MONASH University

Does single-session anodal tDCS over frontoparietal network affect motor sequence learning?

Submitted in total fulfilment of the requirements of

Doctor of philosophy

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December 2016

Dedicate to

My lovely parents

And

All people who believe in it

“"True success is overcoming the fear of being unsuccessful", Paul Sweeney

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Thesis abstract

Does single-session anodal tDCS of frontoparietal network sites affect motor sequence learning?

Application of anodal transcranial direct current stimulation (a-tDCS) has been extensively used as a novel technique to modulate neuroplasticity induced by motor sequence learning tasks. Although there is a large number of studies providing evidence for efficacy of multiple-sessions a-tDCS over the primary motor cortex (M1) in motor sequence tasks, such as serial reaction time tasks (SRTT) and sequential visual isometric pinch task (SVIPT) (Reis et al., 2009a; Saucedo Marquez et al., 2013; Schambra et al., 2011; Waters-Metenier et al., 2014), the efficacy of single-session M1 a-tDCS remains controversial. Regardless of the role of M1 in acquisition and recall of motor sequences, stimulation of other cortical sites of brain beyond M1 might be more effective for fast stage of sequence learning.

Neuroimaging studies have shown that other functionally connected sites of the frontoparietal network (FPN), including the dorsolateral prefrontal cortex (DLPFC), and the posterior parietal cortex (PPC), have greater involvement than M1 in the fast stage of sequential learning (Koch et al., 2008a; Koch et al., 2008b; Sakai et al., 1998). While, not much is known about utilisation of a-tDCS over DLPFC and PPC at fast stage of motor sequence learning, therefore, the studies introduced in this thesis are motivated by the need to identify the optimal stimulation site for enhancement of motor sequence at fast stage of learning in the trained dominant hand. In addition, we also determined transfer learning into the untrained hand at the initial stage of sequence learning. To explore the underlying mechanisms behind the efficacy of a-tDCS technique, we measured corticospinal excitability (CSE), short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) of M1 using a single and paired-pulse transcranial magnet stimulation (TMS) paradigms. To the best of our

knowledge this is the first study to identify the effects of single-session a-tDCS of the superficial sites of the FPN (DLPFC, M1 and PPC) on cortical and behavioural outcomes in both the trained and untrained hands. Even though the results indicated no significant differences between sham and a-tDCS groups, we found that temporal and spatial processing in SVIPT was differentially affected by a-tDCS groups. The finding suggest that the left PPC is more involved in temporal processing while the DLPFC seems to be associated in spatial processing at initial stage of learning. Transfer of learning into the non-dominant hand was observed in all stimulation groups for outcomes which improved in the dominant hand but not for those showed no improvement in the right trained hand.

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 four original papers published in peer reviewed journals. The last paper has not been submitted for publication yet.

The core theme of the thesis is the effects of a single session of anodal transcranial direct current stimulation of the frontoparietal networks on motor sequence learning and transfer learning into the untrained hand. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the Department of Physiotherapy, Faculty of Medicine, Nursing and Health Sciences under the primary supervision of Dr Shapour Jaberzadeh as well as co-authors Prof Paul B Fitzgerald and Dr Maryam Zoghi. The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research. The studies in this thesis were granted ethics approval from the Monash University Human Ethics Committee CF13/3302 - 2013001720 (Appendix 1).

In case of Chapters 2 to 7 (studies 1-5), my contribution to the work involved the following:

Thesis Chapter	Publication title	Publication status	Nature and extent of candidate's contribution
2	*The effect of anodal transcranial direct current stimulation on motor sequence learning in healthy individuals*: A systematic review and meta-analysis	Published	80% Developed study design, review of appropriate literature, securing ethics approval, recruitment of participants, data collection and analysis, manuscript synthesis and preparation
3	*Reliability of motor evoked potentials induced by transcranial magnetic stimulation: The effects of initial motor evoked potentials removal.*	Published	80%
5	*The effects of inter-trial interval on implicit learning of sequential visual isometric pinch task*	Published	80%
6	*Single-session anodal tDCS with small-size stimulating electrodes over frontoparietal superficial sites does not affect motor sequence learning*	Published	80%

I have presented all manuscripts, either submitted or published, and renumbered sections of submitted or published papers in order to generate a consistent presentation within the main body of the thesis. Published and submitted manuscripts in pdf format are included in the thesis.

Signed: ……...

Date: ……...

Acknowledgments

I am indebted to many people who provided me with support and assistance whilst undertaking this degree.

First and foremost, I want to thank my supervisor Dr Shapour Jaberzadeh for his supervision, advice and guidance. He is so generous with his time and knowledge. I appreciate for every single thing he has done for me over the course of my PhD. I am also thankful my co-supervisors Professor Paul B Fitzgerald and Dr Maryam Zoghi for their valuable feedbacks and kind help throughout the work.

I would also to thank all of my collaborative colleagues for their support and encouragements especially Andisheh Bastani, who was the first PhD student in the non-invasive brain stimulation lab, her attempts made some things easier for future students like me and helped me a lot.

My thanks go to the staff of Physiotherapy Department at Monash University especially Jenny Keating. She is such a brilliant researcher and I have learnt so much from Jenny. Thanks also to Prue Murgan, Alison Francis-Cracknell, Mick Storr, Narelle Dalwood, Robin Hutchinson, Ross Illes, Sophie Paynter, Stephan Maloney, Kim McGregor and Sheila Young. I am grateful to all of them for their willing, support and attention.

I would especially like to thank Sebastain White for his assistance to set up a number of macros in Power Lab and also my dear sister, Masoumeh, for her help to develop some macros in MATLAB software for automation of data analysis. I would also extend my thanks to Dr Allie ford who kindly proofread my thesis and prompted additional insights.

This thesis would not be possible without the help of 101 subjects who participated in the experiments and donated their time for some strange experiments on their brain, many of whom I now call friends they are fantastic and adventure. Special thanks to the Australian Postgraduate Award and Endeavour International Postgraduate Research Scholarship (IPRS) for supporting my research. Without your help, it would not have been possible so thank you for investing this research. All the people that encouraged and helped me in any way to get to this point. I thank you

My thanks go to my lovely sisters Fatemeh, Fatiheyeh, Hamideh and Masoumeh and my dear brothers Ali and Mohammad for their incredible support and encouragements.

My deep gratitude goes to my dear dad, Hosein, for his endless support, encouragement and love. ***To my dear mum***, Sedigheh, who raised me with a love of science and supported me in all my pursuit. It is difficult for me to find a word for saying how I thank you. You are the love of my life and I dedicate this work to you.

Above all, I give thanks to my Lord, Allah, who is always with me. When my dear mum was pass away at the beginning of this journey, nobody could help me to continue this journey except you. Thank you.

Thesis outputs

Published

Fahimeh Hashemirad, Maryam Zoghi, Paul B. Fitzgerald, Shapour Jaberzadeh. *The effect of anodal transcranial direct current stimulation on motor sequence learning in healthy individuals*: A systematic review and meta-analysis, Volume 102, Pages 1-12, February 2016.

Fahimeh Hashemirad, Maryam Zoghi, Paul B. Fitzgerald, Shapour Jaberzadeh. *Reliability of motor evoked potentials induced by transcranial magnetic stimulation: The effects of initial motor evoked potentials removal. Basic and Clinical Neurosciences Journal. April 2016.*

Fahimeh Hashemirad, Paul B. Fitzgerald, Maryam Zoghi, Masoumeh Hashemirad, Shapour Jaberzadeh. *Inter-trial interval and implicit learning of sequential visual isometric pinch task. Journal of Bodywork and Movement therapies. Nov. 2016.*

Fahimeh Hashemirad, Paul B. Fitzgerald, Maryam Zoghi, Shapour Jaberzadeh. *Single-session anodal tDCS with small-size stimulating electrodes over frontoparietal superficial sites does not affect motor sequence learning. Journal of Frontiers Human Neuroscience. April 2017.*

Oral presentation

Fahime Hashemirad, Maryam Zoghi, Paul B. Fitzgerald, Shapour Jaberzadeh. "The effects of anodal transcranial direct current stimulation of movement related functional areas of the brain on motor sequence learning".4th Basic and Clinical Neuroscience Congress, 23-25 Dec 2015, Razi Hall, Tehran, Iran.

Fahime Hashemirad, Maryam Zoghi, Paul B. Fitzgerald, Shapour Jaberzadeh "Non-invasive brain stimulation and motor skill learning" 16th specific spinal congress, 23-24 Dec 2015, USWR, Iran.

Fahimeh Hashemirad Monash Peninsula Postgraduate Conference, Peninsula Campus, Peninsula Campus, 29 October 2015.

Fahimeh Hashemirad 3 Min thesis Faculty of Medicine, Nursing and Health Sciences, Monash University Peninsula Campus 4 Aug 2015.

Fahime Hashemirad 3 Min thesis Faculty of Medicine, Nursing and Health Sciences, Monash University Peninsula Campus 28 July 2014.

Fahime Hashemirad, Maryam Zoghi, Paul B. Fitzgerald, Shapour Jaberzadeh "Implicit motor sequence training enhances motor learning in both training and non-training sides". The 15th Specific Spinal Physical Therapy, 24 & 25 December 2014, Iran.

Fahimeh Hashemirad Maryam Zoghi, Paul B. Fitzgerald, Shapour Jaberzadeh "The Effect of tDCS on motor skill learning -Seminar presentation". SPHC Research and Education Festival, Peninsula Campus, Monash University Australia, 3 December 2013.

Fahimeh Hashemirad, Maryam Zoghi, Paul B. Fitzgerald, Shapour Jaberzadeh "The effects of non-invasive brain stimulation on motor skill learning: A systematic review and meta-analysis". 23 Jun HDR festival

Poster presentation

Fahime Hashemirad, Maryam Zoghi, Paul B. Fitzgerald, Masoumeh Hasemirad, Shapour Jaberzadeh. "Does a single session of a-tDCS of fronto-parietal network sites affect motor sequence learning and transfer learning". Second Australasian Brain Stimulation Meeting, 28-29 July 2016 Melbourne.

Fahime Hashemirad, Maryam Zoghi, Paul B. Fitzgerald, Shapour Jaberzadeh "The effects of inter-trial interval on implicit motor sequence learning". 6th Annual Victorian student Research Symposium. Royal Melbourne Hospital Parkville, Victoria. Poster presentation, 15 May 2015.

Fahime Hashemirad, Maryam Zoghi, Paul B. Fitzgerald, Shapour Jaberzadeh "The effects of non-invasive brain stimulation on motor skill learning: A systematic review and meta-analysis". ASMR Symposium, 30 May 2014.

Fahimeh Hashemirad, Maryam Zoghi, Paul B. Fitzgerald, Shapour Jaberzadeh "The priming effect of transcranial direct current stimulation on spinal stabilization training: A methodological consideration"- The 15th Specific Spinal Physical Therapy, Iran, 2013.

Abbreviations

Abbreviations	Definition
ANOVA	analysis of variance
a-tDCS	Anodal transcranial direct current stimulation
c-tDCS	Cathodal transcranial direct current stimulation
DLPFC	Dorsolateral prefrontal cortex
EBM reviews	Evidence based medicine reviews
EMG	Electromyography
FDI	First dorsal interosseous muscle
fMRI	Functional magnetic resolution imaging
FPN	frontoparietal network
ICC	Intra-Class Correlation
ICF	Intracortical facilitation
SICI	Short-interval intracortical inhibition
M1	Primary motor area
MEPs	Motor evoked potentials
MUHREC	Monash university human research ethics committee
MVC	Maximum voluntary contraction
NMDA	N-methyl-D-Aspertic acid
GABA	Gamma Aminobutyric Acid
PET	Positron Emission Tomography
PPT	Posterior parietal cortex
RCTs	Randomized control studies
RMT	Resting motor threshold
rTMS	Repeated transcranial magnetic stimulation
SD	Standard deviation
SE	Standard error
SMC	Supplementary motor cortex
SMD	Standard mean difference
SQTAP	Sequential finger tapping task
SRTT	Serial reaction time task
SPSS	Statistical Package For Social Sciences
SVIPT	Sequential visual isometric pinch task
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation

Thesis outline

This thesis contains eight chapters (Figure 1-1), which present the results of a body of work over the period of my PhD candidacy:

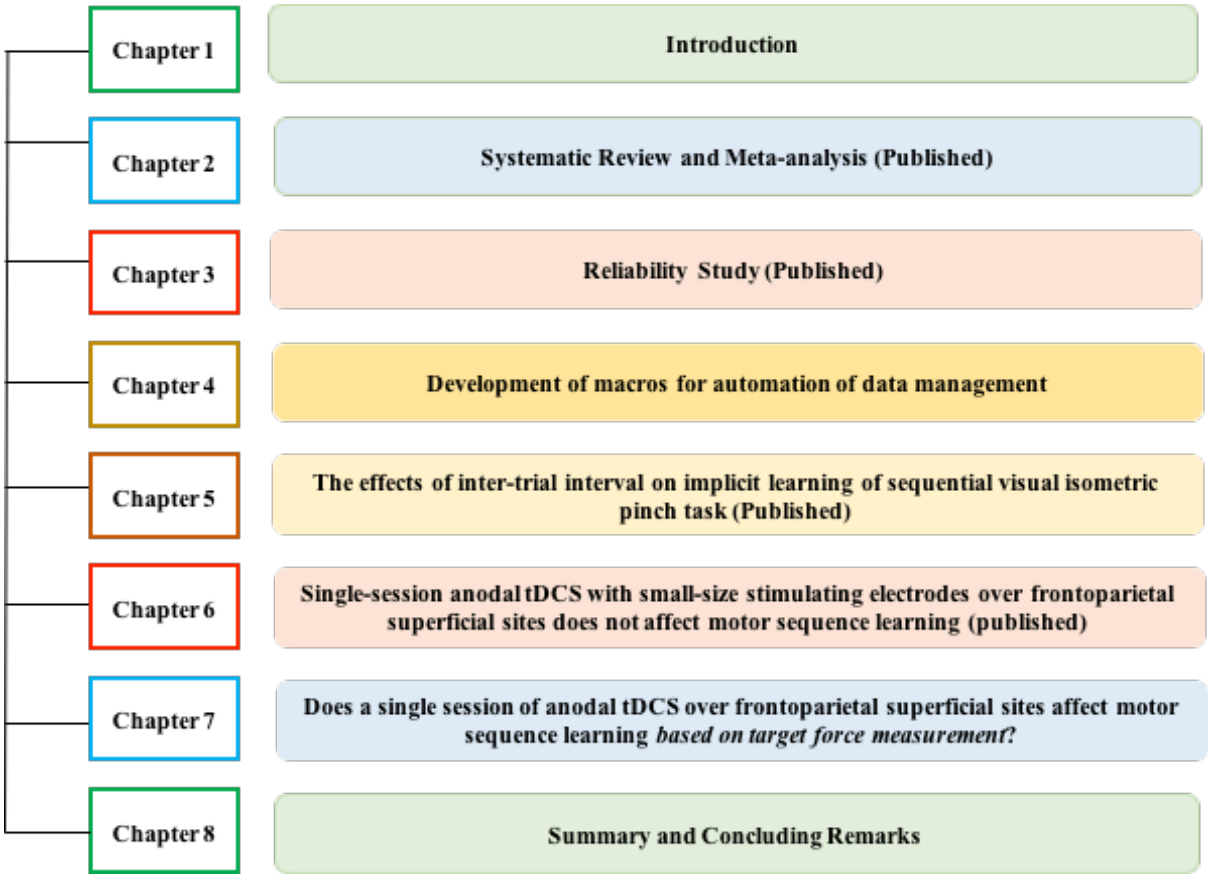

Figure 1-1: Thesis structure

Chapter 1. General Introduction

Motor sequence learning

Motor sequence learning is defined as the capacity to acquire sequential movement patterns, which has a crucial role in everyday life (Katschnig-Winter et al., 2014). This inherent ability in humans is responsible for myriad skills, from simple tasks such as pressing a key to compound tasks such as playing a piano. Anodal transcranial direct current stimulation (a-tDCS) is one of the experimental approaches which have been suggested to enhance motor sequence learning. Although literature demonstrated that repeated sessions of motor training concurrent with a-tDCS of M1, facilitates learning over multiple days through an enhancement of consolidation during motor sequence tasks (Reis et al., 2009b; Saucedo Marquez et al., 2013; Schambra et al., 2011), application of a single session of a-tDCS does not lead to a significant improvement for complex tasks (Boggio et al., 2006 ; Buttkus et al., 2011).

The two types of sequence learning tasks which have been extensively used in tDCS studies are serial reaction time tasks (SRTT) and sequential visual isometric pinch tasks (SVIPT). In SRTT, visual cues or stimuli in the form of numbers or shapes are represented in a repeated sequence order at any of four locations horizontally from left to right on a computer screen, and participants respond by pressing a corresponding key on a response pad (Robertson, 2007) (Figure 1-2).

Figure 1-2: Serial reaction time tasks (SRTT). A series of numbers from 1 to 4. are presented horizontally on the computer screen. Participants are required to press a number on keyboard with the corresponding finger as quickly and accurately as possible.

In contrast, during SVIPT, participants are presented with a number of target forces as visual cues and they are instructed to control their force on a transducer to move a cursor on a computer screen in order to meet different target forces; these forces appear in a sequenced order (Reis et al., 2009a; Saucedo Marquez et al., 2013; Schambra et al., 2011) (Figure 1-3).

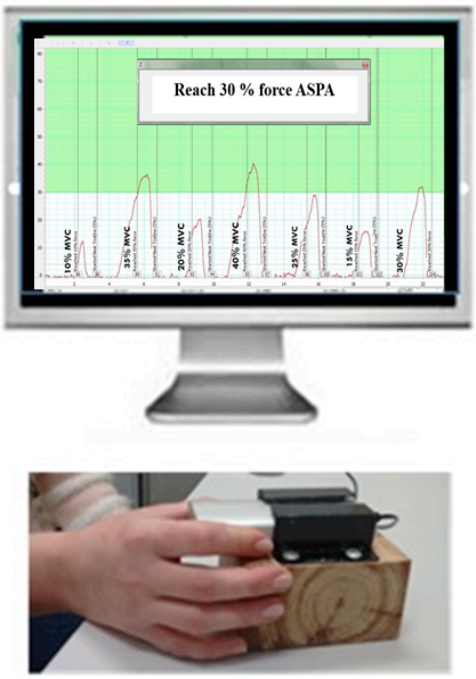

Figure 1-3: Sequential visual isometric pinch task (SVIPT). A series of target forces are presented in a sequence order on the computer screen. Participants are instructed to squeeze the force transducer to hit each target as quickly and accurately as possible.

Repeating sequenced movements, in both tasks, can result in improvement in performance, so participants respond more quickly and accurately after training.

According to awareness of participants of sequence movements, two main memory and learning systems operate in the brain: one system is the declarative or explicit and another one is the non-declarative or implicit system. Therefore, sequence learning can be categorized into explicit and implicit types of learning.

Implicit and explicit learning

Explicit sequence learning happens with awareness of sequenced movements (Robertson, 2007). In contrast, implicit motor sequence learning occurs when the learner is not aware of the order of sequenced stimuli (Daskalakis et al., 2002; Robertson, 2007).

There are several differences in the neural substrates that implement implicit and explicit learning. Implicit sequence learning is mainly related to activity in the contralateral M1 brain region (Kantak et al., 2012; Pascual-Leone et al., 1994). In contrast, with establishment of the explicit knowledge and conscious recall of the sequence, the premotor cortex, DLPFC and supplementary motor area (SMA) are mainly activated in the brain (Honda et al., 1998; Vidoni & Boyd, 2007).

For both types of learning, participants are required to learn how to produce accurate responses to sequentially ordered stimuli. Behavioural gains in explicit sequence learning are measured based on explicit knowledge or conscious recall, obtained after training, compared to the baseline; whereas implicit sequence learning is measured under conditions of unawareness that participants are not able to explicitly recall the order of stimuli. In this situation, implicit sequence learning is either measured as the differences between behavioural outcomes in sequence blocks before and after training (Curran & Keele, 1993; Willingham et al., 1989), or the differences between sequence and random blocks. In random blocks, the visual cues are presented in a random order (Bahrick et al., 1954; Grafton et al., 2002; Nissen & Bullemer, 1987). Based on the time course of learning, implicit or explicit sequence learning can be also categorized into fast and slow stages of learning.

Stages of motor sequence learning

As shown in Figure 1-4, improvement in performance can occur within-session (or 'online') in a single training session, which is called fast stage learning. These improvements can also occur 'offline' or between training sessions, when training has ended. This positive offline effect shows motor memory consolidation and skill stabilization (Doyon & Benali, 2005; Korman et al., 2005; Muellbacher et al., 2002; Robertson et al., 2004; Walker et al., 2002). After the completion of training, online and offline effects can be retained over hours (short-term retention), or even weeks to months (long-term retention)(Savion-Lemieux & Penhune, 2005). Therefore, gains in performance continue after practice has ended in retention phase (Dayan & Cohen, 2011).

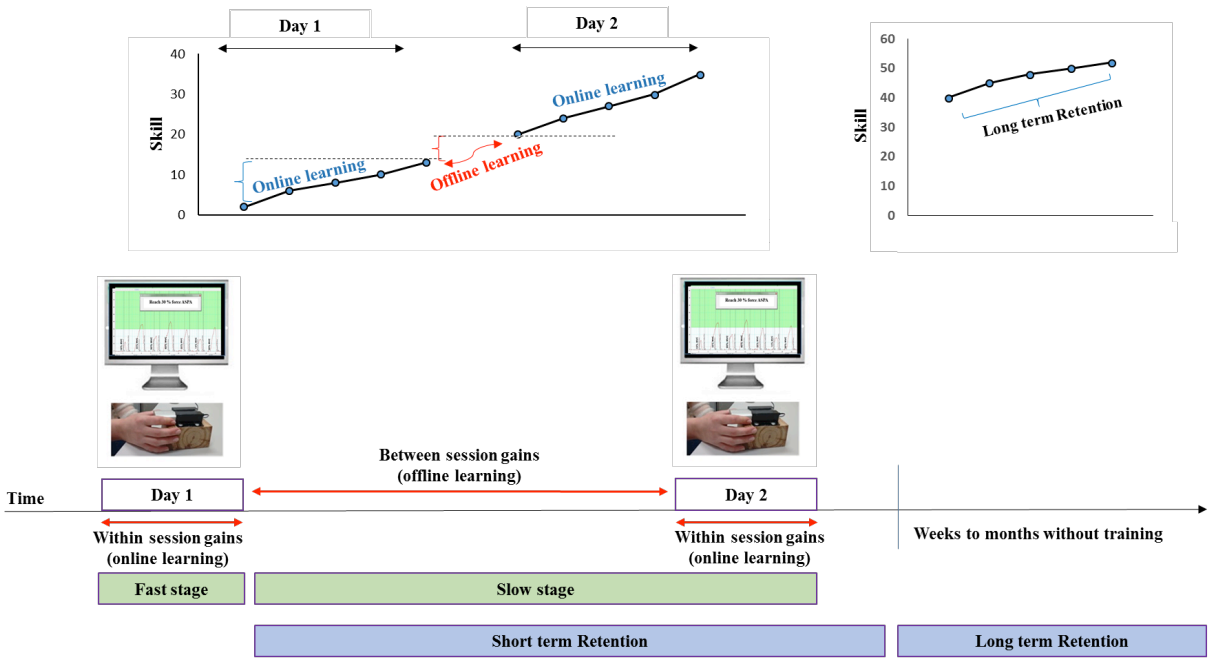

Figure 1-4: The different stages of motor sequence learning. Substantial enhancements can be seen within a single training session, which is considered 'fast stage'. Further gains can be achieved across multiple sessions of practice, which is called 'slow stage learning'. Therefore, performance improvements can happen not only during practice (online learning), but also between sessions, with no additional training (offline learning). Skill can be retained after training has ended over hours (short-term retention) or even weeks and months (long-term retention) (Dayan & Cohen, 2011).

The achievement of sequence learning in different stages of motor learning requires changes in various areas of the brain which collectively is called neuroplasticity.

Mechanism of neuroplasticity

Changes in neuronal activity and excitability in different areas of the brain following sequence learning known as neuroplasticity. This neuroplasticity involves a range of different processes including strengthening existing synapses, growing new synapses, and consolidation of the most efficient synapses, which drive successful performance of a task (Platz et al., 2012).

In both animals and humans, two recognized processes that have been studied extensively across various species are long-term potentiation (LTP) and long-term depression (LTD) (Bliss & Lømo, 1973; Ito, 1989). LTP is a long-lasting improvement in synaptic efficiency that follows certain kinds of electrical stimulation, and it was first recognized by Bliss and Lomo in 1973. Unlike LTP, LTD involves a prolonged inhibition of synaptic transmission and was introduced by Ito and his colleagues in 1989. The evidence supports the idea that learning is related to modification of LTP and LTD.

Motor learning induces increases in neurotransmitter release, the density of post synaptic receptors, and the number of presynaptic invaginations. During training, when a single synapse is repeatedly stimulated, calcium entry through postsynaptic N-methyl-D-aspartate (NMDA) receptors can initiate LTP (Figure 1-5A). Indeed, LTP requires activation of postsynaptic NMDA receptors by synaptically released glutamate. Glutamate is the main excitatory neurotransmitter located in the brain (Aoyama & Nakaki, 2013; Castro-Alamancos & Borrell, 1993). NMDA, kainate, and a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are known as ionotropic glutamate receptors (Furukawa et al., 2005).

AMPA and kainate receptors respond to glutamates by opening Na+ channels and initiating an action potential in postsynaptic neurons (Perkinton & Sihra, 1999). NMDA receptors have an internal voltage-dependent site to bind with Mg^{2+} ions to block the receptor. Binding the glutamate and NMDA removes the Mg^{2+} and open the NMDA receptors which leads to increase the permeability of the membrane to Ca^{2+} (Paoletti & Neyton, 2007; Song & Huganir, 2002). The flow of Ca^{2+} results in the induction of more action potentials and activation of AMPA receptors, which results in modifying the strength of the synaptic connection (Figure 1-5B).

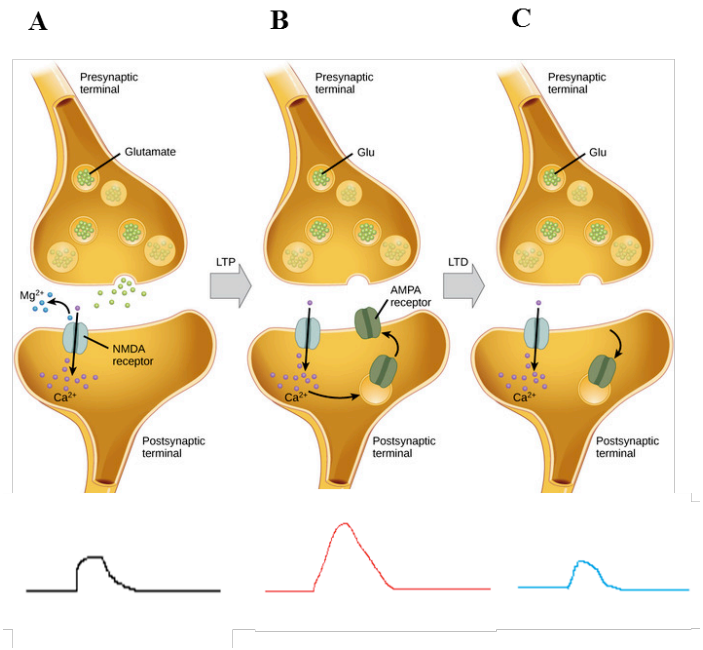

Figure 1-5. Long-term potentiation (LTP) and long-term depression (LTD). The NMDA receptor is activated by glutamate bindings (A). After depolarization, Mg^{+2} are removed for NMDA receptors and Ca^{+2} can enter the cell. In response to an increase in intracellular Ca^{+2}, some AMPA receptors are present in the membrane in LTP phase (B). The AMPA receptor is removed from the membrane and the nerve cell becomes less responsive to glutamate in the LTD phase (C). Adapted from https://www.boundless.com/biology/textbooks/boundless-biology-textbook/the-nervous-system-35/how-neurons-communicate-200/synaptic-plasticity-765-11998/

In contrast to LTP, LTD occurs when a low firing rate of a presynaptic neuron leads to lower transmission efficacy. Indeed, an insufficient number of glutamate molecules binding to NMDA receptors makes the postsynaptic neuron less responsive to glutamate released from the presynaptic neuron (Figure 1-5C).

In addition to glutamate receptors, changes in gamma-aminobutyric acid (GABA) receptors (Castro-Alamancos & Connors, 1996; Castro-Alamancos et al., 1995) have been reported during learning. Bazemore described the function of GABA for the first time (Bazemore et al., 1956). There are three types of GABA receptors termed $GABA_A$, $GABA_B$, and $GABA_C$ (Kahsai et al., 2012). The activation of these receptors increases the permeability of chloride and bicarbonate (HCO^{3-}) ions (Momiyama & Koga, 2001) at post synaptic neurons and result in neuronal inhibition (Figure 1-6).

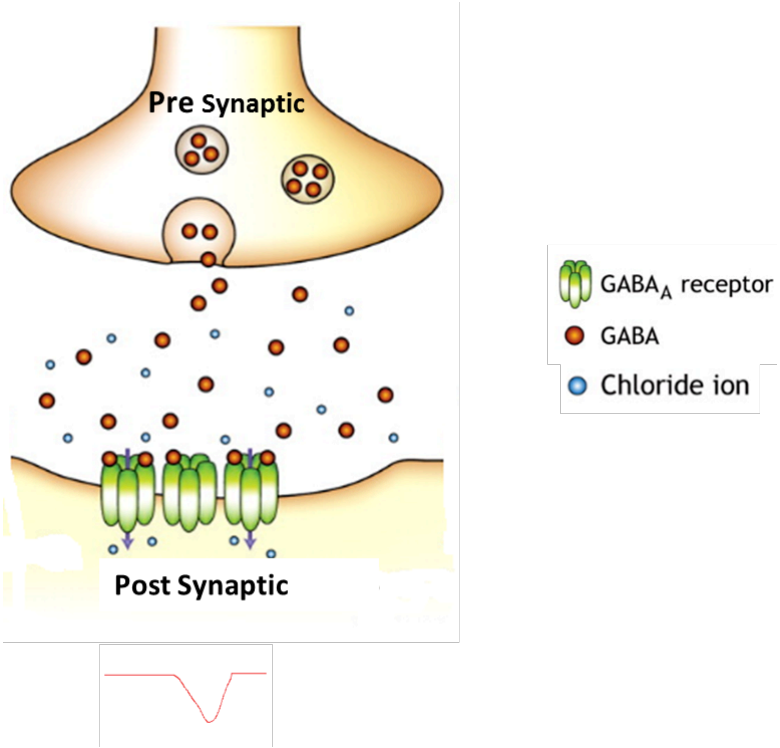

Figure 1-6: Gamma-aminobutyric acid (GABA) receptors. Adapted from http://www.cmaj.ca/content/178/2/185/F1.expansion.html

Reduction in GABA inhibition, or increase in the number of glutamate receptors, facilitate LTP-like activity in the brain (Anggono & Huganir, 2012; Castro-Alamancos & Connors, 1996; Castro-Alamancos et al., 1995; Henley & Wilkinson, 2013; Song & Huganir, 2002). Therefore, plasticity of the human cortex is modulated by changes in these receptors. Pharmacological studies support the essential role of GABA and glutamate during the process of motor leaning (Bütefisch et al., 2000; Pleger et al., 2003). $GABA_A$ receptor agonists, such as lorazepam diminishes motor learning (Blin et al., 2001), whereas agents such as amphetamines facilitate excitatory receptors and improve motor learning (Bütefisch et al., 2002).

Neuroplasticity has been reported in different cortical and subcortical areas of the brain. The following section describes various cortical and subcortical areas of the brain, which are responsible for motor sequence learning.

Neuroplasticity in cortical and subcortical areas

Neural plasticity has been reported in various cortical and subcortical areas of the brain (Figure 1-7A & B). Two distinct cortical-subcortical circuits are activated in the acquisition and the retrieval of learned sequences of movements; theses circuits include cortico-basal ganglia-thalamo-cortical loop and cortico-cerebello-thalamo-cortical loop (Figure 1-7C) (Doyon et al., 2003).

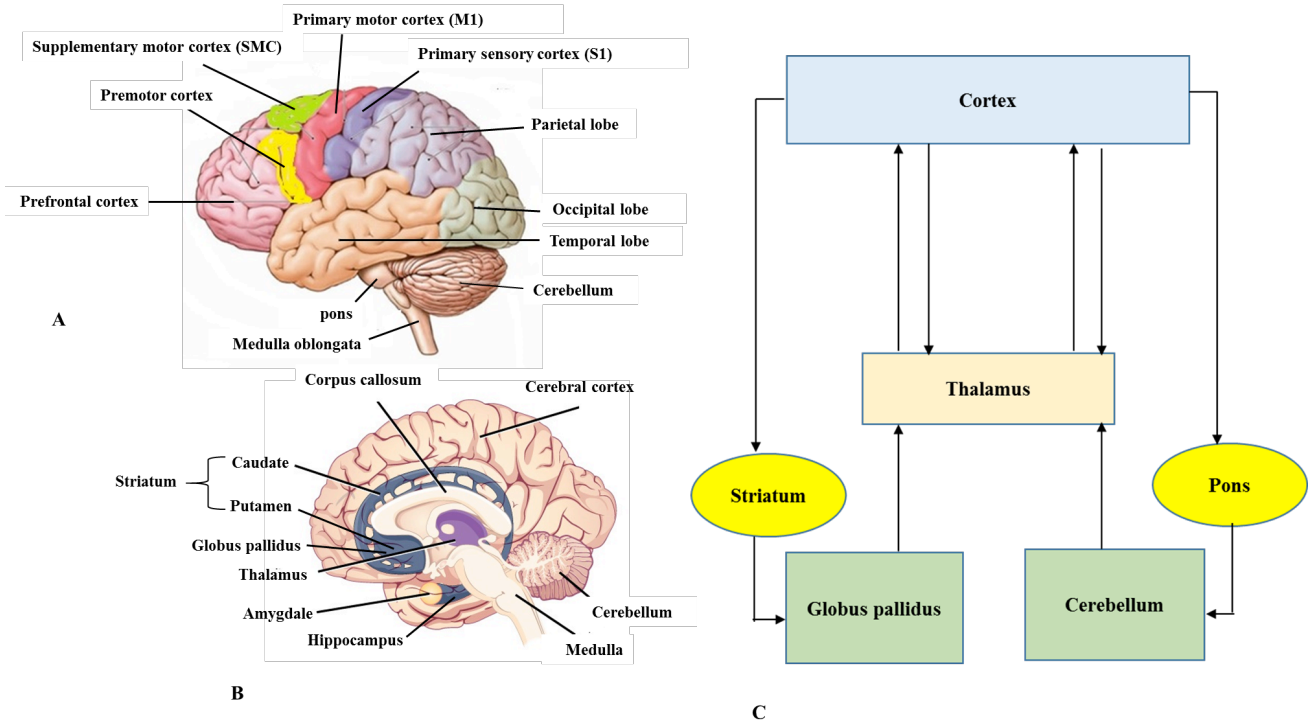

Figure 1-7: Cortical (A) and subcortical (B) areas of the brain as well as two principal circuits: a cortico-striato-thalamo-cortical loop and a cortico-cerebello-thalamo-cortical loop (C) (Doyon et al., 2003). Adapted from http://www2.highlands.edu/academics/divisions/scipe/biology/faculty/harnden/2121/notes/cns.htm. Subcortical areas of the brain adapted (A), https://cnx.org/resources/8a02925f230a5cb626a4b939d5fb287eec4d4100/Figure_35_03_02b.jpg (B).

Neuroplasticity in subcortical areas of the brain

Basal ganglion

The basal ganglion is located deep in each hemisphere and contains four subcortical nuclei: 1) striatum (caudate and putamen nuclei), 2) globus pallidus, 3) sub thalamic nucleus, 4) substantial nigra. (Figure 1-8A & B). Changes of activity have been detected in the basal ganglia at different phases of the acquisition of motor sequence skills (Harvey et al., 2005; Lehericy et al., 2005). The striatum is the major part of the basal ganglia, which manages the input information of this circuit. Therefore, it has a crucial role in learning and cognitive processes, as well as establishing alterations in neuronal activity when a specific behavioral task is being learned.

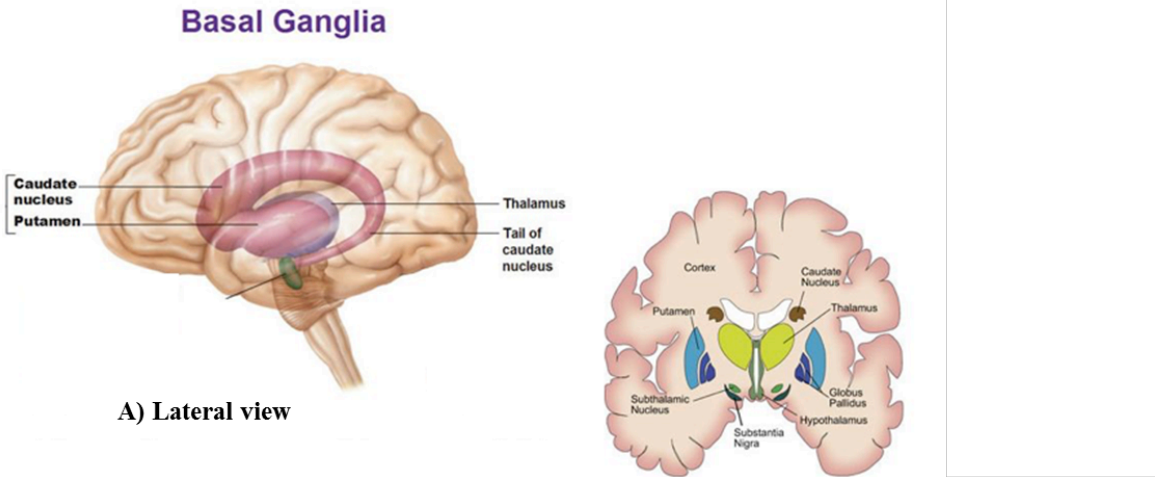

Figure 1-8: lateral (A) and frontal (B) view of basal ganglia. Adapted from http://antranik.org/cerebral-white-matter-and-gray-matter-and-basal-ganglia/ (A). http://kin450-neurophysiology.wikispaces.com/basal+Ganglia+11 (B).

Cerebellum

The cerebellum is usually considered to adjust movements indirectly by regulating the output information received from the motor system (Brooks & Watts, 1988; Gilman, 1985; Glickstein & Yeo, 1990; Nowak et al., 2007; Thach, 1996). The cerebellum is responsible for correction of movements, when they differ from the intended movements. Therefore, this area modifies central motor commands in order to perform subsequent movements with less prediction errors. Consequently, the cerebellum is more involved in adaptation tasks rather than sequence tasks. In adaptation tasks, participants learn how to return to a former level of performance when environmental changes occur, such as driving a new car, while motor sequence learning is defined as an advanced or higher level of performance, which is learned after practice.

Involvement of the frontoparietal network (FPN) in sequence learning

As mentioned, several cortical regions have been assumed to be essential for the achievement or maintenance of motor sequence behaviours. There is a connection among cortical areas of the brain through a network referred as to the frontoparietal network (FPN) (He et al., 2007; Zanto & Gazzaley, 2013).

The FPN includes different cortical areas, such as the prefrontal cortex (PFC) (Grafton et al., 1995; Hazeltine et al., 1997; I. H. Jenkins et al., 1994; Sakai et al., 1998; Sakai et al., 2002), the premotor cortex (Grafton et al., 1995; Hazeltine et al., 1997; I. H. Jenkins et al., 1994; Jueptner et al., 1997), M1 (Grafton et al., 1995; Hazeltine et al., 1997; Karni et al., 1995), supplementary motor area (SMA) (Feurra et al., 2011; Filmer et al., 2014; I. H. Jenkins et al., 1994) and the parietal cortex (I. H. Jenkins et al., 1994; Kuo et al., 2008; Sakai et al., 1998).

Here, we briefly explain the roles of different areas of the FPN in the process of motor sequence learning.

Prefrontal cortex (PFC)

The prefrontal cortex (PFC) is a frontal area of the brain, which is involved in extremely varied processes, ranging from cognition, emotion, motivation and complex motor activity to social interactions (Briand et al., 2007; Courtin et al., 2013; Diamond, 2011; Goto et al., 2010; Ray & Zald, 2012). The PFC is typically subdivided into four regions: medial PFC (MPFC), anterior PFC (APFC), ventrolateral PFC (VLPFC) and dorsolateral PFC (DLPFC) (Figure 1-9A& B).

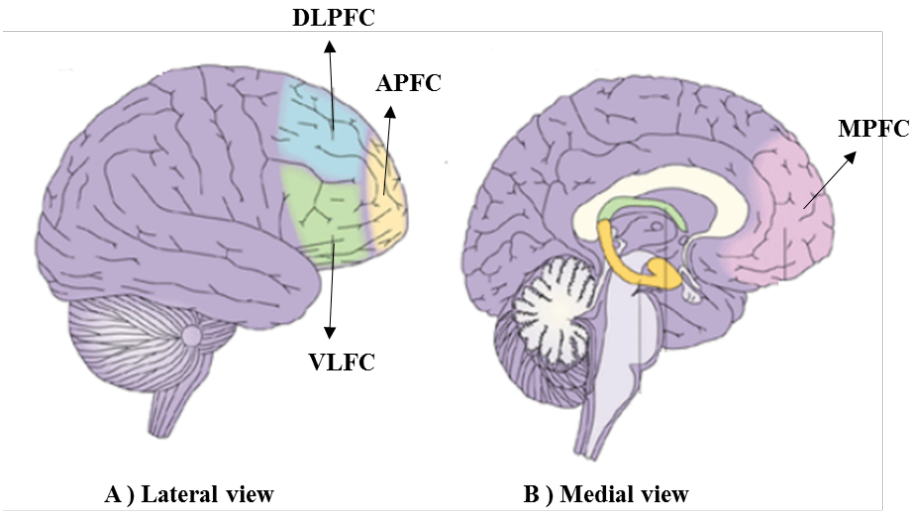

Figure 1-9: Lateral (A) and medial (B) view of prefrontal cortex (PFC). Medial PFC (MPFC), Anterior PFC (APFC) Ventrolateral PFC (VLPFC) and Dorsolateral PFC (DLPFC). Adapted from http://www.nature.com/nrn/journal/v4/n8/fig_tab/nrn1178_F1.html.

