under electrodes [50, 51] as well as adverse effects including headache and pain during and after stimulation. All participants rated the unpleasantness of any scalp sensation using numeric analogue scales (NAS) (e.g., 0 = no tingling to 10 = worst tingling imaginable).

M1 excitability measurement

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 2002 (Magstim Company Limited, Whiteland, Wales, UK) stimulator with a flat 70mm figure-of-eight standard magnetic coil (peak magnitude field 2.2T). The vertex (Cz) point was measured and marked to be used as a reference [52]. 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. A scalp site optimal for evoking an MEP in the first dorsi interosseous (FDI) muscle of the right hand was found and marked as a reference. The coil position and orientation were constantly checked during the experiment to ensure that no changes occurred.

MEP resting threshold (RT) was tested in steps of 2% maximum stimulator output [35], and defined as the lowest intensity for which five of ten successive MEPs exceed 50μV (rest) peak-to-peak amplitude [53–55]. For all further MEP measurements, the TMS intensity was set at 120% of each individual's RT. Fifteen stimuli were elicited to assess corticospinal excitability of M1 at each time point. The stimulus intensity remained constant throughout the study session for each participant.

Surface EMG was recorded from the right FDI muscle using bipolar Ag/AgCl disposable surface electrodes with an inter electrode distance of 3cm (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 [52, 56]. The location of FDI was determined based on anatomical landmarks [57] and also observation of muscle response in the testing position (abduction of index finger toward the thumb) [58]. The accuracy of EMG electrode placement was verified by asking the participant to contract the muscle of interest while the investigator monitored online EMG activity. A ground electrode was placed ipsilaterally on the styloid process of the ulnar bone [59, 60]. The electrodes were secured by hypoallergenic tape (Micropore, USA). All raw EMG signals were band pass filtered (10–1000Hz), amplified (61000) and sampled at 2000Hz and collected on a PC running commercially available software (ChartTM software, ADinstrument, Australia) via a laboratory analogue-digital interface (The PowerLab 8/30, ADinstrument, Australia). Peak-to-peak MEP amplitude was detected and measured automatically using a custom-designed macro in Powerlab 8/30 software after each magnetic stimulus.

S1 excitability measurement

SEP were recorded following electrical stimulation of the right median nerve at the wrist level at 2Hz with pulse width of 0.2ms [61]. The intensity of stimulation was fixed at the motor threshold [61]. At this stimulus intensity, SEPs were recorded from S1 using electroencephalography (EEG) electrodes. One electrode was located over the C′3 (2cm behind C3) and the reference electrode was placed over the mid-frontal (Fz) position [20, 62]. The electrical potentials were recorded in epochs from 0 to 200ms after the stimulus. A total of 500 stimulus-related epochs were recorded. Peak-to-peak amplitude of N20-P25 responses generated in S1 were measured and compared before and after tDCS stimulation in different areas of PNM [63].

Measurement of STh and PTh

All evaluations were performed at T_{pre}, T_0, and T_{30} by a blinded rater. The primary outcomes were STh and PTh to electrical stimulation. Electrical stimulation was applied by a pen electrode (model: 2762CC, Chattanooga, USA) to the right median nerve (pulse duration: 200μs)

at wrist level. Current supply started at 0mA and was increased in steps of 0.1mA until the participant reported sensation and pain. The intensity of current at which perception of the electrical stimulus was first reported was taken as the STh; the intensity of current at which participants first reported pain was taken as the PTh and then averaged for analysis.

Measurement of P_pTh

Pressure was induced using a pressure algometer (model: FDX 50, Wagner, USA; capacity: 50× 0.05Ibf, accuracy: ±0.3% of full scale) with a flat circular metal probe dressed in a plastic cover. Force was displayed digitally in increments of 0.1N. The algometer was mounted vertically. For each measurement the algometer was calibrated to enable force to be applied at a controlled and steady rate. P_pTh was defined as the amount of force required to elicit a sensation of pain distinct from pressure or discomfort [64]. The P_pTh measurement point was marked in the middle of the belly of the FDI muscle [65].

Participants were instructed in the application of the algometer and given a demonstration. They then underwent two practice measurements on their non-dominant side. Participants were asked to say "stop" immediately when a discernible sensation of pain, distinct from pressure of discomfort, was felt; at this point, the experimenter retracted the algometer [66, 67]. The digital display continued to show the value of pressure applied at the moment the algometer was retracted. The algometer was applied perpendicularly to the skin and lowered at a rate of a rate of approximately 5N/s until P_pTh was reached [64], as detected by participants' verbal report. At each time point, three P_pThs were measured approximately 10–15s apart and then averaged for analysis.

Data analysis

The data were analyzed, blinded to experimental conditions. The post-intervention means were normalized to intra-individually and are given as ratios of the baseline [54]. Using one-way repeated measures ANOVA at T_{pre} of all conditions, we sought to detect any carry-over effect at the starting point of each session.

A two-way repeated measures ANOVA was used to assess the effects of two independent variables, experimental conditions (a-tDCS of S1, M1, DLPFC, sham, and no-tDCS) and time points (T_{pre}, T_0, and T_{30}), on MEPs, SEPs, STh, PTh and P_pTh. 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 [68]. Additionally, to test whether the baseline value of each stimulation site differed significantly from post-intervention time points (T_0, T_{30}), a paired-sample t-test was applied.

A significance level of $P = 0.05$ was adopted for all comparisons. A post-hoc test (Bonferroni) was performed where indicated. Means are reported ±SE. Statistical analyses were performed using SPSS software version 22.

Results

Comparison of baseline values

One-way repeated measure ANOVA showed that baseline values of dependent variables (peak-to-peak amplitude of MEPs and of N20-P25 of SEPs) remained unchanged in multiple sessions of assessment for all experimental conditions. This indicates that the wash-out period was adequate and refuses any possibility of carry-over effects from previous interventions on same participants.

The effects of a-tDCS of M1, S1, and DLPFC on M1 excitability

The two-way repeated measures ANOVA revealed significant main effects for the stimulation site, time, and site × time variables (Table 1). Post hoc comparisons showed significant difference between M1/S1, DLPFC/S1, M1/Sham or no-tDCS, DLPFC/Sham or no-tDCS, and S1/no-tDCS at both T_0 and T_{30} (Fig. 3). Based on the results, there was no significant difference between M1-DLPFC and S1-sham at T_0 or T_{30}. No significant difference was found between sham and no-tDCS conditions. A two-tailed, paired sample t-test with an alpha level of 0.01 was used to compare baseline values with T_0 and T_{30} in each experimental condition. The results indicated that a-tDCS of both M1 and DLPFC increased the size of MEPs at T_0 and T_{30} significantly. Significant difference was observed between T_0-T_{30} but not T_{pre}-T_0 or T_{pre}-T_{30} following a-tDCS of S1 (Table 2).

The effects of a-tDCS of M1, S1, and DLPFC on S1 excitability

The results of two-way repeated measures ANOVA showed significant effects for the site of stimulation, time, and site×time variables (Table 1). Post hoc comparisons indicated that there was a significant difference between M1-S1, M1-sham/no-tDCS, and S1-sham/no-tDCS at T_0. No significant change was found in the amplitude of N_{20}-P_{25} 30 minutes after a-tDCS. The results also showed that there was no difference between sham and no-tDCS. Paired sample

Table 1. ANOVA results for the effects of a-tDCS on the MEP and SEP sizes and the level of STh, PTh, and P_pTh.

		df	F-value	P-value
MEP size	**Stimulation site**	4	12.8	0.001
	Time	2	29.2	0.0008
	Stimulation site× Time	8	9.1	0.0001
	MEP sizes at T_0	4	12.4	0.0008
	MEP sizes at T_{30}	4	10.1	0.002
SEP Size	**Stimulation site**	4	4.3	0.005
	Time	2	0.37	0.03
	Stimulation site× Time	8	2.4	0.02
	SEP sizes at T_0	4	5.8	0.001
	SEP sizes at T_{30}	4	0.19	0.90
STh	**Stimulation site**	4	4.3	0.005
	Time	2	9.5	0.009
	Stimulation site× Time	8	3.6	0.001
	STh sizes at T_0	4	2.8	0.35
	STh sizes at T_{30}	4	6.0	0.001
PTh	**Stimulation site**	4	3.6	0.01
	Time	2	20.6	0.0008
	Stimulation site× Time	8	2.5	0.01
	PTh sizes at T_0	4	1.8	0.17
	PTh sizes at T_{30}	4	5.1	0.009
P_pTh	**Stimulation site**	4	3.0	0.02
	Time	2	29.6	0.0009
	Stimulation site× Time	8	2.8	0.009
	P_pTh sizes at T_0	4	2.9	0.13
	P_pTh sizes at T_{30}	4	27.8	0.0009

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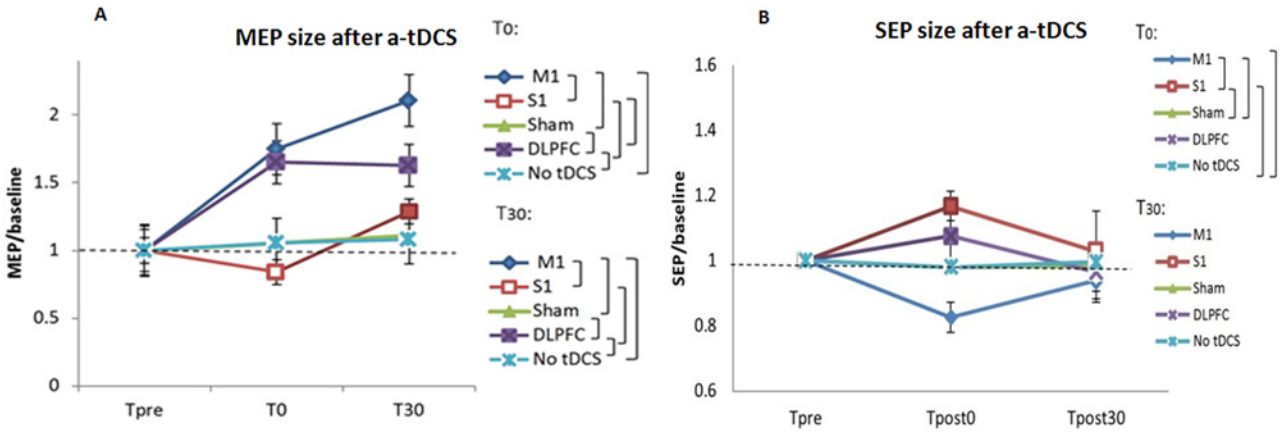

Fig 3. The effects of a-tDCS over different stimulation sites on MEP and SEP sizes. The peak-to-peak amplitude of MEPs (A), and the peak-to-peak amplitude of N20-P25 of SEPs (B) are illustrated following a-tDCS of primary motor cortex (M1), primary sensory cortex (S1), dorsolateral prefrontal cortex (DLPFC), and sham a-tDCS over time. Filled symbols indicate significant deviation of the post transcranial stimulation MEP and SEP amplitudes relative to the baseline; the brackets show significant differences between different testing conditions. Data are reported as mean ± SEM.

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t-tests indicated that there was a significant difference in the size of SEPs at T_{pre} and T_0 following a-tDCS of M1 and S1 (Fig. 3). No significant difference was found between SEP sizes at T_{pre} and T_{30} (Table 2).

The effects of a-tDCS of M1, S1, and DLPFC on STh

STh analysis showed significant main effects of stimulation site, time, and site × time (Table 1). As displayed in Fig. 3, there was no significant difference between M1, S1, and DLPFC at T_0 and T_{30}. The result indicated a significant difference between M1/sham or no-tDCS at T_0 and T_{30}, and DLPFC/sham or no-tDCS at T_{30}. With regard to the STh level after a-tDCS of superficial regions of the PNM at each time point No significant STh changes were found between sham and no-tDCS conditions (Fig. 4). Furthermore, comparing baseline values, the results indicated that STh were significantly increased after a-tDCS of M1 and DLPF at both T_0 and T_{30}. Paired t-test results also showed that there is a significant STh increase after a-tDCS of S1 at T_{30} but not T_0 (Table 2).

The effects of a-tDCS of M1, S1, and DLPFC on PTh

The repeated measures ANOVA revealed significant main effects of the stimulation site, time, and stimulation site×time on PTh following a-tDCS of superficial areas of the PNM (Table 1). As shown by the post hoc tests, there was no significant difference between experimental conditions at T_0. Significant differences were found between S1/sham or no-tDCS, M1/no-tDCS, DLPFC/no-tDCS, while there was no significant difference in PTh between sham and a-tDCS of M1 and DLPFC. The results also revealed no significant difference between sham and no-tDCS conditions (Fig. 4). Comparing baseline PTh with the values at T_0 and T_{30} indicated that PTh increased immediately and 30 minutes after a-tDCS of S1. PTh increase was also found after a-tDCS of DLPFC and M1 at T_{30} but not T_0 ($P < 0.01$) (Table 2).

The effects of a-tDCS of M1, S1, and DLPFC on P_pTh

A two-way repeated measures ANOVA was used to compare the effects of five different conditions on brain excitability at three time points. The results revealed significant main effects of time, stimulation site, and stimulation × time interaction. Post hoc comparison indicated that

Table 2. The effects of different experimental conditions on the size of MEPs/SEPs and the level of STh, PTh, P_pTh.

		MEP	SEP	STh	PTh	P_pTh
M_1 tDCS	**T_0**	1.74±0.11	0.82±0.04	1.49 ± 0.12	1.27 ± 0.09	1.18 ± 0.07
	T_{30}	2.11±0.13	0.94±0.06	1.59 ± 0.12	1.45 ± 0.11	1.34 ± 0.08
	P-Value (T_{pre}-T_0)	0.002	0.003	0.003	0.012	0.02
	P-Value (T_{pre}-T_{30})	0.002	0.40	0.004	0.002	0.001
	P-Value (T_0-T_{30})	0.01	0.08	0.12	0.09	0.001
S_1 tDCS	**T_0**	0.84±0.09	1.16±0.04	1.87 ± 0.51	1.37 ± 0.11	1.63 ± 0.28
	T_{30}	1.3±0.15	1.03±0.12	1.98 ± 0.37	1.49 ± 0.09	1.56 ± 0.25
	P-Value (T_{pre}-T_0)	0.11	0.004	0.105	0.009	0.04
	P-Value (T_{pre}-T_{30})	0.08	0.81	0.025	0.000	0.01
	P-Value (T_0-T_{30})	0.005	0.09	0.48	0.107	0.17
DLPFC tDCS	**T_0**	1.65±0.15	1.07±0.09	1.60 ± 0.2	1.25 ± 0.12	1.05 ±. 06
	T_{30}	1.62±0.07	0.96±0.08	1.75 ± 0.16	1.37 ± 0.09	1.22 ± 0.12
	P-Value (T_{pre}-T_0)	0.002	0.44	0.01	0.07	0.41
	P-Value (T_{pre}-T_{30})	.000	0.63	0.001	0.002	0.11
	P-Value (T_0-T_{30})	0.87	0.09	0.021	0.38	0.12
Sham tDCS	**T_0**	1.05±0.05	0.97±0.01	1.08 ± 0.08	1.19 ± 0.16	1.28 ± 0.06
	T_{30}	1.1±0.02	0.98±0.02	1.14 ± 0.30	1.19 ± 0.11	1.29 ± 0.07
	P-Value (T_{pre}-T_0)	0.06	0.23	0.30	0.26	0.07
	P-Value (T_{pre}-T_{30})	0.053	0.45	0.14	0.09	0.03
	P-Value (T_0-T_{30})	0.11	0.77	0.35	0.98	0.90
No-tDCS	**T_0**	1.05±0.06	0.98±0.03	0.99 ± 0.04	0.97 ± 0.01	1.01 ± 0.03
	T_{30}	1.08±0.02	0.99±0.01	1.03 ± 0.03	1.00 ± 0.02	0.99 ± 0.02
	P-Value (T_{pre}-T_0)	0.09	0.11	0.96	0.051	0.67
	P-Value (T_{pre}-T_{30})	0.07	0.83	0.32	0.059	0.93
	P-Value (T_0-T_{30})	0.12	0.17	0.14	0.15	0.60

The effect of a-tDCS of M1, S1, DLPFC, sham, and no condition on MEPs, SEPs,sensory (STh) and pain (PTh), and pressure pain threshold (P_pTh) changes are illustrated at T0 and T30. T0 and T30 rows show Mean ± SE changes compared to baseline values. As the mean values normalized to baseline, the mean and post-intervention values are given as ratios of the baseline, the value of T_{pre} is considered as 1.

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there were no significant changes at any sites of stimulation at T_0. The results also showed significant differences in P_pTh between M1/no-tDCS and DLPFC/no-tDCS conditions. No significant difference between sham and no-tDCS conditions was detected (Fig. 4). Compared to baseline values, pairwise comparison showed significant P_pTh changes following a-tDCS of M1 at T_{30} (Table 2).

Safety and side effects of a-tDCS

All participants tolerated the applied currents in different conditions very well and there was no interruption of experimental procedures due to adverse or side effects of the applied currents. Table 3 summarizes the means ± SEM for reported side effects under the anode and cathode for each of the experimental sessions. Itching is a side effect of a-tDCS, which was experienced by all participants in active and sham sessions. There were no side effects reported by participants after the end of stimulation. No reports of burning sensations, headaches, or pain were recorded during or after stimulation.

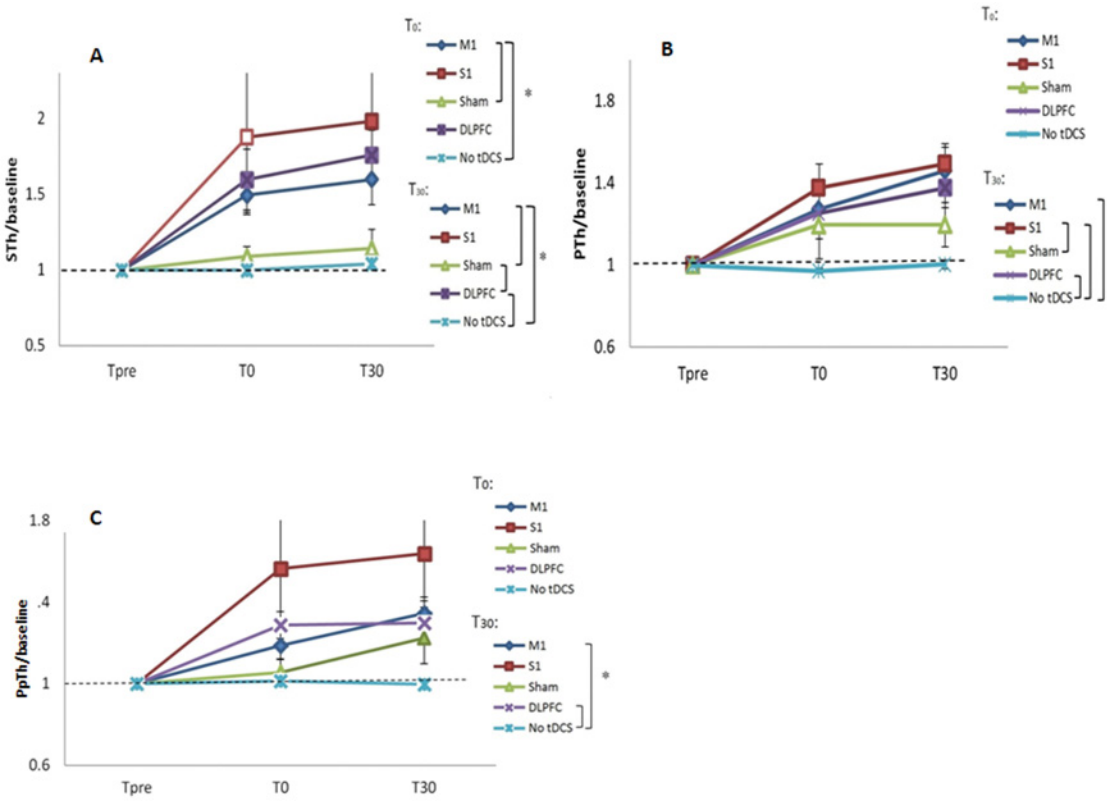

Fig 4. The effects of a-tDCS over different stimulation sites on sensory and pain threshold. Sensory threshold (STh) (A), pain threshold (PTh) (B), and pressure pain threshold (P_pTh) (C) changes are illustrated following a-tDCS of primary motor cortex (M1), primary sensory cortex (S1), dorsolateral prefrontal cortex (DLPFC), and sham a-tDCS over time. Filled symbols indicate significant deviation of the post transcranial stimulation STh, PTh, P_pTh relative to the baseline; the brackets show significant differences between different testing conditions. Data are reported as mean±SEM.

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Discussion

The effects of a-tDCS of M1, S1, and DLPFC on M1 excitability

Our results indicate that there are significant differences between active and sham/no-tDCS in all conditions at T_0 (except a-tDCS of S1), and at T_{30}. Hence, we can conclude that our findings are due to the real effects of active a-tDCS, not placebo effects. M1 excitability increases by a-tDCS of both M1 and DLPFC, whereas a-tDCS of S1 produces an opposite effect immediately after intervention (at T_0). Interestingly, the MEP sizes significantly increased 30 minutes after completion of S1 a-tDCS.

The findings in current study are in agreement with several other studies in which MEP increases after application of a-tDCS over the M1 [7, 35, 40, 43, 69]. Although compared to other studies, the electrode size and current intensity are different in our study; the current density is kept identical. Furthermore, as can be seen in Fig. 3, the M1 and DLPFC stimulation induced similar M1 corticospinal changes immediately after a-tDCS. Although there is no evidence showing the effects of a-tDCS of the DLPFC on M1 excitability yet, some anatomical studies suggest that the premotor cortex is divided into dorsal and ventral parts and the dorsal part sends its output to the M1 and spinal cord and receives prominent input from DLPFC [70, 71]. DLPFC activity is important during pain for maintenance of information in short-term memory and governing efficient performance control in the presence of painful stimuli by modulation of attention [72, 73]. The attention modulation signals from the DLPFC and motor

Table 3. Numeric sensation score by participants during experimental conditions.

		Anode electrode				Reference electrode			
		M1	S1	DLPFC	Sham	M1	S1	DLPFC	Sham
Tingling sensation	Beginning	4.6 ± 0.28	5.1 ± 0.42	4.3 ± 0.48	2.8 ± 0.26	2.1 ± 0.16	3.2 ± 0.11	2.7 ± 0.21	1.7 ± 0.09
	Middle	3.6 ± 0.23	3.9 ± 0.34	2.7 ± 0.19	0.9 ± 0.31	2.0 ± 0.21	2.6 ± 0.12	2.0 ± 0.06	0.8 ± 0.10
	End	1.7 ± 0.15	1.3 ± 0.11	1.8 ± 0.22	0.5 ± 0.09	1.1 ± 0.18	1.8 ± 0.12	1.4 ± 0.17	0.3 ± 0.08
Itching sensation	Beginning	3.2 ± 0.17	3.9 ± 0.38	2.7 ± 0.36	1.1 ± 0.20	2.7 ± 0.21	3.0 ± 0.17	3.1 ± 0.18	1.2 ± 0.12
	Middle	1.8 ± 0.13	2.2 ± 0.35	2.1 ± 0.28	0.5 ± 0.11	2.1 ± 0.18	1.7 ± 0.19	1.9 ± 0.16	1.0 ± 0.08
	End	2.1 ± 0.21	1.0 ± 0.09	1.2 ± 0.12	0.3 ± 0.10	1.5 ± 0.20	1.3 ± 0.06	0.9 ± 0.07	0.1 ± 0.03
Burning sensation	Beginning	-	-	-	-	-	-	-	-
	Middle	-	-	-	-	-	-	-	-
	End	-	-	-	-	-	-	-	-
Not tolerated	Beginning	-	-	-	-	-	-	-	-
	Middle	-	-	-	-	-	-	-	-
	End	-	-	-	-	-	-	-	-

The values are rated using Numeric Analogue Scale (NAS) 0 is rated as no sensation and 10 rated as the worst sensation imaginable. The sensations are recorded during three phases of stimulation: Beginning (0 to 7 minutes of stimulation), Middle (7 to 14 minutes of stimulation), End (14 to 20 minute of stimulation). Sensations under both active (anode) and reference (cathode) electrodes were recorded during a-tDCS of primary motor cortex (M1), primary sensory cortex (S1), dorsolateral prefrontal cortex (DLPFC), and sham tDCS. Scores are reported as mean ± SEM.

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preparation information from the dorsal part of the premotor cortex are received by the M1. These functional connections findings can explain why increasing the level of DLPFC stimulation leads to increased M1 excitability. As a result, based on the functional [74, 75] and anatomical [74, 76] relationship between DLPFC and M1, we conclude that a-tDCS of the DLPFC may activate the DLPFC-premotor-primary motor pathway and increase M1 excitability.

The mechanisms underlying these changes remain unclear. TDCS affects the stimulated area by a number of different mechanisms and is able to induce changes in different functional areas of brain [77]. TDCS induces physiological changes that result in local and distant plastic changes. Furthermore, the immediate after-effect of a-tDCS is associated with changes in neuronal membrane channels, such as sodium and calcium transporters [78–80]. After-effects of a-tDCS are also influenced by the potentiation of synaptic glutamatergic receptors [36].

After a-tDCS of S1, the level of M1 excitability remained unchanged immediately after stimulation and only increased significantly after a 30-minute delay. The cortico-cortical interconnections in superficial layers of the M1 and S1 are likely to be crucial for sensory input processing in S1 and sensorimotor integration [81–83]. It has been found that S1-projecting M1 pyramidal neurons strongly recruit a type of interneuron named Vasointestinal Peptide (VIP). VIP interneurons are inhibitory and fast-spiking. They account for the most *gamma*-Aminobutyric acid (GABAergic) interneurons in S1 and, are located in the superficial layers of S1 and target the distal dentrities of pyramidal cells in M1 [84–86]. A-tDCS of S1 might activate VIP interneurons, suppressing M1 excitability for a short period. It is likely that M1 excitability gradually increases after 30 minutes due to M1 and S1 projection [84] and due to short-term potentiation (STP) [87] and early long-term potentiation (e-LTP) [88] mechanisms. E-LTP depends on activation of calcium-dependent kinases, which control the trafficking of α-amino-3-hydroxy-5methyl-4isoxazolepropionic acid, and activation of N-methyl-D-aspartate—a subtype of glutamate receptor [89–91].

The effects of a-tDCS of M1, S1, and DLPFC on S1 excitability

Our results show that there were significant differences between sham/no-tDCS and active a-tDCS of M1 and S1. Surprisingly, converse behavior was found following a-tDCS of M1 and S1; the N20-P25 component of the SEPs increased immediately after a-tDCS of S1, but decreased immediately after a-tDCS of M1.

In agreement with other SEP studies, application of a-tDCS over S1 increased the level of S1 excitability [20, 43]. In contrast with SEP studies in which it is reported that SEP facilitation lasted longer than one hour after 10 minutes of a-tDCS of S1 [20, 43], in our study 20 minutes of a-tDCS only increased the level of S1 excitability immediately after a-tDCS. Ion channel alteration in S1 is the probable mechanism behind the immediate effects of a-tDCS [78]. The current study is the first report of the effects of M1 and DLPFC stimulation on S1 excitability.

Few studies have investigated the integration of information between multiple cortical regions, including M1 and S1, in pain conditions [92, 93]. Some of these studies produced similar evidence of reduced MEP amplitude following S1 excitability reduction with experimental pain [92], whereas others failed to do so [94–96]. Schabrun et al. concluded that following pain induced S1 excitability reduction, M1 excitability and motor outputs also reduced, although there is no relationship between these measures. Schabrun et al. also indicated that S1 excitability reduction influences M1 processes, but the underlying mechanisms are not understood [92]. There are three possible explanations for the differences between the results; first, the individual variation is high [97], which may conceal or reveal excitability or perception changes in the studies. Second, different methodologies were used in the studies, which may influence the activities of different parts of the brain. Third, the active areas of the brain in experimental subjects experiencing pain are different to those in healthy subjects.

Suppression of N20-P25 amplitude after M1 stimulation may be explained by activation of the projections from motor to sensory cortex [6, 86]. These projections are mainly affect areas 1 and 2 of the sensory cortex. Any changes in these areas of S1 could be easily assessed by P25 and N33 amplitudes [98]. Moreover, some neuroimaging studies demonstrated that a-tDCS of M1 induces widespread bi-directional changes in regional neuronal activities, including thalamic nuclei [1]. Therefore any changes in the N20 component of SEP could be partially explained by activation of the sensory cortex by thalamo-cortical fibers [98]. Although, there is a possibility for contribution of cortico-subcortico-cortical reentry loops to the suppression of the sensory cortex, it is more likely that the observed suppression is produced by cortico-cortical effect from the motor cortex to sensory cortex.

The effects of a-tDCS of M1, S1, and DLPFC on STh

In the current study, no significant changes were found between sham/no-tDCS conditions and the active ones. An immediate effect was observed after a-tDCS of M1. A significant increase was also observed 30 minutes after a-tDCS of M1 and DLPFC.

Our results are in line with those of Boggio et al. (2008), who concluded that a-tDCS of M1 but not DLPFC immediately increases STh [61]. Similarly, our recent meta-analysis showed that a-tDCS of both M1 and S1 increases STh immediately after a-tDCS [99]. However, we found that a-tDCS of DLPFC significantly increased STh after a 30 minute delay, while Boggio et al. found no STh increase [61]. This discrepancy may be explained by differences in methodologies in these studies, such as use of different electrode sizes (5×7cm by Boggio et al. vs. 1.5×2cm in our study), and current intensity (2mA vs. 0.3mA respectively) [46]. In addition, an a-tDCS study by Antal et al. demonstrated that both anodal and sham stimulations had no effect on STh [100]. Ragert et al. also found that a-tDCS of S1 enhances tactile spatial acuity rather than suppresses it [16].

Likewise, there is some evidence indicating that stimulation of M1, may increase the activity of insula and thalamus [101–103]. As a result, the insular-thalamus pathway activation following a-tDCS of M1 may be a possible explanation for the modulation of sensory/pain processing, leading to a STh increase.

The effects of a-tDCS of M1, S1, and DLPFC on PTh

The significant difference between sham and no-tDCS conditions, and lack of significant difference between all three sites of stimulation and sham tDCS suggest that a-tDCS may have strong placebo effects on behavioural aspects of pain processing such as PTh. Compared to the no-tDCS condition, PTh significantly increased after 30 minutes of a-tDCS of M1, S1, DLPFC, as well as following sham tDCS.

Our results demonstrate that there is no difference between stimulation of superficial areas of PNM and a-tDCS of all three sites of stimulation increases PTh in healthy individuals. Therefore, it can be concluded that that PTh modulation with a-tDCS of M1, S1, and DLPFC may results in the inhibition of thalamic and brainstem nuclei activity, decreasing the hyperactivity in these areas that underlies chronic pain [61, 104]. Indeed, neuroimaging studies demonstrate that anodal M1 [1] and S1 [15] tDCS induces widespread bidirectional changes in regional neuronal activities, including thalamic nuclei. Therefore, in this study a-tDCS may be modulating thalamic inhibitory connections such that a bigger magnitude of stimulus is required to generate a perception response [102].

Our result revealed that a-tDCS at 0.3mA is associated with effective blinding when compared with the no-tDCS condition. Complete blinding is important in clinical studies with a subjective outcome measure [105], thus both assessor and participants were blinded in our study and it is highly likely that the sensory effects of active stimulation were similar to those of sham tDCS with electrode size of 1.5×2cm and amplitude of 0.3mA. The effects of sham and active tDCS on subjective measurements have been reported in other studies [12, 13, 106] and their results are in agreement with the results of current study. In contrast, some tDCS studies have shown no placebo effect; in these studies the electrodes were large ($35cm^2$) and thus lacked the focal effect of small electrodes ($3cm^2$ in the current study) [20], which possibly affected these studies' results.

The effects of a-tDCS of M1, S1, and DLPFC on P_pTh

The results of applying a-tDCS over M1, S1, and DLPFC revealed no significant immediate effect on P_pTh. A-tDCS of M1 and DLPFC resulted in a significant P_pTh increase after 30 minutes. In addition, we found no significant difference between either active/sham tDCS or sham/no-tDCS conditions. Furthermore, no significant differences between sham and control or all three sites of stimulation and sham tDCS suggest that a-tDCS has placebo effects on mechanical pain processing. Our results are consistent with previous studies [32, 33] in demonstrating that a-tDCS of M1 and S1 has no significant effect on P_pTh.

Mechanoreceptive inputs from large A-beta and small A-delta fibres are ended at the thalamic ventral caudal nucleus via dorsal column medial pathways [107]. As a result, it is possible that a bigger magnitude of stimulus is required to generate a mechanical pain perception [102].

Peripheral electrical stimulation recruits axons based on their diameter, starting with large-diameter fibers (Aß fibers) [108]; in contrast, mechanoreceptors excite myelinated large A-beta and small A-delta fibers and may be processed through anatomically different pathways. It seems that a-tDCS of M1 and DLPFC alters both pathways, resulting in PTh and P_pTh increase after a-tDCS of M1 and DLPFC.

Safety and side effects of a-tDCS

The findings of the current study suggest that the use of a-tDCS with small electrodes leads to minimal side effects in healthy individuals. The participants' tolerance for a-tDCS with the small electrode size was compatible with that for the conventional electrode size at all sites of stimulation. No adverse effect such as seizure, headache, or nausea were recorded, and general discomfort (itching and/or tingling) was the most often reported side effect of both active and sham tDCS.

Limitations of the study

Our findings must be interpreted in the context of several limitations. First, the data were obtained from a healthy population with no pain history; therefore the results may not necessarily be extrapolated to people with different types of pain. Second, the effects were evaluated in young participants (under 35 years); older individuals may respond differently to a-tDCS. Third, some studies have reported gender differences in responses to tDCS [109–111]; as most of our participants were women, it is possible that gender influenced our results. Fourth, the current study utilized a conventional electrode montage with the active electrode (anode) over target stimulation areas and the passive electrode (cathode) over contralateral supraorbital area (subgenual cortex). Therefore the findings in this study should be interpreted in light of the fact that all target stimulation sites received anodal stimulation and the subgenual cortex under the passive electrode received cathodal stimulation. This may become significant depending on the nature of the functional connectivity between the subgenual cortex and the PNM sites stimulated in this study.

Suggestions for future research

Our study did not assess the effects of tDCS on M1 and S1 excitability beyond 30 minutes. Further studies are required to fully characterize the effects of tDCS over superficial PNM regions. The effects of a-tDCS application time, current intensity, and electrode size should be systematically studied to improve our understanding in this field. Furthermore, to reveal the mechanisms of action of a-tDCS of superficial PNM regions on the excitability of M1 and S1, M1 excitability should be studied by measuring silent period, intracortical inhibition and facilitation to indirectly assess the role of GABAa, GABAb and glutamergic receptors. Additional pharmacological experiments using receptor agonists/antagonists are needed to determine if a-tDCS of different areas of the PNM has different or similar mechanisms.

Further studies are also recommended to investigate the effects of cathodal tDCS of PNM regions on brain excitability and pain perception. In addition, the current study can be a pilot for further hypothesis generation regarding the complex mechanisms that are involved in response to brain stimulation. To increase homogeneity, controlling for hormonal status in female participants is recommended in future studies.

Conclusion

In summary, the results of this study suggest that compared to a-tDCS of S1, a-tDCS of M1 and DLPFC are better techniques to enhance the excitability of M1. Furthermore, there is no site-specific effect on behavioral aspects of pain processing and a-tDCS of all superficial areas of PNM increased STh and PTh. Our findings can be employed to develop a-tDCS protocols for clinical applications of pain modulation. Our study provides valuable information about the best site of stimulation for future therapeutic strategies in neurorehabilitation and pain studies.

Supporting Information

S1 CONSORT Checklist.
(DOC)

S1 Protocol. Original protocol of study.
(DOCX)

Author Contributions

Conceived and designed the experiments: BV SJ MZ. Performed the experiments: BV. Analyzed the data: BV. Contributed reagents/materials/analysis tools: BV SJ. Wrote the paper: BV SJ MZ. Designed the study: BV SJ MZ. Data collection: BV. Data analysis: BV SJ. Writing and editing the drafts: BV SJ MZ.

References

1. Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain. 2005; 9: 463–484. PMID: 15979027
2. Peyron R, Laurent B, Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol Clin. 2000; 30: 263–288. PMID: 11126640
3. Luedtke K, Rushton A, Wright C, Geiss B, Juergens TP, May A. Transcranial direct current stimulation for the reduction of clinical and experimentally induced pain: a systematic review and meta-analysis. Clin J Pain. 2012; 28: 452–461. doi: 10.1097/AJP.0b013e31823853e3 PMID: 22569218
4. Kulkarni B, Bentley DE, Elliott R, Youell P, Watson A, Derbyshire SW, et al. Attention to pain localization and unpleasantness discriminates the functions of the medial and lateral pain systems. Eur J Neurosci. 2005; 21: 3133–3142. PMID: 15978022
5. Ostrowsky K, Magnin M, Ryvlin P, Isnard J, Guenot M, Mauguiere F. Representation of pain and somatic sensation in the human insula: a study of responses to direct electrical cortical stimulation. Cereb Cortex. 2002; 12: 376–385. PMID: 11884353
6. Chen JL, Carta S, Soldado-Magraner J, Schneider BL, Helmchen F. Behaviour-dependent recruitment of long-range projection neurons in somatosensory cortex. Nature. 2013; 499: 336–340. doi: 10.1038/nature12236 PMID: 23792559
7. Lang N, Nitsche MA, Paulus W, Rothwell JC, Lemon RN. Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. Exp Brain Res. 2004; 156: 439–443. PMID: 14745467
8. O'Connell NE, Wand BM, Marston L, Spencer S, Desouza LH. Non-invasive brain stimulation techniques for chronic pain. A report of a Cochrane systematic review and meta-analysis. Eur J Phys Rehabil Med. 2011; 47: 309–326. PMID: 21494222
9. Vaseghi B, Zoghi M, Jaberzadeh S. Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study. Clin Neurophysiol. 2014; 125: 1847–1858. doi: 10.1016/j.clinph.2014.01.020 PMID: 24555922
10. Casey KL. Forebrain mechanisms of nociception and pain: analysis through imaging. Proc Natl Acad Sci U S A. 1999; 96: 7668–7674. PMID: 10393878
11. Ploner M, Schnitzler A. [Cortical representation of pain]. Nervenarzt. 2004; 75: 962–969. PMID: 15184984
12. Talbot JD, Marrett S, Evans AC, Meyer E, Bushnell MC, Duncan GH. Multiple representations of pain in human cerebral cortex. Science. 1991; 251: 1355–1358. PMID: 2003220
13. Garcia-Larrea L, Peyron R. Motor cortex stimulation for neuropathic pain: From phenomenology to mechanisms. Neuroimage. 2007; 37 Suppl 1: S71–79. PMID: 17644413
14. Lima MC, Fregni F. Motor cortex stimulation for chronic pain: systematic review and meta-analysis of the literature. Neurology. 2008; 70: 2329–2337. doi: 10.1212/01.wnl.0000314649.38527.93 PMID: 18541887
15. Vogt BA, Sikes RW. The medial pain system, cingulate cortex, and parallel processing of nociceptive information. Prog Brain Res. 2000; 122: 223–235. PMID: 10737061
16. Tang JS, Qu CL, Huo FQ. The thalamic nucleus submedius and ventrolateral orbital cortex are involved in nociceptive modulation: a novel pain modulation pathway. Prog Neurobiol. 2009; 89: 383–389. doi: 10.1016/j.pneurobio.2009.10.002 PMID: 19819292

17. Iannetti GD, Mouraux A. From the neuromatrix to the pain matrix (and back). Exp Brain Res. 2010; 205: 1–12. doi: 10.1007/s00221-010-2340-1 PMID: 20607220

18. Lang N, Siebner HR, Ward NS, Lee L, Nitsche MA, Paulus W, et al. How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? Eur J Neurosci. 2005; 22: 495–504. PMID: 16045502

19. Baudewig J, Nitsche MA, Paulus W, Frahm J. Regional modulation of BOLD MRI responses to human sensorimotor activation by transcranial direct current stimulation. Magnetic Resonance in Medicine. 2001; 45: 196–201. PMID: 11180425

20. Matsunaga K, Nitsche MA, Tsuji S, Rothwell JC. Effect of transcranial DC sensorimotor cortex stimulation on somatosensory evoked potentials in humans. Clin Neurophysiol. 2004; 115: 456–460. PMID: 14744588

21. Schabrun SM, Ridding MC, Galea MP, Hodges PW, Chipchase LS. Primary sensory and motor cortex excitability are co-modulated in response to peripheral electrical nerve stimulation. PLoS One. 2012; 7: e51298. doi: 10.1371/journal.pone.0051298 PMID: 23227260

22. Coghill RC, Talbot JD, Evans AC, Meyer E, Gjedde A, Bushnell MC, et al. Distributed processing of pain and vibration by the human brain. J Neurosci. 1994; 14: 4095–4108. PMID: 8027764

23. Casey KL, Minoshima S, Morrow TJ, Koeppe RA. Comparison of human cerebral activation pattern during cutaneous warmth, heat pain, and deep cold pain. J Neurophysiol. 1996; 76: 571–581. PMID: 8836245

24. Laurent B, Peyron R, Garcia Larrea L, Mauguiere F. [Positron emission tomography to study central pain integration]. Rev Neurol (Paris). 2000; 156: 341–351. PMID: 10795010

25. Jones AK, Brown WD, Friston KJ, Qi LY, Frackowiak RS. Cortical and subcortical localization of response to pain in man using positron emission tomography. Proc Biol Sci. 1991; 244: 39–44. PMID: 1677194

26. Keeser D, Meindl T, Bor J, Palm U, Pogarell O, Mulert C, et al. Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. J Neurosci. 2011; 31: 15284–15293. doi: 10.1523/JNEUROSCI.0542-11.2011 PMID: 22031874

27. Ploghaus A, Becerra L, Borras C, Borsook D. Neural circuitry underlying pain modulation: expectation, hypnosis, placebo. Trends Cogn Sci. 2003; 7: 197–200. PMID: 12757820

28. Wager TD, Rilling JK, Smith EE, Sokolik A, Casey KL, Davidson RJ, et al. Placebo-induced changes in FMRI in the anticipation and experience of pain. Science. 2004; 303: 1162–1167. PMID: 14976306

29. Fernandez-de-Las-Penas C, Madeleine P, Martinez-Perez A, Arendt-Nielsen L, Jimenez-Garcia R, Pareja JA. Pressure pain sensitivity topographical maps reveal bilateral hyperalgesia of the hands in patients with unilateral carpal tunnel syndrome. Arthritis Care Res (Hoboken). 2010; 62: 1055–1064. doi: 10.1002/acr.20189 PMID: 20235189

30. Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C, Buchel C. Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. Brain. 2002; 125: 1326–1336. PMID: 12023321

31. Giesecke T, Gracely RH, Williams DA, Geisser ME, Petzke FW, Clauw DJ. The relationship between depression, clinical pain, and experimental pain in a chronic pain cohort. Arthritis Rheum. 2005; 52: 1577–1584. PMID: 15880832

32. Grundmann L, Rolke R, Nitsche MA, Pavlakovic G, Happe S, Treede RD, et al. Effects of transcranial direct current stimulation of the primary sensory cortex on somatosensory perception. Brain Stimul. 2011; 4: 253–260. doi: 10.1016/j.brs.2010.12.002 PMID: 22032740

33. Bachmann CG, Muschinsky S, Nitsche MA, Rolke R, Magerl W, Treede RD, et al. Transcranial direct current stimulation of the motor cortex induces distinct changes in thermal and mechanical sensory percepts. Clin Neurophysiol. 2010; 121: 2083–2089. doi: 10.1016/j.clinph.2010.05.005 PMID: 20570558

34. O'Connell NE, Wand BM, Marston L, Spencer S, Desouza LH. Non-invasive brain stimulation techniques for chronic pain. Cochrane Database Syst Rev. 2014; 4: CD008208. doi: 10.1002/14651858.CD008208.pub3 PMID: 24729198

35. Nitsche Paulus. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol. 2000; 527 Pt 3: 633–639. PMID: 10990547

36. Nitsche MA, Fricke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N, et al. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. J Physiol. 2003; 553: 293–301. PMID: 12949224

37. Stagg CJ, Nitsche MA. Physiological basis of transcranial direct current stimulation. Neuroscientist. 2011; 17: 37–53. doi: 10.1177/1073858410386614 PMID: 21343407

38. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia. 1971; 9: 97–113. PMID: 5146491

39. Nitsche MA, Doemkes S, Karakose T, Antal A, Liebetanz D, Lang N, et al. Shaping the effects of transcranial direct current stimulation of the human motor cortex. J Neurophysiol. 2007; 97: 3109–3117. PMID: 17251360

40. Uy J, Ridding MC. Increased cortical excitability induced by transcranial DC and peripheral nerve stimulation. J Neurosci Methods. 2003; 127: 193–197. PMID: 12906948

41. Brunoni AR, Amadera J, Berbel B, Volz MS, Rizzerio BG, Fregni F. A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. Int J Neuropsychopharmacol. 2011; 14: 1133–1145. doi: 10.1017/S1461145710001690 PMID: 21320389

42. Poreisz C, Boros K, Antal A, Paulus W. Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. Brain Res Bull. 2007; 72: 208–214. PMID: 17452283

43. Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology. 2001; 57: 1899–1901. PMID: 11723286

44. Pellicciari MC, Brignani D, Miniussi C. Excitability modulation of the motor system induced by transcranial direct current stimulation: a multimodal approach. Neuroimage. 2013; 83: 569–580. doi: 10.1016/j.neuroimage.2013.06.076 PMID: 23845429

45. Parazzini M, Rossi E, Ferrucci R, Liorni I, Priori A, Ravazzani P. Modelling the electric field and the current density generated by cerebellar transcranial DC stimulation in humans. Clin Neurophysiol. 2013. doi: 10.1016/j.clinph.2013.12.101 PMID: 24508190

46. Bastani A, Jaberzadeh S. a-tDCS differential modulation of corticospinal excitability: the effects of electrode size. Brain Stimul. 2013; 6: 932–937. doi: 10.1016/j.brs.2013.04.005 PMID: 23664681

47. Kanda M, Nagamine T, Ikeda A, Ohara S, Kunieda T, Fujiwara N, et al. Primary somatosensory cortex is actively involved in pain processing in human. Brain Res. 2000; 853: 282–289. PMID: 10640625

48. Miranda PC, Lomarev M, Hallett M. Modeling the current distribution during transcranial direct current stimulation. Clin Neurophysiol. 2006; 117: 1623–1629. PMID: 16762592

49. Mylius V, Jung M, Menzler K, Haag A, Khader PH, Oertel WH, et al. Effects of transcranial direct current stimulation on pain perception and working memory. Eur J Pain. 2012; 16: 974–982. doi: 10.1002/j.1532-2149.2011.00105.x PMID: 22337597

50. George MS, Aston-Jones G. Noninvasive techniques for probing neurocircuitry and treating illness: vagus nerve stimulation (VNS), transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). Neuropsychopharmacology. 2010; 35: 301–316. doi: 10.1038/npp.2009.87 PMID: 19693003

51. Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: State of the art 2008. Brain Stimul. 2008; 1: 206–223. doi: 10.1016/j.brs.2008.06.004 PMID: 20633386

52. Schwartz MS, Andrasik FE. Biofeedback: A practitioner's guide. 2003: Guilford Press.

53. Hallett M. Transcranial magnetic stimulation: a useful tool for clinical neurophysiology. Ann Neurol. 1996; 40: 344–345. PMID: 8797523

54. Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol. 1994; 91: 79–92. PMID: 7519144

55. Wassermann E, Epstein C, Ziemann U. Oxford handbook of transcranial stimulation. 2008: Oxford University Press.

56. Gilmore KL, Meyers JE. Using surface electromyography in physiotherapy research. Aust J Physiother. 1983; 29: 3–9. doi: 10.1016/S0004-9514(14)60659-0 PMID: 25026429

57. Perotto AO, Delagi EF. Anatomical guide for the electromyographer: the limbs and trunk. 2005: Charles C Thomas Publisher.

58. Kendall FP, McCreary EK, Provance PG, Abeloff DK. Muscles: testing and function. 1993.

59. Oh SJ. Clinical electromyography: nerve conduction studies. 2003: Lippincott Williams & Wilkins.

60. Basmajian JV, De Luca C. Muscles alive. Proceedings of The Royal Society of Medicine. 1985; 278: 126.

61. Boggio PS, Zaghi S, Lopes M, Fregni F. Modulatory effects of anodal transcranial direct current stimulation on perception and pain thresholds in healthy volunteers. European Journal of Neurology. 2008; 15: 1124–1130. doi: 10.1111/j.1468-1331.2008.02270.x PMID: 18717717

62. Fukuda S. Somatosensory evoked potential]. Masui. The Japanese journal of anesthesiology. 2006; 55: 280.

63. Ragert P, Becker M, Tegenthoff M, Pleger B, Dinse HR. Sustained increase of somatosensory cortex excitability by 5 Hz repetitive transcranial magnetic stimulation studied by paired median nerve stimulation in humans. Neurosci Lett. 2004; 356: 91–94. PMID: 14746871

64. Fischer AA. Pressure algometry over normal muscles. Standard values, validity and reproducibility of pressure threshold. Pain. 1987; 30: 115–126. PMID: 3614975

65. Chesterton LS, Barlas P, Foster NE, Lundeberg T, Wright CC, Baxter GD. Sensory stimulation (TENS): effects of parameter manipulation on mechanical pain thresholds in healthy human subjects. Pain. 2002; 99: 253–262. PMID: 12237203

66. Fischer AA. Pressure threshold meter: its use for quantification of tender spots. Arch Phys Med Rehabil. 1986; 67: 836–838. PMID: 3778185

67. Fischer AA. Pressure Threshold Measurement for Diagnosis of Myofascial Pain and Evaluation of Treatment Results. The Clinical Journal of Pain. 1986; 2: 207–214.

68. Meyers LS, Gamst G, Guarino AJ. Applied multivariate research: Design and interpretation. 2006: SAGE Publications, Incorporated.

69. Fricke K, Seeber AA, Thirugnanasambandam N, Paulus W, Nitsche MA, Rothwell JC. Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. J Neurophysiol. 2011; 105: 1141–1149. doi: 10.1152/jn.00608.2009 PMID: 21177994

70. Dum RP, Strick PL. The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci. 1991; 11: 667–689. PMID: 1705965

71. He SQ, Dum RP, Strick PL. Topographic organization of corticospinal projections from the frontal lobe: motor areas on the lateral surface of the hemisphere. J Neurosci. 1993; 13: 952–980. PMID: 7680069

72. Bunge SA, Ochsner KN, Desmond JE, Glover GH, Gabrieli JD. Prefrontal regions involved in keeping information in and out of mind. Brain. 2001; 124: 2074–2086. PMID: 11571223

73. Van Ryckeghem DM, De Houwer J, Van Bockstaele B, Van Damme S, De Schryver M, Crombez G. Implicit associations between pain and self-schema in patients with chronic pain. Pain. 2013; 154: 2700–2706. doi: 10.1016/j.pain.2013.07.055 PMID: 23932910

74. Hoshi E. Functional specialization within the dorsolateral prefrontal cortex: A review of anatomical and physiological studies of non-human primates. Neuroscience Research. 2006; 54: 73–84. PMID: 16310877

75. Kolb B, Whishaw IQ. Fundamentals of human neuropsychology. 2009: Macmillan.

76. Bracht T, Federspiel A, Schnell S, Horn H, Hofle O, Wiest R, et al. Cortico-cortical white matter motor pathway microstructure is related to psychomotor retardation in major depressive disorder. PLoS One. 2012; 7: e52238. doi: 10.1371/journal.pone.0052238 PMID: 23284950

77. Medeiros LF, de Souza IC, Vidor LP, de Souza A, Deitos A, Volz MS, et al. Neurobiological effects of transcranial direct current stimulation: a review. Front Psychiatry. 2012; 3: 110. doi: 10.3389/fpsyt.2012.00110 PMID: 23293607

78. Kuo MF, Paulus W, Nitsche MA. Boosting focally-induced brain plasticity by dopamine. Cereb Cortex. 2008; 18: 648–651. PMID: 17591596

79. Monte-Silva K, Liebetanz D, Grundey J, Paulus W, Nitsche MA. Dosage-dependent non-linear effect of L-dopa on human motor cortex plasticity. J Physiol. 2010; 588: 3415–3424. doi: 10.1113/jphysiol.2010.190181 PMID: 20660568

80. Nitsche MA, Kuo MF, Grosch J, Bergner C, Monte-Silva K, Paulus W. D1-receptor impact on neuroplasticity in humans. J Neurosci. 2009; 29: 2648–2653. doi: 10.1523/JNEUROSCI.5366-08.2009 PMID: 19244540

81. Diamond ME, von Heimendahl M, Knutsen PM, Kleinfeld D, Ahissar E. 'Where' and 'what' in the whisker sensorimotor system. Nat Rev Neurosci. 2008; 9: 601–612. doi: 10.1038/nrn2411 PMID: 18641667

82. Petreanu L, Gutnisky DA, Huber D, Xu NL, O'Connor DH, Tian L, et al. Activity in motor-sensory projections reveals distributed coding in somatosensation. Nature. 2012; 489: 299–303. doi: 10.1038/nature11321 PMID: 22922646

83. Xu NL, Harnett MT, Williams SR, Huber D, O'Connor DH, Svoboda K, et al. Nonlinear dendritic integration of sensory and motor input during an active sensing task. Nature. 2012; 492: 247–251. doi: 10.1038/nature11601 PMID: 23143335

84. Lee S, Kruglikov I, Huang ZJ, Fishell G, Rudy B. A disinhibitory circuit mediates motor integration in the somatosensory cortex. Nat Neurosci. 2013; 16: 1662–1670. doi: 10.1038/nn.3544 PMID: 24097044

85. Rudy B, Fishell G, Lee S, Hjerling-Leffler J. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Dev Neurobiol. 2011; 71: 45–61. doi: 10.1002/dneu.20853 PMID: 21154909

86. Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B. The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. J Neurosci. 2010; 30: 16796–16808. doi: 10.1523/JNEUROSCI.1869-10.2010 PMID: 21159951

87. Samii A, Wassermann EM, Ikoma K, Mercuri B, Hallett M. Characterization of postexercise facilitation and depression of motor evoked potentials to transcranial magnetic stimulation. Neurology. 1996; 46: 1376–1382. PMID: 8628485

88. Monte-Silva K, Kuo MF, Hessenthaler S, Fresnoza S, Liebetanz D, Paulus W, et al. Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. Brain Stimul. 2013; 6: 424–432. doi: 10.1016/j.brs.2012.04.011 PMID: 22695026

89. Aroniadou VA, Keller A. Mechanisms of LTP induction in rat motor cortex in vitro. Cereb Cortex. 1995; 5: 353–362. PMID: 7580127

90. Castro-Alamancos MA, Donoghue JP, Connors BW. Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. J Neurosci. 1995; 15: 5324–5333. PMID: 7623155

91. Kirkwood A, Dudek SM, Gold JT, Aizenman CD, Bear MF. Common forms of synaptic plasticity in the hippocampus and neocortex in vitro. Science. 1993; 260: 1518–1521. PMID: 8502997

92. Schabrun SM, Jones E, Kloster J, Hodges PW. Temporal association between changes in primary sensory cortex and corticomotor output during muscle pain. Neuroscience. 2013; 235: 159–164. doi: 10.1016/j.neuroscience.2012.12.072 PMID: 23357117

93. Schabrun SM, Hodges PW. Muscle pain differentially modulates short interval intracortical inhibition and intracortical facilitation in primary motor cortex. J Pain. 2012; 13: 187–194. doi: 10.1016/j.jpain.2011.10.013 PMID: 22227117

94. Le Pera D, Graven-Nielsen T, Valeriani M, Oliviero A, Di Lazzaro V, Tonali PA, et al. Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. Clin Neurophysiol. 2001; 112: 1633–1641. PMID: 11514246

95. Svensson P, Miles TS, McKay D, Ridding MC. Suppression of motor evoked potentials in a hand muscle following prolonged painful stimulation. Eur J Pain. 2003; 7: 55–62. PMID: 12527318

96. Martin PG, Weerakkody N, Gandevia SC, Taylor JL. Group III and IV muscle afferents differentially affect the motor cortex and motoneurones in humans. J Physiol. 2008; 586: 1277–1289. PMID: 17884925

97. Tsao H, Tucker KJ, Hodges PW. Changes in excitability of corticomotor inputs to the trunk muscles during experimentally-induced acute low back pain. Neuroscience. 2011; 181: 127–133. doi: 10.1016/j.neuroscience.2011.02.033 PMID: 21333720

98. Jones EG, Coulter JD, Hendry SH. Intracortical connectivity of architectonic fields in the somatic sensory, motor and parietal cortex of monkeys. J Comp Neurol. 1978; 181: 291–347. PMID: 99458

99. Vaseghi B, Zoghi M, Jaberzadeh S. Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study. Clin Neurophysiol. 2014. doi: 10.1016/j.clinph.2014.09.029 PMID: 25631611

100. Antal A, Brepohl N, Poreisz C, Boros K, Csifcsak G, Paulus W. Transcranial direct current stimulation over somatosensory cortex decreases experimentally induced acute pain perception. Clin J Pain. 2008; 24: 56–63. doi: 10.1097/AJP.0b013e318157233b PMID: 18180638

101. Craig AD, Chen K, Bandy D, Reiman EM. Thermosensory activation of insular cortex. Nat Neurosci. 2000; 3: 184–190. PMID: 10649575

102. DaSilva AF, Volz MS, Bikson M, Fregni F. Electrode positioning and montage in transcranial direct current stimulation. J Vis Exp. 2011. doi: 10.3791/2958 PMID: 22439017

103. Mesulam MM, Mufson EJ. Insula of the old world monkey. III: Efferent cortical output and comments on function. J Comp Neurol. 1982; 212: 38–52. PMID: 7174907

104. Fregni F, Pascual-Leone A. Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. Nat Clin Pract Neurol. 2007; 3: 383–393. PMID: 17611487

105. Wood L, Egger M, Gluud LL, Schulz KF, Juni P, Altman DG, et al. Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study. Bmj. 2008; 336: 601–605. doi: 10.1136/bmj.39465.451748.AD PMID: 18316340

106. O'Connell NE, Cossar J, Marston L, Wand BM, Bunce D, Moseley GL, et al. Rethinking clinical trials of transcranial direct current stimulation: participant and assessor blinding is inadequate at intensities of 2mA. PLoS One. 2012; 7: e47514. doi: 10.1371/journal.pone.0047514 PMID: 23082174

107. Ohara S, Weiss N, Lenz FA. Microstimulation in the region of the human thalamic principal somatic sensory nucleus evokes sensations like those of mechanical stimulation and movement. J Neurophysiol. 2004; 91: 736–745. PMID: 14573561

108. Stieglitz T. Diameter-dependent excitation of peripheral nerve fibers by multipolar electrodes during electrical stimulation. Expert Rev Med Devices. 2005; 2: 149–152. PMID: 16293051

109. Knops A, Nuerk HC, Sparing R, Foltys H, Willmes K. On the functional role of human parietal cortex in number processing: How gender mediates the impact of a 'virtual lesion' induced by rTMS. Neuropsychologia. 2006; 44: 2270–2283. PMID: 16828812

110. Boggio PS, Rocha RR, da Silva MT, Fregni F. Differential modulatory effects of transcranial direct current stimulation on a facial expression go-no-go task in males and females. Neurosci Lett. 2008; 447: 101–105. doi: 10.1016/j.neulet.2008.10.009 PMID: 18926878

111. Chaieb L, Antal A, Paulus W. Gender-specific modulation of short-term neuroplasticity in the visual cortex induced by transcranial direct current stimulation. Vis Neurosci. 2008; 25: 77–81. doi: 10.1017/S0952523808080097 PMID: 18282312

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.