MPFC is involved in the regulation of an extensive range of emotional behaviours, such as fear (Milad & Quirk, 2002; Milad et al., 2007). Damage to this area has been associated with psychiatric situations such as post-traumatic stress disorders (Pitman et al., 2012; Shin & Liberzon, 2010). APFC is responsible for problem-solving and planning, which is mainly well developed in humans compared with other primates (Koechlin et al., 1999; Ramnani & Owen, 2004), and damage to this region results in particular difficulty with planning and problem solving (Shallice, 1982). VLPFC has a crucial role in social-threat processing (Fuster, 1988; He et al., 2007) and disturbances in this area are associated with increased occurrence of social anxiety (Adolphs, 2003; Guyer et al., 2008). DLPFC is more involved in motor activity compared to other areas of the PFC, is and also responsible for the executive control of information processing, behavioural expression, working memory, inhibition of irrelevant stimuli to produce the best response to stimuli, as well as attention to stimuli (Brazovskaya et al., 1972). This area is functionally connected to motor areas of the cortex, such as M1, and subcortical areas of the brain, such as the dorsal caudate nucleus of the basal ganglia, thalamus, and hippocampus (Brazovskaya et al., 1972; Morrell, 1961).

Primary motor area (M1)

The primary motor cortex, M1, is the area of the brain which is responsible for motor skill acquisition and consolidation (Muellbacher et al., 2002) (Figure 1-10A). The organization of the Ml area in the cortex involves a distorted map of the body (Homunculus), in which larger parts are devoted to regions characterized by fine movements such as hands, while smaller parts are related to the body regions characterized by gross movements, such as legs (Geyer et al., 1996) (Figure 1-10B). The process of gaining a motor skill through the continued learning of compound movements is linked with the neural plasticity of the M1 area. This area is critically involved in memory formation, motor execution and consolidation of motor skills in both humans and animals (Chan & Nicholson, 1986; Muellbacher et al., 2002; Rioult-Pedotti et al., 2000). Rapid cortical plasticity, including decreased inhibition or increased excitability, occurs following learning in this area. Indeed, the activity of the M1 region can be modulated by both facilitative and inhibitory approaches during the process of sequential learning (Kim et al., 2004).

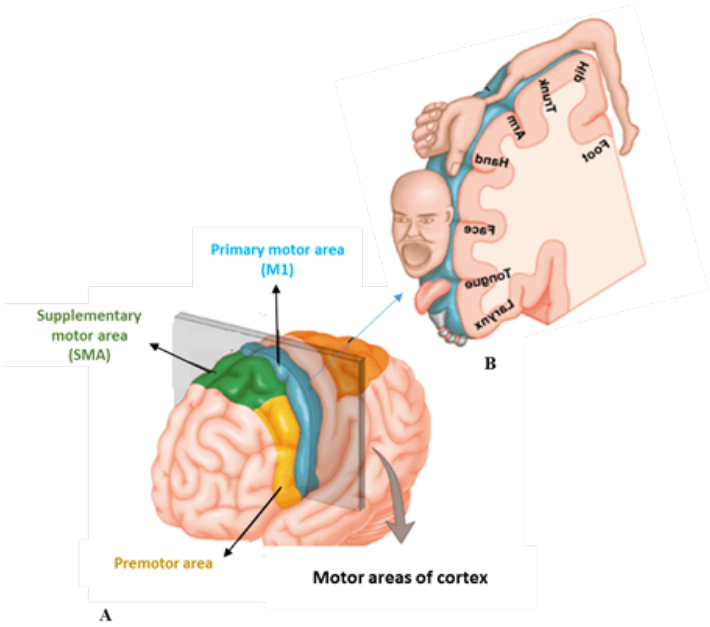

Figure 1-10: Motor areas of cortex (A) and the homunculus of the primary motor cortex (Ml) (B). Adapted from http://brainconnection.brainhq.com/2013/03/05/the-anatomy-of-movement.

Supplementary motor area (SMA)

The SMA, as the second main motor area in the brain (Fulton, 1935) (Figure 1-10A), is engaged in organizing sequential motor movements (Boecker et al., 1994; I. Jenkins et al., 1994; Matelli et al., 1993; Sakai et al., 1999). This part of the brain is divided into two distinct subcomponents: rostral (anterior) and caudal areas (Boecker et al., 1998). These two areas are differentially activated dependent on the complexity and phase of sequence learning. The rostral part of SMA (Pre-SMA) is mostly involved in complex motor planning and control of movement, whereas the caudal SMA, which is closely linked to the M1 region, is involved in movement execution. Therefore, this part is mainly activated during task performance due to its role in motor executive function (Deiber et al., 1991; Playford et al., 1992).

Premotor cortex

The premotor cortex is a region in the frontal lobe which is densely connected with M1, and is considered to be the third part of the motor area of the brain (Richard A Andersen & Christopher A Buneo, 2002; Exner et al., 2002; Morecraft & van Hoesen, 1993) (Figure 1-10A). This area is involved in *cognitive functions* and has a critical role in coding space and associative learning, in which learning occurs through links between information, so it is difficult to recall information in isolation (Gallese et al., 1996; Rizzolatti et al., 1996). For example, we can easily recall eyebrows as a part of the face rather than as an isolated part without any link to a whole face. the premotor cortex is also essential for memory consolidation and encoding of the learning (Exner et al., 2002; Grafton et al., 1998; Honda et al., 1998; Maquet, 2000; Nitsche et al., 2010).

Parietal cortex

The parietal cortex has a crucial role in many cognitive tasks, particularly in the sensory control of action. The parietal cortex forms parts of a dorsal and ventral visual pathway that are involved in encoding spatial locations and object recognition (Goodale & Milner, 1992; Mishkin & Ungerleider, 1982). The posterior parietal cortex (PPC) is located between the somatosensory cortex, in the postcentral gyrus, and the visual cortex in the occipital lobe. Thus, it is well positioned to collect both somatosensory and visual input and send output to motor areas and the premotor and frontal cortices. PPC is historically known as the "association cortex", which integrates information across modalities. PPC is anatomically divided by the intraparietal sulcus (IPS) into the superior parietal lobule (SPL) and inferior parietal lobule (IPL) (Figure 1-11A). On the medial view, there is a region, the precuneus (PCu), which is anterior to the parietooccipital sulcus, and separates the parietal lobule from the occipital cortex (Figure 1-11B).

The PPC collects input from the three sensory systems: the visual, the auditory, and the somatosensory system. Most of the output from this area goes to the DLPFC and then to the various areas of the motor cortex. The PPC seems to be responsible for two key functions including anticipatory motor control and integration of multisensory information (Cohen & Andersen, 2002; Desmurget et al., 1999; Snyder et al., 1997). Information from this area may be sent back to the cerebellum to update and regulate the internal model in favor of succeeding actions.

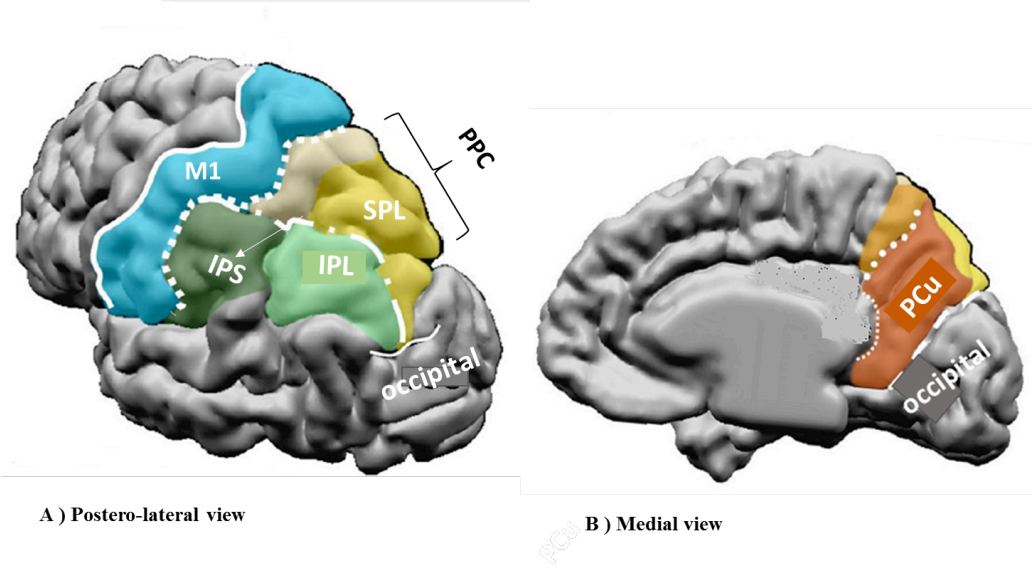

Figure 1-11: Anatomical illustration of the postero-lateral (a) and medial (b) views of the posterior parietal cortex (PPC) including the superior parietal lobule (SPL), inferior parietal lobule (IPL) and the precuneus (PCu). Adapted from Culham et al. 2006.

DLPFC, M1 and PPC as three superficial FPN sites which are differentially engaged in the slow and fast stages of sequential learning.

FPN involvement in different stages of learning

Three superficial sites of the FPN, are differentially involved in the process of sequential learning (Grafton et al., 1995; Hazeltine et al., 1997; I. H. Jenkins et al., 1994; Sakai et al., 1998) (Figure 1-12). The activity of the DLPFC is crucial for cognitive control, executive functions and working memory (Hasan et al., 2013). The PPC is strongly associated with sensorimotor integration for perception and action (Rivera-Urbina et al., 2015) and the M1 region is considered as the final relay in the motor learning process that converts motor programs into the desired movements.

During the early stages of learning, the DLPFC, premotor and parietal cortices show relatively greater activity (Grafton et al., 2002; Grafton et al., 1992; Jueptner et al., 1997).

Increased activations in the basal ganglia and cerebellum nuclei illustrate later phases of short-term motor learning (Doyon & Ungerleider, 2002). Although increases of activation in M1 have been reported during the fast stage of learning (Grafton et al., 1997; Hazeltine et al., 1997), decreasing or unchanged activation in this region has been also reported (Doyon & Ungerleider, 2002; I. H. Jenkins et al., 1994; Toni et al., 1998).

In contrast to the fast stage of learning, the slow stage is strongly connected with increased activation in the M1 area (Floyer-Lea & Matthews, 2005), SMA (Lehericy et al., 2005), and putamen (Floyer-Lea & Matthews, 2005; Lehericy et al., 2005).

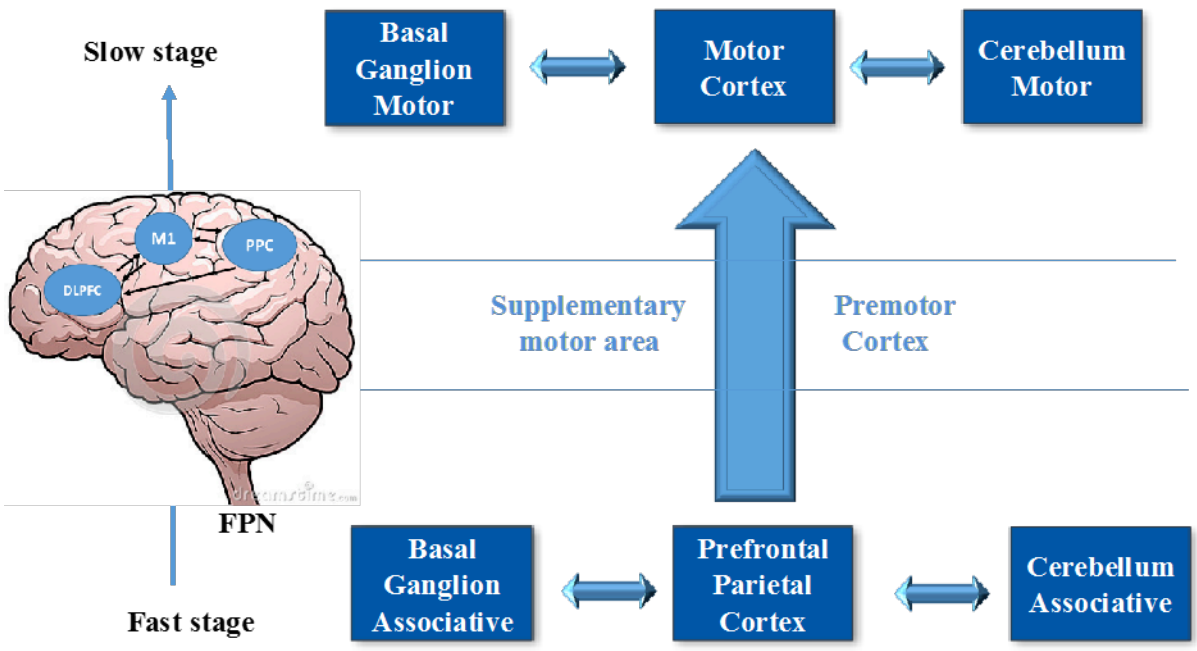

Figure 1-12: Frontoparietal network (FPN) contribute differentially in fast and slow stages of learning.

Regarding the importance of transfer learning in both healthy participants and neurological patients such as stroke patients, especially for a fine force control task, we aimed to

investigate whether the left untrained hand differentially benefited from right hand training during stimulation of three superficial FPN sites (DLPFC, PPC and M1) or not.

Here we briefly explained transfer learning and mechanisms of related to transfer learning.

Transfer Learning

Practicing a new task with one hand typically facilitates subsequent performance of the other hand (Parlow & Kinsbourne, 1989; Taylor & Heilman, 1980). This phenomenon is known as intermanual transfer, indicating that acquired gains in one hand following training are not effector-specific and can be transferred into the opposite, untrained, side. Intermanual transfer has been reported for an extensive range of manual tasks (Taub & Goldberg, 1973; Thut et al., 1996; Wieg, 1932). It has also been observed in motor sequence tasks such as SRTT and SVIPT (Camus et al., 2009; Grafton et al., 2002; Japikse et al., 2003; Perez et al., 2007). Neuroimaging techniques have identified the underlying neuronal system of intermanual transfer by showing cerebellar activation of the FPN in both sides, which would contribute to intermanual transfer.

Underlying mechanism for intermanual transfer

The corpus callosum, which links the two hemispheres, is responsible for transfer of knowledge and information between two hemispheres. Damage to this area in patients with split-brain and acallosal conditions results in less intermanual transfer (Bloom & Hynd, 2005; de Guise et al., 1999; Lassonde et al., 1995).

The corpus callosum can be divided into two primary parts (Figure 1-13): anterior and posterior parts. The anterior part joins the frontal lobes. The posterior part joins the temporal,

parietal and occipital lobes (Pandya et al., 1971; Pandya & Seltzer, 1986).

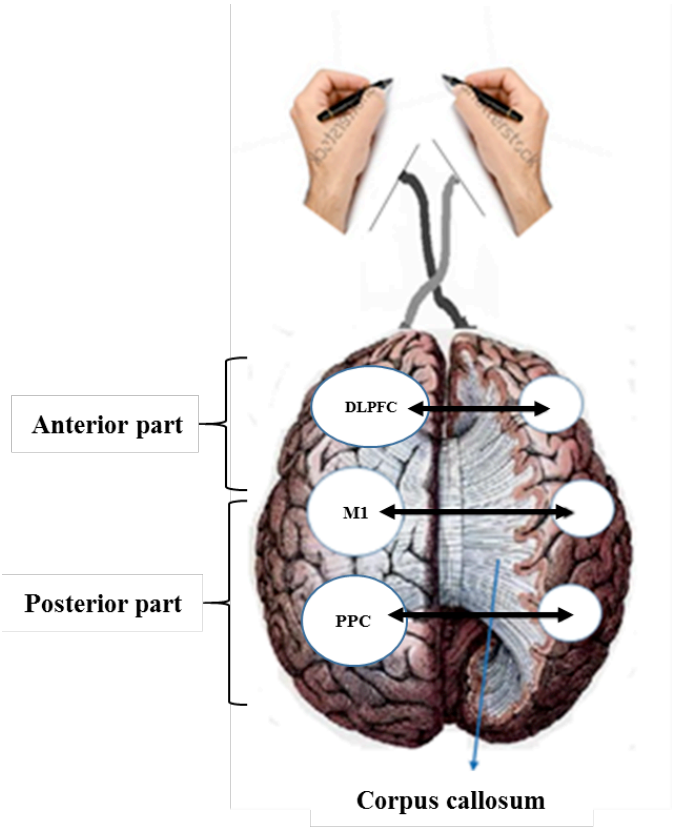

Figure 1-13: The corpus callosum connects the two hemispheres including two primary parts: the anterior and posterior parts. The anterior part joins the frontal lobes and the posterior part links the temporal, parietal and occipital lobes. Adapted from http://hubel.med.harvard.edu/book/b34.htm.

Neuroimaging studies revealed bilateral M1 activation when participants performed SRTT training (Bischoff-Grethe et al., 2004; Daselaar et al., 2003), which might reflect practice of movements in one hand leading to excitatory or inhibitory activity in both hemispheres (Almeida & Stetter, 2002; Tinazzi & Zanette, 1998).

In the following sections, an overview of the non-invasive brain stimulation techniques (NIBS) are provided.

Non-invasive brain stimulation techniques (NIBS)

NIBS techniques can be used for study of inhibitory or excitatory mechanisms of brain during motor function. NIBS include transcranial magnetic stimulation (TMS) and transcranial electrical stimulation (tES). tES is an umbrella term used for tDCS, transcranial random noise stimulation (tRNS) and transcranial alternative current stimulation (tACS). In contrast to tDCS, which induce a change in neuronal membrane potentials and is dependent on polarity of current flow (anodal versus cathodal), tRNS and tACS are the methods which are not polarity dependent.

TMS is another NIBS technique can be used therapeutically to modulate neural excitability. A number of repetitive transcranial magnetic stimulation (rTMS) and theta burst stimulation (TBS) as recent advances introduced in TMS methods. In general, TMS frequencies of 1 and less than one reduces while the frequencies above 1 increases corticospinal excitability.

TMS as the main assessment tool used in the experiments within this thesis to evaluate cortical excitability. In the following section, we further explain tDCS as the intervention of interest and TMS as the assessment tool used in this thesis.

Transcranial direct current stimulation (tDCS)

During the past decades, non-invasive brain stimulation has been successfully used to test the role of specific brain areas for particular behaviour. Most of the non-invasive brain stimulation studies so far have mainly focused on the role of the primary motor cortex (M1) in motor skill learning. For example, facilitating activity in M1 has been consistently shown to improve motor function and skill learning. However, brain-imaging studies revealed that motor skill learning is associated with the recruitment of large-scale neuronal circuits beyond

M1, involving supplementary motor area

Historical perspectives

Modulation of the brain function using electrical stimulation is a very old technique (Adolphs, 2003; Kellaway, 1946; Shallice, 1982). There is evidence to indicate that some ancient physicians used *electric fish* over the scalp for treatment of different disorders. The physician of the Roman Emperor Claudius, Scribonius Largus, was the first man to describe how headache can be ameliorated through delivering an electric current by placing a live torpedo fish over the scalp (Adolphs, 2003; Kellaway, 1946). Ibn-Sinah, the great Iranian physician, used a live electric catfish for treatment of epilepsy in the eleventh century (Kellaway, 1946).

After invention of batteries by the early years of the eighteenth century, electricity was produced by machines on demand. Using a battery, Luigi Galvani (1791) showed that an exposed nerve can be activated and produced a muscle contraction. Aldini (1804), Galvani's nephew, performed a series of experiments on corpses of decapitated prisoners. He could produce jaw movements by electrical stimulation on recently decapitated humans. Bartellow (1874) stimulated *the exposed cortex* in a conscious patient (*invasive stimulation*), who suffered from head tumour, he reported arm and leg movements in the right side of body after stimulation of the left cortex with direct currents. Direct current, in a form of *non-invasive stimulation* through a pair of electrodes over the scalp, has been used by Priori et al. (1998). This technique was followed by Nitsche and Paulus (2000), as transcranial direct current stimulation (tDCS), to modulate the brain.

Clinical applications of tDCS

Nitsche and Paulus (2000) found that the effects of tDCS on the brain are polarity dependent. This concept is established using changes in CSE recorded by TMS. The anode (positive charged electrode) directs current into the brain and increases CSE by neuronal depolarisation, while the cathode (negative charged electrode) hyperpolarises cortical neurons and may decrease CSE (Nitsche & Paulus, 2000, 2001; Priori et al., 1998) (Figure 1-14). However, some recent studies have shown that anodal tDCS can decrease excitability when the stimulation time is increased (Monte-Silva et al., 2013), and cathodal tDCS can increase excitability when intensity is increased (Batsikadze et al., 2013). Consequently, the relationship between tDCS and CSE is not dependent on just the polarity but also the duration and intensity of stimulation applied through the experiment.

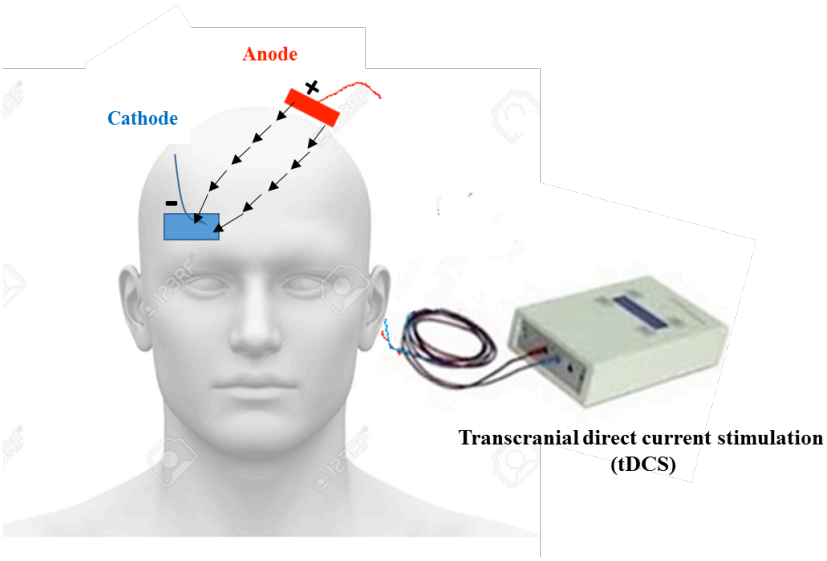

Figure 1-14: Transcranial direct current stimulation (tDCS). The anode electrode enters currents towards the cathode electrode through the brain.

Over the past two decades, tDCS has attracted growing interest for investigation into the brain function in healthy individuals as well as patients with psychiatric or neurological

disorders such as stroke (de Guise et al., 1999; Obayashi, 2004; Pandya et al., 1971; Pandya & Seltzer, 1986; Parlow & Kinsbourne, 1989; Taylor & Heilman, 1980; Thut et al., 1996; Wieg, 1932) or Parkinson's disease, (Briand et al., 2007; Courtin et al., 2013; Diamond, 2011; Goto et al., 2010; Livingston & Ingersoll, 2008; Ray & Zald, 2012; Viviani & Lacquaniti, 2015).

Mechanisms of action of tDCS

Systematic animal and human studies have provided valuable information on the underlying mechanisms for the effects of tDCS on the brain cells (Bishop & O'leary, 1950; Chan et al., 1988; Fritsch et al., 2010; Morrell, 1961). The proposed mechanism behind tDCS is polarity-dependent shifts of the resting membrane potential, which directly leads to facilitation or inhibition of the cortical neurons in the superficial regions of brain, which may also indirectly affect deeper and more remote areas (Lorenz et al., 2003; Willis & Westlund, 1997). Three principles are defined to interpret the behavioural effects of tDCS (De Xivry & Shadmehr, 2014; Morrell, 1961): modulation of firing rate; strengthening of newly formed association of synapses, and modulation of new firing patterns.

I. Modulation of firing rate

TDCS has the capability to change neuronal membrane polarity. However, tDCS cannot produce action potentials; it is able to change both spontaneous and evoked discharge rates (Liebetanz et al., 2002; Nitsche & Paulus, 2001; Stagg et al., 2011; Stagg & Nitsche, 2011). During anodal stimulation, increases in firing rate reflect the depolarization of neurons, while cathodal stimulation through decreased firing rate results in hyperpolarization of neurons (Brazovskaya et al., 1972; Creutzfeldt et al., 1962; Morrell, 1961) (Figure 1-15).

Consequently, changes in excitability of the targeted neurons observed following modulation the firing rate can facilitate or inhibit neurons to respond the stimulus (Ardolino et al., 2005; Bindman et al., 1962; Liebetanz et al., 2002; Purpura & McMurtry, 1965; Stagg & Nitsche, 2011).

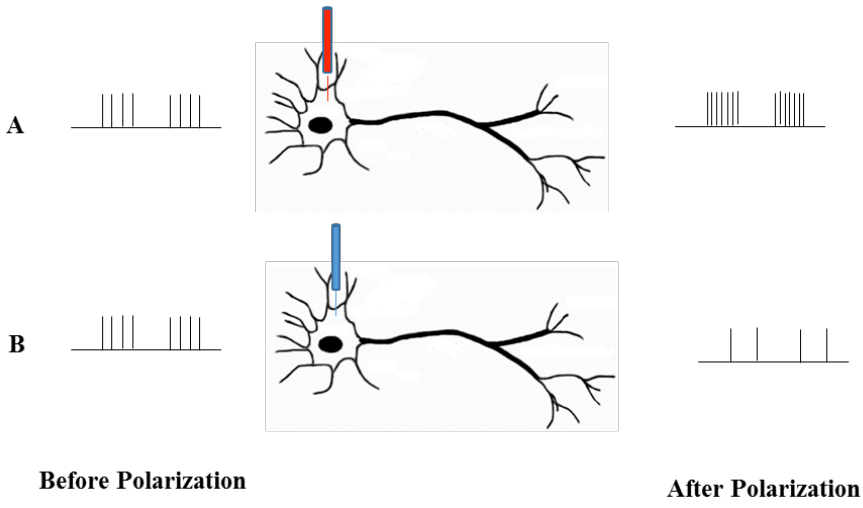

Figure 1-15: Increases in firing rate and depolarization of neurons occur after anodal stimulation (A) and decreases in the firing rate and hyperpolarization of neurons occur after cathodal stimulation (B).

Increases in the firing rate following anodal polarization can sensitize neurons of the region, in that the stimulus can fire neurons more easily and increase the opportunity for neurons to produce an appropriate behavioural response to stimulus with a lower threshold (Brazovskaya et al., 1972; Creutzfeldt et al., 1962; Morrell, 1961). Thus, through this mechanism, application of a-tDCS above motor regions of the brain during the learning process, is able to enhance behavioural responses to the stimulus.

II. Strengthening of newly formed associations

Changes in activity of neurons following DC polarization remained 20 min or even hours after the current is switched off (Bindman et al., 1962; Morrell, 1961; Sokolov, 1977). The

long-lasting effects are related to modulation of synaptic activity and protein synthesis via neurotransmitters, which is accompanied by modifications of calcium levels and intracellular cAMP. A-tDCS inhibits synaptic neurotransmission by GABA, while cathodal tDCS inhibits synaptic neurotransmission via glutamate. Therefore, this modality has the potential to stabilize newly formed synaptic connections through the modulation of neurotransmitters in the stimulated cortex and modulate synaptic plasticity through the well-characterized phenomena of LTP and LTD.

III. Modulation of new firing patterns

The last mechanism is related to the effects produced in firing patterns of the brain. Using a-tDCS during learning may lead to formation of new and stronger synaptic connections between activated neurons (Liebetanz et al., 2002). Morell et al. (1961) showed that if visual cortex neurons are stimulated with a flash of light at a frequency of 3 Hz during anodal polarization, neurons respond at the same frequency to a single flash of light over the next 20 minutes after anodal stimulation.

TDCS safety

Any application of tDCS over the scalp requires consideration of safety. Although the current applied to the brain tissues should be large enough to modulate the brain, this current should be in a safe range to reduce the induced electrochemical effects under electrodes (Geddes & Roeder, 2004). The production of electrochemical substances at the electrode-tissue interface may lead to tissue damage under the electrodes (Agnew & McCreery, 1987). Under the anode, increase of negatively charged ions, such as chloride ions, may produce an alkaline reaction, while under the cathode, acidic reactions may happen due to the excess of positive ions such as sodium ions. These chemical reactions could cause sensory side effects of tDCS such as

itching, burning and tingling sensations. Using non-metallic and conductive rubber electrodes can diminish chemical reactions induced at the electrode–skin interface during tDCS stimulation. These electrodes should be completely covered by saline-soaked sponges (Nitsche et al., 2003).

TDCS parameters

Some parameters such as the *amplitude* of applied current, *electrode size* and *duration of the stimulation* (Iyer et al., 2005; Nitsche et al., 2003; Nitsche & Paulus, 2000, 2001; Priori et al., 1998) can affect tDCS safety. Therefore, *current density* and *total charge*, determined from the mentioned parameters, can be used as indeces to determine safety limits of tDCS (Agnew & McCreery, 1987; Nitsche et al., 2003). These parameters are measured by the following formulas:

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

$$Total\ charge\ \left(\frac{Coulomb\ (C)}{cm^2}\right) = Current\ density\ (mA/cm^2)\ \times\ total\ stimulus\ duration\ (s)$$

Current intensity of more than 25 mA/cm^2 or the total charge of more than 216 C/cm^2 can induce tissue damage (McCreery et al., 1990; Yuen et al., 1981). Therefore, the applied current dosage should be below these limits to avoid any adverse on the activated areas of the brain.

The results of some safety studies in healthy adults and patients (Gandiga et al., 2006; Iyer et al., 2005; Nitsche et al., 2003; Poreisz et al., 2007) show no evidence of harmful effects

following application of tDCS. Nitscheh et al. (2003), applied tDCS in approximately 500 subjects. Participants reported no side-effects, except a slight tingling feeling during the first seconds of stimulation under the electrodes. A total of 103 healthy participants was evaluated by Iyer et al. (2005). They also found no adverse effects on function, cognition and electroencephalography (EEG) following application of 1 or 2 mA tDCS with electrode size 25 cm^2 over the left frontal cortex for 20 min.

In this thesis, a commercial stimulator (Intelect Advanced Therapy System, Chattanooga, TN, USA) (Figure 1-16) was used to deliver a direct current with intensity of 0.3 mA for a duration of 20 min during SVIPT. Two conductive rubber electrodes, which were covered completely by saline-soaked sponges, were used to stimulate the target areas. An active electrode (3 cm^2) was placed over the left M1, DLPFC, or PPC region and the return electrode (12 cm^2) was positioned over the contralateral supraorbital region.

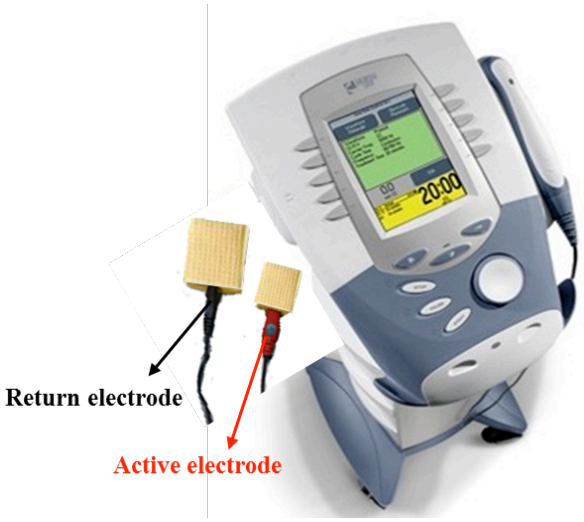

Figure 1-16: Intelect Advanced Therapy System, Chattanooga, TN, USA with active electrode (3 cm^2) and return electrode (12 cm^2), which was used in this thesis.

Current intensity of 0.3 mA has been shown to increase CSE (Bastani & Jaberzadeh, 2013; Vaseghi et al., 2015). This low intensity allowed us to reduce the size of the active electrode (3 cm^2) while still keeping the current density (0.1 mA/cm^2) in a safe range (McCreery et al., 1990; Nitsche et al., 2003). We used a small electrode size of 3 cm^2 (1.5 x 2 cm) in order to selectively stimulate M1 and rule out the possibility of stimulation of nearby areas, such as the premotor cortex or SMA. The location of M1 was identified using TMS and the location of two other areas were determined by the international 10-20 system (Steinmetz et al., 1989). Therefore, the stimulating electrodes for DLPFC or PPC were placed over F3 and P3, respectively (Figure 1-17).

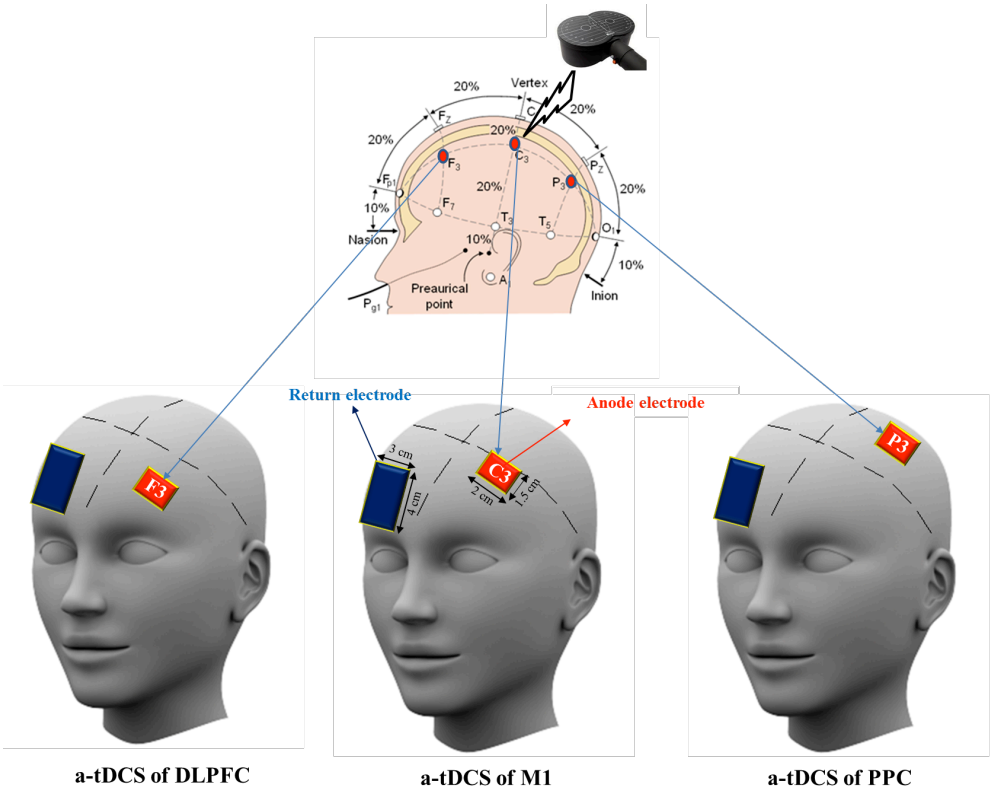

Figure 1-17: a-tDCS over three areas of the FPN including DLPFC, M1 and PPC.

In the current thesis, the guideline suggested by Koechlin et al. (1999) was followed throughout the experiments to make sure the applied current dosage induced minimum or no

side or adverse effects (Koechlin et al., 1999) (Appendix 2). All participants endured the applied currents during the experiments and no one required to stop the experiment due to adverse or side effects of the applied currents.

Transcranial magnetic stimulation (TMS)

TMS was introduced as a painless and non-invasive technique to stimulate the human motor cortex by Barker et al. (1985). This method has been used extensively in human motor cortical research to detect normal or abnormal functions of different brain areas.

TMS induces a magnetic pulse by a simple magnetic coil which is placed over the brain. This transient magnetic field passes through the tissues and scalp and produces electrical current in nearby conducting material (Figure 1-18). The induced electrical current can depolarize the cell membranes of cortical motor neurons and interneurons and stimulate these neurons if the depolarization exceeds a threshold level.

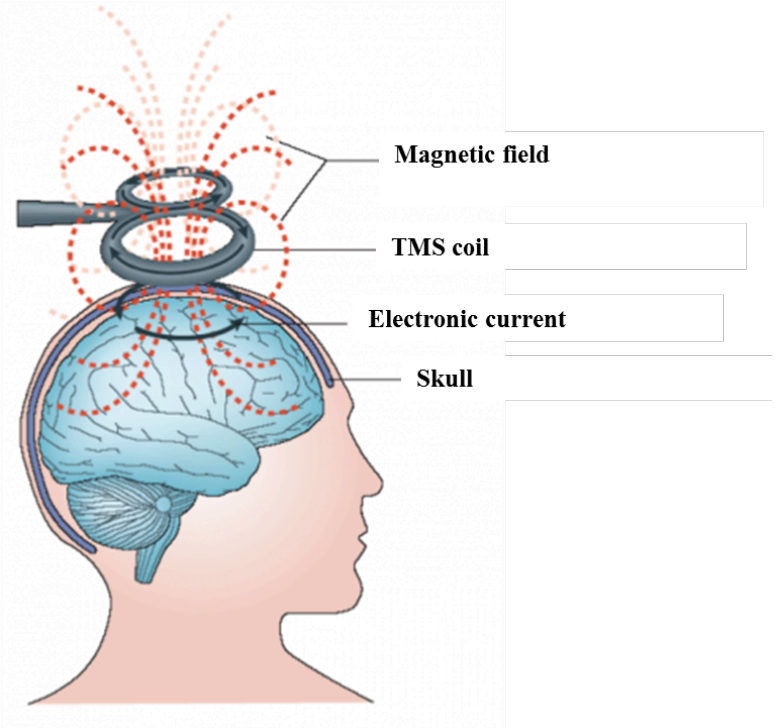

Figure 1-18: Magnetic field is induced by TMS coil after passing through the skull converts an electrical currents which can stimulate the brain. https://www.psychologytoday.com/blog/reading-between-the-headlines/201401/transcranial-magnetic-stimulation-tms-treats-depression

TMS can be used as a research/diagnostic device to assess the activity of the corticospinal tract. The corticospinal tract conducts impulses from the M1 area to the spinal cord. Single- and paired-pulse TMS have been widely used to evaluate the corticospinal tract and intracortical interneurons in clinical and physiological studies. The output of stimulating nerve cells in M1 can be simply evaluated in the form of a motor-evoked potential (MEP) by using surface electromyography (EMG) from the target muscle (Figure 1-19).

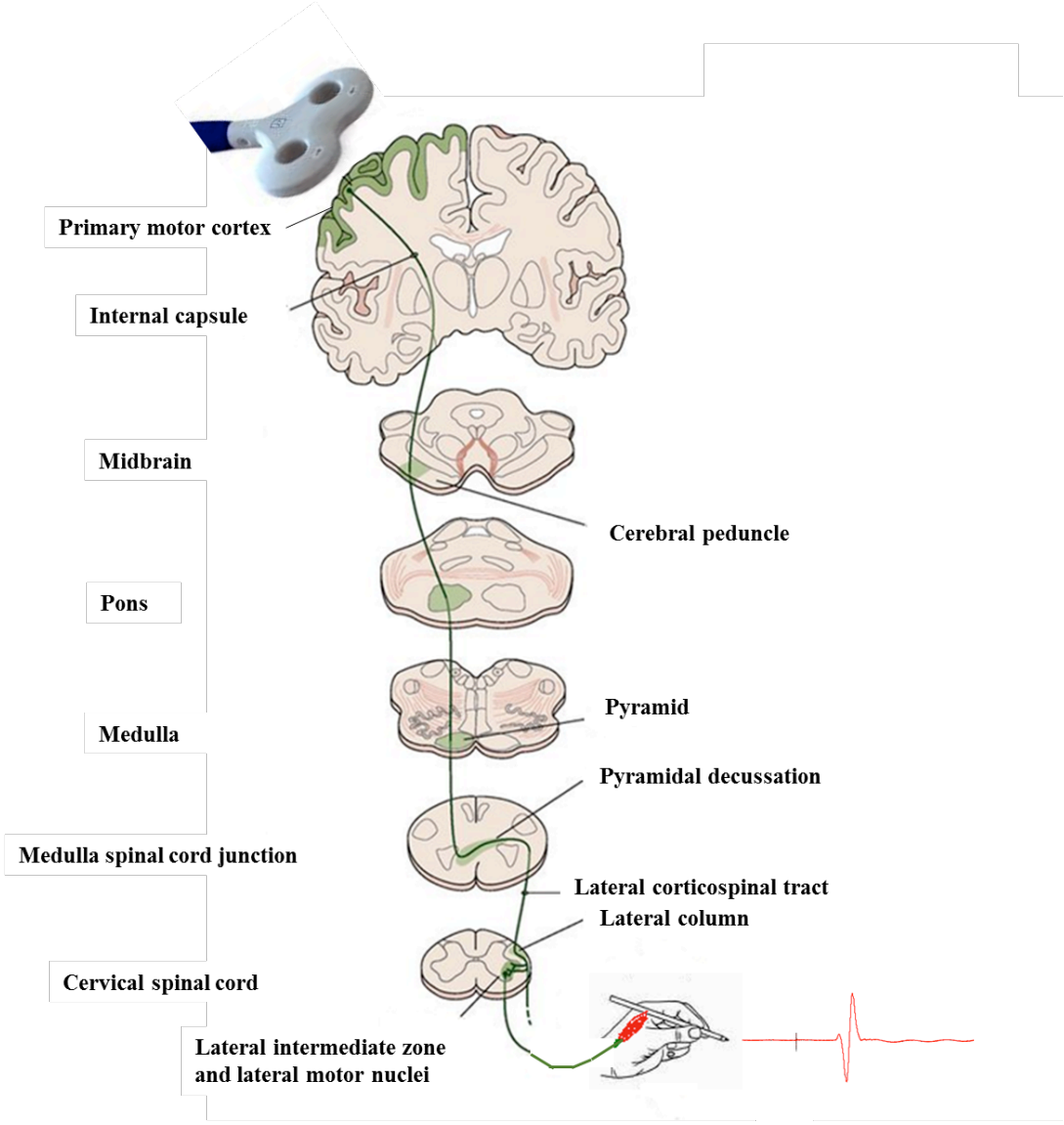

Figure 1-19: Corticospinal tract begin from primary motor cortex and pass through the medullary pyramids and terminate at the spinal cord. Adapted from http://www.unifr.ch/neuro/rouiller/assets/files/THESE-MASTERS/these-eric-schmidlin-final.pdf

In the present thesis, single and paired-pulse TMS were used to assess the level of CSE, SICI and ICF. These two TMS paradigms are described in more detail in the following sections.

Single-pulse TMS

Assessment CSE

CSE is frequently evaluated by TMS for clinical and research purposes (Rossini & Rossi, 2007). For assessment of CSE, single pulse TMS (Mag Pro R30 stimulator (Mag Venture) was used to record MEPs from the right first dorsal interosseous muscles (FDI) (Figure 1-20).

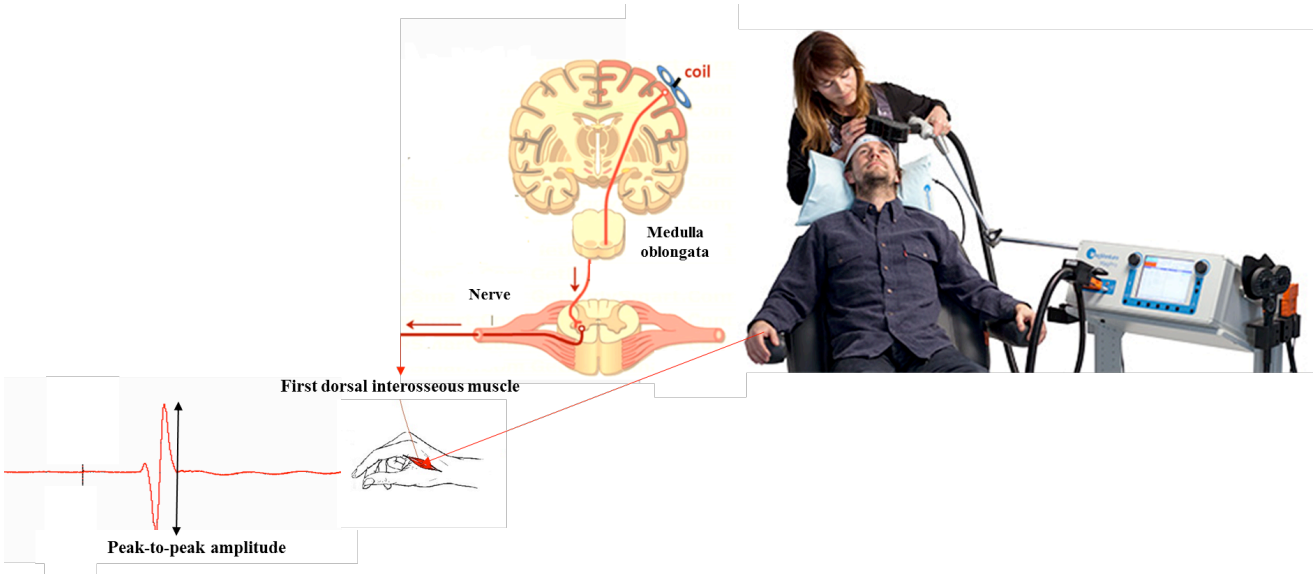

Figure 1-20: MEP recording from FDI muscles by a single pulse TMS.

MEP responses were recorded using surface Ag/AgCl electrodes located above the right FDI in a belly-tendon arrangement. A ground electrode was placed on the styloid process of the ulnar bone. To reduce skin resistance and ensure good surface contact, a standard skin preparation procedure of cleaning was completed for each electrode position. The accuracy of

EMG electrode placement was confirmed by asking the subjects to maximally contract this muscle by pressing their index finger to thumb while the researcher checked online EMG activity. To determine the hot spot for the right FDI, the coil was set tangentially to the skull with an angle of 45º to the midline in a posterior-anterior direction on the left M1 region, which has been suggested to be the most effective approach to stimulate CSE trans-synaptically (Adams, 1952). The coil was then moved until the largest MEPs could be consistently recorded from the target muscle. The position of this optimal site, referred to as the 'hot spot' for the target muscle, was marked with a semipermanent pen on the scalp to guide accurate coil orientation throughout the experiment. To minimize a large number of confounding variables, the guideline checklist suggested by Chipcase et al (2013) was followed in this thesis.

After determination of the hot spot, the resting motor threshold (RMT) was measured. The RMT was defined as the lowest TMS output (intensity) required to produce an MEP peak-to-peak amplitude that exceeds 50 μV in at least five of ten consecutive trials (Sokolov, 1977). The RMT was obtained based on the guideline suggested by Rotwell et al. (1998) in which the TMS intensity starts with a suprathreshhold intensity and decreases in steps of 2% or 5 % of stimulator output. This decrease continues until a level is reached, below which consistent or reliable responses disappear. RMT reveals the total excitability of the motor pathway, consisting of huge pyramidal cells, cortical excitatory and inhibitory interneurons, and spinal motor neurons (Ziemann, 2004). Therefore, RMT indicates excitability of cortico-cortical axons and is largely mediated by voltage-gated sodium channels (Savion-Lemieux & Penhune, 2005). In both research and clinical studies, it is essential to accurately estimate the RMT (Vidoni & Boyd, 2007) because inaccurate estimation of RMT can lead to over stimulation of a participant cortex, which can increase the possibility of TMS-induced seizures (Purpura & McMurtry, 1965).

All raw EMG signals, were amplified, filtered (20 Hz -10 kHz) and recorded with a PC running a commercially available data acquisition and automated-analysis package (PowerLab^{TM} ADInstrument 4/35 with LabChart^{TM}, Australia) for offline analysis (Figure 1-21).

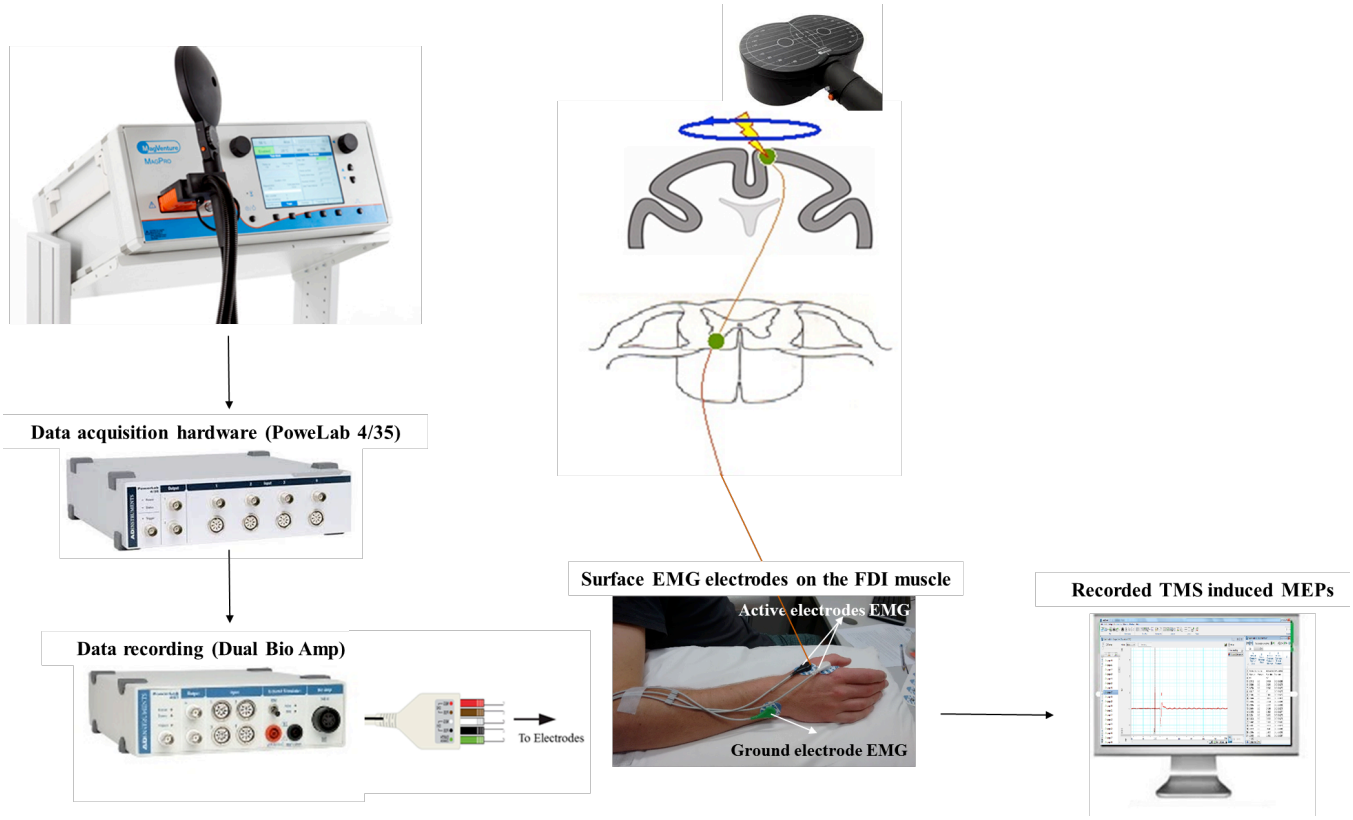

Figure 1-21: MEP recording using TMS. EMG electrodes were attached on belly FDI muscles and ground electrodes was placed on the styloid process of the ulnar bone to record MEPs. Power lab 4/35 was used for data acquisition.

As shown in Figure 1-22, MEP amplitude from FDI is measured as peak-to-peak amplitude. The average of 20 peak-to-peak amplitudes were calculated for measurement of M1 CSE in this thesis.

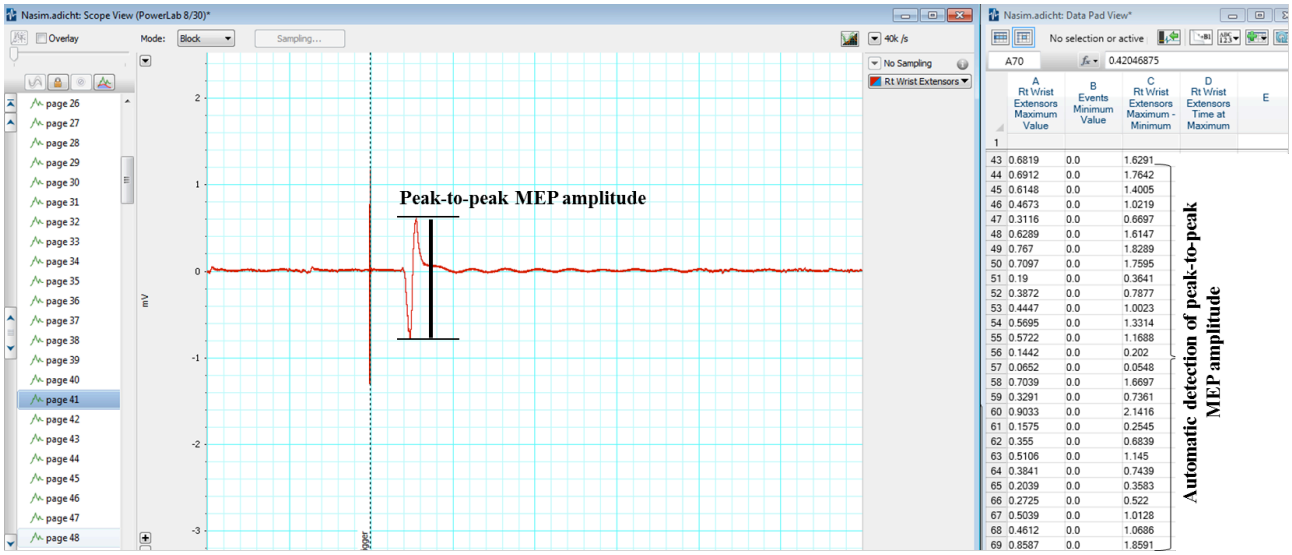

Figure 1-22: peak-to-peak amplitude from FDI muscle which was detected automatically using LabChart software from the ADInstrument Company.

Paired-pulse TMS

Assessment of SICI and ICF

A paired-pulse TMS is used to obtain SICI and ICF in order to assess inhibitory or excitatory connections of interneurons. To record SICI and ICF, a subthreshold conditioning stimulus (CS) is applied prior to the suprathreshhold test stimulus (TS) with varying inter-stimulus intervals (ISI) (Kujirai et al., 1993). The CS was set to an intensity of 80% of the RMT which does not induce changes of excitability in the spinal cord (Kujirai et al., 1993; Picard & Strick, 1996). The TS was adjusted to produce MEPs of 1 mV peak-to-peak amplitude. The recorded MEP is suppressed compared to the single-pulse induced MEPs, when the CS is delivered 3 msec prior to the TS or increased when the CS is delivered 7-10 msec before the TS (Kujirai et al., 1993) (Figure 1-23).

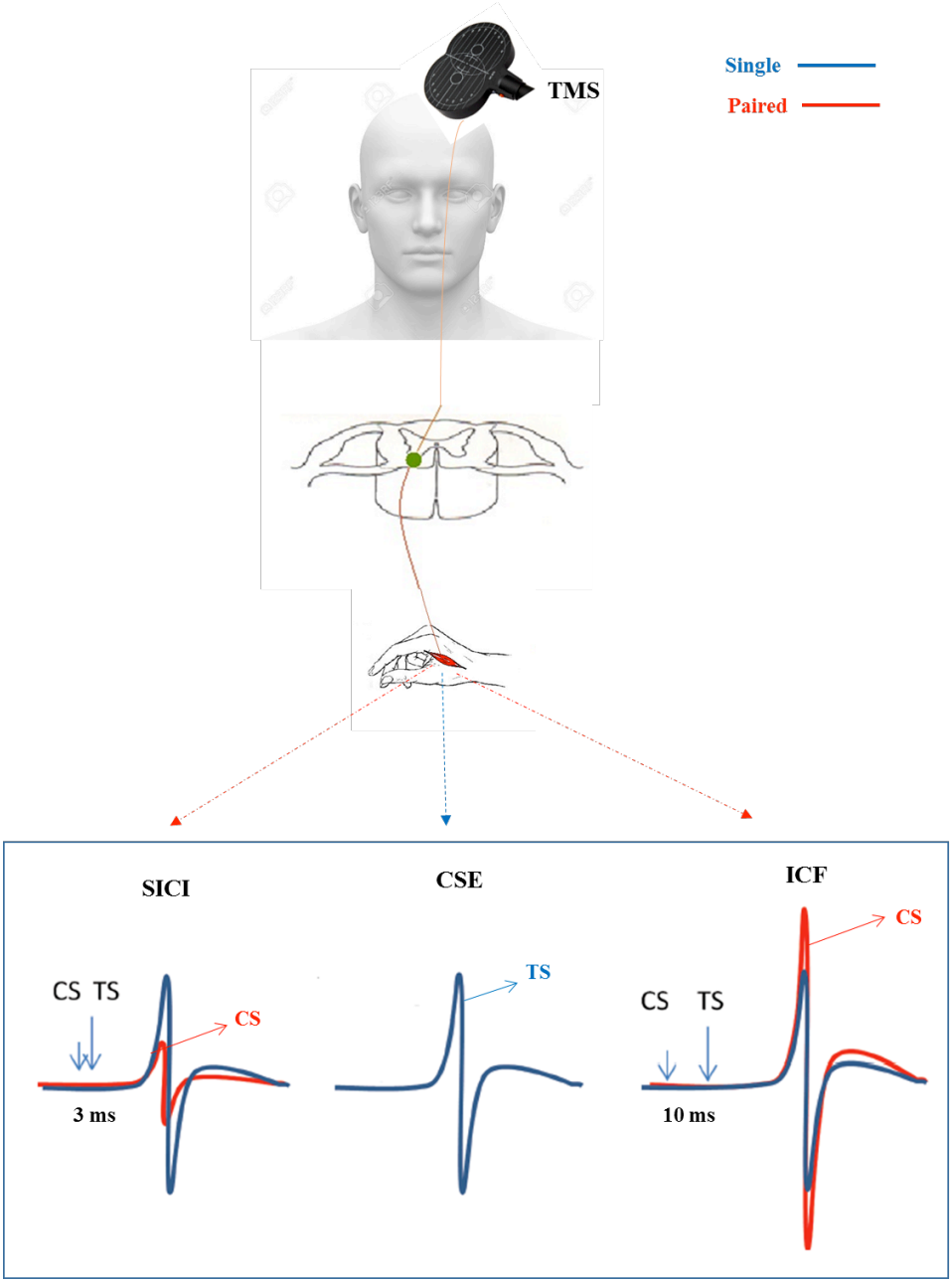

Figure 1-23: Using single- and paired pulsed transcranial magnetic stimulation (TMS) over motor cortex to assess corticospinal excitability (CSE) (A), short-interval intracortical inhibition (SICI) (B) and intracortical facilitation (ICF) (C). SICI and ICF involve comparing MEP amplitude of a single, suprathreshold test stimulus (TS) to a paired-pulse condition with a subthreshold conditioning stimulus (CS)(Thomas, 2002). In SICI, the recorded MEP is suppressed compared to the single-pulse induced MEPs, when the CS is delivered 3 msec prior to the TS. In ICF, the recorded MEP is increased, when the CS is delivered 10 msec before the TS.

ICF is used as an index for the evaluation glutamate receptors (function of excitatory circuits) in the motor cortex (Ziemann et al., 1996). In contrast, SICI has been related with the activity

of $GABA_A$ receptors and used as an index for the evaluation of function of inhibitory circuits (Kujirai et al., 1993) (Figures 1-24A & B).

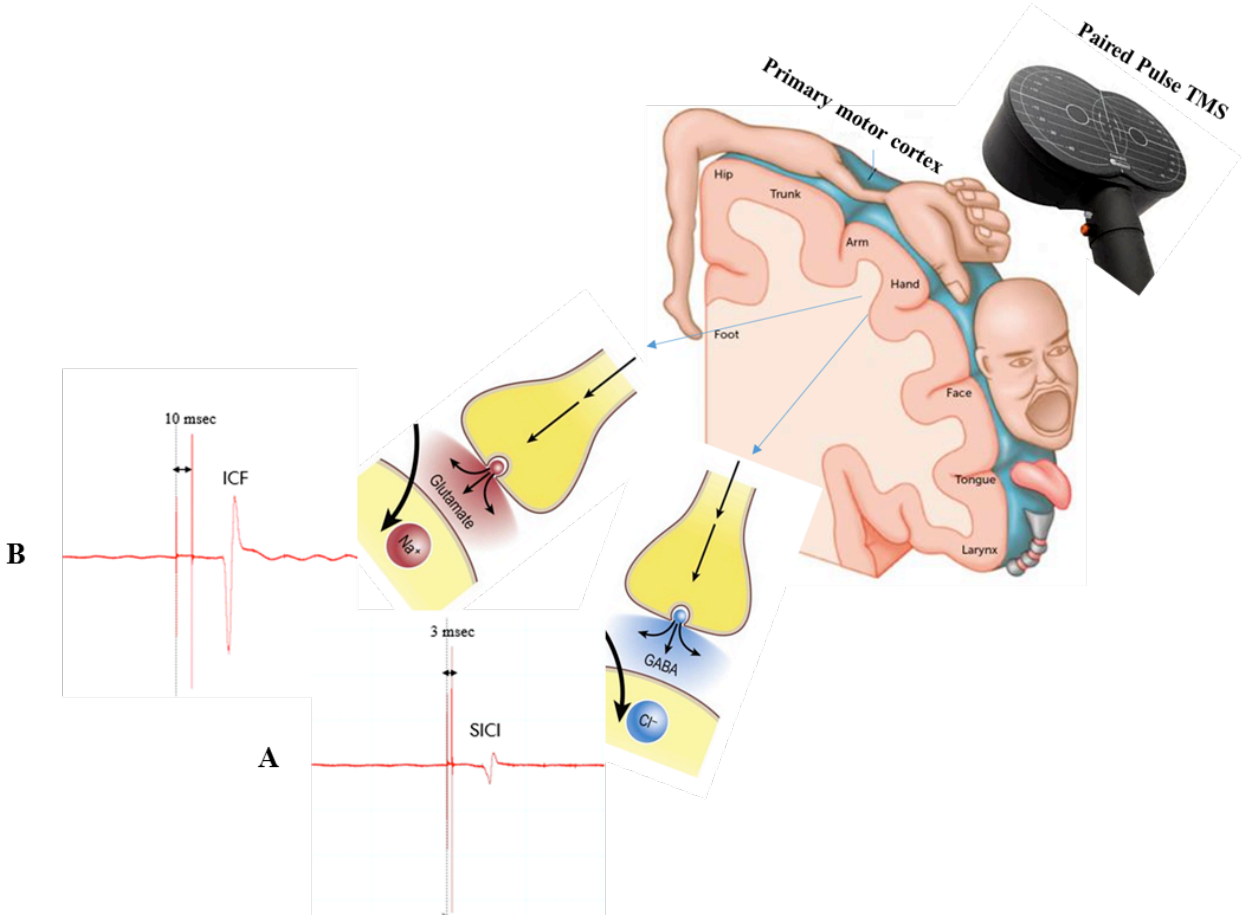

Figure 1-24: Paired-pulsed TMS was used to record short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) by the inter-stimulus interval (ISI) of 3 and 10 msec, respectively.

The whole set up, which was used in this thesis, is shown in Figure 1-25. The FPN was stimulated with a-tDCS during training using SVIPT. TMS was also used to assess cortical changes after interventions.

Figure 1-25: Study set up design. TMS was used as assessment too and tDCS stimulator was used as an intervention during training with SVIPT. Active electrodes was placed over the target area and return electrodes was placed on the right supraorbital area. MEP responses were recorded using surface Ag/AgCl electrodes located above the right FDI in a belly-tendon arrangement. A ground electrode was placed on the styloid process of the ulnar bone.

In this chapter, we briefly explained motor sequence learning, transfer learning and the contribution of different areas of the brain during sequential learning. In addition, this chapter presented an introduction to non-invasive brain stimulation including tDCS and TMS.

Research aims

The primary aim of this thesis was to determine the effects of single-session a-tDCS over superficial sites of the FPN during SVIPT using the dominant hand. The secondary aim was then to examine transfer of learning into the untrained hand. To address this aims, a number of studies were designed and carried out on healthy participants.

To explore the underlying mechanisms behind the efficacy of a-tDCS technique, we measured CSE, SICI and ICF of M1 using single and paired-pulse TMS paradigms.

Thesis overview

Chapter 2 presents a systematic review and meta-analysis published in Brain and Cognition Journal, titled "The effect of anodal transcranial direct current stimulation on motor sequence learning in healthy individuals: A systematic review and meta-analysis". This study evaluated the literature on the effects on motor sequence learning of single-or multiple-sessions a-tDCS over M1. The results of this meta-analysis showed that multiple sessions compared to a single session of a-tDCS, induces significant improvements in motor sequence learning at post intervention time points in both tasks; SRTT and SVIPT. Indeed, application of a-tDCS over M1 across the three or five consecutive days induces significant improvement in performance of motor sequence learning while no improvement was found after a single session of stimulation over M1.

A problem with multi session of treatment using a-tDCS, is the fact that patients should attend health care providers for a number of time. The cost of treatment in these multi session treatment protocols is very high. Therefore, our aim is to develop a protocol to achieve enhanced motor learning within a session of treatment. Therefore, in this thesis we aimed to

find the optimal stimulation sites to achieve aforementioned improvement within a single session of treatment. Indeed, the results of our meta-analysis suggest that attention must be directed to the optimization of stimulation sites especially for fast stage of learning. To achieve this, we decided to check the effects of other cortical sites of FPN, DLPFC and PPC, which are more engaged at fast stage of learning. Finding optimal stimulation sites can have a profound impact on tDCS efficacy for enhancement of motor sequence learning in both trained and untrained hand. In most of tDCS studies (Reis et al., 2015; Reis et al., 2009a; Saucedo Marquez et al., 2013; Schambra et al., 2011), M1 has been widely stimulated to affect cortical and behavioural outcomes during sequential movements using SVIPT. In addition, neuroimaging studies have also shown that a broad network in cortical and subcortical areas of the brain such as FPN (Hasan et al., 2013; Honda et al., 1998; Karni et al., 1998; Miller & Cohen, 2001; Muellbacher et al., 2002; Rioult-Pedotti et al., 2000; Ungerleider et al., 2002), which are involved for perception and production of accurate actions in sequential learning with both trained and untrained hands. Application of tDCS on functionally connected sites of FPN including DLPFC, and PPC which have essential roles in sequential learning especially at initial stage of learning are not completely investigated. The studies introduced in this thesis are motivated by the need for exploring functional connectivity of FPN sites in a precision control task such as SVIPT using a-tDCS in both trained and untrained hands.

Before conducting the main tDCS studies, we carried out a TMS reliability study. Since TMS was used to assess M1 CSE within experiments in this thesis, we needed to conduct a reliability study to examine the intra- and intersession reliability of the TMS induced motor evoked potentials (MEPs) to assure that any changes that will be observed in the tDCS experiments are due to physiological changes after therapeutic intervention within the subject, and not due to errors arising from methodological inconsistencies. Chapter 3 outlines the

TMS reliability study, published in Basic and Clinical Neurosciences Journal titled "Reliability of motor evoked potentials induced by transcranial magnetic stimulation: The effects of initial motor evoked potentials". The results of this study revealed high degree of reliability of elicited MEP responses for myself as a rater. We also found that the removal of the first three or five MEPs did not affect the reliability of TMS outcome.

Regarding the growing interest in using SVIPT as a visuomotor sequence task in clinical and neuroscience research, we chose this task for experimental studies in this thesis. Chapter 4 explains development of macros in order to automate data analysis in different studies in this thesis (Chapters 5-7).

Chapter 5 presents a research study, published in the Journal of Bodywork and Movement therapies, titled "The effects of inter-trial interval on implicit learning of sequential visual isometric pinch task". This chapter explains that manipulation of inter-trial intervals does not affect implicit motor sequence learning using SVIPT.

Chapter 6 reports a research study, published in the Journal of Frontiers Human Neuroscience, titled "Single-session anodal tDCS with small-size stimulating electrodes over superficial the FPN sites does not affect motor sequence learning". This chapter examines the effects of single-session a-tDCS over superficial the FPN sites (DLPFC, M1 or PPC) on behavioural and cortical outcome measures. We found no significant differences in behavioural and cortical outcome measures in any a-tDCS groups. We concluded that, the trial-based nature of the outcome measures in this study and lack of sensitivity to detect small changes may be the reason behind the finding in this study. In the trial-based data handling method, which was used in most of tDCS studies, three outcome measures were considered: movement time, error rate and skill (Reis et al., 2015; Reis et al., 2009a; Saucedo Marquez et al., 2013; Schambra et al., 2011).

Therefore, the data in this study also handled and analysed based on event (force) based analysis of response time, reaction time and force deviations for both the trained and untrained hand blocks. In this method the above outcomes were measured for each target force across the whole block. The results were then presented in Chapter 7, titled: “Does single-session a-tDCS of FPN superficial sites affect motor sequence learning: an event based assessment of outcomes”. This chapter examines the effects of single-session a-tDCS over the DLPFC, M1 or PPC on response time, reaction time and force deviations for both the trained and untrained hands.

Finally, Chapter 8 provides a summary of key findings and general discussion of the thesis, including implications and future directions for research. The findings of this thesis increase our understanding into using a-tDCS over FPN sites for fast stage of learning in a pinch force control task such as SVIPT, within healthy participants. Such an understanding may help in development of NIBS protocols as a safe and non-invasive technique for improvement of motor sequence learning at initial stage of learning.

Declaration for Chapter 2

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

Nature of the contribution	Extent of the contribution (%)
Identification and 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 the contribution
Shapour Jaberzadeh	Guidance in framing of the manuscript, reviewing and provision of feedback on the manuscript drafts.
Paul B Fitzgerald	Reviewing and provision of feedback on the manuscript drafts.
Maryam Zoghi	Determining a quality score for the included articles as the second independent reviewer and provision of feedback on the manuscript drafts.

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

Candidate's name

Signature Date

Signature Date

Preamble to Chapter 2

Chapter 2 provides a systematic review and meta-analysis to determine the effects of single and multiple sessions of a-tDCS on two different tasks: the sequential finger tapping task/serial reaction time task (SEQTAP/SRTT) and the sequential visual isometric pinch task (SVIPT).

Aim:

To synthesize available evidence examining whether M1 a-tDCS has beneficial effect on improvement of motor sequence learning following fast (a single session) and slow (multiple sessions) stages of learning in healthy individuals.

Chapter 2. The effect of anodal transcranial direct current stimulation on motor sequence learning in healthy individuals: A systematic review and meta-analysis

The format of this chapter is consistent with the Journal of Brain and Cognition. Supplementary tables for this chapter are provided in Appendices 3-5.

Brain and Cognition 102 (2016) 1–12

Contents lists available at ScienceDirect

Brain and Cognition

journal homepage: www.elsevier.com/locate/b&c

The effect of anodal transcranial direct current stimulation on motor sequence learning in healthy individuals: A systematic review and meta-analysis

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ARTICLE INFO

Article history:
Received 6 July 2015
Revised 13 November 2015
Accepted 17 November 2015
Available online 9 December 2015

Keywords:
Transcranial direct current stimulation (tDCS)
Primary motor cortex
Motor sequence learning
Corticospinal excitability

ABSTRACT

A large number of studies have indicated the effect of anodal transcranial direct current stimulation (a-tDCS) on the primary motor cortex (M1) during motor skill training. The effects of a-tDCS on different stages of motor sequence learning are not yet completely understood. The purpose of this meta-analysis was to determine the effects of single and multiple sessions of a-tDCS on two different tasks: the sequential finger tapping task/serial reaction time task (SEQTAP/SRTT) and the sequential visual isometric pinch task (SVIPT). We searched electronic databases for M1 a-tDCS studies. Thirteen studies met the inclusion criteria. The results indicate that application of multiple sessions of a-tDCS, compared to single session a-tDCS induced a significant improvement in skill in both SEQTAP/SRTT and SVIPT. Retention after a single day and multiple days of a-tDCS was statistically significant for the SEQTAP/SRTT task but not for SVIPT. Therefore, our findings suggest that application of M1 a-tDCS across the three or five consecutive days can be helpful to improve motor sequence learning.

1. Introduction

Motor sequence learning is defined as an inherent ability in humans to learn sequential actions, which has essential role in everyday life. This ability help us to learn numerous human skills from simple tasks such as pressing a button to complex activities like playing a piano (Keele, Ivry, Mayr, Hazeltine, & Heuer, 2003). Sequence motor learning can be categorized into two groups: explicit and implicit. In explicit motor sequence, learning occurs with awareness of sequential ordering of stimuli while in implicit motor sequence learning participants are not aware of this sequential ordering (Robertson, 2007).

A number of tasks have been developed to investigate different aspects of motor sequence learning. A frequently used paradigm is serial reaction time task (SRTT) in which participants respond to visual cue that appeared in one of four horizontal locations on a computer screen by pressing a key that corresponded to the stimulus locations (Keele et al., 2003; Robertson, 2007). Another commonly used task is sequential finger tapping task (SEQTAP) in which participants respond to a series of numbers from 1 to 4 displayed on a computer screen by pressing the corresponding button with the corresponding finger (Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002). Another paradigm have been used to assess learning a sequence of forces is visual isometric pinch force task (SVIPT), in which participants learn how to control precisely their fingertip forces in a sequenced order of different target forces. Changes in movement speed, accuracy as well as skill, which are measured by combination of both speed and accuracy, could be considered as behavioural outcome measures to monitor improvement following motor sequence tasks.

In contrast to motor sequence learning, sensory-motor adaptation is the trial-and-error process of adjusting movement to new demands in which participants learn how to adapt a known movement to individuals or environmental changes such as driving a new car, adapting to perturbation caused by altered visual feedback on a computer screen or adapting to physical changes following an injury (Hill, Davey, & Kennard, 2000; Penhune & Steele, 2012). Therefore, performance improvements in motor adaption tasks occur as participants learn to return to a former level of performance whereas in motor sequence learning tasks, a higher level of skill acquired.

Improvement in outcome measures of motor learning can be occurred during training (online) but also after the training has

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http://dx.doi.org/10.1016/j.bandc.2015.11.005
0278-2626/

ended (offline). Online and offline skill gains can be retained over time, resulting in long-term retention (Romano, Howard, & Howard, 2010). Therefore, motor sequence learning is characterized by fast and slow stages of learning. Fast learning occurs early on, within a single training session, and slow stage learning occurs later, in which incremental gains are achieved over multiple sessions of practice (Dayan & Cohen, 2011).

In the process of motor sequence learning, the functional properties of different brain areas can change as a result of practice and experience (Karni et al., 1998). Animal (Rioult-Pedotti, Friedman, & Donoghue, 2000) and human studies (Rosenkranz, Kacar, & Rothwell, 2007; Stefan et al., 2006; Ziemann, Ilic, Pauli, Meintzschel, & Ruge, 2004) have shown a strong link between motor learning and brain neuroplasticity. The process of motor skill learning involves the strengthening of synaptic connectivity. Long-term potentiation (LTP) has been identified as the likely physiological basis of learning (Rioult-Pedotti, Friedman, & Donoghue, 2000; Stefan et al., 2006; Ziemann et al., 2004). Depending on the task and the learning phase, different brain regions are engaged (Dayan & Cohen, 2011; Doyon & Ungerleider, 2002; Karni et al., 1998). One area of the brain, which is engaged in motor learning, is the primary motor cortex (M1) (Classen, Liepert, Wise, Hallett, & Cohen, 1998; Karni et al., 1995; Nudo, Milliken, Jenkins, & Merzenich, 1996; Pascual-Leone et al., 1995). This area has a crucial role in acquisition and consolidation of motor learning (Muellbacher et al., 2002; Nitsche et al., 2003).

Imaging studies demonstrated that M1 is differentially modulated during fast and slow stages of learning (Dayan & Cohen, 2011; Floyer-Lea & Matthews, 2005). There is no consensus on the activity of M1 during the fast or early stage of motor learning. Some studies showed decreased M1 activity (Downs & Black, 1998; Doyon & Ungerleider, 2002; Toni, Krams, Turner, & Passingham, 1998), while other researches showed increased activity. A number of studies did not show any changes in the activity of M1 in this phase (Downs & Black, 1998; Toni et al., 1998). In contrast to the fast stage of motor learning, there is a consensus on increased activation of M1 during the slow phase of learning (Dayan & Cohen, 2011; Floyer-Lea & Matthews, 2005; Karni et al., 1998). Due to the role of plastic changes in the cortical areas of the brain during motor skill learning (Pascual-Leone, Grafman, & Hallett, 1994; Pascual-Leone et al., 1995), non-invasive neuromodulatory techniques hold promise for enhancement motor learning through changes in corticospinal excitability (CSE).

Transcranial direct current stimulation (tDCS) is a safe and non-invasive technique to modulate CSE in a polarity-dependent manner (Nitsche et al., 2008; Priori, Berardelli, Rona, Accornero, & Manfredi, 1998). Anodal-tDCS (a-tDCS) leads to increased CSE (Nitsche & Paulus, 2000), while cathodal tDCS (c-tDCS) may results in decreased CSE (Nitsche et al., 2008; Priori et al., 1998). In a number of studies, a-tDCS was applied over M1 to boost the effects of training during variety of task paradigms such as SRTT (Kang & Paik, 2011; Kantak, Mummidisetty, & Stinear, 2012; Nitsche et al., 2003), SEQTAP (Kantak et al., 2012; Saucedo Marquez, Zhang, Swinnen, Meesen, & Wenderoth, 2013; Tecchio et al., 2010), SVIPT (Reis et al., 2009; Saucedo Marquez et al., 2013; Schambra et al., 2011), adaptation tasks (Kaski, Quadir, Patel, Yousif, & Bronstein, 2012), tracking tasks (Prichard, Weiller, Fritsch, & Reis, 2014) as well as other tasks such as Jebsen–Taylor Hand Function (Butts, Kolar, & Newman-Norlund, 2014).

Regarding to task specific effect of a-tDCS on motor learning (Saucedo Marquez et al., 2013), we focus on motor sequence tasks in this systematic review and meta-analysis. Although beneficial effects of a-tDCS over M1 for improvement of motor sequences have been identified (Cuypers et al., 2013; Nitsche et al., 2003; Reis et al., 2009; Saucedo Marquez et al., 2013; Schambra et al., 2011; Vines, Cerruti, & Schlaug, 2008; Vines, Nair, & Schlaug, 2008), the exact nature of involvement of M1 during application of single and multiple sessions a-tDCS at different stages of motor sequence learning is not yet understood. Therefore, the aim of this systematic review and meta-analysis was to investigate the effects of M1 a-tDCS on behavioural outcomes following single or multiple sessions of a-tDCS in both SEQTAP/SRTT and SVIPT.

2. Methods

2.1. Literature search

PubMed, Ovid Medline, Scopus, PROQuest, CINAHL, EMBASE, EBM reviews, Cochrane Library, Physiotherapy Evidence Database (PEDro) and SPORT Discuss were searched for appropriate studies published any time before February 2015. We also searched reference lists of all retrieved papers for additional references. Key search terms were: transcranial direct current stimulation, tDCS, non-invasive brain stimulation, corticospinal excitability, motor skill learning, motor sequence learning, transcranial magnetic stimulation, and TMS. This process identified 1708 articles and, after discarding duplicates, 1287 remaining articles were screened for suitability for inclusion in this meta-analysis.

2.2. Selection criteria

2.2.1. Inclusion criteria

Articles were included if they met the following criteria: (1) application of a-tDCS over M1, with conventional or other montages such as dual-hemisphere M1 stimulation or using an extra cephalic reference electrode, during motor sequence learning tasks; (2) having a control group (sham plus training or training only); (3) measurement of behavioural changes (such as movement speed, accuracy and skill) or CSE changes; (4) healthy individuals, and (5) published in peer-reviewed journals in English.

2.2.2. Exclusion criteria

In this systematic review, we focused on concurrent application of M1 a-tDCS during sequence motor learning tasks in upper limb. Therefore, we excluded articles if they applied a-tDCS during other tasks such as tracking tasks (Prichard et al., 2014), cognition tasks such as games, or adaptation tasks (Galea, Vazquez, Pasricha, Orban de Xivry, & Celnik, 2011; Hunter, Sacco, Nitsche, & Turner, 2009) and other tasks (Galea & Celnik, 2009; Minarik, Sauseng, Dunne, Berger, & Sterr, 2015). Studies that applied a-tDCS with a combination of therapeutic interventions, such as mental practice, motor imagery and pharmacological interventions (Kuo et al., 2008) were also excluded. Application of M1 a-tDCS before (Kuo et al., 2008) or after (Cantarero, Tang, O'Malley, Salas, & Celnik, 2013; Tecchio et al., 2010) motor sequence tasks were not included. Animal studies (Fritsch et al., 2010), reviews, case reports and letters were also excluded.

2.3. Quality assessment

Two researchers independently reviewed each included article and determined a quality score using the Physiotherapy Evidence Database (PEDro scale) (Moseley, Herbert, Sherrington, & Maher, 2002; Möcks, Gasser, & Tuan, 1984). The PEDro scale contains some items to assess the external and internal validity of the article, graded on a "yes/no" scale. The PEDro scale results in total scores from 0 to 10, with a higher PEDro score providing a surrogate indication of higher quality.

2.4. Experimental tasks in the included studies

We included studies that evaluated the effect of applying a-tDCS over M1 on behavioural outcomes during two different motor sequence tasks: Sequential key pressing tasks (SEQTAP/SRTT) and sequential force tasks (SVIPT).

2.5. Sequential finger tapping (SEQTAP/SRTT)

SEQTAP is a finger sequence pressing task using the dominant or non-dominant hand, in which subjects press different keys on a keyboard after being shown a series of sequenced numbers from 1 to 4 on a computer screen. In this task, participants are instructed to press the corresponding number on the keyboard with the corresponding finger, for example: index finger for number 1, middle finger for number 2, ring finger for number 3 and little finger for number 4. Each key press produced a black dot on the screen appeared below the corresponding number indicating pressing a given number independently of its correctness. (Cuypers et al., 2013; Saucedo Marquez et al., 2013; Vines, Cerruti, et al., 2008; Vines, Nair, et al., 2008; Zimerman et al., 2013). In SRTT, a visual cue such as asterisk can appear in any of the four position on the computer screen which horizontally spaced from left to right and the participants are instructed to press a corresponding key to the position of asterisk on a key response numbered from 1 to 4. For example, for the most left position of asterisk pressing button 1 with index finger and for the second left side press button 2 with middle finger and so on (Kang & Paik, 2011; Kantak et al., 2012; Nitsche et al., 2003). Therefore, the SRTT is a four-choice reaction time task in which participants learn a sequence of visual cue positions while SEQTAP is independent of location of stimulus in which participants learn a sequence of numeric numbers from 1 to 4. In both task, participants are instructed to respond to visual cues by pressing a corresponding button with corresponding finger. Thus, in this review and meta-analysis we use the term SEQTAP/SRTT for both types of sequential key pressing tasks.

2.6. Sequential visual isometric pinch task (SVIPT)

SVIPT is a force task in which participants are instructed to control a cursor displayed on a computer monitor with a force transducer, which held between the thumb and index finger. The participants move the cursor between the home position and target zones by altering the pinch force exerted onto the transducer (Reis et al., 2009, 2015; Saucedo Marquez et al., 2013; Schambra et al., 2011). However, SVIPT and SEQTAP/SRTT tasks are both useful paradigms to examine different aspects of motor sequence learning, their actual task demand differ; SVIPT have greater motor demands and emphasize precisely control of pinch force, while key pressing tasks have relatively minimal motor demands and focus on cognitive functions recruited during sequential motor behaviour. Therefore, we analyzed data in both tasks separately in this systematic review and meta-analysis.

2.7. Outcome measures

Changes in movement speed, accuracy and acquired skill can be considered as outcome measures for assessment of motor sequence learning.