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	Supervisory input on study design, Guidance in the framing of the manuscript, Review and provision of feedback on final manuscript draft	5 %

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

Candidate's Signature: [redacted] Date: 15-Sep- 2015

Signature: [redacted] Date: 15-Sep-2015

Signature: [redacted] Date: 15-Sep-2015

Preamble to Chapter 6

Based on the findings in the second systematic review and meta-analysis (Chapter 3), c-tDCS of the M1, S1, and DLPFC increases STh/PTh. In Chapter 6, the effects of c-tDCS of these cortical sites on M1/S1 excitability and STh/PTh are evaluated. The current study is the first which collectively evaluates these effects. The data in Chapter 6 provides evidence for functional connectivities between these cortical sites.

Chapter 6: Differential effects of cathodal-tDCS of prefrontal, motor, and somatosensory cortices on cortical excitability and pain perception: a double-blind randomised sham-controlled study

The format of this chapter is consistent with the European Journal of Neuroscience.

The setup system used in this study, Ethics approval, TMS safety, Personal health history form, and Edinburg handedness questionnaires and consent form are provided in Appendices 7-13.

EJN European Journal of Neuroscience

FENS Federation of European Neuroscience Societies

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Differential effects of cathodal transcranial direct current stimulation of prefrontal, motor and somatosensory cortices on cortical excitability and pain perception – a double-blind randomised sham-controlled study

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Abstract

The primary aim of this study was to assess the effects of cathodal transcranial direct current stimulation (c-tDCS) over cortical regions of the pain neuromatrix, including the primary motor (M1), sensory (S1) and dorsolateral prefrontal (DLPFC) cortices on M1/S1 excitability, sensory (STh), and pain thresholds (PTh) in healthy adults. The secondary aim was to evaluate the placebo effects of c-tDCS on induced cortical and behavioural changes. Before, immediately after and 30 min after c-tDCS the amplitude of N20–P25 components of somatosensory evoked potentials (SEPs) and peak-to-peak amplitudes of motor evoked potentials (MEPs) were measured under four different experimental conditions. STh and PTh for peripheral electrical and mechanical stimulation were also evaluated. c-tDCS of 0.3 mA was applied for 20 min. A blinded assessor evaluated all outcome measures. c-tDCS of M1, S1 and DLPFC significantly decreased the corticospinal excitability of M1 ($P < 0.05$) for at least 30 min. Following the application of c-tDCS over S1, M1 and DLPFC, the amplitude of the N20–P25 component of SEPs decreased for at least 30 min ($P < 0.05$). Compared with baseline values, significant STh and PTh increases were observed after c-tDCS of these three sites. Decreasing the level of S1 and M1 excitability, following S1, M1 and DLPFC stimulation, confirmed the functional connectivities between these cortical sites involved in pain processing. Furthermore, increasing the level of STh/PTh after c-tDCS of these sites indicated that stimulation of not only M1 but also S1 and DLPFC could be considered a technique to decrease the level of pain in patients.

Introduction

The processing of painful stimuli is a complex phenomenon in which a network of brain areas is activated (Almeida *et al.*, 2004; Anderson *et al.*, 2006; Iannetti & Mouraux, 2010). Primary (S1) and secondary (S2) somatosensory cortices, the thalamus and the posterior part of the insula – collectively called the lateral pain system – are responsible for sensory discrimination of pain (Chen *et al.*, 2013). In contrast, the anterior cingulate cortex (ACC) and anterior part of the insula – the medial pain system – are involved in the affective-motivation processing of pain (Lang *et al.*, 2004; Kulkarni *et al.*, 2005; Vaseghi *et al.*, 2014). The cognitive aspect of pain is related to dorsolateral prefrontal cortex (DLPFC) activity (Apkarian *et al.*, 2005). Recent studies have shown that the primary motor cortex (M1) is also involved in pain modulation (Talbot *et al.*, 1991; Casey, 1999; Vogt & Sikes, 2000; Ploner & Schnitzler, 2004; Garcia-Larrea & Peyron, 2007; Tang *et al.*, 2009). Some other areas of the brain, including the periaqueductal grey matter system and nucleus cuneiformis, also play a major role in pain modulation (Chen *et al.*, 2013). Involvement of these areas in pain processing occurs in a large distributed neural network called the pain neuromatrix (PNM) (Iannetti & Mouraux, 2010). Although functional magnetic resonance imaging (fMRI) studies indicate functional connectivities between different areas of the PNM (Peyron *et al.*, 2000), it is unclear how alteration of excitability in one of these areas affects excitability in the others.

Cathodal transcranial direct current stimulation (c-tDCS) is a non-invasive neuromodulatory technique, which conventionally reduces excitability of cortical areas as well as specific superficial areas of the PNM such as S1, M1 and DLPFC. As fMRI studies indicate that experience of pain coincides with hyperactivity of both cortical and subcortical PNM sites (Peyron *et al.*, 2000; Bornhovd *et al.*,

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2002; Apkarian *et al.*, 2005), it is rational to hypothesise that any reduction in excitability of these sites by c-tDCS will reduce pain (Koyama *et al.*, 2005; Anderson *et al.*, 2006). c-tDCS has been applied to M1 and S1 in healthy individuals (Terney *et al.*, 2008; Csifcsak *et al.*, 2009; Bachmann *et al.*, 2010; Grundmann *et al.*, 2011), resulting in decreased brain excitability under the stimulated areas (Nitsche & Paulus, 2000; Matsunaga *et al.*, 2004) and increased sensory (STh) and pain (PTh) threshold perception in healthy individuals (Bachmann *et al.*, 2010; Grundmann *et al.*, 2011). In addition, some evidence shows the efficacy of c-tDCS on pain reduction in patients with chronic (Antal *et al.*, 2011; Naylor *et al.*, 2014) and acute pain (Antal *et al.*, 2008) following c-tDCS of M1 and DLPFC. However, new evidence suggests that inhibitory effects of c-tDCS may shift to excitatory effects by modulation of c-tDCS parameters such as current intensity and duration (Batsikadze *et al.*, 2013), site of stimulation (Boros *et al.*, 2008), and repetition (Monte-Silva *et al.*, 2010).

Overall, the physiological interactions between the different cortical pain-related areas of the brain (M1, S1 and DLPFC) are poorly understood. Schabrun *et al.* (2013) conducted one of the few studies to address this issue, investigating the temporal relationship between M1 and S1; they found non-linear activation of M1 and S1 during and after acute pain (Schabrun *et al.*, 2013). However, no research exists on the effects of c-tDCS of DLPFC on M1 and S1 excitability and pain processing (Laurent *et al.*, 2000; Kulkarni *et al.*, 2005).

Pain is a continuous experience interpreted by some behavioural responses (Tracey & Mantyh, 2007) such as STh and PTh (Bornhovd *et al.*, 2002; Giesecke *et al.*, 2005; Fernandez-de-Las-Penas *et al.*, 2010). STh and PTh are not closely correlated (Wolff, 1964). Some tDCS researchers have reported that the inhibitory effects of c-tDCS increased the level of STh and/or PTh and decreased pain level in patients (Apkarian *et al.*, 2005; Bachmann *et al.*, 2010; Grundmann *et al.*, 2011; Ahumada *et al.*, 2013), while others found no such effects (Bachmann *et al.*, 2010; Grundmann *et al.*, 2011). The effect of c-tDCS of DLPFC on STh/PTh is yet to be definitively explained.

In the current study, we aimed to explore the effects of c-tDCS of M1, S1 and DLPFC on M1 and S1 excitability, STh, and PTh level. The secondary aim was to evaluate the placebo effects of c-tDCS on these cortical sites. Given the basic mechanisms behind the efficacy of c-tDCS (Terney *et al.*, 2008; Medeiros *et al.*, 2012), we hypothesised that c-tDCS of M1, S1 and DLPFC would decrease S1 and M1 excitability levels. The results of a meta-analysis study conducted by our group (Vaseghi *et al.*, 2015) led us to hypothesise that c-tDCS of these cortical sites would increase both STh and PTh levels.

Method

Study design

We conducted a double-blind, randomised, sham-controlled crossover study to determine the site-specific effect of a single session of c-tDCS on M1 and S1 excitability, and STh and PTh in healthy volunteers. This study conformed to the ethical standards of the *Declaration of Helsinki* and was approved by the institutional ethics committee of Monash University, Australia. It was registered as a clinical trial on the Australian New Zealand Clinical Trial (registry number: ACTRN12614000817640, http://www.anzctr.org.au/) after enrolment of all participants. The authors confirm that all ongoing and related tDCS studies are also registered with this body.

Participants

Between October and December 2013, we conducted 60 experiments involving 12 healthy volunteers (four men and eight women, all Monash University students) with a mean age of 23.6 $\pm$ 5.3 years (age range 20–34 years). All were right-handed as determined by the Edinburgh Handedness Inventory (10-item version, mean laterality quotient = 87.9 $\pm$ 10.5) (Oldfield, 1971). Eligibility criteria were: age between 18 and 35 years; no clinically significant or unstable medical, neuropsychiatric or chronic pain disorders; no history of substance misuse or dependence; no use of central nervous system-effective medication; and no history of brain surgery, tumour or intracranial metal implantation. All participants were interviewed and examined by a physician prior to enrolment in the study and provided written, informed consent. The progress of the clinical trial through various phases (Enrolment, Allocation, Follow-up and Analysis) is shown in Fig. 1.

Experimental procedures

All 12 healthy participants received intervention with tDCS under each of four different experimental conditions, namely c-tDCS of M1, S1 and DLPFC, and sham in a random order (Fig. 2). At least 72 h was allowed between experimental sessions for each participant to avoid any interference or carry-over effect. The experiments were also completed at the same time of day (mornings or early afternoon) to avoid diurnal variation. The participants were exposed to the tDCS for 20 min in all experiments. Motor evoked potentials (MEPs), somatosensory evoked potentials (SEPs), STh, PTh to peripheral electrical stimulation, and PTh to pressure stimulation (P_pTh) were assessed before (T_{pre}), immediately after (T_0) and 30 min after (T_{30}) each intervention. Participants were blinded to the tDCS conditions (sham or active). Two researchers were involved in the current study, one as an assessor and the other as tDCS administrator. The assessor, responsible for data collection and data analysis, was blinded to all experimental conditions, the stimulated targets in sham and active conditions, and the feelings of participants during the intervention. The tDCS administrator was not aware of the active tDCS/sham conditions and the stimulation targets in each case.

c-tDCS of superficial regions of PNM

c-tDCS was administered through an active saline-soaked surface sponge electrode (2 $\times$ 1.5 cm) over the target area and a reference electrode (3 $\times$ 4 cm) over the right contralateral supraorbital area (Nitsche & Paulus, 2000). The larger size for the reference electrode decreases current density (CD) and reduces side effects under the indifferent electrode due to more focused density under the cathode (Nitsche *et al.*, 2007). The tDCS stimulator (Intelect® Advanced Therapy System; Chattanooga, Vista, CA, USA) was programmed to deliver 0.3 mA direct current for 20 min, with 10 s of linear fade in and fade out. The electrodes were fixed with two straps, one horizontal and one perpendicular.

Current intensity was set at 0.3 mA, which enabled us to considerably decrease the size of the active electrode (Uy & Ridding, 2003; Bastani & Jaberzadeh, 2013) while keeping the CD in a safe range with limited side effects (Poreisz *et al.*, 2007; Brunoni *et al.*, 2011) and highly focused stimulation (Bastani & Jaberzadeh, 2013). This intensity level provides superior induction of corticospinal excitability (CSE) (Nitsche & Paulus, 2001; Brunoni *et al.*, 2011; Pellicciari *et al.*, 2013; Parazzini *et al.*, 2014).

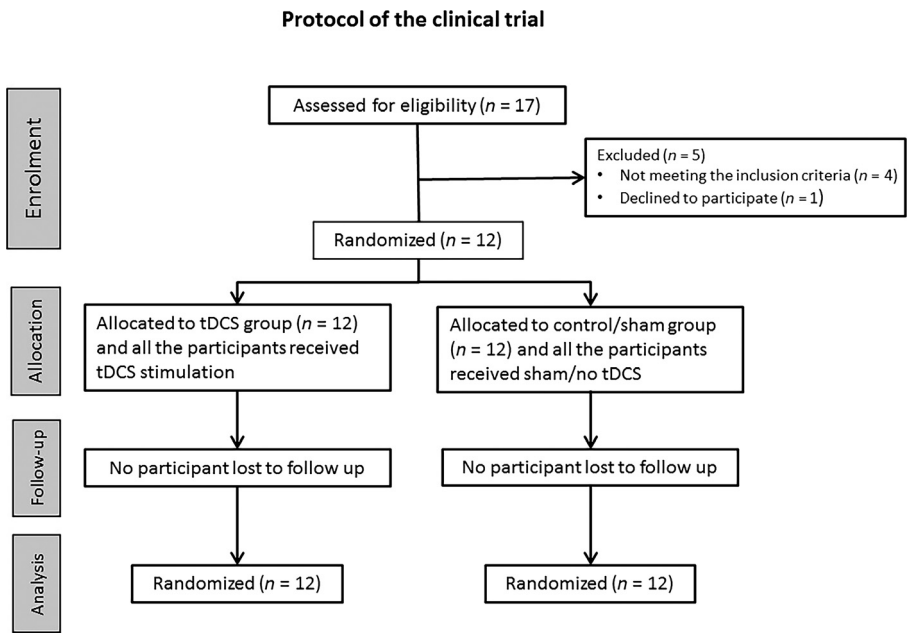

FIG. 1. Flow diagram of the progress through the phases (Enrolment, Allocation, Follow-up, and Analysis) of the randomised clinical trial of transcranial direct current stimulation (tDCS) and sham/control groups.

For c-tDCS of M1 and S1, the cathode was placed over C3 and C′3 (2 cm posterior to C3). For c-tDCS of DLPFC, the cathode was placed over F3. The reference electrode (anode) was conventionally placed over the contralateral supraorbital area with the assumption of no or negligible neuromodulatory effects on the subgenual cortex. All participants received the sham condition, while the electrodes were randomly placed in the same positions as for active M1 or S1 stimulation. Eight participants received sham c-tDCS over M1 and the other four participants received it over S1. The assessor was unaware of the stimulation targets in the sham condition. The tDCS administrator turned the machine on, using identical tDCS parameters, and turned it off after 30 s of stimulation without participants' knowledge, keeping the machine's display outside participants' sight.

As skin redness was the key indicator for the assessor to distinguish between the active and sham conditions, the tDCS administrator marked all the stimulation targets and supraorbital area with a red marker and tried to spread out all marks, using a skin cream immediately after removing the electrodes. As a result, skin redness under the electrodes, which could be used by the assessor to distinguish between the active and sham conditions, was masked. All pre- and post-stimulation evaluations followed an identical process.

Measurement of side effects

To record adverse or side effects of stimulation, all participants were asked to complete a questionnaire during and after all experimental conditions. The questionnaire contained rating scales for the presence and severity of side effects such as itching, tingling and burning sensations under electrodes (Nitsche *et al.*, 2008; George & Aston-Jones, 2010) and other adverse effects, including headache and pain during and after stimulation. All participants rated the unpleasantness of any scalp sensation using numeric analog scales (e.g. 0 = no tingling to 10 = worst tingling imaginable). The participants were asked to speculate if they received active or sham stimulation in each condition to assess the integrity of blinding in participants.

M1 excitability measurement

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 2002 (Magstim Company, Whiteland, UK) stimulator with a flat 70-mm figure-of-eight standard magnetic coil (peak magnitude field 2.2 T). The vertex (Cz) point was measured and marked to be used as a reference (Schwartz & Andrasik, 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. A scalp site optimal for evoking an MEP in the first dorsi interosseous (FDI) muscle of the right hand was found and marked as a reference. The coil position and orientation were constantly checked during the experiment to ensure that no changes occurred.

MEP resting threshold (RT) was tested in steps of 2% maximum stimulator output (Nitsche & Paulus, 2000), and defined as the lowest intensity for which five of 10 successive MEPs exceeded 50 μV (rest) peak-to-peak amplitude (Rossini *et al.*, 1994; Hallett, 1996; Wassermann *et al.*, 2008). For all further MEP measurements, the transcranial magnetic stimulation (TMS) intensity was set at 120% of each individual's RT. Fifteen stimuli (10 s apart) were elicited to assess corticospinal excitability of M1 at each time point. The stimulus intensity remained constant throughout the study session for each participant.

Surface electromyogram (EMG) was recorded from the right FDI 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 (Gilmore & Meyers, 1983; Schwartz & Andrasik, 2003). The location of FDI was determined based on anatomical landmarks (Perotto & Delagi, 2005) and also observation of muscle response in the testing position (abduction of index finger) (Kendall *et al.*, 1993). The accuracy of EMG electrode placement was verified by asking the participant to contract the muscle of interest while the investigator monitored

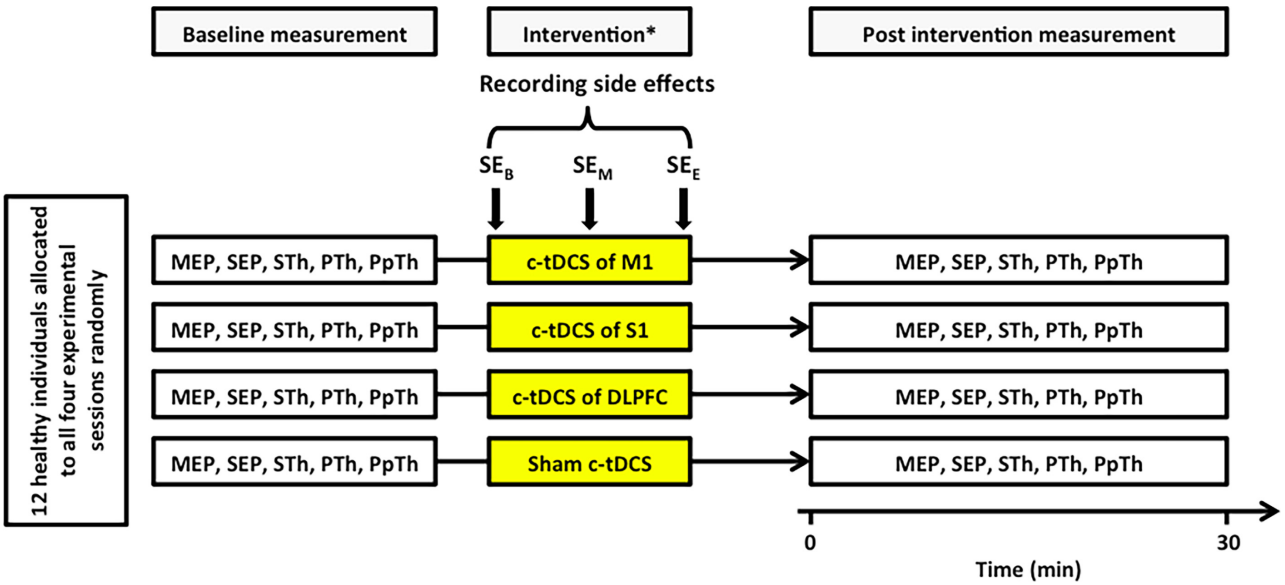

FIG. 2. Experimental design. *Cathodal tDCS with current intensity of 0.3 mA, active electrode size of 3 cm^2, reference electrode size of 12 cm^2, and duration of 20 min. MEP, motor evoked potential recorded by single-pulse transcranial magnetic stimulation; SEP, sensory evoked potential; STh, sensory threshold; PTh, pain threshold; PpTh, pressure pain threshold; c-tDCS, cathodal transcranial direct current stimulation; M1, primary motor cortex; S1, primary sensory cortex; DLPFC, dorsolateral prefrontal cortex; $SE_{(B, M, E)}$, side effects at beginning, middle, and end of intervention.

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, 3M Health Care Ltd, Leicestershire, UK). 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, ADInstruments, Bella Vista, NSW, Australia) via a laboratory analog–digital interface (The PowerLab 8/30; ADInstruments). Peak-to-peak MEP amplitude was detected and measured automatically using a custom-designed macro in Powerlab 8/30 software after each magnetic stimulus.

S1 excitability measurement

SEPs were recorded following electrical stimulation of the right median nerve at the wrist level at 2 Hz with pulse width of 0.2 ms (Boggio *et al.*, 2008b). The stimulus intensity was fixed at the motor threshold (Boggio *et al.*, 2008b). At this intensity, SEPs were recorded from S1 using electroencephalography electrodes. One electrode was located over the C′3 and the reference electrode was placed over Fz (Matsunaga *et al.*, 2004; Fukuda, 2006). The electrical potentials were recorded in epochs from 0 to 200 ms after the stimulus. A block of 500 stimulus-related epochs was recorded at each time point. The average peak-to-peak amplitudes of N20–P25 responses were compared before and after tDCS stimulation in different areas of the PNM (Ragert *et al.*, 2004).

To expose the S1 and M1 areas for application of c-tDCS and to record TMS-induced MEPs, the SEP recording electrodes were removed at each time point. The reliability of SEP electrode placement and reproducibility of SEP responses were tested in a pilot study before starting the main study.

Measurement of STh and PTh

All evaluations were performed at T_{pre}, T_0 and T_{30} by a blinded rater. The primary outcomes were STh and PTh for electrical stimulation. Electrical stimulation was applied by a pen electrode (model: 2762CC; Chattanooga) to the right median nerve (pulse duration – 200 μs) at wrist level. Current supply started at 0 mA and was increased in steps of 0.1 mA until the participant reported sensation and pain. The intensity of current at which perception of the electrical stimulus was first reported was taken as STh; the intensity of current at which participants first reported pain was taken as PTh. These measurements were averaged for analysis.

Measurement of P_pTh

Pressure was induced using a pressure algometer (model: FDX 50; Wagner, Greenwich, CT, USA; capacity: 50 × 0.05 lbf, accuracy: ± 0.3% of full scale) with a flat circular metal probe dressed in a plastic cover. Force was displayed digitally in increments of 0.1 N. The algometer was mounted vertically. For each measurement the algometer was calibrated to enable force to be applied at a controlled and steady rate. P_pTh was defined as the amount of force required to elicit a sensation of pain distinct from pressure or discomfort (Fischer, 1987). The P_pTh measurement point was marked in the middle of the belly of the FDI muscle (Chesterton *et al.*, 2002).

Participants were instructed in the application of the algometer and given a demonstration. They then underwent two practice measurements on their non-dominant side. Participants were asked to say 'stop' immediately when a discernible sensation of pain, distinct from pressure of discomfort, was felt; at this point, the experimenter retracted the algometer (Fischer, 1986a,b). The digital display continued to show the value of pressure applied at the moment the algometer was retracted. The algometer was applied perpendicularly to the skin and lowered at approximately 5 N/s until P_pTh was reached (Fischer, 1987), as detected by participants' verbal report. At each time point, three PpTh values were measured approximately 10–15 s apart and then averaged for analysis.

Data analysis

The researchers who recorded the outcome measures and analysed the data were blinded to the experimental conditions. The post-inter-

vention means were normalized intra-individually and are given as ratios of the baseline (Rossini *et al.*, 1994). One-way repeated measures analysis of variance (ANOVA) at T_{pre} of all conditions was used to detect any carry-over effect at the starting point for each session.

A two-way repeated measures ANOVA was used to assess the effects of two independent variables, experimental conditions (c-tDCS of S1, M1, DLPFC and sham) and time points (T_{pre}, T_0 and T_{30}), on MEPs, SEPs, STh, PTh and P_pTh. Mauchly's test was used to assess the validity of the sphericity assumption for repeated-measures ANOVA. Greenhouse–Geisser corrected significance values were used when sphericity was lacking (Meyers *et al.*, 2006). Additionally, to test whether the baseline value of each stimulation site differed significantly from post-intervention time points (T_0, T_{30}), a one-way repeated-measures ANOVA was applied. In case of significant main effects, *post-hoc* comparisons were performed, using Bonferroni. Means are reported ± SE. To assess whether participants were successfully blinded to the stimulation conditions (active or sham), Pearson's chi-square test was used. In addition, a one-way ANOVA was carried-out on the averaged rating scale scores recorded in the questionnaires to assess any significant differences between the participants' feelings during active and sham conditions. Statistical analyses were performed using SPSS software version 22.

Results

Safety and side effects of c-tDCS

All participants tolerated the applied currents in different conditions very well and there was no interruption of experimental procedures due to adverse or side effects of the applied currents. Table 1 summarises the numerical values for the reported side effects under anode and cathode electrodes in all experimental sessions. Itching and tingling were experienced by all participants in active sessions. Tingling and itching were also felt by all participants at the beginning and in the middle of sham condition. No participant reported side effects after the end of stimulation. No reports of burning sensations, headaches or pain were recorded during or after stimulation.

The participants' judgment on the stimulation conditions is summarized in Table 2. Pearson's chi-square test showed no significant differences between the active and sham conditions [χ^2_6 (n = 12) = 1.95, P = 0.17] in the majority of participants, and just two participants (14% of conditions) guessed the active conditions correctly (excluding the 'Cannot say' responses).

The results of the one-way ANOVA indicated that sensations were significantly different across the conditions ($F_{3,44}$ = 33.71, P < 0.001). The *post hoc* comparisons showed that there was no significant difference between sensations experienced by participants in sham and active conditions under the cathode electrode [except between sham and S1 stimulation (P = 0.003) at T_{30}]. Under the reference electrode, there was no significant difference between active and sham conditions.

Comparison of baseline values

One-way repeated-measures ANOVA showed that baseline values of dependent variables (peak-to-peak amplitude of MEPs and of N20–P25 of SEPs) remained unchanged in multiple sessions of assessment for all experimental conditions. This indicates that the washout period was adequate and refutes any possibility of carry-over effects from previous interventions on the same participants.

The effects of c-tDCS of M1, S1 and DLPFC on M1 excitability

The two-way repeated-measures ANOVA results indicate a significant main effect for the site of stimulation and time. *Post hoc* comparisons showed that c-tDCS of M1 and S1 significantly decreased the size of MEPs at T_0 and T_{30}. c-tDCS of DLPFC resulted in reduction of MEP sizes immediately after stimulation (Table 3). The post-hoc comparison results also demonstrated that there was no significant difference between the three sites of stimulation at T_0 (Table 4). Comparing MEP size following c-tDCS of M1, S1 and DLPFC at T_{30} demonstrated significant differences between M1-DLPFC and S1-DLPFC. In addition, one-way repeated-measures ANOVA was used

TABLE 1. Numerical sensation score by participants during experimental conditions

	Cathode electrode				Reference electrode			
	M1	S1	DLPFC	Sham	M1	S1	DLPFC	Sham
Tingling sensation								
Beginning	3.2 ± 0.16	3.1 ± 0.27	4.3 ± 0.21	2.1 ± 0.18	2.1 ± 0.16	3.2 ± 0.11	2.7 ± 0.21	1.7 ± 0.09
Middle	2.4 ± 0.25	2.1 ± 0.38	1.8 ± 0.25	0.8 ± 0.43	2.0 ± 0.21	2.6 ± 0.12	2.0 ± 0.06	0.8 ± 0.10
End	0.7 ± 0.15	0.6 ± 0.12	0.9 ± 0.20	0.1 ± 0.01	1.1 ± 0.18	1.8 ± 0.12	1.4 ± 0.17	0.3 ± 0.08
Itching sensation								
Beginning	2.7 ± 0.11	3.2 ± 0.21	2.1 ± 0.08	1.2 ± 0.31	1.3 ± 0.19	1.7 ± 0.16	1.5 ± 0.09	0.8 ± 0.10
Middle	1.3 ± 0.19	1.2 ± 0.2	1.3 ± 0.12	0.2 ± 0.05	0.7 ± 0.11	0.3 ± 0.08	0.5 ± 0.06	0.2 ± 0.01
End	0.4 ± 0.07	0.3 ± 0.09	0.4 ± 0.1	–	0.4 ± 0.10	–	0.2 ± 0.02	–
Burning sensation								
Beginning	–	–	–	–	–	–	–	–
Middle	–	–	–	–	–	–	–	–
End	–	–	–	–	–	–	–	–
Not tolerated								
Beginning	–	–	–	–	–	–	–	–
Middle	–	–	–	–	–	–	–	–
End	–	–	–	–	–	–	–	–

The values are rated using the numeric analog scale. 0 is rated as no sensation and 10 rated as the worst sensation imaginable. The sensations are recorded during three phases of stimulation: Beginning (0–7 min of stimulation), Middle (7–14 min of stimulation), End (14–20 min of stimulation). Sensations under both active (anode) and reference (cathode) electrodes were recorded during a-tDCS of primary motor cortex (M1), primary sensory cortex (S1), dorsolateral prefrontal cortex (DLPFC) and sham tDCS. Scores are reported as mean ± SEM

TABLE 2. The judgements of participants on the stimulation condition

	Actual testing conditions (n = 12)				
Perceived stimulation	c-tDCS of M1	c-tDCS of S1	c-tDCS of DLPFC	Sham	Total
Active	2	2	4	2	10
Sham	2	3	2	1	8
Cannot say	8	7	6	9	30
Total	12	12	12	12	48

to compare baseline values with those at T_0 and T_{30}, showing significant differences immediately after c-tDCS of M1, S1 and DLPFC. The results also demonstrated a significant decrease 30 min after c-tDCS of M1 and S1. The post-hoc comparisons also detected a significant difference between sham and active conditions (Fig. 3A).

To assess the lasting effects of MEP changes, a one-way repeated-measures ANOVA was carried out for each condition. The results indicated significant lasting effects following c-tDCS of M1 ($F_{3,33}$ = 6.07, P = 0.01), S1 ($F_{3,33}$ = 4.25, P = 0.03) and DLPFC ($F_{3,33}$ = 7.63, P = 0.02). The results of post-hoc comparisons are summarized in Table 4.

The effects of c-tDCS of M1, S1 and DLPFC on S1 excitability

A two-way repeated-measures ANOVA revealed significant main effects of stimulation site and time (Table 3). The result of *post hoc* comparisons showed that the size of N20–P25 amplitude decreased immediately and 30 min after c-tDCS of M1, S1 and DLPFC (Fig. 3B). In addition, the results indicated a significant difference between c-tDCS of M1-DLPFC at T_0, and M1-S1 and S1-DLPFC at T_{30}. The post-hoc comparisons also showed a significant difference between sham and active conditions (Fig. 3B).

Furthermore, the result of one-way repeated-measures ANOVA indicated significant N20–P25 amplitude changes following M1 c-tDCS ($F_{3,33}$ = 4.68, P = 0.02), S1 ($F_{3,33}$ = 9.27, P = 0.01) and DLPFC ($F_{3,33}$ = 2.36, P = 0.02). The results of the post-hoc comparison are summarized in Table 4.

The effects of c-tDCS of M1, S1, DLPFC on STh

STh analysis showed significant main effects of stimulation site, time and interaction of site by time (Table 2). STh was significantly increased by c-tDCS of all sites of stimulation at T_0 and T_{30} (Fig. 4). Comparing the level of STh following c-tDCS of M1, S1 and DLPFC at T_0 and T_{30} revealed a significant difference between S1 and DLPFC at T_{30}.

As can be seen in Fig. 4, STh values following active c-tDCS of all sites were significantly higher than those for sham tDCS.

The effects of c-tDCS of M1, S1, DLPFC on PTh

PTh analysis showed significant main effects of stimulation site and time (Table 3). Post-hoc comparisons revealed no significant difference between c-tDCS of M1, S1 and DLPFC at T_0, while at T_{30} there was a significant difference in PTh between M1-DLPFC and S1-DLPFC (Fig. 4).

Compared with baseline values, a significant PTh increase was observed immediately and 30 min after c-tDCS of three superficial areas of the PNM, except c-tDCS of DLPFC at T_{30} (Table 4). In addition, a statistically significant PTh difference was detected between active and sham tDCS (except 30 min after c-tDCS of DLPFC).

TABLE 3. ANOVA results for the effects of c-tDCS on the MEP and SEP sizes and the level of STh, PTh, and P_pTh

	d.f.	F-value	P-value
MEP size			
Stimulation site	4	17.8	0.001
Time	2	26.2	0.000
Stimulation site × Time	8	8.3	0.000
MEP sizes at T_0	4	25.4	0.000
MEP sizes at T_{30}	4	13.3	0.002
SEP size			
Stimulation site	4	7.3	0.005
Time	2	8.7	0.03
Stimulation site × Time	8	9.4	0.001
SEP sizes at T_0	4	7.8	0.001
SEP sizes at T_{30}	4	8.34	0.002
STh			
Stimulation site	4	3.6	0.01
Time	2	17.6	0.000
Stimulation site × Time	8	2.5	0.01
STh sizes at T_0	4	4.4	0.01
STh sizes at T_{30}	4	8.2	0.002
PTh			
Stimulation site	4	4.6	0.03
Time	2	10.4	0.005
Stimulation site × Time	8	7.4	0.01
PTh sizes at T_0	4	5.2	0.02
PTh sizes at T_{30}	4	9.8	0.004
P_pTh			
Stimulation site	4	3.1	0.21
Time	2	1.4	0.09
Stimulation site × Time	8	2.8	0.09
P_pTh sizes at T_0	4	2.9	0.13
P_pTh sizes at T_{30}	4	2.8	0.11

The effects of c-tDCS of M1, S1 and DLPFC on P_pTh

Two-way repeated-measures ANOVA was conducted to compare the effects of four different conditions on P_pTh at three time points. The results revealed no significant main effects of time, site of stimulation, and interaction of stimulation site by time (Table 3). *Post hoc* comparison results also showed no significant difference between sham and active c-tDCS for any stimulation site (Table 4).

Discussion

Safety and side effects of c-tDCS

We hypothesised that the c-tDCS parameters would be adequate for a successful blinding with minimum side effects. The findings support this hypothesis. We found that the use of c-tDCS with small electrodes (1.5 × 2 cm) and current intensity of 0.3 mA (current density of 0.1 mA/cm^2) caused minimal or no side effects in healthy individuals. Similar to previous tDCS studies (Poreisz *et al.*, 2007; Nitsche *et al.*, 2008; Brunoni *et al.*, 2011), the most prevalent side effects following the stimulation of M1, S1 and DLPFC were itching and tingling. No adverse effects such as seizure, headaches or nausea were recorded.

Participants were not able to distinguish between the active or sham conditions under the active electrode (except in S1 stimulation condition at T_{30}). This indicates that the blinding was successful. In addition, the lack of a significant difference in rating scales under the reference electrode in sham and active conditions demonstrated that blinding was adequate during the conditions. Based on the results of some studies (Wood *et al.*, 2008; Hrobjartsson & Gotzsche, 2010), inadequate blinding may lead to exaggeration in subjective outcomes.

TABLE 4. The effects of different experimental conditions (c-tDCS of M1, S1, DLPFC, sham and no condition) on the size of MEPs/SEPs and the level of STh, PTh, P_pTh

	MEP	SEP	STh	PTh	P_pTh
M_1 tDCS					
T_0*	0.6 ± 0.06	0.87 ± 0.17	1.41 ± 0.23	1.57 ± 0.17	1.2 ± 0.37
T_{30}*	0.53 ± 0.11	0.76 ± 0.26	1.52 ± 0.28	1.46 ± 0.21	1.32 ± 0.38
P (T_{pre}–T_0)	0.002	0.03	0.02	0.012	0.41
P (T_{pre}–T_{30})	0.002	0.02	0.02	0.002	0.23
P (T_0–T_{30})	0.07	0.12	0.35	0.12	0.74
S_1 tDCS					
T_0	0.57 ± 0.07	0.78 ± 0.11	1.44 ± 0.37	1.44 ± 0.08	1.27 ± 0.68
T_{30}	0.54 ± 0.05	0.69 ± 0.18	2.1 ± 0.41	1.55 ± 0.09	1.31 ± 0.75
P (T_{pre}–T_0)	0.01	0.03	0.04	0.000	0.23
P (T_{pre}–T_{30})	0.01	0.01	0.03	0.000	0.62
P (T_0–T_{30})	0.52	0.04	0.78	0.07	0.72
DLPFC tDCS					
T_0	0.51 ± 0.05	0.72 ± 0.05	1.60 ± 0.2	1.25 ± 0.12	1.05 ± 0.06
T_{30}	1.01 ± 0.07	0.82 ± 0.07	1.45 ± 0.16	1.26 ± 0.09	1.22 ± 0.12
P (T_{pre}–T_0)	0.000	0.01	0.02	0.02	0.41
P (T_{pre}–T_{30})	0.22	0.03	0.03	0.06	0.11
P (T_0–T_{30})	0.000	0.07	0.21	0.21	0.12
Sham tDCS					
T_0	1.05 ± 0.05	0.97 ± 0.01	1.08 ± 0.08	1.17 ± 0.2	1.08 ± 0.12
T_{30}	1.1 ± 0.02	0.98 ± 0.02	1.14 ± 0.30	1.18 ± 0.17	1.21 ± 0.31
P (T_{pre}–T_0)	0.06	0.23	0.30	0.14	0.26
P (T_{pre}–T_{30})	0.053	0.45	0.14	0.08	0.05
P (T_0–T_{30})	0.11	0.77	0.35	0.34	0.06

*Mean ± SE changes compared with baseline values. As the mean values are normalized to baseline, the mean and post-intervention values are given as ratios of the baseline, and the value of *T*pre is considered as 1.
c-tDCS, cathodal transcranial direct current stimulation; DLPFC, dorsolateral prefrontal cortex; MEPs, motor evoked potentials; SEPs, somatosensory evoked potentials; STh, sensory threshold; PTh, pain threshold; P_pTh, pressure pain threshold.

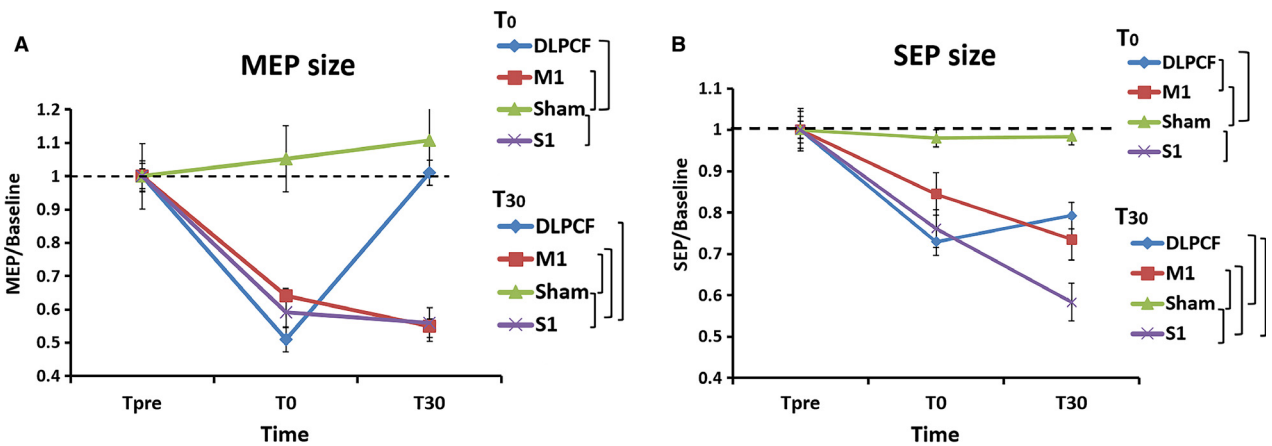

FIG. 3. The effects of c-tDCS over different stimulation sites on the peak-to-peak amplitude of MEPs (A), and the peak-to-peak amplitude of N20–P25 of SEPs (B) over time. Filled symbols indicate significant deviation of the post-transcranial stimulation motor evoked potential and SEP amplitudes relative to the baseline; the brackets show significant differences between different testing conditions. Data are reported as mean ± SEM.

Furthermore, regarding the significant difference of participants' feeling in sham and S1 stimulation conditions, it is recommended to interpret the impact of c-tDCS on STh/PTh in healthy adults.

Comparison of active vs. sham tDCS

Comparison of all three active c-tDCS conditions with the sham condition indicated that c-tDCS has no placebo effects on either M1/S1 excitability or STh/PTh at any time point. This indicates that the observed changes following application of active conditions are real effects of c-tDCS.

The effects of c-tDCS of M1, S1 and DLPFC on M1 excitability

The results of the present study partially support our hypothesis in which we assumed that the application of c-tDCS over M1, S1 and DLPFC reduces M1 excitability. M1 excitability reduction is in agreement with similar studies in this area (Monte-Silva *et al.*, 2010; Nitsche & Paulus, 2011; Medeiros *et al.*, 2012) that show the inhibitory effect of c-tDCS is caused by local changes in ionic concentration and alteration of transmembrane proteins under the stimulated area (Ardolino *et al.*, 2005). However, some studies show

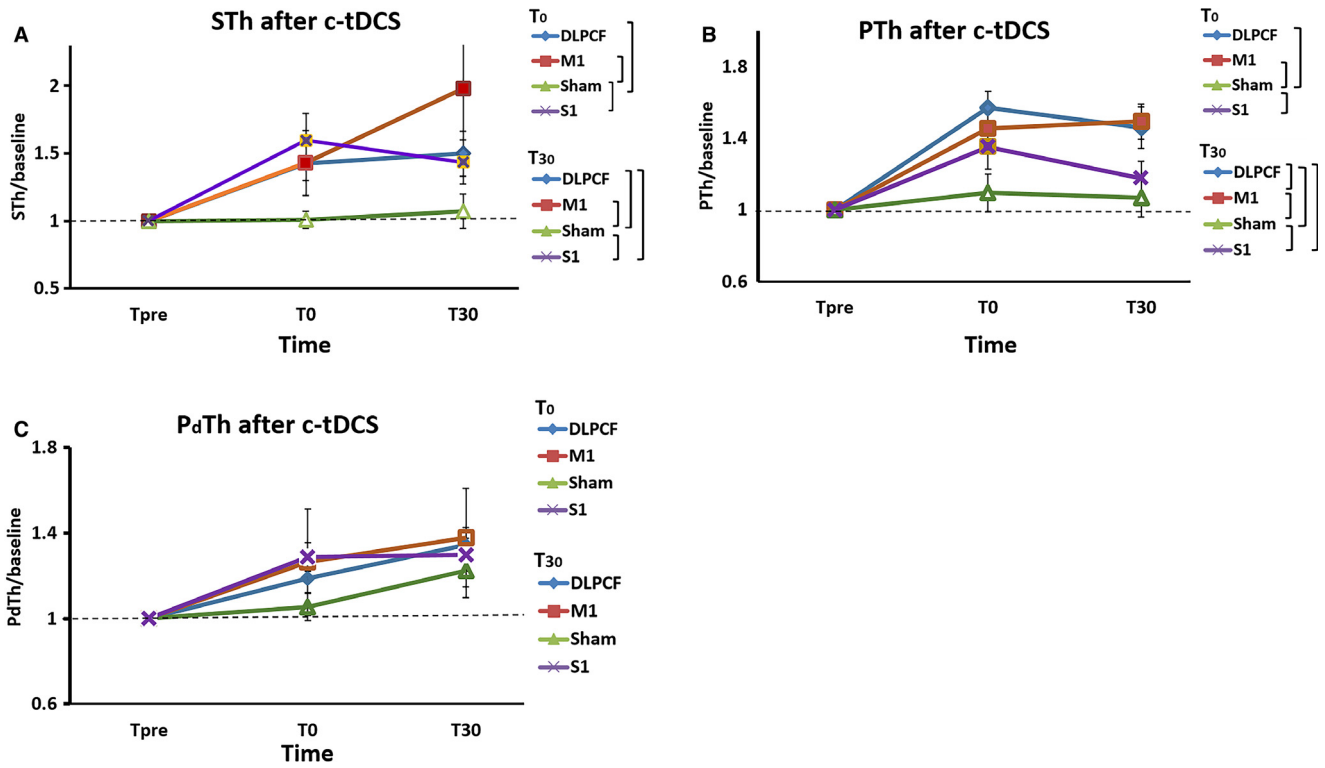

FIG. 4. The effects of c-tDCS over different stimulation sites on the sensory threshold (STh) (A), pain threshold (PTh) (B) and pressure pain threshold (P_pTh) (C) over time. Filled symbols indicate significant deviation of the post-transcranial stimulation STh, PTh, P_pTh relative to the baseline; the brackets show significant differences between different testing conditions. Data are reported as mean ± SEM.

the opposite effects following c-tDCS of M1 (Nitsche *et al.*, 2005; Medeiros *et al.*, 2012; Batsikadze *et al.*, 2013). As shown in some methodological studies, different tDCS approaches such as different current intensity/density (Batsikadze *et al.*, 2013) or application time (Pirulli *et al.*, 2014) may induce both facilitatory and inhibitory effects.

M1 CSE reduction, which lasted at least for 30 min after S1 c-tDCS, also supports our hypothesis. To the best of our knowledge, no tDCS study has investigated the functional connectivities between S1 and M1. However, M1 and S1 excitability reduction following experimental pain are observed in some pain studies (Valeriani *et al.*, 1999; Suppa *et al.*, 2013). The cortico-cortical interconnections in superficial layers of M1 and S1 are likely to be crucial for sensory input processing in M1 and sensorimotor integration (Basmajian & De Luca, 1985; Oh, 2003). The other probable explanation for the M1 CSE reduction following S1 stimulation is the integration of information between cortical and subcortical sites of PNM. For instance, the contribution of thalamus and basal ganglia in the thalamo-cortico-basal ganglia loop has been identified in pain condition (Bingel *et al.*, 2002; Borsook *et al.*, 2010). As a result, it is possible that S1 c-tDCS affected the function of subcortical sites of PNM, which indirectly modulated the M1 excitability level.

The M1 CSE suppression was observed only immediately after c-tDCS of the DLPFC. As a result, our findings support our hypothesis in part. The suppression was short-lived; after 30 min, M1 CSE had returned to its baseline value. Based on some anatomical studies, the premotor cortex is divided into dorsal and ventral parts and the dorsal part sends its output to M1 and the spinal cord and receives prominent input from DLPFC (Hallett, 1996; Schwartz & Andrasik, 2003). Due to functional (Bantick *et al.*, 2002; Pellicciari *et al.*, 2013) and anatomical (Bantick *et al.*, 2002; Parazzini *et al.*, 2014) connectivities, c-tDCS of DLPFC may activate the DLPFC–premotor–primary motor pathway, which may lead to reduction of M1 excitability immediately after stimulation. Lack of lasting effect following c-tDCS of DLPFC may be explained by the role of DLPFC in short-term memory maintenance and executive functions (Schwartz & Andrasik, 2003; Nitsche *et al.*, 2007).

The effects of c-tDCS of M1, S1 and DLPFC on S1 excitability

Decreasing the N20–P25 amplitude after S1, DLPFC and M1 c-tDCS supports our hypothesis that c-tDCS of these cortical sites increased the S1 excitability level. We demonstrated that S1 c-tDCS decreases the amplitude of the N20–P25 component. The S1 excitability depression is in line with previous studies in which it was indicated that c-tDCS had inhibitory effects over the stimulated area (Dieckhofer *et al.*, 2006). However, in one study SEP components were not affected by 10 min of c-tDCS of S1 (Matsunaga *et al.*, 2004). Regarding the methodological differences in these studies, there are some possible reasons explaining the conflicting findings. First, the prolonged constant c-tDCS (20 vs. 10 min) increased the ionic concentration and inhibitory effects of c-tDCS under the active electrode (cathode). Second, the small size of the active electrode utilised in the current study (1.5 × 2 cm) compared with Matsunaga *et al.*'s (2004) study (5 × 7 cm) allowed more focused stimulation of the targeted cortical area. In Matsunaga *et al.*'s (2004) study, bigger electrodes are used in adjacent cortical areas, which may have had diverse effects on S1 excitability.

This study is the first to examine the impact of M1 c-tDCS on the level of S1 excitability in healthy individuals. Although there is no study to support or negate the results, there is some evidence showing that experimental pain leads to excitability reduction in

both S1 and M1 (Schabrun *et al.*, 2013; Suppa *et al.*, 2013). However, there is also some evidence of opposing results (Le Pera *et al.*, 2001; Svensson *et al.*, 2003; Martin *et al.*, 2008). In a pain study, Schabrun *et al.* (2013) concluded that S1 excitability decreases following painful conditions and S1 excitability reduction affects M1 processes, but the underlying mechanisms are not understood (Schabrun *et al.*, 2013). In addition, the current study is the first report of the effects of DLPFC c-tDCS on S1 excitability. In the present study, c-tDCS of DLPFC resulted in S1 excitability reduction. Suppression of S1 excitability after c-tDCS of M1 and DLPFC may indicate functional connectivities between different areas of the PNM (Peyron *et al.*, 2000; Iannetti *et al.*, 2005; Koyama *et al.*, 2005; Tracey & Mantyh, 2007), including cortico-subcortico-cortical and cortico-cortical loops. Decreased S1 excitability may have bidirectional inhibitory effects on cortico-thalamic fibres and consequently affect the thalamo-cortical activities (Jones *et al.*, 1978).

Effects of c-tDCS of M1, S1 and DLPFC on STh

Based on the result of a recent systematic review conducted by our group (Vaseghi *et al.*, 2015), we hypothesised that STh is increased after M1, DLPFC and S1 c-tDCS. The results demonstrated that c-tDCS of M1 and S1 led to increased STh for at least 30 min. This finding is in line with those of several other studies (Csifcsak *et al.*, 2009; Bachmann *et al.*, 2010; Grundmann *et al.*, 2011). Grundmann and colleagues reported that c-tDCS of S1 decreased the sensitivity of innocuous cold but not warm stimuli, which implies greater sensitivity of small unmyelinated fibres (Aδ) compared with other fibres carrying other thermal qualities (Ziegler *et al.*, 1988). In addition, Bachmann *et al.* (2010) showed that c-tDCS of M1 increases STh for both cold and mechanical stimuli (Bachmann *et al.*, 2010). Some repetitive TMS studies (Summers *et al.*, 2004; Oliviero *et al.*, 2005) found that stimulation of M1 increased the threshold for cold detection but not for warmth perception.

Our hypothesis was also supported by the STh increase following DLPFC stimulation. The current study is the first to report the effects of DLPFC c-tDCS on pain threshold. It seems that the functional connectivity of DLPFC with the insular cortex, ACC and other cortical areas of PNM (Peyron *et al.*, 2000; Casey *et al.*, 2001; Boggio *et al.*, 2008b; Heinze *et al.*, 2014) is a possible mechanism behind the efficacy of c-tDCS of DLPFC on STh increase.

Effects of c-tDCS of M1, S1 and DLPFC on PTh

Increasing the level of PTh following the application of c-tDCS over M1, S1 and DLPFC supported our hypothesis in which we hypothesised that stimulation of these cortical sites results in PTh increase. Previous studies found that c-tDCS of M1 increases mechanical PTh and laser-induced heat pain and has no effect on cold pain (Antal *et al.*, 2008; Bachmann *et al.*, 2010). In contrast, PTh remains unaltered following c-tDCS of M1, although warmth and cold detection thresholds increase significantly (Grundmann *et al.*, 2011). Few researchers have applied c-tDCS over S1 to investigate PTh changes in healthy individuals. Our results are supported by some studies, in which it is indicated that c-tDCS of S1 reduces subjective pain perception (Antal *et al.*, 2008; Antal & Paulus, 2010). In contrast, Grundmann *et al.* (2011) found that c-tDCS of S1 has no effect on PTh in healthy individuals (Grundmann *et al.*, 2011). To our knowledge, our study is the first to demonstrate that c-tDCS over DLPFC is able to increase PTh in healthy participants, although the mechanism behind its efficacy for PTh is unclear. Overall, the results indicate that the effect of c-tDCS is not site-specific. In contrast, the results of a recent meta-analysis demonstrated a site-specific effect of c-tDCS over cortical sites of PNM on STh/PTh modulation in healthy adults (Vaseghi *et al.*, 2015). Vaseghi *et al.* (2015) indicated that there are a limited number of studies investigating the inhibitory effects of c-tDCS on pain reduction in patients with different pathologies. Clearly, more investigations must be taken into consideration to crystalise the modulatory effects of c-tDCS on the function of the cortical and subcortical sites of PNM.

The results suggest no site-specific effect on STh and PTh following c-tDCS of M1, S1 and DLPFC. Among all cortical and subcortical sites of PNM involved in processing painful stimuli (Almeida *et al.*, 2004; Tracey & Mantyh, 2007), the involvement of the thalamus in sensory inputs (Craig *et al.*, 2000) and painful stimuli (Davis *et al.*, 1999; Casey *et al.*, 2001) processing has been shown by some positron emission tomography scan studies. As a result, it can be concluded that the thalamus is the main structure for sensory input processing (Summers *et al.*, 2004). Furthermore, regarding the extensive connections between S1, M1 and DLPFC and insula, and involvement of insula in sensory and painful input processing (Mesulam & Mufson,1982), activity modulation of the insular cortex by c-tDCS is another possible mechanism behind the STh and PTh enhancement with no site-specific effect.

Effects of c-tDCS of M1, S1 and DLPFC on P_pTh

The results of the current study revealed that applying c-tDCS over M1, S1 and DLPFC had no significant effect on P_pTh in healthy individuals. So, our hypothesis, in which we assumed that c-tDCS of these cortical sites increases P_pTh, was not supported by the results. These findings are in line with the results of other c-tDCS studies assessing the effects of M1 and S1 c-tDCS on mechanical quantitative sensory testing parameters such as mechanical pain sensitivity, pressure pain threshold and wind-up ratio (Rogalewski *et al.*, 2004; Bachmann *et al.*, 2010; Moloney & Witney, 2014). In contrast, Bachmann *et al.* (2010) reported that mechanical pain threshold significantly increased over baseline values following c-tDCS of M1. Temporarily impaired tactile detection following c-tDCS of M1 has also been reported (Rogalewski *et al.*, 2004; Bachmann *et al.*, 2010). Several reasons for these conflicting results exist. First, peripheral electrical stimulation recruits axons based on their diameter, starting with large-diameter fibres (Aβ fibres) (Stieglitz, 2005), whereas mechanoreceptors excite myelinated large Aβ and small Aδ fibres and may be processed through anatomically different pathways (Ohara *et al.*, 2004), which explains why c-tDCS of M1, S1 and DLPFC increased electrical STh and PTh but had no effect on P_pTh. Second, mechanoreceptive inputs from large Aβ and small Aδ fibres end at the thalamic ventral caudal nucleus via dorsal column medial pathways (Ohara *et al.*, 2004). As a result, it is likely that c-tDCS will affect P_pTh with higher current intensity or other methodological alterations.

Limitations of the study

Our study has some limitations – first, the correlation between the outcome measures was not evaluated, i.e. the correlations between cortical (MEP/SEP) and behavioural (STh/PTh) changes were not assessed. After the experiments and following consultation with a qualified statistician, we found that recruitment of more 39 participants is needed to measure the correlation between the cortical and behavioural changes. Given the number of conditions (four) and the length of each experiment (3 h) in the current study, and regarding the difficult nature of tDCS/TMS/SEP studies, it was practically

impossible to recruit the required number of participants. Therefore, assessment of correlation between these cortical and behavioural outcome measures has had to be postponed for future studies. Secondly, the data were obtained from a healthy population with no pain history, so the results may not necessarily be applicable to patients with pain conditions. Thirdly, the effects were evaluated in young participants (less than 35 years). Older individuals may respond differently to c-tDCS. Fourthly, some studies have reported gender differences in responses to c-tDCS (Knops *et al.*, 2006; Boggio *et al.*, 2008a; Chaieb *et al.*, 2008); as most of our participants were women, it is possible that gender influenced our results. In addition to this, the findings should be interpreted in light of the fact that the current study utilised a conventional electrode montage with an active electrode (cathode) over target areas and passive electrode (anode) over the contralateral supraorbital area (subgenual cortex) (Nitsche *et al.*, 2002; Ardolino *et al.*, 2005). Although the reference electrode was four times larger than the active one (the density was four times less) to minimise neuromodulatory effects under the reference electrode, in reality the subgenual cortex may still be affected by the very low density of currents under the anode (Kanda *et al.*, 2000; Miranda *et al.*, 2006; Mylius *et al.*, 2012).

Suggestions for future research

The present study was a pilot study to investigate the effects of c-tDCS of M1, S1 and DLPFC on M1/S1 excitability and the STh/PTh in healthy adults. As the relationship between these cortical and behavioural changes is extremely valuable, additional investigation is recommended to address this relationship. Our study did not assess the effects of tDCS on M1 and S1 excitability beyond 30 min. As long-lasting effects are clinically important, further studies are required to fully understand the effects of c-tDCS over superficial regions of the PNM. Moreover, due to the limited number of c-tDCS studies on pain modulation, more systematic investigations on c-tDCS parameters including application time, current intensity, electrode size and site of stimulation in both healthy participants and patients are required to provide optimal c-tDCS parameters for pain reduction. Furthermore, to reveal the mechanisms of action of c-tDCS of superficial PNM regions on the excitability of M1 and S1, the CSE of M1 should be studied by measuring silent period, intracortical inhibition and facilitation to indirectly assess the role of $GABA_A$, $GABA_B$ and glutamergic receptors. In addition, it is necessary to replicate our study using only male participants to investigate the potential influence of female hormones on our results.

Conflict of interests

This manuscript is based on research conducted by Bita Vaseghi, a PhD candidate at Monash University, Melbourne, Australia. This project had no external funding, and no financial or other relationships pose a conflict of interest.

Abbreviations

ACC, anterior cingulate cortex; ANOVA, analysis of variance; CD, current density; CSE, corticospinal excitability; c-tDCS, cathodal transcranial direct current stimulation; DLPFC, dorsolateral prefrontal cortex; EMG, electromyogram; FDI, first dorsi interosseous; fMRI, functional magnetic resonance imaging; M1, primary motor cortex; MEP, motor evoked potential; PTh, pain threshold; RT, resting threshold; S1, primary sensory cortex; SEP, somatosensory evoked potential; STh, sensory threshold; TMS, transcranial magnetic stimulation.

References

Ahumada, J., Fernandez de Sevilla, D., Couve, A., Buno, W. & Fuenzalida, M. (2013) Long-term depression of inhibitory synaptic transmission induced by spike-timing dependent plasticity requires coactivation of endocannabinoid and muscarinic receptors. *Hippocampus*, **23**, 1439–1452.

Almeida, T.F., Roizenblatt, S. & Tufik, S. (2004) Afferent pain pathways: a neuroanatomical review. *Brain Res.*, **1000**, 40–56.

Anderson, W.S., O'Hara, S., Lawson, H.C., Treede, R.D. & Lenz, F.A. (2006) Plasticity of pain-related neuronal activity in the human thalamus. *Prog. Brain Res.*, **157**, 353–364.

Antal, A. & Paulus, W. (2010) Effects of transcranial theta-burst stimulation on acute pain perception. *Restor. Neurol. Neuros.*, **28**, 477–484.

Antal, A., Brepohl, N., Poreisz, C., Boros, K., Csifcsak, G. & Paulus, W. (2008) Transcranial direct current stimulation over somatosensory cortex decreases experimentally induced acute pain perception. *Clin. J. Pain*, **24**, 56–63.

Antal, A., Kriener, N., Lang, N., Boros, K. & Paulus, W. (2011) Cathodal transcranial direct current stimulation of the visual cortex in the prophylactic treatment of migraine. *Cephalalgia*, **31**, 820–828.

Apkarian, A.V., Bushnell, M.C., Treede, R.D. & Zubieta, J.K. (2005) Human brain mechanisms of pain perception and regulation in health and disease. *Eur. J. Pain-London*, **9**, 463–484.

Ardolino, G., Bossi, B., Barbieri, S. & Priori, A. (2005) Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. *J. Physiol.-London*, **568**, 653–663.

Bachmann, C.G., Muschinsky, S., Nitsche, M.A., Rolke, R., Magerl, W., Treede, R.D., Paulus, W. & Happe, S. (2010) Transcranial direct current stimulation of the motor cortex induces distinct changes in thermal and mechanical sensory percepts. *Clin. Neurophysiol.*, **121**, 2083–2089.

Bantick, S.J., Wise, R.G., Ploghaus, A., Clare, S., Smith, S.M. & Tracey, I. (2002) Imaging how attention modulates pain in humans using functional MRI. *Brain*, **125**, 310–319.

Basmajian, J.V. & De Luca, C. (1985) *Muscles Alive*. Williams and Wilkins, Baltimore, MD.

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

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M.F. & Nitsche, M.A. (2013) Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *J. Physiol.-London*, **591**, 1987–2000.

Bingel, U., Quante, M., Knab, R., Bromm, B., Weiller, C. & Buchel, C. (2002) Subcortical structures involved in pain processing: evidence from single-trial fMRI. *Pain*, **99**, 313–321.

Boggio, P.S., Rocha, R.R., da Silva, M.T. & Fregni, F. (2008a) Differential modulatory effects of transcranial direct current stimulation on a facial expression go-no-go task in males and females. *Neurosci. Lett.*, **447**, 101–105.

Boggio, P.S., Zaghi, S., Lopes, M. & Fregni, F. (2008b) Modulatory effects of anodal transcranial direct current stimulation on perception and pain thresholds in healthy volunteers. *Eur. J. Neurol.*, **15**, 1124–1130.

Bornhovd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C. & Buchel, C. (2002) Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. *Brain*, **125**, 1326–1336.

Boros, K., Poreisz, C., Munchau, A., Paulus, W. & Nitsche, M.A. (2008) Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. *Eur. J. Neuorsci.*, **27**, 1292–1300.

Borsook, D., Upadhyay, J., Chudler, E.H. & Becerra, L. (2010) A key role of the basal ganglia in pain and analgesia–insights gained through human functional imaging. *Mol. Pain.*, **6**, 27.

Brunoni, A.R., Amadera, J., Berbel, B., Volz, M.S., Rizzerio, B.G. & Fregni, F. (2011) A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *Int. J. Neuropsychop.*, **14**, 1133–1145.

Casey, K.L. (1999) Forebrain mechanisms of nociception and pain: analysis through imaging. *Proc. Natl. Acad. Sci. USA*, **96**, 7668–7674.

Casey, K.L., Morrow, T.J., Lorenz, J. & Minoshima, S. (2001) Temporal and spatial dynamics of human forebrain activity during heat pain: analysis by positron emission tomography. *J. Neurophysiol.*, **85**, 951–959.

Chaieb, L., Antal, A. & Paulus, W. (2008) Gender-specific modulation of short-term neuroplasticity in the visual cortex induced by transcranial direct current stimulation. *Visual Neurosci.*, **25**, 77–81.

Chen, J.L., Carta, S., Soldado-Magraner, J., Schneider, B.L. & Helmchen, F. (2013) Behaviour-dependent recruitment of long-range projection neurons in somatosensory cortex. *Nature*, **499**, 336–340.
Chesterton, L.S., Barlas, P., Foster, N.E., Lundeberg, T., Wright, C.C. & Baxter, G.D. (2002) Sensory stimulation (TENS): effects of parameter manipulation on mechanical pain thresholds in healthy human subjects. *Pain*, **99**, 253–262.
Craig, A.D., Chen, K., Bandy, D. & Reiman, E.M. (2000) Thermosensory activation of insular cortex. *Nat. Neurosci.*, **3**, 184–190.
Csifcsak, G., Antal, A., Hillers, F., Levold, M., Bachmann, C.G., Happe, S., Nitsche, M.A., Ellrich, J. & Paulus, W. (2009) Modulatory effects of transcranial direct current stimulation on laser-evoked potentials. *Pain Med.*, **10**, 122–132.
Davis, K.D., Lozano, R.M., Manduch, M., Tasker, R.R., Kiss, Z.H. & Dostrovsky, J.O. (1999) Thalamic relay site for cold perception in humans. *J. Neurophysiol.*, **81**, 1970–1973.
Dieckhofer, A., Waberski, T.D., Nitsche, M., Paulus, W., Buchner, H. & Gobbele, R. (2006) Transcranial direct current stimulation applied over the somatosensory cortex – differential effect on low and high frequency SEPs. *Clin. Neurophysiol.*, **117**, 2221–2227.
Fernandez-de-Las-Penas, C., Madeleine, P., Martinez-Perez, A., Arendt-Nielsen, L., Jimenez-Garcia, R. & Pareja, J.A. (2010) Pressure pain sensitivity topographical maps reveal bilateral hyperalgesia of the hands in patients with unilateral carpal tunnel syndrome. *Arthrit. Care Res.*, **62**, 1055–1064.
Fischer, A.A. (1986a) Pressure threshold measurement for diagnosis of myofascial pain and evaluation of treatment results. *Clin. J. Pain*, **2**, 207–214.
Fischer, A.A. (1986b) Pressure threshold meter: its use for quantification of tender spots. *Arch. Phys. Med. Rehab.*, **67**, 836–838.
Fischer, A.A. (1987) Pressure algometry over normal muscles. Standard values, validity and reproducibility of pressure threshold. *Pain*, **30**, 115–126.
Fukuda, S. (2006) Somatosensory evoked potential. *Masui*, **55**, 280.
Garcia-Larrea, L. & Peyron, R. (2007) Motor cortex stimulation for neuropathic pain: from phenomenology to mechanisms. *NeuroImage*, **37**(Suppl 1), S71–S79.
George, M.S. & Aston-Jones, G. (2010) Noninvasive techniques for probing neurocircuitry and treating illness: vagus nerve stimulation (VNS), transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). *Neuropsychopharmacology*, **35**, 301–316.
Giesecke, T., Gracely, R.H., Williams, D.A., Geisser, M.E., Petzke, F.W. & Clauw, D.J. (2005) The relationship between depression, clinical pain, and experimental pain in a chronic pain cohort. *Arthritis Rheum.*, **52**, 1577–1584.
Gilmore, K.L. & Meyers, J.E. (1983) Using surface electromyography in physiotherapy research. *Aust. J. Physiother.*, **29**, 3–9.
Grundmann, L., Rolke, R., Nitsche, M.A., Pavlakovic, G., Happe, S., Treede, R.D., Paulus, W. & Bachmann, C.G. (2011) Effects of transcranial direct current stimulation of the primary sensory cortex on somatosensory perception. *Brain Stimul.*, **4**, 253–260.
Hallett, M. (1996) Transcranial magnetic stimulation: a useful tool for clinical neurophysiology. *Ann. Neurol.*, **40**, 344–345.
Heinze, K., Ruh, N., Nitschke, K., Reis, J., Fritsch, B., Unterrainer, J.M., Rahm, B., Weiller, C., Kaller, C.P. (2014) Transcranial direct current stimulation over left and right DLPFC: lateralized effects on planning performance and related eye movements. *Biol. Psychol.*, **102c**, 130–140.
Hrobjartsson, A., Gotzsche, P.C. (2010) Placebo interventions for all clinical conditions. *Cochrane Db. Syst. Rev.*, CD003974.
Iannetti, G.D. & Mouraux, A. (2010) From the neuromatrix to the pain matrix (and back). *Exp. Brain Res.*, **205**, 1–12.
Iannetti, G.D., Niazy, R.K., Jezzard, P., Brooks, J.C., Zambreanu, L., Vennart, W., Matthews, P.M. & Tracey, I. (2005) Simultaneous recording of laser-evoked brain potentials and continuous, high-field functional magnetic resonance imaging in humans. *NeuroImage*, **28**, 708–719.
Jones, E.G., Coulter, J.D. & Hendry, S.H. (1978) Intracortical connectivity of architectonic fields in the somatic sensory, motor and parietal cortex of monkeys. *J. Comp. Neurol.*, **181**, 291–347.
Kanda, M., Nagamine, T., Ikeda, A., Ohara, S., Kunieda, T., Fujiwara, N., Yazawa, S., Sawamoto, N., Matsumoto, R., Taki, W. & Shibasaki, H. (2000) Primary somatosensory cortex is actively involved in pain processing in human. *Brain Res.*, **853**, 282–289.
Kendall, F.P., McCreary, E.K., Provance, P.G. & Abeloff, D.K. (1993) *Muscles: Testing and Function*. Williams & Wilkins, Baltimore, MD.
Knops, A., Nuerk, H.C., Sparing, R., Foltys, H. & Willmes, K. (2006) On the functional role of human parietal cortex in number processing: how gender mediates the impact of a 'virtual lesion' induced by rTMS. *Neuropsychologia*, **44**, 2270–2283.
Koyama, T., McHaffie, J.G., Laurienti, P.J. & Coghill, R.C. (2005) The subjective experience of pain: where expectations become reality. *Proc. Natl. Acad. Sci. USA*, **102**, 12950–12955.
Kulkarni, B., Bentley, D.E., Elliott, R., Youell, P., Watson, A., Derbyshire, S.W., Frackowiak, R.S., Friston, K.J. & Jones, A.K. (2005) Attention to pain localization and unpleasantness discriminates the functions of the medial and lateral pain systems. *Eur. J. Neuorsci.*, **21**, 3133–3142.
Lang, N., Nitsche, M.A., Paulus, W., Rothwell, J.C. & Lemon, R.N. (2004) Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. *Exp. Brain Res.*, **156**, 439–443.
Laurent, B., Peyron, R., Garcia Larrea, L. & Mauguiere, F. (2000) [Positron emission tomography to study central pain integration]. *Rev. Neurol.-France*, **156**, 341–351.
Le Pera, D., Graven-Nielsen, T., Valeriani, M., Oliviero, A., Di Lazzaro, V., Tonali, P.A. & Arendt-Nielsen, L. (2001) Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. *Clin. Neurophysiol.*, **112**, 1633–1641.
Martin, P.G., Weerakkody, N., Gandevia, S.C. & Taylor, J.L. (2008) Group III and IV muscle afferents differentially affect the motor cortex and motoneurones in humans. *J. Physiol.-London*, **586**, 1277–1289.
Matsunaga, K., Nitsche, M.A., Tsuji, S. & Rothwell, J.C. (2004) Effect of transcranial DC sensorimotor cortex stimulation on somatosensory evoked potentials in humans. *Clin. Neurophysiol.*, **115**, 456–460.
Medeiros, L.F., de Souza, I.C., Vidor, L.P., de Souza, A., Deitos, A., Volz, M.S., Fregni, F., Caumo, W. & Torres, I.L. (2012) Neurobiological effects of transcranial direct current stimulation: a review. *Front. Psychiatry*, **3**, 110.
Mesulam, M.M. & Mufson, E.J. (1982) Insula of the old world monkey. III: Efferent cortical output and comments on function. *J. Comp. Neurol.*, **212**, 38–52.
Meyers, L.S., Gamst, G. & Guarino, A.J. (2006) *Applied Multivariate Research: Design and Interpretation*. SAGE Publications, New York.
Miranda, P.C., Lomarev, M. & Hallett, M. (2006) Modeling the current distribution during transcranial direct current stimulation. *Clin. Neurophysiol.*, **117**, 1623–1629.
Moloney, T.M. & Witney, A.G. (2014) Pressure pain thresholds increase after preconditioning 1 Hz repetitive transcranial magnetic stimulation with transcranial direct current stimulation. *PLoS One*, **9**, e92540.
Monte-Silva, K., Kuo, M.F., Liebetanz, D., Paulus, W. & Nitsche, M.A. (2010) Shaping the optimal repetition interval for cathodal transcranial direct current stimulation (tDCS). *J. Neurophysiol.*, **103**, 1735–1740.
Mylius, V., Jung, M., Menzler, K., Haag, A., Khader, P.H., Oertel, W.H., Rosenow, F. & Lefaucheur, J.P. (2012) Effects of transcranial direct current stimulation on pain perception and working memory. *Eur. J. Pain-London*, **16**, 974–982.
Naylor, J.C., Borckardt, J.J., Marx, C.E., Hamer, R.M., Fredrich, S., Reeves, S.T. & George, M.S. (2014) Cathodal and anodal left prefrontal tDCS and the perception of control over pain. *Clin. J. Pain*, **30**, 693–700.
Nitsche, M. & Paulus, W. (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.-London*, **527**, 633–639.
Nitsche, M.A. & Paulus, W. (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology*, **57**, 1899–1901.
Nitsche, M.A. & Paulus, W. (2011) Transcranial direct current stimulation – update. *Restor. Neurol. Neuros.*, **29**, 463–492.
Nitsche, M.A., Liebetanz, D., Tergau, F. & Paulus, W. (2002) [Modulation of cortical excitability by transcranial direct current stimulation]. *Nervenarzt*, **73**, 332–335.
Nitsche, M.A., Seeber, A., Frommann, K., Klein, C.C., Rochford, C., Nitsche, M.S., Fricke, K., Liebetanz, D., Lang, N., Antal, A., Paulus, W. & Tergau, F. (2005) Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *J. Physiol.-London*, **568**, 291–303.
Nitsche, M.A., Doemkes, S., Karakose, T., Antal, A., Liebetanz, D., Lang, N., Tergau, F. & Paulus, W. (2007) Shaping the effects of transcranial direct current stimulation of the human motor cortex. *J. Neurophysiol.*, **97**, 3109–3117.
Nitsche, M.A., Cohen, L.G., Wassermann, E.M., Priori, A., Lang, N., Antal, A., Paulus, W., Hummel, F., Boggio, P.S., Fregni, F. & Pascual-Leone, A. (2008) Transcranial direct current stimulation: State of the art 2008. *Brain Stimul.*, **1**, 206–223.
Oh, S.J. (2003) *Clinical Electromyography: Nerve Conduction Studies*. Lippincott Williams & Wilkins, Philadelphia, PA.

Ohara, S., Weiss, N. & Lenz, F.A. (2004) Microstimulation in the region of the human thalamic principal somatic sensory nucleus evokes sensations like those of mechanical stimulation and movement. *J. Neurophysiol.*, **91**, 736–745.

Oldfield, R.C. (1971) The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, **9**, 97–113.

Oliviero, A., Esteban, M.R., de la Cruz, F.S., Cabredo, L.F. & Di Lazzaro, V. (2005) Short-lasting impairment of temperature perception by high frequency rTMS of the sensorimotor cortex. *Clin. Neurophysiol.*, **116**, 1072–1076.

Parazzini, M., Rossi, E., Ferrucci, R., Liorni, I., Priori, A. & Ravazzani, P. (2014) Modelling the electric field and the current density generated by cerebellar transcranial DC stimulation in humans. *Clin. Neurophysiol.*, **125**, 577–584.

Pellicciari, M.C., Brignani, D. & Miniussi, C. (2013) Excitability modulation of the motor system induced by transcranial direct current stimulation: a multimodal approach. *NeuroImage*, **83**, 569–580.

Perotto, A.O. & Delagi, E.F. (2005) *Anatomical Guide for the Electromyographer: the Limbs and Trunk*. Charles C Thomas Publisher, Springfield, IL.

Peyron, R., Laurent, B. & Garcia-Larrea, L. (2000) Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiol. Clin.*, **30**, 263–288.

Pirulli, C., Fertonani, A. & Miniussi, C. (2014) Is neural hyperpolarization by cathodal stimulation always detrimental at the behavioral level? *Front. Behav. Neurosci.*, **8**, 226.

Ploner, M. & Schnitzler, A. (2004) Cortical representation of pain. *Nervenarzt*, **75**, 962–969.

Poreisz, C., Boros, K., Antal, A. & Paulus, W. (2007) Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain Res. Bull.*, **72**, 208–214.

Ragert, P., Becker, M., Tegenthoff, M., Pleger, B. & Dinse, H.R. (2004) Sustained increase of somatosensory cortex excitability by 5 Hz repetitive transcranial magnetic stimulation studied by paired median nerve stimulation in humans. *Neurosci. Lett.*, **356**, 91–94.

Rogalewski, A., Breitenstein, C., Nitsche, M.A., Paulus, W. & Knecht, S. (2004) Transcranial direct current stimulation disrupts tactile perception. *Eur. J. Neuorsci.*, **20**, 313–316.

Rossini, P.M., Barker, A.T., Berardelli, A., Caramia, M.D., Caruso, G., Cracco, R.Q., Dimitrijević, M.R., Hallett, M., Katayama, Y., Lücking, C.H., Maertens de Noordhout, A.L., Marsden, C.D., Rothwell, J.C., Swash, M. & Tomberg, C. (1994) Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroen. Clin. Neuro.*, **91**, 79–92.

Schabrun, S.M., Jones, E., Kloster, J. & Hodges, P.W. (2013) Temporal association between changes in primary sensory cortex and corticomotor output during muscle pain. *Neuroscience*, **235**, 159–164.

Schwartz, M.S. & Andrasik, F.E. (2003) *Biofeedback: A Practitioner's Guide*. Guilford Press, New York.

Stieglitz, T. (2005) Diameter-dependent excitation of peripheral nerve fibers by multipolar electrodes during electrical stimulation. *Expert Rev. Med. Devic.*, **2**, 149–152.

Summers, J., Johnson, S., Pridmore, S. & Oberoi, G. (2004) Changes to cold detection and pain thresholds following low and high frequency transcranial magnetic stimulation of the motor cortex. *Neurosci. Lett.*, **368**, 197–200.

Suppa, A., Biasiotta, A., Belvisi, D., Marsili, L., La Cesa, S., Truini, A., Cruccu, G. & Berardelli, A. (2013) Heat-evoked experimental pain induces long-term potentiation-like plasticity in human primary motor cortex. *Cereb. Cortex*, **23**, 1942–1951.

Svensson, P., Miles, T.S., McKay, D. & Ridding, M.C. (2003) Suppression of motor evoked potentials in a hand muscle following prolonged painful stimulation. *Eur. J. Pain-London*, **7**, 55–62.

Talbot, J.D., Marrett, S., Evans, A.C., Meyer, E., Bushnell, M.C. & Duncan, G.H. (1991) Multiple representations of pain in human cerebral cortex. *Science*, **251**, 1355–1358.

Tang, J.S., Qu, C.L. & Huo, F.Q. (2009) The thalamic nucleus submedius and ventrolateral orbital cortex are involved in nociceptive modulation: a novel pain modulation pathway. *Prog. Neurobiol.*, **89**, 383–389.

Terney, D., Bergmann, I., Poreisz, C., Chaieb, L., Boros, K., Nitsche, M.A., Paulus, W. & Antal, A. (2008) Pergolide increases the efficacy of cathodal direct current stimulation to reduce the amplitude of laser-evoked potentials in humans. *J. Pain Symptom Manag.*, **36**, 79–91.

Tracey, I. & Mantyh, P.W. (2007) The cerebral signature for pain perception and its modulation. *Neuron*, **55**, 377–391.

Uy, J. & Ridding, M.C. (2003) Increased cortical excitability induced by transcranial DC and peripheral nerve stimulation. *J. Neurosci. Meth.*, **127**, 193–197.

Valeriani, M., Restuccia, D., Di Lazzaro, V., Oliviero, A., Profice, P., Le Pera, D., Saturno, E. & Tonali, P. (1999) Inhibition of the human primary motor area by painful heat stimulation of the skin. *Clin. Neurophysiol.*, **110**, 1475–1480.

Vaseghi, B., Zoghi, M. & Jaberzadeh, S. (2014) Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study. *Clin. Neurophysiol.* **125**, 1847–1858.

Vaseghi, B., Zoghi, M. & Jaberzadeh, S. (2015) A meta-analysis of site-specific effects of cathodal transcranial direct current stimulation on sensory perception and pain. *PloS One*, **10**, e0123873.

Vogt, B.A. & Sikes, R.W. (2000) The medial pain system, cingulate cortex, and parallel processing of nociceptive information. *Prog. Brain Res.*, **122**, 223–235.

Wassermann, E., Epstein, C. & Ziemann, U. (2008) *Oxford Handbook of Transcranial Stimulation*. Oxford University Press, Oxford.

Wolff, B.B. (1964) The relationship of experimental pain tolerance to pain threshold: a critique of GELFAND's paper. *Can. J. Psychology*, **18**, 249–253.

Wood, L., Egger, M., Gluud, L.L., Schulz, K.F., Juni, P., Altman, D.G., Gluud, C., Martin, R.M., Wood, A.J. & Sterne, J.A. (2008) Empirical evidence of bias in treatment effect estimates in controlled trials with different interventions and outcomes: meta-epidemiological study. *Brit. Med. J.*, **336**, 601–605.

Ziegler, D., Mayer, P. & Gries, F.A. (1988) Evaluation of thermal, pain, and vibration sensation thresholds in newly diagnosed type 1 diabetic patients. *J. Neurol. Neurosur. Ps.*, **51**, 1420–1424.

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.

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	Supervisory input on study design, Guidance in the framing of the manuscript, Review and provision of feedback on final manuscript draft	5 %

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

Candidate's Signature:  Date: 15-Sep- 2015

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Preamble to Chapter 7

The application of a-tDCS over the M1 is an established technique to enhance M1 excitability and reduce pain perception in pain studies. The findings in Chapter 5 indicate that a-tDCS of not only the M1, but also the DLPFC and the S1, may affect M1 excitability. Such data provides evidence for the existence of functional connectivities between these cortical sites. Chapter 7 is a methodological study which investigates the potential effects of concurrent a-tDCS of these functionally connected cortical sites on the M1 CSE. This novel technique is named unihemispheric concurrent dual-site a-tDCS (a-tDCS$_{UHCDS}$). To assess the mechanisms behind the efficacy of this technique, intracortical inhibition and facilitation are measured by using the TMS paired-pulse paradigm. This chapter introduces a more effective technique for the induction of excitability changes in the M1, compared to the conventional single-site a-tDCS. The effects of this novel technique on STh/PTh changes is presented in Chapter 9.

Chapter 7: The effects of anodal-tDCS on corticospinal excitability and its after-effects: conventional versus unihemispheric concurrent dual-site stimulation

The format of this chapter is consistent with the Frontiers in Human Neuroscience.

The setup system used in this study, Ethics approval, TMS safety, Personal health history form, and Edinburg handedness questionnaires and consent form are provided in Appendices 7-13.

frontiers in Human Neuroscience

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The effects of anodal-tDCS on corticospinal excitability enhancement and its after-effects: conventional vs. unihemispheric concurrent dual-site stimulation

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Previous researchers have approved the ability of anodal transcranial direct current stimulation (a-tDCS) of the primary motor cortex (M1) to enhance corticospinal excitability (CSE). The primary aim of the current study was to investigate the effect of concurrent stimulation of M1 and a functionally connected cortical site of M1 on CSE modulation. This new technique is called unihemispheric concurrent dual-site a-tDCS (a-tDCS$_{UHCDS}$). The secondary aim was to investigate the mechanisms underlying the efficacy of this new approach in healthy individuals. In a randomized crossover study, 12 healthy right-handed volunteers received a-tDCS under five conditions: a-tDCS of M1, a-tDCS$_{UHCDS}$ of M1-dorsolateral prefrontal cortex (DLPFC), a-tDCS$_{UHCDS}$ of M1-primary sensory cortex (S1), a-tDCS$_{UHCDS}$ of M1-primary visual cortex (V1), and sham a-tDCS$_{UHCDS}$. Peak-to-peak amplitude of transcranial magnetic stimulation (TMS) induced MEPs, short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were assessed before and four times after each condition. A-tDCS$_{UHCDS}$ conditions induced larger MEPs than conventional a-tDCS. The level of M1 CSE was significantly higher following a-tDCS$_{UHCDS}$ of M1-DLPFC than other a-tDCS$_{UHCDS}$ conditions ($p < 0.001$), and lasted for over 24 h. The paired-pulse TMS results after a-tDCS of M1-DLPFC showed significant facilitatory increase and inhibitory change. A-tDCS$_{UHCDS}$ of M1-DLPFC increases M1 CSE twofold that of conventional a-tDCS. A-tDCS$_{UHCDS}$ of M1-DLPFC enhances the activity of glutamergic mechanisms for at least 24 h. Such long-lasting M1 CSE enhancement induced by a-tDCS$_{UHCDS}$ of M1-DLPFC could be a valuable finding in clinical scenarios such as learning, motor performance, or pain management. The present study has been registered on the Australian New Zealand Clinical Trial at http://www.anzctr.org.au/ with registry number of ACTRN12614000817640.

Keywords: unihemispheric concurrent dual-site anodal transcranial direct current stimulation, primary motor cortex, corticospinal excitability, pain neuromatrix, neuroplasticity, long-lasting effect

Introduction

Anodal transcranial direct current stimulation (a-tDCS) of the primary motor cortex (M1) is a well-known technique (Nitsche and Paulus, 2000, 2011; Stagg and Nitsche, 2011) for modulating the resting membrane potentials of neurons, resulting in alteration of the endogenous excitability of brain neural circuits and networks (Medeiros et al., 2012). Recent fMRI studies showed that a-tDCS increases corticospinal excitability (CSE) of both local stimulated and distant areas, probably through interconnections between them (Meyerson et al., 1993; Lang et al., 2005). Literature indicates that tDCS induces CSE enhancement in M1, which could be used as a priming or stand-alone technique in therapeutic scenarios including improvement of motor function (Goodwill et al., 2013; Williams et al., 2013; Dutta et al., 2014; Filmer et al., 2014; Ludemann-Podubecka et al., 2014), motor learning (Kuo et al., 2008; Stagg and Nitsche, 2011; Zimerman et al., 2012; Karok and Witney, 2013; Vollmann et al., 2013; Meinzer et al., 2014; Parasuraman and Mckinley, 2014), and pain management (Bolognini et al., 2013; Bae et al., 2014; Foerster et al., 2015; Hagenacker et al., 2014; Moloney and Witney, 2014; Vaseghi et al., 2014; Wang et al., 2014; Zmigrod, 2014).

The focus of a large number of tDCS studies is to identify the optimal a-tDCS parameters for induction of larger CSE with longer lasting effect compared to conventional tDCS approach. Despite some promising results from previous studies, which investigated the effects of current densities/intensities (Furubayashi et al., 2008; Moliadze et al., 2014; Murray et al., 2014), electrode size (Nitsche et al., 2007; Kronberg and Bikson, 2012; Bastani and Jaberzadeh, 2013), the number of within-session repetitions of a-tDCS (Bastani and Jaberzadeh, 2012), and the duration of tDCS application (Nitsche and Paulus, 2001; Furubayashi et al., 2008), additional exploratory studies are needed to refine the existing parameter and to introduce a novel tDCS approach. One important tDCS parameter is the electrode montage. Conventional tDCS montage involves the application of the anode over a presumed target (e.g., M1 for CSE enhancement) and the cathode over an indifferent cortical site, i.e., contralateral supraorbital area. In addition to the conventional electrode montage, some clinical researchers introduced a single channel bi-hemispheric montage (Vines et al., 2008). In this montage, the reference electrode (cathode) is located over the contralateral M1. The aim is to reduce inhibition from the contralateral M1 and to induce larger CSE under the anode (Vines et al., 2008; Kidgell et al., 2013b; Park et al., 2014; Koyama et al., 2015). However, due to the reduction of M1 CSE under the cathode, the applicability of this approach for cortical (Mordillo-Mateos et al., 2012; O'Shea et al., 2014) or behavioral (O'Shea et al., 2014) modifications has not been widely accepted yet.

Apart from M1 stimulation for induction of CSE changes, research interest has shifted toward stimulation of cortical sites, which are functionally connected to M1, including dorsolateral prefrontal cortex (DLPFC), primary sensory cortex (S1), or premotor cortex. This new approach is backed by the result of some fMRI studies, which showed that the excitability modulation induced by a-tDCS is not limited to the stimulated sites; functionally connected areas are also affected (Lang et al., 2005; Kwon et al., 2008; Keeser et al., 2011). For instance, a-tDCS of the premotor cortex (Boros et al., 2008), S1 (Kirimoto et al., 2011), or DLPFC (Vaseghi et al., 2015b) increases M1 CSE. In addition, literature approved he involvement of both S1 and DLPFC in two networks involved in planning, execution, and control of movements (Kandel, 2000; Miller, 2000; Saper et al., 2000; Miller and Cohen, 2001; Hasan et al., 2013; Borich et al., 2015) and pain management (Apkarian et al., 2005; Iannetti and Mouraux, 2010). The results of these studies provide evidence for functional relationship among these cortical sites.

Therefore, unilateral concurrent stimulation of M1 and its functionally connected cortical sites would be a possible alternative electrode montage for induction of larger M1 CSE with longer lasting effects compared to conventional a-tDCS electrode montage. This new technique was called unihemispheric concurrent dual-site a-tDCS (a-tDCS$_{UHCDS}$). The rationale behind the superiority of this new approach is that a-tDCS$_{UHCDS}$ intensifies the mutual communications between M1 and its functionally connected sites (Luft et al., 2014). Therefore, this pilot study aimed to compare the potential effects of a-tDCS$_{UHCDS}$ of M1-S1 and M1-DLPFC with the conventional M1 a-tDCS on CSE enhancement and its lasting effects.

We hypothesised that a-tDCS$_{UHCDS}$ induces larger and longer-lasting CSE than conventional electrode montage of M1 a-tDCS. Due to the novelty of the proposed technique, we also aimed to investigate the possible mechanisms behind the a-tDCS$_{UHCDS}$-induced CSE changes. Drawing on the basic mechanisms behind the efficacy of conventional a-tDCS (Liebetanz et al., 2002; Hummel et al., 2005, 2010; Nitsche et al., 2005; Paulus et al., 2008; Medeiros et al., 2012; Kidgell et al., 2013a), we hypothesised that a-tDCS$_{UHCDS}$ of M1 and the other functionally connected site of M1 decreases short-interval intracortical inhibition (SICI), and increases ICF. Similar to conventional a-tDCS, a-tDCS$_{UHCDS}$ involves the application of low-amplitude current via surface electrodes, which is expected to be tolerable for the participants. However, as tDCS$_{UHCDS}$ is a new neuromodulatory approach, we also aimed to assess its possible side effects.

Material and Methods

Study Design

We implemented a sham-controlled crossover study to determine the effect of a-tDCS$_{UHCDS}$ on M1 CSE in healthy individuals. All experimental procedures were approved by the Monash University Human Research Ethics Committee and conformed to the Declaration of Rohrich (2007). The current study is registered as a clinical trial on the Australian New Zealand Clinical Trial (registry number: ACTRN12614000817640)[1].

[1]http://www.anzctr.org.au/

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Participants

Twelve healthy (nine women and three men, all Monash University students) with mean age of 25 ± 1.31 (age range 19–36 years) participated in all experimental sessions. The sample size was calculated (with power of 80%) based on the data generated from the first six participants. All were right-handers as determined by the Edinburgh Handedness Inventory (10-item version, mean laterality quotient = 89 ± 9.3; Oldfield, 1971). None of the participants reported contraindications to transcranial magnetic stimulation (TMS) or tDCS, current use of any medications, or history of neurological or psychiatric disease. The health condition of participants was assessed before written informed consent was sought and provided. All volunteers were blinded to the purpose of the experiments.

Assessment of CSE of M1

CSE of M1 was measured by the peak-to-peak amplitude of TMS-induced motor-evoked potentials (MEPs) of the right first dorsal interossei (FDI) muscle. Single- and paired-pulse magnetic stimuli were delivered by a MagPro R30 (MagOption) stimulator (MagVenture, Denmark) with an angulated figure-of-eight coil (max. initial dB/dt 28 KT/s near the coil surface). The coil was placed over left M1, contralateral to the target muscles, with a posterior-anterior orientation, and set at angle of 45° to the midline. The area of stimulation with largest MEPs was defined as the hotspot and marked on the scalp to be used throughout the tests to ensure consistency of the coil placement. Resting motor threshold (RMT) was defined as the minimal stimulator output needed to elicit five MEPs in a series of 10 with minimum amplitude of 50–100 μV in the relaxed FDI muscle (Rossini et al., 1994; Hallett, 1996; Wassermann et al., 2008). Single-pulse MEPs were recorded with the TMS intensity adjusted to elicit ∼1 mV peak-to-peak amplitude at baseline. Stimulation intensity was kept constant for the post-intervention assessments.

Assessment of Intracortical Inhibition and Facilitation

In order to evaluate the function of intracortical inhibition and facilitation circuits in M1, paired-pulse TMS was used to measure SICI and intracortical facilitation (ICF; Kujirai et al., 1993). In this method, a subthreshold TMS stimulus is followed by a suprathreshold TMS pulse with an inter-stimulus interval (ISI) of 1–5 ms or 8–15 ms to measure SICI or ICF respectively (Kujirai et al., 1993). In the present study, conditioning stimulus intensity was applied as 80% of RMT (0.8 × RMT), followed by a suprathreshold test stimulus (Di Pino et al., 2014). The test stimulus intensity was adjusted to achieve a baseline MEP of around 1 mV (Zoghi et al., 2003; Kothari et al., 2014). The ISI was set at 3 ms to measure SICI and 10 ms to measure ICF (Di Pino et al., 2014; Opie and Semmler, 2014). Five blocks of ISI were designed to deliver both single- and paired-pulse TMS randomly. Each block contained 20 single-pulse and 40 paired-pulse TMS (20 ISI of 3 and 20 ISI of 10 ms). One of five blocks was randomly selected in each time point of measurement to minimize the bias induced by the order of stimuli. Blocks of MEPs in which the muscle was not relaxed were excluded from the analysis. In order to avoid any profound effect of inter-pulse interval on MEP size, a ten-second interval was applied between stimulations (Vaseghi et al., 2015a).

tDCS Characteristics

Participants received tDCS under each of five different conditions in random order: a-tDCS of M1, a-$tDCS_{UHCDS}$ of M1-S1, a-$tDCS_{UHCDS}$ of M1-DLPFC, a-$tDCS_{UHCDS}$ of M1-V1, and sham a-$tDCS_{UHCDS}$. Direct current was applied through active saline-soaked surface sponge electrodes (1.5 × 2 cm) over target areas including M1, S1 and DLPFC, and reference electrodes (2 × 6 cm) over the contralateral supraorbital area (Bikson et al., 2010; **Figure 1**). The small size of active electrode produces a highly focused DC current over the target areas, which enabled us to stimulate M1 and S1 with two separated anode electrodes separately. Based on the result of some computational modeling studies, the effects of tDCS can be more focalized by smaller electrodes (Nitsche et al., 2007; Bikson et al., 2010). In addition, recent experimental investigations on human brain illustrated that utilizing smaller active electrodes over M1 resulted in larger CSE (Nitsche et al., 2007; Bastani and Jaberzadeh, 2013; Vaseghi et al., 2015b).

The tDCS stimulators were set to deliver 0.3 mA direct current for 20 min, with 10 s of linear fade in and fade out. Current intensity of 0.3 mA allowed us to considerably decrease the size of electrodes (Uy and Ridding, 2003) while keeping the current density in a safe range (0.1 mA/cm^2) with limited side effect (Poreisz et al., 2007; Brunoni et al., 2011). The superiority of lower intensities in induction of larger CSE has been shown by some tDCS studies (Nitsche and Paulus, 2001; Brunoni et al., 2011; Parazzini et al., 2013; Pellicciari et al., 2013).

Two channels of a tDCS device were used for stimulation of the target areas in a-$tDCS_{UHCDS}$ conditions. Current intensity of 0.3 mA and density of 0.1 mA/cm^2 were identical in each active electrode during all experimental conditions.

Using a similar electrode montage as conventional tDCS protocols, the narrow shaped cathodal reference electrodes were placed over contralateral supraorbital area over subgenual cortex (**Figure 1**). To reduce the neuromodulatory effects of these electrodes, the size of them were kept four times larger than the active electrodes. This arrangement considerably reduces the density under these electrodes.

The anode was placed over the left M1 for the right FDI muscle as identified by TMS. For stimulation of S1, the anode was identified based on the international 10–20 system and the anode was placed over C′3 (2 cm posterior to C3). For a-tDCS of DLPFC and the primary visual cortex (V1), the anode was placed over F3 and Oz respectively (**Figure 1**). The reference electrode (cathode) was conventionally placed over the contralateral supraorbital area with the assumption of no or negligible neuromodulatory effects on the subgenual cortex. As V1 is not directly connected to M1, a-$tDCS_{UHCDS}$ of M1–V1 was a control condition to assess whether the changes following a-$tDCS_{UHCDS}$ of M1-DLPFC or M1–S1 are due to stimulation of the brain with twice the current density of conventional a-tDCS or concurrent stimulation of M1 and a functionally connected site to the M1. In the sham condition, the electrodes

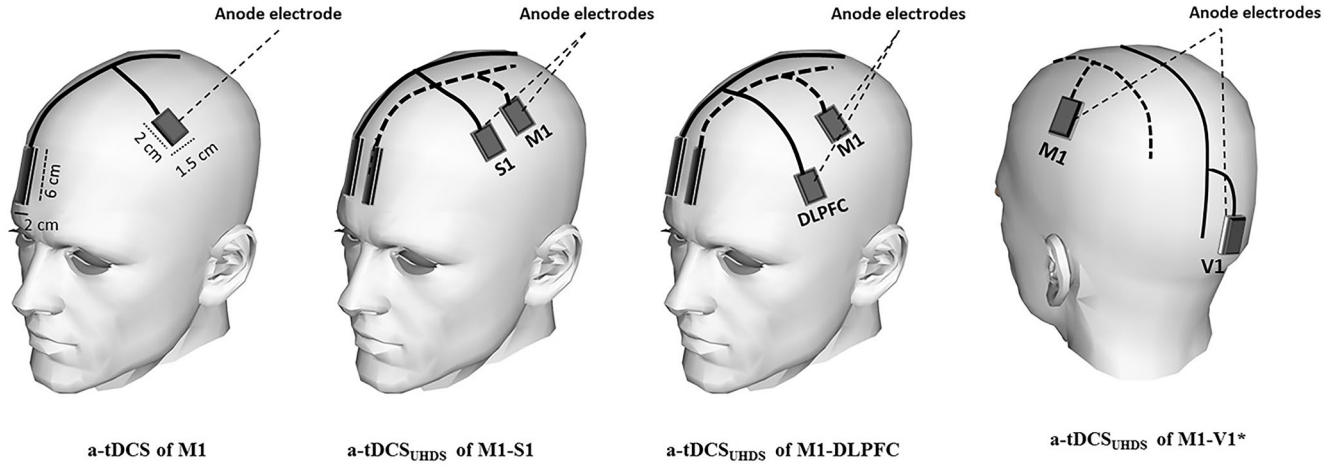

FIGURE 1 | Schematic illustration of electrode montage in conventional a-tDCS and a-tDCS$_{UHCDS}$; the active electrodes were positioned over M1, dorsolateral prefrontal cortex (DLPFC), S1, and V1. The reference electrodes were placed over the contralateral supraorbital area in all conditions. In the sham condition, the electrodes were placed in the same positions as for M1-S1 or M1-DLPFC stimulation. *The reference electrodes are not shown in a-tDCS$_{UHCDS}$ of M1-V1.

were placed in the same positions as for M1-S1 or M1-DLPFC stimulation randomly, but the stimulator was turned off after 30 s of stimulation. All pre and post evaluations were identical to those in other conditions.

Experimental Procedure

Using a cross-over study design, each participant was randomly assigned to receive all active and sham conditions. We allocated a code for each participant and experimental condition. Using a random number table, the sequence of experimental conditions was assigned for 12 participants and placed in opaque envelops to ensure the concealment of the allocation. Then, one envelop was allocated to each participants' code in a random order. The volunteers were comfortably seated in a fully adjustable treatment chair (MagVenture, Denmark) with head and arm rests. First, the hotspot of M1 FDI was identified by single-pulse TMS and marked. Then the stimulus intensity was adjusted to elicit single-pulse MEPs with peak-to-peak amplitudes of an average of 1 mV. After determination of RMT, 80% of RMT was calculated as the subthreshold test stimulus. Twenty single-pulse MEPs and 40 MEPs induced by paired-pulse TMS, including 20 MEPs with ISI of 3 and 20 MEPs with ISI of 10 ms, were recorded. The single- and paired-pulse TMS with ISI of 3 and 10 ms were applied in a random order.

Based on the participant code and the assigned sequence, tDCS was applied in each experimental session. The experimental sessions were separated by at least 7 days to avoid interference or carry-over effects of tDCS, and completed at the same time of the day (late mornings or early afternoon) to avoid diurnal variation. The duration of tDCS application was 20 min in all experiments. All the outcome measures were measured before (T_{pre}), immediately after (T_0), 30 min (T_{30}) and 60 (T_{60}) minutes after each intervention. TMS measurements were conducted 24 h after the end of the intervention (T_{day2}; **Figure 2**). To control the effect of female hormonal fluctuation on the size of MEPs, the experimental sessions were carried out between the 7th and 21st day of women's menstrual cycles. Participants were blinded to the condition of tDCS (sham or active).

Measurement of Side Effects

To record side or adverse effects of stimulation, all participants were asked to complete a questionnaire during all experimental conditions. The questionnaire contained rating scales for the presence and severity of side effects such as itching, tingling, burning sensations under electrodes (Poreisz et al., 2007; Boros et al., 2008; George and Aston-Jones, 2010) and other adverse effects including headache and pain during and after stimulation. All participants rated the unpleasantness of any scalp sensation using numeric analogue scales (NAS; e.g., 0 = no tingling to 10 = worst tingling imaginable).

Data Management and Statistical Analysis

Peak-to-peak amplitude of 20 single-pulse MEPs were automatically calculated and averaged online for each time point of measurement, using a custom designed macro. Area under the curve of MEPs was also quantified off-line from the digitized averages of rectified EMG for conditioned and unconditioned stimuli in each trial by using a custom designed macro in Powerlab 8/30 software. The size of the conditioned MEP was expressed as a percentage of the unconditioned test MEPs in order to evaluate the effectiveness of ICI or ICF.

The differences in RMT recorded at the starting point of each experimental condition (T_{pre}) were analyzed with one-way repeated measures analysis of variance (ANOVA) to detect any carry over effect. A two-way repeated measures

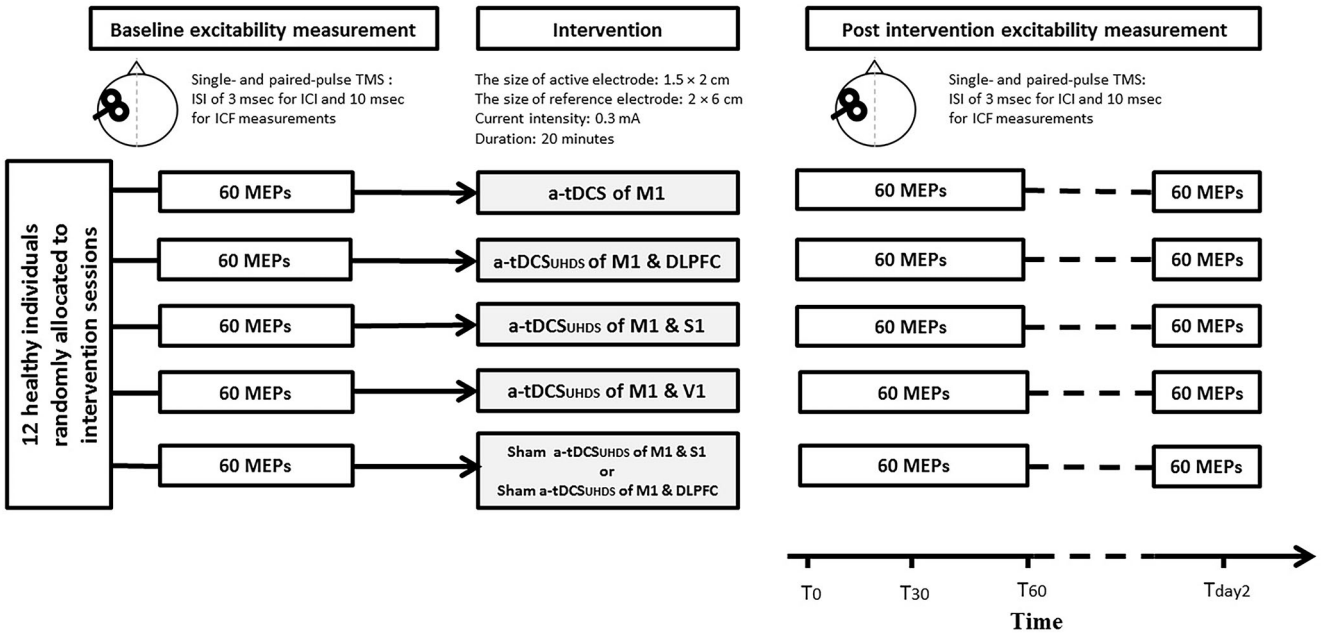

FIGURE 2 | Experimental design for the comparison of conventional a-tDCS and a-tDCS$_{UHCDS}$; in each time point of measurement, 20 single pulse transcranial magnetic stimulation (TMS), 20 paired pulse TMS with inter-stimulus interval (ISI)s of 3 ms, and 20 paired-pulse TMS with ISIs of 10 ms were delivered to measure corticospinal excitability (CSE), short interval intracortical inhibition (SICI) and ICF respectively.

ANOVA was performed to assess the effects of two independent variables (the peak-to-peak amplitude of MEPs, SICI, and IFC): experimental conditions with five levels, and measurement time with five levels on induced MEP amplitude. Mauchly's test was used to assess the validity of the sphericity assumption for repeated measures ANOVA. Greenhouse-Geisser corrected significance values were used because sphericity could not be assumed (Meyers et al., 2006). In case of significant main effect, *post hoc* paired-sample two-tailed *t*-tests were performed using the least significant difference adjustment for multiple comparisons to evaluate the MEP, SICI, and ICF changes following the intervention at different time points of measurement and to compare baseline values with post-intervention measurements.

In order to assess whether participants were successfully blinded to the stimulation conditions (active or sham), Pearson's chi-square was used. In addition, a one-way ANOVA was carried out on the mean values of rating scale recorded by questionnaire to assess any significant differences between the participants' feelings during active and sham conditions. Statistical analyses were performed using SPSS software version 22. Means are reported ± standard error of measurement (SEM).

Results

Comparison of Baseline Values

One-way repeated measures ANOVA showed that there was no significant difference between baseline RMT at the starting point of all experimental conditions (F_4 = 2.97, p = 0.09).

The Effects of a-tDCS and a-tDCSUHCDS on M1 CSE

Two-way repeated measures ANOVA indicated significant main effects of experimental conditions (F_4 = 18.41, $p < 0.001$), time (F_4 = 33.55, $p < 0.001$), and the interaction of condition and time (F_{16} = 9.19, $p < 0.001$). MEP amplitude increased significantly following a-tDCS$_{UHCDS}$ of M1-DLPFC compared to other experimental conditions in all time points of measurements (**Figure 3A**). As can be seen in **Table 1**, *t*-tests revealed a significant difference in MEP amplitude between stimulation of M1 and a-tDCS$_{UHCDS}$ of M1–V1 condition at T_0, T_{30}, and T_{60}, whilst no significant difference was found between sham and a-tDCS$_{UHCDS}$ of M1–V1 condition. Similarly, no significant difference was detected between a-tDCS$_{UHCDS}$ of M1–S1 and a-tDCS of M1 at one hour after intervention. The *post hoc* comparison also revealed a significant difference in MEP amplitude between a-tDCS$_{UHCDS}$ of both M1–S1 and M1–DLPFC and other conditions at T_{day2} (**Table 1**). Comparing sham and four other experimental conditions revealed significant differences between all active tDCS conditions (except a-tDCS$_{UHCDS}$ of M1–V1) and sham tDCS (**Table 1**).