2.7.1. Movement speed

Decrease in execution time (time from first key press to last key release) or reaction time (time from stimulus to any finger force above resting range) during the SEQTAP/SRTT task provides evidence that learning has occurred. In some studies (Kang & Paik, 2011; Kantak et al., 2012; Nitsche et al., 2003), in order to differentiate sequence learning from general training effects, a block with a random order or a new sequence of numbers was inserted between sequence blocks. Difference in reaction time between sequence and random blocks serves as an index for sequence learning (Abrahamse, Jimenez, Verwey, & Clegg, 2010). In this meta-analysis, normalized reaction time and response time were considered as outcome measures for improvement of speed in the SEQTAP/SRTT tasks. Normalized reaction time is obtained from mean sequence-block time divided by mean random-block time (Kang & Paik, 2011; Kantak et al., 2012; Nitsche et al., 2003). Response time was obtained from first key press to the last key press. In the current study, we used the term of movement speed for both reaction time and response time.

2.7.2. Accuracy

An increase in the number of correct sequences represents accuracy improvement in motor skill learning. It can be calculated from the difference between the numbers of correct sequences relative to the baseline (Zimerman et al., 2013) or the percentage change in the total number of correct sequential keystrokes over some trials (Karok & Witney, 2013; Vines, Cerruti, et al., 2008; Vines, Nair, et al., 2008). This calculation, whether positive or negative, preserves the sign of performance change (Vines, Nair, et al., 2008).

2.7.3. Skill

Most motor tasks are affected by the so-called speed–accuracy trade-off, i.e., accuracy decreases when speed increases and vice versa. A skill index considers two parameters: speed and accuracy. In two articles, the index was obtained by dividing the percentage of correct sequences by the average time per trial (Cuypers et al., 2013; Saucedo Marquez et al., 2013). In contrast, Waters-Metenier et al. (2014) measured execution time while participants were instructed to keep the error rate at a constant level. Because there is a correlation between skill and execution time Waters-Metenier' measurements can also be considered as a surrogate for skill (Waters-Metenier et al., 2014).

In SVIPT studies (Reis et al., 2009, 2015; Saucedo Marquez et al., 2013; Schambra et al., 2011), skill can be considered as an outcome measure and obtained from the combination of response time and error rate

The following formula was applied:

$$\text{Skill} = \frac{1 - \text{error rate}}{\text{error rate } (\ln(\text{duration})^b}$$

where error rate and duration are averaged over some trials. The b is a dimensionless parameter which was estimated by non-linear least squares regression. This value has been set to $b = 5.424$ in some studies (Reis et al., 2009; Schambra et al., 2011) but when individually determined, values ranged from 2.9 to 8.1 in one study (Saucedo Marquez et al., 2013).

2.8. Data extraction

The following data were extracted from each article: study design, sample size, characteristics of a-tDCS intervention, task paradigms and measured outcomes (Table 1). Mean and standard deviations (*SD*) of outcome measures in different temporal component motor learning (during intervention, post intervention and retention) were extracted for each article whenever possible. Where the required data were not reported, we contacted the corresponding author(s) to request the original data. If the authors did not respond, a JAVA-based Plot Digitizer (Joseph, 2010) was used to directly estimate mean and *SD* from graphs.

Table 1
Characteristics of included studies.

Included	Study design	Sample size (mean ± *SD*)	Task	Performed hand	Practice trials (training time)	Training sessions	Outcome	Data extraction	PEDro/10
Schambra et al. (2011)	Single blinded sham controlled	tDCS LM1 (14): 7F (27.1 ± 1.3) shamLM1 (14): 7F (29.9 ± 1.7 tDCS RM1 (15): 9F (29.6 ± 2.5) shamRM1 (14): 7F (26.5 ± 1.2	SVIPT	Right Left	6 blocks of 200 trials (45 min)	3 consecutive days	Skill	Post intervention	7
Reis et al. (2009)	Doubled blinded sham controlled	tDCS (12): 7F (28.3 ± 2.2) Sham (12): 5F (30.8 ± 3.0)	SVIPT	Right	6 blocks of 200 trials (45 min)	5 consecutive days	Skill	Post intervention Retention 3 days after	8
Saucedo Marquez et al. (2013)	Doubled blinded sham controlled cross over	27 (12F) right handed tDCS (14): 6F (23.14 ± 2.6) Sham (13): 6F (24.85 ± 3.51)	SVIPT	Left	7 blocks (20 min)	3 consecutive days	Skill	Post 20 Retention: 1 week	8
Reis et al. (2015)	Doubled blinded sham controlled study	34 (18F) right handed tDCS (17): 10F (27.1 ± 1) Sham (17): 8F (27.2 ± 1)	SVIPT	Right	5 blocks of 160 trials (40 min)	3 consecutive days	Skill	Post 15	8
Saucedo Marquez et al. (2013)	Doubled blinded sham controlled cross over	27 (12F) right handed tDCS (14): 6F (23.14 ± 2.6) Sham (13): 6F (24.85 ± 3.51)	SEQTAP (five digits)	Left	20 trials (20 min)	3 consecutive days	Skill	Post intervention: 20 min after Retention: 1 week after	8
Cuypers et al. (2013)	Doubled blinded sham controlled cross over	13 (6F) (19.92 ± 1.12)	SEQTAP (eight digits)	Dominant hand	20 training blocks (20 min)	Single day	Skill	Post intervention	7
Kantak et al. (2012)	Randomized Cross over design	12	Implicit SRTT (10 digits)	Left	600 trials (15 min)	Single day	Ratio of reaction time (S/R)	Post intervention (5 min after) Retention: 24 h after	5
Kang and Paik (2011)	Randomized Cross over design	11 (8F) (26.3 ± 3.6) right handed	Implicit SRTT (12 digits)	Right	20 blocks of 120 trials (20 min)	Single day	Ratio of reaction time (S/R)	During stimulation: Block S6 Post: Immediately after Retention: 24 h after	6
Nitsche et al. (2003)	Cross over design	20 (23–34) Right and left handed	Implicit SRTT (12 digits)	Right	8 blocks of 120 trials (15 min)	Single day	Standardized response time (S/R)	During stimulation	6
Waters-Metenier et al. (2014)	Sham controlled	24 (10F) (21.90 ± 0.4) a tDCS: 12 Sham: 12 right handed	SEQTAP (5 digits)	Left	384 trials (60 min)	4 consecutive days	Execution time	Post intervention Retention: 1 week after	7
Zimerman et al. (2013)	Doubled blinded sham controlled cross over	10 (25.2 ± 2.9) right handed	SEQTAP (5 digits)	Right	Five blocks (25 min)	Single day	Number of correct sequence, Block 5–Block 1	Post intervention	7
Vines, Cerruti, et al. (2008)	Cross over design	16 (27.6 ± 3.6) right handed	SEQTAP (5 digits)	Left	Three trials (4 min)	Single day	The percentage of change in the number of correct, from pre tDCS to post tDCS	Post intervention Immediately after	5

Table 1 (*continued*)

Included	Study design	Sample size (mean ± *SD*)	Task	Performed hand	Practice trials (training time)	Training sessions	Outcome	Data extraction	PEDro/10
Vines, Nair, et al. (2008)	Cross over design	17 right handed	SEQTAP (5 digits)	Right and Left	Three trials (4 min)	Single day	The percentage of change in the number of correct keystrokes, from pre tDCS to post tDCS	Post intervention Immediately after	5
Karok and Witney (2013)	Sham controlled cross over	20 (25.6 ± 4.5) right handed	SEQTAP (6 digits)	Left	12 trials (4 min)	Single day		Post intervention Immediately after	6

F: female; SVIPT: sequential visual isometric pinch task; SRTT: serial reaction time tasks; SEQTAP: sequential finger tapping task; B: Block; S: sequence; R: Random.

In instances where *SD*s was not provided, we calculated or imputed them based on available data. The following formula was used to obtain *SD*s.

$$SD = SE\sqrt{n}$$

where *SE* = standard error and *n* = number of subjects in each group.

In studies with more than two groups eligible to be included in the same meta-analysis, the sham group was split into two groups with smaller sample sizes to overcome a unit of analysis error (Higgins & Green, 2008).

2.9. Data analysis

Cochrane Collaboration Revman (V 5.2) software was used to calculate effect sizes of a-tDCS intervention in motor sequence learning. We used the standardized mean difference (SMD) to estimate the effect size of the intervention in each study relative to the sham group. In meta-analysis, SMD is used to standardize the results of the studies when the studies all assess the same outcome but measure it in a variety of ways. SMD, which is most frequently used in meta-analysis, can be statistically pooled from different studies as a best estimate of the size of the effect of a particular intervention. The effect size calculation was based on the mean, *SD* and size of the studied samples (Hedges & Olkin, 1985; Wolf, Lecraw, Barton, & Jann, 1989). In this review, effect sizes were obtained using the difference between the mean of the a-tDCS group and the sham group, divided by the pooled *SD*s of both groups. We employed a fixed effect analysis where heterogeneity was not substantial, guided by recommendations by Higgins, Thompson, Deeks, and Altman (2003). Data were pooled in the meta-analysis using a random effect model if statistical heterogeneity was detected between trials.

The I^2 statistic was used to quantify statistical heterogeneity. This indicates the percentage of variability due to heterogeneity rather than to chance alone. An I^2 of 0% indicates no heterogeneity; greater values indicate increasing heterogeneity, and values greater than 50% imply substantial heterogeneity (Higgins et al., 2003). We also used χ^2 tests for homogeneity. The assumption of homogeneity was deemed not valid if $p < 0.1$.

The meta-analysis was conducted with alpha set at 0.05 for differences between groups expressed as a pooled effect size. According to Cohen (1988), an effect size of 0.2 demonstrates a small effect, 0.5 a moderate effect, and 0.8 and above indicate large intervention effects (Cohen, 1988). Where the pooled difference between a-tDCS and sham was significant at any time point (during intervention, post intervention or retention), we concluded that a-tDCS was effective in improving motor sequence learning.

3. Results

3.1. Literature search

As shown in Fig. 1, the electronic search identified 1708 records. After removal of duplicates, the remaining 1287 studies were manually reviewed. Abstract examination for eligibility excluded 1147 articles that did not satisfy pre-established inclusion criteria. Of the 140 articles fully examined, 127 articles were excluded for various reasons. The most common reasons for exclusion were applying different intervention tasks (adaptation and cognition), and lack of sham or control groups. Fourteen studies met all inclusion criteria; one article (Stagg et al., 2011) was subsequently excluded due to insufficient data. Thirteen articles were therefore selected for data extraction and meta-analysis, but as seven of them provided two comparisons, the total number of comparisons was 20.

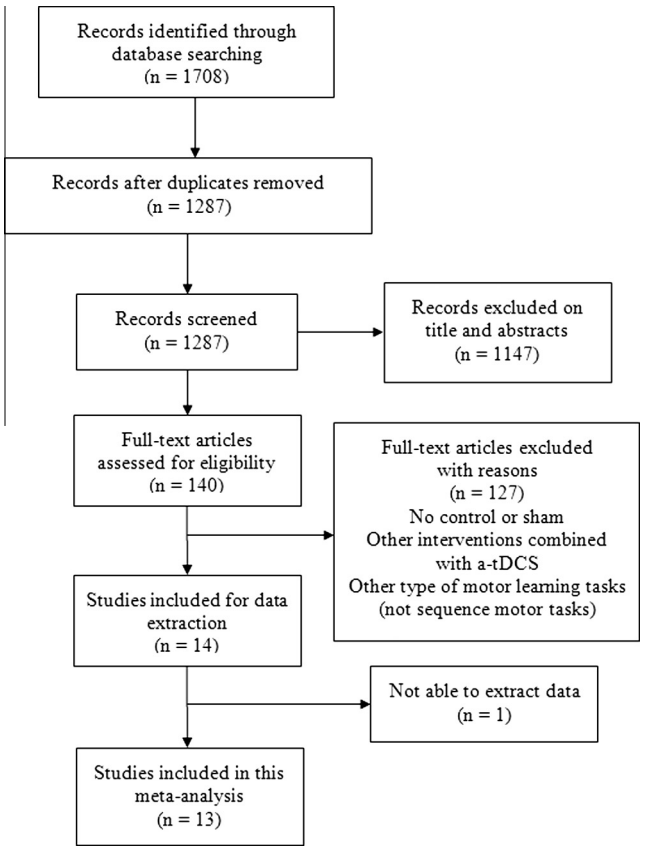

Fig. 1. Flow chart of systematic review.

3.2. Quality analysis

The PEDro score of included studies ranged between 5 and 8 with a mean score of 6.64 out of 10. The homogeneity in quality scores suggests that most included studies used a similar design. The greatest source of bias was blinding and randomization. Only six studies included random allocation (Kang & Paik, 2011; Kantak et al., 2012; Karok & Witney, 2013; Nitsche et al., 2003; Reis et al., 2015; Saucedo Marquez et al., 2013) and five studies used double blinding (Cuypers et al., 2013; Reis et al., 2015; Saucedo Marquez et al., 2013; Waters-Metenier et al., 2014; Zimerman et al., 2013), while one study used single blinding (Schambra et al., 2011). The baseline skills in both groups (a-tDCS and Sham) were similar in all the studies included in our meta-analysis.

3.3. Study characteristics

The 13 articles meeting the inclusion criteria had a total of 292 healthy participants with range of mean age between 19.92 and 30.8 years. Table 1 shows the characteristics of the included studies. Eight studies (Cuypers et al., 2013; Kang & Paik, 2011; Kantak et al., 2012; Karok & Witney, 2013; Nitsche et al., 2003; Vines, Cerruti, et al., 2008; Vines, Nair, et al., 2008; Zimerman et al., 2013) applied a-tDCS during a single session and five studies (Reis et al., 2009, 2015; Saucedo Marquez et al., 2013; Schambra et al., 2011; Waters-Metenier et al., 2014) used anodal stimulation over multiple sessions ranging from 3 to 5 days.

Four studies used interventions during SVIPT (Reis et al., 2009, 2015; Saucedo Marquez et al., 2013; Schambra et al., 2011), while the others used stimulation during the SEQTAP/SRTT task. Sequence length varied in the included studies. Four studies (Cuypers et al., 2013; Kang & Paik, 2011; Kantak et al., 2012; Nitsche et al., 2003) used an SEQTAP/SRTT task with sequence length ranging from 8 to 12 digits. Participants' awareness about the sequence repetition was formally examined by a question at the end of the experiments (Kang & Paik, 2011; Kantak et al., 2012; Nitsche et al., 2003). This confirmed that implicit nature of learning in these studies. Five studies (Karok & Witney, 2013; Vines, Cerruti, et al., 2008; Vines, Nair, et al., 2008; Waters-Metenier et al., 2014; Zimerman et al., 2013) used shorter sequences of 5 or 6 digits such shorter sequences are probably linked to more explicit learning. The types of learning was explicitly reported in studies (Karok & Witney, 2013; Vines, Nair, et al., 2008; Waters-Metenier et al., 2014; Zimerman et al., 2013).

In most of the included studies a-tDCS was applied with a classical montage, the active electrode (anode) was fixed over the M1 contralateral to the performing hand and the reference electrode was applied over the contralateral supraorbital area (Cuypers et al., 2013; Kang & Paik, 2011; Nitsche et al., 2003; Reis et al., 2009, 2015; Schambra et al., 2011; Vines, Nair, et al., 2008; Zimerman et al., 2013). In two articles, the reference electrode was applied over the ipsilateral shoulder (Saucedo Marquez et al., 2013; Schambra et al., 2011). In four studies (Kang & Paik, 2011; Karok & Witney, 2013; Vines, Cerruti, et al., 2008; Waters-Metenier et al., 2014) the reference electrode was applied over the ipsilateral hemisphere to investigate the effects of dual-hemisphere stimulation. Nine studies stimulated the right M1 while participants trained with the left hand (non-dominant hand). In two studies, participants used either their right or left hands (Schambra et al., 2011; Vines, Nair, et al., 2008) while a-tDCS stimulated the contralateral M1 and the other studies applied a-tDCS on the left M1 during training of the right hand. A-tDCS characteristics are shown in Table 2. Current densities varied from 0.04 to 0.125 mA/cm^2 and durations ranged from 10 to 25 min in all included studies.

In eight studies, transcranial magnetic stimulation (TMS) was used to locate the M1 for the first dorsal interosseous (FDI) muscle (Cuypers et al., 2013; Kantak et al., 2012; Karok & Witney, 2013; Reis et al., 2009, 2015; Schambra et al., 2011; Waters-Metenier et al., 2014; Zimerman et al., 2013). In other studies, the stimulation site of a-tDCS electrodes was determined using the international 10–20 system. In all included studies, behavioural outcomes were measured to determine the effects of a-tDCS on motor sequence learning. Four articles measured ratios of reaction time (Kang & Paik, 2011; Kantak et al., 2012; Nitsche et al., 2003) or execution time (Waters-Metenier et al., 2014). Four studies measured accuracy (Karok & Witney, 2013; Vines, Cerruti, et al., 2008; Vines, Nair, et al., 2008; Zimerman et al., 2013) and five articles calculated skill as an outcome measure (Cuypers et al., 2013; Reis et al., 2009, 2015; Saucedo Marquez et al., 2013; Schambra et al., 2011). Participants were similar in terms of behavioural outcomes such as reaction time, percentage of errors and skill and no significant differences were reported between sham and a-tDCS groups at baseline. As shown in Table 2, behavioural outcomes were measured during intervention, post intervention and during a retention phase. The required data for meta-analysis were directly derived from three studies (Karok & Witney, 2013; Schambra et al., 2011; Vines, Cerruti, et al., 2008). In four studies, the corresponding author provided the original data (Cuypers et al., 2013; Kang & Paik, 2011; Kantak et al., 2012; Reis et al., 2009). In the remaining studies, the data were extracted using plot digitizer software.

All included articles compared the effects of a-tDCS and sham, except for one study that compared a-tDCS and a non-current stimulation group (Nitsche et al., 2003). For the sham a-tDCS, participants received the current in a ramp-like fashion but

Table 2
Characteristics of a-tDCS.

	Electrode montage	Electrode size	Intensity current/duration	Intensity/density	Time of training	TMS
Schambra et al. (2011)	A = Left M1 and Right M1 R = Ipsilateral shoulder	A = 25 cm^2 R = 25 cm^2	1 mA 20 min	0.04 mA/cm^2	During training	FDI Hot spot
Reis et al. (2009)	A = Left M1 R = Right supraorbital	A = 25 cm^2 R = 25 cm^2	1 mA 20 min	0.04 mA/cm^2	During training	FDI Hot spot
Saucedo Marquez et al. (2013)	A = Right M1 R = Ipsilateral shoulder	A = 25 cm^2 R = 99 cm^2	1 mA 20 min	0.04 mA/cm^2	During training	
Reis et al. (2015)	A = Left M1 R = Right supraorbital	A = 16 cm^2 R = 16 cm^2	1 mA 20 min	0.062 mA/cm^2	During training	FDI Hot spot
Cuypers et al. (2013)	A = M1 (contralateral of dominant hand) R = Contralateral supraorbital	A = 25 cm^2 R = 50 cm^2	1 mA and 1.5 mA 20 min	0.04 mA/cm^2 0.06 mA/cm^2	During training	FDI Hot spot
Kantak et al. (2012)	A = Right M1 R = Left supraorbital	A = 8 cm^2 R = 48 cm^2	1 mA 15 min	0.125 mA/cm^2	During training	FDI Hot spot
Kang and Paik (2011)	Uni tDCS: (A = Left M1; R = Right supraorbital) BitDCS: (A = Left M1; R = Right M1)	A = 25 cm^2 R = 25 cm^2	2 mA 20 min	0.08 mA/cm^2	During training	
Nitsche et al. (2003)	A = Left M1 R = Right supraorbital	A = 35 cm^2 R = 35 cm^2	1 mA 15 min	0.028 mA/cm^2	During training	
Waters-Metenier et al. (2014)	A = Right M1 R = Left M1	A = 35 cm^2 R = 35 cm^2	2 mA 25 min	0.05 mA/cm^2	60 min training	FDI Hot spot
Zimerman et al. (2013)	A = Left M1 R = Right supraorbital	A = 25 cm^2 R = 25 cm^2	1 mA 20 min	0.04 mA/cm^2	During training	FDI Hot spot
Vines, Cerruti, et al. (2008)	Uni tDCS (A = Right M1 and R = left supraorbital) Dual tDCS (A = Right M1 and R = Left M1)	A = 16.3 cm^2 R = 30 cm^2	1 mA 20 min	0.06 mA/cm^2	During training	
Vines, Nair, et al. (2008)	A = Right and Left M1 R = Contralateral supraorbital	A = 16.3 cm^2	1 mA	0.06 mA/cm^2	During training	
Karok and Witney (2013)	Uni tDCS (A = Right M1 and R = Left supraorbital) Dual tDCS (A = Right M1 and R = Left M1)	A = 25 cm^2 R = 35 cm^2	1.5 mA 10 min	0.06 mA/cm^2	During training	FDI Hot spot

A: Active electrode; R: reference electrode; TMS: transcranial magnetic stimulation; FDI: first dorsal interosseous muscle.

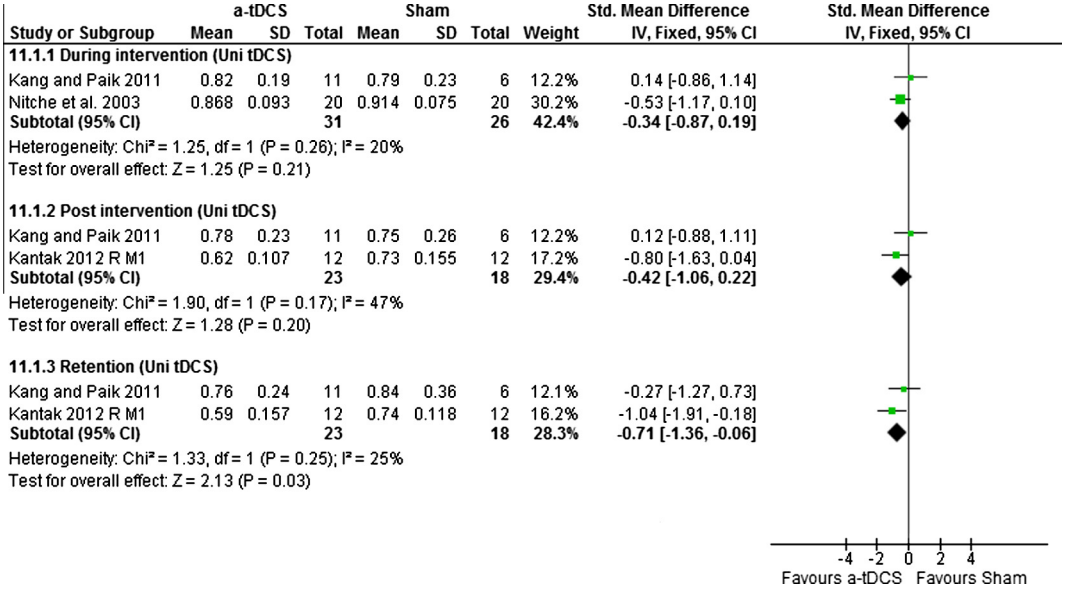

Fig. 2. Meta-analysis of single session uni-hemisphere stimulation on movement speed in SEQTAP/SRTT task.

stimulation faded out slowly after 10 (Kantak et al., 2012) or 30 s (Reis et al., 2009; Vines, Cerruti, et al., 2008; Vines, Nair, et al., 2008). For the non-current group no stimulation was used at all.

For four studies (Kang & Paik, 2011; Karok & Witney, 2013; Vines, Cerruti, et al., 2008; Vines, Nair, et al., 2008), in which control data were compared to data for two different interventions in the same meta-analysis, the control group sample size was halved.

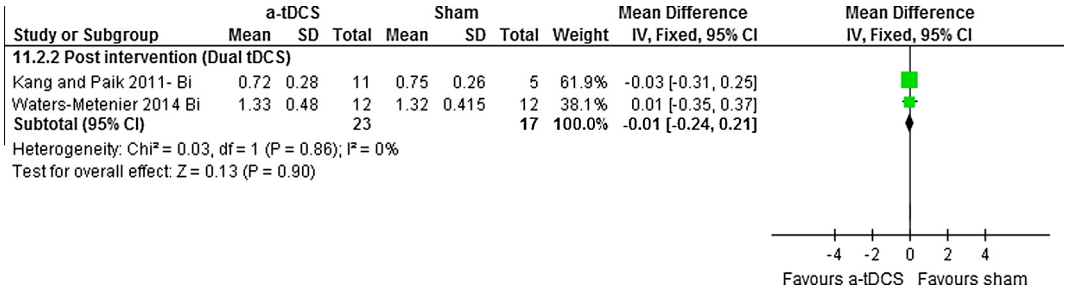

Fig. 3. Meta-analysis of single session dual-hemisphere stimulation on movement speed in SEQTAP/SRTT task.

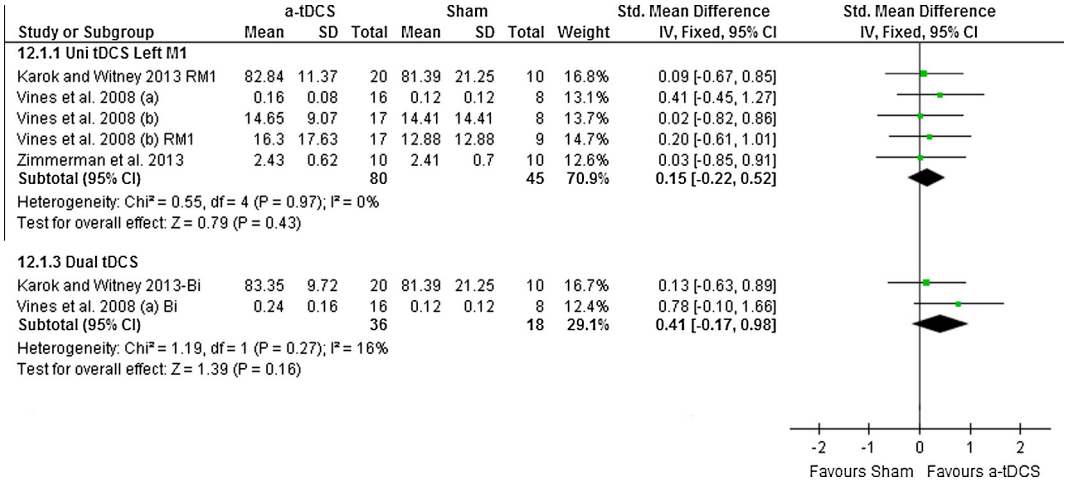

Fig. 4. Meta-analysis of single session a-tDCS on accuracy in SEQTAP/SRTT task.

3.4. Pooled data analysis

The 13 articles, describing a total of 20 studies, enabled a total of 36 comparisons. Performance was evaluated during both single and multiple a-tDCS, for two different motor sequence tasks separately. Three outcome measures were evaluated: movement speed, accuracy, and skill. We sub-grouped data based on uni- and dual-hemispheric montage in movement speed and accuracy during SEQTAP/SRTT task. Given the limited number of studies in multiple sessions we pooled data uni- and dual-tDCS in two studies in the SEQTAP/SRTT (Saucedo Marquez et al., 2013; Waters-Metenier et al., 2014). All studies in this meta-analysis applied M1 a-tDCS with a classical montage except two studies (Saucedo Marquez et al., 2013; Schambra et al., 2011), which M1 a-tDCS were employed with an extracephalic montage. Where data from different types of intervention were pooled, we evaluated the impact on overall results by excluding each individual study in turn. No significant changes were observed on the general results after removing individual studies with different types of intervention.

3.5. Effects of single session a-tDCS on movement speed in SEQTAP/SRTT

Subgroup analysis showed no significant changes in movement speed during or following single session uni-hemisphere stimulation in the SEQTAP/SRTT task while a significant change was seen at retention with the SMD of −0.71 (95% CI, −1.36 to −0.06, $p = 0.02$) (Fig. 2).

As shown in Fig. 3, no significant change was seen after application of dual-hemisphere stimulation on movement speed in motor sequence learning.

3.6. Effects of single session a-tDCS on accuracy during SEQTAP/SRTT

The effects of single session uni- and dual-hemisphere stimulation on accuracy were examined in five articles (Karok & Witney, 2013; Vines, Cerruti, et al., 2008; Vines, Nair, et al., 2008; Waters-Metenier et al., 2014; Zimerman et al., 2013). The results have shown no significant changes on accuracy in motor sequence learning after application of either uni- or dual-hemisphere stimulation. The pooled SMDs using the fixed effects model after uni and dual tDCS were 0.15 (95% CI: −0.19, 0.52, $p = 0.43$) and 0.41(95% CI: −0.17, 0.98, $p = 0.16$) respectively (Fig. 4).

3.7. Effects of single session a-tDCS on skill in SEQTAP/SRTT and SVIPT

The pooled analysis showed no significant changes in skill measure after single session a-tDCS in both tasks, the SEQTAP/SRTT and SVIPT (Fig. 5).

3.8. Effects of multiple session a-tDCS on skill in SEQTAP/SRTT

The results of application of multiple session a-tDCS indicate that application of three or four days of a-tDCS led to a significant result at post-intervention and retention time points. The SMD using a fixed effects model was 0.97 (95% CI: 0.39, 1.56,

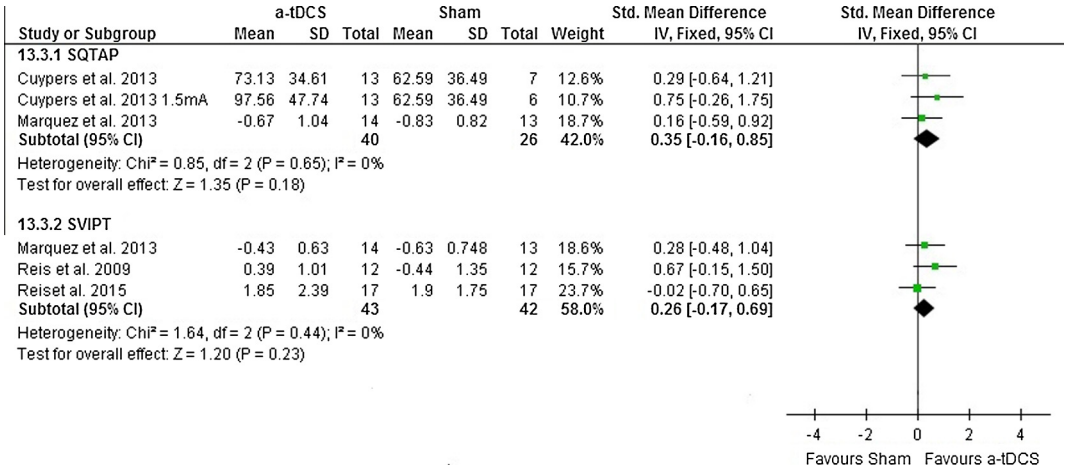

Fig. 5. Meta-analysis of single session a-tDCS on skill measure in SEQTAP/SRTT and SVIPT.

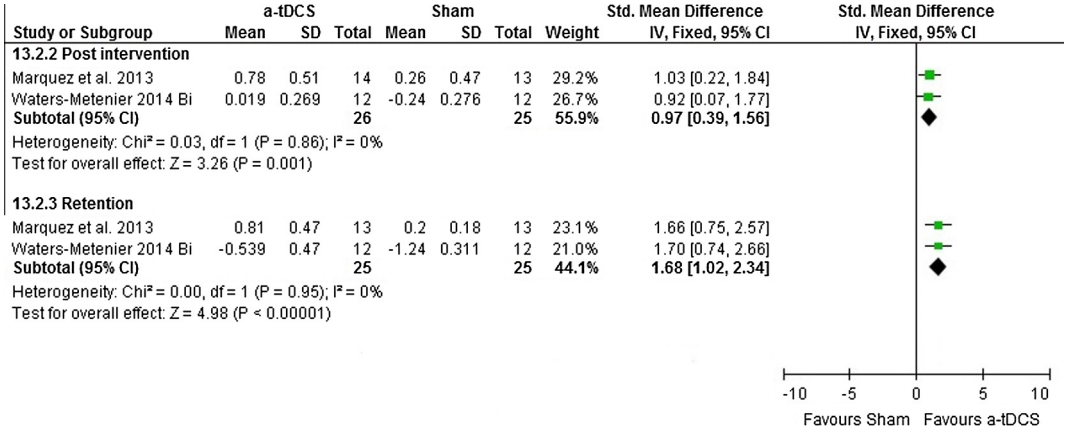

Fig. 6. Meta-analysis of multiple session a-tDCS on skill measure in SEQTAP/SRTT.

$p = 0.001$) at post intervention and 1.68 (95% CI: 1.02, 2.34, $p < 0.0001$) at retention (Fig. 6).

3.9. Effects of multiple session a-tDCS on skill in SVIPT

The results of application of multiple sessions of a-tDCS indicate a significant change at post intervention, SMD: 0.64 (95% CI: 0.3, 0.98, $p = 0.0002$), but not at the retention time point, SMD: 0.51 (95% CI: −0.05, 1.07, $p = 0.08$) (Fig. 7).

4. Discussion

The purpose of this systematic review and meta-analysis was to determine the efficacy of single and multiple sessions of M1 a-tDCS on behavioural changes such as movement speed, accuracy and skill outcomes, during two different motor sequence learning tasks: SEQTAP/SRTT and SVIPT. To the best of our knowledge this is the first meta-analysis shedding light on the effects of single and multiple session a-tDCS on sequence motor learning. Our results showed that application of multiple sessions of a-tDCS over M1, compared to single session a-tDCS, induces significant changes in behavioural outcomes of both SQTAP/SRTT and SVIPT learning tasks at post intervention time. We also observed a significant improvement at retention time point after a single day and multiple day M1 a-tDCS in SEQTAP/SRTT, for SVIPT no such improvement was present.

4.1. Single session M1 a-tDCS

The results indicate that one session of a-tDCS seems not to be enough to induce significant changes in behavioural outcomes during and immediately after stimulation. However, the results indicate a significant improvement 24 h after a single session of M1 a-tDCS, compared to sham interventions in the SEQTAP/SRTT task.

The failure of single session M1 a-tDCS to induce behavioural changes at fast stages of learning is in line with some imaging studies have shown that, M1 activity decreases during the fast stage of learning (Doyon & Ungerleider, 2002; Floyer-Lea & Matthews, 2005; Hardwick, Rottschy, Miall, & Eickhoff, 2013; Karni et al., 1998; Toni et al., 1998). However, increased activity and involvement of M1 during motor learning has been shown in some studies (Fritsch et al., 2010; Rioult-Pedotti et al., 2000). Contrary to our results, a significant improvement in the rate of learning for SRTT was observed following a single-session a-tDCS over M1 in a study by Nitsche et al. (2003). In this study, they used large electrode sizes (35 cm^2), which may have stimulated nearby functional sites, such as the primary sensorimotor area, premotor and supplementary motor areas, which are also involved in the fast stage of learning (Floyer-Lea & Matthews, 2005; Grafton et al., 1992; Hardwick

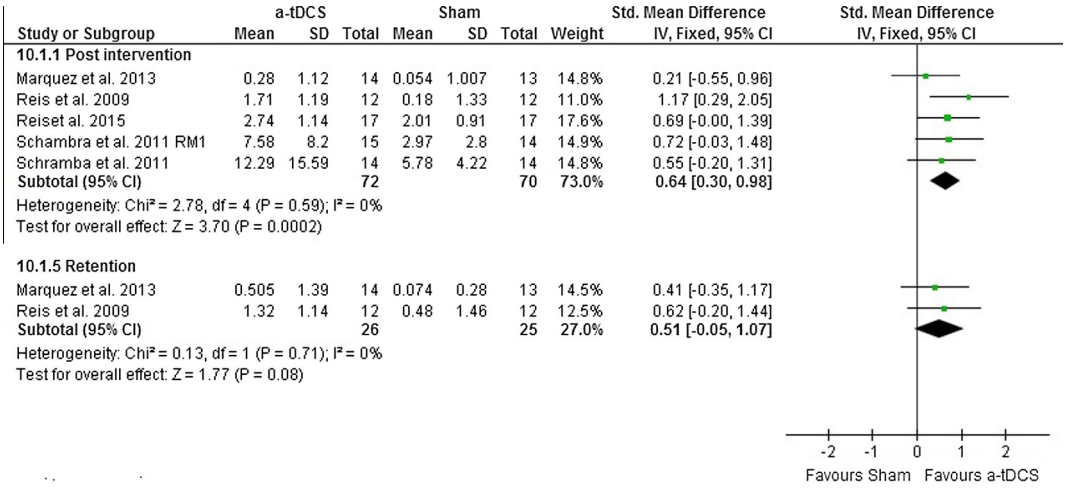

Fig. 7. Meta-analysis of multiple session a-tDCS on skill measure in SVIPT.

et al., 2013; Honda et al., 1998). Therefore, application of single session a-tDCS over other areas of the brain could have improved motor sequence in the early stages of learning.

Another reason for the observed failure of single-session anodal stimulation could be related to inadequacy of a-tDCS protocols applied over M1 during the fast stage of motor skill learning. The optimum protocol for a-tDCS has not yet been determined. It is possible to enhance the effects of a-tDCS on M1 in a single session by changing a-tDCS parameters such as the intensity, electrode size, duration and type of stimulation. In this meta-analysis, the majority of included studies used an intensity of 1 mA a-tDCS with different electrode sizes, which induced a range of different current densities from 0.028 to 0.125 mA/cm^2. In the present meta-analysis, the biggest effect size for single session a-tDCS was found in the study by Kantak et al. (2012), who applied the smallest electrode size (8 cm^2) with highest current density (0.125 mA/cm^2). They revealed statistically significant improvement at retention assessment (24 h after completion of stimulation). Our results also revealed statistically significant improvement at retention assessment, which is consistent with studies showing that M1 plays a central role in early consolidation and retention of motor learning (Muellbacher et al., 2002; Reis et al., 2015; Richardson et al., 2006).

Regarding to results of study of Reis et al. (2015), retest interval time after the end of training can be considered as a factor to detect of a-tDCS-induced offline effect in a single session a-tDCS study. They found that retest intervals should be latter than 15 min after the end of training to detect offline effects of a single practice session of a-tDCS. In the current study, post intervention time points were immediately or after 15 or 20 min after the end of experiment indicating this interval time is not enough to record offline effects of a single session a-tDCS. Thus besides some tDCS parameters, retest interval time after the end of training can be considered as a critical factor to record of offline gains in a single session a-tDCS study.

We sub grouped data based on uni- and dual-hemisphere M1 stimulation during the SEQTAP/SRTT. The results have shown no difference between uni- and dual-tDCS on improvement of performance in the SEQTAP/SRTT. Some evidence indicates that bilaterally stimulating both motor cortices induces greater improvement compared to the conventional unilateral montage (Karok & Witney, 2013; Vines, Cerruti, et al., 2008). However, Kang and Paik (2011) found no difference between uni- and dual-hemisphere stimulation. It should be noted this discrepancy may be related to the different task paradigms used in these studies. In current study, we found no difference between uni- and dual tDCS on behavioural outcome measures in motor sequence learning.

4.2. Multiple session M1 a-tDCS

Our results revealed a significant improvement in skill measure with multiple session a-tDCS over M1 at post intervention in both SEQTAP/SRTT and SVIPT tasks. The effect of a-tDCS over M1 at retention time seems to be task specific, because significant changes in behavioural measurement were only observed in the SEQTAP/SRTT task. However, it should be noted the SMD for the SVIPT task at retention time was near to significant ($p = 0.08$) (Waters-Metenier et al., 2014). Therefore, it is likely that the increase in sample size leads to different results at retention time for both tasks.

The improvement seen in behavioural measures of learning with application of a-tDCS over M1 might occur via activation of the *N*-methyl-D-aspartate (NMDA) receptor in the context of a decreased GABAergic tone (Liebetanz, Nitsche, Tergau, & Paulus, 2002). The development of such connectivity has been shown to correlate with motor skill learning (Okano, Hirano, & Balaban, 2000). Imaging studies have shown that with extended practice of a motor sequence, there is an increase in activation and enlargement of cortical maps in M1 (Floyer-Lea & Matthews, 2005). This area is actively engaged in post-practice processes and helps to stabilize or enhance sequence performance over the retention interval. Increasing excitability of M1 using a-tDCS can raise the chance of forming more effective and stronger synaptic connections between activated neurons during motor sequence learning. The present review indicates that, to enhance skill learning in SEQTAP/SRTT and SVIPT in healthy individuals, application of multiple session a-tDCS is needed to induce effective changes in brain activity.

There are some limitations in this review. There was a large variety of different stimulation techniques across studies. Given the limited number of studies available for inclusion in this meta-analysis, we were forced to combine different types of a-tDCS and training paradigms to investigate the effects of single and multiple session a-tDCS on motor sequence learning. We were therefore unable to resolve the intervention and training heterogeneity completely. Regarding to this fact that these stimulation parameters might have a major influence on efficacy of a-tDCS, overall findings and statistical significance should be interpreted

with caution. In addition, we measured behavioural changes in young healthy individuals; therefore the results cannot be generalized to patients or older people. Given the increasing interest in the potential clinical utility of a-tDCS for improving motor skills, more studies are needed to evaluate the effects of a-tDCS parameters in both healthy individuals and patients with pathological conditions. Besides behavioural changes, measuring cortical changes could be helpful to deepen our understanding of how a-tDCS affects motor sequence learning.

5. Conclusions

We conclude that the effects of a-tDCS over M1 on motor sequence learning may depend on the stages of motor sequence learning and the type of acquired task. Our findings indicate that application of multiple sessions of a-tDCS over M1, compared to single session a-tDCS, induces significant changes in behavioural outcomes of both SQTAP/SRTT and SVIPT learning tasks at post intervention time. The effects of a-tDCS over M1 on retention time might be task specific because significant improvement was only observed in the SEQTAP/SRTT task but not for SVIPT.

Conflict of interest statement

This work was supported by Monash University and there is no conflict of interest regarding the publication of this paper. Paul B. Fitzgerald is supported by an NHMRC Practitioner Fellowship (606907). He has received equipment for research from MagVenture A/S, Medtronic Ltd, Cervel Neurotech and Brainsway Ltd and funding for research from Cervel Neurotech.

Acknowledgments

The authors gratefully thank Prof. Jennifer L. Keating for her assistance in statistical analysis and valuable comments on the manuscript. We also appreciate the authors of included studies who sent us the original data for inclusion in meta-analysis.

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Declaration for Chapter 3

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

Nature of the contribution	Extent of the 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 the contribution
Shapour Jaberzadeh	Supervisoly input on study design, guidance in framing of the manuscript, reviewing and provision of feedback on the manuscript drafts.
Paul B Fitzgerald	Reviewing and provision of feedback on the manuscript drafts.
Maryam Zoghi	Reviewing and provision of feedback on the manuscript drafts.

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

Candidate's name

Signature Date

Signature Date

Preamble to Chapter 3

Reliability is one of the most important issues in clinical studies where TMS used as an assessment tool to make sure that the changes following interventions are not due to systematic or methodological errors. Chapter 3 examines the effects of removal of the initial MEPs elicited using two techniques including 120% RMT and 1mV on reliability scores.

Aim

To evaluate the effects of removal of initial MEPs on the reliability of TMS induced MEPs from the first dorsal interosseous muscles (FDI) in healthy individuals.

Chapter 3: The effects of initial motor evoked potentials removal on TMS reliability

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

TMS safety, consent form and Edinburg handedness questionnaires are provided in Appendices 6-8.

Reliability of Motor Evoked Potentials Induced by Transcranial Magnetic Stimulation: The Effects of Initial Motor Evoked Potentials Removal

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Citation: Hashemirad, F., Zoghi, M., Fitzgerald, P. B., & Jaberzadeh, Sh. (2017). Reliability of Motor Evoked Potentials Induced by Transcranial Magnetic Stimulation: The Effects of Initial Motor Evoked Potentials Removal. *Journal of Basic and Clinical Neuroscience, 8*(1), 43-50.http://dx.crossref.org/10.15412/J.BCN.03080106

doi: http://dx.crossref.org/10.15412/J.BCN.03080106

Article info:
Received: 02 January 2016
First Revision: 29 March 2016
Accepted: 16 April 2016

Key Words:
Transcranial magnetic stimulation, Reliability, Evoked response variability, First dorsal interosseous muscles

ABSTRACT

Introduction: Transcranial magnetic stimulation (TMS) is a useful tool for assessment of corticospinal excitability (CSE) changes in both healthy individuals and patients with brain disorders. The usefulness of TMS-elicited motor evoked potentials (MEPs) for the assessment of CSE in a clinical context depends on their intra-and inter-session reliability. This study aimed to evaluate if removal of initial MEPs elicited by using two types of TMS techniques influences the reliability scores and whether this effect is different in blocks with variable number of MEPs.

Methods: Twenty-three healthy participants were recruited in this study. The stimulus intensity was set at 120% of resting motor threshold (RMT) for one group while the stimulus intensity was adjusted to record MEPs up to 1 mV for the other group. Twenty MEPs were recorded at 3 time points on 2 separate days. An intra-class correlation coefficient (ICC) reliability with absolute agreement and analysis of variance model were used to assess reliability of the MEP amplitudes for blocks with variable number of MEPs.

Results: A decrease in ICC values was observed with removal of 3 or 5 MEPs in both techniques when compared to all MEP responses in any given block. Therefore, removal of the first 3 or 5 MEPs failed to further increase the reliability of MEP responses.

Conclusion: Our findings revealed that a greater number of trials involving averaged MEPs can influence TMS reliability more than removal of the first trials.

1. Introduction

Transcranial magnetic stimulation (TMS) is a useful tool for assessment of corticospinal excitability (CSE) changes in both healthy individuals and patients with brain disorders (Barker et al., 1987; Rossini et al., 1994; Liepert et al., 2000). The magnetic pulses induced by TMS over the contralateral primary motor cortex (M1) can pass through the scalp and induce a response known as "motor evoked potential" (MEP) in the target muscle. This response is recorded using surface electromyogra-

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phy (EMG) electrodes placed over the muscle of interest (Malcolm et al., 2006).The peak-to-peak amplitude of the elicited MEPs is an indication of changing CSE. Smaller amplitudes indicate lower excitability, while larger amplitudes suggest higher CSE (Chipchase et al., 2012).

Literature review indicates that there is a high degree of variability in the TMS-induced resting MEPs (Kiers et al., 1993; Ellaway et al., 1998).This variability could result from technical factors such as orientation, location, and stability of the TMS coil (Barker et al., 1987; Hill et al., 2000; Chipchase et al., 2012). However, variability in MEP responses remains even after controlling these factors. This inherent variability could result from neurophysiological changes in the CSE pathway (Truccolo et al., 2002). More variability might be expected in the amplitude of the first few MEPs due to changes in regional cerebral flow (Mochizuki et al., 2006) and changes in excitatory synaptic drive to corticospinal neurons (Ellaway et al., 1998).

The first few MEP responses might be larger than the subsequent MEPs (Brasil-Neto et al., 1994), and the increased variability in initial MEPs can affect TMS reliability (Schmidt et al., 2009).Therefore, removal of the first, more fluctuating MEPs might increase the averaged reliability scores. In TMS studies, CSE could be assessed using 2 different techniques. In the first technique, the test stimulus is calculated as a ratio of a resting motor threshold (RMT) such as 120% RMT. In the second technique, the test stimulus is adjusted to produce MEP responses up to 1 mV, which is commonly used in paired-pulse TMS studies. Since there is an inverse relationship between variability of MEP responses and TMS stimulus intensity (Kiers et al., 1993), the MEPs evoked by the 1 mV technique are less subject to variability, which may be less affected by more variable and fluctuating initial MEPs.

The literature suggests that increasing the number of evoked MEPs increases the TMS reliability (Ellaway et al., 1998; Truccolo et al., 2002; Kamen, 2004; Bastani & Jaberzadeh, 2012). Little is known about how removal of the first few MEPs affects the reliability scores of TMS techniques. In this study, we investigate the effects of removal of the initial elicited MEPs on reliability scores, and also whether this effect is different in blocks with different MEP numbers. We hypothesised that removal of three or five initial MEPs should increase reliability. We also hypothesised that the removal of the initial MEPs should have more profound effects on enhancement of reliability than the number of MEPs in each block.

2. Methods

2.1. Participants

Twenty three healthy participants were recruited in thisstudy and divided into two groups to assess the reliability of MEPs responses induced by two types of TMS techniques. Thirteen participants (11 females and two males with the mean [SD] age of 26.5[9.9] y) were included in one group where the test stimulus was considered at 120% RMT. In the other group (11 females and two mals, with the mean [SD] age of 24[3.7] y), the test stimulus was adjusted at 1 mV. Handedness of the participants was assessed using the Edinburgh Handedness Questionnaire (Oldfield, 1971). The dominant hand was tested in each participant. Of 23 participants, 21 were right-hand dominant. Participants were screened for contraindication to TMS applications. They provided their written informed consent prior to the experiments. All protocols used were approved by the Human Research Ethics Committees at Monash University and conformed to the Declaration of Helsinki.

2.2. Measurements

2.2.1. Electromyography

Participants were tested in a sitting position with forearm supported in a pronated position. A standard skin preparation (Gilmore & Meyers, 1983) procedure was performed for each electrode placement site. EMG electrodes were placed on the first dorsal interosseous (FDI) muscle of the dominant hand with an inter-electrode distance of 2 cm. A ground electrode was placed ipsilaterally over the styloid process of the ulna bone. All EMG signals were filtered, amplified (10 Hz–500 Hz x 1000), and sampled at 1000 Hz. All data were recorded on a PC via a commercially available software (Chart™ software, ADInstrument, Australia) and a laboratory analogue-digital interface (The Power Lab 8/30, ADInstrument, Australia) for later off-line analysis.

2.2.2. Motor evoked potentials

Single pulse magnetic stimuli were delivered using two stimulators with a figure-of-eight coil. A Magstim 2002 (Magstim Company Limited, UK) stimulator was used for recording MEPs with intensity of 120% RMT in group 1, and a MagPro R30 (MagOption) stimulator (MagVenture Denmark) was used for recording MEPs using the second technique in group 2. In both groups, the coil was placed over the dominant M1, i.e. contralateral to the muscle of interest. The orientation of the coil

was set at an angle of 45° to the midline and tangential to the scalp. In this orientation, the induced current flow is directed from posterior to anterior. The coil was moved around the M1 of the FDI muscle to determine the optimal site of stimulation. After localizing this site, known as a hot spot, the coil position was marked on the scalp as a reference. Coil position and orientation were constantly assessed throughout the experiment to minimize technical inconsistencies.

After localizing the hot spot, RMT was measured. RMT is defined as the lowest intensity to induce at least 5 MEPs larger than 50 μV in peak-to-peak amplitude out of 10 consecutive stimuli to find RMT, also the intensity of the stimulator was decreased in steps of 2% of the maximum stimulator output. The test stimulus was set at 120% of each individual's RMTs in group 1 and adjusted up to produce MEP responses of about 1 mV in group 2.

2.3. Procedure

Each participant was tested in two separate testing sessions. The first session involved two sets of data collection. FDI muscle MEPs were recorded before and immediately after a 20-minute break in which subjects were recommended to do activities such as reading books or magazines. During each testing session, 20 MEPs with interpulse intervals of 10 seconds (Vaseghi et al., 2015) were recorded. A follow-up session was held at least 72 hours after the first session. All participants were assessed at the same time of day in both sessions to avoid diurnal variations.

2.4. Data analysis

In both groups, 20 stimuli were delivered, with 10 seconds interstimulus interval. The averaged MEPs at each time point were calculated for the first 10 (Block 1), first 15 (Block 2), and all 20 MEPs (Block 3). Then the averaged MEPs were also calculated after removal of the first 3 and the first 5 MEPs in each block. The effects of removal of the first 3 and the first 5 MEPs in each block were evaluated using intraclass correlation coefficients (ICCs) with absolute agreement and a 2-way mixed model. Repeated measures analysis of variance (ANOVA) was used to detect any differences between the averaged MEPs across 3 time points at any given block.

SPSS (version 20) was used for the data analysis. A significance level of $P<0.05$ was adopted for all conditions. Post hoc tests (Student t test with Bonferroni correction) were performed where indicated.

3. Results

A total of 23 individuals were recruited for this study. Three subjects took part in both groups while the rest of the subjects participated in only one group. In group 1 (n=13), stimulus intensity was delivered at 120% RMT. In group 2 (n=13), the average stimulus intensity required to produce MEPs of about 1 mV was 139% RMT (with Min and Max 104% and 185 %

Table 1. The results of ICCs and F test in three blocks 10, 15, and 20 MEPs in three types of conditions (all trials, after removal of the first three or five MEPs) at three time points across the two sessions (MEPs 120% RMT).

Test Intensity=120% RMT N=13	T1 Session 1 (Mean±SD)	T2 Session 1 (Mean±SD)	T1 Session 2 (Mean±SD)	F (2, 24)	P	ICCs	P
Block 1a (1-10 MEPs)	0.78±0.47	0.70±0.66	0.68±0.50	0.325	0.726	0.851	0.000
Block 1b (4-10 MEPs)	0.71±0.39	0.65±0.69	0.63±0.45	0.197	0.864	0.754	0.002
Block 1c (6-10 MEPs)	0.71±0.43	0.64±0.68	0.68±0.49	0.134	0.875	0.830	0.000
Block 2a (1-15 MEPs)	0.74±0.41	0.71±0.64	0.70±0.41	0.069	0.934	0.897	0.000
Block 2b (4-15 MEPs)	0.69±0.35	0.69±0.66	0.67±0.36	0.022	0.978	0.839	0.000
Block 2c (6-15 MEPs)	0.68±0.37	0.69±0.66	0.70± 0.37	0.009	0.991	0.881	0.000
Block 3a (1-20 MEPs)	0.72±0.40	0.77±0.65	0.69±0.40	0.397	0.677	0.922	0.000
Block 3b (4-20 MEPs)	0.67±0.35	0.76±0.67	0.68±0.37	0.514	0.605	0.893	0.000
Block 3c (6-20 MEPs)	0.67±0.38	0.77±0.67	0.69±0.38	0.521	0.601	0.895	0.000

Significant results are bold.

Table 2. The results of ICCs and f tests in three blocks 10, 15 and 20 MEPs in three types of conditions (all trials, after removal of the first three or five MEPs) at three time points across the two sessions (MEPs~1 mV).

Stimulus Intensity=MEPs 1mV N=13	T1 Session 1 (Mean±SD)	T2 Session 1 (Mean±SD)	T1 Session 2 (Mean±SD)	F (2, 24)	P	ICCs	P
Block 1a (1-10 MEPs)	1.09±0.24	1.12±0.35	1.01±0.19	0.791	0.465	0.533	0.056
Block 1b (4-10 MEPs)	1.05±0.37	1.102±0.39	1.05±0.28	0.094	0.911	0.422	0.135
Block 1c (6-10 MEPs)	1±0.42	1.17± 0.48	1.07±0.42	0.699	0.510	0.564	0.043
Block 2a (1-15 MEPs)	1.06±0.20	1.07±0.32	1.05±0.25	0.073	0.930	0.721	0.005
Block 2b (4-15 MEPs)	1.03±0.34	1.03±0.38	1.1±0.32	0.267	0.768	0.609	0.029
Block 2c (6-15 MEPs)	0.99±0.30	1.08±0.38	1.09±0.36	0.527	0.597	0.694	0.008
Block 3a (1-20 MEPs)	1.03±0.21	1.06±0.29	1.04±0.25	0.082	0.94	0.770	0.002
Block 3b (4-20 MEPs)	1.02±0.31	1.02±0.34	1.1±0.30	4.37	0.651	0.684	0.009
Block 3c (6-20 MEPs)	0.98±0.303	1.06±0.33	1.07±0.33	0.56	0.578	0.733	0.003

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RMT). The average (SD) handedness scores were 79.4(25.2) and 86.7(9.8) in groups 1 and 2, respectively.

In Table 1, the results of the ICCs and F tests values in all blocks with different number of trials are shown for group 1. The ICC values ranged from 0.75 to 0.92 in blocks 1, 2, and 3 indicating that increasing the number of trials can lead to an increase in ICC values. The results of ICCs in all blocks with removal of the first 3 or 5 MEPs revealed slightly decreased reliability for the FDI MEP responses. More reduction in ICC values was observed with removing the first 3 MEPs in all blocks, compared to removal of the first 5 MEPs (Table 1). No differences were observed in the averages of MEP sizes in blocks with different number of trials between any time points across two sessions. As shown in Table 2, similar results were observed in group 2 with test intensity of up to 1 mV. The range of the ICCs in this group was lower than that in group 1, but similar pattern wasfound in the results of the ICC values. ICCs in all blocks with removal of the first 3 or 5 MEPs revealed slightly decreased reliability for the FDI MEP responses. More reduction in ICCs was obtained with removing the first 3 MEPs in all blocks, compared to removal of the first 5 MEPs (Table 2). There were no significant differences in the average MEP size at any time points in any given block (Table 2).

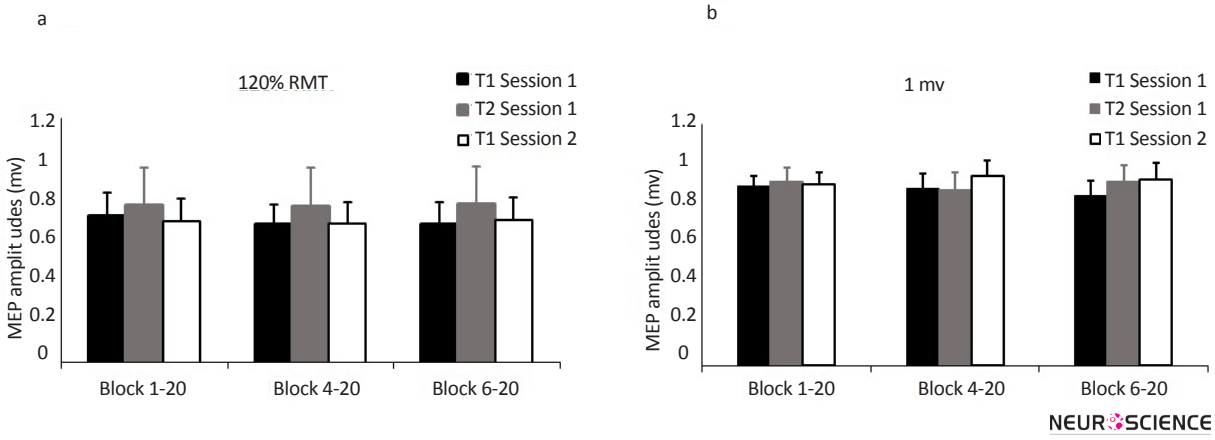

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Figure 1. Comparison of MEPs amplitude in blocks of 20 MEPs in3 conditions (all trials, after removal of the first 3 and 5 MEPs at 3 time points across two sessions. a) group 120% RMT, b) group 1 mV.

The ICC values for this group ranges from 0.42 to 0.77 in blocks 1, 2, and 3 indicating that raising the number of trials can lead to an increase in ICC values.

Figure 1 shows the results of comparison of MEPs amplitude in block 20 MEP responses in 3 conditions (all trials, after removal of the first 3 and 5 MEPs) for two types of TMS methods, 120% RMT and intensity to elicit 1 mV MEPs.

4. Discussion

In this study, we assessed the reliability of TMS induced MEP, using two types of TMS techniques (120% RMT and 1 mV), and considering removal of data for the first few trials in each block. The hypothesis that the removal of initial MEPs would increase the MEP reliability was refuted by the results. Our results have shown that reliability scores decrease with removal of the first 3 or 5 MEPs in each block, except for block 10 at 1 mV intensity in which removing the first 5 trials slightly increased ICCs compared to all 10 MEPs. In both techniques, we observed more reduction in ICC values with removing the first 3 MEPs in all blocks, compared to removal of the first 5 MEPs. The results also indicate that, compared to removal of the first few MEPs, the number of MEPs in each block has a more profound effect on the enhancement of reliability in both techniques.

The patterns of variability of MEP size and the mechanisms responsible for this variability have not been completely determined. Changes in the level of synchrony of neuronal pulse activity and spontaneous changes in motor neuron excitability are often identified as the sources of such variability (Srinivasan et al., 1999; Sankarasubramanian et al., 2015; Livingston & Ingersoll, 2008; Lopez-Alonso et al., 2015; Möcks et al., 1987; Truccolo et al., 2001). Large changes in CSE might result in greater fluctuations in MEP amplitude during the first few trials of TMS (Brasil-Neto et al., 1994; Ellaway et al., 1998), which can affect overall reliability of elicited MEPs. However, our finding demonstrated that removal of the first few trials resulted in lower values of MEP reliability when compared to removing all trials in any given block. The ICC values recorded for all three blocks of 10, 15, and 20 MEPs showed a rise in reliability score with increasing the number of trials, which is in agreement with those results suggesting that there is a relationship between the number of trials and reliability score (Kiers et al., 1993; Kamen, 2004; Christie et al., 2007; Bastani & Jaberzadeh, 2012).

In the current study, different impacts on reliability scores are achieved by removing the first 3 or 5 MEPs. Different values of ICCs in a given block with removal of the first 3 or 5 trials indicated that not only the number of MEPs, but also the number of removed initial trials can influence reliability of this response. In the current study, a slight increase in ICC values was observed in blocks with the first 5 trials removed, compared to exclusion of the first 3 trials. This finding can be explained by the increased homogeneity in MEP amplitudes being expected after the first 5 MEPs, which is line with some studies that reported ICC values above 0.6 for blocks of 5 MEPs (Kamen, 2004; Christie et al., 2007; Bastani and Jaberzadeh, 2012).

Similar patterns in reliability scores were found between two types of TMS techniques. The only difference was found in block 10 MEPs using TMS technique 1 mV. In this case, by removing the first 5 trials, ICCs slightly increased. In addition, there is a clear trend that, after removal of first few trials, the SD of MEP increased in the 1 mV technique more than that in the 120% RMT technique. This increase was larger for the removal of 5 trials than the removal of 3 trials, indicating the first 3 or 5 MEPs were very close to the mean value of all trials.

Taken together, to receive reliable responses, increasing the number of trials might be more effective than removing the first few trials. Therefore, using 20 MEPs allows us to accurately measure mean MEP amplitude as a valid outcome. More studies are needed to find out factors which contribute to MEP variability and the reliability of MEP responses.

There are some limitations in this research. Healthy young participants were assessed in this study, therefore, our results cannot be extrapolated to other populations such as patients or elderly people. Furthermore, the intensity of the stimuli was set at 120% of RMT or 1 mV at rest condition, therefore the findings could not be generalized to other TMS intensities and active conditions. Future studies must be conducted on patients, on other age ranges, and for active and rest conditions at different TMS intensities.

This study demonstrated that a greater number of trials involving averaged MEPs can influence TMS reliability more than removal of the first few trials in a given block. On the other hand, removal of more variable and fluctuating initial MEPs did not have a significant impact on overall reliability of TMS-induced MEPs between two techniques (1 mV and 120% RMT).

Acknowledgments

The authors gratefully thank Dr. Allie Ford for her proofreading on this manuscript. This paper had no financial support.

Conflict of Interest

The authors declared no conflict of interests.

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Chapter 4: Development of macros for automation of data management

The main purpose of this chapter is to briefly explain the development of a number of macros, a group of recorded commands, which were used for automation of data recording and analysis.

Preamble to Chapter 4

In this chapter, all the steps and procedures in order to automate data management for using during the sequential visual isometric pinch task (SVIPT) are briefly explained.

Aim

To explain the development of a number of macros used for automation of data recording and analysis during SVIPT.

Sequential visual isometric pinch task (SVIPT)

In this thesis, a force transducer (ADInstrument MLT004/ST, NSW, Australia) was used for SVIPT. This force transducer is an isometric dynamometer, which converts mechanical inputs to voltage signals (Figures 4-1A &B). A wooden support was made in order to fix this force transducer in place throughout the experiments (Figure 4-1C).

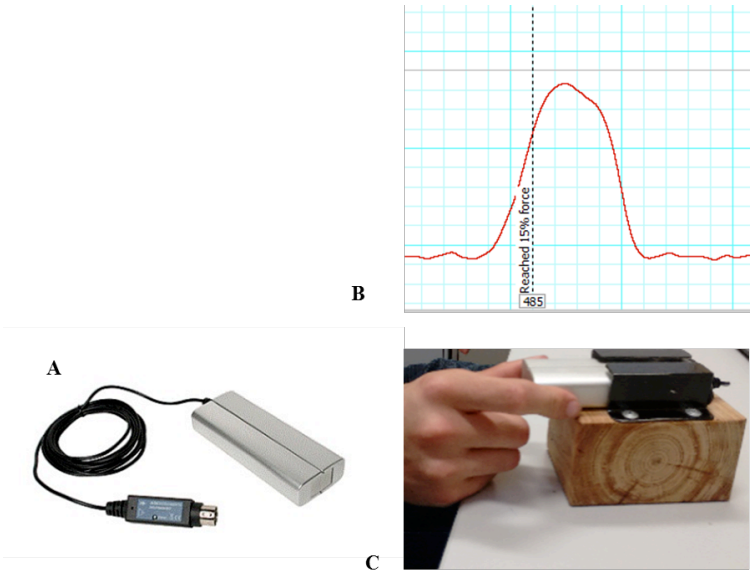

Figure 4-1: The force transducer (ADInstrument MLT004/ST) (A). When participants squeezed the force transducer, mechanical signals converted to voltage signals (B). A wooden support was made in order to fix this force transducer in place (C).

Using this force transducer for SVIPT requires the following systems (Figure 4-2):

1- PowerLab™ (Hardware)

2- LabChart™ (Software)

3- MATLAB (Software)

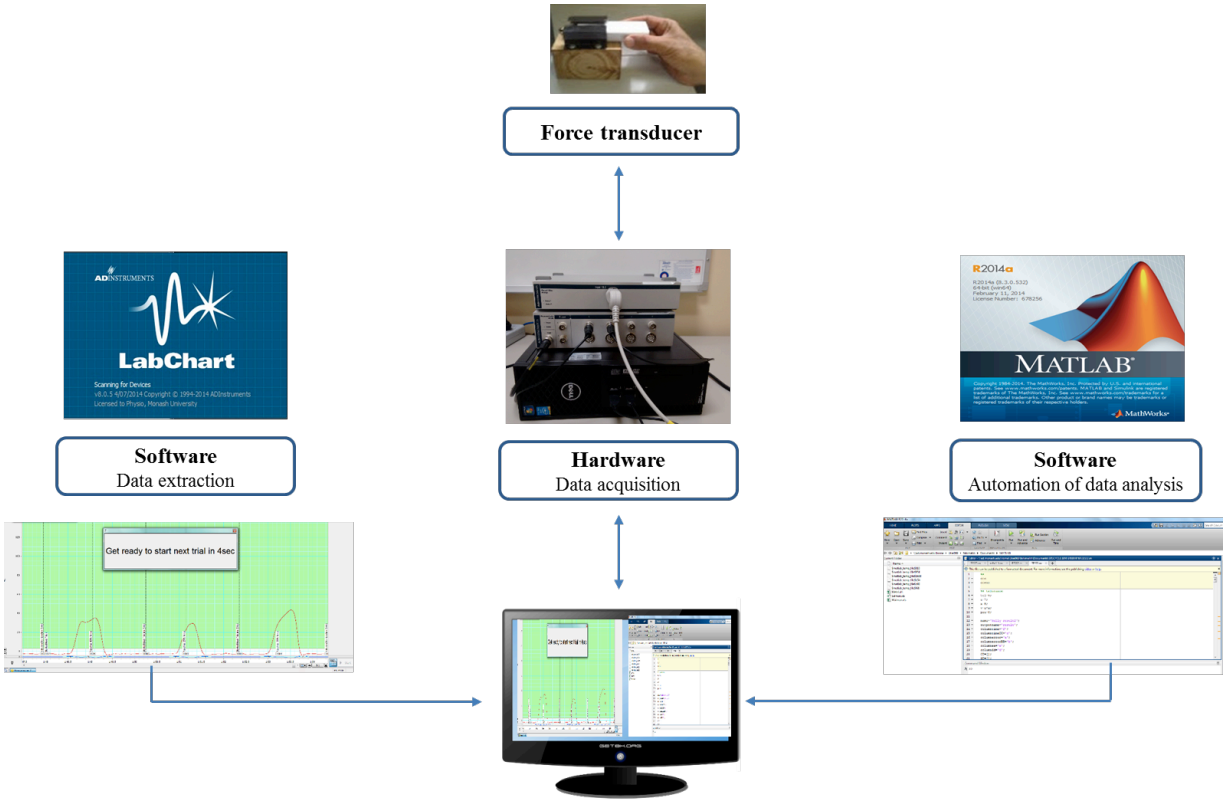

Figure 4-2: Systems used for data recording and processing obtained from the force transducer.

PowerLab™

The force transducer was directly connected to a PowerLab™ (4/35), which is reliable data acquisition hardware and converts voltage signals received from the force transducer to digital signals. Resulting signals from PowerLab™ were stored in the attached computer.

LabChart™ Software

LabChart™ software was used for off-line analysis data recorded from the PowerLab™. Using LabChart™, data were displayed on the computer screen and allowed for advanced data handling and calculations.

The macro in LabChart™ consisted of three parts including calibration, grip protocol and data pad, which are explained in the following sections.

MATLAB software

MATLAB software was used for automated analysis of data from PowerLab™ and LabChart™. As shown in Figure 4-3, four macros were developed, two in LabChart and another two in MATLAB environment. These macros, which are explained in the following sections, enabled us to achieve all requirements for using this force transducer for the studies (Chapter 5-7).

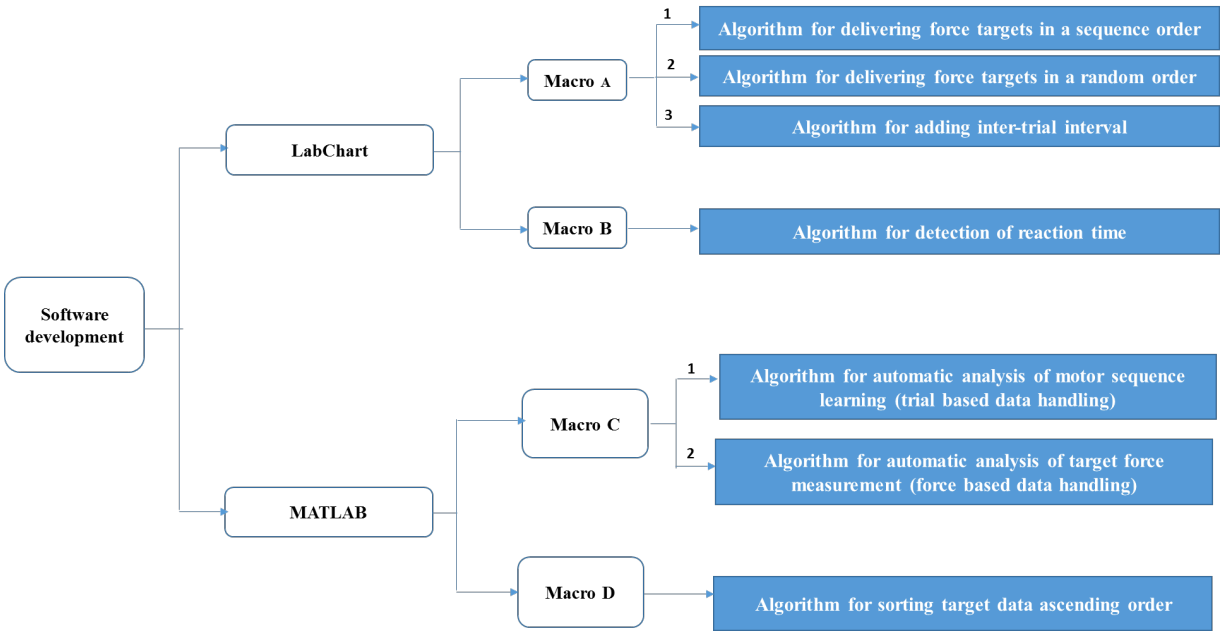

Figure 4-3: Four different macros were developed, two in LabChart™ and, two in MATLAB, to achieve all requirements for employment of the force transducer for SVIPT in this thesis.

Development of Macros in LabChart™

Two macros (A& B) were developed in LabChart™.

Macro A (Appendix 11)

Macro A was modified in three steps based on the following requirements:

Requirement 1: An algorithm for delivering target forces in a sequence order

The reason behind the development of this macro was to design a program for delivering visual targets to be met using the force transducer in a sequence order. In this program, a series of eight trials with seven different target forces in a sequence order were presented on the computer screen. Here we briefly explain three parts of this macro including calibration, grip protocol and data pad.

I. Calibration

The program needed to be calibrated for each participant before starting the experiment. For calibration, participants were asked to squeeze the force transducer between their thumb and index fingers as much as they could, and then they were asked to release the force transducer to return the cursor to the baseline (Figure 4-4). Squeezing the force transducer caused the cursor to move up on the computer screen proportional to the level of force exerted on the force transducer. After producing maximum contraction, participants released the force on the transducer and the cursor returned to the baseline.

Figure 4-4: For calibration, participants were asked to hold the force transducer between their thumb and index fingers and squeezed it as much as they could. Proportion to the force produced, a cursor moved up on the computer screen and then they were asked to release the force on the transducer to return the cursor to the baseline.

A two-point calibration was used for each participant in which the difference in voltage between the first point (maximum contraction) and the second point (baseline) was considered as the maximum voluntary contraction (MVC) (Figure 4-5).

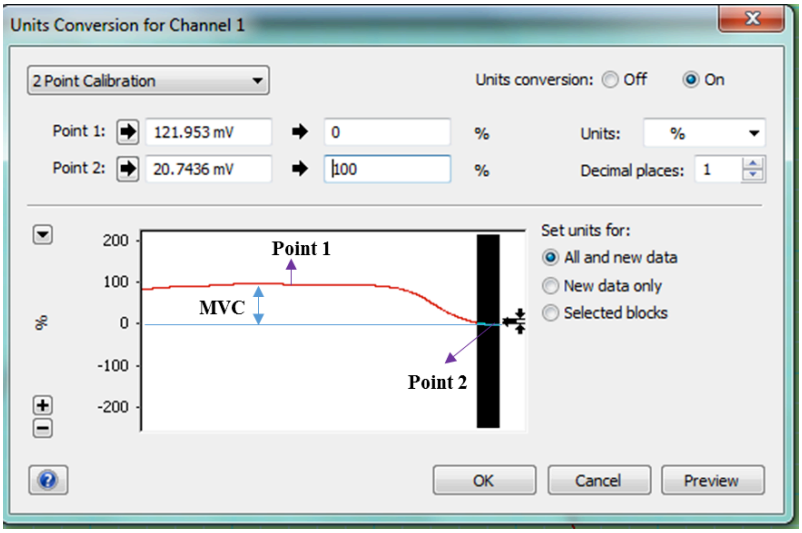

Figure 4-5: Two-point calibration which was used in LabChart™ in which the difference in voltage between maximum contraction (point 1) and the baseline (point 2) was considered as the maximum voluntary contraction (MVC).

II. Grip protocol

After calibration, the grip protocol was run (Figure 4-6). A set of seven target forces appeared on the computer screen from 10% to 40% of MVC in a sequence order (10, 35, 20, 40, 25, 15 and 30% of MVC). A visual green line, as well as a numerical value in a text box on the screen, indicated the level of required target force.

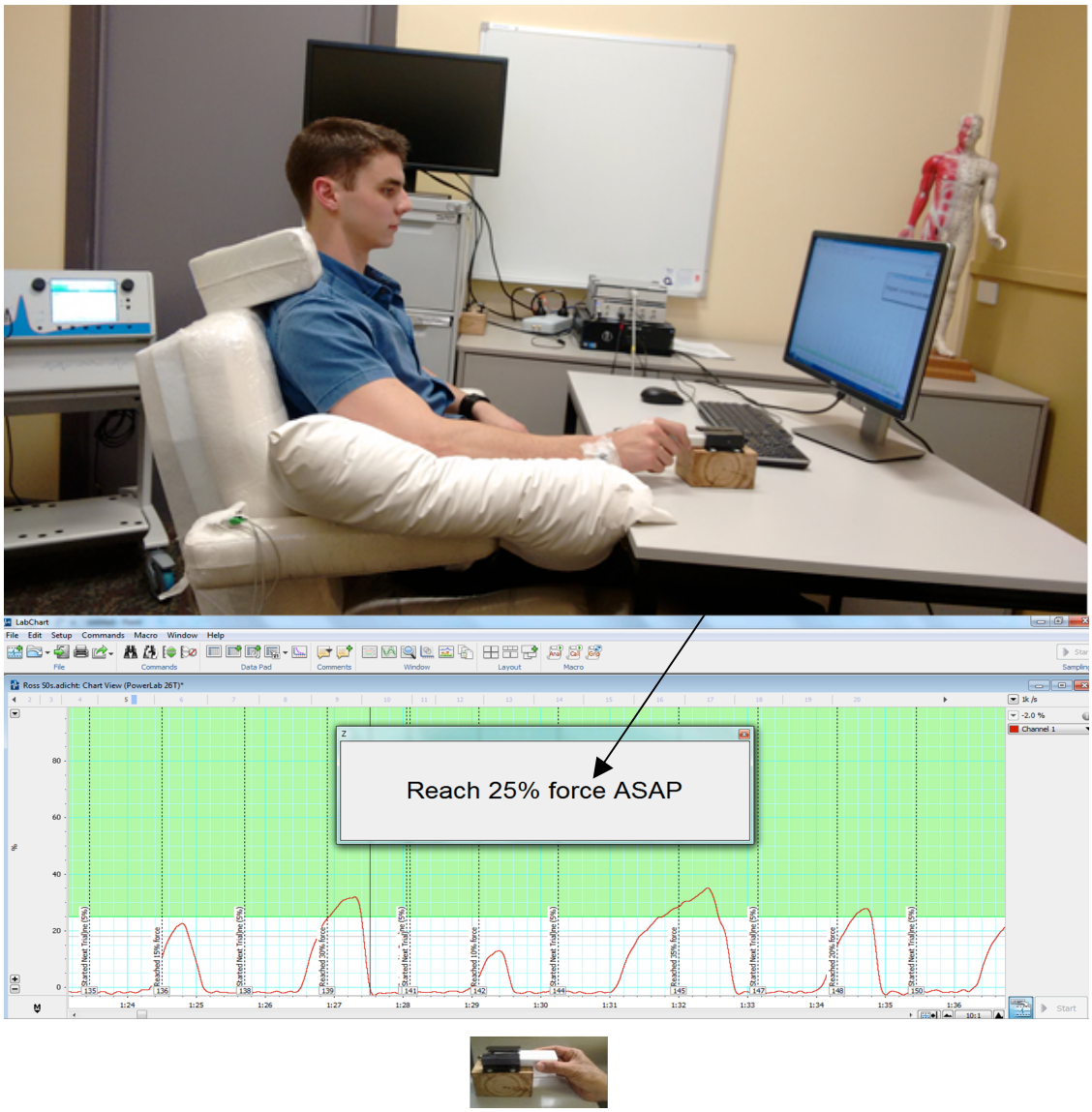

Figure 4-6: Different target forces appeared on the computer screen. A visual green line, as well as a numerical value in a text box on the screen, indicated the level of the required target force. Participants were asked to squeeze the force transducer to hit the target force as quickly and accurately as possible.

Participants were asked to complete each block (consisting of eight trials) as quickly and accurately as possible. They squeezed the force transducer to reach each target, a range of 5%

of each target was acceptable (target force ± 5% MVC). Over or under this range were considered as overshoot or undershot, respectively (Figure 4-7).

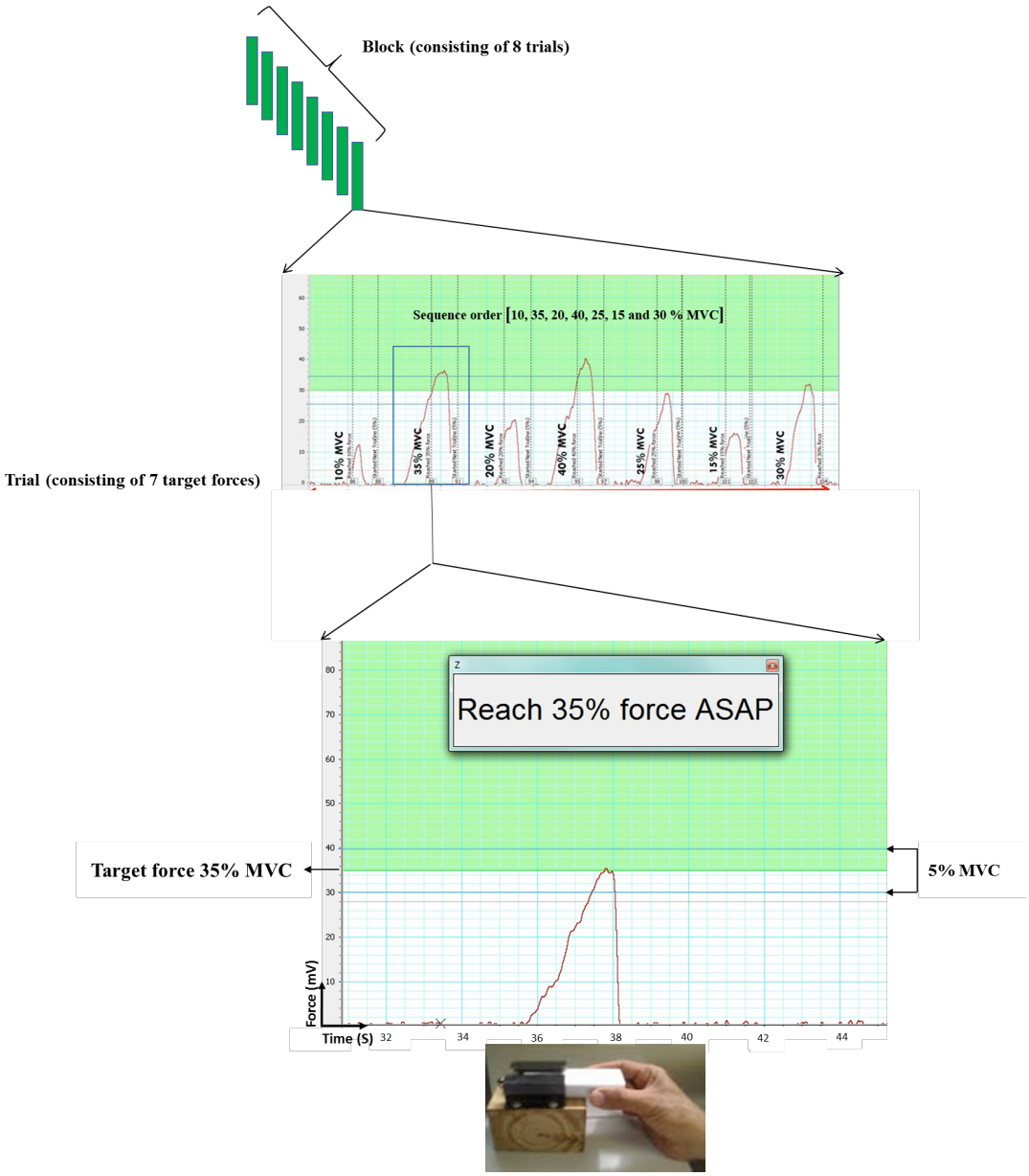

Figure 4-7: Each block was consisted of eight trials with a set of seven target forces from 10% to 40% of MVC in a sequence order (10, 35, 20, 40, 25, 15 and 30% of MVC) appeared on the computer screen.

After completion of each experiment, the results were displayed in a Data Pad. As shown in Figure 4-8A, the Data Pad display consisted of six columns including centre error, time to

maximum, selection start, target force, force reached and time at maximum, which provided some information about force targets.