Comparing the MEP amplitudes baseline and post-intervention time points of measurement, the *post hoc* comparisons showed that there was significant differences

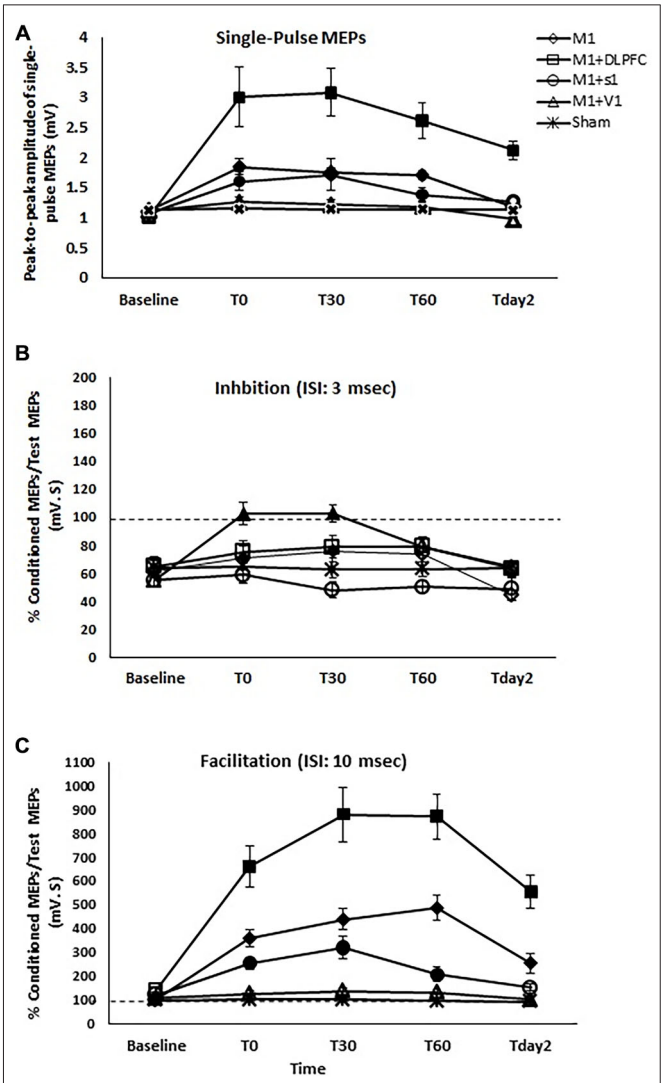

FIGURE 3 | The effects of different stimulation sites on the peak-to-peak amplitude of MEPs (A), SICI with ISI of 3 ms (B), and intracortical facilitation (ICF) (C) following a-tDCS of primary motor cortex (M1), a-tDCS$_{UHCDS}$ of M1 and DLPFC, M1and primary sensory cortex (S1), M1and primary visual cortex (V1), and sham. Filled symbols indicate significant deviation of the post-intervention MEP amplitude, SICI, and ICF compared to the baseline. Data are reported as mean ± SEM.

between T_{Pre}–T_0 (p = 0.002), T_{Pre}–T_{30} (p = 0.004), T_{Pre}–T_{60} (p = 0.004) following a-tDCS$_{UHCDS}$ of M1–S1, T_{Pre}–T_0 ($p < 0.005$), T_{Pre}–T_{30} ($p < 0.005$), T_{Pre}–$T6_0$ ($p < 0.005$), and T_{pre}–T_{day2} ($p < 0.005$) following a-tDCS$_{UHCDS}$ of M1–DLPFC, and T_{Pre}–T_0 (p = 0.001), T_{Pre}–T_{30} (p = 0.003), T_{Pre}–$T6_0$ (p = 0.003) following M1 a-tDCS. The results of *post hoc* comparisons are summarized in **Figure 3A**.

The Effects of a-tDCS and a-tDCSUHCDS on SICI

Two-way repeated measures ANOVA showed significant effects of condition (F_4 = 5.99, p = 0.001), Time (F_4 = 21.24, $p < 0.001$), and interaction of time and condition (F_{16} = 6.55, $p < 0.001$) on SICI. *Post hoc* comparisons revealed no significant difference between a-tDCS$_{UHCDS}$ of M1–DLPFC and a-tDCS of M1 at T_0, T_{30}, T_{60}, and T_{day2} (**Table 1**). Significant differences in SICI were found between a-tDCS of M1 and a-tDCS$_{UHCDS}$ of M1–S1 at T_0 and T_{30} and between a-tDCS of M1 and sham tDCS at all post-intervention time points (**Table 1**). There was no significant SICI difference between a-tDCS$_{UHCDS}$ of M1–V1 and sham condition in any time points of measurement.

Post hoc comparison also demonstrated that there was a significant difference between T_{Pre}–T_0 (p = 0.001) and T_{Pre}–T_{30} (p = 0.001) following a-tDCS$_{UHCDS}$ of M1–S1, and between T_{Pre}–T_0 (p = 0.004) and T_{Pre}–T_{30} (p = 0.004) following M1 a-tDCS. No significant SICI alteration was found at any time-points following a-tDCS$_{UHCDS}$ of M1–DLPFC, M1–V1, or sham condition (**Figure 3B**).

The Effects of a-tDCS and a-tDCSUHCDS on ICF

Two-way repeated measures ANOVA found significant main effects of condition (F_4 = 36.74, $p < 0.001$), time (F_4 = 65.31, $p < 0.001$), and interaction of condition × time (F_{16} = 21.29, $p < 0.001$) on ICF. *Post hoc* comparisons revealed significant ICF differences between a-tDCS$_{UHCDS}$ of M1–DLPFC and all other conditions at all time points of measurement (**Table 1**). Significant differences in ICF were also found between a-tDCS$_{UHCDS}$ of M1–S1 and a-tDCS of M1 at T_{30} and T_{60} (**Table 1**). There was a significant difference in ICF between sham and other active conditions except a-tDCS$_{UHCDS}$ of M1–V1 (**Table 1**).

Comparing post-intervention and baseline values, the result showed significant difference between T_{Pre}–T_0 (p = 0.001), T_{Pre}–T_{30} (p = 0.001), T_{Pre}–$T6_0$ ($p < 0.005$), and T_{pre}–T_{day2} (p = 0.001) following a-tDCS$_{UHCDS}$ of M1–S1. Significant differences were also found between T_{Pre}–T_0 ($p < 0.005$), T_{Pre}–T_{30} ($p < 0.005$), T_{Pre}–$T6_0$ ($p < 0.005$), and T_{pre}–T_{day2} ($p < 0.005$) following a-tDCS$_{UHCDS}$ of M1–DLPFC, and T_{Pre}–T_0 (p = 0.004), T_{Pre}–T_{30} (p = 0.003), T_{Pre}–$T6_0$ (p = 0.004) following M1 a-tDCS. No significant difference in ICF was found following a-tDCS$_{UHCDS}$ of M1–V1 or sham condition at any time point (**Figure 3C**).

Safety and Side Effects of a-tDCSUHCDS

Participants' experiences were recorded at the beginning, during and at the end stage of the intervention. The averaged sensation score recorded during the intervention is summarized in **Table 2**. The only reported sensations related to the anode were itching and tingling. Based on the result, the most sever tingling (4.3 ± 0.2) and itching (3.1 ± 0.64) were recorded under the anode electrode at the beginning of M1–S1 condition. Itching and tingling under the cathode electrode were also the most commonly reported side effects. One of the participants reported a burning sensation at the beginning of a-tDCS$_{UHCDS}$ of M1–S1. No adverse effect related to a-tDCS$_{UHCDS}$ or a-tDCS was detected during the follow-up measurements.

The participant's judgment on the stimulation conditions is summarized in **Table 3**. Pearson's chi square showed no significant differences between the active and sham conditions ($\chi^2(4, n = 12) = 6.75$, p = 0.15), demonstrating that participants were not able to determine the type of stimulation. The majority

TABLE 1 | Summary of post hoc comparisons of means differences at each time-point of measurement for the effects of conventional a-tDCS of M1 and unihemispheric concurrent dual-site a-tDCS on M1 corticospinal excitability dual a-tDCS stimulations on CSE of M1.

		1–2	1–3	1–4	1–5	2–3	2–4	2–5	3–4	3–5	4–5
Single-pulse TMS	T_{pre}	0.04	0.42	0.91	0.66	0.14	0.08	0.03	0.34	0.27	0.08
T_0	0.004*	0.38	0.001*	0.03	0.000*	0.001*	0.000*	0.001*	0.02	0.34	
T_{30}	0.003*	0.91	0.000*	0.000*	0.016	0.001*	0.001*	0.005*	0.002*	0.49	
T_{60}	0.004*	0.06	0.001*	0.000*	0.001*	0.001*	0.000*	0.08	0.001*	0.82	
T_{day2}	0.000*	0.23	0.01	0.39	0.000*	0.000*	0.000*	0.004*	0.03	0.006	
SICI (ISI: 3 msec)	T_{pre}	0.96	0.40	0.23	0.58	0.45	0.33	0.005	0.98	0.28	0.122
T_0	0.67	0.004*	0.26	0.31	0.047	0.168	0.001*	0.001*	0.006	0.32	
T_{30}	0.03	0.003*	0.02	0.004*	0.003*	0.03	0.17	0.000*	0.000*	0.005	
T_{60}	0.35	0.58	0.06	0.002*	0.96	0.003*	0.05	0.001*	0.08	0.07	
T_{day2}	0.001*	0.01	0.23	0.004*	0.001*	0.001*	0.000*	0.97	0.91	0.02	
ICF (ISI: 10 msec)	T_{pre}	0.04	0.09	0.12	0.14	0.01	0.04	0.07	0.09	0.17	0.32
T_0	0.03	0.06	0.02	0.003*	0.001*	0.000*	0.000*	0.007	0.002*	0.05	
T_{30}	0.000*	0.001*	0.003*	0.000*	0.000*	0.000*	0.000*	0.07	0.004*	0.06	
T_{60}	0.000*	0.003*	0.003*	0.000*	0.000*	0.002*	0.000*	0.002*	0.004*	0.07	
T_{day2}	0.000*	0.07	0.12	0.034	0.001*	0.000*	0.000*	0.067	0.21	0.17	

The asterisks denote significant differences ($p < 0.005$). 1: Stimulation of M12: Stimulation of M1-DLPFC3: Stimulation of M1-S14: Stimulation of M1-V15: Sham tDCS.

of participants were properly blinded and the active or sham conditions were correctly guessed just in 16% of conditions (excluding the "Cannot say" responses).

The results of one-way ANOVA indicated that sensations were significantly different across the conditions ($F_{(4,47)} = 7.36$, $p = 0.01$). The *post hoc* comparisons showed that there was no significant difference between sensation of participants in sham and active conditions under the cathode electrode (except between sham and active a-$tDCS_{UHCDS}$ of M1–S1 stimulation ($p = 0.004$) at the End stage of stimulation). Under the reference electrode, there was no significant difference between active and sham conditions."

Discussion

Comparison of Baseline Values

All baseline RMT values remained unchanged at the starting point of all experimental conditions, meaning the washout period was adequate and any possibility of carry over effect from previous interventions on the same participants is refuted.

The Effects of a-tDCSUHCDS on M1 CSE

Our study was designed to assess the effects of concurrent stimulation of ipsilateral M1 and DLPFC on M1 CSE. Compared to a-tDCS of M1, we found that a-$tDCS_{UHCDS}$ of M1–DLPFC

TABLE 2 | Participant's sensation scores during experimental conditions.

		Anode electrode					Reference electrode				
		M1	**M1-DLPFC**	**M1-S1**	**M1-V1**	**Sham**	**M1**	**M1-DLPFC**	**M1-S1**	**M1-V1**	**Sham**
Tingling	Beginning	3.6 ± 0.21	3.9 ± 0.34	4.3 ± 0.2	2.9 ± 0.27	2.1 ± 0.16	1.5 ± 0.13	1.8 ± 0.12	2.1 ± 0.13	1.7 ± 0.22	1.4 ± 0.19
	Middle	2.1 ± 0.18	2.8 ± 0.15	1.4 ± 0.31	2.1 ± 0.14	1.4 ± 0.10	1.1 ± 0.18	0.7 ± 0.10	1.4 ± 0.10	1.2 ± 0.16	1.0 ± 0.07
	End	–	1.1 ± 0.19	1.1 ± 0.45	1.7 ± 0.21	0.8 ± 0.10	0.5 ± 0.27	0.6 ± 0.11	0.8 ± 0.24	0.9 ± 0.19	0.5 ± 0.1
Itching	Beginning	2.9 ± 0.09	3.0 ± 0.36	3.1 ± 0.64	1.3 ± 0.29	1.2 ± 0.21	1.1 ± 0.12	1.1 ± 0.09	1.2 ± 0.15	1.8 ± 0.11	1.1 ± 0.08
	Middle	1.3 ± 0.28	1.9 ± 0.03	2.6 ± 0.12	0.9 ± 0.15	0.8 ± 0.14	0.4 ± 0.16	0.6 ± 0.25	0.6 ± 0.17	1.2 ± 0.15	0.8 ± 0.12
	End	–	0.7 ± 0.1	1.2 ± 0.52	0.7 ± 0.23	–	0.1 ± 0.20	–	0.3 ± 0.06	0.9 ± 0.12	0.6 ± 0.09
Burning	Beginning	–	–	0.45 ± 0.1	–	0.23 ± 0.07		–	–	–	–
	Middle	–	–	0.31 ± 0.07	–	0.2 ± 0.03		–	–	–	–
	End	–	–	–	–			–	–	–	–
Not tolerated	Beginning	–	–	–	–			–	–	–	–
	Middle	–	–	–	–			–	–	–	–
	End	–	–	–	–			–	–	–	–

The values are rated using the NAS. 0 is rated as no sensation and 10 rated as the worst sensation imaginable. The sensations are recorded during three phases of stimulation: Beginning (0–7 min of stimulation), Middle (7–14 min of stimulation), End (14–20 min of stimulation). Sensations under both active (anode) and reference (cathode) electrodes were recorded during a-tDCS of M1, S1, DLPFC and sham a-tDCS. Scores are reported.

TABLE 3 | The judgements of participants on the stimulation condition.

		Actual testing conditions (n = 12)					
		a-tDCS of M1	a-tDCS$_{UHCDS}$ of M1-DLPFC	a-tDCS$_{UHCDS}$ of M1–S1	a-tDCS$_{UHCDS}$ of M1–V1	Sham	Total
Perceived stimulation	Active	2	2	3	1	4	12
	Sham	4	4	4	4	2	18
	Cannot say	6	6	5	7	6	30
	Total	12	12	12	12	12	60

induces larger M1 CSE (∼1.5 times) which lasted at least 24 h. We also found that a-tDCS$_{UHCDS}$ of M1–S1 increased CSE of M1 for 30 min, whilst the effects of a-tDCS on M1 lasted for one hour; there was no significant change in the size of MEPs in these two conditions. We hypothesized that concurrent stimulation of M1 and the other sites of the same hemisphere considerably increases M1 CSE. Our findings support this hypothesis in part. The results are in line with those of previous studies, which reported that M1 a-tDCS increased M1 CSE for one hour (Nitsche and Paulus, 2000, 2001; Bastani and Jaberzadeh, 2013). Compared to M1 stimulation, a-tDCS$_{UHCDS}$ of M1-DLPFC increased the size of MEPs for at least for 24 h. This study is the first to assess the effects of unihemispheric concurrent dual site stimulation of target areas of the brain, so further research is needed to support or disprove our results. However, considerable larger MEPs following a-tDCS$_{UHCDS}$ of M1-DLPFC, lasting for at least 24 h, is an extremely valuable clinical finding and should be explored further in future studies.

Comparison of the results from the conventional M1 a-tDCS and a-tDCS$_{UHCDS}$ of M1–DLPFC and other functionally connected pairs indicated that concurrent stimulation of M1-DLPFC is a more effective technique to increase M1 CSE. The efficacy of a-tDCS$_{UHCDS}$ of M1–DLPFC on CSE enhancement is more likely to be site specific, which is caused due to the effect of concurrent stimulation of functionally connected sites of M1. With some reasons, the findings in this study rule out the doubling of total charge as a driven source for the observed changes. First, a-tDCS$_{UHCDS}$ of M1–V1 had no effect on M1 CSE. Second, there was no significant difference between active and sham a-tDCS$_{UHCDS}$. Third, a-tDCS$_{UHCDS}$ of M1–S1 had similar effects on the size of MEPs to those of standard a-tDCS but with reduced durability. In a recent study conducted by our group, we applied a-tDCS over M1, S1, and DLPFC separately and found that CSE of M1 was significantly increased by a-tDCS of M1 or DLPFC (Vaseghi et al., 2015b). Moreover, some anatomical studies indicate that the premotor cortex is divided into dorsal and ventral parts and the dorsal part sends its output to the M1 and spinal cord and receives prominent input from DLPFC (Dum and Strick, 1991; He et al., 1993). The attention modulation signals from the DLPFC and motor preparation information from the dorsal part of the premotor cortex are received by the M1 (Bunge et al., 2001; Nitsche and Paulus, 2001; Van Ryckeghem et al., 2013). As a result, compared to stimulation of M1, a-tDCS$_{UHCDS}$ of M1–DLPFC may activate the DLPFC-premotor-primary motor pathway (Hoshi, 2006; Bracht et al., 2012) and increase M1 excitability. In contrast, inhibitory and fast-spiking interneurons named Vasointestinal Peptides (VIPs) in the superficial layers of S1 project to M1 pyramidal neurons; they account for the most GABAergic interneurons in S1 and target the distal dendrites of pyramidal cells in M1 (Lee et al., 2010, 2013; Rudy et al., 2011). It is possible that concurrent stimulation of M1 and S1 with a-tDCS$_{UHCDS}$ might activate VIP interneurons that in turn increase the size of MEPs and promote their long-lasting effects. The effect of excitability changes in V1 on CSE of M1 has not been investigated; however, Pirulli et al. (2014) found that V1 excitability changes have opposite effects on motor performance. They applied cathodal tDCS on V1, which led to motor performance improvement, and concluded that possible inhibitory compensatory circuits in V1 are inhibited by c-tDCS, resulting in motor performance improvement (O'Shea et al., 2007; Jacobson et al., 2012). Consequently, it is possible that in our experiments stimulation of V1 with M1 increased the inhibitory effects of those inhibitory circuits, which led to suppression of the effects of stimulation of M1, and subsequently a-tDCS$_{UHCDS}$ of M1–V1 had no effects on CSE of M1.

The Effects of a-tDCSUHCDS on SICI

In our study, SICI reduced for 60 min after a-tDCS of M1, which supports our hypothesis and is consistent with some previous studies of the effects of a-tDCS of M1 (Liepert et al., 1998; Hummel et al., 2005; Nitsche et al., 2005; Kidgell et al., 2013b). Few researchers have described the effects of a-tDCS on the GABAergic inhibitory system (Nitsche et al., 2005; Hummel et al., 2010) but many researchers are studying different approaches to find the most efficient method with a reasonably long-lasting effect. Nitsche et al. (2005) found a significant increase in SICI lasted for at least 30 min following 13 min of 1 mA a-tDCS (Nitsche et al., 2005). In contrast, another recent study suggested that SICI reduces for 30 min following a-tDCS of M1 (Kidgell et al., 2013a). The authors applied a-tDCS over M1 with a range of current intensities, and concluded that a-tDCS of M1 reduces SICI independently of current intensity. Yet again, Batsikadze et al. (2013) observed no significant changes in SICI following 20 min of 2 mA a-tDCS.

We demonstrated that SICI was reduced for at least 30 min after a-tDCS$_{UHCDS}$ of M1–S1 and there was significant difference between this condition and a-tDCS of M1, supporting our hypothesis. These finding may suggest that both conventional a-tDCS and a-tDCS$_{UHCDS}$ of M1–S1 can increase the excitability of intracortical inhibitory interneurons and as a result, reduce SICI. It can be concluded that CSE enhancement is independent of stimulation site in the dominant hemisphere. In addition,

significant differences between sham and conventional a-tDCS of M1, a-tDCS$_{UHCDS}$ of M1–S1 indicate that the results observed are due to the real effects of conventional a-tDCS and a-tDCS$_{UHCDS}$.

We also found that a-tDCS$_{UHCDS}$ of M1–V1 induced no significant changes in SICI and there was no significant difference between sham and a-tDCS$_{UHCDS}$ of M1–V1. It is suggested that increasing the current intensity in the same hemisphere is not the main reason behind the CSE enhancement of M1; functional connectivities probably play an important role in this regard.

This study is the first to investigate the effect of concurrent stimulation of M1 and another site in the same hemisphere on the M1 CSE. It seems that conventional stimulation of M1 and a-tDCS$_{UHCDS}$ of M1–DLPFC and M1–S1 reduce GABAergic intracortical inhibition, which can be interpreted as disinhibition of corticospinal neurons, resulting in increased CSE.

The Effects of a-tDCSUHCDS on ICF

We showed that a-tDCS of M1 increased the level of ICF in the stimulated area. In addition, comparing single- and double-site conditions showed that a-tDCS$_{UHCDS}$ of M1–DLPFC increased the level of ICF to triple that of a-tDCS of M1, and this effect lasted for 24 h after the intervention. This finding supports our hypothesis. Moreover, significant differences between active and sham conditions demonstrated that the results are not due to the placebo effect.

Some evidence supports an increase of ICF after a-tDCS of M1 (Chen, 2004; Nitsche et al., 2005; Batsikadze et al., 2013). In line with our results, it has been shown that ICF increases immediately after a-tDCS lasted for 90 min (Batsikadze et al., 2013). In contrast, Ogata et al. (2007) reported that a-tDCS of M1 has no significant influence on ICF or SICI (Ogata et al., 2007). Differences between Ogata et al.'s methods of conditioning and test stimulus intensity and our own probably explain these results.

Given that glutamate and NMDA receptors are involved in mediating ICF (Ziemann et al., 1996, 1998; Chen et al., 1998), it can be concluded that glutamergic and NMDA receptor concentration in the M1 intensifies following a-tDCS of M1. Since the present study is the first to investigate a-tDCS$_{UHCDS}$ effects on CSE, the results cannot be compared to other studies directly. However, regarding the role of DLPFC in motor functions (Bedwell et al., 2014; Van Snellenberg et al., 2014; Harding et al., 2015), it can be suggested that a-tDCS$_{UHCDS}$ M1–DLPFC might stabilize the tDCS-induced NMDA-receptor-dependent excitability enhancement in M1, resulting in raised ICF.

As can be seen in **Figure 3**, the level of ICF decreased following a-tDCS$_{UHCDS}$ of M1–S1 compared to a-tDCS of M1. Therefore, our hypothesis is not supported. No researchers have investigated the effects of concurrent stimulation of M1 and S1 in the same hemisphere, but in a recent study, M1 CSE enhancement was found with 30 min delay following a-tDCS of S1 (Vaseghi et al., 2015b). These authors concluded that the inhibitory effects of VIP interneurons on M1 probably increased after a-tDCS of S1 (Vaseghi et al., 2015b). Thus, one possible explanation for our own results is that increasing the activity of inhibitory VIP interneurons in S1 has an effect on CSE of M1, which controls the excitability enhancement of M1 following a-tDCS$_{UHCDS}$ of M1–S1.

Safety and Side Effects of a-tDCSUHCDS

Based on the results, participants were successfully blinded to the experimental conditions. They were not able to distinguish the active or sham conditions (except in ending stage of active M1-S1 a-tDCS$_{UHCDS}$ condition). No significant difference in rating scales was also found under the reference electrodes in sham and active conditions. In addition, minimal side effects following a-tDCS$_{UHCDS}$ suggest that stimulation of two functional areas of the same hemisphere with two separated tDCS devices is a safe approach in healthy individuals. The participants' tolerance for a-tDCS$_{UHCDS}$ with small electrodes was comparable with that for the conventional approach with larger electrodes. Similar to previous studies (Gandiga et al., 2006; Brunoni et al., 2011), general discomfort (itching/tingling) was the most frequently recorded side effect and just one participant of 12 reported a slight burning feeling. In addition, Poreisz et al. (2007) investigated the tDCS side effects over a large number of participants in both healthy and patient groups, while M1, S1, DLPFC, and visual cortex were stimulated. The results demonstrated that mild tingling and tingling were the most common sensations in healthy adults and there was no significant difference between participants' sensation after stimulation of different cortical targets (Poreisz et al., 2007).

Limitations of the Study

Our study has some limitations. First, the duration of the CSE stimulation effect of a-tDCS$_{UHCDS}$ was only assessed up to 24 h after intervention. Longer follow-up is required to properly evaluate the lasting effect of a-tDCS$_{UHCDS}$ of M1–DLPFC, and such data will be valuable for future studies investigating an optimal approach to enhance CSE of M1. Second, the effects were evaluated in young participants (less than 35 years); older individuals may respond differently to a-tDCS$_{UHCDS}$. Third, we utilized a conventional electrode montage with active electrodes (anode) over target stimulation areas and reference electrodes (cathode) over the contralateral supraorbital area (subgenual cortex). Regarding the functional connectivity between the subgenual cortex and the stimulated sites in this study, it is possible that the position of reference electrodes affect the level of CSE.

Suggestions for Future Research

Our results and the known functional connectivities between M1 and other cortical areas of the brain involved in motor learning, including the posterior parietal cortex, premotor cortex and supplementary motor area, suggest that the effect of a-tDCS$_{UHCDS}$ of these areas on M1 CSE should be investigated. In addition, more studies are required to fully characterize the effects of a-tDCS$_{UHCDS}$ on CSE of M1. For instance, the effects of a-tDCS$_{UHCDS}$ application time, current intensity, and electrode size should be systematically studied to improve

our understanding of these phenomena and their interactions. Furthermore, additional pharmacological experiments using receptor agonists/antagonists are needed to determine the exact mechanism behind the efficacy of a-tDCS$_{UHCDS}$. It is also recommended that the effects of cathodal tDCS$_{UHCDS}$ on CSE of M1 be investigated. Such data will clarify the connectivities of the cortical areas of the brain.

Conclusion

We found that a-tDCS$_{UHCDS}$ of M1-DLPFC not only considerably enhances M1 CSE (three fold) compared to the conventional a-tDCS approach, but extends the effects for at least 24 h. Further development of this new approach is likely to produce an efficient therapeutic neurorehabilitation strategy for pain treatment in patients with chronic pain or for motor performance improvement in stroke or multiple sclerosis patients.

Author Contributions

The Corresponding author of this manuscript is "BV", and "SJ" and "MZ" contribute in preparation of the current manuscript. The current study is a part of PhD thesis of the corresponding author. So, "SJ", as the main supervisor, and "MZ", as the co-supervisor, helped the corresponding author to design the study, interpret the results of study, and provide feedback on final conclusion and final draft of the manuscript.

With the submission of this manuscript, I would like to undertake that:

- All authors of this research paper have directly participated in the planning, execution, or analysis of this study;
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- The contents of this manuscript have not been copyrighted or published previously;
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- My Institute's (Monash University) representative is fully aware of this submission.

Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnhum.2015.00533/abstract

References

Apkarian, A. V., Bushnell, M. C., Treede, R. D., and Zubieta, J. K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *Eur. J. Pain.* 9, 463–484. doi: 10.1016/j.ejpain.2004.11.001

Bae, S. H., Kim, G. D., and Kim, K. Y. (2014). Analgesic effect of transcranial direct current stimulation on central post-stroke pain. *Tohoku J. Exp. Med.* 234, 189–195. doi: 10.1620/tjem.234.189

Bastani, A., and Jaberzadeh, S. (2012). Correction: 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. *PLoS One* 7:e47582. doi: 10.1371/journal.pone.0047582

Bastani, A., and Jaberzadeh, S. (2013). a-tDCS differential modulation of corticospinal excitability: the effects of electrode size. *Brain Stimul.* 6, 932–937. doi: 10.1016/j.brs.2013.04.005

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M. F., and Nitsche, M. A. (2013). Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *J. Physiol.* 591, 1987–2000. doi: 10.1113/jphysiol.2012.249730

Bedwell, S. A., Billett, E. E., Crofts, J. J., and Tinsley, C. J. (2014). The topology of connections between rat prefrontal, motor and sensory cortices. *Front. Syst. Neurosci.* 8:177. doi: 10.3389/fnsys.2014.00177

Bikson, M., Datta, A., Rahman, A., and Scaturro, J. (2010). Electrode montages for tDCS and weak transcranial electrical stimulation: role of "return" electrode's position and size. *Clin. Neurophysiol.* 121, 1976–1978. doi: 10.1016/j.clinph.2010.05.020

Bolognini, N., Spandri, V., Olgiati, E., Fregni, F., Ferraro, F., and Maravita, A. (2013). Long-term analgesic effects of transcranial direct current stimulation of the motor cortex on phantom limb and stump pain: a case report. *J. Pain Symptom Manage.* 46, e1–e4. doi: 10.1016/j.jpainsymman.2013.06.014

Borich, M. R., Brodie, S. M., Gray, W. A., Ionta, S., and Boyd, L. A. (2015). Understanding the role of the primary somatosensory cortex: Opportunities for rehabilitation. *Neuropsychologia* doi: 10.1016/j.neuropsychologia.2015.07.007 [Epub ahead of print].

Boros, K., Poreisz, C., Münchau, A., Paulus, W., and Nitsche, M. A. (2008). Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. *Eur. J. Neurosci.* 27, 1292–1300. doi: 10.1111/j.1460-9568.2008.06090.x

Bracht, T., Federspiel, A., Schnell, S., Horn, H., Höfle, O., Wiest, R., et al. (2012). Cortico-cortical white matter motor pathway microstructure is related to psychomotor retardation in major depressive disorder. *PLoS One* 7:e52238. doi: 10.1371/journal.pone.0052238

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., and Fregni, F. (2011). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *Int. J. Neuropsychopharmacol.* 14, 1133–1145. doi: 10.1017/s1461145710001690

Bunge, S. A., Ochsner, K. N., Desmond, J. E., Glover, G. H., and Gabrieli, J. D. (2001). Prefrontal regions involved in keeping information in and out of mind. *Brain.* 124, 2074–2086. doi: 10.1093/brain/124.10.2074

Chen, R. (2004). Interactions between inhibitory and excitatory circuits in the human motor cortex. *Exp. Brain Res.* 154, 1–10. doi: 10.1007/s00221-003-1684-1

Chen, R., Tam, A., Bütefisch, C., Corwell, B., Ziemann, U., Rothwell, J. C., et al. (1998). Intracortical inhibition and facilitation in different representations of the human motor cortex. *J. Neurophysiol.* 80, 2870–2881.

Di Pino, G., Pellegrino, G., Assenza, G., Capone, F., Ferreri, F., Formica, D., et al. (2014). Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat. Rev. Neurol.* 10, 597–608. doi: 10.1038/nrneurol.2014.162

Dum, R. P., and Strick, P. L. (1991). The origin of corticospinal projections from the premotor areas in the frontal lobe. *J. Neurosci.* 11, 667–689.

Dutta, A., Boulenouar, R. S., Guiraud, D., and Nitsche, M. A. (2014). Delineating the effects of anodal transcranial direct current stimulation on myoelectric control based on slow cortical potentials. *Conf. Proc. IEEE. Eng. Med. Biol. Soc.* 2014, 3094–3097. doi: 10.1109/embc.2014.6944277

Filmer, H. L., Dux, P. E., and Mattingley, J. B. (2014). Applications of transcranial direct current stimulation for understanding brain function. *Trends Neurosci.* 37, 742–753. doi: 10.1016/j.tins.2014.08.003

Foerster, B. R., Nascimento, T. D., Deboer, M., Bender, M. A., Rice, I. C., Truong, D. Q., et al. (2015). Excitatory and Inhibitory Brain Metabolites as Targets and Predictors of Effective Motor Cortex tDCS Therapy in Fibromyalgia. *Arthritis Rheumatol.* 67, 576–581. doi: 10.1002/art.38945

Furubayashi, T., Terao, Y., Arai, N., Okabe, S., Mochizuki, H., Hanajima, R., et al. (2008). Short and long duration transcranial direct current stimulation (tDCS) over the human hand motor area. *Exp. Brain Res.* 185, 279–286. doi: 10.1007/s00221-007-1149-z

Gandiga, P. C., Hummel, F. C., and Cohen, L. G. (2006). Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clin. Neurophysiol.* 117, 845–850. doi: 10.1016/j.clinph.2005.12.003

George, M. S., and Aston-Jones, G. (2010). Noninvasive techniques for probing neurocircuitry and treating illness: vagus nerve stimulation (VNS), transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). *Neuropsychopharmacology* 35, 301–316. doi: 10.1038/npp.2009.87

Goodwill, A. M., Reynolds, J., Daly, R. M., and Kidgell, D. J. (2013). Formation of cortical plasticity in older adults following tDCS and motor training. *Front. Aging Neurosci.* 5:87. doi: 10.3389/fnagi.2013.00087

Hagenacker, T., Bude, V., Naegel, S., Holle, D., Katsarava, Z., Diener, H. C., et al. (2014). Patient-conducted anodal transcranial direct current stimulation of the motor cortex alleviates pain in trigeminal neuralgia. *J. Headache Pain.* 15:78. doi: 10.1186/1129-2377-15-78

Hallett, M. (1996). Transcranial magnetic stimulation: a useful tool for clinical neurophysiology. *Ann. Neurol.* 40, 344–345. doi: 10.1002/ana.410400303

Harding, I. H., Yücel, M., Harrison, B. J., Pantelis, C., and Breakspear, M. (2015). Effective connectivity within the frontoparietal control network differentiates cognitive control and working memory. *Neuroimage.* 106, 144–153. doi: 10.1016/j.neuroimage.2014.11.039

Hasan, A., Galea, J. M., Casula, E. P., Falkai, P., Bestmann, S., and Rothwell, J. C. (2013). Muscle and timing-specific functional connectivity between the dorsolateral prefrontal cortex and the primary motor cortex. *J. Cogn. Neurosci.* 25, 558–570. doi: 10.1162/jocn_a_00338

He, S. Q., Dum, R. P., and Strick, P. L. (1993). Topographic organization of corticospinal projections from the frontal lobe: motor areas on the lateral surface of the hemisphere. *J. Neurosci.* 13, 952–980.

Hoshi, E. (2006). Functional specialization within the dorsolateral prefrontal cortex: a review of anatomical and physiological studies of non-human primates. *Neurosci. Res.* 54, 73–84. doi: 10.1016/j.neures.2005.10.013

Hummel, F., Celnik, P., Giraux, P., Floel, A., Wu, W. H., Gerloff, C., et al. (2005). Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain.* 128, 490–499. doi: 10.1093/brain/awh369

Hummel, F. C., Heise, K., Celnik, P., Floel, A., Gerloff, C., and Cohen, L. G. (2010). Facilitating skilled right hand motor function in older subjects by anodal polarization over the left primary motor cortex. *Neurobiol. Aging.* 31, 2160–2168. doi: 10.1016/j.neurobiolaging.2008.12.008

Iannetti, G. D., and Mouraux, A. (2010). From the neuromatrix to the pain matrix (and back). *Exp. Brain Res.* 205, 1–12. doi: 10.1007/s00221-010-2340-1

Jacobson, L., Koslowsky, M., and Lavidor, M. (2012). tDCS polarity effects in motor and cognitive domains: a meta-analytical review. *Exp. Brain Res.* 216, 1–10. doi: 10.1007/s00221-011-2891-9

Kandel, E. R. (2000). "From nerve cells to cognition: The internal cellular representation required for perception and action," in *Principles of neural science.* 5th Edn, Vol. 1, eds E. R. Kandel, J. H. Schwartz, and T. M. Jessel (New York: McGraw-Hill), 381–403.

Karok, S., and Witney, A. G. (2013). Enhanced motor learning following task-concurrent dual transcranial direct current stimulation. *PLoS One* 8:e85693. doi: 10.1371/journal.pone.0085693

Keeser, D., Meindl, T., Bor, J., Palm, U., Pogarell, O., Mulert, C., et al. (2011). Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. *J. Neurosci.* 31, 15284–15293. doi: 10.1523/jneurosci.0542-11.2011

Kidgell, D. J., Daly, R. M., Young, K., Lum, J., Tooley, G., Jaberzadeh, S., et al. (2013a). Different current intensities of anodal transcranial direct current stimulation do not differentially modulate motor cortex plasticity. *Neural Plast.* 2013, 1–9. doi: 10.1155/2013/603502

Kidgell, D. J., Goodwill, A. M., Frazer, A. K., and Daly, R. M. (2013b). Induction of cortical plasticity and improved motor performance following unilateral and bilateral transcranial direct current stimulation of the primary motor cortex. *BMC Neurosci.* 14:64. doi: 10.1186/1471-2202-14-64

Kirimoto, H., Ogata, K., Onishi, H., Oyama, M., Goto, Y., and Tobimatsu, S. (2011). Transcranial direct current stimulation over the motor association cortex induces plastic changes in ipsilateral primary motor and somatosensory cortices. *Clin. Neurophysiol.* 122, 777–783. doi: 10.1016/j.clinph.2010.09.025

Kothari, M., Svensson, P., Nielsen, J. F., and Baad-Hansen, L. (2014). Influence of position and stimulation parameters on intracortical inhibition and facilitation in human tongue motor cortex. *Brain Res.* 1557, 83–89. doi: 10.1016/j.brainres.2014.02.017

Koyama, S., Tanaka, S., Tanabe, S., and Sadato, N. (2015). Dual-hemisphere transcranial direct current stimulation over primary motor cortex enhances consolidation of a ballistic thumb movement. *Neurosci. Lett.* 588, 49–53. doi: 10.1016/j.neulet.2014.11.043

Kronberg, G., and Bikson, M. (2012). Electrode assembly design for transcranial Direct Current Stimulation: a FEM modeling study. *Conf. Proc. IEEE. Eng. Med. Biol Soc.* 2012, 891–895. doi: 10.1109/EMBC.2012.6346075

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., et al. (1993). Corticocortical inhibition in human motor cortex. *J. Physiol.* 471, 501–519. doi: 10.1113/jphysiol.1993.sp019912

Kuo, M. F., Unger, M., Liebetanz, D., Lang, N., Tergau, F., Paulus, W., et al. (2008). Limited impact of homeostatic plasticity on motor learning in humans. *Neuropsychologia.* 46, 2122–2128. doi: 10.1016/j.neuropsychologia.2008.02.023

Kwon, Y. H., Ko, M. H., Ahn, S. H., Kim, Y. H., Song, J. C., Lee, C. H., et al. (2008). Primary motor cortex activation by transcranial direct current stimulation in the human brain. *Neurosci. Lett.* 435, 56–59. doi: 10.1016/j.neulet.2008.02.012

Lang, N., Siebner, H. R., Ward, N. S., Lee, L., Nitsche, M. A., Paulus, W., et al. (2005). How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? *Eur. J. Neurosci.* 22, 495–504. doi: 10.1111/j.1460-9568.2005.04233.x

Lee, S., Hjerling-Leffler, J., Zagha, E., Fishell, G., and Rudy, B. (2010). The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. *J. Neurosci.* 30, 16796–16808. doi: 10.1523/jneurosci.1869-10.2010

Lee, S., Kruglikov, I., Huang, Z. J., Fishell, G., and Rudy, B. (2013). A disinhibitory circuit mediates motor integration in the somatosensory cortex. *Nat. Neurosci.* 16, 1662–1670. doi: 10.1038/nn.3544

Liebetanz, D., Nitsche, M. A., Tergau, F., and Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain.* 125, 2238–2247. doi: 10.1093/brain/awf238

Liepert, J., Classen, J., Cohen, L. G., and Hallett, M. (1998). Task-dependent changes of intracortical inhibition. *Exp. Brain Res.* 118, 421–426. doi: 10.1007/s002210050296

Ludemann-Podubecka, J., Bösl, K., Rothhardt, S., Verheyden, G., and Nowak, D. A. (2014). Transcranial direct current stimulation for motor recovery of upper limb function after stroke. *Neurosci. Biobehav. Rev.* 47, 245–259. doi: 10.1016/j.neubiorev.2014.07.022

Luft, C. D., Pereda, E., Banissy, M. J., and Bhattacharya, J. (2014). Best of both worlds: promise of combining brain stimulation and brain connectome. *Front Syst Neurosci.* 8:132. doi: 10.3389/fnsys.2014.00132

Medeiros, L. F., de Souza, I. C., Vidor, L. P., de Souza, A., Deitos, A., Volz, M. S., et al. (2012). Neurobiological effects of transcranial direct current stimulation: a review. *Front. Psychiatry.* 3:110. doi: 10.3389/fpsyt.2012.00110

Meinzer, M., Jahnigen, S., Copland, D. A., Darkow, R., Grittner, U., Avirame, K., et al. (2014). Transcranial direct current stimulation over multiple days improves learning and maintenance of a novel vocabulary. *Cortex.* 50, 137–147. doi: 10.1016/j.cortex.2013.07.013

Meyers, L. S., Gamst, G., and Guarino, A. J. (2006). *Applied multivariate research: Design and interpretation.* New Delhi: SAGE Publications.

Meyerson, B. A., Lindblom, U., Linderoth, B., Lind, G., and Herregodts, P. (1993). Motor cortex stimulation as treatment of trigeminal neuropathic pain. *Acta. Neurochir. Suppl. (Wien).* 58, 150–153. doi: 10.1007/978-3-7091-9297-9_34

Miller, E. K. (2000). The prefrontal cortex and cognitive control. *Nat. Rev. Neurosci.* 1, 59–65. doi: 10.1038/35036228

Miller, E. K., and Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annu. Rev. Neurosci.* 24, 167–202. doi: 10.1146/annurev.neuro.24.1.167

Moliadze, V., Schmanke, T., Andreas, S., Lyzhko, E., Freitag, C. M., and Siniatchkin, M. (2014). Stimulation intensities of transcranial direct current stimulation have to be adjusted in children and adolescents. *Clin. Neurophysiol.* 126, 1392–1399. doi: 10.1016/j.clinph.2014.10.142

Moloney, T. M., and Witney, A. G. (2014). Pressure pain thresholds increase after preconditioning 1 Hz repetitive transcranial magnetic stimulation with transcranial direct current stimulation. *PLoS One* 9:e92540. doi: 10.1371/journal.pone.0092540

Mordillo-Mateos, L., Turpin-Fenoll, L., Millàn-Pascual, J., Núñez-Pérez, N., Panyavin, I., Gómez-Arguelles, J. M., et al. (2012). Effects of simultaneous bilateral tDCS of the human motor cortex. *Brain Stimul.* 5, 214–222. doi: 10.1016/j.brs.2011.05.001

Murray, L. M., Edwards, D. J., Ruffini, G., Labar, D., Stampas, A., Pascual-Leone, A., et al. (2014). Intensity dependent effects of tDCS on corticospinal excitability in chronic Spinal Cord Injury. *Arch. Phys. Med. Rehabil.* 96, S114–S121. doi: 10.1016/j.apmr.2014.11.004

Nitsche, M. A., Doemkes, S., Karakose, T., Antal, A., Liebetanz, D., Lang, N., et al. (2007). Shaping the effects of transcranial direct current stimulation of the human motor cortex. *J. Neurophysiol.* 97, 3109–3117. doi: 10.1152/jn.01312.2006

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 3, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nitsche, M. A., and Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology.* 57, 1899–1901. doi: 10.1212/wnl.57.10.1899

Nitsche, M. A., and Paulus, W. (2011). Transcranial direct current stimulation—update 2011. *Restor. Neurol. Neurosci.* 29, 463–492. doi: 10.3233/RNN-2011-0618

Nitsche, M. A., Seeber, A., Frommann, K., Klein, C. C., Rochford, C., Nitsche, M. S., et al. (2005). Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *J. Physiol.* 568, 291–303. doi: 10.1113/jphysiol.2005.092429

O'Shea, J., Boudrias, M. H., Stagg, C. J., Bachtiar, V., Kischka, U., Blicher, J. U., et al. (2014). Predicting behavioural response to TDCS in chronic motor stroke. *Neuroimage.* 85, 924–933. doi: 10.1016/j.neuroimage.2013.05.096

O'Shea, J., Johansen-Berg, H., Trief, D., Gobel, S., and Rushworth, M. F. (2007). Functionally specific reorganization in human premotor cortex. *Neuron.* 54, 479–490. doi: 10.1016/j.neuron.2007.04.021

Ogata, K., Yamasaki, T., and Tobimatsu, S. (2007). "Modulation of Intracortical Inhibition and Facilitation of Motor Cortex Through Transcranial Direct Current Stimulation", in: *Complex Medical Engineering, 2007. CME 2007. IEEE/ICME International Conference* (New York: IEEE). doi: 10.1109/ICCME.2007.4382099

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia.* 9, 97–113. doi: 10.1016/0028-3932(71)90067-4

Opie, G. M., and Semmler, J. G. (2014). Modulation of short- and long-interval intracortical inhibition with increasing motor evoked potential amplitude in a human hand muscle. *Clin. Neurophysiol.* 125, 1440–1450. doi: 10.1016/j.clinph.2013.11.015

Parasuraman, R., and Mckinley, R. A. (2014). Using noninvasive brain stimulation to accelerate learning and enhance human performance. *Hum. Factors.* 56, 816–824. doi: 10.1177/0018720814538815

Parazzini, M., Rossi, E., Ferrucci, R., Liorni, I., Priori, A., and Ravazzani, P. (2013). Modelling the electric field and the current density generated by cerebellar transcranial DC stimulation in humans. *Clin. Neurophysiol.* 125, 577–584. doi: 10.1016/j.clinph.2013.09.039

Park, E., Kim, Y. H., Chang, W. H., Kwon, T. G., and Shin, Y. I. (2014). Interhemispheric modulation of dual-mode, noninvasive brain stimulation on motor function. *Ann. Rehabil. Med.* 38, 297–303. doi: 10.5535/arm.2014.38.3.297

Paulus, W., Classen, J., Cohen, L. G., Large, C. H., Di Lazzaro, V., Nitsche, M., et al. (2008). State of the art: Pharmacologic effects on cortical excitability measures tested by transcranial magnetic stimulation. *Brain Stimul.* 1, 151–163. doi: 10.1016/j.brs.2008.06.002

Pellicciari, M. C., Brignani, D., and Miniussi, C. (2013). Excitability modulation of the motor system induced by transcranial direct current stimulation: a multimodal approach. *Neuroimage.* 83, 569–580. doi: 10.1016/j.neuroimage.2013.06.076

Pirulli, C., Fertonani, A., and Miniussi, C. (2014). Is neural hyperpolarization by cathodal stimulation always detrimental at the behavioral level? *Front. Behav. Neurosci.* 8:226. doi: 10.3389/fnbeh.2014.00226

Poreisz, C., Boros, K., Antal, A., and Paulus, W. (2007). Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain Res. Bull.* 72, 208–214. doi: 10.1016/j.brainresbull.2007.01.004

Rohrich, R. J. (2007). Ethical approval of clinical studies, informed consent, and the Declaration of Helsinki: what you need to know. *Plast. Reconstr. Surg.* 119, 2307–2309. doi: 10.1097/01.prs.0000229193.31894.d0

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., et al. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr. Clin. Neurophysiol.* 91, 79–92. doi: 10.1016/0013-4694(94)90029-9

Rudy, B., Fishell, G., Lee, S., and Hjerling-Leffler, J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Dev. Neurobiol.* 71, 45–61. doi: 10.1002/dneu.20853

Saper, C. B., Iversen, S., and Frackowiak, R. (2000). Integration of sensory and motor function: the association areas of the cerebral cortex and the cognitive capabilities of the brain. *Principles of neural science.* 4, 349–380.

Stagg, C. J., and Nitsche, M. A. (2011). Physiological basis of transcranial direct current stimulation. *Neuroscientist.* 17, 37–53. doi: 10.1177/1073858410386614

Uy, J., and Ridding, M. C. (2003). Increased cortical excitability induced by transcranial DC and peripheral nerve stimulation. *J. Neurosci. Methods.* 127, 193–197. doi: 10.1016/s0165-0270(03)00142-0

Van Ryckeghem, D. M., De Houwer, J., Van Bockstaele, B., Van Damme, S., De Schryver, M., and Crombez, G. (2013). Implicit associations between pain and self-schema in patients with chronic pain. *Pain.* 154, 2700–2706. doi: 10.1016/j.pain.2013.07.055

Van Snellenberg, J. X., Slifstein, M., Read, C., Weber, J., Thompson, J. L., Wager, T. D., et al. (2014). Dynamic shifts in brain network activation during supracapacity working memory task performance. *Hum. Brain. Mapp.* 36, 1245–6410. doi: 10.1002/hbm.22699

Vaseghi, B., Jaberzadeh, S., and Zoghi, M. (2015a). Inter-pulse Interval Affects the Size of Single-pulse TMS-induced Motor Evoked Potentials: a Reliability Study. *Basic and Clinical Neuroscience.* 6, 44–51.

Vaseghi, B., Zoghi, M., and Jaberzadeh, S. (2014). Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study. *Clin. Neurophysiol.* 125, 1847–1858. doi: 10.1016/j.clinph.2014.01.020

Vaseghi, B., Zoghi, M., and Jaberzadeh, S. (2015b). How does anodal transcranial direct current stimulation of the pain neuromatrix affect brain excitability and pain perception? A randomised, double-blind, sham-control study. *PLoS One* 10:e0118340. doi: 10.1371/journal.pone.0118340

Vines, B. W., Cerruti, C., and Schlaug, G. (2008). Dual-hemisphere tDCS facilitates greater improvements for healthy subjects' non-dominant hand compared to uni-hemisphere stimulation. *BMC Neurosci.* 9:103. doi: 10.1186/1471-2202-9-103

Vollmann, H., Conde, V., Sewerin, S., Taubert, M., Sehm, B., Witte, O. W., et al. (2013). Anodal transcranial direct current stimulation (tDCS) over supplementary motor area (SMA) but not pre-SMA promotes short-term visuomotor learning. *Brain Stimul.* 6, 101–107. doi: 10.1016/j.brs.2012.03.018

Wang, J., Wang, Y., Hu, Z., and Li, X. (2014). Transcranial direct current stimulation of the dorsolateral prefrontal cortex increased pain empathy. *Neuroscience.* 281C, 202–207. doi: 10.1016/j.neuroscience.2014.09.044

Wassermann, E., Epstein, C., and Ziemann, U. (2008). *Oxford Handbook of Transcranial Stimulation.* Oxford: Oxford University Press.

Williams, P. S., Hoffman, R. L., and Clark, B. C. (2013). Preliminary evidence that anodal transcranial direct current stimulation enhances time to task failure of a sustained submaximal contraction. *PLoS One* 8:e81418. doi: 10.1371/journal.pone.0081418

Ziemann, U., Chen, R., Cohen, L. G., and Hallett, M. (1998). Dextromethorphan decreases the excitability of the human motor cortex. *Neurology.* 51, 1320–1324. doi: 10.1212/wnl.51.5.1320

Ziemann, U., Rothwell, J. C., and Ridding, M. C. (1996). Interaction between intracortical inhibition and facilitation in human motor cortex. *J. Physiol.* 496, 873–881. doi: 10.1113/jphysiol.1996.sp021734

Zimerman, M., Heise, K. F., Hoppe, J., Cohen, L. G., Gerloff, C., and Hummel, F. C. (2012). Modulation of training by single-session transcranial direct current stimulation to the intact motor cortex enhances motor skill acquisition of the paretic hand. *Stroke.* 43, 2185–2191. doi: 10.1161/strokeaha.111.645382

Zmigrod, S. (2014). The role of the parietal cortex in multisensory and response integration: evidence from transcranial direct current stimulation (tDCS). *Multisens Res.* 27, 161–172. doi: 10.1163/22134808-00002449

Zoghi, M., Pearce, S. L., and Nordstrom, M. A. (2003). Differential modulation of intracortical inhibition in human motor cortex during selective activation of an intrinsic hand muscle. *J. Physiol.* 550, 933–946. doi: 10.3410/f.1014208.193059

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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.

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	Supervisory input on study design, Guidance in the framing of the manuscript, Review and provision of feedback on final manuscript draft	5 %

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

Candidate's Signature:  Date: 15-Sep- 2015

Signature:  Date: 15-Sep-2015

Signature:  Date: 15-Sep-2015

Preamble to Chapter 8

The application of c-tDCS over the M1 is an established technique to reduce M1 excitability while reducing STh/PTh. The findings in Chapter 6 indicate that c-tDCS of not only the M1 but also the DLPFC and the S1 may also affect M1 excitability. Such data provides evidence for the existence of functional connectivities between these cortical sites. Chapter 8 is a methodological which investigates the potential effects of concurrent c-tDCS of these functionally connected cortical sites on the M1 CSE. This novel technique is named unihemispheric concurrent dual-site c-tDCS (c-tDCS$_{UHCDS}$). To assess the mechanisms behind the efficacy of this novel technique, intracortical inhibition and facilitation are also measured by the paired-pulse TMS paradigm. This chapter provides evidence for an unexpected observation which indicates that compared to the conventional single-site a-tDCS, concurrent c-tDCS of these cortical sites induces no CSE reduction. The effects of this novel technique on STh/PTh changes is presented in Chapter 9.

Chapter 8: Unihemispheric concurrent dual-site c-tDCS: the effects on corticospinal excitability

This study is under review at the European Journal of Neuroscience. The format of this chapter is consistent with the European Journal of Neuroscience.

The setup system used in this study, Ethics approval, TMS safety, Personal health history form, and Edinburg handedness questionnaires and consent form are provided in Appendices 7-13.

Behavioural Neuroscience

Unihemispheric concurrent dual-site cathodal transcranial direct current stimulation: the effects on corticospinal excitability

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Running title: Unihemispheric concurrent dual-site c-tDCS and corticospinal excitability

Abstract

We aimed to assess the effects of concurrent cathodal transcranial direct current stimulation (c-tDCS) of two targets in a hemisphere, termed unihemispheric concurrent dual-site cathodal tDCS (c-tDCS$_{UHCDS}$), on the size of M1 corticospinal excitability and its lasting effect. Secondary aims were to identify the mechanisms behind the efficacy of c-tDCS$_{UHCDS}$ and to evaluate the side effects of this new technique. Twelve healthy volunteers received 20 min c-tDCS under five conditions in a random order: M1 c-tDCS, c-tDCS$_{UHCDS}$ of M1-dorsolateral prefrontal cortex (DLPFC), M1–primary sensory cortex (S1), M1– primary visual cortex (V1), and sham. The M1 corticospinal excitability of the first dorsal interossei muscle was assessed before, immediately after, 30 min, 60 min, and 24 hours after the interventions. Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were also assessed, using paired-pulse paradigm. Compared to conventional M1 c-tDCS, CSE significantly increased following c-tDCS$_{UHCDS}$ of M1-DLPFC and M1-V1 for up to 24 hours ($P = 0.001$). Significant increases in ICF were observed following c-tDCS$_{UHCDS}$ of M1-DLPFC ($P = 0.005$) and M1-V1 ($P = 0.002$). Compared to baseline values, ICF and SICI significantly increased at T_{60} ($P < 0.001$) and T_{24h} ($P < 0.001$) following the concurrent c-tDCS of M1 and V1. Sham c-tDCS$_{UHCDS}$ did not induce any significant alteration. The corticospinal excitability increase mainly accompanied with ICF increase, which indirectly indicates the activity of glutamergic mechanisms. The findings may help us to understand the brain function and develop future motor learning studies. No significant excitability change induced by sham c-tDCS$_{UHCDS}$ which suggests that there is no placebo effect associated with this new tDCS technique.