A

Ross program 2 Sebastain_Fixed.adicht: Data Pad View*

Active point at t=23.74

A660 =E660-D660

	A Centre Error	B Time to Max (s)	C Selection Start	D Target Force (%)	E Force Reached	F Channel 1 Time at Maximum	G	H
1	-0.4904	0.00	0:00:23.74		-0.4904	0:00:23.74		
2				Channel 1	Channel 1	Channel 1		
3			Sel Start	Extract Nos	Maximum	Max Time		
4					%			
5	6.8522	0.46	0:00:00.705	10	16.8522	0:00:01.162		
6	-0.6864	2.07	0:00:02.888	35	34.3136	0:00:04.955		
7	1.1582	0.60	0:00:06.793	20	21.1582	0:00:07.394		
8	1.9753	2.10	0:00:09.035	40	41.9753	0:00:11.131		
9	7.1458	1.48	0:00:11.402	25	32.1458	0:00:12.884		
10	3.4558	0.48	0:00:14.666	15	18.4558	0:00:15.146		
11	6.333	1.89	0:00:16.815	30	36.333	0:00:18.704		
12	8.5152	2.06	0:00:19.155	10	18.5152	0:00:21.219		
13	-1.1912	1.05	0:00:22.927	35	33.8088	0:00:23.976		
14	0.0891	0.65	0:00:25.638	20	20.0891	0:00:26.291		
15	-2.0931	1.38	0:00:27.905	40	37.9069	0:00:29.288		
16	6.9082	0.55	0:00:30.99	25	31.9082	0:00:31.538		
17	6.4254	0.53	0:00:33.194	15	21.4254	0:00:33.729		
18	-2.5462	1.23	0:00:35.339	30	27.4538	0:00:36.568		
19	7.2086	0.45	0:00:38.392	10	17.2086	0:00:38.845		
20	-0.6864	1.15	0:00:40.559	35	34.3136	0:00:41.708		
21	5.4344	0.55	0:00:43.657	20	25.4344	0:00:44.209		
22	6.8455	0.93	0:00:45.862	40	46.8455	0:00:46.79		
23	-2.5055	1.20	0:00:48.501	25	22.4945	0:00:49.702		
24	8.3557	0.44	0:00:51.405	15	23.3557	0:00:51.845		
25	-0.6159	0.98	0:00:53.488	30	29.3841	0:00:54.471		
26	3.0214	0.34	0:00:56.005	10	13.0214	0:00:56.34		
27	3.0257	1.28	0:00:57.885	35	38.0257	0:00:59.163		
28	-0.5048	0.55	0:01:00.666	20	19.4952	0:01:01.219		

B

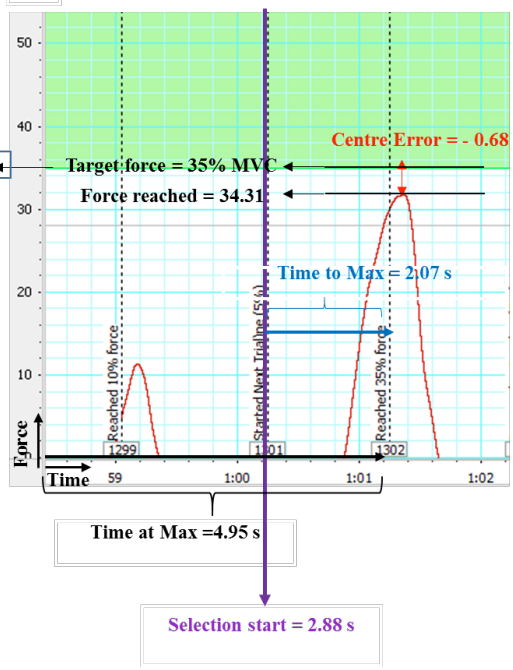

Figure 4-8: The raw data for the target forces displayed in Data Pad (A). For example, six variables including centre error, time to maximum, selection start, target force, force reached and time at maximum shown for the target force of 35% MVC (B).

For example, *centre error* (column A) represented the difference between the force produced (column E) by the participant and the given target force (column D). *Selection start* (column C) was defined as the time appearing of stimuli on the computer screen. *Time to max (column B) or response time was* the period from appearing of stimuli (selection start) to reach the maximum level of force target. *Time at maximum* (column F) was defined as the time from the beginning of each trial to reach the maximum level of the given force target. For instance, all variables have been shown for the target force 35% of MVC (Figure 4-8B).

Requirement 2: An algorithm for delivering target forces in a random order

To modify the first macro in order to allow force targets to be displayed in either random or sequence order, two codes of *zero (sequence)* and *one (random)* were defined. Therefore, target forces appeared in a random order when the number *one* was chosen in the trial types as shown in the following command.

```
'%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

'Change Parameters in Here

'Target Parameters
Baseline = 5 'Enter baseline % to return to after each contraction
Undershoot = 7 'Enter amount of room to leave for possible
undershoot (%)

'Parameters about each trial
No targets = 7 'Enter number of trails you wish to run
Ordered targets = Array (10, 35, 20, 40, 25, 15, 30) 'List of
targets in order, length must be the same as no targets

'Parameters about repeating each of the above trials
No trials = 8 ' How many times to repeat the above mentioned trials
Trial types = array (1, 1, 1, 1, 1, 1, 1) 'Which trials to
randomise, 1 means randomise, zero means ordered) length must be the
same as no trials

'%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

Requirement 3: An algorithm for adding inter-trial interval (ITI)

Although, force targets appeared in two types of sequence or random order in each trial in macro A, there was no interval between trials in each block. The following command was then added to macro A to allow us to insert a pause or interval between each trial.

```
counter = counter + 1
```

```
fahime=fahime+1

if fahime=7 then
fahime=0
Call Doc.SetDataPadValue(1, 1, 26, "-------------")
Call WaitFor (0, 0, 1)

end if
```

With this command, a dashed line appeared in the indicator box at the end of each trial (Figure 4-9) that lasted according to the determined ITI. We investigated the effects of ITIs in a range from 1 to 4 sec. on the acquired knowledge using SVIPT in study 3 (Chapter5).

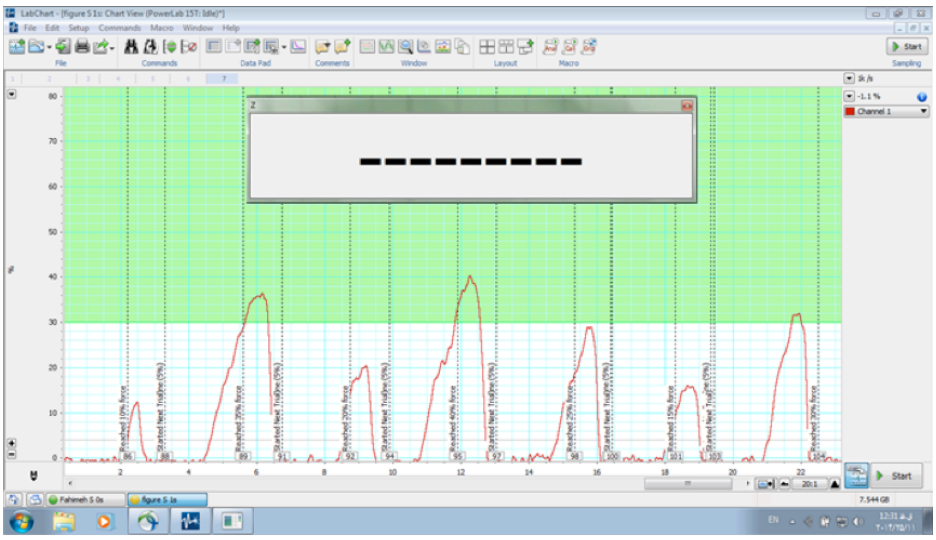

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Figure 4-9: A dashed line in the indicator box was presented at the end of each trial according to the determined inter-trial interval (ITI) to notify one trial had ended and the next trial was going to be started.

Macro B (Appendix 12)

Requirement: An algorithm for automatic detection of reaction time

As explained earlier, *the selection start* in macro A was defined as the time of appearing of the visual cue on the screen (Figure 4-8). There is an interval between seeing a visual target on the computer screen to reach the target which was measured as *response time*. In macro B, *the selection start* was changed from the appearance stimulus on the computer screen to the time of initiation of any movement by participants. By changing selection start in macro B, we were able to measure *moving time*, which was obtained from the new selection start to the time at maximum (Figure 4-10).

Two variables of response time and moving time, gave us the opportunity to calculate *reaction time*. Reaction time is defined the time from the stimulus appeared to the moment any movement started. Therefore, we were able to achieve this variable from the difference between response time (macro A) and moving time (macro B) (Figure 4-10).

A

Data Pad (macro A)

Seq Mooly 11-3-2015: Data Pad View

1,801 points from t=8.049 to t=9.85

A1

	Overshoot (%)	Time to Max (s)	C Selection Start	Target Force (%)	Force Reached	F Channel 1 Time at Maximum	G
1	-40.0	1.42	0:00:08.049	40	38.4836	0:00:08.91	
2				Channel 1	Channel 1	Channel 1	
3			Sel Start	Extract Nos	Maximum	Max Time	
4					%		
5	5.0096	0.3	0:00:00.0	10	15.0096	0:00:03.0	
6	6.3227	2.97	0:00:01.999	35	41.3227	0:00:04.97	
7	3.1459	1.20	0:00:05.949	20	23.1459	0:00:07.15	
8	-1.5164	0.86	0:00:08.049	40	38.4836	0:00:08.91	
9	-0.0538	0.85	0:00:09.949	25	24.9462	0:00:10.799	
10	0.1827	0.96	0:00:11.799	15	15.1827	0:00:12.755	
11	-3.288	1.00	0:00:13.799	30	26.712	0:00:14.796	
12	-0.3915	0.86	0:00:15.799	10	9.6085	0:00:16.662	
13	1.3024	0.88	0:00:17.699	35	36.3024	0:00:18.58	
14	-2.8784	1.04	0:00:19.649	20	17.1216	0:00:20.694	
15	2.5691	0.94	0:00:21.699	40	42.5691	0:00:22.639	
16	4.2048	1.15	0:00:23.649	25	29.2048	0:00:24.795	
17	4.5105	0.92	0:00:25.899	15	19.5105	0:00:26.817	
18	18.628	0.89	0:00:27.899	30	48.628	0:00:28.789	
19	0.9588	0.93	0:00:29.849	10	10.9588	0:00:30.777	
20	-4.9643	0.90	0:00:31.799	35	30.0357	0:00:32.694	
21	0.203	0.86	0:00:33.849	20	20.203	0:00:34.712	

B

Data Pad (macro B)

Mooly recationtime_SebLC7 (1): Data Pad View

1,900 points from t=0 to t=1.9

A1

	Overshoot (%)	Moving time	C Selection Start	Target Force (%)	Force Reached	F Channel 1 Time at Maximum	G
1	-4.6239	0.00	0:00:00.0	10	15.0096	0:00:00.0	
2				Channel 1	Channel 1	Channel 1	
3			Sel Start	Extract Nos	Maximum	Max Time	
4					%		
5	5.0096	0.15	0:00:00.84	10	15.0096	0:00:00.99	
6	6.3227	2.27	0:00:02.704	35	41.3227	0:00:04.97	
7	3.1459	0.42	0:00:06.728	20	23.1459	0:00:07.15	
8	-1.5164	0.32	0:00:08.594	40	38.4836	0:00:08.91	
9	-0.0538	0.28	0:00:10.522	25	24.9462	0:00:10.799	
10	0.1827	0.24	0:00:12.516	15	15.1827	0:00:12.755	
11	-3.288	0.32	0:00:14.473	30	26.712	0:00:14.796	
12	-0.3915	0.28	0:00:16.381	10	9.6085	0:00:16.662	
13	1.3024	0.26	0:00:18.322	35	36.3024	0:00:18.58	
14	-2.8784	0.22	0:00:20.469	20	17.1216	0:00:20.694	
15	2.5691	0.24	0:00:22.397	40	42.5691	0:00:22.639	
16	4.2048	0.23	0:00:24.562	25	29.2048	0:00:24.795	
17	4.5105	0.23	0:00:26.588	15	19.5105	0:00:26.817	
18	18.628	0.24	0:00:28.546	30	48.628	0:00:28.789	
19	0.9588	0.23	0:00:30.549	10	10.9588	0:00:30.777	
20	-4.9643	0.23	0:00:32.459	35	30.0357	0:00:32.694	
21	0.203	0.22	0:00:34.496	20	20.203	0:00:34.712	

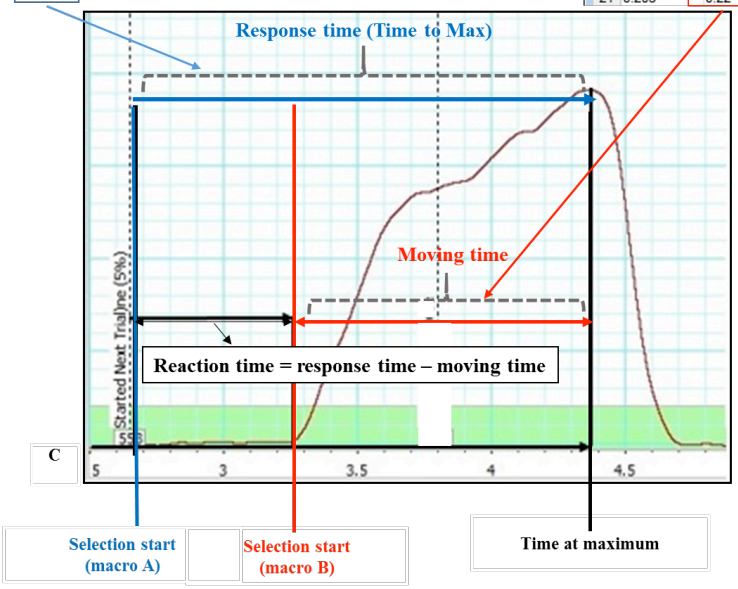

Figure 4-10: Reaction time was calculated from the difference between response time (column B, macro A) and moving time (column B, macro B).

Although these macros (A & B) in LabChart™ enabled us to collect raw data from force transducer in each block, due to the large amount of data recorded by Data Pad, two other macros (C & D) were developed in MATLAB for automation of data analysis.

Developing macros in MATLAB software

Macro C (Appendix 13)

Requirement: an algorithm for automatic analysis of sequence learning (movement time, error rate and skill)

Macro C enabled us to achieve the required variables for sequence learning based on *trial based*, including movement time, error rate and skill, which obtained based on the average data in each trial (Reis et al., 2015; Reis et al., 2009a; Saucedo Marquez et al., 2013; Schambra et al., 2011) (Figure 4-11).

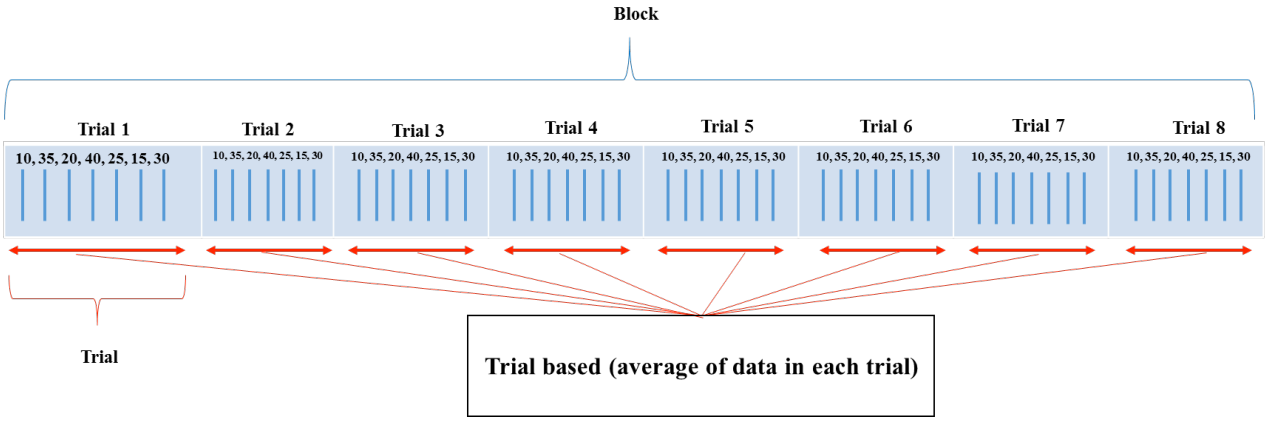

Figure 4-11: Average of data for eighth trials were calculated across a block in trial based data handling,.

Movement time

Movement time was defined as the whole time for completion of a trial with different target forces (Figure 4-12). In this thesis series of eight trials were completed by participants in each block. Movement time in each block was measured as the average of movement for eight trials which was taken from onset for the first force target to cessation of movement after the final target (Reis et al., 2009a).

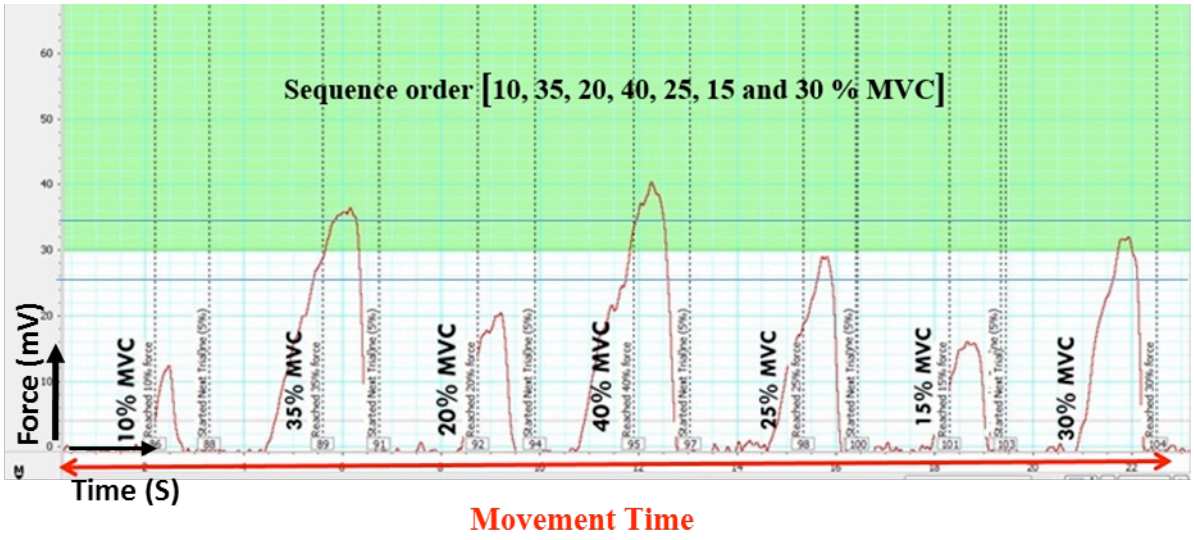

Figure 4-12: Movement time is defined as the whole time for completion of a trial.

Error rate

The error rate was calculated as the proportion of trials with at least one error. Participants should meet all seven targets in each trial correctly to be scored as accurate for that trial. For example, the error rate for a block was 5/8 equals 0.625, if participants had at least one or more over- or undershoot target in five trials out of eight trials of that block.

Skill

Skill is a combination of both parameters of movement time and error rate and represent changes in the speed-accuracy trade off following motor sequence learning. Skill was obtained from the following formula suggested by Reis et al. (2009).

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

As shown in Figure 4-13, an Excel file obtained from MATLAB program automatically measured movement time, error rate and skill in per block. This file consisted of six columns

including block number, undershoot, overshoot, movement time, error rate and skill. The average of movement times and error rates for the eight trials in a given block were presented in columns 4 and 5, respectively. The last column gave us skill measurement, which was obtained from formula suggested by Reise et al. (2009).

1	2	3	4	5	6
Block	under shoot	overshoot	movement time	error rate	skill
1	3	2			
1	1	2			
1	1	2			
1	0	3			
1	0	3			
1	0	4			
1	0	4			
1	0	2	18.5	1	0
2	0	2			
2	1	1			
2	0	2			
2	0	1			
2	0	1			
2	0	1			
2	1	1			
2	0	3	15.75	1	0
3	0	2			
3	0	1			
3	0	3			
3	0	3			
3	0	1			
3	0	2			
3	0	2			
3	0	2	16.875	1	0
4	0	1			
4	0	1			
4	0	0			
4	1	2			
4	0	0			
4	0	3			
4	0	0			
4	0	1	16.5	0.625	0.03946

Figure 4-13: Behavioural outcomes including movement time, error rate and skill were automatically analysed for each block using MATLAB program. For example, in block 4, the average movement time for 8 trials was 16.5 sec. The error rate was 5 / 8 or 0.625 because 5 trials had at least one error and skill was 0.039.

Since changes in each target force are not determined by trial based data handling, we also measured data based on a *force-target-by-force target basis* (force based data handling) (Figure 4-14).

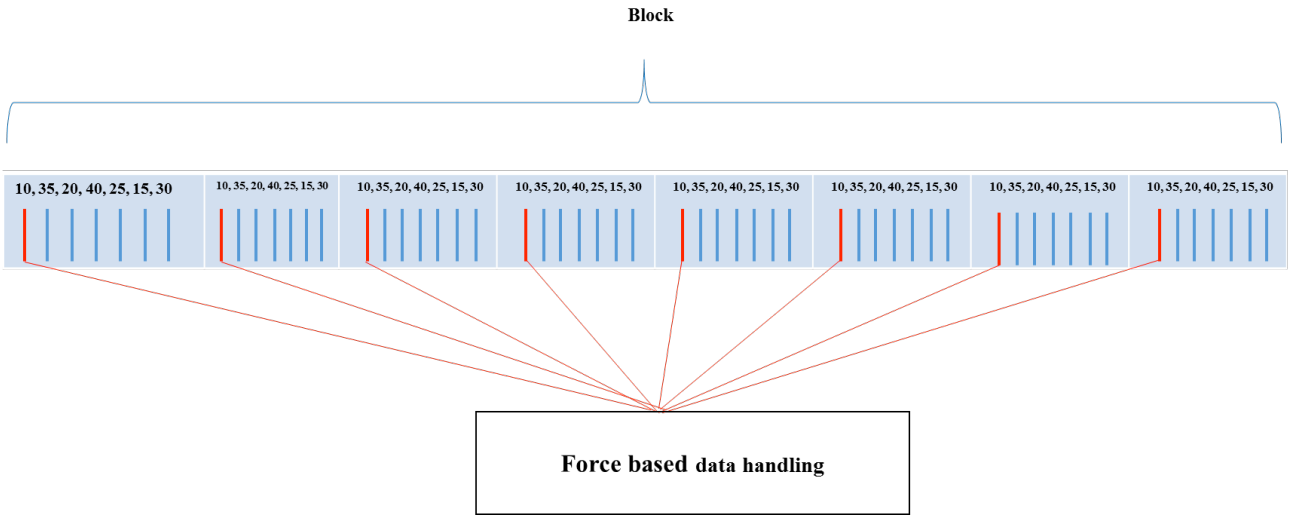

Figure 4-14: In force based data handling, the average of the eight repetitions of the same force target were measured in per block.

Macro C was then modified in order to automatic analysis of data obtained base on *target force measurement* (force based data handling).

Requirement 2: An algorithm for automatic analysis of target force measurement

Macro C enable us to automate analysis of force deviations, response time and reaction time on a force-target-by-force target basis.

Force deviations

As shown in Figure 4-15, the raw data from Data Pad was imported into MATLAB. MATLAB Outputs were exported as two Excel file sheets in which the variance of the eight repetitions of the same target forces from 10% to 40% were measured for each force target individually. For example, force deviations for force 10% of MVC in a set of eight trials in a given block was calculated from the following formula, where a = force production, subscript indicate the trial number, and n = 8.

$$\sqrt{\frac{(a_1 - 10)^2 + (a_2 - 10)^2 + ... + (a_n - 10)^2)}{n}}$$

Data pad

Seq Mooly 11-3-2015: Data Pad View*

1,801 points from t=8.049 to t=9.85

L20

	A Center Error	Time to Max (s)	C Selection Start	D Target Force (%)	E Force Reached	F Channel 1 Time at Maximum
1	-1.5164	1.42	0:00:08.049	40	38.4836	0:00:08.91
2				Channel 1	Channel 1	Channel 1
3	E – D		Sel Start	Extract Nos	Maximum	Max Time
4					%	
5	5.0096	0.3	0:00:00.0	10	15.0096	0:00:03.0
6	6.3227	2.97	0:00:01.999	35	41.3227	0:00:04.97
7	3.1459	1.20	0:00:05.949	20	23.1459	0:00:07.15
8	-1.5164	0.86	0:00:08.049	40	38.4836	0:00:08.91
9	-0.0538	0.85	0:00:09.949	25	24.9462	0:00:10.799
10	0.1827	0.96	0:00:11.799	15	15.1827	0:00:12.755
11	-3.288	1.00	0:00:13.799	30	26.712	0:00:14.796
12	-0.3915	0.86	0:00:15.799	10	9.6085	0:00:16.662
13	1.3024	0.88	0:00:17.699	35	36.3024	0:00:18.58
14	-2.8784	1.04	0:00:19.649	20	17.1216	0:00:20.694
15	2.5691	0.94	0:00:21.699	40	42.5691	0:00:22.639
16	4.2048	1.15	0:00:23.649	25	29.2048	0:00:24.795
17	4.5105	0.92	0:00:25.899	15	19.5105	0:00:26.817
18	18.628	0.89	0:00:27.899	30	48.628	0:00:28.789
19	0.9588	0.93	0:00:29.849	10	10.9588	0:00:30.777
20	-4.9643	0.90	0:00:31.799	35	30.0357	0:00:32.694

block	trial	center error	number	Force deviation
1	1	5.0096	10	
1	2	-0.3915	10	
1	3	0.9588	10	
1	4	-2.4689	10	
1	5	-2.9882	10	
1	6	1.7897	10	
1	7	2.0667	10	
1	8	0.1971	10	1.9838125

MATLAB (Excel file sheet 3)

block	number	mean	SD	VV	Mean VV
1	10	10.521662	2.5781925	2.4674529	1.9838125
1	15	12.495125	4.0435196	4.5365948	4.122
1	20	17.835665	3.4854656	3.9133449	3.2254125
1	25	27.863175	5.0305296	5.5082411	4.1153
1	30	31.256185	7.4460832	7.0775450	4.4830375
1	35	34.168787	5.3683627	5.0899722	4.8168625
1	40	39.65645	3.1583041	2.9742311	2.685825

MATLAB (Excel file sheet 4)

Figure 4-15: Automatic analysis of force deviations in MATLAB (sheet 3 & 4) using the raw data from Data Pad. The force deviations for target force of 10% MVC was 1.98 in block 1.

Response time

The mean of response time for the eight repetitions of the same target force across a block was automatically calculated in MATLAB using the raw data from Data Pad (Figure 4-16). The following formula was used to compute of response time for each force target where d = response time and n = 8.

$$Response\ time = \sum_{n=8} \left(\frac{d1 + d2\ \ldots\ldots\ldots dn}{n} \right)$$

Data Pad

Seq Mooly 11-3-2015: Data Pad View*

1,801 points from t=8.049 to t=9.85

L20

	Centre error	Time to Max (s)	C Selection Start	Target Force (%)	Force Reached	F Channel 1 Time at Maximum
1	-1.5164	1.42	0:00:08.049	40	38.4836	0:00:08.91
2				Channel 1	Channel 1	Channel 1
3			Sel Start	Extract Nos	Maximum	Max Time
4					%	
5	5.0096	0.3	0:00:00.0	10	15.0096	0:00:03.0
6	6.3227	2.97	0:00:01.999	35	41.3227	0:00:04.97
7	3.1459	1.20	0:00:05.949	20	23.1459	0:00:07.15
8	-1.5164	0.86	0:00:08.049	40	38.4836	0:00:08.91
9	-0.0538	0.85	0:00:09.949	25	24.9462	0:00:10.799
10	0.1827	0.96	0:00:11.799	15	15.1827	0:00:12.755
11	-3.288	1.00	0:00:13.799	30	26.712	0:00:14.796
12	-0.3915	0.86	0:00:15.799	10	9.6085	0:00:16.662
13	1.3024	0.88	0:00:17.699	35	36.3024	0:00:18.58
14	-2.8784	1.04	0:00:19.649	20	17.1216	0:00:20.694
15	2.5691	0.94	0:00:21.699	40	42.5691	0:00:22.639
16	4.2048	1.15	0:00:23.649	25	29.2048	0:00:24.795
17	4.5105	0.92	0:00:25.899	15	19.5105	0:00:26.817
18	18.628	0.89	0:00:27.899	30	48.628	0:00:28.789
19	0.9588	0.93	0:00:29.849	10	10.9588	0:00:30.777
20	-4.9643	0.90	0:00:31.799	35	30.0357	0:00:32.694

MATLAB

block	trial	center error	Time to max	Selection Start	Target Force	Mean Response Time
1	1	5.0096	0.3	0	10	
1	2	0.3915-	0.86	15.799	10	
1	3	0.9588	0.93	29.849	10	
1	4	2.4689-	1.07	44.649	10	
1	5	2.9882-	0.88	59.549	10	
1	6	1.7897	0.81	73.949	10	
1	7	2.0667	0.83	89.749	10	
1	8	0.1971	0.89	105.249	10	0.85875
1	1	0.1827	0.96	11.799	15	
1	2	4.5105	0.92	25.899	15	
1	3	5.2184-	0.75	39.649	15	
1	4	5.7031-	0.96	55.499	15	
1	5	5.53-	0.91	70.099	15	
1	6	5.3569-	0.84	85.949	15	
1	7	4.6991-	0.82	101.449	15	
1	8	1.7753	0.84	116.549	15	0.875
1	1	3.1459	1.2	5.949	20	
1	2	2.8784-	1.04	19.649	20	

Figure 4-16: Automatic aanalysis of response time in MATLAB using the raw data of Data Pad (column B). For example, the mean response time for target force of 10% of MVC was 0.85 sec in block 1.

Reaction time:

The mean of the eight repetitions of reaction time in same force target across a block was automatically calculated using MATLAB. For instance, the mean of reaction time for the eight repetitions of the target force of 10% for block 1 was 0.6 sec (Figure 4-17).

block	trial	center error	Reaction time	Selection start 2	Target Force	Mean Reaction time
1	1	5.0096	0.15	0.84	10	
1	2	0.3915-	0.58	16.381	10	
1	3	0.9588	0.7	30.549	10	
1	4	2.4689-	0.84	45.484	10	
1	5	2.9882-	0.67	60.219	10	
1	6	1.7897	0.58	74.527	10	
1	7	2.0667	0.62	90.366	10	
1	8	0.1971	0.68	105.922	10	0.6025
1	1	0.1827	0.72	12.516	15	
1	2	4.5105	0.69	26.588	15	
1	3	5.2184-	0.53	40.182	15	
1	4	5.7031-	0.74	56.242	15	
1	5	5.53-	0.69	70.782	15	
1	6	5.3569-	0.62	86.569	15	
1	7	4.6991-	0.6	102.051	15	
1	8	1.7753	0.62	117.166	15	0.65125
1	1	3.1459	0.78	6.728	20	
1	2	2.8784-	0.82	20.469	20	
1	3	0.203	0.64	34.496	20	

Figure 4-17: The results of automatic measurement of reaction time in MATLAB.

Macro D (Appendix 14)

Requirement: program for sorting target data

When blocks were randomized, it was necessary to reorder the collected data into a consistent sequence to facilitate analysis. Therefore, macro D was developed to sort target data ascending order for each block (Figure 4-18).

Random block

Centre error	Time to max	Selection start	Target force	Force reached	Time
4.6276	2.24	00:00.0	15	19.6276	00:02.2
2.2086	3.13	00:02.7	40	42.2086	00:05.9
10.5123	1.78	00:06.9	10	20.5123	00:08.7
0.9697	3.69	00:09.2	35	35.9697	00:12.9
-3.7428	1.68	00:13.9	25	21.2572	00:15.6
17.9819	2.99	00:16.5	30	47.9819	00:19.5
2.8868	2.36	00:19.7	20	22.8868	00:22.1
11.8361	1.53	00:23.7	30	41.8361	00:25.3
6.5831	1.88	00:26.0	15	21.5831	00:27.9
6.0527	1.88	00:28.5	20	26.0527	00:30.4
2.4495	1.63	00:31.1	25	27.4495	00:32.8
-3.006	1.84	00:33.6	40	36.994	00:35.5
-2.4756	2.57	00:36.5	35	32.5244	00:39.1
6.2754	1.71	00:39.8	10	16.2754	00:41.6
2.5143	1.19	00:43.4	20	22.5143	00:44.6
20.6074	1.94	00:45.4	25	45.6074	00:47.3
3.2491	1.83	00:47.6	10	13.2491	00:49.5
1.6013	1.65	00:50.1	15	16.6013	00:51.8

Sort targets

MATLAB (macro D)

Centre error	Time to max	Selection start	Target force	Force reached	Time
10.5123	1.78	7.985E-05	10	20.5123	0.0001
4.6276	2.24	0	15	19.6276	2.6E-05
2.8868	2.36	0.000228	20	22.8868	0.000255
3.7428-	1.68	0.00016087	25	21.2572	0.00018
17.9819	2.99	0.00019096	30	47.9819	0.000226
0.9697	3.69	0.00010647	35	35.9697	0.000149
2.2086	3.13	3.1817E-05	40	42.2086	6.8E-05
6.2754	1.71	0.00046122	10	16.2754	0.000481
6.5831	1.88	0.00030149	15	21.5831	0.000323
6.0527	1.88	0.00033043	20	26.0527	0.000352
2.4495	1.63	0.00036052	25	27.4495	0.000379
11.8361	1.53	0.00027487	30	41.8361	0.000293
2.4756-	2.57	0.00042244	35	32.5244	0.000452
3.006-	1.84	0.00038946	40	36.994	0.000411
3.2491	1.83	0.00055149	10	13.2491	0.000573
1.6013	1.65	0.00058043	15	16.6013	0.0006
2.5143	1.19	0.0005023	20	22.5143	0.000516
20.6074	1.94	0.00052545	25	45.6074	0.000548

Figure 4-18: Sorting of target data ascending order trial by trial in a random block using macro D.

In summary, this chapter provided an in-depth background of how macros were setup and how data was collected in order to automate data management during SVIPT.

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 the contribution	Extent of the contribution (%)
Review of literature, study 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 the contribution
Shapour Jaberzadeh	Study design, guidance in framing of the manuscript, reviewing and provision of feedback on the manuscript drafts.
Paul B Fitzgerald	Reviewing and provision of feedback on the manuscript drafts.
Maryam Zoghi	Reviewing and provision of feedback on the manuscript drafts.
Masoumeh Hashemirad	Developing some macros in MATLAB in order to automation of data analysis.

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

Candidate's name

Signature Date

Signature Date

Preamble to Chapter 5

The following chapter was accepted to be published in the Journal of Bodywork and Movement therapies on 23th November 2016. Chapter 5 evaluates the influence of varying interval between sequenced trials on the acquisition of implicit sequence learning during SVIPT. In this chapter, Tables and Figures have been inserted into the manuscript for ease of reading.

Aim

To investigate the effects of inter-trial interval on implicit motor sequence learning using SVIPT.

Chapter 5: The effects of inter-trial interval on implicit learning of sequential visual isometric pinch task

The format of this chapter is consistent with the Journal of Bodywork and Movement therapies.

Journal of Bodywork & Movement Therapies xxx (2016) 1–7

Contents lists available at ScienceDirect

Journal of Bodywork & Movement Therapies

journal homepage: www.elsevier.com/jbmt

Original research study

The effects of inter-trial interval on implicit learning of sequential visual isometric pinch task

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ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form
7 November 2016
Accepted 23 November 2016

Keywords:
Sequential visual isometric pinch tasks (SVIPT)
Implicit motor sequence learning
Inter-trial interval
Pinch force control

ABSTRACT

Sequential visual isometric pinch task (SVIPT) has been recently used as a visuomotor sequence task in clinical research. The influence of varying intervals between sequenced trials on the acquisition of implicit sequence learning is not yet determined for SVIPT. The aim of this study was to investigate the effects of inter-trial interval (ITI) on implicit motor sequence learning using SVIPT. A total of 32 healthy participants with mean age 31.3 ± 4.5 years participated in this study. Participants were randomly assigned to one of four ITI groups; (1, 2, 3 and 4 s). They were instructed to control their force on a force transducer to reach a number of targets which appeared on the computer screen by changing the pinch force exerted onto the transducer. In this study, outcome measures were movement time, error rate and skill, which were measured before and after training. Our results indicated that motor sequence learning similarly affected various ITIs. Indeed, all participants exhibited same improvement in implicit learning of SVIPT even though the ITIs varied from 1 to 4 s. Our findings suggest that implicit learning of SVIPT is independent of ITI within this range in healthy individuals.

1. Introduction

The inherent ability of humans to acquire sequential movement patterns through practice is defined as motor sequence learning (Katschnig-Winter et al., 2014). This kind of learning has a crucial role in everyday life, from simple activities such as pressing a button to complex tasks such as playing a piano (Waters-Metenier et al., 2014). Sequence learning can be categorized into explicit and implicit types of learning. Implicit motor sequence learning occurs when the learner is not aware of the repeating sequence. (Robertson, 2007). In contrast, explicit sequence learning occurs with awareness of sequenced movements (Robertson, 2007).

Extensive research in humans and animals has dealt with the importance of temporal factors on motor sequence learning (Deffains et al., 2011; Destrebecqz and Cleeremans, 2003; Dominey, 1998). In sequential movement patterns, stimuli are usually delivered in a number of trials and clustered to form a block of training. Repeating sequential stimuli in each trial, with or without awareness of the sequence, can result in changes in behavioural outcome measures, such as speed and accuracy, as well as skill (Reis et al., 2009, 2015; Saucedo Marquez et al., 2013; Schambra et al., 2011).

Several experimental approaches have been established to better understand motor sequence learning. In this regard, two types of tasks have been extensively used: serial reaction time tasks (SRTT) and sequential visual isometric pinch tasks (SVIPT). In SRTT, visual cues or stimuli are represented in a repeated order at any of four positions horizontally on a computer monitor, and participants respond by pressing a corresponding button, from 1 to 4 on a response pad (Robertson, 2007). In SVIPT, participants are required to pinch force on a force transducer to precisely meet different levels of visual target forces that appear in a sequence on the computer screen (Reis et al., 2009, 2015; Saucedo Marquez et al., 2013; Schambra et al., 2011).

Knowledge gained through sequential learning can be affected by varying temporal factors such as inter-trial intervals (ITI) or response-stimulus intervals (RSI) (Buonomano et al., 2009; Cheng

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http://dx.doi.org/10.1016/j.jbmt.2016.11.014
1360-8592/

et al., 2013; Frensch and Miner, 1994; Koegel et al., 1980; Ohashi, 1994; Wolach, 1970). Some behavioural studies have shown that sequence elements are chunked or grouped together during sequential learning (Tremblay et al., 2009, 2010; Wymbs et al., 2012). A chunk is defined as several clusters of information which are separated from each other by intervals or long time gaps (Miller, 1956). Intervals ranging from milliseconds to a few seconds have been investigated in the majority of studies (Buhusi and Meck, 2005; Ivry and Spencer, 2004; Mauk and Buonomano, 2004; van Wassenhove, 2009).

There is some evidence for the effects of temporal factors on implicit learning of SRTT (Curran and Keele, 1993; Frensch and Miner, 1994; Keele and Jennings, 1992; Koch and Hoffmann, 2000; Rosenbaum et al., 1983; Stadler, 1992, 1993, 1995), Inserting a pause between successive trials may lead to enhancement of sequence learning, through the chunking of the series of information that subsequently helps the sequence to be stored and retrieved more easily (Bourne Jr. and Bunderson, 1963; Stadler, 1995). However, sequence learning could be impaired by longer ITIs, due to impairment of memory formation (Frensch and Miner, 1994; Loess and Waugh, 1967; Stadler, 1992, 1993; Willingham et al., 1997), especially during tasks in which participants are given feedback after the completion of each trial (Bourne et al., 1965).

The length of this interval may be important to learning (Bourne et al., 1965; Weinberg et al., 1964; Willingham et al., 1997). Weinberg et al. (1964) investigated the effects of intervals of 1, 5, 10, and 20 s on learning a simple motor task. They found that increasing intervals facilitate performance up to an optimum interval of 5 s. Koegel et al. (1980) suggested the range from 1 to 4 s improve learning more than the long intervals i.e. 5–26 s. Complexity and type of task may be important in determination of optimum ITIs in order to increase learning. Decreased fatigue effect can be considered as another reason for enhancement of learning following inserting intervals during training (Jerome et al., 1958). Early signs of fatigue were reported for ITI of 1 and 3.5 s for work periods from 5 to 30 min. An interval of 7.5 s appeared optimal to decrease fatigue effects for the same work periods (Jerome et al., 1958).

Even though there is some evidence for the effects of temporal factors on implicit learning of SRTT (Curran and Keele, 1993; Frensch and Miner, 1994; Keele and Jennings, 1992; Koch and Hoffmann, 2000; Rosenbaum et al., 1983; Stadler, 1992, 1993, 1995), these effects have not been studied in SVIPT. Unlike SRTT which involves with a great deal of cognitive demand, SVIPT is a fine-motor control task, with greater motor demands. Regarding the growing interest in using SVIPT as a visuomotor sequence task in research, understanding important influences, such as temporal factors on acquired learning in this task would be of great value. Therefore, we designed this study to investigate the effects of ITIs on implicit learning in SVIPT.

For this study, participants were divided into four groups, with various intervals from 1 to 4 s to explore the influence of ITIs on implicit learning using SVIPT. We hypothesized that participants in the 4-sec interval group would exhibit more improvement in implicit learning than other ITIs groups, as increased time between trials should allow more opportunity to chunk each sequence.

2. Methods and materials

2.1. Participants

A total of 32 healthy participants (24 females, 8 males, 31.3 ± 4.5 years) participated in this randomised single-blind study. All participants were healthy with normal or corrected-to- normal vision. Participants were excluded if they had any disability with their fingers, hands, or with wrist movements, or significant experience with computer gaming or current usage of drugs known to influence motor behavior or cognition. Ethics committee approval and written informed consent from the participants were obtained prior to the experiment. All participants completed the Edinburgh Handedness Inventory (Oldfield, 1971), and were also given a questionnaire about their sleep hours, sleep quality, attention and fatigue, using numerical rating scale (NRS). The participants were instructed to select a number from 0 (lowest) to 10 (highest) to describe their subjective feelings of fatigue, attention and sleep quality (Martin et al., 2009; Waters-Metenier et al., 2014; Wewers and Lowe, 1990). About the sleep hours, we asked them "how many hours did you sleep the night before?" In all 32 participants, sleep hours were from 3 to 10 h with the mean ± SD (7.28 ± 1.44).

2.2. Experimental design

2.2..1. Sequential visual isometric pinch task (SVIPT)

SVIPT is a pinch force task in which participants are asked to modulate their precision force on the force transducer (AD instrument MLT004/ST, NSW, Australia) to reach a target force displayed on the computer screen (Fig. 1). This force transducer is an isometric dynamometer which converts biological signals into electrical analog signals. The analog signal was then digitized using Power Lab data acquisition hardware.

2.2.2. Procedure

Thirty-two participants were randomly assigned to one of the four ITI groups with intervals from 1 to 4 s. Participants were seated in front of a computer and asked to hold the force transducer between the thumb and index fingers of their dominant hand. At the beginning of each experiment, maximum isometric contraction (MVC) was determined individually in each participant. The MVC was used to calibrate the force transducer within the Power Lab data acquisition system. After two familiarization trials, participants performed a pre-test block (consisting of seven trials) with their dominant hand. Then, eight blocks were carried out with dominant hand. This lasted approximately 20 min with a one min rest interval between each block. After training, participants performed one block with their dominant hand as a post-test assessment. Each block consisted of eight trials and each trial included seven different target forces in a sequenced order (10, 35, 20, 40, 25, 15, and 30% MVC) (Fig. 2).

During the task, participants were presented with a visual and numerical indication of the target forces on the screen. Participants were asked to squeeze the force transducer in order to move the cursor toward the target levels. Each target level was defined as the target force and a range between plus and minus of 5% of MVC for each target was acceptable (Target force ± 5% MVC). Any movement of the cursor above or below this range was determined as error and considered an overshoot or undershoot, respectively. After reaching each target, participants released the force on the transducer and the cursor returned to the baseline. Each release triggered the system to show the next target. When the next target appeared, the visual and numerical indication on the computer screen changed accordingly. Participants were instructed to reach target forces by squeezing the force transducer as quickly and accurately as they could.

After completion of all seven target forces in each trial, a dashed line appeared in the numerical text box, which indicated that the current trial had ended. The next trial then started after an interval of 1–4 s, depending on which ITI group the participant was in. Participants were blinded to the length of the ITI or sequenced order. Since some studies have shown ITIs more than 4 s can disturb

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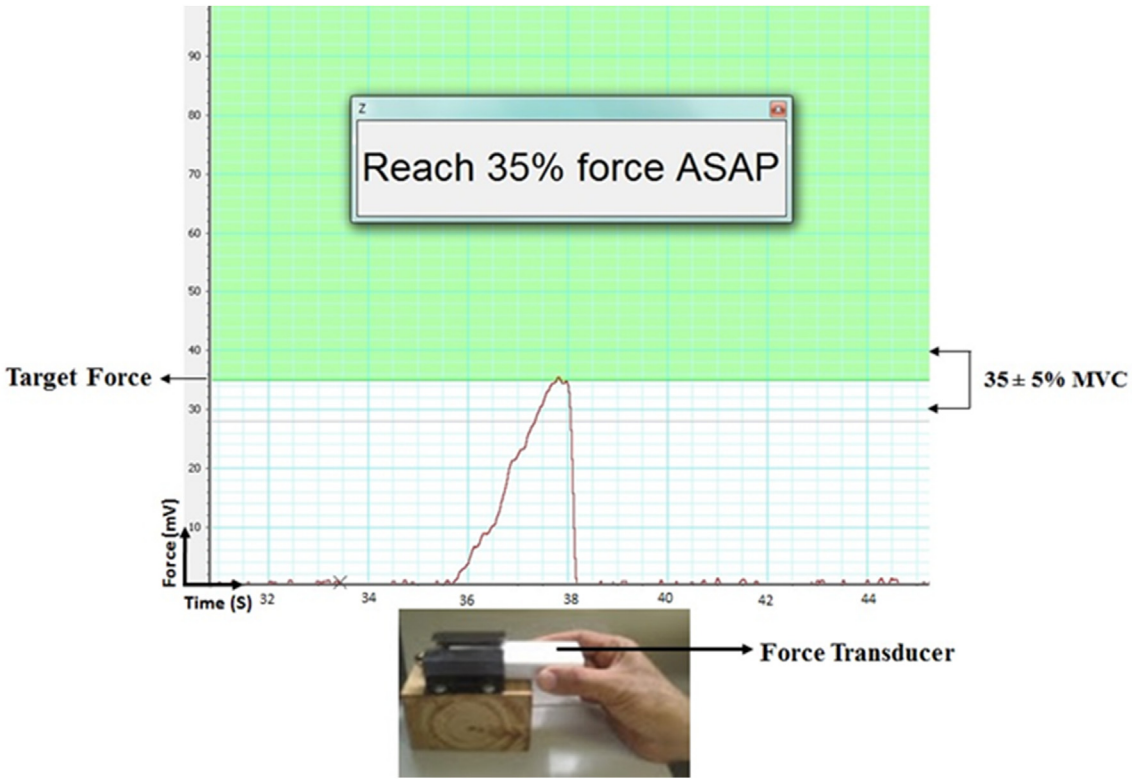

Fig. 1. Pinch grip protocol using SVIPT.

learning (Carnine, 1976; Koegel et al., 1980; Weinberg et al., 1964), we chose a limited range of ITIs up to 4 s, which was roughly the half of the time for completion of each trial.

After completion of the experiment, explicit learning was assessed directly via participants' own recognition and recall (Nissen et al., 1989; Vidoni and Boyd, 2007; Zhuang et al., 1998). The participants were asked whether they had noticed that the stimuli had been presented in a repeating order or not. The participants were asked two questions. 1- "whether you had noticed that the stimuli had been presented in a repeating order or not". If they said yes, we asked them the second question 2- Can you recall the sequence? If they could correctly recall five or more successive target forces of the sequence (i.e. 10, 35, 20, 40 and 25% of MVC), we considered their learning was explicit and their data were excluded. This was done because if participants received explicit knowledge and became aware that the targets occur in a repeating sequence they would show more sequence knowledge compared to participants with implicit knowledge (Keele et al., 2003).

2.3. Outcome measures

2.3.1. Movement time

Movement time in each trial was measured from movement onset for the first target to cessation of movement after the final target. The average movement time of eight trials in each block was taken as the movement time for that given block (Reis et al., 2009).

2.3.2. Error rate

The error rate was calculated as the proportion of trials with at least one over- or undershoot within each block (Reis et al., 2009). Each block consisted of eight trials (seven target forces) and participants should meet all seven targets in each trial correctly to be scored as accurate for that trial. For example, the error rate for a block was 4/8 if participants had at least one or more over- or undershot target in four out of eight trials in that block.

2.3.3. Skill

Skill was calculated by considering movement time and error rate and obtained from the following formula (Reis et al., 2009):

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

2.4. Data analysis

Data were analysed using MATLAB (R2014a) and SPSS (version 22). The normality of data was assessed using Kolmogorov-Smirnov (K-S) test. One-way ANOVA was used to determine differences in demographic characteristics and parameters such as sleep hours, quality sleep, attention, computer game experience, fatigue as well as MVC across four groups with different ITIs.

For all normal distributed variables, a mixed-design ANOVA (Repeated-measure) with the factor Time (pre vs. post) as a within-subjects factor and factor Group (1, 2, 3 and 4 s) as between-subjects factor was used to determine differences in measured data among the groups over time. If the assumption of Mauchly's sphericity test was violated, a Greenhouse-Geisser was applied in order to correct non-sphericity. Post hoc tests (Bonferroni correction) were performed as appropriate to determine where differences occurred.

For non-normal distributed data, log transformation was used to equalize variances and validly perform parametric statistical analyses. If normal distribution were not corrected by logarithmic transformation and skewness scores were still more than one, non-

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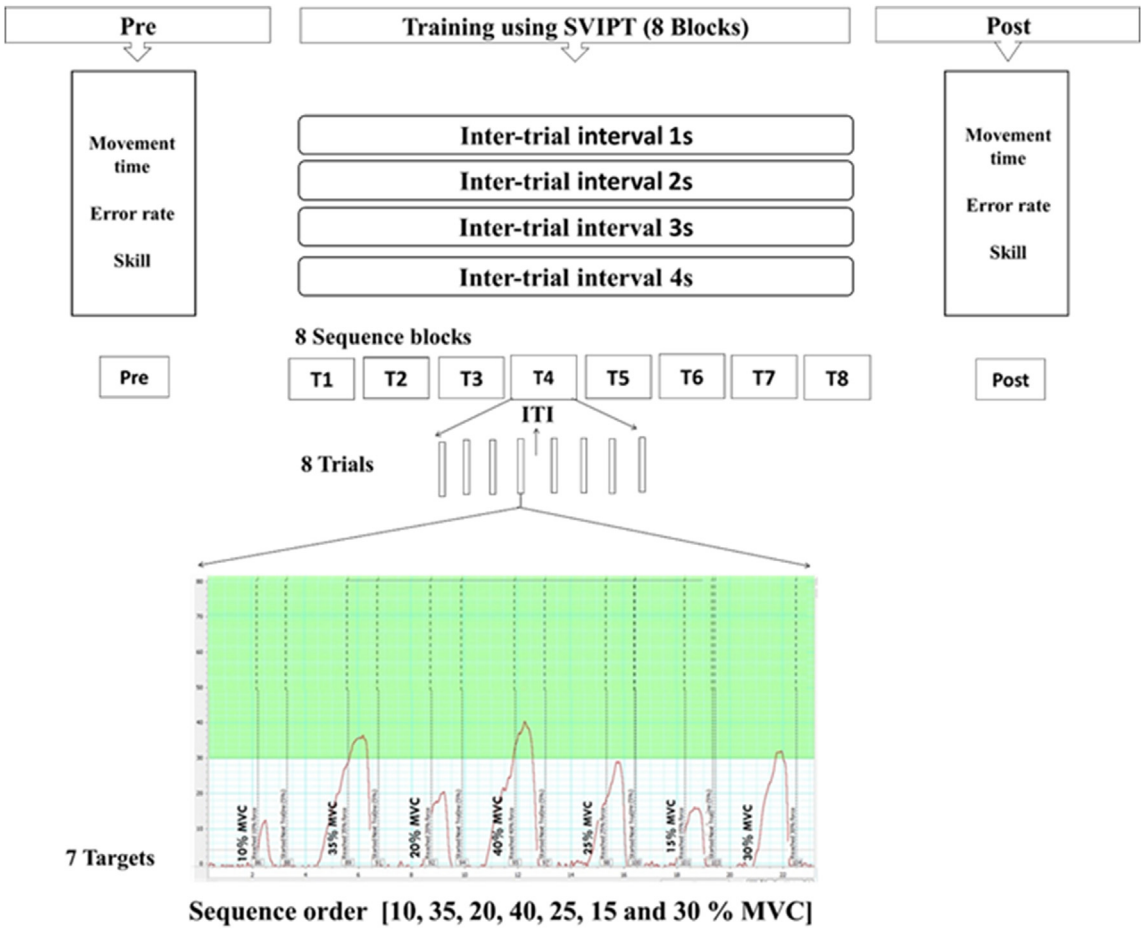

Fig. 2. Experimental set up for implicit motor sequence learning using SVIPT. Participants were required to implement precise pinch force on a force transducer to meet different levels of visual target forces that appeared in a sequence order on the computer screen. Each block consisted of eight trials and each trial included seven different target forces in a sequenced order (10, 35, 20, 40, 25, 15, and 30% MVC). After completion of all seven target forces in each trial depending on which inter-trial interval (ITI) group the participant was, an interval of 1–4 s was inserted before the next trial started.

parametric tests were conducted.

A Related-Samples Wilcoxon Signed Rank Test was used to investigate the median of differences between pre- and post-test for non-normal distributed data. Independent-Samples Median Test was applied to evaluate changes in medians of variables across the groups. Post hoc analysis (Bonferroni correction) were performed as appropriate to determine where differences occurred. The level of significant was set at $p < 0.05$ in all data analysis.

3. Results

The results of one-way ANOVA showed that there were no significant differences in demographics and parameters including MVC, sleep hours, sleep quality, attention, computer game experience and sequence knowledge among the four groups (Table 1). No participants could recall five consecutive target forces so no participants received explicit knowledge during training. In addition, no participants reported fatigue during training.

Results of K-S normality test indicated that movement time was normal distributed ($p = 2$) while error rate ($p < 0.001$) and skill ($p < 0.001$) were non-normally distributed. Error rate with skewness (1.64) and SE (0.41) and skill with skewness (1.71) and SE (0.41) were not corrected by log transformation (K-S, $p < 0.001$). Therefore, non-parametric analysis was applied for error rate and

Table 1
Demographic characteristics and psychological measures.

	Group 1s Mean ± SD	Group 2s Mean ± SD	Group 3s Mean ± SD	Group 4s Mean ± SD	ANOVA F (3,28)	p
Number (Female/Male)	8 (5/3)	8 (8/0)	8 (4/4)	8 (7/1)		
Age	35.25 ± 10.49	28.12 ± 7.05	36.87 ± 9.35	30.25 ± 10.36	1.52	0.22
Handedness (Edinburgh)	89.15 ± 9.16	80.33 ± 20.77	61 ± 48.31	71.25 ± 48.53	0.898	0.45
MVC	56.7 ± 21.12	64.88 ± 16.55	80.59 ± 36.20	61.51 ± 17.35	1.46	0.24
Computer game (Hour)	0.125 ± 0.35	0.25 ± 0.46	0.125 ± 0.35	0.375 ± 1.06	0.288	0.83
Sleep hour	6.75 ± 1.66	8 ± 0.92	7.12 ± 1.8	7.25 ± 1.16	1.06	0.38
Sleep quality (0:10)	7.37 ± 1.18	7.87 ± 1.24	6.62 ± 2.06	7.12 ± 1.39	1.134	0.35
Attention (0:10)	7.37 ± 1.18	6.75 ± 1.03	7.25 ± 1.48	7.5 ± 0.53	0.694	0.56
Awareness (the number of recall)	0.625 ± 0.91	0.625 ± 0.74	0.625 ± 0.91	0.53 ± 0.46	0.46	0.71

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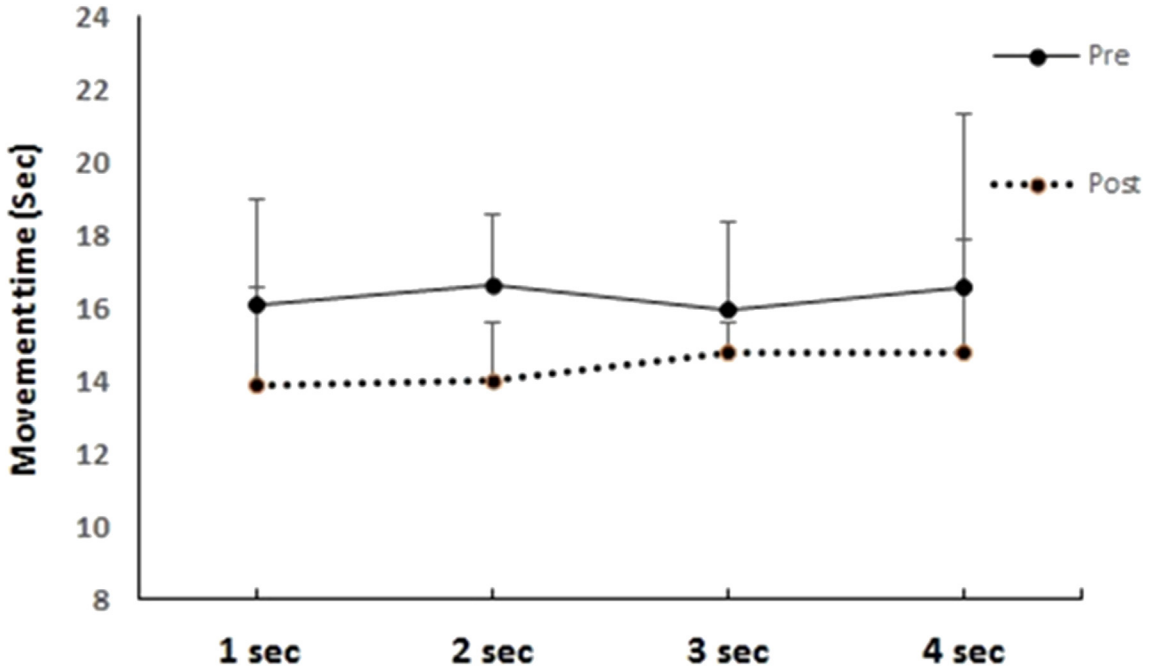

Fig. 3. The results of mixed-design ANOVA on movement time before (Pre) and after (Post) training among all four groups with various inter-trial interval (ITI) from 1 to 4 s. Data are presented at mean (SD).

skill while movement time was analysed using a mixed-design ANOVA.

No baseline differences were found in behavioural outcome measures including movement time ($p = 0.96$), error rate ($p = 0.74$) and skill ($p = 0.62$) among the four groups.

Movement time decreased from 16.2 ± 3.04 s (mean ± SD) at pre-test to 14.3 ± 2.15 s at post-test. The Results of a mixed-design ANOVA on movement time revealed a significant reduction in factor Time ($F(1, 28) = 31.8$, $p < 0.001$) while no significant differences were found in factor Group ($F(3, 28) = 0.106$, $p = 0.95$). Therefore, movement time significantly improved in all four groups after training in compared to the baseline (Fig. 3). No interaction effect was observed between Group × Time ($F(3, 28) = 0.77$, $p = 0.51$).

Results of Related-Samples Wilcoxon Signed Rank Test revealed significant improvement in error rate ($Z = -2.88$ ($n = 32$), $p = 0.004$) and skill ($Z = 3.09$ ($n = 32$), $p = 0.002$) over times from pre-to post-test. Mean rank of error rate and skill changed significantly from pre- (1.66, 1.25) to post-test (1.34, 1.75), respectively.

The results of Independent-Samples Test indicated no differences in error rate ($\chi2(3) = 1.24$, $p = 0.71$) and skill ($\chi2(3) = 1.74$, $p = 0.62$) at the baseline measurement among the four groups (Fig. 4A and B). Similar results were found on the medians of error rate ($\chi2(3) = 4.26$, $p = 0.23$) and skill ($\chi2(3) = 1$, $p = 0.8$) after training (Fig. 4C and D). Indeed, improvement in error rate and skill after training were similarly observed in all ITI groups with various range of ITI from 1 to 4 s.

4. Discussion

The present study was designed to examine whether implicit learning in SVIPT is influenced under conditions where the ITIs were manipulated from 1 to 4 s. The results demonstrate that despite differences in ITIs, the participants exhibited similar improvements in behavioural outcome measures after training with SVIPT.

Observed independence of behavioural outcomes from ITIs can be related to the structure of implicit learning used in this study. In implicit learning, information about each trial is stored automatically and participants do not even know that memory is involved during training (Stadler, 1995; Willingham et al., 1997). The amount of attention required for the implicit learning is dependent on the structure of sequences or repetition of stimuli (Cohen et al., 1990; Curran and Keele, 1993). Therefore, two forms of implicit learning are defined: attentional and non-attentional (Curran and Keele, 1993). When stimuli are represented in different orders and there is no linear association between items in a sequence order, attentional implicit learning can occur. The role of attention is more dominant for learning in this situation since successive stimuli are coded hierarchically by parsing the structure into subgroups. In this type of learning, any factor that influences on attentional capacity during learning such as sequence intervals or distractors can produce changes in the acquisition of the implicit learning. In simple structures, such as where there is a linear relationship between stimuli, in which one stimulus is uniquely associated with another (e.g. stimulus A is always followed by stimulus B), learning is relatively free from attention. In the current study, the simple sequence of stimuli was repeated in each trial, implicit learning might have occurred based on a simple associative mechanism, according to Cohen et al. (1990). Inserting a consistent interval from 1 to 4 s at the begging of each trial in such a simple sequence not expected to have any effect in performance of SVIPT. Therefore, our hypothesis was not supported as the similar results were found.

Sensitivity to the measured variables can be another reason to explain the observed independence of implicit learning from the ITIs. In the current study, we measured behavioural outcomes such as movement time, error rate and skill, which have usually been assessed following sequence learning in SVIPT studies (Reis et al., 2009, 2015; Saucedo Marquez et al., 2013; Schambra et al., 2011). Some studies have shown that reaction time is a more reliable index for sequence learning than movement time or execution time (Deffains et al., 2011; Moisello et al., 2009). Therefore, further

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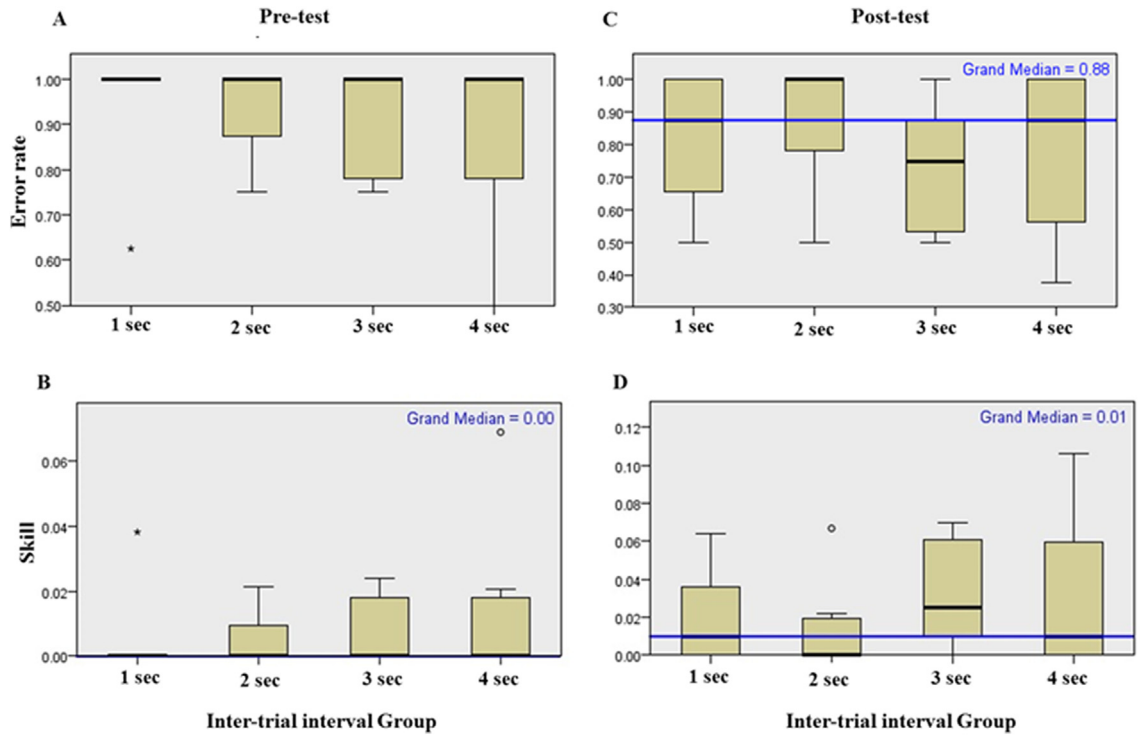

Fig. 4. The results of Independent-Samples on error rate (A &C) and skill (B & D) before and after training, respectively. No significant effects were found at any stimulation conditions.

research is needed to measure some variables, such as reaction time, during SVIPT in order to determine the effects of manipulation of temporal factors on this variable (this is the subject of a forthcoming publication). In addition, in this study, we measured implicit sequence learning based on participants' own recognition of the presence of a sequence (Curran and Keele, 1993; Nitsche et al., 2003; Willingham et al., 1989) and compared differences between sequence blocks before and after training. However, comparing assessment blocks before and after training under circumstances in which subjects are unaware of sequence order can provide information about implicit learning. Measuring a specific index for implicit learning, i.e. the difference in outcome measures between sequence and random blocks, could be a more reliable index for measuring implicit learning (Bahrick et al., 1954; Nissen and Bullemer, 1987).

It should be noted that, in most ITI studies, participants were given feedback during intervals while in the current SVIPT study, participants received no feedback, because we did not intend memory to become involved. To the best of our knowledge this is the first study shedding light on the effects of ITIs within SVIPT. Further research is needed to investigate the effects of temporal factors in situations, both with and without feedback, in SVIPT.

4.1. Limitations and suggestions

There are some limitations in this study. We measured behavioural outcomes in young healthy participants, therefore, our findings cannot be generalized to patients or elderly people. The sample size is quite small, so our results should be used cautiously because of the width of the confidence intervals. More studies need to be performed using larger sample sizes to improve precision in testing temporal variables on sequential learning. The influences of other temporal factors such as ISI or ITI on implicit learning during SVIPT with different sequence structures (attentional and non-attentional) need to be investigated in the future. No participants reported fatigue during training indicating the type and length of practice was moderate in this study. It should be noted that different levels of force or different length of practice can change sensitivity to the temporal factors in any given task. In the current study, improvements in behavioural outcomes were determined after the end of a single session of training. We therefore do not know the effects of ITIs on long-term training in this task, so it is worthwhile investigating the effects of ITIs on other stages of motor learning, such as long-term retention or off-line consolidation. In this study, we focused on implicit learning. However, it is likely that temporal variables such as ITI can have significant impact on explicit motor sequence learning in which memory is more engaged than for implicit learning. Further research need to be investigated the effects of temporal factors on explicit motor sequence learning in SVIPT.

5. Conclusion

Our results suggest that implicit learning of SVIPT is not dependent on the ITI within a range from 1 to 4 s. Indeed, participants showed implicit sequence learning for SVIPT despite differences in ITI from 1 to 4 s following repetition of a series of organised trials.

Acknowledgements

The authors gratefully thank Dr Allie Ford for her proofreading on this manuscript. We also appreciate the financial support of Monash University which made this project possible.

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Please cite this article in press as: Hashemirad, F., et al., The effects of inter-trial interval on implicit learning of sequential visual isometric pinch task, Journal of Bodywork & Movement Therapies (2016), http://dx.doi.org/10.1016/j.jbmt.2016.11.014

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Declaration for Chapter 6

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

Nature of the contribution	Extent of the contribution (%)
Review of literature, study 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 the contribution
Shapour Jaberzadeh	Study design, guidance in framing of the manuscript, reviewing and provision of feedback on the manuscript drafts.
Paul B Fitzgerald	Reviewing and provision of feedback on the manuscript drafts.
Maryam Zoghi	Reviewing and provision of feedback on the manuscript drafts.

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

Candidate's name

Signature Date

Signature Date

Preamble to Chapter 6

Chapter 6 examines the effects of single-session a-tDCS over M1, DLPFC or PPC on implicit sequential learning of SVIPT in both the trained and untrained hands. Explanatory statement, tDCS questionnaire and sample size calculation are provided in Appendices 13-15.

Aim

To investigate whether the application of a single session of a-tDCS over frontoparietal network (FPN) could enhance the effects of training of SVIPT on behavioural outcome measures (movement time, error rateand skll) in both trained and untrained hands.