Key words: Unihemispheric dual-site cathodal transcranial direct current stimulation, functional connectivity, Primary motor cortex, corticospinal excitability, Lasting effect

1. Introduction

Cathodal transcranial direct current stimulation (c-tDCS) has been known as a neuromodulatory technique for reduction of corticospinal excitability (Nitsche and Paulus, 2000; Mendonca et al., 2011). Literature indicates, 10 min c-tDCS induces reversible hyperpolarization of neurons in the target area, for up to 60 minutes (Nitsche and Paulus, 2000; 2001). This technique has been applied for different therapeutic purposes in neurological or psychological conditions such as neurological pain (Mendonca et al., 2011; O'Connell et al., 2011; Riberto et al., 2011; Ngernyam et al., 2015), migraine (Antal et al., 2008b; Antal et al., 2011; Dasilva et al., 2012), stroke (Hummel et al., 2005; Boggio et al., 2007; Schlaug et al., 2008), depression (Fregni et al., 2006a; Boggio et al., 2008a), and focal epilepsy (Fregni et al., 2006b; Liebetanz et al., 2006).

Conventionally, 1 mA c-tDCS of primary motor cortex (M1) with duration of up to 13 min reduces corticospinal excitability for up to one hour (Nitsche and Paulus, 2000; 2001; Schretlen et al., 2014). Many studies have attempted to optimize this polarity driven effect in terms of both size and its lasting. A number of studies showed that, higher-intensity or longer stimulation durations enhance the inhibitory effects of c-tDCS (Apkarian et al., 2005; Ahumada et al., 2013; Accornero et al., 2014; Vaseghi Bita, 2015). However, new evidence suggests that depending on the c-tDCS parameters such as current intensity, duration of application, c-tDCS can reduce or enhance corticospinal excitability (Boros et al., 2008; Monte-Silva et al., 2010; Batsikadze et al., 2013). In contrary to an implicit assumption that more intensive protocol may enhance the efficacy of the stimulation, Batsikadze et al. (2013) indicated that stimulation parameters have a non-linear effect on corticospinal excitability modulation. They found that 20 min c-tDCS with intensity of 1 mA resulted in a significant decrease in corticospinal

excitability, whereas 2 mA c-tDCS, with the same duration, increased the corticospinal excitability (Batsikadze et al., 2013).

Despite some promising results from above studies, more investigations are needed to optimize c-tDCS effects. An alternative way, which may optimize the effect of c-tDCS on corticospinal excitability modulation is concurrent stimulation of M1 and one of its functionally connected sites in the same hemisphere. The functional connectivity of M1 and dorsolateral prefrontal cortex (DLPFC), primary sensory cortex (S1), and premotor cortex has been evidenced by some tDCS/fMRI studies (Lang et al., 2005; Kwon et al., 2008; Keeser et al., 2011). In addition, the co-activation of M1 and S1 (Ghai et al., 2000; Boggio et al., 2008b) and DLPFC (Jefferys, 1981; Bikson et al., 2004; Ragert et al., 2008) in planning, execution, and control of movement has been well established. This co-activation is also exists during pain processing (Peyron et al., 2000; Apkarian et al., 2005). The rationale behind the superiority of this novel technique, unihemispheric concurrent dual-site c-tDCS (c-tDCS$_{UHCDS}$), is based upon Hebbian principle of synaptic plasticity and manipulation of functional connectivities in a network (Rizzo et al., 2009; Koch et al., 2013). Based on the Hebbian principle, if two connected neurons (or two sites of a network) are activated simultaneously the connection strengthens and the connected neurons will be strongly activated together in the future with same stimulus intensity (Arai et al., 2011; Koch et al., 2013). As a result, it is hypothesized that c-tDCS$_{UHCDS}$ intensifies the mutual communications between M1 and its functionally connected sites (Luft et al., 2014). Regarding the direct relationship between the level of excitability-related changes and motor performance (Aihara et al., 2015) and cognition (Ahmed et al., 2012) improvement, current study may provide primitive evidence to optimize c-tDCS effects in motor performance, cognition, or pain management studies.

In the present study, primary sensory cortex (S1) and dorsolateral prefrontal cortex (DLPFC) are chosen as two functionally connected cortical sites of M1. The primary aim was to

investigate the effect of c-tDCS$_{UHCDS}$ of M1-DLPFC and M1-S1 on M1 corticospinal excitability and its lasting effects, compared to conventional M1 c-tDCS. We hypothesized that c-tDCS$_{UHCDS}$ of M1-S1 and M1-DLPFC is more efficient than the conventional M1 c-tDCS in corticospinal excitability reduction. Due to the novelty of the proposed approach, and the need for better understanding of mechanisms behind the efficacy of c-tDCS$_{UHCDS}$, short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were also measured. It is hypothesized that corticospinal excitability enhancement in c-tDCS$_{UHCDS}$ of M1-S1 and M1-DLPFC conditions is accompanied with increasing the level of SICI and decreasing the level of ICF. As c-tDCS$_{UHCDS}$ is a new technique, we also aimed to systematically assess its side effects. We hypothesized that concurrent stimulation of two sites in a same hemisphere is a safe with minimum side effects.

2. Methods

2.1.Study design

The study was conducted as a randomized, sham-controlled, cross-over design study with at least seven days of wash-out. All experimental procedures were approved by the Monash University Human Research Ethics Committee and conformed to the Declaration of Helsinki (1964). The current study is registered as a clinical trial on the Australian New Zealand Clinical Trial (registry number: ACTRN12614000817640, http://www.anzctr.org.au/).

2.2.Participants

We recruited 12 healthy volunteers (eight women and four men, all Monash University students) with mean age of 24 ± 2.11 (age range 19-34). The sample size was calculated (with power of 80%) based on the result of a pilot study on six participants. All participants were right-handers as determined by the Edinburgh Handedness Inventory (10-item version, mean laterality quotient = 89 ± 9.3) (Valle et al., 2009). The method and procedure of the study were explained

to the participants before they received the written informed consent form. None of the participants reported contraindications to Transcranial magnetic stimulation (TMS) or tDCS, and all were medication-free before and during the study. None declared a history of neurological or psychiatric disease or pain during the three months before the study.

2.3.M1 corticospinal excitability assessment

Participants were comfortably seated in a fully adjustable treatment chair (MagVenture, Denmark) with head and arm rests. Single-pulse TMS of the left M1, using a MagPro ×30 (MagOption) stimulator (MagVenture, Denmark) with a flat 70 mm figure-of-eight coil (max. initial dB/dt 28 KT/s near the coil surface) was applied to record motor-evoked potentials (MEPs) from the right first dorsal interossei (FDI) muscle. The coil was placed over the left M1 with a posterior-anterior orientation and set at angle of 45° to the midline. The area of stimulation with largest MEP responses was defined as the hotspot and marked on the scalp to be used throughout the tests to ensure the consistency of the coil placement. Resting motor threshold (RMT) was defined as the minimal stimulator output needed to elicit five MEPs in a series of 10 with minimum amplitude of 50-100 μV in the relaxed FDI muscle (Rossini et al., 1994; Hallett, 1996; Wassermann et al., 2008). Single-pulse MEPs were recorded with the TMS intensity adjusted to elicit ~1 mV peak-to-peak amplitude at baseline. Stimulation intensity was kept constant for the post-intervention assessments.

2.4.Intracortical inhibition and facilitation assessment

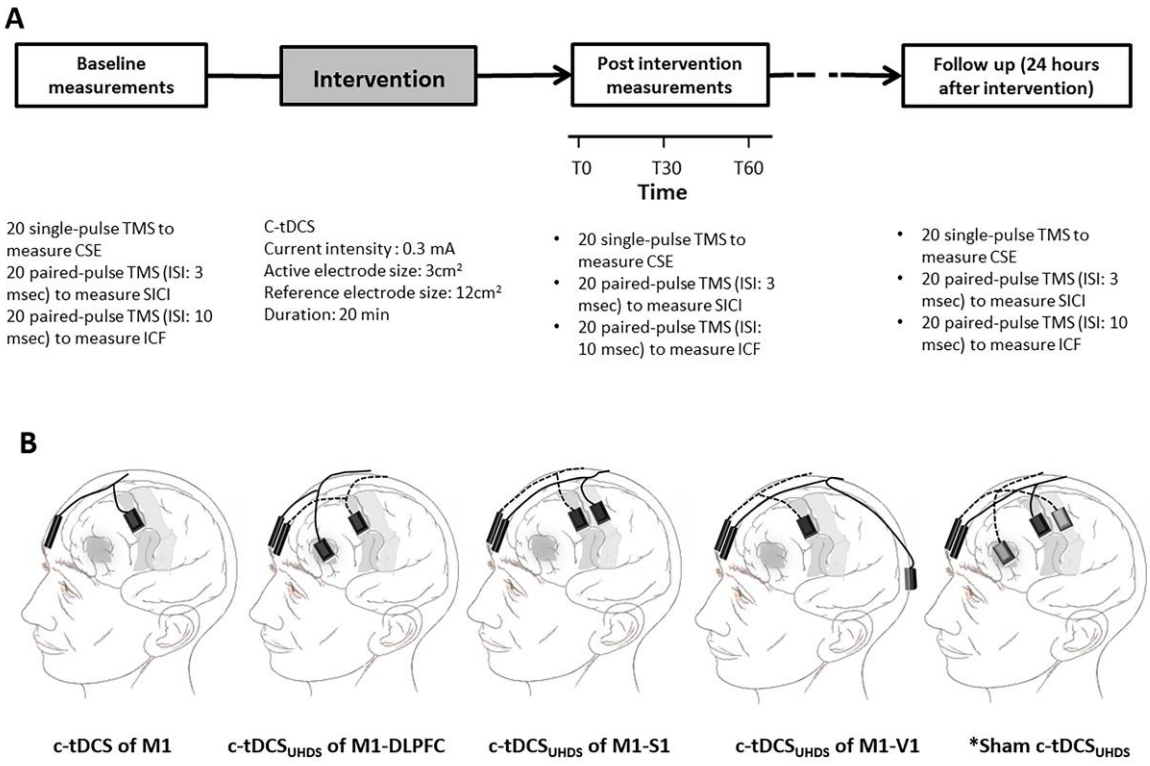
In order to evaluate the function of intracortical inhibition and facilitation circuits in the M1, paired-pulse TMS was used to measure SICI and ICF (Kujirai et al., 1993). In this method, a subthreshold TMS stimulus is followed by a suprathreshold TMS pulse with an inter-stimulus interval (ISI) of 1-5 msec or 8-15 msec to measure SICI or ICF respectively (Kujirai et al.,

1993). In the present study, subthreshold test stimulus intensity was applied as 80% of RMT (0.8 × RMT), followed by a suprathreshold test stimulus (Di Pino et al., 2014). The test stimulus intensity was adjusted to achieve a baseline MEP of around 1 mV (Zoghi et al., 2003; Kothari et al., 2014). The ISI was set at 3 msec to measure SICI and 10 msec to measure ICF (Di Pino et al., 2014; Opie and Semmler, 2014). Five blocks of ISI were designed to deliver both single- and paired-pulse TMS randomly. Each block of ISI contained 20 single-pulse and 40 paired-pulse TMS (20 ISI of 3 and 20 ISI of 10 msec). One of five blocks was randomly selected in each time point of measurement to minimize the bias induced by the order of stimuli. Blocks of MEPs in which the muscle was not relaxed were excluded from the analysis. In order to avoid any profound effect of inter-pulse interval on MEP size, a ten-second interval was applied between Stimulations (Vaseghi et al., 2015).

2.5.Experimental procedure

Volunteers were comfortably seated in a fully adjustable treatment chair (MagVenture, Denmark) with head and arm rests. After finding the hotspot of M1 FDI by single-pulse TMS, the stimulus intensity was adjusted to elicit single-pulse MEPs with averaged amplitude of 1 mV. 80% of RMT was calculated as the subthreshold test stimulus. In the next stage, 20 single-pulse TMS-induced MEPs, 20 pair-pulsed induced MEPs with ISI of 3 msec, and 20 with ISI of 10 msec were recorded in a random order as a baseline measurement. Then participants received tDCS under each of five different experimental conditions in random order: c-tDCS of M1, c-tDCS$_{UHCDS}$ of M1-S1, c-tDCS$_{UHCDS}$ of M1-DLPFC, c-tDCS$_{UHCDS}$ of M1- primary visual cortex (V1), and sham c-tDCS$_{UHCDS}$. The experimental sessions were separated by at least seven days to avoid interference or carry-over effects of tDCS, and completed at the same time of day to avoid diurnal variation (late mornings or early afternoon). Duration of intervention was 20 minutes in both active and sham conditions. All outcome measures were

measured before (T_{pre}), immediately after (T_0), 30 minutes (T_{30}), 60 minutes (T_{60}) and 24 hours (T_{24h}) after each intervention (Fig. 1).



Participants were blinded to the condition of tDCS (sham or active) and the purposes of the study. To control the effect of female hormonal fluctuation on the size of MEPs, the experimental sessions were carried out between the 7th and 21st day of women's menstrual cycles.

2.6.Active and sham transcranial direct current stimulation conditions

A direct current stimulator (Intelect® Advanced Therapy System, Chattanooga, USA) was set to deliver 0.3 mA (Bastani and Jaberzadeh, 2013) direct current for 20 minutes, with 10 seconds of linear fade in and fade out. Current intensity of 0.3 mA allowed us to considerably decrease the size of electrodes (Uy and Ridding, 2003), while keeping the current density (0.1 mA/cm^2) in a safe range with limited side effects (Nitsche and Paulus, 2000; Antal et al., 2008a). The

superiority of low intensity in induction of larger corticospinal excitability is supported by numerous tDCS studies (Nitsche and Paulus, 2001; Brunoni et al., 2011; Parazzini et al., 2013).

C-tDCS was applied through active and reference saline-soaked surface sponge electrodes sized 2 × 1.5 cm (3cm^2) and 2 × 6 cm (12 cm^2) respectively (Fig. 1). The small size of active electrodes produces a highly focused DC current over the target areas, which enabled us to stimulate M1 and S1 with two separated cathode electrodes. Based on the result of some computational modeling studies, the effects of tDCS can be focused by smaller electrodes (Nitsche et al., 2007; Bikson et al., 2010). In addition, recent anodal tDCS studies illustrated that utilizing the smaller active electrodes over M1 resulted in larger corticospinal excitability even when current density is held constant (Nitsche et al., 2007; Bastani and Jaberzadeh, 2013). However, some computational modelling studies indicated that current intensity to electrode size ratio (current density) is the main factor for induction of larger excitability (Miranda et al., 2009; Roth, 2009; Faria et al., 2011).

The reference electrodes (anode) were conventionally positioned over the contralateral supraorbital region with the assumption of no or negligible neuromodulatory effects on the subgenual cortex. To reduce the neuromodulatory effects of reference electrodes on the subgenual cortex, we kept them four times as large as the active electrodes (i.e., the density was four times less) (Nitsche et al., 2007). Considering the narrow shape of reference electrodes, two reference electrodes administered over the contralateral supraorbital region. The electrodes were fixed with two horizontal and perpendicular straps. The density under each active electrode was 0.1 mA/cm^2 in all experimental conditions.

The cathode was placed over the left M1 for the right FDI muscle. The location of M1 was identified by TMS. The international 10-20 system was used to position the active electrode accurately on S1, and DLPFC. For the c-tDCS$_{UHCDS}$ M1-S1condition, the cathode was placed

over C3 and C′3 (2 cm posterior to C3) and for stimulation of DLPFC, the cathode was placed over F3. V1 is not part of the network involved in movement planning or pain processing. Therefore, to investigate whether the changes induced by c-tDCS$_{UHCDS}$ are site specific or simply due to stimulation of the brain by doubled current density, c-tDCS$_{UHCDS}$ of M1–V1 was included as a control condition. In the sham condition, the electrodes were placed in the same positions as for active M1-S1 or M1-DLPFC conditions, but the stimulator was turned off after 30 seconds of stimulation. All pre- and post-intervention evaluations were kept identical to the ones in other conditions. To stimulate V1, the cathode electrode was placed over Oz.

2.7.Measurement of side effects

During and after all experiments, participants were asked to complete a questionnaire to record their feelings and any side effects. The questionnaire contained rating scales for the presence and severity of side effects such as itching, tingling, burning sensations under electrodes (Boros et al., 2008; George and Aston-Jones, 2010) and other adverse effects, including headache and pain during and after stimulation. All participants rated the unpleasantness of any scalp sensation using numeric analogue scales (NAS) (e.g., 0 = no tingling to 10 = worst tingling imaginable).

2.8.Data management and statistical analysis

Peak-to-peak amplitude of 20 single-pulse MEPs were automatically calculated and averaged online for each time point of measurement, using a custom designed macro. Area under the curve of MEPs was also quantified off-line from the digitized averages of rectified EMG for conditioned and unconditioned stimuli in each trial by using a custom designed macro in Powerlab 8/30 software. The size of the conditioned MEP was expressed as a percentage of the unconditioned test MEPs in order to evaluate the effectiveness of ICI or ICF.

To detect any carry-over effect, one-way repeated measures ANOVA was carried out on the RMT recorded at the starting point of each experimental condition (T_{pre}). A two-way repeated measures ANOVA was performed to assess the effects of two independent variables, experimental conditions with 5 levels (c-tDCS, c-tDCS$_{UHCDS}$ of M1-S1, M1-V1, M1-DLPFC, and sham c-tDCS$_{UHCDS}$) and time with 5 levels (T_{pre}, T_0, T_{30}, T_{60}, and T_{24h}), on corticospinal excitability, SICI, and ICF as independent variables. Mauchly's test was used to assess the validity of the sphericity assumption for repeated measures ANOVA. Greenhouse-Geisser corrected significance values were used when sphericity was lacking (Meyers et al., 2006). When ANOVA produced significant results, post hoc comparisons were performed using t-tests (paired samples, two-tailed, $P < 0.05$) adjusted for multiple comparisons to compare MEP, SICI, ICF changes following the intervention at each time point of measurement. Additionally, to test whether the baseline value of each stimulation site differed significantly from post-intervention time points (T_0, T_{30}, T_{60}, and T_{24h}), a one-way repeated measure ANOVA was applied. In case of any significant main effect, post hoc comparisons were performed, adjusted for multiple comparisons.

In order to assess whether participants were successfully blinded to the stimulation conditions (active or sham), Pearson's chi-square was used on rating scales recorded by questionnaire. In addition, a one-way ANOVA was carried out on the mean values of rating scale recorded by questionnaire to assess any significant differences between the participants' feelings during active and sham conditions. Means are reported ± standard error (SE). Statistical analyses were performed using SPSS software version 22.

3. Results

3.1.Comparison of baseline values

One-way repeated measures ANOVA illustrated that the RMT elicited by single-pulse TMS at the starting point of each experimental condition were comparable and there was no significant difference between the baseline RMTs ($F_4 = 1.94$, $P = 0.17$) in all conditions.

3.2.Corticospinal excitability changes following c-tDCS and c-tDCS$_{UHCDS}$

Two-way repeated measures ANOVA showed significant main effects of condition ($F_4 = 13.8$; $P < 0.001$), time ($F_4 = 2.11$; $P= 0.04$), and interaction of condition and time ($F_{16} = 3.53$; $P= 0.001$). Pairwise comparisons indicated that at T_0 there was a significant difference between c-tDCS of M1 and c-tDCS$_{UHCDS}$ M1-DLPFC and M1-V1, and also between c-tDCS$_{UHCDS}$ M1-DLPFC and c-tDCS$_{UHCDS}$ of M1-S1 (Table 1). Significant differences were observed between sham and c-tDCS of M1 and c-tDCS$_{UHCDS}$ of M1-DlPFC at T_0 (Table 1). Post-hoc comparison showed no significant differences between c-tDCS of M1 and c-tDCS$_{UHCDS}$ of M1-S1 and M1-V1 at T_0 (Table 1).

Table 1 Summary of pairwise comparison of means differences at each time-point of measurement for the effects of dual cathodal transcranial direct current stimulations on corticospinal excitability (CSE) of M1measured by sing-pulse TMS

		1-2	**1-3**	**1-4**	**1-5**	**2-3**	**2-4**	**2-5**	**3-4**	**3-5**	**4-5**
Single-pulse TMS	T_{pre}	0.26	0.84	0.44	0.6	0.74	0.46	0.55	0.67	0.07	0.89
	T_0	0.001*	0.25	0.002*	0.000*	0.001*	0.22	0.001*	0.005	0.08	0.03
	T_{30}	0.001*	0.43	0.005*	0.001*	0.002*	0.17	0.004*	0.007	0.01	0.03
	T_{60}	0.000*	0.001*	0.002*	0.004*	0.003*	0.05	0.003*	0.004*	0.31	0.002*
	T_{24h}	0.003*	0.40	0.001*	0.15	0.07	0.27	0.003*	0.19	0.01	0.001*

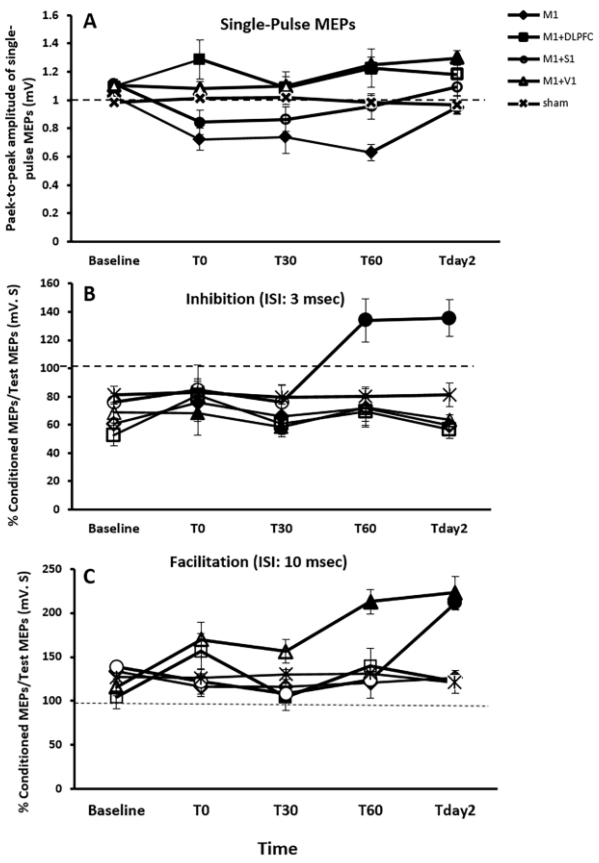
The asterisks represent the adjusted p value in multiple comparisons.
1: Stimulation of M1
2: Stimulation of M1-DLPFC
3: Stimulation of M1-S1
4: Stimulation of M1-V1
5: Sham tDCS

Pairwise comparisons at T_{30} revealed significant differences in corticospinal excitability between c-tDCS of M1 and c-$tDCS_{UHCDS}$ of M1-DLPFC, M1-S1, and M1-V1 and between c-$tDCS_{UHCDS}$ of M1-DLPFC and M1-V1 (Table 1). We measured significant differences between sham and c-tDCS of M1 and c-$tDCS_{UHCDS}$ of M1-DLPFC. There was no significant difference between sham and c-$tDCS_{UHCDS}$ of M1-S1 or M1-V1 at T_{30} (Table 1).

At T_{60}, there were significant differences in corticospinal excitability between c-tDCS of M1 and all other conditions (Table 1). At this time point, there were also significant differences between c-$tDCS_{UHCDS}$ of M1-DLPFC and M1-S1, c-$tDCS_{UHCDS}$ of M1-S1 and M1-V1 at T_{60} (Table 1). The results showed significant differences in corticospinal excitability between sham and all other active conditions except c-$tDCS_{UHCDS}$ of M1-S1 (Table 1).

At T_{24h} there was a significant difference between stimulation of M1 and c-$tDCS_{UHCDS}$ of M1-DLPFC and M1-V1 and also between sham and these two c-$tDCS_{UHCDS}$ conditions (Table 1).

Comparing post-intervention MEP amplitudes with baseline values showed that there was a statistically significant difference between time-points of measurements following c-$tDCS_{UHCDS}$ of M1-V1 ($F_{4} = 3.59$, $P= 0.01$) and c-tDCS of M1 ($F_{4} = 2.50$, $P= 0.002$). The results of post-hoc tests are summarized in Figure 2. Based on these results, M1 c-tDCS significantly reduced MEPs at T_0, T_{30}, and T_{60} (Fig. 2). Corticospinal excitability of M1 increased immediately after the c-$tDCS_{UHCDS}$ of M1-DLPFC, whist no significant change was found at T_{30}, T_{60}, or T_{24h} (Fig. 2A). In addition, c-$tDCS_{UHCDS}$ of M1-S1 resulted in significant immediate reduction in the size of MEPs. However, compared to baseline value, no significant change was found 30 min, 60 min, and 24 hours after c-$tDCS_{UHCDS}$ of M1-S1 (Fig. 2A). C-$tDCS_{UHCDS}$ of M1-V1 showed significant increase with 60 min delay.



Comparing baseline values and post-test values showed significant increase in the size of MEPs at T_{24h} following c-tDCS$_{UHCDS}$ of M1-V1 (Fig. 2A). There was no significant change in the size of MEPs following sham c-tDCS$_{UHCDS}$ (Fig. 2A).

3.3.SICI changes following c-tDCS$_{UHCDS}$

Two-way repeated measures ANOVA showed significant effects of condition ($F_4 = 4.43$, $P = 0.004$), time ($F_4 = 2.77$, $P = 0.01$), and interaction of time and condition ($F_{16} = 4.20$, $P = 0.007$) on SICI. No statistically significant differences were found between conditions at T_0 and T_{30} ($P > 0.005$) (Table 2). Significant differences were found between c-tDCS$_{UHCDS}$ of M1-V1 and c-tDCS$_{UHCDS}$ of M1-DLPFC, M1-S1, and c-tDCS of M1 at T_{60} and T_{24h} (Table 2). The results

also indicated that there were significant differences between sham and c-tDCS$_{UHCDS}$ of M1-V1 at T_{60} and T_{24h}.

One-way repeated measures ANOVA results showed that there were statistically significant differences in corticospinal excitability between time-points of measurement following c-tDCS$_{UHCDS}$ of M1-V1 (F_4 = 12.74, P= 0.002) and c-tDCS$_{UHCDS}$ of M1-DLPFC (F_4 = 4.06, P = 0.01). No significant SICI alteration was found at any time-points of measurements following c-tDCS$_{UHCDS}$ of M1-S1, c-tDCS M1 and sham. The results of post hoc comparisons are summarized in Figure 2B.

Table 2 Summary of pairwise comparison of means differences at each time-point of measurement for the effects of dual cathodal transcranial direct current stimulations on short interval intracortical inhibition (SICI) and intracortical facilitation (ICF)

		1-2	**1-3**	**1-4**	**1-5**	**2-3**	**2-4**	**2-5**	**3-4**	**3-5**	**4-5**
SICI (ISI: 3 msec)	T_{pre}	0.27	0.29	0.06	0.02	0.01	0.02	0.27	0.23	0.51	0.81
	T_0	0.81	0.72	0.67	0.61	0.42	0.87	0.90	0.42	0.24	0.09
	T_{30}	0.22	0.27	0.24	0.09	0.21	0.11	0.76	0.13	0.02	0.12
	T_{60}	0.52	0.31	0.000*	0.07	0.41	0.000*	0.21	0.001*	0.61	0.000*
	T_{24h}	0.27	0.31	0.001*	0.45	0.76	0.000*	0.07	0.002*	0.01	0.000*
ICF (ISI: 10 msec)	T_{pre}	0.32	0.46	0.06	0.35	0.10	0.48	0.16	0.08	0.23	0.33
	T_0	0.005*	0.01	0.002*	0.23	0.004*	0.17	0.07	0.005	0.03	0.003*
	T_{30}	0.07	0.06	0.004*	0.25	0.67	0.000*	0.12	0.001*	0.09	0.003*
	T_{60}	0.12	0.28	0.001*	0.31	0.44	0.000*	0.62	0.000*	0.45	0.000*
	T_{24h}	0.05	0.003*	0.000*	0.67	0.85	0.000*	0.33	0.000*	0.48	0.000*

The asterisks represent the adjusted p value in multiple comparisons.
ISI: Inter-stimulus interval
1: Stimulation of M1
2: Stimulation of M1-DLPFC
3: Stimulation of M1-S1
4: Stimulation of M1-V1
5: Sham tDCS

3.4. ICF changes following c-tDCS$_{UHCDS}$

The results of two-way repeated measures ANOVA indicated significant effect of condition ($F_4 = 5.84$, $P = 0.001$) and time ($F_4 = 4.43$, $P = 0.004$). The interaction of time and condition was also significant ($F_{16} = 6.79$, $P < 0.001$). Post hoc comparisons revealed a significant difference in ICF between c-tDCS of M1 and c-tDCS$_{UHCDS}$ of M1-DLPFC and M1-V1 at T_0 (Table 2). The result also showed that there were significant differences between M1-V1 and M1-S1 and sham conditions at T_0. The results of pairwise comparison at T_{30} and T_{60} were similar; there were significant differences between stimulation of M1 and M1-DLPFC, M1 and M1-V1, M1-S1 and M1-V1, and M1-V1 and sham (Table 2). At T_{24h}, the results showed significant differences between c-tDCS$_{UHCDS}$ of M1-V1 and M1-DLPFC, M1-S1, c-tDCS of M1, and sham (Table 2).

Compared to baseline values, one-way repeated measure ANOVA results revealed that there were significant ICF changes after c-tDCS$_{UHCDS}$ of M1-DLPFC ($F_4 = 4.03$, $P = 0.007$), c-tDCS$_{UHCDS}$ of M1-S1 ($F_4 = 2.13$, $P = 0.04$), and c-tDCS$_{UHCDS}$ of M1-V1 ($F_4 = 13.81$, $P < 0.001$). Figure 2C summarizes the post-hoc comparison results.

3.5. Safety and side effects of a-tDCS$_{UHCDS}$

Participants' experiences were recorded at the beginning, middle and end of the 20 min intervention. The averaged sensation score recorded during the intervention is summarized in Table 3. The only reported sensations related to the cathode were itching and tingling. Redness, itching and tingling under the anode electrodes were the most commonly reported side effects. No participants reported a burning sensation or headache during c-tDCS$_{UHCDS}$ application. No adverse effect related to c-tDCS$_{UHCDS}$ or conventional c-tDCS was detected during the follow-up measurements (Table 3).

Table 3 Participants' numeric sensation scores during experimental conditions

		Cathode electrode					**Reference electrode**				
		M1	M1-DLPFC	M1-S1	M1-V1	Sham	M1	M1-DLPFC	M1-S1	M1-V1	Sham
Tingling	Beginning	3.1 ± 0.24	4.1 ± 0.18	3.9 ± 0.41	3.1 ± 0.24	1.9 ± 0.25	1.6 ± 0.19	2.5 ± 0.13	2.3 ± 0.32	2.5 ± 0.29	1.6 ± 0.15
sensation	Middle	2.7 ± 0.14	2.4 ± 0.23	2.3 ± 0.17	1.6 ± 0.15	1.2 ± 0.14	0.8 ± 0.21	1.3 ± 0. 14	1.7 ± 0.28	2.1 ± 0.15	1.0 ± 0.04
	End	1.3 ± 0.34	0.9 ± 0.14	1.0 ± 0.21	0.7 ± 0.18	0.6 ± 0.16	0.32 ± 0.1	0.9 ± 0.11	1.1 ± 0.41	1.4 ± 0.16	0.4 ± 0.03
Itching	Beginning	2.7 ± 0.11	2.6 ± 0.26	3.1 ± 0.64	1.3 ± 0.29	1.7 ± 0. 17	1.6 ± 0.15	1.2 ± 0.1	1.5 ± 0.2	1.0 ± 0.12	1.3 ± 0.12
sensation	Middle	0.6 ± 0.47	1.2 ± 0.19	2.6 ± 0.12	0.9 ± 0.15	0.5 ± 0.14	0.9 ± 0.21	1.0 ± 0.2	1.1 ± 0.14	0.8 ± 0.27	0.9 ± 0.09
	End	0.3 ± 0.19	0.8 ± 0.27	1.0 ± 0.31	0.7 ± 0.23	-	0.7 ± 0.36	0.4 ± 0.53	0.7 ± 0.09	0.6 ± 0.37	0.3 ± 0.54
Burning	Beginning	-	0.18 ± 0.07	0.52 ± 0.2	-	0.23 ± 0.07		-	-	-	-
sensation	Middle	-	-	0.12± 0.17	-	0.14 ± 0.01		-	-	-	-
	End	-	-	-	-	-		-	-	-	-
						-					
Not	Beginning	-	-	-	-	-		-	-	-	-
tolerated	Middle	-	-	-	-	-		-	-	-	-
	End	-	-	-	-	-		-	-	-	-

The values are rated using the NAS. 0 is rated as no sensation and 10 rated as the worst sensation imaginable. The sensations are recorded during three phases of stimulation: Beginning (0 to 7 minutes of stimulation), Middle (7 to 14 minutes of stimulation), End (14 to 20 minute of stimulation). Sensations under both active (anode) and reference (cathode) electrodes were recorded during a-tDCS of primary motor cortex (M1), primary sensory cortex (S1), dorsolateral prefrontal cortex (DLPFC), and sham tDCS. Scores are reported in Mean ± SE

The participants' judgment on the stimulation conditions is summarized in Table 4. Pearson's chi square showed no significant differences between the active and sham conditions (χ^2 (4, n = 12) = 8.12, P = 0.09), demonstrating that participants were not able to determine the type of stimulation. The majority of participants were properly blinded and the active or sham conditions were correctly guessed just in 15 % of conditions (excluding the "Cannot say" responses).

Table 4 The judgements of participants on the stimulation conditions

		Actual testing conditions (n = 12)					
		c-tDCS of M1	**c-tDCS$_{UHCDS}$ of M1-DLPFC**	**c-tDCS$_{UHCDS}$ of M1–S1**	**c-tDCS$_{UHCDS}$ of M1–V1**	**Sham**	**Total**
Perceived stimulation	**Active**	2	1	3	2	**4**	**12**
	Sham	3	6	3	4	**2**	**18**
	Cannot say	7	5	6	6	**6**	**30**
	Total	12	12	12	12	**12**	**60**

The results of one-way ANOVA indicated that sensations were significantly different across the conditions (F (4, 47) = 6.37, P = 0.03). The post hoc comparisons only showed significant difference between sham and active c-tDCS$_{UHCDS}$ of M1-S1 stimulation (P = 0.002) at the end stage of stimulation. No significant difference was found between sensation of participants in sham and active conditions under the cathode electrode. Under the reference electrode, there was no significant difference between active and sham conditions.

4. Discussion

4.1.Comparison of baseline values

The similarity of the baseline RMTs in each experimental condition indicates that the washout period was adequate and participants experienced no carry-over effect from one intervention to the next.

4.2.Corticospinal excitability changes following M1 c-tDCS

The results showed that 20 min M1 c-tDCS reduced corticospinal excitability, lasting for 60 min. Likewise, it has been previously shown that 13 min c-tDCS with current intensity of 1

mA reduces M1 corticospinal excitability for one hour (Nitsche and Paulus, 2000; 2011; Medeiros et al., 2012; Monte-Silva et al., 2013). In contrast, in some other studies M1 c-tDCS resulted in opposite effects (Nitsche et al., 2005; Batsikadze et al., 2013). As smaller electrodes induce more focused electrical fields over target areas (Bikson et al., 2004; Nitsche et al., 2007), the discrepancy in the result could be easily explained by methodological differences. The second postulated reason behind the opposite effect is the length of c-tDCS application. Batsikadze et al. (2013) compared the effect of 9 and 20 min c-tDCS with intensity of 1 mA on corticospinal excitability (Batsikadze et al., 2013), demonstrating that 20 min c-tDCS induces excitatory effects. All these studies indicated a non-linear relationship between current density/electrode size and direction of induced changes in corticospinal excitability modulation.

4.3.Corticospinal excitability changes following c-tDCS$_{UHCDS}$

Unlike conventional M1 c-tDCS, concurrent stimulation of M1 and DLPFC increased corticospinal excitability immediately after stimulation. The finding does not support our hypothesis in which we assumed that concurrent stimulation of two functionally connected sites decrease the M1 excitability more efficient than conventional M1 c-tDCS. The results also showed significant differences between the inhibitory effect of conventional M1 c-tDCS and excitatory effects of M1-DLPFC c-tDCS$_{UHCDS}$ at all time-points. Basic pharmacological studies indicate that postsynaptic alpha (2)-adrenergic receptors (alpha (2)-ARs) located in the membrane of DLPFC neurons inhibit control of M1 function (Lowe et al., 2000; Rissman et al., 2004; Egorova et al., 2015; Lee and Grafton, 2015). These receptors mediate the function of voltage-gated calcium channels (Boggio et al., 2008b) and decrease the level of free intracellular Ca^{2+} ions (Jefferys, 1981; Ghai et al., 2000). Therefore, the inhibitory effects of c-tDCS may block or disinhibit the inhibitory effects of DLPFC on M1, which increased corticospinal excitability.

The other possible mechanism is activation of homeostatic mechanisms following the doubling of the electrical charges received by target areas. It has been recently indicated that changing the conventional c-tDCS parameters may modulate the Ca^{2+} channels' activity and excitatory mechanisms resulted in opposite cortical (Batsikadze et al., 2013) and behavioral responses (Pirulli et al., 2014). In fact, L-Type voltage-gated Ca^{2+} channels (L-VGCC) are involved in homeostatic plasticity (Kong et al., 2006; Mansouri et al., 2009) to avoid destabilizing and overpowering changes in the brain and maintain equilibrium (Zubieta et al., 2005; Benedetti, 2008). Hence, it can be hypothesized that concurrent stimulation enhances the activity of L-VGCC and the level of intracellular Ca^{2+} level, activating homeostatic mechanisms which lasted at least for 24 hours. This non-linear response was also found in other noninvasive brain stimulation. Moliadze et al. (2012) found that increasing the intensity of transcranial random noise stimulation (tRNS) switched its inhibitory to excitatory effects (Moliadze et al., 2012).

Similar to M1 c-tDCS, c-$tDCS_{UHCDS}$ of M1-S1 resulted in immediate M1 corticospinal excitability reduction, which gradually returned to the baseline value. The finding supports our finding in part. There was no statistical difference between inhibitory effects of M1 stimulation and M1-S1 c-$tDCS_{UHCDS}$. The integrated signals from S1 are projected to M1 through a cortico-cortical interconnection located in superficial layers of the cortex (Diamond et al., 2008; Petreanu et al., 2012; Xu et al., 2012). These projected neurons recruit a type of fast-spiking inhibitory interneurons named Vasointestinal Peptides (VIPs), which are strongly GABAergic interneurons (Lee et al., 2010; Rudy et al., 2011; Lee et al., 2013). Therefore, it can be hypothesized that disinhibition of VIP leads by concurrent stimulation of M1 and S1 results in retuning of corticospinal excitability to baseline value shortly after the immediate corticospinal excitability diminishing.

C-$tDCS_{UHCDS}$ of M1-V1 produced no immediate changes but after a delay of 60 minutes, we observed significant excitatory changes that lasted for at least 24 hours. Such findings are

opposite with our hypothesis in which we assumed that concurrent stimulation of two functionally connected sites decrease the M1 excitability more efficient than conventional M1 c-tDCS. There were significant differences between inhibitory effects of M1 c-tDCS and excitatory effects of concurrent stimulation of M1 and V1 at all time-points of measurement except T_{30}. There is contradictory evidence about the effects of c-tDCS of the visual cortex on visuomotor tasks. Antal et al. (2004) indicated that c-tDCS of V5, the motion-sensitive cortical area, has a facilitatory effect on visuomotor learning. Due to the controlling effect of the visual cortex in complex motor tasks (Antal et al., 2004a; Antal et al., 2004d), they concluded that c-tDCS of V1 disinhibits the inhibitory effects of c-tDCS on M1 (Antal et al., 2004d). In contrast, in another study conducted by Antal et al. (2004), it was observed that V5 c-tDCS has no effect on visuomotor tasks (Antal et al., 2004b). As inhibitory role of V5 occurs only in the early phase of visuo-motor coordination, V5 c-tDCS induces no change in M1 corticospinal excitability. One possible mechanism for the excitatory effects of c-tDCS$_{UHCDS}$ of M1-V1 may be disinhibition of the prominent transluminal inhibitory interneurons, which project from V1 to M1 (Gilbert and Wiesel, 1979; Katzel et al., 2011; Kätzel et al., 2011). Another alternative mechanism behind the excitatory effects of c-tDCS$_{UHCDS}$ of M1-V1 is activation of a homeostatic mechanism to restore the equilibrium in the brain.

Some animal model research demonstrated that low postsynaptic Ca^{2+} enhancement induces long-term depression and increasing the level of calcium causes long-term potentiation (LTP) (Cho et al., 2001; Lisman, 2001). Therefore, one possible mechanism behind the long-lasting effect of c-tDCS$_{UHCDS}$ of M1-V1 is the increase of intracellular Ca^{2+} level and activation of L-VGCC induced by doubled current density. However, more studies are required to discover why concurrent stimulation of M1 and V1, but not other conditions, resulted in an effect lasting 24 hours.

4.4.Physiological mechanisms behind the effects of c-tDCS on M1 corticospinal excitability

The corticospinal excitability reduction immediately after M1 c-tDCS coincided with immediate SICI reduction and no significant ICF change. However, we observed a tendency toward ICF reduction. In line with our results, Nitsche et al. (2005) applied c-tDCS over M1 with intensity of 1 mA and showed immediate SICI reduction. Antal et al. (2005) also indicated that 7 min c-tDCS with intensity of 1 mA reduced corticospinal excitability and SICI. In line with our study, there was no significant ICF alteration after the stimulation (Nitsche et al., 2005). In contrast, Batsikadze et al. (2013) demonstrated that conventional M1 c-tDCS with intensity of 2 mA and electrode size of 35 cm^2 enhanced corticospinal excitability, which coincided with immediate SICI reduction and gradual ICF enhancement lasting for 7–8 hours (Batsikadze et al., 2013). However, they indicated that stimulation of M1 with intensity of 1 mA suppresses the corticospinal excitability and ICF and increases SICI. Batsikadze et al. concluded that intensive parameters like doubling the intensity of stimulation probably overshoot the ceiling targets to keep the neuronal functions at an optimal steady state.

Regardless of decreasing trends in ICF and SICI, the corticospinal excitability reduction at T_{30} and T_{60} was associated with no significant change in SICI and ICF. This result is not accordance with those of previous studies. The reason for these effects is unclear at present, but might be related to different c-tDCS parameters. In addition, it was shown that not only do $GABA_A$ and glutamate-based mechanisms, which were indirectly assessed in the present study, play an important role in the corticospinal excitability level, but involvement of other excitatory and inhibitory neurotransmitters like serotonin and dopamine affect the result (Poreisz et al., 2007; Brunoni et al., 2011; Medeiros et al., 2012). However, more pharmacological and methodological studies are needed to shed light on the mechanisms behind the efficacy of c-tDCS of M1 on its corticospinal excitability.

4.5.Physiological mechanisms behind the effects of c-tDCS$_{UHCDS}$ on M1 corticospinal excitability

The immediate corticospinal excitability enhancement following concurrent DLPFC and M1 c-tDCS was associated with immediate SICI reduction and ICF enhancement by which our hypothesis is not supported. Corticospinal excitability returning to baseline value also coincided with the return of SICI and ICF to baseline values. Compared to M1 c-tDCS, there was a significant ICF increase with no SICI alteration following c-tDCS$_{UHCDS}$ of M1-DLPFC. Such results indicate that the excitatory effect of c-tDCS$_{UHCDS}$ of M1-DLPFC is the result of increasing the level of glutamate immediately after the stimulation. Glutamate is released by pyramidal neuron synapses located in layer V of the cortex and thalamic synaptic inputs (Ragert et al., 2008). These synapses are sensitive to electrical fields (Bikson et al., 2004; Ragert et al., 2008; Bikson et al., 2010; Marquez-Ruiz et al., 2012). As a result, it can be speculated that concurrent stimulation of M1 and DLPFC increase the total charge received by pyramidal cells and activate the homeostatic mechanisms to invert the effect of excessive inhibition induced by c-tDCS$_{UHCDS}$. As the level of glutamate has an opposing effect on the level of $GABA_A$ (Ragert et al., 2008; Kabakov et al., 2012), the enhancement of glutamate mechanisms decreases the level of $GABA_A$ and consequently decreases SICI.

For the c-tDCS$_{UHCDS}$ of M1-S1 condition, the results showed no effect on either SICI or ICF although immediate corticospinal excitability reduction was observed after the stimulation. As a result, the results cannot support our hypothesis in which we assumed that c-tDCS$_{UHCDS}$ is more efficient than conventional M1 c-tDCS in SICI enhancement and ICF reduction. The reason behind this result is unclear, but it seems that other mechanisms (in addition to mediating the level of $GABA_A$ and glutamate) are involved in corticospinal excitability reduction following the concurrent stimulation of M1 and S1 in the same hemisphere.

Comparing M1 c-tDCS and c-tDCS$_{UHCDS}$ of M1-S1 revealed no significant difference in SICI and ICF at any time point of measurement other than T_{24h}.

SICI reduction and ICF enhancement peaked 60 min after c-tDCS$_{UHCDS}$ of M1-V1 and lasted at least for 24 hours, consequently enhancing corticospinal excitability. The results do not support the hypothesis. In addition, there were significant differences between ICF changes following concurrent M1 and V1 c-tDCS and the conventional M1 c-tDCS at all time-points of measurement. The finding could be explained by reduction of $GABA_A$ activity and increasing the glutamate level following c-tDCS$_{UHCDS}$ of M1-V1. Regarding the controlling role of V1 on M1 via the visuomotor tract (Antal et al., 2004d), it is possible that V1 c-tDCS blocked/disinhibited the $GABA_A$ receptors (Antal et al., 2004b; Antal et al., 2004c) and activated the glutamergic mechanisms (Kabakov et al., 2012). Another possible mechanism is transient homeostatic metaplasticity (Kuo et al., 2007). Overpowering the inhibition induced by c-tDCS$_{UHCDS}$ may activate the LTP mechanisms (Turrigiano and Nelson, 2004; Turrigiano, 2008), which convert the conventional inhibitory effects of c-tDCS to excitatory ones. Activation of LTP mechanisms may cause the long-lasting effects. The prolonged SICI reduction and ICF enhancement following c-tDCS$_{UHCDS}$ of M1-V1 is a valuable finding for future studies investigating new approaches for prolonging the tDCS effect.

The results of sham c-tDCS$_{UHCDS}$ revealed that c-tDCS$_{UHCDS}$ has no placebo effect on corticospinal excitability, SICI, and ICF, and strongly implies all modulations are due to real effects of intervention.

4.6.Safety and side effects of c-tDCS$_{UHCDS}$

Based on the results, participants were successfully blinded to the experimental conditions.

They were not able to distinguish between active or sham conditions (except in ending stage of active M1-S1 c-tDCS$_{UHCDS}$ condition). No significant difference in rating scales was also found under the reference electrodes in sham and active conditions. Similar to M1 c-tDCS, concurrent c-tDCS produced minimal side effects, this new tDCS technique is safe for healthy adults. Itching and tingling were the most frequently reported sensations. Two of 12 participants reported burning and one reported a headache feeling. The reported side effects in this study are comparable with those from conventional single site c-tDCS studies (Davis and Whalen, 2001; LeDoux, 2003; Phelps, 2006; Antal et al., 2008a; Turi et al., 2014). Based on these studies, the tDCS-related cutaneous discomfort varies with tDCS parameters, including electrode size and current intensity. In addition, Poreisz et al. (2007) demonstrated that mild tingling and tingling are the most common sensations in healthy adults and there was no significant difference between participants' sensation after stimulation of different cortical targets. (Poreisz et al., 2007). Small electrodes induce less electro-chemical effect and consequently less inconvenient feelings are received by the sensory cortex (Kirouac et al., 2004; McRae et al., 2010; Kanske and Kotz, 2011), resulting in less spatial summation and cutaneous discomfort (Kanske and Kotz, 2011; Ochsner et al., 2012). In addition, Martinesen and colleagues (2004) (McRae et al., 2010) illustrated that current intensity but not current density modifies the perceptual threshold for direct currents. As a result, it can be assumed that utilizing two small active electrodes in c-tDCS$_{UHCDS}$ is associated with mild cutaneous discomfort, which is comparable with the conventional approach of c-tDCS.

4.7.Limitations

In our study the effect of c-tDCS was only assessed until 24 hours after completion of stimulation, which limits our understanding regarding possible further lasting effects.

Corticospinal excitability was evaluated in healthy young adults; older adults or patients with neurological or psychological conditions may respond to c-tDCS$_{UHCDS}$ differently, so the results might not be generalizable to elders or patients. Furthermore, it should be noted that the rater in the current study was not blinded to the experimental conditions which may increase the risk of "experimenter bias" in the current study (Sackett, 1979).

4.8.Suggestions for future studies

Future studies could investigate the impact of c-tDCS$_{UHCDS}$ of M1 on other functionally connected sites of the brain, including the posterior parietal cortex, premotor cortex or supplementary motor area (Ghosh and Porter, 1988; Fink et al., 1997; Munchau et al., 2002; Boros et al., 2008) on M1 excitabilty. Such data would help us to better understand the characteristics of c-tDCS$_{UHCDS}$. The effects of application time, current intensity and electrode size during c-tDCS$_{UHCDS}$ should also be systematically studied to improve our understanding. Pharmacological studies would help to shed light on the mechanisms underlying the efficacy of c-tDCS$_{UHCDS}$ on M1 excitability modulation. To have a better understanding of current flow and penetrated current levels in the s, computational/ fMRI studies are also suggested for the future studies. Moreover, considering the excitatory effects of c-tDCS$_{UHCDS}$ we demonstrated, investigating the efficacy of c-tDCS$_{UHCDS}$ on modulation of pain threshold, working memory, and motor learning is recommended.

5. Conclusion

C-tDCS$_{UHCDS}$ of M1-DLPFC and M1-V1 are new and effective approaches to increasing M1 excitability. As c-tDCS$_{UHCDS}$ is painless, safe and has minimal side effects, it is a promising

tool for treatment of conditions in which enhancement of M1 excitability is a therapeutic goal. Modulation of pain and priming of the effects in therapeutic approaches, such as motor training, are potential uses.

Conflict of interest

This manuscript is based on research conducted by Bita Vaseghi, a PhD candidate at Monash University, Melbourne, Australia. This project had no external funding, and no financial or other relationships pose a conflict of interest.

References

Accornero, N., Capozza, M., Pieroni, L., Pro, S., Davi, L.&Mecarelli, O. (2014) EEG mean frequency changes in healthy subjects during prefrontal transcranial direct current stimulation (tDCS). *Journal of neurophysiology*.

Ahmed, M. A., Darwish, E. S., Khedr, E. M., El Serogy, Y. M.&Ali, A. M. (2012) Effects of low versus high frequencies of repetitive transcranial magnetic stimulation on cognitive function and cortical excitability in Alzheimer's dementia. *Journal of neurology*, **259**, 83-92.

Ahumada, J., Fernandez de Sevilla, D., Couve, A., Buno, W.&Fuenzalida, M. (2013) Long-term depression of inhibitory synaptic transmission induced by spike-timing dependent plasticity requires coactivation of endocannabinoid and muscarinic receptors. *Hippocampus*, **23**, 1439-1452.

Aihara, T., Yamamoto, S., Mori, H., Kushiro, K.&Uehara, S. (2015) Observation of interactive behavior increases corticospinal excitability in humans: A transcranial magnetic stimulation study. *Brain and cognition*, **100**, 1-6.

Antal, A., Brepohl, N., Poreisz, C., Boros, K., Csifcsak, G.&Paulus, W. (2008a) Transcranial direct current stimulation over somatosensory cortex decreases experimentally induced acute pain perception. *The Clinical journal of pain*, **24**, 56-63.

Antal, A., Kincses, T. Z., Nitsche, M. A., Bartfai, O.&Paulus, W. (2004a) Excitability changes induced in the human primary visual cortex by transcranial direct current stimulation: direct electrophysiological evidence. *Investigative ophthalmology & visual science*, **45**, 702-707.

Antal, A., Kriener, N., Lang, N., Boros, K.&Paulus, W. (2011) Cathodal transcranial direct current stimulation of the visual cortex in the prophylactic treatment of migraine. *Cephalalgia : an international journal of headache*, **31**, 820-828.

Antal, A., Lang, N., Boros, K., Nitsche, M., Siebner, H. R.&Paulus, W. (2008b) Homeostatic metaplasticity of the motor cortex is altered during headache-free intervals in migraine with aura. *Cerebral cortex (New York, N.Y. : 1991)*, **18**, 2701-2705.

Antal, A., Nitsche, M. A., Kincses, T. Z., Kruse, W., Hoffmann, K. P.&Paulus, W. (2004b) Facilitation of visuo-motor learning by transcranial direct current stimulation of the motor and extrastriate visual areas in humans. *The European journal of neuroscience*, **19**, 2888-2892.

Antal, A., Nitsche, M. A., Kruse, W., Kincses, T. Z., Hoffmann, K.-P.&Paulus, W. (2004c) Direct Current Stimulation over V5 Enhances Visuomotor Coordination by Improving Motion Perception in Humans. *Journal of cognitive neuroscience*, **16**, 521-527.

Antal, A., Nitsche, M. A., Kruse, W., Kincses, T. Z., Hoffmann, K. P.&Paulus, W. (2004d) Direct current stimulation over V5 enhances visuomotor coordination by improving motion perception in humans. *Journal of cognitive neuroscience*, **16**, 521-527.

Apkarian, A. V., Bushnell, M. C., Treede, R. D.&Zubieta, J. K. (2005) Human brain mechanisms of pain perception and regulation in health and disease. *European journal of pain (London, England)*, **9**, 463-484.

Arai, N., Muller-Dahlhaus, F., Murakami, T., Bliem, B., Lu, M. K., Ugawa, Y.&Ziemann, U. (2011) State-dependent and timing-dependent bidirectional associative plasticity in the human SMA-M1 network. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **31**, 15376-15383.

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

Batsikadze, G., Moliadze, V., Paulus, W., Kuo, M. F.&Nitsche, M. A. (2013) Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. *The Journal of physiology*, **591**, 1987-2000.

Benedetti, F. (2008) Mechanisms of placebo and placebo-related effects across diseases and treatments. *Annu Rev Pharmacol Toxicol*, **48**, 33-60.

Bikson, M., Datta, A., Rahman, A.&Scaturro, J. (2010) Electrode montages for tDCS and weak transcranial electrical stimulation: role of "return" electrode's position and size. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **121**, 1976-1978.

Bikson, M., Inoue, M., Akiyama, H., Deans, J. K., Fox, J. E., Miyakawa, H.&Jefferys, J. G. (2004) Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *The Journal of physiology*, **557**, 175-190.

Boggio, P. S., Nunes, A., Rigonatti, S. P., Nitsche, M. A., Pascual-Leone, A.&Fregni, F. (2007) Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients. *Restorative neurology and neuroscience*, **25**, 123-129.

Boggio, P. S., Rigonatti, S. P., Ribeiro, R. B., Myczkowski, M. L., Nitsche, M. A., Pascual-Leone, A.&Fregni, F. (2008a) A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, **11**, 249-254.

Boggio, P. S., Zaghi, S., Lopes, M.&Fregni, F. (2008b) Modulatory effects of anodal transcranial direct current stimulation on perception and pain thresholds in healthy volunteers. *European Journal of Neurology*, **15**, 1124-1130.

Boros, K., Poreisz, C., Munchau, A., Paulus, W.&Nitsche, M. A. (2008) Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans. *The European journal of neuroscience*, **27**, 1292-1300.

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G.&Fregni, F. (2011) A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, **14**, 1133-1145.

Cho, K., Aggleton, J. P., Brown, M. W.&Bashir, Z. I. (2001) An experimental test of the role of postsynaptic calcium levels in determining synaptic strength using perirhinal cortex of rat. *The Journal of physiology*, **532**, 459-466.

Dasilva, A. F., Mendonca, M. E., Zaghi, S., Lopes, M., Dossantos, M. F., Spierings, E. L., Bajwa, Z., Datta, A., Bikson, M.&Fregni, F. (2012) tDCS-induced analgesia and electrical fields in pain-related neural networks in chronic migraine. *Headache*, **52**, 1283-1295.

Davis, M.&Whalen, P. J. (2001) The amygdala: vigilance and emotion. *Molecular psychiatry*, **6**, 13-34.

Di Pino, G., Pellegrino, G., Assenza, G., Capone, F., Ferreri, F., Formica, D., Ranieri, F., Tombini, M., Ziemann, U., Rothwell, J. C.&Di Lazzaro, V. (2014) Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nature reviews. Neurology*, **10**, 597-608.

Diamond, M. E., von Heimendahl, M., Knutsen, P. M., Kleinfeld, D.&Ahissar, E. (2008) 'Where' and 'what' in the whisker sensorimotor system. *Nature reviews. Neuroscience*, **9**, 601-612.

Egorova, N., Yu, R., Kaur, N., Vangel, M., Gollub, R. L., Dougherty, D. D., Kong, J.&Camprodon, J. A. (2015) Neuromodulation of conditioned placebo/nocebo in heat pain: anodal vs. cathodal transcranial direct current stimulation to the right dorsolateral prefrontal cortex. *Pain*.

Faria, P., Hallett, M.&Miranda, P. C. (2011) A finite element analysis of the effect of electrode area and inter-electrode distance on the spatial distribution of the current density in tDCS. *Journal of neural engineering*, **8**, 066017.

Fink, G. R., Frackowiak, R. S., Pietrzyk, U.&Passingham, R. E. (1997) Multiple nonprimary motor areas in the human cortex. *Journal of neurophysiology*, **77**, 2164-2174.

Fregni, F., Boggio, P. S., Nitsche, M. A., Rigonatti, S. P.&Pascual-Leone, A. (2006a) Cognitive effects of repeated sessions of transcranial direct current stimulation in patients with depression. *Depression and anxiety*, **23**, 482-484.

Fregni, F., Thome-Souza, S., Nitsche, M. A., Freedman, S. D., Valente, K. D.&Pascual-Leone, A. (2006b) A controlled clinical trial of cathodal DC polarization in patients with refractory epilepsy. *Epilepsia*, **47**, 335-342.

George, M. S.&Aston-Jones, G. (2010) Noninvasive techniques for probing neurocircuitry and treating illness: vagus nerve stimulation (VNS), transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, **35**, 301-316.

Ghai, R. S., Bikson, M.&Durand, D. M. (2000) Effects of applied electric fields on low-calcium epileptiform activity in the CA1 region of rat hippocampal slices. *Journal of neurophysiology*, **84**, 274-280.

Ghosh, S.&Porter, R. (1988) Corticocortical synaptic influences on morphologically identified pyramidal neurones in the motor cortex of the monkey. *The Journal of physiology*, **400**, 617-629.

Gilbert, C. D.&Wiesel, T. N. (1979) Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex. *Nature*, **280**, 120-125.

Hallett, M. (1996) Transcranial magnetic stimulation: a useful tool for clinical neurophysiology. *Annals of neurology*, **40**, 344-345.

Hummel, F., Celnik, P., Giraux, P., Floel, A., Wu, W. H., Gerloff, C.&Cohen, L. G. (2005) Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. *Brain : a journal of neurology*, **128**, 490-499.

Jefferys, J. G. (1981) Influence of electric fields on the excitability of granule cells in guinea-pig hippocampal slices. *The Journal of physiology*, **319**, 143-152.

Kabakov, A. Y., Muller, P. A., Pascual-Leone, A., Jensen, F. E.&Rotenberg, A. (2012) Contribution of axonal orientation to pathway-dependent modulation of excitatory transmission by direct current stimulation in isolated rat hippocampus. *Journal of neurophysiology*, **107**, 1881-1889.

Kanske, P.&Kotz, S. A. (2011) Emotion speeds up conflict resolution: a new role for the ventral anterior cingulate cortex? *Cerebral cortex (New York, N.Y. : 1991)*, **21**, 911-919.

Katzel, D., Zemelman, B. V., Buetfering, C., Wolfel, M.&Miesenbock, G. (2011) The columnar and laminar organization of inhibitory connections to neocortical excitatory cells. *Nature neuroscience*, **14**, 100-107.

Kätzel, D., Zemelman, B. V., Buetfering, C., Wölfel, M.&Miesenböck, G. (2011) The columnar and laminar organization of inhibitory connections to neocortical excitatory cells. *Nature neuroscience*, **14**, 100-107.

Keeser, D., Meindl, T., Bor, J., Palm, U., Pogarell, O., Mulert, C., Brunelin, J., Moller, H. J., Reiser, M.&Padberg, F. (2011) Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **31**, 15284-15293.

Kirouac, G. J., Li, S.&Mabrouk, G. (2004) GABAergic projection from the ventral tegmental area and substantia nigra to the periaqueductal gray region and the dorsal raphe nucleus. *The Journal of comparative neurology*, **469**, 170-184.

Koch, G., Ponzo, V., Di Lorenzo, F., Caltagirone, C.&Veniero, D. (2013) Hebbian and anti-Hebbian spike-timing-dependent plasticity of human cortico-cortical connections. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **33**, 9725-9733.

Kong, J., Gollub, R. L., Rosman, I. S., Webb, J. M., Vangel, M. G., Kirsch, I.&Kaptchuk, T. J. (2006) Brain activity associated with expectancy-enhanced placebo analgesia as measured by functional magnetic resonance imaging. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **26**, 381-388.

Kothari, M., Svensson, P., Nielsen, J. F.&Baad-Hansen, L. (2014) Influence of position and stimulation parameters on intracortical inhibition and facilitation in human tongue motor cortex. *Brain research*, **1557**, 83-89.

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., Wroe, S., Asselman, P.&Marsden, C. D. (1993) Corticocortical inhibition in human motor cortex. *The Journal of physiology*, **471**, 501-519.

Kuo, M. F., Grosch, J., Fregni, F., Paulus, W.&Nitsche, M. A. (2007) Focusing effect of acetylcholine on neuroplasticity in the human motor cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **27**, 14442-14447.

Kwon, Y. H., Ko, M. H., Ahn, S. H., Kim, Y. H., Song, J. C., Lee, C. H., Chang, M. C.&Jang, S. H. (2008) Primary motor cortex activation by transcranial direct current stimulation in the human brain. *Neuroscience letters*, **435**, 56-59.

Lang, N., Siebner, H. R., Ward, N. S., Lee, L., Nitsche, M. A., Paulus, W., Rothwell, J. C., Lemon, R. N.&Frackowiak, R. S. (2005) How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? *The European journal of neuroscience*, **22**, 495-504.

LeDoux, J. (2003) Emotion circuits in the brain.

Lee, S., Hjerling-Leffler, J., Zagha, E., Fishell, G.&Rudy, B. (2010) The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **30**, 16796-16808.

Lee, S., Kruglikov, I., Huang, Z. J., Fishell, G.&Rudy, B. (2013) A disinhibitory circuit mediates motor integration in the somatosensory cortex. *Nature neuroscience*, **16**, 1662-1670.

Lee, T. G.&Grafton, S. T. (2015) Out of control: diminished prefrontal activity coincides with impaired motor performance due to choking under pressure. *NeuroImage*, **105**, 145-155.

Liebetanz, D., Klinker, F., Hering, D., Koch, R., Nitsche, M. A., Potschka, H., Loscher, W., Paulus, W.&Tergau, F. (2006) Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. *Epilepsia*, **47**, 1216-1224.

Lisman, J. E. (2001) Three Ca2+ levels affect plasticity differently: the LTP zone, the LTD zone and no man's land. *The Journal of physiology*, **532**, 285.

Lowe, M. J., Dzemidzic, M., Lurito, J. T., Mathews, V. P.&Phillips, M. D. (2000) Correlations in low-frequency BOLD fluctuations reflect cortico-cortical connections. *NeuroImage*, **12**, 582-587.

Luft, C. D. B., Pereda, E., Banissy, M.&Bhattacharya, J. (2014) Best of both worlds: Promise of combining brain stimulation and brain connectome. *Frontiers in Systems Neuroscience*, **8**.

Mansouri, F. A., Tanaka, K.&Buckley, M. J. (2009) Conflict-induced behavioural adjustment: a clue to the executive functions of the prefrontal cortex. *Nature reviews. Neuroscience*, **10**, 141-152.

Marquez-Ruiz, J., Leal-Campanario, R., Sanchez-Campusano, R., Molaee-Ardekani, B., Wendling, F., Miranda, P. C., Ruffini, G., Gruart, A.&Delgado-Garcia, J. M. (2012) Transcranial direct-current stimulation modulates synaptic mechanisms involved in associative learning in behaving rabbits. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 6710-6715.

McRae, K., Hughes, B., Chopra, S., Gabrieli, J. D., Gross, J. J.&Ochsner, K. N. (2010) The neural bases of distraction and reappraisal. *Journal of cognitive neuroscience*, **22**, 248-262.

Medeiros, L. F., de Souza, I. C., Vidor, L. P., de Souza, A., Deitos, A., Volz, M. S., Fregni, F., Caumo, W.&Torres, I. L. (2012) Neurobiological effects of transcranial direct current stimulation: a review. *Frontiers in psychiatry*, **3**, 110.

Mendonca, M. E., Santana, M. B., Baptista, A. F., Datta, A., Bikson, M., Fregni, F.&Araujo, C. P. (2011) Transcranial DC stimulation in fibromyalgia: optimized cortical target supported by high-resolution computational models. *The journal of pain : official journal of the American Pain Society*, **12**, 610-617.

Meyers, L. S., Gamst, G.&Guarino, A. J. (2006) *Applied multivariate research: Design and interpretation*. SAGE Publications, Incorporated.

Miranda, P. C., Faria, P.&Hallett, M. (2009) What does the ratio of injected current to electrode area tell us about current density in the brain during tDCS? *Clinical Neurophysiology*, **120**, 1183-1187.

Moliadze, V., Atalay, D., Antal, A.&Paulus, W. (2012) Close to threshold transcranial electrical stimulation preferentially activates inhibitory networks before switching to excitation with higher intensities. *Brain stimulation*, **5**, 505-511.

Monte-Silva, K., Kuo, M. F., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W.&Nitsche, M. A. (2013) Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain stimulation*, **6**, 424-432.

Monte-Silva, K., Kuo, M. F., Liebetanz, D., Paulus, W.&Nitsche, M. A. (2010) Shaping the optimal repetition interval for cathodal transcranial direct current stimulation (tDCS). *Journal of neurophysiology*, **103**, 1735-1740.

Munchau, A., Bloem, B. R., Irlbacher, K., Trimble, M. R.&Rothwell, J. C. (2002) Functional connectivity of human premotor and motor cortex explored with repetitive transcranial magnetic stimulation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **22**, 554-561.

Ngernyam, N., Jensen, M. P., Arayawichanon, P., Auvichayapat, N., Tiamkao, S., Janjarasjitt, S., Punjaruk, W., Amatachaya, A., Aree-Uea, B.&Auvichayapat, P. (2015) The effects of transcranial direct current stimulation in patients with neuropathic pain from spinal cord injury. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **126**, 382-390.

Nitsche, M. A., Doemkes, S., Karakose, T., Antal, A., Liebetanz, D., Lang, N., Tergau, F.&Paulus, W. (2007) Shaping the effects of transcranial direct current stimulation of the human motor cortex. *Journal of neurophysiology*, **97**, 3109-3117.

Nitsche, M. A.&Paulus, W. (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of physiology*, **527 Pt 3**, 633-639.

Nitsche, M. A.&Paulus, W. (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology*, **57**, 1899-1901.

Nitsche, M. A.&Paulus, W. (2011) Transcranial direct current stimulation--update 2011. *Restorative neurology and neuroscience*, **29**, 463-492.

Nitsche, M. A., Seeber, A., Frommann, K., Klein, C. C., Rochford, C., Nitsche, M. S., Fricke, K., Liebetanz, D., Lang, N., Antal, A., Paulus, W.&Tergau, F. (2005) Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *The Journal of physiology*, **568**, 291-303.

O'Connell, N. E., Wand, B. M., Marston, L., Spencer, S.&Desouza, L. H. (2011) Non-invasive brain stimulation techniques for chronic pain. A report of a Cochrane systematic review and meta-analysis. *European journal of physical and rehabilitation medicine*, **47**, 309-326.

Ochsner, K. N., Silvers, J. A.&Buhle, J. T. (2012) Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. *Annals of the New York Academy of Sciences*, **1251**, E1-E24.

Opie, G. M.&Semmler, J. G. (2014) Modulation of short- and long-interval intracortical inhibition with increasing motor evoked potential amplitude in a human hand muscle. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **125**, 1440-1450.

Parazzini, M., Rossi, E., Ferrucci, R., Liorni, I., Priori, A.&Ravazzani, P. (2013) Modelling the electric field and the current density generated by cerebellar transcranial DC stimulation in humans. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*.

Petreanu, L., Gutnisky, D. A., Huber, D., Xu, N. L., O'Connor, D. H., Tian, L., Looger, L.&Svoboda, K. (2012) Activity in motor-sensory projections reveals distributed coding in somatosensation. *Nature*, **489**, 299-303.

Peyron, R., Laurent, B.&Garcia-Larrea, L. (2000) Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiologie clinique = Clinical neurophysiology*, **30**, 263-288.

Phelps, E. A. (2006) Emotion and cognition: insights from studies of the human amygdala. *Annu. Rev. Psychol.*, **57**, 27-53.

Pirulli, C., Fertonani, A.&Miniussi, C. (2014) Is neural hyperpolarization by cathodal stimulation always detrimental at the behavioral level? *Frontiers in behavioral neuroscience*, **8**, 226.

Poreisz, C., Boros, K., Antal, A.&Paulus, W. (2007) Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain research bulletin*, **72**, 208-214.

Ragert, P., Vandermeeren, Y., Camus, M.&Cohen, L. G. (2008) Improvement of spatial tactile acuity by transcranial direct current stimulation. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **119**, 805-811.

Riberto, M., Marcon Alfieri, F., Monteiro de Benedetto Pacheco, K., Dini Leite, V., Nemoto Kaihami, H., Fregni, F.&Rizzo Battistella, L. (2011) Efficacy of transcranial direct current stimulation coupled with a multidisciplinary rehabilitation program for the treatment of fibromyalgia. *The open rheumatology journal*, **5**, 45-50.

Rissman, J., Gazzaley, A.&D'Esposito, M. (2004) Measuring functional connectivity during distinct stages of a cognitive task. *NeuroImage*, **23**, 752-763.

Rizzo, V., Siebner, H. S., Morgante, F., Mastroeni, C., Girlanda, P.&Quartarone, A. (2009) Paired associative stimulation of left and right human motor cortex shapes interhemispheric motor inhibition based on a Hebbian mechanism. *Cerebral cortex (New York, N.Y. : 1991)*, **19**, 907-915.

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., Dimitrijevic, M. R., Hallett, M., Katayama, Y., Lucking, C. H.&et al. (1994) Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalography and clinical neurophysiology*, **91**, 79-92.

Roth, B. J. (2009) What does the ratio of injected current to electrode area not tell us about tDCS? *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **120**, 1037-1038.

Rudy, B., Fishell, G., Lee, S.&Hjerling-Leffler, J. (2011) Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Developmental neurobiology*, **71**, 45-61.

Sackett, D. L. (1979) Bias in analytic research. *J Chronic Dis*, **32**, 51-63.

Schlaug, G., Renga, V.&Nair, D. (2008) Transcranial direct current stimulation in stroke recovery. *Archives of neurology*, **65**, 1571-1576.

Schretlen, D. J., van Steenburgh, J. J., Varvaris, M., Vannorsdall, T. D., Andrejczuk, M. A.&Gordon, B. (2014) Can Transcranial Direct Current Stimulation Improve Cognitive Functioning in Adults with Schizophrenia? *Clin Schizophr Relat Psychoses*, 1-27.

Turi, Z., Ambrus, G. G., Ho, K. A., Sengupta, T., Paulus, W.&Antal, A. (2014) When size matters: large electrodes induce greater stimulation-related cutaneous discomfort than smaller electrodes at equivalent current density. *Brain stimulation*, **7**, 460-467.

Turrigiano, G. G. (2008) The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell*, **135**, 422-435.

Turrigiano, G. G.&Nelson, S. B. (2004) Homeostatic plasticity in the developing nervous system. *Nature reviews. Neuroscience*, **5**, 97-107.

Uy, J.&Ridding, M. C. (2003) Increased cortical excitability induced by transcranial DC and peripheral nerve stimulation. *Journal of neuroscience methods*, **127**, 193-197.

Valle, A., Roizenblatt, S., Botte, S., Zaghi, S., Riberto, M., Tufik, S., Boggio, P. S.&Fregni, F. (2009) Efficacy of anodal transcranial direct current stimulation (tDCS) for the treatment of fibromyalgia: results of a randomized, sham-controlled longitudinal clinical trial. *Journal of pain management*, **2**, 353-361.

Vaseghi, B., Jaberzadeh, S.&Zoghi, M. (2015) Inter-pulse Interval Affects the Size of Single-pulse TMS-induced Motor Evoked Potentials: a Reliability Study. *Basic and Clinical Neuroscience*, **6**, 44-51.

Vaseghi Bita, Z. M., Jaberzadeh Shapour (2015) A meta-analysis of site-specific effects of cathodal transcranial direct current stimulation on sensory perception and pain *PloS one*.

Wassermann, E., Epstein, C.&Ziemann, U. (2008) *Oxford handbook of transcranial stimulation*. Oxford University Press.

Xu, N. L., Harnett, M. T., Williams, S. R., Huber, D., O'Connor, D. H., Svoboda, K.&Magee, J. C. (2012) Nonlinear dendritic integration of sensory and motor input during an active sensing task. *Nature*, **492**, 247-251.

Zoghi, M., Pearce, S. L.&Nordstrom, M. A. (2003) Differential modulation of intracortical inhibition in human motor cortex during selective activation of an intrinsic hand muscle. *The Journal of physiology*, **550**, 933-946.

Zubieta, J. K., Bueller, J. A., Jackson, L. R., Scott, D. J., Xu, Y., Koeppe, R. A., Nichols, T. E.&Stohler, C. S. (2005) Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **25**, 7754-7762.

Figure legends

Figure 1. Experimental design: Comparing corticospinal excitability (CSE), short interval intracortical inhibition (SICI), and intracortical facilitation (ICF) of left M1 following conventional and unihemispheric dual-site c-tDCS (c-tDCS$_{UHCDS}$) by single- and paired pulse TMS (A); the electrode montage over the targets in both active and sham conditions (B). * The grey electrodes in sham condition indicate that the electrodes were placed in the

Figure 2. The effects of different stimulation sites on the peak-to-peak amplitude of MEPs (A), short interval intracortical inhibition (SICI) (B), and intracortical facilitation (ICF) (C) following c-tDCS of primary motor cortex (M1), unihemispheric concurrent dual-site c-tDCS of M1- dorsolateral prefrontal cortex (DLPFC), M1 – primary sensory cortex (S1), M1 – primary visual cortex (V1), and sham.

Filled symbols indicate significant deviation of the post transcranial stimulation MEP amplitudes relative to the baseline. Data are reported as mean

Declaration for Chapter 9

In the case of Chapter 9, 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.

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	Supervisory input on study design, Guidance in the framing of the manuscript, Review and provision of feedback on final manuscript draft	5 %

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

Candidate's Signature : [redacted] Date: 15-Sep- 2015

Signature: [redacted] Date: 15-Sep-2015

Signature: [redacted] Date: 15-Sep-2015

Preamble to Chapter 9

In the two preceding chapters, the effects of unihemispheric concurrent dual-site a- and c-tDCS on the M1 CSE are investigated. Compared to conventional single-site tDCS, the results in Chapters 7 and 8 provides evidence for the efficacy of these novel techniques on induction of M1 CSE changes. In Chapter 9, the effects of these techniques on STh/PTh are evaluated in comparison to conventional single-site M1 tDCS.

Chapter 9: Unihemispheric concurrent dual-site tDCS: The effects on sensory and pain thresholds in healthy adults

This study is under review at the European Journal of Pain. The format of this chapter is consistent with the European Journal of Pain.

The setup system used in this study, Ethics approval, TMS safety, Personal health history form, and Edinburg handedness questionnaires and consent form are provided in Appendices 7-13.

Original article

Unihemispheric concurrent dual-site transcranial direct current stimulation: The effects on sensory and pain thresholds in healthy adults

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Running title: The effect of $tDCS_{UHCDS}$ on pain perception

Conflict of interest

This manuscript is based on research conducted by Bita Vaseghi, a PhD candidate at Monash University, Melbourne, Australia. This project had no external funding, and no financial or other relationships pose a conflict of interest.

Abstract

Background: The primary aim of the present study was to assess the effects of unihemispheric concurrent dual-site transcranial direct current stimulation ($tDCS_{UHCDS}$) over cortical regions of the pain neuromatrix, including the primary motor cortex (M1), primary sensory cortex (S1), and dorsolateral prefrontal cortex (DLPFC) on sensory (STh) and pain (PTh) thresholds.

Methods: In a single-blinded randomized study, 36 healthy volunteers were randomized into three "tDCS Mode" groups: anodal, cathodal and sham tDCS. Participants in all groups received 20 min tDCS under three conditions: stimulation of M1, M1-DLPFC, and M1-S1. STh and PTh to peripheral electrical stimulation of the median nerve at wrist level were assessed before and three times after each intervention.

Results: Compared to M1 a-tDCS, a-$tDCS_{UHCDS}$ of M1-DLPFC significantly increased both STh and PTh for at least 24 hours ($P < 0.001$). Following a-$tDCS_{UHCDS}$ of M1-S1, STh increased for at least 24 hours ($P < 0.001$), whilst PTh was enhanced for one hour. Compared to M1 c-tDCS, $tDCS_{UHCDS}$ of M1-S1 and M1-DLPFC increased STh for one hour ($P < 0.001$) and had no effect on PTh. Sham $tDCS_{UHCDS}$ had no effect on either STh or PTh.

Conclusions: $tDCS_{UHCDS}$ increased STh and PTh more than conventional single-site M1 stimulation. Significant differences were found between a- and c-$tDCS_{UHCDS}$ of M1-S1 and M1-DLPFC conditions, suggesting that a-$tDCS_{UHCDS}$ of M1-DLPFC is the best technique to enhance STh and PTh for at least 24 hours. Our results provide new insights into the efficient use of tDCS to manage pain.

Keywords: Unihemispheric concurrent dual-site transcranial direct current stimulation, Pain neuromatrix, Sensory threshold, Pain threshold.

Introduction

Transcranial direct current stimulation (tDCS) has emerged as a promising neuromodulatory technique for inducing excitability changes in the cortical target areas (Nitsche and Paulus, 2000; 2001). Application of anode (a-tDCS) over target areas generally depolarizes the neurons in the stimulated area, increasing excitability, whereas cathode (c-tDCS) hyperpolarizes the stimulated neurons, decreasing excitability (Nitsche and Paulus, 2000; Medeiros et al., 2012). Conventionally, tDCS has been applied over the primary motor cortex (M1) for therapeutic purposes including pain management.

Painful stimuli are processed in an extensive network of cortical and subcortical areas of the brain called the pain neuromatrix (PNM) (Clark, 1976). In the PNM, painful stimuli can be processed and operationalised by some key variables like sensory threshold (STh) and pain threshold (PTh) in healthy individuals (Bornhovd et al., 2002; Giesecke et al., 2005; Fernandez-de-Las-Penas et al., 2010). Recent tDCS studies revealed that both a-tDCS and c-tDCS of cortical sites of the PNM, including the M1, primary sensory cortex (S1), and dorsolateral prefrontal cortex (DLPFC), increase STh/PTh in healthy individuals (Apkarian et al., 2005; Bachmann et al., 2010; Grundmann et al., 2011; Ahumada et al., 2013). In addition, site-specific enhancement of STh/PTh due to a- and c-tDCS have been demonstrated in two recent systematic reviews (Vaseghi et al., 2014; 2015d). In spite of these positive findings, some studies have shown no effect on STh/PTh (O'Connell et al., 2011; Csifcsak and Antal, 2012).

Functional MRI (fMRI) studies have demonstrated that the DLPFC is involved in painful stimuli perception (Peyron et al., 2000; Apkarian et al., 2005; Kirimoto et al., 2011). Vaseghi et al. (2015) demonstrated that both a-tDCS (Vaseghi et al., 2015c) and c-tDCS (Vaseghi et al., 2015d) of DLPFC increased STh/PTh in healthy adults and patients with chronic pain (Vaseghi et al., 2014).

Despite the initial success in STh/PTh increase by single-site stimulation of M1, S1 or DLPFC, additional exploratory studies are needed to refine the existing tDCS techniques. In a recent study, two functionally connected sites of the PNM in the same hemisphere were concurrently stimulated by

two separated tDCS channels, termed unihemispheric concurrent dual-site tDCS ($tDCS_{UHCDS}$) (Fig. 1). Vaseghi et al. (2015) demonstrated that a-$tDCS_{UHCDS}$ of M1-DLPFC is more efficient than conventional single-site stimulation in increasing corticospinal excitability, an effect which lasted for at least 24 hours (Vaseghi et al., 2015b). In addition, in a pilot study conducted by our group, concurrent c-tDCS of the M1, S1 or DLPFC showed excitatory but not inhibitory effects on corticospinal excitability. Such results raise a crucial question: what is the impact of concurrent stimulation of two functionally connected cortical sites of the PNM on STh/PTh?

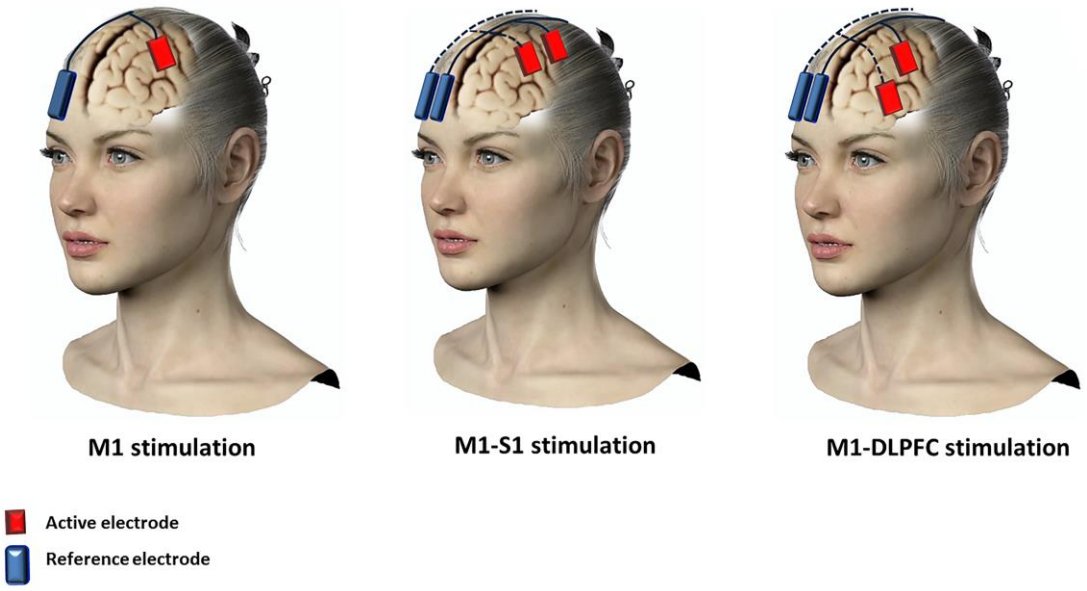


Figure 1. Schematic illustration of the electrode montage in $tDCS_{UHCDS}$: the active electrodes were positioned over M1 and S1 (A) and over M1 and DLPFC (B). The reference electrodes were placed over the contralateral supraorbital area in all experimental conditions.

To answer this question, we compared the effects on STh/PTh modulation of both a- and c-$tDCS_{UHCDS}$ of M1-S1 and M1-DLPFC with the effects of conventional M1 stimulation. We hypothesised that $tDCS_{UHCDS}$ induce larger STh/PTh increases with longer-lasting effects than conventional M1 tDCS. We also aimed to investigate the placebo effects of a- and c-$tDCS_{UHCDS}$ on STh/PTh, hypothesizing that they do not exist.

Methods

Study design

We implemented a randomized, sham-controlled study in healthy adults. All experimental procedures were approved by Monash University's Human Ethics Committee and conformed to the Declaration of Helsinki (1964).

Participants

Thirty-six healthy participants were recruited from Monash University to be involved in this experimental study. The sample size was calculated (with power of 80%) based on the results of a pilot study conducted on eight healthy individuals. Participants were recruited if they fulfilled the following criteria: (1) aged between 18 and 35 years, (2) right-handed, (3) no medical conditions or chronic/acute pain disorders, (4) not taking medication, (5) no history of substance abuse or dependence, (6) no contraindication for receiving tDCS, (7) no chronic pain in the last six months, and (8) no history of neurological or psychiatric disease.

Experimental Procedures

Participants who met the inclusion criteria were randomly assigned into one of the tDCS groups (a-tDCS, c-tDCS and sham). In each group, three different stimulation sites including the M1, M1-S1 and M1-DLPFC were randomly stimulated (Fig. 2).

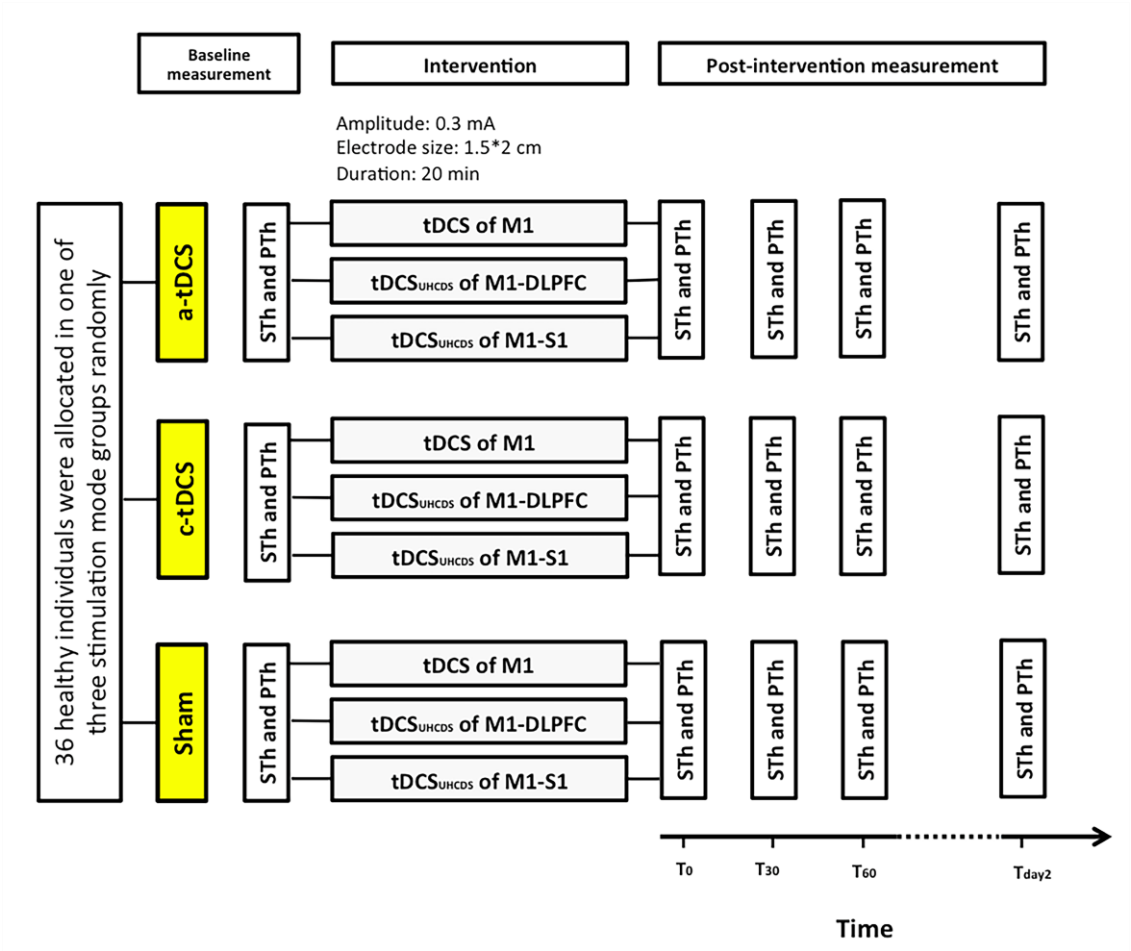


Figure 2. Experimental design for the comparison of conventional tDCS and $tDCS_{UHCDS}$ of cortical areas of PNM on Sensory threshold (STh); Pain threshold (PTh)

Participants were seated in a fully adjustable treatment chair (MagVenture, Denmark) with head and armrest, while their hand was at rest in supination position. At the beginning of each experimental condition, both STh and PTh were measured (T_{pre}) as baseline values; then the target site was stimulated by a-tDCS or c-tDCS for 20 minutes. In the sham condition, the stimulator was switched off after 30 seconds (Gandiga et al., 2006). STh and PTh were measured immediately (T_0), 30 min (T_{30}), 60 min (T_{60}) and 24 hours after intervention by the same rater (Fig. 2).

The experimental sessions were separated by at least seven days to avoid any interference or carry-over effects of tDCS. To avoid diurnal variation, Individuals were always tested at the same time of

day (in either mornings or early afternoons). Experiments were conducted between November 2014 and April 2015.

TDCS characteristics

Direct current was delivered with amplitude of 0.3 mA (Bastani and Jaberzadeh, 2013a) with 10 seconds of linear fade in and fade out. The current intensity of 0.3 mA allowed us to considerably decrease the size of the electrodes (Bastani and Jaberzadeh, 2013b), while keeping the current density (0.1 mA/cm^2) in a safe range with limited side effects (Poreisz et al., 2007; Brunoni et al., 2011). In order to provide highly focused DC stimulation over the target areas (Bastani and Jaberzadeh, 2013b), as indicated in computational modelling studies (Nitsche et al., 2007; Bikson et al., 2010), we employed small active electrodes (1.5 × 2cm). The small size of active electrodes also enabled us to focally stimulate the M1 and S1 with two separate electrodes during concurrent stimulation of the brain sites in tDCS$_{UHCDS}$ conditions.

The reference electrodes were conventionally positioned over the contralateral supraorbital region with the assumption of no or negligible neuromodulatory effects on the subgenual cortex. To minimize any neuromodulatory effects, we used reference electrodes four times larger than the active electrodes (making the current density four times less) (Fig. 1) (Nitsche et al., 2007). The narrow shape of the reference electrodes allowed them to be easily fixed over the contralateral supraorbital region with two horizontal and perpendicular straps.

Current density of 0.1 mA/cm^2 was identical under each active electrode during all experimental conditions. Two identical stimulators (Intelect® Advanced Therapy System, Chattanooga, USA) delivered 20 minutes of direct current over the target areas.

The target cortical stimulation sites were identified using the international 10-20 system of EEG electrode placement. The active electrode was placed over C3 and C′3 (2cm posterior to C3) to stimulate the M1 and/or S1 respectively. For stimulation of the DLPFC, the active electrode was

positioned over the F3. Reference electrodes were conventionally placed over the contralateral supraorbital area (Fig. 1).

In the sham condition, the electrodes were placed in the same positions as for the experimental groups but the stimulator was switched off after 30 seconds of stimulation (Gandiga et al., 2006). All pre- and post-evaluations were identical to those in other conditions.

The order of stimulation sites were also randomised for each participant. Randomization and allocation concealment were carried out by a collaborator who did not participate in data collection or data analysis. All participants were blinded to the purpose of the experiments. All participants provided written informed consent.

Measurement of STh and PTh

STh and PTh were assessed using rectangular electrical pulses applied by a pen electrode (model: 2762CC, Chattanooga, USA) to the right median nerve (pulse duration: 200μs) at wrist level (Vaseghi et al., 2015c). Current intensity started from 0mA and increased gradually in steps of 0.1mA. The intensity of current at which perception of the electrical stimulus was first reported was taken as the STh. The intensity of current at which participants reported the first painful sensation was taken as the PTh. These measurements were repeated three times at each time point and averaged for further analysis. All post-stimulation values were intra-individually normalized to baseline and given as the ratios of the baseline.

Data analysis

To assess differences in demographic variables at baseline, we used Pearson's chi-squared test (χ^2) for categorical characteristics and t-test (adjusted for multiple comparison correction) for continuous variables. At baseline, an independent sample t-test (adjusted for multiple comparison correction) was

performed on the STh/PTh levels of participants to detect any significant carry-over effect between experimental conditions.

A three-way repeated measures ANOVA was used for both STh and PTh values to evaluate the effects of time (T_{pre}, T_0, T_{30}, T_{60}, T_{24h}), stimulation site (M1, M1-DLPFC and M1-S1), and tDCS mode (anodal, cathodal and sham tDCS). The Greenhouse-Geisser correction was used when necessary to correct for non-sphericity. In case of any significant main effect, the least significance difference post-hoc tests were conducted.

Additionally, to evaluate the lasting effect of intervention, a one-way repeated measure ANOVA was applied for each experimental condition. A post-hoc test (Bonferroni) was performed where indicated. A *P*-value of <0.05 was considered significant for all statistical analyses. All results are given as mean and standard error of mean (SEM) and statistical analyses were performed using SPSS software version 22.

Results

Baseline value measurements

As can be seen in Table 1, there was no statistically significant difference between the demographic characteristics or STh/PTh values of the three tDCS mode groups. Handedness was assessed by the Edinburgh Handedness Inventory (10-item version, mean laterality quotient = 94 ± 5.71) (Oldfield, 1971). A Kolmogorov-Smirnov test showed data were normally distributed.

Table 1. Comparison of demographic variables for three different tDCS mode groups

	a-tDCS group			c-tDCS group			Sham group			Test statistics			
	n	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**Mean**	**SD**	**df**	**F**	**χ^2**	**P**
Categorical Variables													
Gender: male/female	3/9			4/8			3/9			1		0.14	0.81
Edinburg handedness inventory, Right/left	12/0			12/0			12/0			1		0.00	0.99
Frequent Alcohol or tobacco, No / Yes	12/0			12/0			11/1			1		0.00	0.82
Continuous Variables													
Age (year)		23.6	4.3		25.2	4.1		23.8	5.1	2.53	0.21		0.17
BMI (kg/m^2)		23.2	1.12		22.67	1.4		24.1	0.95	2.53	0.63		0.52

a-tDCS: anodal transcranial direct current stimulation, c-tDCS: cathodal transcranial direct current stimulation, SD: standard deviation, df: Degree of freedom

The effect of tDCS$_{UHCDS}$ on STh

The three-way repeated measures ANOVA was conducted for recorded STh values to assess the effects of stimulation of cortical sites of the PNM by a-tDCS$_{UHCDS}$, c-tDCS$_{UHCDS}$ and sham tDCS$_{UHCDS}$ on STh across five time points of measurement. Analysis revealed significant main effects of stimulation site, tDCS mode and time, and significant interactions in tDCS mode × time, tDCS mode × stimulation site, stimulation site × time, and tDCS mode × stimulation site × time (Table 2). The post-hoc test results also revealed that concurrent stimulation of the M1-S1 by both a-tDCS and c-tDCS similarly induced increased STh at T_0 (Fig. 3A), T_{30} (Fig. 3B), and T_{60} (Fig. 3C); there was no significant difference between these two stimulation modes. Significant STh increase was found between active stimulation of the M1-S1 and the sham condition at these time points of measurements. After 24 hours, significant STh change was found between a-tDCS$_{UHCDS}$ and two other tDCS modes (c-tDCS$_{UHCDS}$ and sham). No significant STh change was found between sham and active c-

$tDCS_{UHCDS}$ (Fig. 3D). M1 stimulation by active a- and c-tDCS similarly increased the level of STh for 60 minutes. No significant difference was found between M1 a-tDCS and c-tDCS at T_0, T_{30}, and T_{60}.

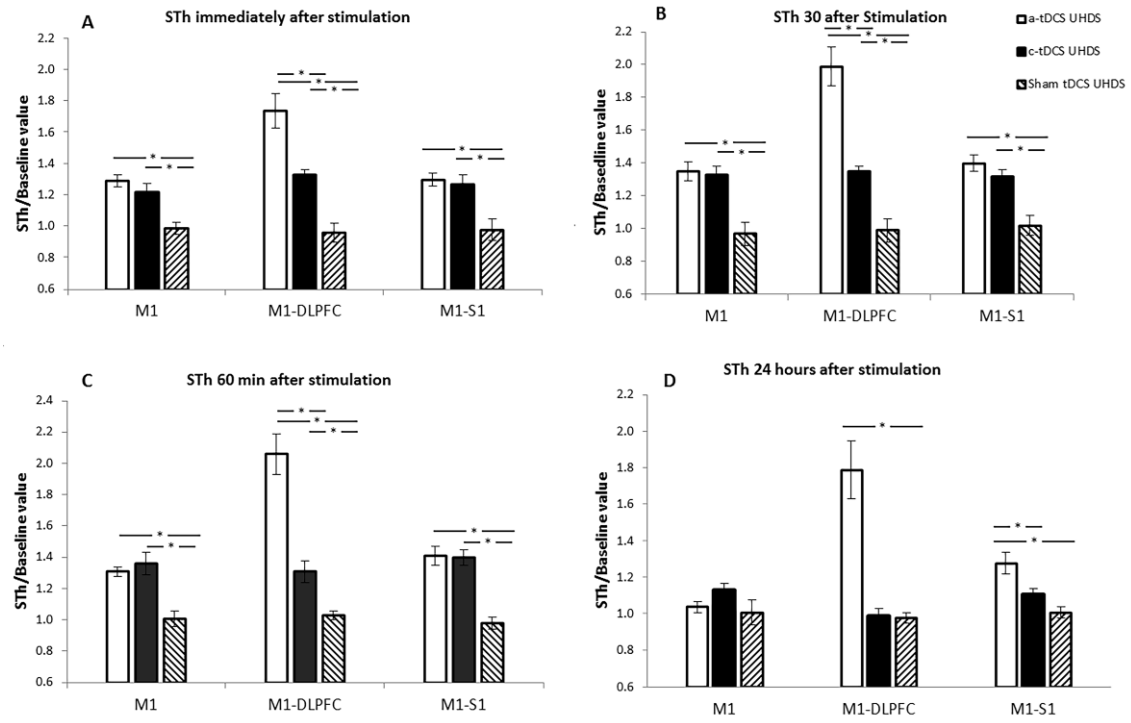


Figure 3. Comparison of the level of sensory threshold (STh) changes following conventional tDCS of M1, $tDCS_{UHCDS}$ of M1-S1 and $tDCS_{UHCDS}$ of M1-DLPFC in three stimulation modes: anodal, cathodal, and sham tDCS. The level of STh of each stimulation mode was compared immediately (T_0) (A), 30 min (T_{30}) (B), 60 min (T_{60}) (C), and one day (T_{24h}) (D) after intervention. The mean values are normalized to baseline. Asterisks indicate significant mean effect. Error bars represent SEM.

The post-hoc comparisons showed significant STh increase at T_0 (Fig. 3A), T_{30} (Fig. 3B) and T_{60} (Fig. 3C) after concurrent stimulation of M1-DLPFC by both a- and $c\text{-}tDCS_{UHCDS.}$ Although both a- and $c\text{-}tDCS_{UHCDS}$ of M1-DLPFC significantly increased STh, there was a significant difference between these two active modes at all time points of measurement (Fig. 3). Significant differences between

both a- and c-tDCS and the sham condition were found at all time points except 24 hours after stimulation, in which there was no significant difference between c-tDCS$_{UHCDS}$ and sham (Fig. 3D).

To assess the duration of the effect of stimulation modes, one-way repeated measures ANOVA was carried out for each condition. The results showed a significant main effect of time following M1 a-tDCS [F (4, 44) = 23.53, P < 0.001, η_P^2 = 0.62], a-tDCS$_{UHCDS}$ of M1-DLPFC [F (4, 44) = 21.00, P = 0.001, η_P^2 = 0.51], and M1-S1 [F (4, 44) = 16.80, P = 0.003, η_P^2 = 0.60]. The result of post-hoc comparison is summarized in Figure 4A. Similar analysis showed a significant main effect of time following M1 c-tDCS [F (4, 44) = 12.18, P < 0.001, η_P^2 = 0.52], c-tDCS$_{UHCDS}$ of M1-DLPFC [F (2.4, 26.3) = 21.03, P = 0.004, η_P^2 = 0.66], and M1-S1 [F (4, 44) = 17.9, P < 0.001, η_P^2 = 0.62]. The post-hoc comparison result can be seen in Figure 4B. No significant main effect of time was found following the application of sham a- and c-tDCS$_{UHCDS}$ conditions.

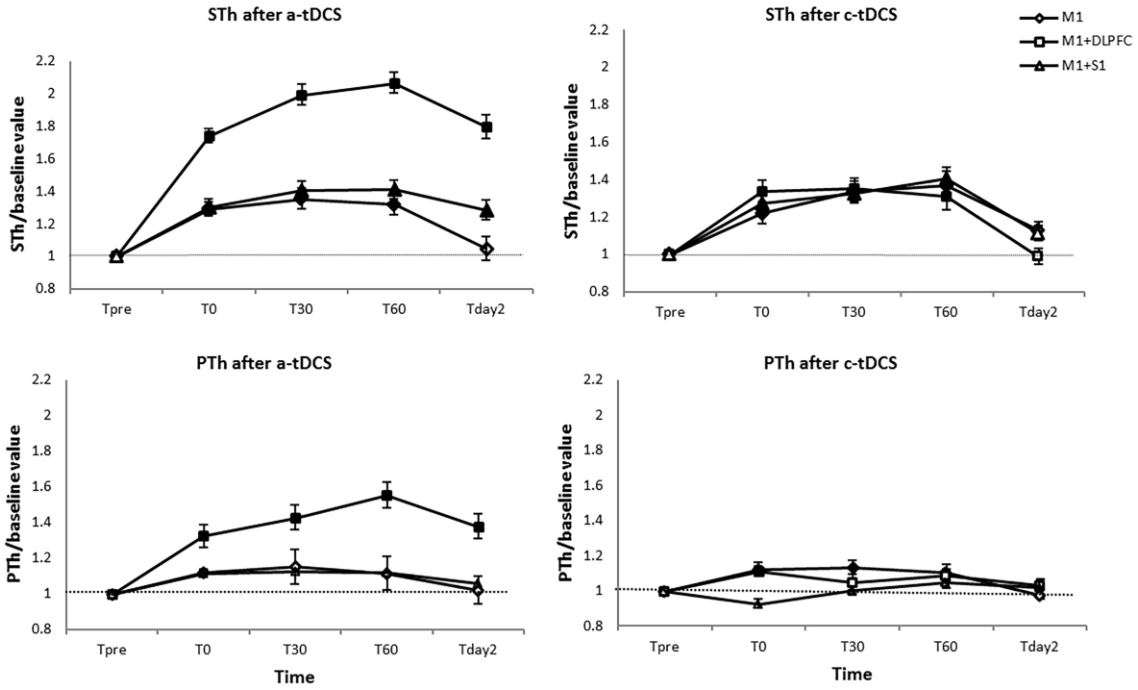


Figure 4. The effect of different anodal and cathodal tDCS experimental conditions on sensory and pain threshold over time; the mean values are normalized to baseline. Filled symbols indicate significant mean effects. Error bars represent SEM.

The effect of tDCS$_{UHCDS}$ on PTh

Three-way repeated measures ANOVA revealed significant main effects of tDCS mode, stimulation site, and time. It also showed significant interactions between tDCS mode × stimulation site, tDCS mode × time, stimulation site × time, and site × time × tDCS mode (Table 2).

Post-hoc comparisons revealed that although there were significant differences between a- and c-tDCS of M1 at T_0, T_{30}, and T_{60}, both conditions increased PTh significantly for at least 60 minutes (Fig. 5A). There were significant differences between active and sham conditions at these three time points. No significant PTh change occurred between tDCS modes.

Post-hoc comparisons also indicated significant differences between a-tDCS$_{UHCDS}$ and c-tDCS$_{UHCDS}$ of M1-DLPFC at all time points. Both these conditions increased the level of PTh for up to 60 minutes (Fig. 5A, B, C). Twenty-four hours after intervention, there was a significant difference between the PTh recorded following concurrent stimulation of M1-DLPFC by a-tDCS and other tDCS modes (c-tDCS and sham) (Fig. 5D).

In addition, PTh significantly increased following concurrent stimulation of M1-DLPFC. Concurrent stimulation of M1-S1 produced significantly greater PTh than concurrent stimulation of a-tDCS and other modes 30 min after stimulation (Fig. 5C). There was no significant difference between the PTh changes induced by the three tDCS modes.

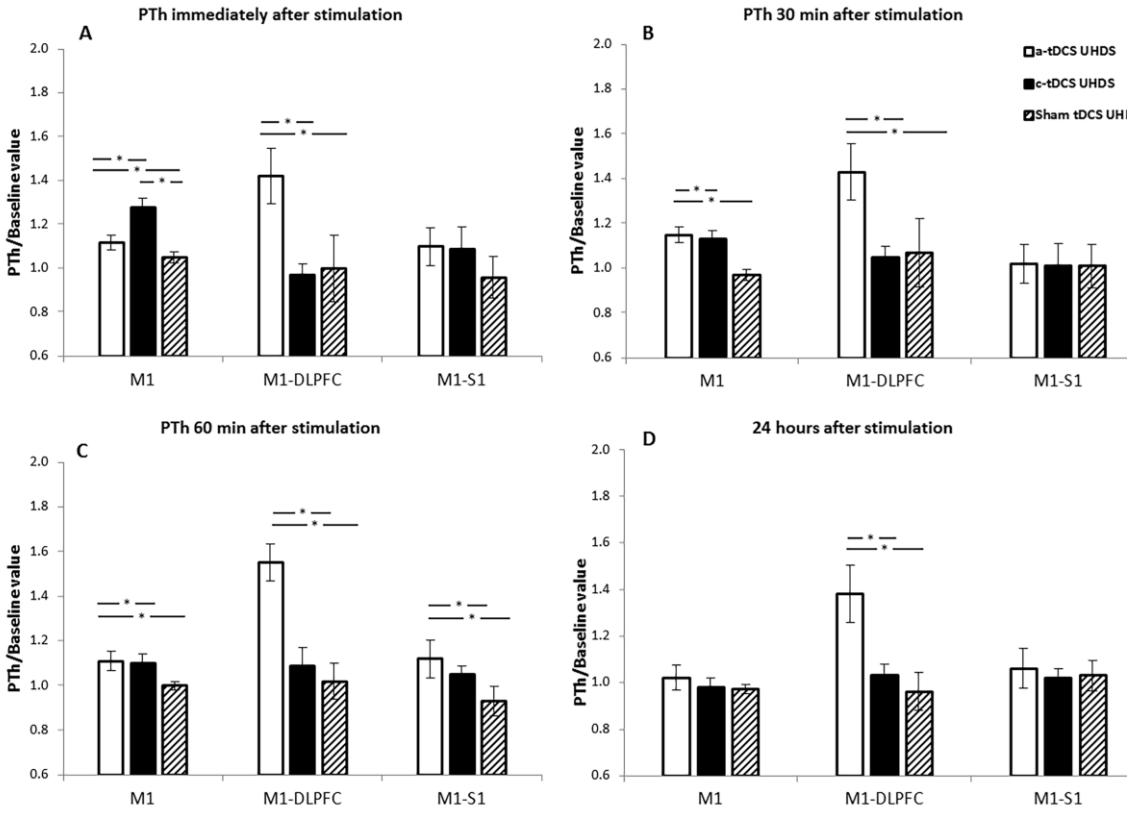


Figure 5. Comparison of the level of PTh changes following conventional tDCS of M1, $tDCS_{UHCDS}$ of M1-S1 and $tDCS_{UHCDS}$ of M1-DLPFC in three stimulation mode: anodal, cathodal, and sham tDCS. The level of PTh of each stimulation mode was compared immediately (T_0) (A), 30 min (T_{30}) (B), 60 min (T_{60}) (C), and one day (T_{24h}) (D) after intervention. The mean values are normalized to baseline. Asterisks indicate significant mean effect. Error bars represent SEM.

One-way repeated measures ANOVA demonstrated a significant main effect of time following M1 a-tDCS [$F(4, 44) = 23.53$, $P < 0.001$, $\eta_P^2 = 0.61$], a-$tDCS_{UHCDS}$ of M1-DLPFC [$F(4, 44) = 61.99$, $P < 0.001$, $\eta_P^2 = 0.85$], and M1-S1 [$F(4, 44) = 4.72$, $P = 0.01$, $\eta_P^2 = 0.47$]. The result of post-hoc comparison is summarised in Figure 4C.

A significant main effect of time was detected for M1 c-tDCS [$F(4, 40) = 4.03$, $P = 0.008$, $\eta_P^2 = 0.29$], c-$tDCS_{UHCDS}$ of M1-DLPFC [$F(4, 40) = 2.72$, $P = 0.04$, $\eta_P^2 = 0.21$], and M1-S1 [$F(1.98, 19.8) = 6.18$, $P = 0.001$, $\eta_P^2 = 0.38$]. The post-hoc comparison result can be seen in Figure 4D.

Discussion

The groups were homogeneous in terms of their demographic and PTh characteristics, so the assumption of independence of the results was supported. Significant changes between sham and active tDCS modes at all time points indicated that the results were real effects of interventions and that $tDCS_{UHCDS}$ has no placebo effect on STh and PTh.

The effect of $tDCS_{UHCDS}$ on STh

We found that similar to conventional M1 a- and c-tDCS, application of $a\text{-}tDCS_{UHCDS}$ and $c\text{-}tDCS_{UHCDS}$ of M1-DLPFC and M1-S1 led to STh increase for 60 minutes. However, there were significant differences between anodal and cathodal modes at T_0, T_{30}, and T_{60}. Interestingly, $a\text{-}tDCS_{UHCDS}$ of M1-DLPFC and M1-S1 had a larger and longer-lasting effect (up to 24 hours).

The results of conventional M1 a- and c-tDCS are in line with previous studies, in which M1 a-tDCS (Nitsche et al., 2008; Bachmann et al., 2010; Vaseghi et al., 2015c) and c-tDCS (Bachmann et al., 2010; Grundmann et al., 2011; Vaseghi et al., 2015d) increased the level of STh in healthy adults. In addition, the results are supported by the results of two recent meta-analyses (Vaseghi et al., 2014; 2015d). Based on the mechanism-based studies, following the application of a-tDCS, the level of activity in inhibitory interneurons decreases (Nitsche et al., 2004; Stagg et al., 2009; Stagg and Nitsche, 2011), which is accompanied with decreased intracortical inhibition and increased intracortical facilitation (Nitsche et al., 2005; Stagg et al., 2009; Stagg and Nitsche, 2011). Kirouac et al. (2004) showed that GABAergic projections from the ventral tegmental area and substantia nigra to the ventrolateral periaqueductal gray and dorsal medullary raphe nucleus modulate the behavioral responses to sensory and pain perception (Kirouac et al., 2004). As a result, we can conclude that in our study concurrent a-tDCS possibly diminished the activity of GABAergic mechanisms and modulated the activity level of GABAergic projection, which indirectly resulted in STh increase. In contrast, non-synaptic mechanisms are the main reasons behind the efficacy of c-tDCS on STh enhancement (Ardolino et al., 2005). Due to c-tDCS-induced water electrolysis and alteration of acid/base balance (Loeb, 1986; Islam et al., 1995; Chesler, 2003), the functions of neuronal membrane

and receptors are strongly affected and the level of Ca^{2+} is decreased (Islam et al., 1995), which indirectly increases the STh.

Ours is the first study to investigate the effect of concurrent stimulation of two interconnected cortical sites of the PNM in one hemisphere on STh alteration and its lasting effect in healthy young adults. The results demonstrated that a-tDCS$_{UHCDS}$ of M1-DLPFC increased STh around 65% more than conventional M1 a-tDCS, and lasted at least 24 hours. c-tDCS$_{UHCDS}$ of M1-DLPFC also increased STh, 14% more than conventional M1 c-tDCS, and the effect lasted for 60 minutes. Some fMRI studies show the involvement of both M1 and DLPFC during painful conditions (Peyron et al., 2000; Apkarian et al., 2005). Stimulation of M1 and DLPFC increases the activity of the insula and thalamus (Mesulam and Mufson, 1982; Craig et al., 2000; DaSilva et al., 2011). As a result, further activity enhancement of the insula-thalamus pathway induced by a-tDCS$_{UHCDS}$ of M1-DLPFC is a possible explanation for the STh increase.

The DLPFC has a controlling effect on the function of the fear-anxiety network, intensifying sensory inputs during painful conditions (Wager, 2005; Kong et al., 2006; Benedetti, 2008). In a different way to a-tDCS, the inhibitory effects of c-tDCS over the DLPFC may diminish its controlling role (Egorova et al., 2015), resulting in STh increase. Further studies are required to determine the mechanisms of STh enhancement following a- and c-tDCS.

The second mechanism behind the efficacy of concurrent stimulation of two functionally connected sites is probably increasing the current flow in c-tDCS$_{UHCDS}$ of M1-DLPFC and induction of dendritic polarization (Jefferys, 1981; Ghai et al., 2000; Bikson et al., 2004). Physiologically, neuronal excitability alteration is defined by axonal orientation relative to the electric field vector, and the homogenous electric field stimulates neurons uniformly (Kabakov et al., 2012). Therefore, stimulation of two sites of PNM increases the area receiving the electrical field and affects dendritic polarization with different neuronal orientation. As some paired associative stimulation studies have shown the inhibitory effects of interneurons in the stimulated area can be changed to excitatory ones (Davare et

al., 2009; Buch et al., 2011), it is possible that c-tDCS$_{UHCDS}$ of M1-DLPFC increases the electrical field, leading to recruitment of other non-target brain regions that indirectly affect sensory detection.

The tDCS mechanism-based studies indicate that the excitatory effects of a-tDCS are associated with alteration in neuronal membrane channels like sodium and calcium channels (Jefferys, 1981; Ghai et al., 2000). In some animal models, low postsynaptic calcium enhancement induces long-term depression and increasing the level of calcium causes long-term potentiation (LTP) (Cho et al., 2001; Lisman, 2001). Compared to conventional methods, doubling the electrical field induced by a-tDCS$_{UHCDS}$ of M1-DLPFC possibly increases the calcium level, leading to further increase in the level of LTP-like plasticity in the neuronal membrane. Therefore, it could be concluded that more LTP induced by a-tDCS$_{UHCDS}$ of M1-DLPFC is the main reason behind the day-long STh increase.

Significant differences between a-tDCS$_{UHCDS}$ of M1-DLPFC and all other conditions, including conventional and c-tDCS$_{UHCDS}$ approaches, at all time points suggest that a-tDCS$_{UHCDS}$ of M1-DLPFC is the most efficient stimulation for increasing STh. Our results also indicate that a-tDCS$_{UHCDS}$ of M1-S1 increases STh for at least 24 hours. No existing experimental evidence can support or refute our results, but the results of some studies of stimulated cortical sites of the PNM are in line with our findings. Many tDCS studies report that a-tDCS of both M1 and S1 increases STh at least for 30 min (Nitsche et al., 2008; Vaseghi et al., 2014; 2015c). Ragert et al. (2008) also found that a-tDCS of S1 enhances rather than suppresses tactile spatial acuity (Ragert et al., 2008). However, Antal et al. (2008) found no significant STh following anodal or sham tDCS of M1 (Antal et al., 2008). This discrepancy in the effect of tDCS on STh changes could be explained by differences in the tDCS parameters used in these studies, such as different electrode sizes (5 × 7cm vs. 1.5 × 2cm), and current intensity (2mA or 1 mA vs. 0.3mA).

Regarding the modulation of calcium level by a-tDCS (Cho et al., 2001; Lisman, 2001), concurrent stimulation of M1 and S1 might enhance the level of calcium release in target areas, which consequently induces the LPT-like plasticity of the neuronal membrane (Mesulam and Mufson, 1982; Craig et al., 2000; DaSilva et al., 2011) and STh increase. Compared to M1 a-tDCS, the long-lasting

after effect of a-tDCS$_{UHCDS}$ of M1-S1 probably increased the level of calcium further, raising STh for at least 24 hours (Csifcsak et al., 2009; Bachmann et al., 2010; Grundmann et al., 2011). The mechanism behind the efficacy of c-tDCS$_{UHCDS}$ of M1-S1 is unclear; more research is needed to identify it. However, it is possible that dendritic polarization (Jefferys, 1981; Ghai et al., 2000; Bikson et al., 2004) of neurons located in the M1 and S1 following c-tDCS$_{UHCDS}$ of M1-S switches the inhibitory effects of c-tDCS to excitatory ones (Davare et al., 2009; Buch et al., 2011). The second probable mechanism is the recruitment of non-target brain regions following the application of a doubled electrical field in c-tDCS$_{UHCDS}$ of M1-S1.

The effect of tDCS$_{UHCDS}$ on PTh

Application of conventional a-tDCS over M1 resulted in an increase in PTh for one hour in healthy adults, in line with previous tDCS studies (Bachmann et al., 2010; Grundmann et al., 2011; Vaseghi et al., 2015c). A systematic review by Rein et al. (2015) showed the efficacy of M1 a-tDCS on PTh enhancement in healthy adults (von Rein et al., 2015). In a previous study, we found that PTh increased with no site-specific effects following conventional a-tDCS of S1, DLPFC or M1 (Vaseghi et al., 2015c). Our current findings are in line with those of Antal et al. (2008), who demonstrated that stimulations of left DLPFC by a-tDCS increased the pain threshold and decreased perceived pain when receiving painful stimuli (Antal et al., 2008). In contrast, Bachmann et al. (2010) indicated that c-tDCS of M1 but not other cortical sites of PNM can affect mechanical PTh (Bachmann et al., 2010); they concluded that different methodological factors, such as transmission of painful stimuli induced by different modalities, is the main reason behind this discrepancy. In a positron emission tomography (PET) scan study, it was found the application of mild cooling stimuli increases the activity of the contralateral insular cortex (the thermo-sensory centre in the human cortex) (Craig et al., 2000), while the contralateral anterior cingulate cortex, contralateral M1 and S1, bilateral secondary sensory cortex, mid-insular cortex, contralateral ventral posterior nucleus in thalamus, medial ipsilateral thalamus, and the vermis and paravermis of the cerebellum are activated during painful heat and cold stimuli

(Davis et al., 1999; Peyron et al., 2000; Casey et al., 2001). The conflicting PTh changes following the application of a-tDCS could be caused by the difference in the provocation of different fiber sizes by different type of stimuli. Peripheral electrical stimulation recruits axons based on their diameter, starting with large-diameter fibers (Aß fibers) (Stieglitz, 2005), whereas mechanoreceptors excite myelinated large Aß and small Aδ fibers and might be processed through anatomically different pathways (Ohara et al., 2004). As a result, the stimulated fibres might activate different parts of the PNM.

Similar to conventional a-tDCS, application of c-tDCS over M1 resulted in PTh enhancement for one hour. The results of previous studies demonstrated that M1 c-tDCS increases mechanical PTh and laser-induced heat pain, while it has no effect on cold pain (Bachmann et al., 2010; Grundmann et al., 2011). In our group's recent study of healthy participants, we found no site-specific effects between c-tDCS of M1, S1, or DLPFC in induction of larger PTh at 30 min post-intervention (Vaseghi et al., 2015a). In contrast Grundmann et al. (2011) revealed that M1 c-tDCS has no effect on PTh alteration, while warmth and cold detection threshold significantly increased (Grundmann et al., 2011). They concluded that the intensity for peripheral nerve stimulation and type of stimulation was the key reason behind the conflicting results.

Mechanism-based studies suggest that c-tDCS-induced hyperpolarization causes inhibition of neurons in the stimulated area (Nitsche and Paulus, 2000; Ardolino et al., 2005). The inhibitory effects of c-tDCS may directly increase the level of PTh in healthy individuals. The other probable mechanism behind PTh enhancement following the application of c-tDCS over M1 is the alteration in the activity of the thalamus (Summers et al., 2004), causing PTh enhancement. fMRI and PET scan studies of all cortical and subcortical sites of the PNM demonstrate that the thalamus is the main structure for painful stimuli processing and pain modulation (Almeida et al., 2004; Apkarian et al., 2005). As a result, induction of widespread bidirectional changes in regional neuronal activities, including in thalamic nuclei (Apkarian et al., 2005) and inhibition of thalamic inhibitory connections (DaSilva et al., 2011), are indirectly responsible for PTh enhancement following c-tDCS application.

Our results indicate that a-tDCS$_{UHCDS}$ of M1-DLPFC increased the level of PTh for more than 24 hours. Concurrent a-tDCS$_{UHCDS}$ stimulation of M1 and DLPFC is more efficient than conventional a-tDCS of M1 in increasing the level of PTh in healthy adults. Psychological researchers have indicated that there are bottom-up circuits which facilitate perceptual processing by directing or biasing attention (Davis and Whalen, 2001; LeDoux, 2003). The bottom-up circuits mainly include the amygdala and a variety of prefrontal regions, specifically DLPFC (Phelps, 2006). In addition, a growing literature shows the involvement of DLPFC in top-down mechanisms (Ochsner and Gross, 2005; McRae et al., 2010; Kanske and Kotz, 2011; Ochsner et al., 2012) involved in cognitive-executive control in the brain (Mansouri et al., 2009). Hence, it is possible that concurrent stimulation of M1 and DLPFC by a-tDCS increases the activity of top-down and/or bottom-up circuits, increasing STh and PTh in healthy adults. Based on the literature, a-tDCS of DLPFC activates the ventral cortical stream, including DLPFC, subgenual cingulate gyrus, ventrolateral cortex, orbitofrontal cortex, amygdala, anterior insula, ventral striatum, medial thalamus, and hippocampus (Zubieta et al., 2005; Kong et al., 2006). The ventral cortical stream is mostly activated in painful stimuli processing, and increasing its activity leads to PTh enhancement or pain reduction (Zubieta et al., 2005; Kong et al., 2006). Therefore, as explained above, concurrent stimulation of M1-DLPFC increases the electrical field in the brain and possibly leads to recruitment of other non-target brain regions and activated dendritic polarization mechanisms (Jefferys, 1981; Ghai et al., 2000; Bikson et al., 2004; Kabakov et al., 2012). All these factors could increase the influx of calcium and LTP-like plasticity (Jefferys, 1981; Ghai et al., 2000), resulting in lasting effects. However, more studies are required to find the reason behind the efficacy of a-tDCS$_{UHCDS}$ of M1-DLPFC but not other tDCS$_{UHCDS}$ conditions on PTh in healthy adults.

Limitation

These findings should be interpreted with some caution due to the following limitations. First, tDCS$_{UHCDS}$ was applied over cortical sites of the PNM in healthy adults and the results cannot be

extrapolated to unwell populations; the neuroplastic changes induced by pain might differ in patients with chronic pain. Second, all participants were young (less than 35 years old) in this study; it is possible that neurobehavioral responses to $tDCS_{UHCDS}$ are different in older individuals. Third, in order to reduce current density and minimize its neuromodulatory effects in the subgenual cortex, we used reference electrodes four times larger than the active ones; however, it is still possible that this electrode montage affects the function of the underlying cortex (Kanda et al., 2000; Miranda et al., 2006; Mylius et al., 2012). Fourth, we only assessed the electrical STh and PTh of healthy individuals. The results for other sensory impulses carried by larger or smaller fibres should be interpreted cautiously for other test stimuli such as pressure or heat.

Suggestions for future research

Regarding the long-lasting effects of a-$tDCS_{UHCDS}$ of M1-DLPFC on both STh and PTh in healthy adults, we recommend the evaluation of the efficacy of this new tDCS approach in patients with chronic pain. Our study was designed as a pilot for future systematic studies of the effects of a-$tDCS_{UHCDS}$ characteristics, including application time, current intensity, and electrode size, on STh/PTh. Clearly, to reveal the mechanisms of action of a-$tDCS_{UHCDS}$ of M1-DLPFC on STh and PTh, fundamental mechanism-based studies are needed. Such investigations will improve our understanding in this field and provide a standardized method allowing future researchers to compare their results.

Author Contributions

All authors of this original article have directly participated in the planning, execution, analysis, and preparing the final version of this manuscript. The contents of this manuscript have not been copyrighted or published previously.

Table 2. ANOVA comparing the effects of $tDCS_{UHDS}$ stimulation over cortical areas of pain neuromatrix on sensory and pain threshold

	1. tDCS mode		2. Time		3. Stimulation site		Interaction 1 by 2		Interaction 1 by 3		Interaction 2 by 3		Interaction 1 by 2 by 3	
	F-value	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**
STh	53.0	0.001	75.2	< 0.001	10.9	< 0.001	20.21	< 0.001	5.25	< 0.001	17.6	< 0.001	8.7	< 0.001
PTh	71.2	< 0.001	44.56	< 0.001	15.72	< 0.001	14.75	< 0.001	10.60	< 0.001	8.64	0.007	10.23	< 0.001

This table compare the effects of conventional tDCS over primary motor cortex (M1) and $tDCS_{UHDS}$ of M1-dorsolateral prefrontal cortex (DLPFC), M1-primary sensory cortex, M1-primary visual cortex, and sham $tDCS_{UHDS}$ of M1-S1 or M1-DLPFC following two modes of tDCS (anodal and cathodal). Both sensory (STh) and pain (PTh) were measured in five time-points of measurement (before, immediately, 30 min, 60 min, and 24 hours after stimulation). P-values are reported for values < 0.05.

References

Ahumada, J., Fernandez de Sevilla, D., Couve, A., Buno, W.&Fuenzalida, M. (2013) Long-term depression of inhibitory synaptic transmission induced by spike-timing dependent plasticity requires coactivation of endocannabinoid and muscarinic receptors. *Hippocampus*, **23**, 1439-1452.

Almeida, T. F., Roizenblatt, S.&Tufik, S. (2004) Afferent pain pathways: a neuroanatomical review. *Brain research*, **1000**, 40-56.

Antal, A., Brepohl, N., Poreisz, C., Boros, K., Csifcsak, G.&Paulus, W. (2008) Transcranial direct current stimulation over somatosensory cortex decreases experimentally induced acute pain perception. *The Clinical journal of pain*, **24**, 56-63.

Apkarian, A. V., Bushnell, M. C., Treede, R. D.&Zubieta, J. K. (2005) Human brain mechanisms of pain perception and regulation in health and disease. *European journal of pain (London, England)*, **9**, 463-484.

Ardolino, G., Bossi, B., Barbieri, S.&Priori, A. (2005) Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. *The Journal of physiology*, **568**, 653-663.

Bachmann, C. G., Muschinsky, S., Nitsche, M. A., Rolke, R., Magerl, W., Treede, R. D., Paulus, W.&Happe, S. (2010) Transcranial direct current stimulation of the motor cortex induces distinct changes in thermal and mechanical sensory percepts. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **121**, 2083-2089.

Bastani, A.&Jaberzadeh, S. (2013a) Differential modulation of corticospinal excitability by different current densities of anodal transcranial direct current stimulation. *PloS one*, **8**, e72254.

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

Benedetti, F. (2008) Mechanisms of placebo and placebo-related effects across diseases and treatments. *Annual review of pharmacology and toxicology*, **48**, 33-60.

Bikson, M., Datta, A., Rahman, A.&Scaturro, J. (2010) Electrode montages for tDCS and weak transcranial electrical stimulation: role of "return" electrode's position and size. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **121**, 1976-1978.

Bikson, M., Inoue, M., Akiyama, H., Deans, J. K., Fox, J. E., Miyakawa, H.&Jefferys, J. G. (2004) Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *The Journal of physiology*, **557**, 175-190.

Bornhovd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C.&Buchel, C. (2002) Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. *Brain : a journal of neurology*, **125**, 1326-1336.

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G.&Fregni, F. (2011) A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, **14**, 1133-1145.

Buch, E. R., Johnen, V. M., Nelissen, N., O'Shea, J.&Rushworth, M. F. (2011) Noninvasive associative plasticity induction in a corticocortical pathway of the human brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **31**, 17669-17679.

Casey, K. L., Morrow, T. J., Lorenz, J.&Minoshima, S. (2001) Temporal and spatial dynamics of human forebrain activity during heat pain: analysis by positron emission tomography. *Journal of neurophysiology*, **85**, 951-959.

Chesler, M. (2003) Regulation and modulation of pH in the brain. *Physiological reviews*, **83**, 1183-1221.

Cho, K., Aggleton, J. P., Brown, M. W.&Bashir, Z. I. (2001) An experimental test of the role of postsynaptic calcium levels in determining synaptic strength using perirhinal cortex of rat. *The Journal of physiology*, **532**, 459-466.

Clark, W. C. (1976) Pain Sensitivity and the Report of Pain: An Introduction to Sensory Decision Theory. In Weisenberg, M.,Tursky, B. (eds) *Pain*. Springer US, pp. 195-222.

Craig, A. D., Chen, K., Bandy, D.&Reiman, E. M. (2000) Thermosensory activation of insular cortex. *Nature neuroscience*, **3**, 184-190.

Csifcsak, G.&Antal, A. (2012) [Non-invasive brain stimulation for relieving acute and chronic pain]. *Ideggyogyaszati szemle*, **65**, 149-160.

Csifcsak, G., Antal, A., Hillers, F., Levold, M., Bachmann, C. G., Happe, S., Nitsche, M. A., Ellrich, J.&Paulus, W. (2009) Modulatory effects of transcranial direct current stimulation on laser-evoked potentials. *Pain medicine (Malden, Mass.)*, **10**, 122-132.

DaSilva, A. F., Volz, M. S., Bikson, M.&Fregni, F. (2011) Electrode positioning and montage in transcranial direct current stimulation. *Journal of visualized experiments : JoVE*.

Davare, M., Montague, K., Olivier, E., Rothwell, J. C.&Lemon, R. N. (2009) Ventral premotor to primary motor cortical interactions during object-driven grasp in humans. *Cortex; a journal devoted to the study of the nervous system and behavior*, **45**, 1050-1057.

Davis, K. D., Lozano, R. M., Manduch, M., Tasker, R. R., Kiss, Z. H.&Dostrovsky, J. O. (1999) Thalamic relay site for cold perception in humans. *Journal of neurophysiology*, **81**, 1970-1973.

Davis, M.&Whalen, P. J. (2001) The amygdala: vigilance and emotion. *Molecular psychiatry*, **6**, 13-34.

Egorova, N., Yu, R., Kaur, N., Vangel, M., Gollub, R. L., Dougherty, D. D., Kong, J.&Camprodon, J. A. (2015) Neuromodulation of conditioned placebo/nocebo in heat pain: anodal vs. cathodal transcranial direct current stimulation to the right dorsolateral prefrontal cortex. *Pain*.

Fernandez-de-Las-Penas, C., Madeleine, P., Martinez-Perez, A., Arendt-Nielsen, L., Jimenez-Garcia, R.&Pareja, J. A. (2010) Pressure pain sensitivity topographical maps reveal bilateral hyperalgesia of the hands in patients with unilateral carpal tunnel syndrome. *Arthritis care & research*, **62**, 1055-1064.

Gandiga, P. C., Hummel, F. C.&Cohen, L. G. (2006) Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **117**, 845-850.

Ghai, R. S., Bikson, M.&Durand, D. M. (2000) Effects of applied electric fields on low-calcium epileptiform activity in the CA1 region of rat hippocampal slices. *Journal of neurophysiology*, **84**, 274-280.

Giesecke, T., Gracely, R. H., Williams, D. A., Geisser, M. E., Petzke, F. W.&Clauw, D. J. (2005) The relationship between depression, clinical pain, and experimental pain in a chronic pain cohort. *Arthritis and rheumatism*, **52**, 1577-1584.

Grundmann, L., Rolke, R., Nitsche, M. A., Pavlakovic, G., Happe, S., Treede, R. D., Paulus, W.&Bachmann, C. G. (2011) Effects of transcranial direct current stimulation of the primary sensory cortex on somatosensory perception. *Brain stimulation*, **4**, 253-260.

Islam, N., Aftabuddin, M., Moriwaki, A., Hattori, Y.&Hori, Y. (1995) Increase in the calcium level following anodal polarization in the rat brain. *Brain research*, **684**, 206-208.

Jefferys, J. G. (1981) Influence of electric fields on the excitability of granule cells in guinea-pig hippocampal slices. *The Journal of physiology*, **319**, 143-152.

Kabakov, A. Y., Muller, P. A., Pascual-Leone, A., Jensen, F. E.&Rotenberg, A. (2012) Contribution of axonal orientation to pathway-dependent modulation of excitatory transmission by direct current stimulation in isolated rat hippocampus. *Journal of neurophysiology*, **107**, 1881-1889.

Kanda, M., Nagamine, T., Ikeda, A., Ohara, S., Kunieda, T., Fujiwara, N., Yazawa, S., Sawamoto, N., Matsumoto, R., Taki, W.&Shibasaki, H. (2000) Primary somatosensory cortex is actively involved in pain processing in human. *Brain research*, **853**, 282-289.

Kanske, P.&Kotz, S. A. (2011) Emotion speeds up conflict resolution: a new role for the ventral anterior cingulate cortex? *Cerebral cortex (New York, N.Y. : 1991)*, **21**, 911-919.

Kirimoto, H., Ogata, K., Onishi, H., Oyama, M., Goto, Y.&Tobimatsu, S. (2011) Transcranial direct current stimulation over the motor association cortex induces plastic changes in ipsilateral primary motor and somatosensory cortices. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **122**, 777-783.

Kirouac, G. J., Li, S.&Mabrouk, G. (2004) GABAergic projection from the ventral tegmental area and substantia nigra to the periaqueductal gray region and the dorsal raphe nucleus. *The Journal of comparative neurology*, **469**, 170-184.

Kong, J., Gollub, R. L., Rosman, I. S., Webb, J. M., Vangel, M. G., Kirsch, I.&Kaptchuk, T. J. (2006) Brain activity associated with expectancy-enhanced placebo analgesia as measured by functional magnetic resonance imaging. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **26**, 381-388.

LeDoux, J. (2003) Emotion circuits in the brain.

Lisman, J. E. (2001) Three Ca2+ levels affect plasticity differently: the LTP zone, the LTD zone and no man's land. *The Journal of physiology*, **532**, 285.

Loeb, G. E. U. o. C. P. (1986) *Electromyography for experimentalists*. University of Chicago Press.

Mansouri, F. A., Tanaka, K.&Buckley, M. J. (2009) Conflict-induced behavioural adjustment: a clue to the executive functions of the prefrontal cortex. *Nature reviews. Neuroscience*, **10**, 141-152.

McRae, K., Hughes, B., Chopra, S., Gabrieli, J. D., Gross, J. J.&Ochsner, K. N. (2010) The neural bases of distraction and reappraisal. *Journal of cognitive neuroscience*, **22**, 248-262.

Medeiros, L. F., de Souza, I. C., Vidor, L. P., de Souza, A., Deitos, A., Volz, M. S., Fregni, F., Caumo, W.&Torres, I. L. (2012) Neurobiological effects of transcranial direct current stimulation: a review. *Frontiers in psychiatry*, **3**, 110.

Mesulam, M. M.&Mufson, E. J. (1982) Insula of the old world monkey. III: Efferent cortical output and comments on function. *The Journal of comparative neurology*, **212**, 38-52.

Miranda, P. C., Lomarev, M.&Hallett, M. (2006) Modeling the current distribution during transcranial direct current stimulation. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **117**, 1623-1629.

Mylius, V., Jung, M., Menzler, K., Haag, A., Khader, P. H., Oertel, W. H., Rosenow, F.&Lefaucheur, J. P. (2012) Effects of transcranial direct current stimulation on pain perception and working memory. *European journal of pain (London, England)*, **16**, 974-982.

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., Paulus, W., Hummel, F., Boggio, P. S., Fregni, F.&Pascual-Leone, A. (2008) Transcranial direct current stimulation: State of the art 2008. *Brain stimulation*, **1**, 206-223.

Nitsche, M. A., Doemkes, S., Karakose, T., Antal, A., Liebetanz, D., Lang, N., Tergau, F.&Paulus, W. (2007) Shaping the effects of transcranial direct current stimulation of the human motor cortex. *Journal of neurophysiology*, **97**, 3109-3117.

Nitsche, M. A., Liebetanz, D., Schlitterlau, A., Henschke, U., Fricke, K., Frommann, K., Lang, N., Henning, S., Paulus, W.&Tergau, F. (2004) GABAergic modulation of DC stimulation-induced motor cortex excitability shifts in humans. *The European journal of neuroscience*, **19**, 2720-2726.

Nitsche, M. A.&Paulus, W. (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of physiology*, **527 Pt 3**, 633-639.

Nitsche, M. A.&Paulus, W. (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology*, **57**, 1899-1901.

Nitsche, M. A., Seeber, A., Frommann, K., Klein, C. C., Rochford, C., Nitsche, M. S., Fricke, K., Liebetanz, D., Lang, N., Antal, A., Paulus, W.&Tergau, F. (2005) Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *The Journal of physiology*, **568**, 291-303.

O'Connell, N. E., Wand, B. M., Marston, L., Spencer, S.&Desouza, L. H. (2011) Non-invasive brain stimulation techniques for chronic pain. A report of a Cochrane systematic review and meta-analysis. *European journal of physical and rehabilitation medicine*, **47**, 309-326.

Ochsner, K. N.&Gross, J. J. (2005) The cognitive control of emotion. *Trends in cognitive sciences*, **9**, 242-249.

Ochsner, K. N., Silvers, J. A.&Buhle, J. T. (2012) Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. *Annals of the New York Academy of Sciences*, **1251**, E1-E24.

Ohara, S., Weiss, N.&Lenz, F. A. (2004) Microstimulation in the region of the human thalamic principal somatic sensory nucleus evokes sensations like those of mechanical stimulation and movement. *Journal of neurophysiology*, **91**, 736-745.

Oldfield, R. C. (1971) The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, **9**, 97-113.

Peyron, R., Laurent, B.&Garcia-Larrea, L. (2000) Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiologie clinique = Clinical neurophysiology*, **30**, 263-288.

Phelps, E. A. (2006) Emotion and cognition: insights from studies of the human amygdala. *Annu. Rev. Psychol.*, **57**, 27-53.

Poreisz, C., Boros, K., Antal, A.&Paulus, W. (2007) Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain research bulletin*, **72**, 208-214.

Ragert, P., Vandermeeren, Y., Camus, M.&Cohen, L. G. (2008) Improvement of spatial tactile acuity by transcranial direct current stimulation. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **119**, 805-811.

Stagg, C. J., Best, J. G., Stephenson, M. C., O'Shea, J., Wylezinska, M., Kincses, Z. T., Morris, P. G., Matthews, P. M.&Johansen-Berg, H. (2009) Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **29**, 5202-5206.

Stagg, C. J.&Nitsche, M. A. (2011) Physiological basis of transcranial direct current stimulation. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*, **17**, 37-53.

Stieglitz, T. (2005) Diameter-dependent excitation of peripheral nerve fibers by multipolar electrodes during electrical stimulation. *Expert review of medical devices*, **2**, 149-152.

Summers, J., Johnson, S., Pridmore, S.&Oberoi, G. (2004) Changes to cold detection and pain thresholds following low and high frequency transcranial magnetic stimulation of the motor cortex. *Neuroscience letters*, **368**, 197-200.

Vaseghi, B., Zoghi, M.&Jaberzadeh, S. (2014) Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, **125**, 1847-1858.

Vaseghi, B., Zoghi, M.&Jaberzadeh, S. (2015a) Differential effects of cathodal-tDCS of prefrontal, motor, and somatosensory cortices on cortical excitability and pain perception: a double-blind randomised sham-controlled study. *The European journal of neuroscience*.

Vaseghi, B., Zoghi, M.&Jaberzadeh, S. (2015b) The effects of anodal-tDCS on corticospinal excitability: conventional versus unihemispheric concurrent dual-site stimulation. *Frrontiers in Human Neuroscience*, **In Press**.

Vaseghi, B., Zoghi, M.&Jaberzadeh, S. (2015c) How does anodal transcranial direct current stimulation of the pain neuromatrix affect brain excitability and pain perception? A randomised, double-blind, sham-control study. *PloS one*, **10**, e0118340.

Vaseghi, B., Zoghi, M.&Jaberzadeh, S. (2015d) A meta-analysis of site-specific effects of cathodal transcranial direct current stimulation on sensory perception and pain. *PloS one*, **10**, e0123873.

von Rein, E., Hoff, M., Kaminski, E., Sehm, B., Steele, C. J., Villringer, A.&Ragert, P. (2015) Improving motor performance without training: The effect of combining mirror visual feedback with transcranial direct current stimulation. *Journal of neurophysiology*, jn.00832.02014.

Wager, T. D. (2005) Expectations and anxiety as mediators of placebo effects in pain. *Pain*, **115**, 225-226.

Zubieta, J. K., Bueller, J. A., Jackson, L. R., Scott, D. J., Xu, Y., Koeppe, R. A., Nichols, T. E.&Stohler, C. S. (2005) Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **25**, 7754-7762.

Preamble to Chapter 10

Chapter 10 summarizes the thesis findings and provides a list of limitations and suggestions for future studies.

Chapter 10: Summary and Concluding Remarks

In the present thesis, the intention is to provide evidence for the existing relationship between cortical sites of PNMs in order to introduce an alternative tDCS technique for the induction of larger CSE with longer lasting effects, compared to current tDCS techniques. In addition, the potential effects of both a- and c-tDCS on increasing the level of STh/PTh in healthy individuals or/and decreasing pain levesl in patients have been investigated. In recent years a large body of research has been focused on a- and c-tDCS effects on M1 CSE as a non-invasive and safe method of pain reduction. Despite the initial success in identifying the optimal a- and c-tDCS parameters, more investigations are needed to systematically find an efficient tDCS technique by which not only STh/PTh are increased, but also CSE is enhanced with longer lasting effects, compared to that created by conventional tDCS techniques.

The primary aim of this thesis is to determine the relationship of M1, S1, and DLPFC following a- and c-tDCS of these functionally connected cortical sites of the PNM. The secondary aim is to establish a novel tDCS technique for the induction of larger and longer lasting CSE and STh/PTh changes. To discuss these aims, and provide concluding remarks as to how they have been satisfied, this section is divided into five parts: (1) two systematic reviews of the literature (Studies 1 and 2), (2) areliability and feasibility study (Study 3), (3) the determination of the relationship between M1, S1, and DLPFC following a- and c-tDCS, (4) the effect of stimulation of these cortical sites on CSE and STh/PTh changes (Studies 4 and 5), and (5) the development of a new tDCS technique for the induction of larger CSE and STh/PTh changes (Studies 6, 7, 8).

A systematic review of the literature

In Chapters 2 and 3, two systematic review and meta-analyses were carried out to verify whether previous studies support the view that a-tDCS (Chapter 2) and c-tDCS (Chapter 3) of cortical sites of the PNM including M1, S1, and DLPFC increases STh and PTh in healthy individual and/or decreases

pain levels in patients with chronic pain. From the findings of meta-analyses in Chapter 2, it can be concluded that a-tDCS of M1 and S1 is effective in increasing STh and PTh in healthy individuals. In addition, the results indicate that a-tDCS of M1 and DLPFC is associated with decreasing the level of pain in patients with chronic pain. Likewise, the results from the second systematic review (Chapter 3) demonstrates that c-tDCS of both M1 and S1 increases STh/PTh in healthy individuals. As described in Chapter 3, there is not enough evidence to consider site-specific effectiveness of c-tDCS in decreasing the level of pain in patients with chronic pain. However, the results showed that c-tDCS is generally successful in decreasing the pain level in chronic pain patients.

These two systematic reviews show the tDCS effectiveness of these cortical sites of the PNM with a STh/PTh increase and a PL decrease. Yet there is not enough evidence to consider the relationship between these cortical sites of the PNM. For instance, no study has investigated the effect of a-tDCS of DLPC on M1 CSE. Such data may provide valuable data to find the most efficient tDCS technique to increase STh/PTh or decrease PL. In addition, there are some systematic reviews and meta-analyses indicating that there is insufficient evidence to make a firm conclusion in efficacy of tDCS in pain relief (O'Connell et al. 2011, Luedtke et al. 2012). However, the results of current systematic reviews and meta-analyses (Chapters 2-3) demonstrated that the effects of both a- and c-tDCS depends on the site of stimulation.

A reliability and feasibility study

Any application of tDCS involves measurement of changes before and after intervention. As, in addition to STh/PTh measurements, we planned to measure CSE changes in Chapters 5-8, a reliability study was conducted to make sure that the changes following interventions are not due to systematic errors and/or methodological inconsistencies in recorded TMS-induced MEPs (Chapter 4). The reliability study is also used to determine the effects of the inter-pulse interval (IPI) of single-pulse TMS on the averaged MEP values and their intra- and inter-session reliability. The higher reliability is achieved by increasing the IPI from 4 sec to 10 sec. In conclusion, while both an IPI of 4 (ICC > 0.87) and 10 (ICC > 0.80) sec resulted in acceptable reliability in FDI muscle, recording MEPs with an IPI of 10 sec induces larger

MEPs. As a result, in this chapter, it is recommended to add length of IPI to the international checklist of considerations for single-pulse TMS application.

Determination of the relationship between M1, S1, and DLPFC during a- and c-tDCS

Determining the relationship between M1, S1, and DLPFC following the application of both a- and c-tDCS has a profound impact on finding the best stimulation site to simultaneously enhance M1 CSE and STh/PTh. Study 4 (Chapter 5) shows that M1 and DLPFC are two proper stimulation sites to enhance M1 CSE. Diverse effects of S1 and M1 a-tDCS on M1 and S1 excitability are also observed in this study. In addition, the results indicate that a-tDCS of all these functionally connected cortical sites increase STh/PTh in healthy individuals. Study 4 is the first to investigate the effect of a-tDCS of these cortical sites on both M1/S1 excitability and STh/PTh. The results provide evidence for an existing relationship between these functionally connected cortical sites of the PNM. Furthermore, for the first time, the effect of a-tDCS of DLPFC on M1 CSE and STh/PTh is investigated (in Study 4).

In Chapter 6 (Study 5), the inhibitory effects of c-tDCS of M1, S1, or DLPFC on M1/S1 excitability and STh/PTh were investigated simultaneously. The findings indicate that c-tDCS of all these three sites decreases M1 and S1 excitability, while STh/PTh are increased. However, there is a significant difference between M1 CSE reduction by M1 c-tDCS and stimulation of S1 and DLPFC. This finding may suggest that due to the functional connectivities existing between these cortical sites, c-tDCS of all cortical sites may result in a STh/PTh increase.

The development of the tDCS technique for the induction of larger CSE and STh/PTh changes.

In Chapter 5 and 6, it is shown that single-site a- and c-tDCS of M1, S1, and DLPFC modify M1 CSE and STh/PTh in healthy adults. However, M1 is the most efficient stimulation site among these three functionally connected cortical sites of the PNM. In Chapter 7, the effects of a novel tDCS technique on the M1 CSE size and its lasting effects is investigated. Indeed, a new technique is designed to take

advantage of concurrent stimulation of two functionally connected cortical sites of the brain – the unihemispheric concurrent dual-site a-tDCS (a-tDCS$_{UHCDS}$). In this chapter, the effects of concurrent a-tDCS of M1, and S1 or DLPFC on the size of M1 CSE changes and their lasting effects are evaluated, and compared to those of conventional single-site M1 a-tDCS. The results indicate that a combination of concurrent a-tDCS of M1 and DLPFC induces larger M1 CSE at least for 24 hours in healthy adults, compared to current conventional a-tDCS techniques. These excitatory effects are accompanied with increasing levels of intracortical facilitation with no significant change in intracortical inhibition in the M1. Similar to the M1 a-tDCS, the results of the participants' feeling rating scale recorded by questionnaire indicated that, similar to single-site a-tDCS, a-tDCS$_{UHCDS}$ is a completely safe and tolerable technique.

In Chapter 8, the effects of concurrent c-tDCS of M1 and S1 or DLPFC on the size and lasting effects of CSE changes are compared with the conventional single-site M1 c-tDCS. Contrary to conventional M1 c-tDCS, CSE enhancement is observed following concurrent c-tDCS of these functionally connected sites, and the result lasts at least 24 hours. Such surprising c-tDCS$_{UHCDS}$ effects are accompanied with significant increases in intracortical facilitation, and no change in intracortical inhibition. The rating scale of feeling during application of c-tDCS$_{UHCDS}$ revealed that, similar to M1 c-tDCS, this new technique is completely safe and tolerable for the participants.

Regarding the efficacy of the tDCS$_{UHCDS}$ technique in CSE enhancement recorded from Studies 6 and 7 (Chapters 7 and 8), Study 8 (Chapter 9) is designed to investigate the effects of unihemispheric concurrent dual-site a- and c-tDCS on STh/PTh levels in healthy adults, compared to that of conventional single-site techniques. Comparing tDCS$_{UHCDS}$ and conventional tDCS, the results imply that tDCS$_{UHCDS}$ of cortical sites of pain neuromatrices is more efficient, than the conventional single-site M1 stimulation, for increasing STh/PTh. Significant differences were found between concurrent a- and c-tDCS$_{UHCDS}$ conditions, suggesting that concurrent a-tDCS of M1 and DLPFC in the same hemisphere could be the most efficient technique to enhance STh and PTh with day-long lasting effects. Such results provide a new insight into future studies investigating an efficient tDCS approach to

manage pain.

Thesis Limitations

Limitations have been mentioned with each study presented in this thesis. To avoid repetition, only the limitations within the framework of multiple studies are presented here. The group under investigation in all experimental studies in this thesis is comprised of healthy young individuals, so findings cannot be extrapolated to older healthy adults or patients with different pathological conditions. As well as this, even though participants from both sexes participated in all studies, gender differences in levels of STh and PTh are not explored. Based on the effect size in cortical (MEP/SEP) and behavioural (STh/PTh) outcome measures and following consultation with a qualified statistician, we found that it is required to recruit 39 more participants in each study to evaluate the correlation between cortical and behavioural changes. As a result, given the number of experimental studies the number of sessions in each study and regarding the difficult nature of tDCS/TMS studies, it was practically impossible to recruit the required number of participants for correlation assessment test. Finally, some studies (Studies 6-8) 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. Concurrent stimulation of two functionally connected cortical sites of the brain is a completely new concept in tDCS studies. This technique has the potential to improve tDCS protocols for different purposes such as CSE enhancement, STh/PTh increase, and pain perception decrease. As a result, further studies are needed to systematically identify the optimal parameters of unihemispheric concurrent dual-site a- and c-tDCS for these purposes. Indeed, the effects of current intensity/density, application time, electrode size, and stimulation sites should be investigated in more detail to determine the optimal parameters for induction of larger and longer CSE enhancement compared to that provided by the conventional single-site tDCS technique. In addition, the efficacy of these optimal parameters for STh/PTh increase or pain perception decrease should be evaluated on both healthy individuals, and patients with different pathological pain disorders. Moreover, exploring the mechanisms of action in

unihemispheric concurrent dual-site a- and c-tDCS can deepen the analysis. Recommendations include pharmacological experiments to compare the physiological mechanisms of a- and c-tDCS$_{UHCDS}$ more directly, using a GABAergic and/or Glutamergic agonist and/or antagonist.

Given the novelty of this new introduced tDCS technique, fMRI and computational modelling studies may be helpful in gaining a realistic picture from the pattern of tDCS current in the concurrent stimulation of two functionally connected cortical sites of the brain. As well, further studies using a larger sample size, long-term follow-ups, multiple tDCS sessions, and larger current density are needed to find out the CSE and STh/PTh changes, and their compatibility with the level of pain perception in patients with different pathological pain.

As any change in different parts of corticospinal tract (e.g. peripheral nerves and spinal cord) may affect the size of MEPs, more studies are required to identify the contribution of different parts of corticospinal tracts on the MEPs changes induced by concurrent stimulation of two functionally connected sites of the brain. Finally, as the relationship between CSE changes and STh/PTh changes is extremely valuable, additional studies are suggested for future investigations to address this relationship.

There is hope that the findings of the present thesis might assist in the development of pain treatment protocols in patients with different pathological or psychological problems. This thesis takes a small step in that direction.

APPENDICES

Appendix 1 Sample size selection

This appendix describes statistical procedures for power analysis and sample size estimation for studies using analysis of variance. Sample size could be easily determined based on the effect size of pilot study. The SPSS reports the effect size index as eta (η^2). The below table give power estimates for different values of the effect size index, *f*, at df_b = 1 to 6, 8, 10 at $\alpha = 0.05$.

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

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

Appendix 2 Quality assessment with 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	*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.
	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	

	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 3 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 4 Checklist for reporting the quality assessment by D&B scale

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?

Yes	1
No	0

If the main outcomes are first mentioned in the Results section, the question should be answered no.

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 generalized to the population from which the study subjects were derived.

11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?

The study must identify the source population for patients and describe how the patients were selected. Patients would be representative if they comprised the entire source population, an unselected sample of consecutive patients, or a random sample. Random sampling is only feasible where a list of all members of the relevant population exists. Where a study

Yes	1
No	0
Unable to determine	0

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?

Yes	1
No	0
Unable to determine	0

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.

13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?

Yes	1
No	0
Unable to determine	0

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 center unrepresentative of the hospitals most of the source population would attend.

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?

Yes	1
No	0
Unable to determine	0

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.

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?

Yes	1
No	0
Unable to determine	0

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.

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.

Yes	1
No	0
Unable to determine	0

Internal validity - confounding (selection bias)

21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?

Yes	1
No	0
Unable to determine	0

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.

22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies)

Yes	1
No	0
Unable to determine	0

For a study which does not specify the time period over which patients were recruited, the question should be answered as unable to determine.

23. Were study subjects randomized to intervention groups?

Studies which state that subjects were randomized should be answered yes except where method of randomization 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 randomized intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?

All non-randomized studies should be answered no. If assignment was concealed from patients but not from staff, it should be answered no.

Yes	1
No	0
Unable to determine	0

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

Yes	1
No	0
Unable to determine	0

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

Yes	1
No	0
Unable to determine	0

Power

27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance isless 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 5 Plot digitizer

Plot or Graph Digitizer is a Java program, which is used to digitize scanned plot of many types of functional data. Often data is found presented in represented 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.

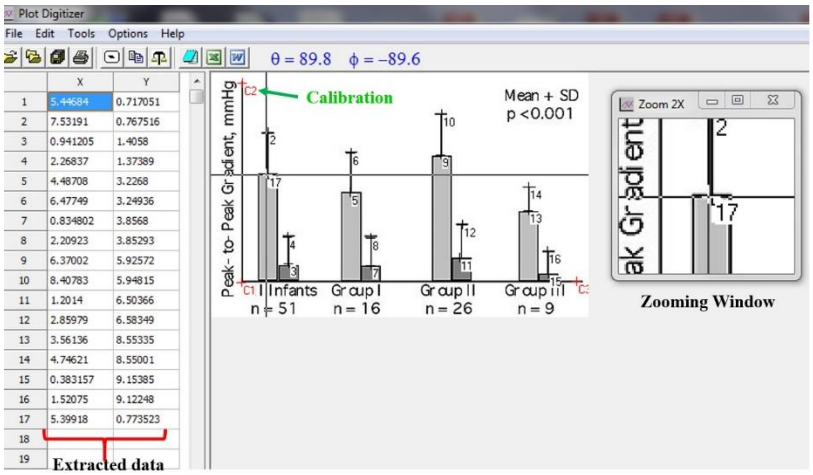
Tis program will allow you to take a scanned image of a plot (in JPEG or Bitmap) and quickly digitize values off the plots just by clicking the mouse on each data point after calibration. Any 3 non-colinear points can be used for calibration. The calibration points **do not need** to be on the axes. Data can be expert to an ASCII, MS Excel, or MS Word files and used wherever 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. Them calibrate the plot by clicking on the calibration option or from "Tool" menu and then digitize the points.

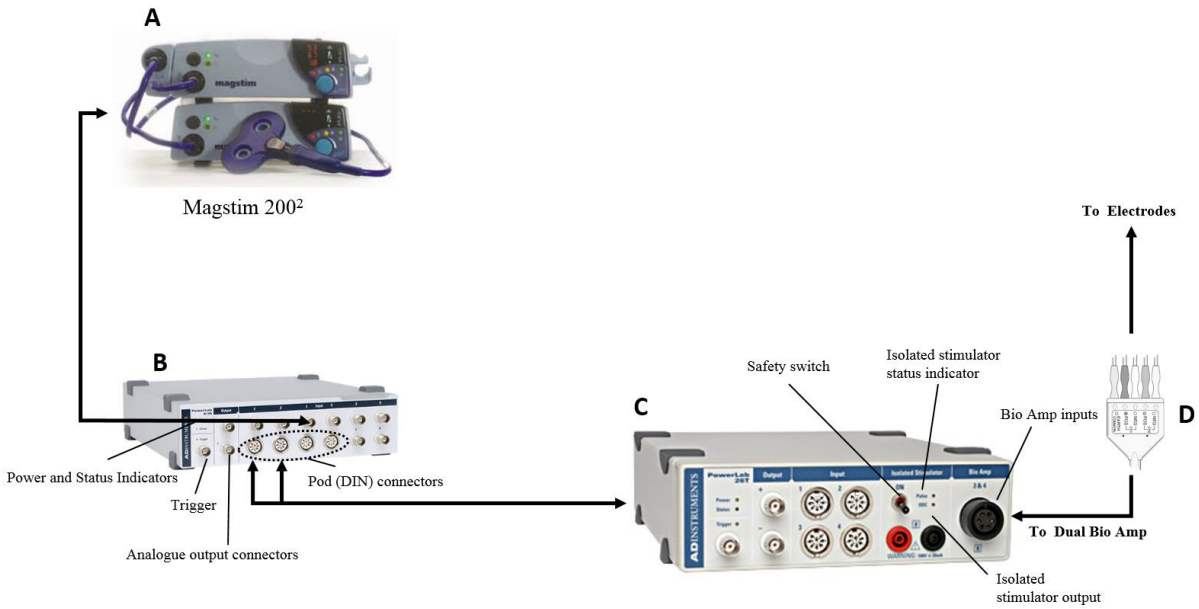
Hint: if you want to digitize the plots from published technical reports that are available

electronically in PDF format, you can copy the image with the snap shot 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

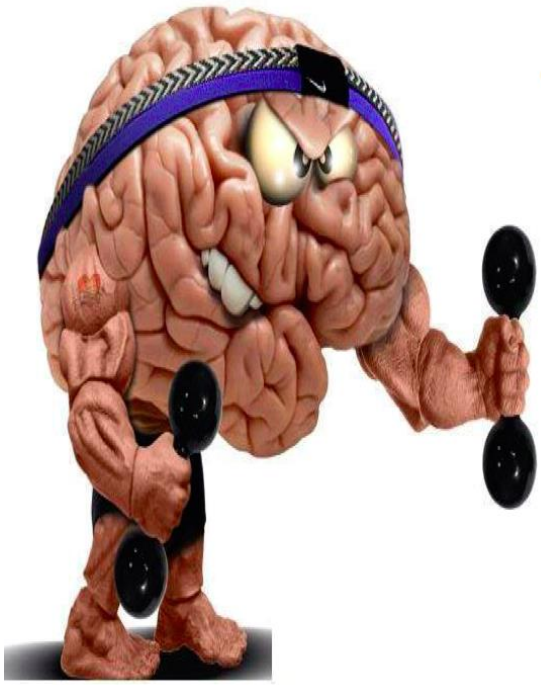
Appendix 6 The set up system used in the present thesis



A) Magstim 200² **B)** The powerlab 8/30 has three indicators at the left frontal panel, one BNC connector for the external trigger, two BNC connectors for analog output and eight BNC connectors (marked input 1-8) with four alternative pod (DIN) connectors for inputs 1 – 4, for recording external signals **C)** Dual Bioamp/stimulator **D)** Cables for recording EMG of the target muscle.

Appendix 7 Sample advertisement

MU global email & faculty e-bulletins & newspaper



MONASH University
Medicine, Nursing and Health Sciences

Healthy volunteers needed

"The effects of tDCS on pain neuromatrix in healthy individual"

- Are you a healthy male or female aged over 18?
- Are you a right-handed person?

If yes

We would like to hear from you

Ethics approval has been granted by the Monash Human Research Ethics Committee (ID number: CF 12/2383-2012001295)

The Non-invasive Brain Stimulation and Neuroplasticity Laboratory (NIBS & NL) in the Department of Physiotherapy (Room B01, Building B, Peninsula Campus) is conducting a series of experiments to assess the therapeutic value of a safe and painless method of brain stimulation (tDCS). After 20 minutes of tDCS, we will look at the function of sensory area of brain by Sensory Evoked Potential (SEP) and the function of motor area by Transcranial Magnetic Stimulation (TMS). Both techniques are safe and painless which are widely used in different laboratories.

You will receive a gift voucher of $ 80 after finishing all sessions as a reimbursement

For more information please contact:

Bita.vaseghi@monash.edu

Appendix 8 Ethics Approval

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

Date:	**13 November 2012**
Project Number:	**CF12/2383 - 2012001295**
Project Title:	**The effects of transcranial direct current stimulation (tDCS) on brain excitability and pain: impact of stimulation site & current amplitude**
Chief Investigator:	**Dr Shapour Jaberzadeh**
Approved:	**From: 13 November 2012 To: 13 November 2017**

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: Dr Maryam Zoghi, Miss Bita Vasehgi

Postal – Monash University, Vic 3800, Australia
Building 3E, Room 111, Clayton Campus, Wellington Road, Clayton
Telephone +61 3 9905 5490 Facsimile +61 3 9905 3831
Email muhrec@monash.edu www.monash.edu/research/ethics/human/index/html
ABN 12 377 614 012 CRICOS Provider #00008C

Appendix 9 Edinburgh Handedness Questionnaire (Oldfield R C, 1971)

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

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 10 TMS safety questionnaire

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 illness (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 head (outside the mouth); any metallic particles in the eye, implant cardiac pacemaker or any intracardiac lines?
☐ Has frequent or sever 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: …….. Tel: ……..
Email: …… Address: …….

Appendix 11Transcranial Magnetic Stimulation Adult Safety Screen

Please circle your response. Have you ever:

1. Had adverse reaction to Transcranial Magnetic Stimulation (TMS)?
Yes/No
2. Had a seizure or epileptic fit? Yes/No
3. Had an electroencephalogram (EEG)? Yes/No
4. Had a stroke? Yes/No
5. Had a head injury or neurosurgery?
Yes/No
6. Do you have any metal in your head (outside 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 pacemaker, medical pumps, or intracardiac lines? Yes/No
8. Do you suffer from frequent or sever headaches?
Yes/No
9. Have you ever had any other brain-related condition?
Yes/No
10. have you ever had any illness that caused brain injury?
Yes/No
11. Are you taking any medications? Yes/No

Please specify:
12. If you are a woman, are you pregnant or is it possible that you may be pregnant?
Yes/No
13. Does anyone in your family have epilepsy?
Yes/No
14. Do you need further explanation of Transcranial Magnetic Stimulation and its associated risks?

Yes/No

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

……

……

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

Participant's name: ……..

Participant's signature: …….

Date: …….

Appendix 12 Explanatory Statement

Explanatory Statement

Dear Participant
Date:
Title:
The effects of transcranial direct current stimulation (tDCS) on brain excitability and pain: impact of stimulation sites & current amplitude

This information sheet is for you to keep.

Student Research Project

My name is Bita Vaseghi and I am conducting a research project with Dr.Shapour Jaberzadeh a senior lecturer in the Department of Physiotherapy towards a PhD at Monash University. This means that I will be writing an article and thesis afterward which is equivalent of a short book.

Why did you choose this particular person/group as participants?

You have been invited to participate because you have responded to the related advertisement and met the following inclusion criteria:

- You are at least 18 years old.
- You can speak, read and understand English.
- Your responses to our screening questions indicate that you met our inclusion criteria to participate in this study.

The aim/purpose of the research

The primary aim of our study is to investigate how application of anodal and cathodal tDCS on different cortical areas may affect corticospinal excitability, sensory perception and pain thresholds and tolerance in healthy individuals. The secondary aim of the current study is to investigate the optimal parameters for applications of a- and c-tDCS to increase corticospinal excitability, sensory perception and pain threshold and tolerance

Possible benefits

There are no direct benefits for the participants from this study. We hope the study will benefit society, especially patients who suffered from chronic pain, by helping us to establish the best parameters of tDCS to reduce pain.

What does the research involve?

A TMS Safety Screening questionnaire will be completed before taking part in the study.

Baseline assessment involves measurement of 1. Induced Peak-peak amplitude of extensor carpi radialis (ECR) muscle response by a magnetic stimulator 2. Amplitude of sensory responses from the brain induced by electrical stimulation of median nerve at wrist, 3. Measurement of Sensory threshold, Pain threshold and pain by application of rectangular pulses using an electrical stimulator and 4. Measurement of reaction time.

Following baseline measurements, participants will be randomly tested under four conditions 1. anodal tDCS (a-tDCS, application of anode over target area), 2. cathodal tDCS (c-tDCS, application of cathode over target area), 3. sham stimulation, and 4. no stimulation (control group). Interventions will be applied in different days at least 48 hours apart. Then all dependent variables will be measured immediately and every 15 minutes following interventions up to 30 minutes.

Then each participant will be tested immediately before and after one of the following conditions as a baseline measurement. In each session participants will be tested before and after application of tDCS.

tDCS is a safe, noninvasive and painless brain stimulation technique which is currently in use in numerous research laboratory on both healthy individuals and patients. TMS is a safe and painless technique which is widely used in different laboratories for both therapeutic and research purposes. TMS will be applied in sitting position through a magnetic coil which will be held over your head. Muscle responses will be recorded from wrist extensor muscles with surface electrodes.

How much time will the research take?

Based on which session you are randomly allocated, the length of sessions will be about 60 minutes in both first and second phases.

Are there any risks to people in this study?

All of the procedures that will be used in this study have been thoroughly tested in previous studies and are used as standard tests of nervous system function in clinical neurophysiology and neurology. As a matter of precaution, we exclude any persons from our study who have had a seizure or suffer from epilepsy, or a family history of epilepsy. Also, anyone who has had a stroke, metal implants in the skull, or cardiac pacemakers is excluded from these experiments. There may be a risk of seizure if there is a pre-existing congenital condition. Please advise the people conducting the study if any of these medical conditions apply.

Who can’t be in this study?

You are unable to participate in this study if you have had a brain injury or if you have had a seizure or suffer from epilepsy, or a family history of epilepsy. Also, anyone who has had a stroke, metal implants in the skull, or cardiac pacemakers is excluded from this study.

Can I withdraw from the study?

Participation in this study is voluntary. You are free to withdraw consent and to discontinue participation in the research at any time. Furthermore, you have the right to request that all traces of your participation be removed from the project records.

How will I know the results of this study?

If you would like to read a summary document of the study, you can request that you are mailed or emailed a summary of the results, discussion and conclusion of the study. This will be mailed within 3 months of study completion.

What will happen to my information?

You will be assigned a code number and all information you volunteer will be coded with this number so that what you tell us or the information we record will not be linked to your identity. For the measurement sessions you will be asked to state your first or preferred name for the purpose of communication. The forms and recorded information will be de-identified and pooled with the data from other participants. All forms and information sheets will be stored in a locked filing cabinet in a locked office for the duration of the study.

Data stored on computers will be protected by security passwords. The results of this study will be the basis part of a PhD thesis that will, in several years' time, probably be available via the internet. Papers arising from the thesis will be submitted for publication in scientific journals and will also be presented at conferences. No publications arising from this work will enable any participant to be identified.

At the completion of the study, all forms and questionnaires (including consent forms) will be filed in a locked cabinet in a locked office for 5 years, after which time they will be destroyed in a confidential manner: paper by shredding, electronic by deleting from the hard drive and back up files. No one other than the research team will have access to these files at any stage. You may request a copy of personal information collected in the course of the research at any stage of the study up to the point where the link between the code and the identity of individuals is broken. This will occur when all information from the other participants have been entered and is anticipated to occur within 4 weeks of the completion of each measurement session. After this point you will only be able to access pooled and de-identified data.

Storage of data

Storage of the data collected will adhere to the University regulations and kept on Monash University premises in a locked cupboard/filing cabinet for 5 years. A report of the study may be submitted for publication, but individual participants will not be identifiable in such a report.

Any questions regarding this project may be directed to

1. Bita Vaseghi, Physiotherapist, PhD Candidate, Physiotherapy Department, School of Primary Health Care, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne – Peninsula Campus
[redacted]

2. Dr Shapour Jaberzadeh, Senior Lecturer Physiotherapy, School of Primary Health Care, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne - Peninsula Campus
[redacted]

Should you have any complaint concerning the manner in which this research is conducted, please do not hesitate to contact the Monash University Human Research Ethics Committee at the following address:

Executive Officer, Human Research Ethics
Monash University Human Research Ethics Committee (MUHREC)
Building 3e Room 111
Research Office
Monash University VIC 3800
[redacted]

Thank you.

Appendix 13 Consent Form

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. Transcranial Direct Current Stimulation (tDCS)

b. Transcranial Magnetic brain Stimulation (TMS)

c. Recording of muscle activity using surface electrodes

d. Recording Sensory evoke potential via EEG electrodes located over cortical sensory area

e. Electrical stimulation of median nerve at wrist.

I understand that I can withdraw all records of my participation in study up till completion of the 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 (please print):_____

Signature: _____Date:

_____

Researcher's name (please print:

_____

Signature: _____Date:

_____

Appendix 14 Standard Monash University Emergency Procedure

Standard Monash University emergency procedures for first-time seizures

In the most unlikely event that a person experiences a first time seizure, according to Victoria Ambulance guidelines, an ambulance is called and they are taken to hospital. Consistent with this, the standard Monash University emergency procedures will be implemented as follows:

Immediate to the event occurring, the research assistant would call the ambulance while the Building first aid officer administers appropriate first aid as per the Victoria Ambulance First Aid Training Guidelines. This includes removing any danger from the person that is, removing or covering anything from the area that may be harmful to them, place something soft under head and loosen any tight clothing. Once the fitting has ceased, the person will be positioned in the recovery position on the floor with a pillow under their head and a blanket over them, while checking for airway, breathing and circulation. Observing and talking to the subject will continue until the ambulance arrives.

The research assistant will phone the security emergency number (an internal phone ext 333 or 990 44318) to request that an ambulance be called, reporting their name, contact phone number, the event, the exact location of the person who experienced the seizure (Human Neuroscience Unit laboratory, Room B1.21, Building B, Peninsula Campus, McMahons Road, Frankston), and an agreed place to meet at the entrance to the building. Security would call an ambulance, the supervisor would go to the university entrance to escort the ambulance to the site and the mobile emergency officer would attend the site immediately. The research assistant would meet the mobile emergency officer at building B entrance and escort them to the Human Neuroscience Unit laboratory.

The procedure outlined is a standard procedure for a first ever seizure, regardless of where it occurs.

References

Ab Aziz CB, Ahmad AH (2006) The Role of the Thalamus in Modulating Pain The Malaysian Journal of Medical Sciences : MJMS 13:11-18

Ackermann H, Scholz E, Koehler W, Dichgans J (1991) Influence of posture and voluntary background contraction upon compound muscle action potentials from anterior tibial and soleus muscle following transcranial magnetic stimulation Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section 81:71-80

Adrendt-Nielsen L, Oberg B, Bjerring P (1990) Quantitative assessment of extradural bupivacaine analgesia British journal of anaesthesia 65:633-638

Agnew W, McCreery D (1987) Considerations for safety in the use of extracranial stimulation for motor evoked potentials Neurosurgery 20:143

Alagona G, Delvaux V, Gérard P, De Pasqua V, Pennisi G, Delwaide PJ, et al. (2001) Ipsilateral motor responses to focal transcranial magnetic stimulation in healthy subjects and acute-stroke patients Stroke; a journal of cerebral circulation 32:1304-1309

Albert DJ (1966) The effect of spreading depression on the consolidation of learning Neuropsychologia 4:49-64

Almeida TF, Roizenblatt S, Tufik S (2004) Afferent pain pathways: a neuroanatomical review Brain Research 1000:40-56 doi:http://dx.doi.org/10.1016/j.brainres.2003.10.073

Amassian V, Maccabee P, Cracco R, Cracco J, Somasundaram M, Rothwell J, et al. (1994) The polarity of the induced electric field influences magnetic coil inhibition of human visual cortex: implications for the site of excitation Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section 93:21-26

Amassian VE, Eberle L, Maccabee PJ, Cracco RQ (1992) Modelling magnetic coil excitation of human cerebral cortex with a peripheral nerve immersed in a brain-shaped volume conductor: the significance of fiber bending in excitation Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section 85:291-301

Aminoff MJ, Eisen AA (1998) AAEM minimonograph 19: somatosensory evoked potentials Muscle & nerve 21:277-290

Anand S, Hotson J (2002) Transcranial magnetic stimulation: neurophysiological applications and safety Brain and cognition 50:366-386

Anderson B, Mishory A, Nahas Z, Borckardt JJ, Yamanaka K, Rastogi K, et al. (2006) Tolerability and safety of high daily doses of repetitive transcranial magnetic stimulation in healthy young men The journal of ECT 22:49

Andy O (1986) Chronic pain as a reticular formation syndrome Pav. J. Biol. Sci. 21:50-59 doi:10.1007/BF02701123

Anggono V, Huganir RL (2012) Regulation of AMPA receptor trafficking and synaptic plasticity Current opinion in neurobiology 22:461-469 doi:10.1016/j.conb.2011.12.006

Aoyama K, Nakaki T (2013) Neuroprotective properties of the excitatory amino acid carrier 1 (EAAC1) Amino acids 45:133-142 doi:10.1007/s00726-013-1481-5

Apkarian AV, Bushnell MC, Treede RD, Zubieta JK (2005) Human brain mechanisms of pain perception and regulation in health and disease European journal of pain (London, England) 9:463-484 doi:10.1016/j.ejpain.2004.11.001

Araki A, Takada A, Yasuhara A, Kobayashi Y (1999) The effects of stimulus rates on the amplitude of median nerve somatosensory evoked potentials: the developmental change Brain & development 21:118-121

Arana GW (2000) An overview of side effects caused by typical antipsychotics J. Clin. Psychiatry 61:5-11

Arendt-Nielsen L, Graven-Nielsen T (2011) Translational musculoskeletal pain research Best Practice & Research Clinical Rheumatology 25:209-226

Australia CP (2010) National Pain Strategy.

Bachmann CG, Muschinsky S, Nitsche MA, Rolke R, Magerl W, Treede RD, et al. (2010) Transcranial direct current stimulation of the motor cortex induces distinct changes in thermal and mechanical sensory percepts Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 121:2083-2089 doi:10.1016/j.clinph.2010.05.005

Bae EH, Schrader LM, Machii K, Alonso-Alonso M, Riviello Jr JJ, Pascual-Leone A, et al. (2007) Safety and tolerability of repetitive transcranial magnetic stimulation in patients with epilepsy: a review of the literature Epilepsy & Behavior 10:521-528

Banoub M, Tetzlaff JE, Schubert A (2003) Pharmacologic and physiologic influences affecting sensory evoked potentials: implications for perioperative monitoring Anesthesiology 99:716-737

Barker AT, Jalinous R, Freeston IL (1985) Non-invasive magnetic stimulation of human motor cortex The Lancet 325:1106-1107

Basile AS (2002) Direct and indirect enhancement of GABAergic neurotransmission by ammonia: implications for the pathogenesis of hyperammonemic syndromes Neurochemistry international 41:115-122

Basmajian JV, De Luca C (1985) Muscles alive vol Proc. R. Soc. Med. Wiliams and Watkins, Baltikmore, USA,

Bassani S, Folci A, Zapata J, Passafaro M (2013) AMPAR trafficking in synapse maturation and plasticity Cellular and molecular life sciences : CMLS 70:4411-4430 doi:10.1007/s00018-013-1309-1

Batsikadze G, Moliadze V, Paulus W, Kuo MF, Nitsche MA (2013) Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans The Journal of physiology 591:1987-2000 doi:10.1113/jphysiol.2012.249730

Baudewig J, Nitsche MA, Paulus W, Frahm J (2001) Regional modulation of BOLD MRI responses to human sensorimotor activation by transcranial direct current stimulation Magnetic resonance in medicine 45:196-201

Bazemore A, Elliott KA, Florey E (1956) Factor I and gamma-aminobutyric acid Nature 178:1052-1053

Berger JR, Blum AS (2007) Somatosensory Evoked Potentials. In: The Clinical Neurophysiology Primer. Springer, pp 485-497

Bindman LJ, Lippold O, Redfearn J (1964) The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects The Journal of physiology 172:369

Boggio PS, Amancio EJ, Correa CF, Cecilio S, Valasek C, Bajwa Z, et al. (2009a) Transcranial DC stimulation coupled with TENS for the treatment of chronic pain: a preliminary study The Clinical journal of pain 25:691-695 doi:10.1097/AJP.0b013e3181af1414

Boggio PS, Nunes A, Rigonatti SP, Nitsche MA, Pascual-Leone A, Fregni F (2007) Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients Restorative neurology and neuroscience 25:123-129

Boggio PS, Zaghi S, Fregni F (2009b) Modulation of emotions associated with images of human pain using anodal transcranial direct current stimulation (tDCS) Neuropsychologia 47:212-217 doi:10.1016/j.neuropsychologia.2008.07.022

Bormann J (1988) Electrophysiology of GABAA and GABAB receptor subtypes Trends in neurosciences 11:112-116

Brasil-Neto JP, Cohen LG, Panizza M, Nilsson J, Roth BJ, Hallett M (1992) Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity Journal of clinical neurophysiology 9:132-136

Bridgers S (1991) The safety of transcranial magnetic stimulation reconsidered: evidence regarding cognitive and other cerebral effects Electroencephalography and clinical neurophysiology. Supplement 43:170

Bridgets SL, Delaney RC (1989) Transcranial magnetic stimulation An assessment of cognitive and other cerebral effects Neurology 39:417-417

Brodal A (1981) Neurological anatomy in relation to clinical medicine. Oxford University Press, USA,

Bushnell MC, Duncan GH (1989) Sensory and affective aspects of pain perception: is medial thalamus restricted to emotional issues? Experimental brain research 78:415-418

Cash DJ, Subbarao K (1987) Desensitization of gamma-aminobutyric acid receptor from rat brain: two distinguishable receptors on the same membrane Biochemistry 26:7556-7562

Castro-Alamancos MA, Borrell J (1993) Motor activity induced by disinhibition of the primary motor cortex of the rat is blocked by a non-NMDA glutamate receptor antagonist Neuroscience letters 150:183-186

Chapman E (2002) Difficult decisions: social and ethical implications of changing medical technology Community genetics 5:110-119 doi:65166

Chen AC, Zhang WT, Han JS (2004) [Transcranial magnetic stimulation (TMS): physiology, psychology, brain mapping and clinical applications] Sheng li ke xue jin zhan [Progress in physiology] 35:102-106

Chen JL, Carta S, Soldado-Magraner J, Schneider BL, Helmchen F (2013) Behaviour-dependent recruitment of long-range projection neurons in somatosensory cortex Nature 499:336-340 doi:10.1038/nature12236

Chipchase L, Schabrun S, Cohen L, Hodges P, Ridding M, Rothwell J, et al. (2012) A checklist for assessing the methodological quality of studies using transcranial magnetic stimulation to study the motor system: An international consensus study Clinical Neurophysiology 123:1698-1704

Chokroverty S, Hening W, Wright D, Walczak T, Goldberg J, Burger R, et al. (1995) Magnetic brain stimulation: safety studies Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control 97:36-42

Cohen AR, Young W, Ransohoff J (1981) Intraspinal localization of the somatosensory evoked potential Neurosurgery 9:157-162

Cohen LG, Hallett M (1987) Cortical stimulation does not cause short-term changes in the electroencephalogrm Annals of neurology 21:512-513

Cohen LG, Ziemann U, Chen R, Classen J, Hallett M, Gerloff C, et al. (1998) Studies of neuroplasticity with transcranial magnetic stimulation Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society 15:305-324

Cornelissen L, Donado C, Kim J, Chiel L, Zurakowski D, Logan DE, et al. (2014) Pain hypersensitivity in juvenile idiopathic arthritis: a quantitative sensory testing study Pediatric rheumatology online journal 12:39 doi:10.1186/1546-0096-12-39

Cotman CW, Monaghan DT (1988) Excitatory amino acid neurotransmission: NMDA receptors and Hebb-type synaptic plasticity Annu. Rev. Neurosci. 11:61-80

Cracco RQ, Cracco JB (1976) Somatosensory evoked potential in man: far field potentials Electroencephalography and clinical neurophysiology 41:460-466

Craig AD, Bushnell MC, Zhang ET, Blomqvist A (1994) A thalamic nucleus specific for pain and temperature sensation Nature 372:770-773

Creutzfeldt OD, Fromm GH, Kapp H (1962) Influence of transcortical dc currents on cortical neuronal activity Exp. Neurol. 5:436-452

Cruccu G, Aminoff MJ, Curio G, Guerit JM, Kakigi R, Mauguiere F, et al. (2008) Recommendations for the clinical use of somatosensory-evoked potentials Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 119:1705-1719 doi:10.1016/j.clinph.2008.03.016

Curtis DR, Johnston GAR, Game CJA, McCulloch RM (1974) Central action of bicuculline Journal of Neurochemistry 23:605-606 doi:10.1111/j.1471-4159.1974.tb06066.x

Cusick JF, Myklebust JB, Larson SJ, Sances A, Jr. (1979) Spinal cord evaluation by cortical evoked responses Archives of neurology 36:140-143

Daskalakis ZJ, Christensen BK, Fitzgerald PB, Chen R (2002) Transcranial Magnetic StimulationA New Investigational and Treatment Tool in Psychiatry The Journal of neuropsychiatry and clinical neurosciences 14:406-415

Datta A, Elwassif M, Bikson M Bio-heat transfer model of transcranial DC stimulation: comparison of conventional pad versus ring electrode. In, 2009. IEEE, pp 670-673

Davidson RJ, Irwin W (1999) The functional neuroanatomy of emotion and affective style Trends in cognitive sciences 3:11-21

Davis KD, Kwan CL, Crawley AP, Mikulis DJ (1998) Functional MRI study of thalamic and cortical activations evoked by cutaneous heat, cold, and tactile stimuli Journal of neurophysiology 80:1533-1546

de Azeredo LA, Marquardt AR, Frazzon AP, Barros HM (2010) Cocaine reverses the changes in GABAA subunits and in glutamic acid decarboxylase isoenzymes mRNA expression induced by neonatal 6-hydroxydopamine Behavioural pharmacology 21:343-352 doi:10.1097/FBP.0b013e32833b33af

DeFelipe J, Fariñas I (1992) The pyramidal neuron of the cerebral cortex: Morphological and chemical characteristics of the synaptic inputs Progress in Neurobiology 39:563-607 doi:http://dx.doi.org/10.1016/0301-0082(92)90015-7

DeLorey TM, Olsen RW (1992) Gamma-aminobutyric acidA receptor structure and function The Journal of biological chemistry 267:16747-16750

Derbyshire SWG (2014) The Use of Neuroimaging to Advance the Understanding of Chronic Pain: From Description to Mechanism Psychosomatic Medicine 76:402-403 doi:10.1097/psy.0000000000000092

Desmedt JE (1987) [Physiology and physiopathology of somatic sensations studied in man by the method of evoked potentials] Journal de physiologie 82:64-136

Desmet J, Hanssens I, van Cauwelaert F (1987) Comparison of the binding of Na+ and Ca2+ to bovine alpha-lactalbumin Biochimica et biophysica acta 912:211-219

Di Lazzaro V, Oliviero A, Meglio M, Cioni B, Tamburrini G, Tonali P, et al. (2000) Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 111:794-799

Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Meglio M, et al. (2004) Direct recording of the output of the motor cortex produced by transcranial magnetic stimulation in a patient with cerebral cortex atrophy Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 115:112-115

Dinse J, Martin P, Schäfer A, Geyer S, Turner R, Bazin P-L (2013) Quantifying Differences Between Primary Cortical Areas in Humans Based on Laminar Profiles in In-Vivo MRI Data. In: Bildverarbeitung für die Medizin 2013. Springer, pp 39-44

Dobkin BH (2003) The clinical science of neurologic rehabilitation. Oxford University Press, USA,

Donoghue JP (1995) Plasticity of adult sensorimotor representations Current opinion in neurobiology 5:749-754

Dougherty PM, Willis WD (1991) Enhancement of spinothalamic neuron responses to chemical and mechanical stimuli following combined micro-iontophoretic application of N-methyl-D-aspartic acid and substance P Pain 47:85-93

Drew CA, Johnston GA, Weatherby RP (1984) Bicuculline-insensitive GABA receptors: studies on the binding of (-)-baclofen to rat cerebellar membranes Neuroscience letters 52:317-321

Du X, Summerfelt A, Chiappelli J, Holcomb HH, Hong LE (2014) Individualized brain inhibition and excitation profile in response to paired-pulse TMS Journal of motor behavior 46:39-48 doi:10.1080/00222895.2013.850401

Düzel E, Hufnagel A, Helmstaedter C, Elger C (1996) Verbal working memory components can be selectively influenced by transcranial magnetic stimulation in patients with left temporal lobe epilepsy Neuropsychologia 34:775-783

Edelman GM, Mountcastle VB (1978) The mindful brain: Cortical organization and the group-selective theory of higher brain function. Massachusetts Inst of Technology Pr,

Emson PC (2007) GABA(B) receptors: structure and function Progress in brain research 160:43-57 doi:10.1016/s0079-6123(06)60004-6

Fagerlund AJ, Hansen OA, Aslaksen PM (2015) Transcranial direct current stimulation as a treatment for patients with fibromyalgia: a randomized controlled trial Pain 156:62-71 doi:10.1016/j.pain.0000000000000006

Fauth C, Meyer B, Prosiegel M, Zihl J, Conrad B (1992) Seizure induction and magnetic brain stimulation after stroke Lancet 339:362

Ferbert A, Mussmann N, Menne A, Buchner H, Hartje W (1991) Short-term memory performance with magnetic stimulation of the motor cortex European archives of psychiatry and clinical neuroscience 241:135-138

Floel A, Rosser N, Michka O, Knecht S, Breitenstein C (2008) Noninvasive brain stimulation improves language learning Journal of cognitive neuroscience 20:1415-1422 doi:10.1162/jocn.2008.20098

Fregni F, Boggio PS, Lima MC, Ferreira MJL, Wagner T, Rigonatti SP, et al. (2006a) A sham-controlled, phase II trial of transcranial direct current stimulation for the treatment of central pain in traumatic spinal cord injury Pain 122:197-209

Fregni F, Gimenes R, Valle AC, Ferreira MJ, Rocha RR, Natalle L, et al. (2006b) A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia Arthritis and rheumatism 54:3988-3998 doi:10.1002/art.22195

Fregni F, Pascual-Leone A (2007) Technology insight: noninvasive brain stimulation in neurology—perspectives on the therapeutic potential of rTMS and tDCS Nature Clinical Practice Neurology 3:383-393

Freye E (2005) Cerebral monitoring in the operating room and the intensive care unit - an introductory for the clinician and a guide for the novice wanting to open a window to the brain. Part II: Sensory-evoked potentials (SSEP, AEP, VEP) Journal of clinical monitoring and computing 19:77-168 doi:10.1007/s10877-005-0713-y

Fuchigami T, Nakayama M, Yoshida S (2015) Development of PET and SPECT Probes for Glutamate Receptors 2015:716514

Fukuda S (2006) Somatosensory evoked potential] Masui. The Japanese journal of anesthesiology 55:280

Furukawa H, Singh SK, Mancusso R, Gouaux E (2005) Subunit arrangement and function in NMDA receptors Nature 438:185-192 doi:10.1038/nature04089

Gallagher M, Chiba AA (1996) The amygdala and emotion Current opinion in neurobiology 6:221-227

Gandiga PC, Hummel FC, Cohen LG (2006) Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation Clinical Neurophysiology 117:845-850

Garey LJ (1994) Brodmann's localisation in the cerebral cortex London: Smith-Gordon:109-129

Gassmann M, Bettler B (2012) Regulation of neuronal GABAB receptor functions by subunit composition Nature reviews. Neuroscience 13:380-394

George MS, Nahas Z, Borckardt JJ, Anderson B, Foust MJ, Burns C, et al. (2007) Brain stimulation for the treatment of psychiatric disorders Current opinion in psychiatry 20:250-254

Geselowitz DB (1998) The zero of potential IEEE engineering in medicine and biology magazine : the quarterly magazine of the Engineering in Medicine & Biology Society 17:128-132

Geyer S, Ledberg A, Schleicher A, Kinomura S, Schormann T, Bürgel U, et al. (1996) Two different areas within the primary motor cortex of man

Geyer S, Schleicher A, Zilles K (1999) Areas 3a, 3b, and 1 of human primary somatosensory cortex: 1. Microstructural organization and interindividual variability NeuroImage 10:63-83

Ghosh S, Brinkman C, Porter R (1987) A quantitative study of the distribution of neurons projecting to the precentral motor cortex in the monkey (M. fascicularis) The Journal of comparative neurology 259:424-444 doi:10.1002/cne.902590309

Ghosh S, Porter R (1988) Morphology of pyramidal neurones in monkey motor cortex and the synaptic actions of their intracortical axon collaterals The Journal of physiology 400:593-615

Gilmore KL, Meyers JE (1983) Using surface electromyography in physiotherapy research Aust J Physiother 29:3-9

Gilmore R (1989) The use of somatosensory evoked potentials in infants and children Journal of child neurology 4:3-19

Gracely RH, Lota L, Walter DJ, Dubner R (1988) A multiple random staircase method of psychophysical pain assessment Pain 32:55-63

Grundmann L, Rolke R, Nitsche MA, Pavlakovic G, Happe S, Treede RD, et al. (2011) Effects of transcranial direct current stimulation of the primary sensory cortex on somatosensory perception Brain Stimul 4:253-260 doi:10.1016/j.brs.2010.12.002

Gugino LD, Rafael Romero J, Aglio L, Titone D, Ramirez M, Pascual-Leone A, et al. (2001) Transcranial magnetic stimulation coregistered with MRI: a comparison of a guided versus blind stimulation technique and its effect on evoked compound muscle action potentials Clinical Neurophysiology 112:1781-1792

Hagelberg N, Jaaskelainen SK, Martikainen IK, Mansikka H, Forssell H, Scheinin H, et al. (2004) Striatal dopamine D2 receptors in modulation of pain in humans: a review European journal of pharmacology 500:187-192 doi:10.1016/j.ejphar.2004.07.024

Hallett M (2000) Transcranial magnetic stimulation and the human brain Nature 406:147-150

Hallett M, Chokroverty S (2005) Magnetic stimulation in clinical neurophysiology. Elsevier Health Sciences,

Henley JM, Wilkinson KA (2013) AMPA receptor trafficking and the mechanisms underlying synaptic plasticity and cognitive aging Dialogues in clinical neuroscience 15:11-27

Henry JL (1976) Effects of substance P on functionally identified units in cat spinal cord Brain Res 114:439-451

Herrero MT, Barcia C, Navarro JM (2002) Functional anatomy of thalamus and basal ganglia Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery 18:386-404 doi:10.1007/s00381-002-0604-1

Hess C, Mills K, Murray N (1986) Magnetic stimulation of the human brain: facilitation of motor responses by voluntary contraction of ipsilateral and contralateral muscles with additional observations on an amputee Neurosci. Lett. 71:235-240

Hess G, Aizenman CD, Donoghue JP (1996) Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex Journal of neurophysiology 75:1765-1778

Hess G, Jacobs KM, Donoghue JP (1994) N-methyl-D-aspartate receptor mediated component of field potentials evoked in horizontal pathways of rat motor cortex Neuroscience 61:225-235

Hill DR (1985) GABAB receptor modulation of adenylate cyclase activity in rat brain slices British journal of pharmacology 84:249-257

Homanics GE, DeLorey TM, Firestone LL, Quinlan JJ, Handforth A, Harrison NL, et al. (1997) Mice devoid of γ-aminobutyrate type A receptor β3 subunit have epilepsy, cleft palate, and hypersensitive behavior Proceedings of the National Academy of Sciences 94:4143-4148

Honoré T, Lauridsen J, Krogsgaard-Larsen P (1982) The Binding of [3H]AMPA, a Structural Analogue of Glutamic Acid, to Rat Brain Membranes Journal of Neurochemistry 38:173-178 doi:10.1111/j.1471-4159.1982.tb10868.x

Horton JC, Adams DL (2005) The cortical column: a structure without a function Philosophical Transactions of the Royal Society B: Biological Sciences 360:837-862

Hoshi E (2006) Functional specialization within the dorsolateral prefrontal cortex: A review of anatomical and physiological studies of non-human primates Neuroscience Research 54:73-84 doi:http://dx.doi.org/10.1016/j.neures.2005.10.013

Hsieh JC, Belfrage M, Stone-Elander S, Hansson P, Ingvar M (1995) Central representation of chronic ongoing neuropathic pain studied by positron emission tomography Pain 63:225-236

Hsieh JC, Hannerz J, Ingvar M (1996) Right-lateralised central processing for pain of nitroglycerin-induced cluster headache Pain 67:59-68

Hufnagel A, Elger C, Durwen H, Böker D, Entzian W (1990) Activation of the epileptic focus by transcranial magnetic stimulation of the human brain Ann. Neurol. 27:49-60

Hummel F, Cohen LG (2005) Improvement of motor function with noninvasive cortical stimulation in a patient with chronic stroke Neurorehabilitation and neural repair 19:14-19 doi:10.1177/1545968304272698

Huntley GW, Jones EG (1991) Relationship of intrinsic connections to forelimb movement representations in monkey motor cortex: a correlative anatomic and physiological study Journal of neurophysiology 66:390-413

Iannetti GD, Mouraux A (2010) From the neuromatrix to the pain matrix (and back) Experimental brain research 205:1-12 doi:10.1007/s00221-010-2340-1

Iyer M, Mattu U, Grafman J, Lomarev M, Sato S, Wassermann E (2005) Safety and cognitive effect of frontal DC brain polarization in healthy individuals Neurology 64:872-875

Jalinous R, Chris H (2006) The guide to magnetic stimulation The MagStim Company Ltd, Whitland, Wales, UK

Ji RR, Kohno T, Moore KA, Woolf CJ (2003) Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci 26:696-705

Johnston GA (1996) GABAc receptors: relatively simple transmitter -gated ion channels? Trends in pharmacological sciences 17:319-323

Johnston GA, Curtis DR, Beart PM, Game CJ, McCulloch RM, Twitchin B (1975) Cis- and trans-4-aminocrotonic acid as GABA analogues of restricted conformation J Neurochem 24:157-160

Jones AK, Brown WD, Friston KJ, Qi LY, Frackowiak RS (1991) Cortical and subcortical localization of response to pain in man using positron emission tomography Proceedings. Biological sciences / The Royal Society 244:39-44 doi:10.1098/rspb.1991.0048

Jones E (1983a) The nature of the afferent pathways conveying short-latency inputs to primate motor cortex Adv. Neurol. 39:263

Jones EG (1983b) The nature of the afferent pathways conveying short-latency inputs to primate motor cortex Advances in neurology 39:263-285

Kahsai L, Carlsson MA, Winther AM, Nassel DR (2012) Distribution of metabotropic receptors of serotonin, dopamine, GABA, glutamate, and short neuropeptide F in the central complex of Drosophila Neuroscience 208:11-26 doi:10.1016/j.neuroscience.2012.02.007

Kandler R (1990) Safety of transcranial magnetic stimulation Lancet 335:469-470

Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, Mickel SJ, et al. (1997) Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors Nature 386:239-246 doi:10.1038/386239a0

Keenan KG, Farina D, Merletti R, Enoka RM (2006) Influence of motor unit properties on the size of the simulated evoked surface EMG potential Experimental Brain Research 169:37-49

Keller A (1993) Intrinsic synaptic organization of the motor cortex Cerebral Cortex 3:430-441

Kendall FP, McCreary EK, Provance PG (2010) Muscles, testing and function: with posture and pain. Williams & Wilkins Baltimore, MD,

Kiers L, Cros D, Chiappa K, Fang J (1993) Variability of motor potentials evoked by transcranial magnetic stimulation Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section 89:415-423

Kothari M, Svensson P, Nielsen JF, Baad-Hansen L (2014) Influence of position and stimulation parameters on intracortical inhibition and facilitation in human tongue motor cortex Brain Res 1557:83-89 doi:10.1016/j.brainres.2014.02.017

Kubota H, Katsurabayashi S, Moorhouse AJ, Murakami N, Koga H, Akaike N (2003) GABAB receptor transduction mechanisms, and cross-talk between protein kinases A and C, in GABAergic terminals synapsing onto neurons of the rat nucleus basalis of Meynert The Journal of physiology 551:263-276 doi:10.1113/jphysiol.2003.046524

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. (1993) Corticocortical inhibition in human motor cortex The Journal of physiology 471:501-519

Kulkarni B, Bentley DE, Elliott R, Youell P, Watson A, Derbyshire SW, et al. (2005) Attention to pain localization and unpleasantness discriminates the functions of the medial and lateral pain systems The European journal of neuroscience 21:3133-3142 doi:10.1111/j.1460-9568.2005.04098.x

Kuner R (2010) Central mechanisms of pathological pain Nat Med 16:1258-1266

Kwan CL, Crawley AP, Mikulis DJ, Davis KD (2000) An fMRI study of the anterior cingulate cortex and surrounding medial wall activations evoked by noxious cutaneous heat and cold stimuli Pain 85:359-374

Labruna L, Fernández-del-Olmo M, Ivry RB (2011) Comparison of different baseline conditions in evaluating factors that influence motor cortex excitability Brain stimul 4:152-155

Lang N, Nitsche MA, Paulus W, Rothwell JC, Lemon RN (2004) Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability Experimental brain research 156:439-443 doi:10.1007/s00221-003-1800-2

Lang N, Siebner HR, Ward NS, Lee L, Nitsche MA, Paulus W, et al. (2005) How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? Eur. J. Neurosci. 22:495-504

Lascano AM, Brodbeck V, Lalive PH, Chofflon M, Seeck M, Michel CM (2009) Increasing the diagnostic value of evoked potentials in multiple sclerosis by quantitative topographic analysis of multichannel recordings Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society 26:316-325 doi:10.1097/WNP.0b013e3181baac00

Liebetanz D, Nitsche MA, Tergau F, Paulus W (2002) Pharmacological approach to the mechanisms of transcranial DC stimulation induced after effects of human motor cortex excitability Brain : a journal of neurology 125:2238

Lindblom U, Verrillo RT (1979) Sensory functions in chronic neuralgia Journal of Neurology, Neurosurgery, and Psychiatry 42:422-435

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Luedtke K, Rushton A, Wright C, Geiss B, Juergens TP, May A (2012) Transcranial direct current stimulation for the reduction of clinical and experimentally induced pain: a systematic review and meta-analysis Clin J Pain 28:452-461 doi:10.1097/AJP.0b013e31823853e3

MacDonald AW, Cohen JD, Stenger VA, Carter CS (2000) Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control Science (New York, N.Y.) 288:1835-1838

Macdonald RL, Olsen RW (1994) GABAA receptor channels Annual review of neuroscience 17:569-602 doi:10.1146/annurev.ne.17.030194.003033

Magistris M, Rösler K, Truffert A, Myers J (1998) Transcranial stimulation excites virtually all motor neurons supplying the target muscle. A demonstration and a method improving the study of motor evoked potentials Brain : a journal of neurology 121:437-450

Matsunaga K, Nitsche MA, Tsuji S, Rothwell JC (2004) Effect of transcranial DC sensorimotor cortex stimulation on somatosensory evoked potentials in humans Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 115:456-460

May A, Bahra A, Buchel C, Frackowiak RS, Goadsby PJ (1998) Hypothalamic activation in cluster headache attacks Lancet 352:275-278 doi:10.1016/s0140-6736(98)02470-2

Mayberg HS (1997) Limbic-cortical dysregulation: a proposed model of depression The Journal of neuropsychiatry and clinical neurosciences 9:471-481

McCreery DB, Agnew WF, Yuen TGH, Bullara L (1990) Charge density and charge per phase as cofactors in neural injury induced by electrical stimulation Biomedical Engineering, IEEE Transactions on 37:996-1001

Medeiros LF, de Souza IC, Vidor LP, de Souza A, Deitos A, Volz MS, et al. (2012) Neurobiological effects of transcranial direct current stimulation: a review Front Psychiatry 3:110 doi:10.3389/fpsyt.2012.00110

Mendonca ME, Santana MB, Baptista AF, Datta A, Bikson M, Fregni F, et al. (2011) Transcranial DC stimulation in fibromyalgia: optimized cortical target supported by high-resolution computational models The journal of pain : official journal of the American Pain Society 12:610-617 doi:10.1016/j.jpain.2010.12.015

Meyer G (1987) Forms and spatial arrangement of neurons in the primary motor cortex of man The Journal of comparative neurology 262:402-428

Miranda PC, Lomarev M, Hallett M (2006) Modeling the current distribution during transcranial direct current stimulation Clinical Neurophysiology 117:1623-1629

Momiyama T (2002) [Dopamine receptors and calcium channels regulating striatal inhibitory synaptic transmission] Nihon yakurigaku zasshi. Folia pharmacologica Japonica 120:61p-63p

Momiyama T, Koga E (2001) Dopamine D(2)-like receptors selectively block N-type Ca(2+) channels to reduce GABA release onto rat striatal cholinergic interneurones The Journal of physiology 533:479-492

Mori F, Codeca C, Kusayanagi H, Monteleone F, Buttari F, Fiore S, et al. (2010) Effects of anodal transcranial direct current stimulation on chronic neuropathic pain in patients with multiple sclerosis The journal of pain : official journal of the American Pain Society 11:436-442 doi:10.1016/j.jpain.2009.08.011

Mtui E, Gruener G, FitzGerald M (2011) Clinical neuroanatomy and neuroscience. Elsevier Health Sciences,

Muakkassa KF, Strick PL (1979) Frontal lobe inputs to primate motor cortex: evidence for four somatotopically organized 'premotor' areas Brain Res 177:176-182

Murray MM, Brunet D, Michel CM (2008) Topographic ERP analyses: a step-by-step tutorial review Brain topography 20:249-264 doi:10.1007/s10548-008-0054-5

Nagel IE, Schumacher EH, Goebel R, D'Esposito M (2008) Functional MRI investigation of verbal selection mechanisms in lateral prefrontal cortex NeuroImage 43:801-807 doi:10.1016/j.neuroimage.2008.07.017

Nakata H, Sakamoto K, Kakigi R (2014) Meditation reduces pain-related neural activity in the anterior cingulate cortex, insula, secondary somatosensory cortex, and thalamus Frontiers in psychology 5:1489 doi:10.3389/fpsyg.2014.01489

Nathan P, Smith MC, Deacon P (1990) The corticospinal tracts in man Brain : a journal of neurology 113:303-324

Nathan SS, Sinha SR, Gordon B, Lesser RP, Thakor NV (1993) Determination of current density distributions generated by electrical stimulation of the human cerebral cortex Electroencephalography and Clinical Neurophysiology 86:183-192

Naylor JC, Borckardt JJ, Marx CE, Hamer RM, Fredrich S, Reeves ST, et al. (2014) Cathodal and anodal left prefrontal tDCS and the perception of control over pain Clin J Pain 30:693-700 doi:10.1097/ajp.0000000000000025

Neggers SF, Langerak TR, Schutter DJ, Mandl RC, Ramsey NF, Lemmens PJ, et al. (2004) A stereotactic method for image-guided transcranial magnetic stimulation validated with fMRI and motor-evoked potentials NeuroImage 21:1805-1817 doi:10.1016/j.neuroimage.2003.12.006

Ni Z, Gunraj C, Wagle-Shukla A, Udupa K, Mazzella F, Lozano AM, et al. (2011) Direct demonstration of inhibitory interactions between long interval intracortical inhibition and short interval intracortical inhibition The Journal of physiology 589:2955-2962 doi:10.1113/jphysiol.2011.207928

Nicoll RA (2004) My close encounter with GABA(B) receptors Biochemical pharmacology 68:1667-1674 doi:10.1016/j.bcp.2004.07.024

Niedermeyer E, da Silva FL (2005) Electroencephalography: basic principles, clinical applications, and related fields. Lippincott Williams & Wilkins,

Nitsche M, Niehaus L, Hoffmann K, Hengst S, Liebetanz D, Paulus W, et al. (2004) MRI study of human brain exposed to weak direct current stimulation of the frontal cortex Clinical Neurophysiology 115:2419-2423

Nitsche M, Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation The Journal of physiology 527:633

Nitsche MA, Boggio PS, Fregni F, Pascual-Leone A (2009) Treatment of depression with transcranial direct current stimulation (tDCS): a review Exp. Neurol. 219:14-19

Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. (2008) Transcranial direct current stimulation: state of the art 2008 Brain stimul 1:206-223

Nitsche MA, Liebetanz D, Lang N, Antal A, Tergau F, Paulus W (2003) Safety criteria for transcranial direct current stimulation (tDCS) in humans Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 114:2220-2222; author reply 2222-2223

Nitsche MA, Paulus W (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans Neurology 57:1899

Nitsche MA, Paulus W (2011) Transcranial direct current stimulation--update 2011 Restor Neurol Neurosci 29:463-492 doi:10.3233/rnn-2011-0618

Nitsche MA, Schauenburg A, Lang N, Liebetanz D, Exner C, Paulus W, et al. (2003b) Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human J. Cogn. Neurosci. 15:619-626

O'Connell NE, Wand BM, Marston L, Spencer S, Desouza LH (2011) Non-invasive brain stimulation techniques for chronic pain. A report of a Cochrane systematic review and meta-analysis Eur J Phys Rehabil Med 47:309-326

O'Connell NE, Wand BM, Marston L, Spencer S, DeSouza LH (2010) Non-invasive brain stimulation techniques for chronic pain Cochrane database of systematic reviews (Online) 9

Oh SJ (2003) Clinical electromyography: nerve conduction studies. Lippincott Williams & Wilkins,

Oliveri M, Caltagirone C, Filippi MM, Traversa R, Cicinelli P, Pasqualetti P, et al. (2000) Paired transcranial magnetic stimulation protocols reveal a pattern of inhibition and facilitation in the human parietal cortex The Journal of physiology 529 Pt 2:461-468

Paoletti P, Neyton J (2007) NMDA receptor subunits: function and pharmacology Current Opinion in Pharmacology 7:39-47 doi:http://dx.doi.org/10.1016/j.coph.2006.08.011

Pascual-Leone A, Cohen L, Shotland L, Dang N, Pikus A, Wassermann E, et al. (1992) No evidence of hearing loss in humans due to transcranial magnetic stimulation Neurology 42:647-647

Pascual-Leone A, Houser C, Reese K, Shotland L, Grafman J, Sato S, et al. (1993) Safety of rapid-rate transcranial magnetic stimulation in normal volunteers Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section 89:120-130

Pascual-Leone A, Tarazona F, Keenan J, Tormos JM, Hamilton R, Catala MD (1998) Transcranial magnetic stimulation and neuroplasticity Neuropsychologia 37:207-217

Pascual-Leone A, Valls-Solé J, Wassermann EM, Hallett M (1994) Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex Brain : a journal of neurology 117:847-858

Pascual-Leone A, Walsh V, Rothwell J (2000) Transcranial magnetic stimulation in cognitive neuroscience--virtual lesion, chronometry, and functional connectivity Current opinion in neurobiology 10:232-237

Paulus W (2011) Transcranial electrical stimulation (tES–tDCS; tRNS, tACS) methods Neuropsychol Rehabil 21:602-617

Perkinton MS, Sihra TS, Williams RJ (1999) Ca(2+)-permeable AMPA receptors induce phosphorylation of cAMP response element-binding protein through a phosphatidylinositol 3-kinase-dependent stimulation of the mitogen-activated protein kinase signaling cascade in neurons The Journal of neuroscience : the official journal of the Society for Neuroscience 19:5861-5874

Perotto A, Delagi EF (2005) Anatomical guide for the electromyographer: the limbs and trunk. Charles C Thomas Pub Ltd,

Petersen NT, Pyndt HS, Nielsen JB (2003) Investigating human motor control by transcranial magnetic stimulation Experimental brain research 152:1-16 doi:10.1007/s00221-003-1537-y

Petrican R, Schimmack U (2008) The role of dorsolateral prefrontal function in relationship commitment Journal of Research in Personality 42:1130-1135

Petrovic P, Petersson KM, Ghatan PH, Stone-Elander S, Ingvar M (2000) Pain-related cerebral activation is altered by a distracting cognitive task Pain 85:19-30

Piccolino M (1997) Luigi Galvani and animal electricity: two centuries after the foundation of electrophysiology Trends in neurosciences 20:443-448

Poreisz C, Boros K, Antal A, Paulus W (2007) Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients Brain research bulletin 72:208-214

Porter R, Lemon R (1993) Corticospinal function and voluntary movement. vol 45. Oxford University Press, USA,

Price DD (2000) Psychological and neural mechanisms of the affective dimension of pain Science (New York, N.Y.) 288:1769-1772

Priori A (2003) Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability Clinical Neurophysiology 114:589-595

Priori A, Berardelli A, Rona S, Accornero N, Manfredi M (1998) Polarization of the human motor cortex through the scalp Neuroreport 9:2257

Purpura DP, McMurtry JG (1965) Intracellular activities and evoked potential changes during polarization of motor cortex J. Neurophysiol. 28:166

Qian A, Johnson JW (2006) Permeant ion effects on external Mg2+ block of NR1/2D NMDA receptors J Neurosci 26:10899-10910

Rademacher J, Caviness V, Steinmetz H, Galaburda A (1993) Topographical variation of the human primary cortices: implications for neuroimaging, brain mapping, and neurobiology Cerebral Cortex 3:313-329

Riberto M, Marcon Alfieri F, Monteiro de Benedetto Pacheco K, Dini Leite V, Nemoto Kaihami H, Fregni F, et al. (2011) Efficacy of transcranial direct current stimulation coupled with a multidisciplinary rehabilitation program for the treatment of fibromyalgia Open Rheumatol J 5:45-50 doi:10.2174/1874312901105010045

Robertson VJ, Robertson V, Low J, Ward A, Reed A (2006) Electrotherapy explained: principles and practice. Butterworth-Heinemann,

Rosen AC, Ramkumar M, Nguyen T, Hoeft F (2009) Noninvasive transcranial brain stimulation and pain Current pain and headache reports 13:12-17

Rossi S, Hallett M, Rossini PM, Pascual-Leone A (2009a) Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 120:2008-2039 doi:10.1016/j.clinph.2009.08.016

Rossini P, Barker A, Berardelli A, Caramia M, Caruso G, Cracco R, et al. (1994) Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee Electroencephalography and Clinical Neurophysiology 91:79-92

Rossini PM, Rossi S (1998) Clinical applications of motor evoked potentials Electroencephalography and clinical neurophysiology 106:180-194

Rossini PM, Rossi S (2007) Transcranial magnetic stimulation: diagnostic, therapeutic, and research potential Neurology 68:484-488 doi:10.1212/01.wnl.0000250268.13789.b2

Rothwell J (1997) Techniques and mechanisms of action of transcranial stimulation of the human motor cortex J. Neurosci. Methods 74:113-122

Rothwell J, Hallett M, Berardelli A, Eisen A, Rossini P, Paulus W (1998) Magnetic stimulation: motor evoked potentials. The International Federation of Clinical Neurophysiology Electroencephalography and clinical neurophysiology. Supplement 52:97-103

Rouiller EM, Babalian A, Kazennikov O, Moret V, Yu XH, Wiesendanger M (1994) Transcallosal connections of the distal forelimb representations of the primary and supplementary motor cortical areas in macaque monkeys Experimental brain research 102:227-243

Rowny S, Lisanby SH (2008) Brain stimulation in psychiatry Psychiatry, Third Edition:2354-2371

Sanger TD, Garg RR, Chen R (2001) Interactions between two different inhibitory systems in the human motor cortex The Journal of physiology 530:307-317

Sato D, Yamashiro K, Yoshida T, Onishi H, Shimoyama Y, Maruyama A (2013) Effects of water immersion on short- and long-latency afferent inhibition, short-interval intracortical inhibition, and intracortical facilitation Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 124:1846-1852 doi:10.1016/j.clinph.2013.04.008

Schabrun SM, Chipchase LS (2012) Priming the brain to learn: The future of therapy? Manual therapy 17:184-186

Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, et al. (1987) Sequence and functional expression of the GABA A receptor shows a ligand-gated receptor super-family Nature 328:221-227 doi:10.1038/328221a0

Schulz R, Gerloff C, Hummel FC (2013) Non-invasive brain stimulation in neurological diseases Neuropharmacology 64:579-587

Schwartz MS (2003) Biofeedback: A practitioner's guide. the guilford press,

Sessle BJ (2000) Acute and chronic craniofacial pain: brainstem mechanisms of nociceptive transmission and neuroplasticity, and their clinical correlates Critical Reviews in Oral Biology & Medicine 11:57-91

Shagass C, Schwartz M (1965) AGE, PERSONALITY, AND SOMATOSENSORY CEREBRAL EVOKED RESPONSES Science (New York, N.Y.) 148:1359-1361

Shellhaas RA, Chang T, Tsuchida T, Scher MS, Riviello JJ, Abend NS, et al. (2011) The American Clinical Neurophysiology Society's guideline on continuous electroencephalography monitoring in neonates Journal of Clinical Neurophysiology 28:611-617

Sloper JJ, Powell TP (1979) An experimental electron microscopic study of afferent connections to the primate motor and somatic sensory cortices Philosophical transactions of the Royal Society of London. Series B, Biological sciences 285:199-226

Song I, Huganir RL (2002) Regulation of AMPA receptors during synaptic plasticity Trends in neurosciences 25:578-588 doi:http://dx.doi.org/10.1016/S0166-2236(02)02270-1

Sontag W, Kalka D (2007) Repetitive transcranial magnetic stimulation does not influence immunological HL-60 cells and neuronal PC12 cells Int. J. Radiat. Biol. 83:603-615

Staphorsius AS, Kreukels BP, Cohen-Kettenis PT, Veltman DJ, Burke SM, Schagen SE, et al. (2015) Puberty suppression and executive functioning: An fMRI-study in adolescents with gender dysphoria Psychoneuroendocrinology 56:190-199 doi:10.1016/j.psyneuen.2015.03.007

Szabo GG, Lenkey N, Holderith N, Andrasi T, Nusser Z, Hajos N (2014) Presynaptic calcium channel inhibition underlies CB(1) cannabinoid receptor-mediated suppression of GABA release The Journal of neuroscience : the official journal of the Society for Neuroscience 34:7958-7963 doi:10.1523/jneurosci.0247-14.2014

Talbot JD, Marrett S, Evans AC, Meyer E, Bushnell MC, Duncan GH (1991) Multiple representations of pain in human cerebral cortex Science (New York, N.Y.) 251:1355-1358

Taylor C (1999) Medical Image Computing and Computer-assisted Intervention. Springer,

Tokuno H, Tanji J (1993) Input organization of distal and proximal forelimb areas in the monkey primary motor cortex: a retrograde double labeling study The Journal of comparative neurology 333:199-209 doi:10.1002/cne.903330206

Toleikis JR (2005) Intraoperative monitoring using somatosensory evoked potentials. A position statement by the American Society of Neurophysiological Monitoring Journal of clinical monitoring and computing 19:241-258 doi:10.1007/s10877-005-4397-0

Turk DC, Okifuji A (1999) Does sex make a difference in the prescription of treatments and the adaptation to chronic pain by cancer and non-cancer patients? Pain 82:139-148

Udupa K, Ni Z, Gunraj C, Chen R (2010) Effect of long interval interhemispheric inhibition on intracortical inhibitory and facilitatory circuits The Journal of physiology 588:2633-2641 doi:10.1113/jphysiol.2010.189548

Valet M, Sprenger T, Boecker H, Willoch F, Rummeny E, Conrad B, et al. (2004) Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain—an fMRI analysis Pain 109:399-408

van de Wassenberg W, van der Hoeven J, Leenders K, Maurits N (2008) Multichannel recording of median nerve somatosensory evoked potentials Neurophysiologie clinique = Clinical neurophysiology 38:9-21 doi:10.1016/j.neucli.2007.08.002

Vaseghi B, Zoghi M, Jaberzadeh S (2013) Inter-Pulse interval affects the size of single-pulse TMS induced motor evoked potentials: a reliability study Manuscript submitted for publication

Vaseghi B, Zoghi M, Jaberzadeh S (2014) Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology doi:10.1016/j.clinph.2014.01.020

Vicario-Abejon C, Collin C, Tsoulfas P, McKay RD (2000) Hippocampal stem cells differentiate into excitatory and inhibitory neurons The European journal of neuroscience 12:677-688

Viisanen H, Ansah OB, Pertovaara A (2012) The role of the dopamine D2 receptor in descending control of pain induced by motor cortex stimulation in the neuropathic rat Brain research bulletin 89:133-143 doi:10.1016/j.brainresbull.2012.08.002

Viisanen H, Pertovaara A (2010) Antinociception by motor cortex stimulation in the neuropathic rat: does the locus coeruleus play a role? Experimental brain research 201:283-296 doi:10.1007/s00221-009-2038-4

Wagner T, Fregni F, Fecteau S, Grodzinsky A, Zahn M, Pascual-Leone A (2007a) Transcranial direct current stimulation: a computer-based human model study NeuroImage 35:1113-1124

Wagner T, Valero-Cabre A, Pascual-Leone A (2007b) Noninvasive human brain stimulation Annu. Rev. Biomed. Eng. 9:527-565

Wassermann E, Epstein CM, Ziemann U (2008) The Oxford handbook of transcranial stimulation. Oxford University Press, USA,

Wassermann EM (1998) Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996 Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section 108:1-16

Wassermann EM (2002) Variation in the response to transcranial magnetic brain stimulation in the general population Clinical Neurophysiology 113:1165-1171

Wiech K, Ploner M, Tracey I (2008) Neurocognitive aspects of pain perception Trends in cognitive sciences 12:306-313 doi:10.1016/j.tics.2008.05.005

Wienbruch C, Candia V, Svensson J, Kleiser R, Kollias SS (2006) A portable and low-cost fMRI compatible pneumatic system for the investigation of the somatosensensory system in clinical and research environments Neuroscience letters 398:183-188 doi:10.1016/j.neulet.2006.01.025

Wiethoff S, Hamada M, and Rothwell JC. Variability in Response to Transcranial Direct Current Stimulation of the Motor Cortex. Brain Stimulation, 2014. 73: p. 468-475.

Willis WD, Westlund KN (1997) Neuroanatomy of the pain system and of the pathways that modulate pain Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society 14:2-31

Woolf CJ, Salter MW (2000) Neuronal plasticity: increasing the gain in pain Science 288:1765-1769

Woolf CJ, Thompson SW (1991) The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states Pain 44:293-299

Xiao-Ming W, Ju-Ming Y (2011) The Clinical Application of Transcranial Magnetic Stimulation in the Study of Epilepsy MANAGEMENT OF EPILEPSY–RESEARCH, RESULTS AND TREATMENT:35

Xu XJ, Dalsgaard CJ, Wiesenfeld-Hallin Z (1992) Intrathecal CP-96,345 blocks reflex facilitation induced in rats by substance P and C-fiber-conditioning stimulation Eur J Pharmacol 216:337-344

Yamada H, Tamaki T, Wakano K-i, Mikami A, Transfeldt EE (1995) Effect of transcranial magnetic stimulation on cerebral function in a monkey model Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control 97:140-144

Yuen TGH, Agnew WF, Bullara LA, Jacques S, McCreery DB (1981) Histological evaluation of neural damage from electrical stimulation: considerations for the selection of parameters for clinical application Neurosurgery 9:292

Z'graggen W, Humm A, Durisch N, Magistris M, Rösler K (2005) Repetitive spinal motor neuron discharges following single transcranial magnetic stimuli: a quantitative study Clinical Neurophysiology 116:1628-1637

Zhang XH, Jia N, Zhao XY, Tang GK, Guan LX, Wang D, et al. (2013) Involvement of pGluR1, EAAT2 and EAAT3 in offspring depression induced by prenatal stress Neuroscience 250:333-341 doi:10.1016/j.neuroscience.2013.04.031

Ziemann U (2004a) TMS and drugs Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 115:1717-1729 doi:10.1016/j.clinph.2004.03.006

Ziemann U (2004b) TMS and drugs Clinical Neurophysiology 115:1717-1729

Ziemann U, Lönnecker S, Steinhoff B, Paulus W (1996a) Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study Annals of neurology 40:367-378

Ziemann U, Rothwell JC, Ridding MC (1996b) Interaction between intracortical inhibition and facilitation in human motor cortex The Journal of physiology 496 (Pt 3):873-881

Ziemann U, Tergau F, Wischer S, Hildebrandt J, Paulus W (1998) Pharmacological control of facilitatory I-wave interaction in the human motor cortex. A paired transcranial magnetic stimulation study Electroencephalography and clinical neurophysiology 109:321-330

Zumsteg D, Wieser HG (2002) Effects of aging and sex on middle-latency somatosensory evoked potentials: normative data Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 113:681-685