Chapter 6: Does single-session anodal tDCS over frontoparietal superficial sites affect motor sequence learning?

The following chapter has been published to the Journal of Frontier Human Neuroscience.

frontiers in Human Neuroscience

ORIGINAL RESEARCH
published: 03 April 2017
doi: 10.3389/fnhum.2017.00153

Single-Session Anodal tDCS with Small-Size Stimulating Electrodes Over Frontoparietal Superficial Sites Does Not Affect Motor Sequence Learning

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Due to the potential of anodal transcranial direct current stimulation (a-tDCS) for enhancement of fine sequenced movements and increasing interest in achieving high level of fine movements in the trained and untrained hands especially at initial stage of learning, we designed this study to investigate whether the application of single-session a-tDCS with small-size stimulating electrodes over FPN sites, such as dorsolateral prefrontal cortex (DLPFC), primary motor cortex (M1) or posterior parietal cortex (PPC) could enhance sequence learning with the trained hand and these effects are transferred into the untrained hand or not. A total of 51 right-handed healthy participants were randomly assigned to one of the four stimulation groups: a-tDCS of left M1, DLPFC, PPC, or sham. Stimulation was applied for 20 min during a sequential visual isometric pinch task (SVIPT). Eight blocks of training using SVIPT were completed with the right hand during stimulation. Two blocks of sequence training with each hand were performed by participants as assessment blocks at three time points: baseline, 15 min and one day following the intervention. Behavioral outcomes including movement time, error rate and skill were assessed in all assessment blocks across three time points. We also measured corticospinal excitability, short-interval intracortical inhibition, and intracortical facilitation using single- and paired-pulse transcranial magnetic stimulation. The results indicated that the behavioral outcomes were significantly improved with the right trained hand, but this learning effect was not modulated by a-tDCS with small-size stimulating electrodes over the FPN. Transfer of learning into the untrained hand was observed in all four groups for movement time but not for the error rate or skill. Our results suggest that sequential learning in SVIPT and its transfer into the untrained hand were not sensitive to a single-session a-tDCS with small-size stimulating electrodes over left M1, DLPFC or PPC in young healthy participants.

Keywords: non-invasive brain stimulation, motor sequence learning, transfer of learning, transcranial magnetic stimulation, primary motor cortex, dorsolateral prefrontal cortex, posterior parietal cortex

OPEN ACCESS

Edited by:
Stephane Perrey,
University of Montpellier, France

Reviewed by:
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Pablo de Olavide University, USA

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Received: *17 December 2016*
Accepted: *15 March 2017*
Published: *03 April 2017*

Citation:
Hashemirad F, Fitzgerald PB, Zoghi M and Jaberzadeh S (2017) Single-Session Anodal tDCS with Small-Size Stimulating Electrodes Over Frontoparietal Superficial Sites Does Not Affect Motor Sequence Learning. Front. Hum. Neurosci. 11:153. doi: 10.3389/fnhum.2017.00153

INTRODUCTION

Learning sequences in fine movements plays a crucial role in everyday life and requires a strong coordination between visual and motor cortex. Finding novel techniques to improve rehabilitation in fine movements in the trained hand as well as transfer of learning into the untrained hand would implicate for patients who struggle with fine motor tasks, such as those with stroke or Parkinson's disease. Recently, anodal transcranial direct current stimulation (a-tDCS), which modulate brain activity, has allowed direct investigation of the role of specific areas of the brain during different stages of sequence learning (Nitsche et al., 2003; Fregni et al., 2005; Reis et al., 2009; Schambra et al., 2011; Zaehle et al., 2011; Javadi and Walsh, 2012; Pope and Miall, 2012; Hoy et al., 2013; Saucedo Marquez et al., 2013; Weiss et al., 2013; Convento et al., 2014; Waters-Metenier et al., 2014; Reis et al., 2015; Rivera-Urbina et al., 2015).

A large body of neuroimaging evidence has revealed that sequence learning is mediated by frontoparietal network (FPN) superficial sites including dorsolateral prefrontal cortex (DLPFC) (Jenkins et al., 1994; Sakai et al., 1998; Miller and Cohen, 2001; Hasan et al., 2013), the primary motor cortex (M1) (Grafton et al., 1995; Karni et al., 1995; Hazeltine et al., 1997; Rioult-Pedotti et al., 2000) and posterior parietal cortex (PPC) (Jenkins et al., 1994; Sakai et al., 1998). The contribution of specific areas of the FPN may change across sequence learning depends on the stage of learning (Karni et al., 1998; Doyon and Ungerleider, 2002; Dayan and Cohen, 2011). M1 known to play an important role in acquisition and consolidation of movements, while rapid improvements gained over the course of a single training session (fast stage of learning) are more associated with the activity of DLPFC or PPC (Sakai et al., 1998; Koch et al., 2008a,b).

Although there is a large number of studies providing evidence for efficacy of multiple-sessions a-tDCS over M1(which links to slow stage of learning) (Reis et al., 2009; Schambra et al., 2011; Saucedo Marquez et al., 2013; Waters-Metenier et al., 2014; Hashemirad et al., 2016), the efficacy of single-session of M1 a-tDCS remains controversial.

Exploring single-session a-tDCS effects over other areas of the FPN, such as DLPFC or PPC, which are more associated with initial stages of learning are necessary to be investigated to determine the optimum stimulation sites to influence sequence learning. In this study, we applied single-session a-tDCS over three different areas of the brain (M1, DLPFC, or PPC) during a sequential visual isometric pinch task (SVIPT) in order to assess the effects of a-tDCS on a fine-motor control task in young healthy individuals at the early stage of learning. We also examined the effects of a-tDCS on transfer learning into the untrained hand by quantifying generalization behavioral outcomes into the untrained hand. To evaluate possible underlying mechanisms which are responsible for the effects of a-tDCS during SVIPT, we also measured changes in M1 corticospinal excitability (CSE), short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) using a single- or paired-pulse transcranial magnetic stimulation (TMS).

To the best of our knowledge, this is the first study to verify the effects of single-session a-tDCS over the FPN sites on implicit motor sequence learning and transfer learning into untrained hand using SVIPT. The aims of this study were to investigate: (1) the effects of a-tDCS of M1, DLPFC, or PPC on cortical and behavioral changes during motor sequence learning using SVIPT, (2) the correlation between behavioral and cortical effects, and (3) whether the acquired behavioral changes during the training are transferable to the untrained hand.

MATERIALS AND METHODS

Study Design

This study was a parallel randomized single-blind sham-controlled study where each participant took part in one of the four stimulation conditions.

Participants

Fifty-one healthy participants (36 females, 15 males; age between 18 and 40 years old with *mean* ± *SD*; 25.82 ± 6.14 were randomly assigned to one of the four stimulation groups: (1) a-tDCS of left M1, (2) a-tDCS of left DLPFC, (3) a-tDCS of left PPC, (4) sham a-tDCS. All participants were right-handed based on the Edinburgh Handedness Inventory (Oldfield, 1971) (Laterality index: 78.83% ± 20.98). Exclusion criteria for participation in the experiments were: (1) having contraindications to be assessed by TMS or for receiving tDCS, e.g., having a seizure or with the family, having any metal in their head, severe headaches and pregnancy, (2) current usage of any medicine which could affect the brain excitability, motor learning or cognition, (3) history of neurological or psychiatric diseases, (4) significant experience with musical instruments or computer games (more than 5 hours of practice in a day or 1000 h of practice during the last six months before the study), (5) disability in fingers, hand or wrist, (6) age above 40 years or less than 18 years. All participants were naive to the purpose of the experiments. All tests were conducted between 8 am and 4 pm. To control for the effect of female hormonal fluctuation on the size of MEPs, the experimental sessions were carried out between the 7th and 23th day of women's menstrual cycles. Information about sleep hours, quality of sleep and experience with computer games were also obtained through a brief questionnaire. This study was carried out in accordance with the recommendations of the Human Ethics Committee at Monash University with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Human Ethics Committee at Monash University.

TMS Measurement

A MagPro R30 stimulator (MagVenture) with a butterfly coil (MC-B70) and dimensions (169 × 112 × 16/33 mm) was used to induce motor-evoked potentials (MEPs) from the right first dorsal interosseous (FDI) muscles. The coil was placed over the left M1 region with a posterior–anterior orientation and set at an 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 ensure consistency of coil placement throughout the

experiment. Resting motor threshold (RMT) was defined as the minimal stimulator output needed to elicit three out of six MEPs with minimum amplitude of 50–100 μV in a relaxed FDI muscle (Rossini and Rossi, 1998). All raw EMG signals, were amplified, filtered (20 Hz–10 kHz) and recorded with a PC running a commercially available data acquisition and automated-analysis package (PowerLab™ ADInstrument 4/35 with LabChart™, Australia) for offline analysis.

Single-Pulse TMS

Single-pulse TMS (MagPro R30 stimulator) was used over the left M1 in order to record MEPs from the right FDI muscle. Test TMS intensity was adjusted to produce a test MEP of about 1mV in FDI muscle at rest. Twenty single-pulse were delivered with 10 s inter pulse interval and 20 MEPs were recorded from the right FDI muscle. Average peak-to-peak amplitudes of 20 MEPs were calculated for each time point (Baseline, post 15 min and post 24 h) to assess CSE of M1.

Paired-Pulse TMS

Paired-pulse TMS (MagPro R30 stimulator) was used to evaluate SICI and ICF in M1. In this paradigm, a sub threshold conditioning stimulus was followed by a supra threshold test stimulus (Kujirai et al., 1993). The amplitude of the conditioning stimulus was set to 80% of the RMT and unconditioned stimulus or test stimulus was adjusted at 1mV. Paired-pulse TMS was delivered randomly in a block of 40 trials with inter-stimulus intervals (ISI) of 3 or 10 ms, respectively. MEP areas were quantified for conditioned and unconditioned stimuli using a custom designed macro in Power Lab 4/35 software. The size of the conditioned MEPs was expressed as a percentage of unconditioned test MEPs at baseline. Test intensity was adjusted to elicit an unconditioned MEP with peak-to-peak amplitudes of 1 mV at the following day.

Transcranial Direct Current Stimulation (tDCS)

A commercially available stimulator (Intelect Advanced Therapy System, Chattanooga, TN, USA) was used to deliver direct current with intensity of 0.3 mA for 20 min through a pair saline-soaked rectangular sponge surface electrodes. The size of active and return electrodes were 2 × 1.5 (3 cm^2) and 4 × 3 (12 cm^2), respectively. The small size of electrodes yield a highly focused direct current over the target areas, which enabled us to stimulate the target areas without stimulating nearby areas (Nitsche et al., 2007; Faria et al., 2011; Vaseghi et al., 2015a,b). In this study, we adjusted the current intensity for the small electrode size (3 cm^2) by keeping the current density (0.1 mA/cm^2) in a safe range (Nitsche and Paulus, 2000; Poreisz et al., 2007), to modulate the excitability of neurons in the target area (Bastani and Jaberzadeh, 2013a,b; Vaseghi et al., 2015a,b). Therefore, the active electrode with size of 3 cm^2 was placed over the target areas (left M1, DLPFC, or PPC) and the return electrode (12 cm^2) was fixed over the contralateral supraorbital region. For the sham group, the active electrode randomly was placed over the three different stimulation areas (M1, DLPFC, or PPC). The distribution for the stimulation conditions was randomly balanced across participants. The current was ramped up to 0.3 mA and then ramped down so that participants felt an initial sensation for 30 s of stimulation.

The locations of M1 was identified using TMS, the location of DLPFC or PPC were determined using the international 10–20 system (Steinmetz et al., 1989). Therefore, the stimulating electrodes for DLPFC or PPC were placed over F3 and P3, respectively. participants were asked to report tDCS side effects such as itching, tingling, burning sensations, headache, pain, and any other sensations (Poreisz et al., 2007). All participants rated the presence and severity of these side effects using numeric analog scales (NAS) (e.g., 0 = no feeling to 10 = worst feeling imaginable). To check the blinding integrity, after completion of the stimulation session, participants were asked to indicate if they thought they had received active or sham stimulation.

Apparatus and Task

A force transducer (AD instrument MLT004/ST, NSW, Australia) was used for induction of SVIPT in this study. SVIPT is a pinch force task in which participants were asked to squeeze the force transducer between their thumb and index finger to move a cursor upward on the computer screen to meet different target forces (**Figure 1**). At the beginning of each experiment, maximum voluntary contraction (MVC) was individually determined for each participant. Two trials were then given as familiarization. After familiarization, two sequence blocks were randomly performed as baseline measurement with each hand. Each sequence block consisted of eight trials and each trial included seven target forces which appeared in a sequence order (10, 35, 20, 40, 25, 15, and 30% MVC) on the computer screen. The inter-trial interval was set at 1 s. Each target force was only presented once in each trial. The level of each target force was determined by a green line or a numerical number in an indicator box on the computer screen. Participants were instructed to squeeze the force transducer to reach the target force in a range of 5% below or above the target force. More or less than this range was considered as an over- or under-shoot error. During training, each participant completed eight blocks of the same sequence order with dominant hand, except for the block 6 which was set in a random order. Inter-block interval was set at 1 min. Each participant completed the training in approximately 20–25 min. Participants were received no feedback during training. They were also not aware of the sequential order of the target forces in each trial. To make sure implicit learning, they were asked to recall target forces to determine the amount of their awareness. If they could recall more than three consecutive target forces, leaning was considered as explicit and their data were excluded from analysis. Fifteen min after completion of the training, participants completed two blocks as a post-test with each hand randomly. One day after training, two blocks were repeated as a retention test with each hand. The number of trials, as well as sequence order of target forces, were the same in the both training and assessment blocks.

The following behavioral outcomes were measured in each assessment block:

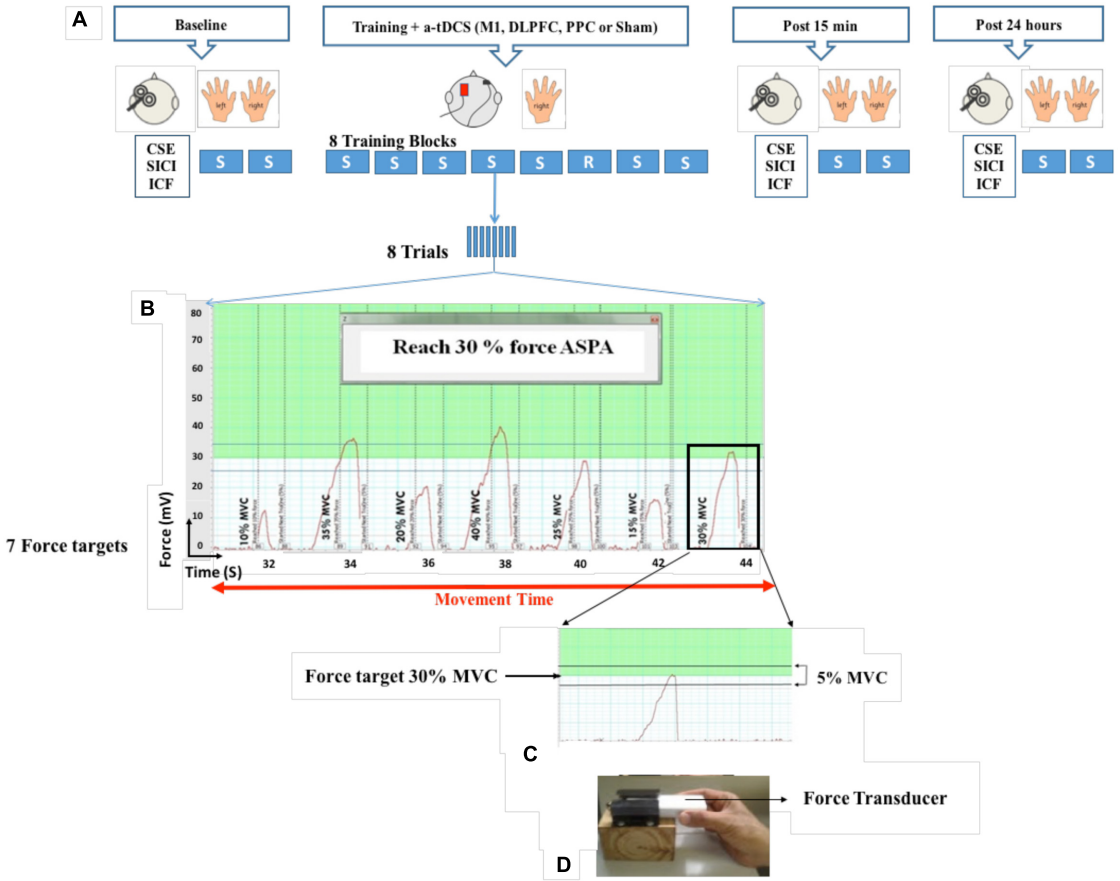

FIGURE 1 | Experimental set-up. Participants held a force transducer between their thumb and index finger and altered their precision force on the force transducer to move a cursor on the computer screen to reach different target forces. During eight blocks of training, a-tDCS (left M1, DLPFC, PPC, and sham) were applied over the left hemisphere contralateral to the performed hand. Each block consisted of eight trials and each trial included seven target forces from 10 to 30 % of MVC which appeared on the computer screen. Cortical and behavioral changes were assessed over three time points at baseline, post 15 min and post 24 h after intervention **(A,B)**. Participants were required to squeeze the force transducer to reach the target force in a range of 5% below or above the target force **(C,D)**. SVIPT: Sequential visual isometric pinch task, A-tDCS: Anodal transcranial direct current stimulation, M1, Primary motor cortex; DLPFC, Dorsolateral prefrontal cortex; PPC, Posterior parietal cortex; S, Sequence block; R, Random block; CSE, Corticospinal excitability; SICI, Short-interval intracortical inhibition; ICF, intra-cortical facilitation (ICF); maximum voluntary contraction (MVC).

Movement Time

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

Error Rate

The error rate was calculated as the proportion of the trials with at least one over- or undershoot (Reis et al., 2009). Participants needed to meet all seven targets in each trial correctly to get the accuracy of that trial.

Skill

Skill, which is defined as a combination of both parameters of movement time and error rate, represents changes in the speed-accuracy trade-off. This variable was obtained from the following formula suggested by Reis et al. (2009).

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

Experimental Procedure

In each experiment, the same procedure was followed: (1) baseline measurements (TMS and SVIPT), (2) training paired with anodal/sham tDCS stimulation, (3) post measurements (TMS and SVIPT) after 15 min and (4) post measurements (TMS and SVIPT) after 24 h (**Figure 1**). To decrease the effects of order, TMS and SVIPT was randomized for each assessment, as was the choice of the performing hand.

Data Analysis

Kolmogorov–Smirnov (K–S) test was used to assess the normality of data. For all normal distributed variables, a mixed-design ANOVA (Repeated-measure) with the factor of Time (baseline,

post 15 min after and post 24 h) as a within-subjects factor and factor of Group (a-tDCS of M1, DLPFC, PPC, or sham) as between-subjects factor was conducted to assess the effects of a-tDCS on motor sequence learning among the four groups over time. This analysis was separately applied for assessment blocks with trained and untrained hands. A Greenhouse–Geisser test was used in order to correct non-sphericity if the assumption of Mauchly's test of sphericity was violated. *Post hoc* tests with Bonferroni correction were performed as appropriate to determine where differences occurred.

For non-normally distributed data, log transformation was performed in order to achieve normal distributions of the data. After the transformation, if normal distribution were not corrected and the skewness of the log data were still more than one, non-parametric tests were conducted. The Friedman two-way analysis of variance by ranks was used to assess differences in mean rank of non-parametric variables across three time points. A K-independent method by median test was conducted to evaluate whether the groups differed in their median or not. A Kruskal–Wallis test one-way analysis by rank was used if median test was not computed when all data were equal or less than median. Equality of deviation of mean rank among the four groups as an assumption for Kruskal–Wallis method was tested by Levin's test of non-parametric variables. Bonferroni correction was used for correction of multiples groups, if differences between groups was determined.

Pearson correlation was conducted to investigate relationship between cortical and behavioral outcomes. SPSS (version 20) and MATLAB (R2014a) were used to analysis the data in this study. Statistical significance was set at $p < 0.05$.

RESULTS

Of the 51 participants enrolled in this study, three subjects were excluded because they could not perform the SVIPT task as instructed.

As shown in **Table 1**, there were no significant difference in participants' characteristics such as age, right-handedness, MVC, and also some other parametric variables including experience with computer games, sleep hours, sleep quality, attention during task, fatigue, and sequence awareness ($p > 0.05$).

There were also no significant differences in cortical outcome measures, including CSE ($p = 0.82$), SICI ($p = 0.32$) and ICF ($p = 0.87$) or behavioral outcomes including movement time ($p = 0.52$), error rate ($p = 0.64$), and skill ($p = 0.49$) among the four groups at the baseline.

There were no significant differences between participants' feeling in all four condition measurements (Supplementary Table S1). Blinding integrity was intact because participants were not able to determine active versus sham a-tDCS in either group based on the results obtained from Pearson's chi-square [$\chi 2$ (4, $n = 48$) = 1.33, $P = 0.24$].

Cortical Outcome Measures

Resting motor threshold and test intensity (mean ± SEM) are reported in **Table 2**, for all four groups at each experimental session. The results of one-way ANOVA showed no significant differences in RMT [$F(3,44) = 1.14$, $p = 0.34$] or test intensity [$F(3,44) = 1.45$; $p = 0.23$] at baseline among the groups. In addition, no significant difference was found for either RMT [$F(1,44) = 3.4$, $p = 0.072$] or test intensity [$F(1,44) = 0.024$, $p = 0.87$] between the two experimental sessions.

Effects of a-tDCS and Training on CSE

Figure 2 shows the mean peak-to-peak amplitude of MEPs before and after interventions over three time points (baseline, post 15 min and post 24 h) in all four groups. The results of ANOVA showed no main effects of Time [$F(1.58,67.9) = 0.031$, $p = 0.94$] or Group [$F(3,43) = 1.41$, $p = 0.25$]. The interaction between Time and Group [$F(4.73,67.9) = 1.55$, $p = 0.18$] on the size of the MEPs was not significant.

TABLE 1 | Participants' characteristic in the four experimental groups.

	Group M1	DLPFC	PPC	Sham	ANOVA	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*F*	*P*
Number (Female/Male)	12 (8/4)	12 (9/3)	12 (9/3)	12 (8/4)		
Age	27.8 ± 5.8	25.1 ± 5.8	24.8 ± 5.9	25.5 ± 7.1	1.52	0.22
Handedness	75.5 ± 27.7	82.4 ± 15.6	77 ± 19.6	85.7 ± 11.4	0.89	0.45
MVC	61.4 ± 26.2	68.7 ± 22.1	61.5 ± 18.6	70.1 ± 26.3	1.46	0.24
Computer game (Hour in a day)	0.78 ± 1.39	0.2 ± 0.44	0.8 ± 1.3	0.5 ± 0.57	0.347	0.79
Sleep hour day1	7.3 ± 1.55	6.8 ± 0.83	6.8 ± 1.24	6.8 ± 1.83	0.68	0.57
Sleep quality day1	7.8 ± 1.74	7.3 ± 1.59	8.13 ± 1.12	7.1 ± 1.83	0.68	0.57
Attention day1	8 ± 1.04	8.13 ± 0.99	7.8 ± 1.12	7.8 ± 1.06	0.1	0.954
Fatigue day1	1.67 ± 1.92	0.75 ± 2.12	0.25 ± 0.707	0.43 ± 0.787	1.58	0.21
Sleep hour day2	7.6 ± 1.5	7.5 ± 1.5	7.4 ± 0.54	7.3 ± 2.05	0.064	0.97
Sleep quality day2	8.1 ± 1.4	8.1 ± 0.75	7.6 ± 1.3	8.5 ± 0.57	0.45	0.71
Attention day2	9.06 ± 0.63	8.5 ± 1.02	8.6 ± 0.54	8.2 ± 0.5	1.33	0.29
Fatigue day2	0	0.67 ± 1.03	1.2 ± 1.7	0	2.09	0.13
Awareness	0.88 ± 1.64	0.4 ± 0.89	0.4 ± 0.54	0.2 ± 0.44	0.43	0.72

TABLE 2 | Mean of resting motor threshold (RMT) and test intensity as % of maximum stimulator output at the two experimental sessions.

Stimulation groups	RMT		Test intensity	
	Session 1	Session 2	Session 1	Session 2
M1	34.5% ± 1.93	34.5% ± 1.83	47.08% ± 2.77	46.08 % ± 2.85
DLPFC	33% ± 1.17	32.4% ± 1.27	43.7% ± 1.58	42.9 % ± 1.28
PPC	37.3% ± 1.65	36.8% ± 1.6	50.9% ± 2.43	51.9% ± 1.95
Sham	36.5% ± 2.4	35.7% ± 2.06	48.4% ± 2.88	49.5% ± 2.57

Data are presented as mean ± SEM.

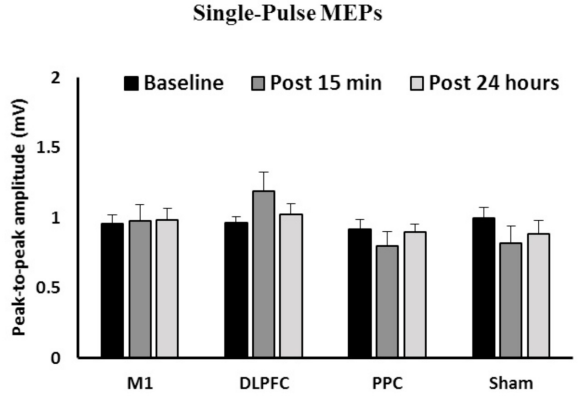

FIGURE 2 | The mean of peak-to-peak amplitudes of MEPs in the right FDI among the four groups over three time points. No significant difference was found in the main factors of Time or Group. Data are presented at Mean ± SEM.

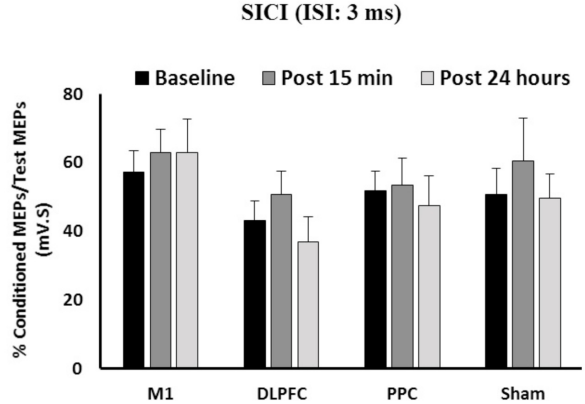

FIGURE 3 | The mean of SICI in M1 after a-tDCS stimulation among the four condition groups over three time points. No significant differences were found in main factors of Time or Group. Data are presented at Mean ± SEM.

Effects of a-tDCS and Training on SICI

The results of a mixed-design ANOVA showed that a-tDCS delivered concurrently with training using SVIPT did not modulate SICI across the three time points [$F(2,86) = 1.58$, $p = 0.21$] (**Figure 3**). Main effects of Group was not significant for SICI [$F(3,43) = 1.45$, $p = 0.24$]. In addition, the interaction between Time and Group on SICI was not significant [$F(6,86) = 0.31$, $p = 0.93$].

Effects of a-tDCS and Training on ICF

The results of a mixed-design ANOVA showed the main effects of Time [$F(2, 86) = 1.82$, $p = 0.16$] or Group [$F(3,43) = 0.61$, $p = 0.6$] was not significant on ICF (**Figure 4**). The interaction between Group and Time was not significant either on ICF [$F(6,86) = 0.43$, $p = 0.85$].

Behavioral Outcome Measures

Movement time was normally distributed so we conducted a mixed-design ANOVA to investigate the effects of interventions on this variable in both the trained and untrained hands. In contrast, the error rate and skill were non-normally distributed. Since their normality were not corrected using log transformation, we conducted nonparametric tests on these variables to test the effects of intervention on these variables with both the trained and untrained hands.

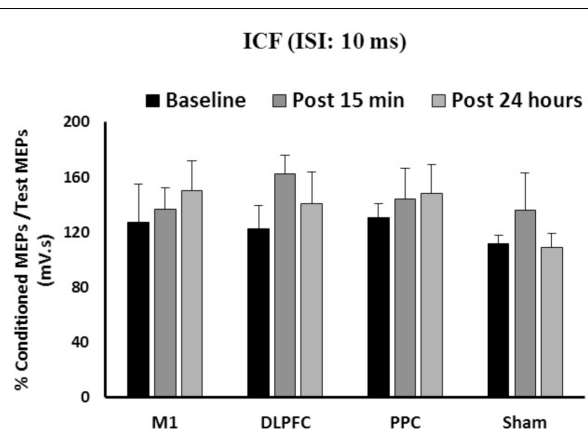

FIGURE 4 | The mean of ICF in M1 after a-tDCS stimulation among four condition groups over three time points. No significant differences were found in main factors of Time or Group. Data are presented at Mean ± SEM.

Movement Time

Trained Hand

Mean movement time was decreased from 19.2 ± 5.6 at baseline to 15.7 ± 2.5 at post 15 min and 15.8 ± 2.5 at post 24 h after intervention. The results of mixed-design ANOVA showed significant improvement in movement time with the right trained

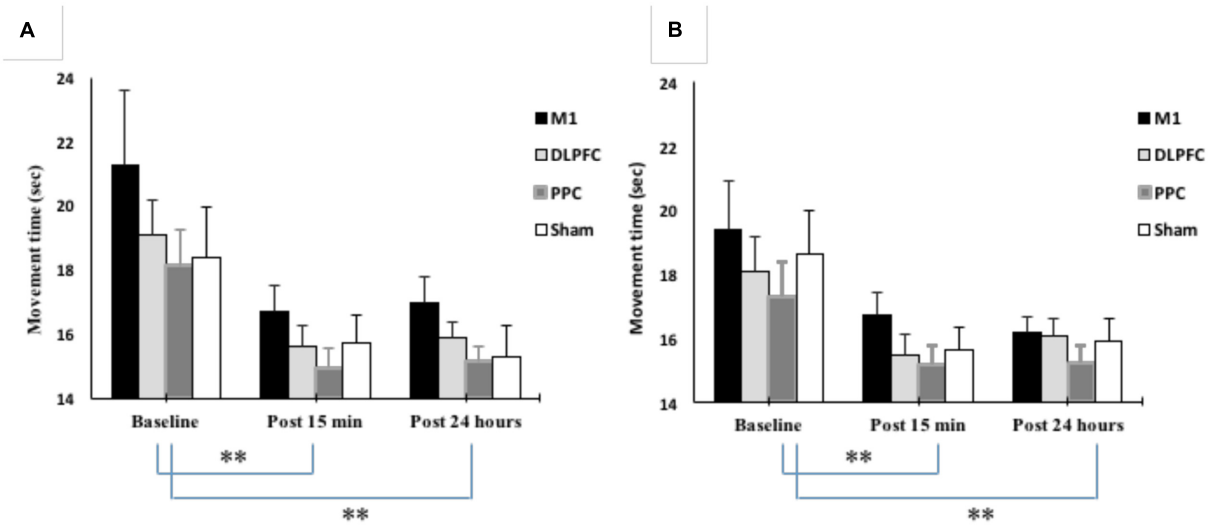

FIGURE 5 | Changes in movement time in blocks of right trained hand (A) and left untrained hand **(B)**. The results showed significant improvement in movement times for all four stimulation groups over three time points. $^{**}P < 0.01$.

hand over the three time points [$F(1.15,50.6) = 20.1$, $p < 0.001$] (**Figure 5A**). *Post hoc* analysis with Bonferoni correction showed that the movement time significantly decreased 15 min and 24 h after intervention compared to baseline ($p < 0.001$). However, there was no significant difference between two post-tests ($p = 1$).

The main effect of the stimulation group was not significant for the trained right hand [$F(3, 44) = 1.302$, $p = 0.28$]. There was also no interaction between Group and Time [$F(3.45, 50.6) = 0.24$, $p = 0.89$].

Untrained Hand

As shown in **Figure 5B**, movement time significantly improved in the left untrained hand [$F(1.26,55.5) = 22.4$, $p < 0.001$]. The results of *post hoc* analysis showed that the movement time significantly decreased at post 15 min (15.7 ± 2.29) compared to baseline (18.3 ± 4.18) ($p < 0.001$). This reduction remained in the following day (15.8 ± 1.94) and significantly different from baseline ($p < 0.001$). However, there was no significant difference between post-tests ($p = 1$).

The main effect of stimulation groups for the left hand was not significant [$F(3,44) = 0.79$, $p = 0.503$]. Interaction between Group and Time was also not significant [$F(3.78,55.5) = 0.27$, $p = 0.88$].

Error Rate

The minimum, maximum, and mean rank as well as median of the error rate are represented in **Table 3** for both the trained and untrained hands over three time points.

Trained Hand

Friedman's test showed a statistically significant decrease in the error rate for the right hand [$\chi 2(2, n = 48) = 17.9$, $p < 0.001$] (**Figure 6A**). The mean rank of the error rate decreased from 2.38 at baseline to 1.95 at post 15 min and to 1.68 at post 24 h. *Post hoc* analysis with a Bonferroni correction showed that there was only a significant difference between baseline and post 24 h ($p = 0.002$). No significant difference was found between baseline and post 15 min ($p = 0.109$) or two post-tests ($p = 0.554$).

The results of K-independent samples showed that there were no significant differences among the four groups at baseline [$\chi^2(3, n = 48) = 0.52$, $p = 0.91$], post 15 min [$\chi^2(3, n = 48) = 5.67$, $p = 0.12$] or post 24 h after intervention [$\chi^2(3, n = 48) = 3.2$, $p = 0.36$] (**Figure 7A**). Therefore, a-tDCS had no site-specific effects on error rate at any stimulation groups over times.

Untrained Hand

Friedman's test indicated no significant decrease in error rate for assessment blocks which performed with the left untrained hand over the three time points [$\chi^2(2, n = 48) = 4.01$, $p = 0.134$] (**Figure 6B**).

Results of K-independent samples showed no significant changes in error rate of the left hand across the groups over the three time points (**Figure 7B**). The effects of a-tDCS and training were the same across the four groups at baseline [$\chi^2(3, n = 48) = 4.08$, $p = 0.25$], post 15 min [$\chi^2(3, n = 48) = 3.02$, $p = 0.38$] as well as post 24 h after intervention [$\chi^2(3, n = 48) = 2.36$, $p = 0.5$].

Skill

Table 4 represents the minimum, maximum and mean rank as well as median of skill for both the trained and untrained hands over three time points.

Trained Hand

The results of Friedman test showed a significant increase in mean rank of skill with the right trained hand [$\chi^2(2, n = 48) = 22.3$, $p < 0.001$] (**Figure 8A**). The mean rank of skill increased from baseline (1.57) to post 15 min (2.03) and post 24 h (2.4). *Post hoc* analysis with Bonferroni correction showed

TABLE 3 | The minimum, maximum, mean rank, and median of the error rate in assessment blocks performed with trained (right) or untrained (left) hand at three time points.

Error rate $N = 48$	Group	Min–Max/Mean rank			Median		
		Baseline	Post 15 min	Post 24 h	Baseline	Post 15 min	Post 24 h
Trained (right)	M1	2.5–32/23.6	8–38/28.6	2.5–39/28.5	1	0.87	0.87
	DLPFC	5.5–32/ 24.6	2–38/18.21	2.5–39/19.3			
	PPC	5.5–32/26.3	5–38/29	2.5–39/26.8			
	Sham	1–32/23.2	2–38/22.1	2.5–39/23.2			
Untrained (left)	M1	4.5–34/23.8	5–37/29.5	7–36/24.2	1	0.87	1
	DLPFC	1–34/19.1	1–37/23.2	1–36/22.5			
	PPC	4.5–34/26.2	5.5–37/25.8	5–36/29.2			
	Sham	13–34/28.7	2.5–37/19.3	3–36/21.9			

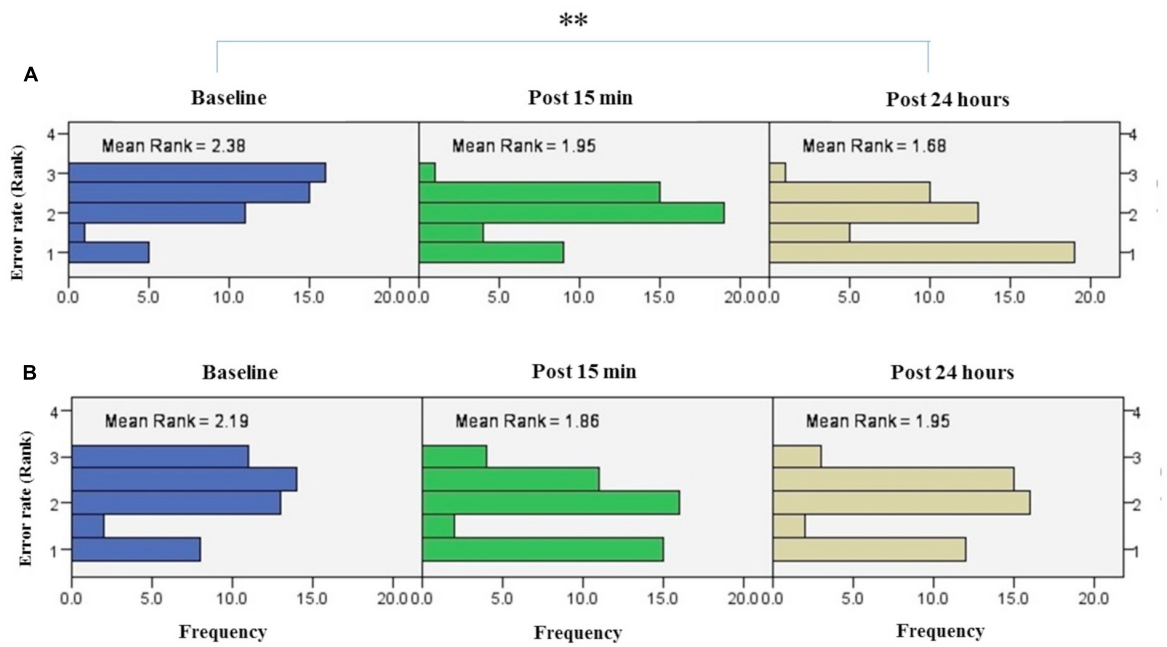

FIGURE 6 | Results of Friedman test and distribution of error rate by rank in both right trained hand (A), left untrained hand **(B)** over three time points. Asterisks indicate significant differences in mean rank across time points. $^{**}P < 0.01$.

that skill significantly improved at post 24 h after intervention compared to the baseline measurement ($p < 0.001$) but this increase was not significant between baseline and 15 min after intervention ($p = 0.074$) or between two post-tests ($p = 0.22$).

The results of K-independent test revealed no significant differences in skill across the four groups in assessment blocks with the dominant right hand (**Figure 9A**). The effects of a-tDCS and training were the same across the four groups at baseline [$\chi^2(3, n = 48) = 0.291$, $p = 0.962$], post 15 min [$\chi^2(3, n = 48) = 6$, $p = 0.112$] as well as post 24 h [$\chi^2(3, n = 48) = 3.33$, $p = 0.343$].

Untrained Hand

The results of Friedman's test in the assessment block with the left untrained hand revealed a trend of improvement in skill over time [$\chi^2(2, n = 48) = 5.62$, $p = 0.06$] (**Figure 8B**).

Results of K-independent samples showed no significant changes in the error rate of left hand across the groups over the three time points (**Figure 9B**). The effects of a-tDCS and training were the same across the four groups at baseline [$\chi^2(3, n = 48) = 3.04$, $p = 0.384$], post 15 min [$\chi^2(3, n = 48) = 4.66$, $p = 0.198$] as well as post 24 h after interventions [$\chi^2(3, n = 48) = 3.59$, $p = 0.309$].

Correlation between Cortical and Behavioral Outcomes

A Pearson correlation test was conducted to determine the relationship between cortical and behavioral outcomes for two experimental sessions. No correlations were found between cortical and behavioral outcomes except for movement time and ICF, which showed a low inverse relationship at the second

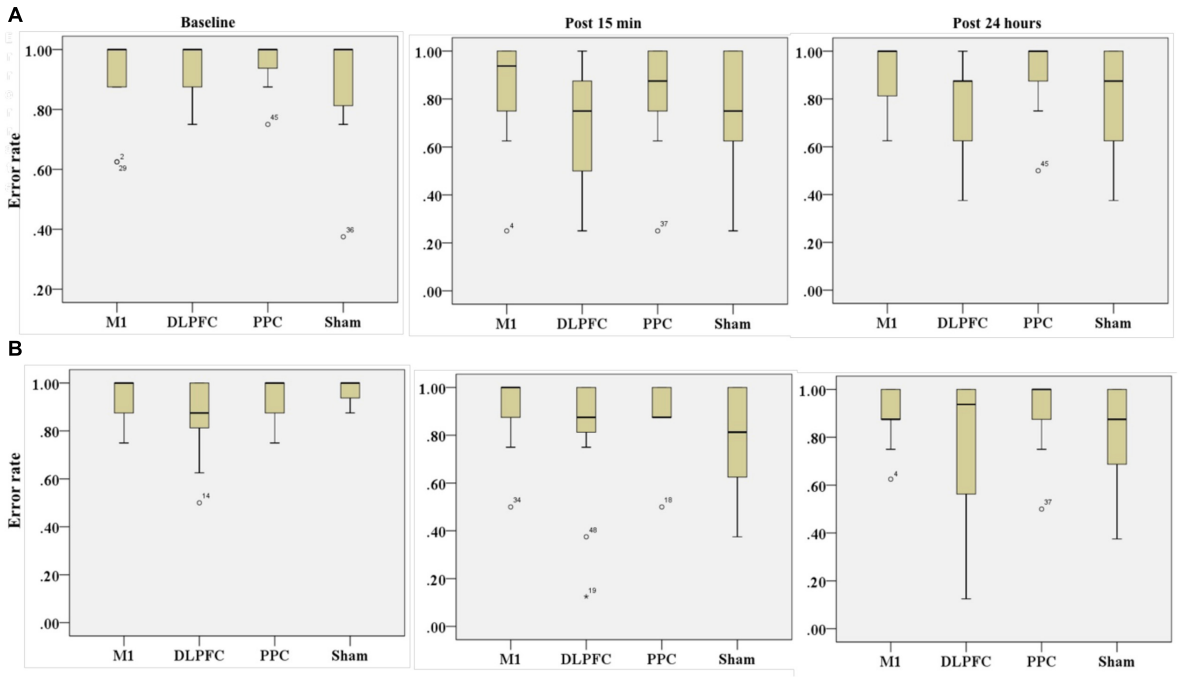

FIGURE 7 | Effects of a-tDCS and training on error rate among the four stimulation groups in the right trained hand (A) and left untrained hand **(B)** at three time points. No significant effects were found between all stimulation conditions.

TABLE 4 | The minimum, maximum, mean rank and median of skill in assessment blocks performed with trained (right) and untrained (left) hand at three time points.

Skill	Group	Min–Max/Mean rank			Median		
		Baseline	Post 15 min	Post 24 hours	Baseline	Post 15 min	Post 24 hours
Trained (right)	M1	17–47/25.7	11–40/19.8	9.5–45/20.7	0E-7	0.0092	0.01
	DLPFC	17–42/24	11–48/31.1	9.5–46/29.4			
	PPC	17–43/22.5	11–44/20.1	9.5–48/21.8			
	Sham	17–48/25.6	11–46/26.8	9.5–47/25.9			
Untrained (left)	M1	15–46/25	11.5–46/19.4	13–42/24.5	0E-7	0.0091	0E-7
	DLPFC	15–48/29.9	11.5–48/25.3	13–48/26.5			
	PPC	15–44/23	11.5–43/23	13–44/19.7			
	Sham	15–41/19.9	11.5–46/30.1	13–45/27.1			

session ($r = -0.41$, $p = 0.003$) (Supplementary Table S2). This result indicates that decrease in movement time for performing SVIPT was correlated by increase in facilitation of interneurons of M1 at one day after intervention.

DISCUSSION

In this study, we applied single-session a-tDCS with small-size stimulating electrodes over M1, DLPFC, PPC, or sham during training with SVIPT in young healthy participants. The effects were investigated on both cortical (CSE, SICI, and ICF) and behavioral (movement time, error rate, and skill) outcome measures. Our findings showed no significant additional effects in implicit motor sequence learning in the trained hand following focal stimulation of a-tDCS over any of the FPN superficial sites compared to sham group. Transfer of learning into the untrained hand were only observed for movement time not for error rate or skill in all different stimulation sites. We also found no significant effects on CSE, SICI, and ICF in M1 area following intervention. There are some possible reasons behind the negative results.

One explanation can be related to the a-tDCS characteristics used in this study. Because we aimed to selectively stimulate M1, not nearby areas, such as premotor cortex, supplementary motor area or primary sensory area, we used a small electrode size of 3 cm^2 in order to adjust the size of the electrode, low intensity stimulation of 0.3 mA was used that produced a current density of 0.1 mA/cm^2. However, some studies have shown that a small electrode size (3 cm^2) or current density (0.1 mA/cm^2)

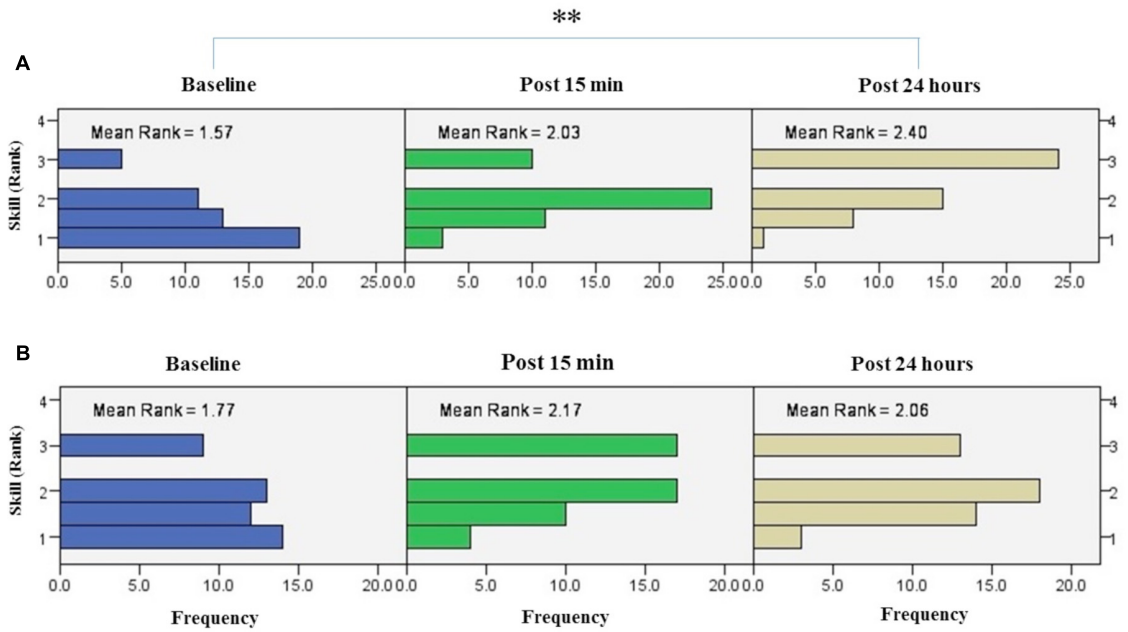

FIGURE 8 | Results of Friedman test and distribution of skill by rank in blocks of the right hand (A) and left hand **(B)** over three time points. Asterisks indicate significant differences in mean rank across time points. $^{**}P < 0.01$.

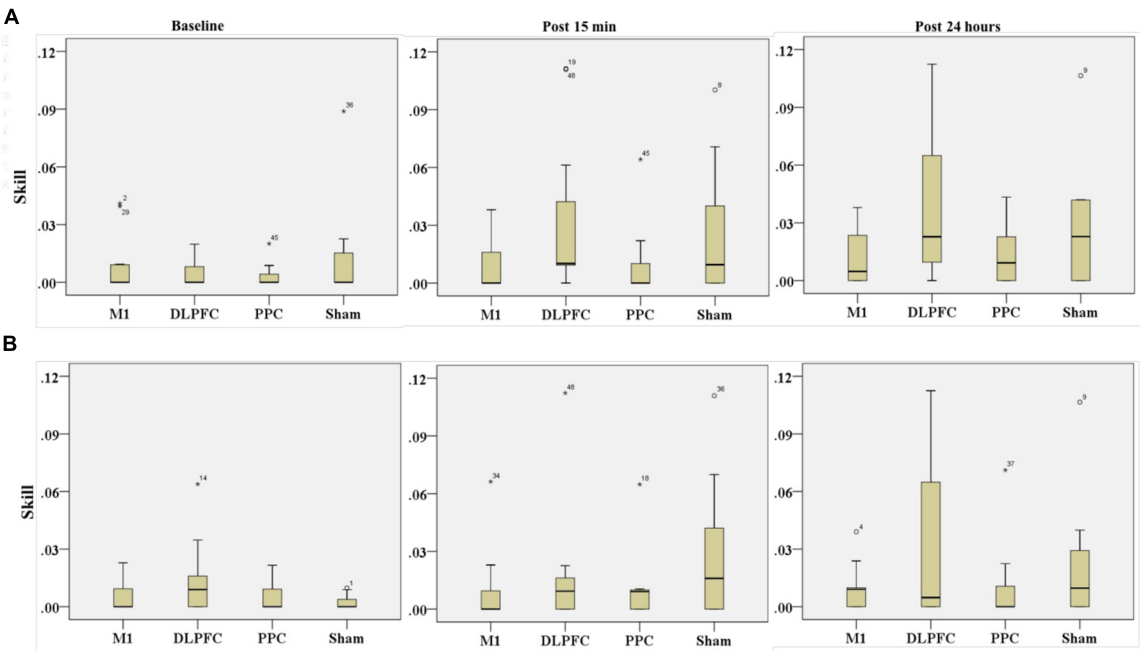

FIGURE 9 | Effects of a-tDCS and training on skill among the four stimulation groups in the right trained hand (A) and left untrained hand **(B)** at three time points. No significant effects were found between all stimulation conditions.

can affect M1 excitability at rest state (Nitsche and Paulus, 2000; Bastani and Jaberzadeh, 2013a; Vaseghi et al., 2015a), we observed no changes in CSE after application a-tDCS during SVIPT. Regarding the issue that performing a cognitive or motor task during stimulation can modulate the effects of tDCS on M1 excitability (Antal et al., 2007), it is likely a-tDCS with these characteristics, when applied during training, do not impact on neurophysiologic outcomes.

In addition, our results indicated no changes in the excitability of M1 following a single session of a-tDCS over DLPFC or

PPC combined with training of SVIPT. Even though, cortical excitability of DLPFC or PPC were not assessed in the current study but we can assume no changes in excitability of DLPFC and PPC following a-tDCS with small-size stimulating electrodes of these sites. This may be considered as a reason behind the absence of a-tDCS effects on the performed task. We also found no significant changes in SICI and ICF of M1 after stimulation, which is consistent with a systematic review that showed tDCS generates little-to-no neurophysiological effects on SICI or ICF (Horvath et al., 2015). However, a significant enhancement in SICI and ICF in M1 area reported in a study by Rivera-Urbina et al. (2015) after application of a-tDCS over PPC at rest state, not concurrently with training (Rivera-Urbina et al., 2015). In line with our findings Uehara et al. (2013) found no direct DLPFC-M1 connectivity during performance of a rhythmic of abduction with the index finger (Uehara et al., 2013).

Although we expected single-session focal stimulation a-tDCS over DLPFC or PPC led to enhance sequential learning, compared to the sham group, due to neuropsychological evidence strongly supports the role of PPC or DLPFC in higher cognitive functions or sensorimotor integration (Bahrick et al., 1954; Seger, 1994; Castro-Alamancos et al., 1995; Castro-Alamancos and Connors, 1996), no specific effects were found on SVIPT. The absence of any effects for DLPFC or PPC a-tDCS in the current study can be explained by tDCS characteristics or task-dependent effects of a-tDCS on learning and memory formation (Saucedo Marquez et al., 2013). A mini review by Ammann et al. (2016) showed that the standard tDCS montage (the current intensity (1-2 mA) and electrode size (25–35 cm^2) on different areas of the brain can lead to significant positive results on motor learning (Ammann et al., 2016). some studies have shown a-tDCS of the left DLPFC (with a range of current density from 0.028 to 0.1 mA/cm^2 and electrode sizes of 25–35 cm^2) could modify different kinds of tasks, such as implicit probabilistic classification learning (Kincses et al., 2004), sequential-letter memory tasks (Fregni et al., 2005), cognitive tasks (Kuo and Nitsche, 2015) as well as mental practice (Foerster et al., 2013). In spite of that, in line with the findings in the current study, literature also indicates that even utilization of standard intensity and electrode size is not sufficient to improve sensorimotor learning of a highly skilled tasks with a single session application in healthy participants (Butefisch et al., 2000; Boggio et al., 2006; Ni et al., 2009; Saiote et al., 2013; Minarik et al., 2015; Hashemirad et al., 2016). In line with our results a study by Convento et al. (2014) showed no improvement in performance of a Jebsen–Taylor Hand Function Test, after single-session left PPC with electrode size of 5 cm $\times$5 cm and intensity of 2 mA (Convento et al., 2014).

Another possible reason can explain our null results is that ceiling effects may be present in healthy and young participants. In addition, inter variability between participants (Lopez-Alonso et al., 2014) might be another reason for negative results obtained in the current study. Regarding to the huge controversy in the results of tDCS studies, further research is needed to compare the effects of different protocols of tDCS in terms of intensity, electrode size as well as stimulation sites on improvement of motor learning in different kinds of motor tasks.

Our results demonstrated that transfer of learning into the untrained hand only occurred for movement time not for the error rate or skill. Contrary to our results, Camus et al. (2009) found transfer learning into the left untrained hand in both movement time and error rate after six blocks of training using SVIPT with the right hand (Camus et al., 2009). There are several factors that may be responsible for this discrepancy. They probably used explicit types of SVIPT due to the number of target forces and feedback was given throughout their experiment, while participants learned SVIPT implicitly in our experiment. In addition, they did not apply a-tDCS during training.

Although we found no between-groups effects following the single-session a-tDCS over the FPN superficial sites, further research is need to find out what specific cortical site is involved in sequence learning as well as transfer learning into the opposite site for a precision control task, such as SVIPT. It should be noted that the method used in the literature for assessment of behavioral outcomes in SVIPT is trial-based. In this method, behavioral outcomes are measured in the span of a trial. This method of data handling is gross and does not able to detect detailed changes which might occurred in each target force at early stage of learning during SVIPT. So, further research is needed to investigate tDCS effects within the span of an individual force (this is the subject of a forthcoming publication). Increasing our knowledge about sequence learning, especially for fine control tasks may have significant implications for rehabilitation of patients who are suffered from neurological disorders, such as a stroke or Parkinson's disease.

Limitations and Suggestions

There are some limitations in this study. We included healthy young individual participants so we cannot extrapolate our results to elderly or patient' populations. Regarding to the lack of effects of a-tDCS on cortical outcomes (CSE, ICF, and SICI), one possible reason for the null findings may be related to the small size of the stimulating electrodes. Further research using larger electrode sizes over the FPN sites is needed to investigate the possible excitatory effects of nearby cortical sites on cortical and behavioral outcomes during a fine motor sequence task such as SVIPT.

We assessed outcome measures only one day after intervention, and long term effects of a-tDCS on behavioral outcome measures were not demonstrated in this study. In the current study, we used a-tDCS and TMS for finding functional connectivity of FPN sites; using new techniques such as double-coil TMS and diffusion tensor imaging (DTI) can be more helpful to find out functional connectivity and specific roles of the FPN sites in motor sequence leaning. We also measured general behavioral outcomes including movement time, error rate and skill in the level of each trial; the measurement of other variables such as reaction time or force deviations in the level of each target force might be more sensitive to motor sequence learning and induced plasticity following intervention such as tDCS.

CONCLUSION

Our results demonstrated that a single session a-tDCS with small-size stimulating electrodes over DLPFC, M1, or PPC combined with training of SVIPT has no significant additional effects on implicit motor sequence learning in the trained hand. We also found no significant changes in M1 excitability, inhibition or facilitation following a-tDCS during SVIPT. Additionally, transfer learning into the untrained hand was seen only for speed but not for accuracy or skill after application of a-tDCS during a fine control task such as SVIPT.

FUNDING

This work was supported by Monash University and there is no conflict of interest regarding the publication of this paper. PBF was supported by a Practitioner Fellowship grant from the National Health and Medical Research Council (NHMRC) (1078567). PF has received equipment for research from Cervel Neurotech, Medtronic Ltd, MagVenture A/S and Brainsway Ltd and funds for research from Cervel Neurotech.

AUTHOR CONTRIBUTIONS

The present article is a part of PhD thesis of the corresponding author FH. The main supervisor SJ and the co-supervisors, PF and MZ, helped the corresponding author to develop the study design.

ACKNOWLEDGMENTS

The authors gratefully thank Sabastian White and Masoumeh Hashemirad for their help to create macros in LabChart ™ and MATLAB software for recording and automation analysis of data obtained from the force transducer. We also appreciate Dr. Allie Ford for her proofreading on this manuscript. The authors are grateful to all participants who participated in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnhum.2017.00153/full#supplementary-material

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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 7

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

Nature of the contribution	Extent of the contribution (%)
Review of literature, study 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 the contribution
Shapour Jaberzadeh	Study design, guidance in framing of the manuscript, reviewing and provision of feedback on the manuscript drafts.
Paul B Fitzgerald	Reviewing and provision of feedback on the manuscript drafts.
Maryam Zoghi	Reviewing and provision of feedback on the manuscript drafts.
Masoumeh Hashemirad	Developing some macros in MATLAB in order to automation of data analysis.

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

Candidate's name

Signature Date

Signature Date

Preamble to Chapter 7

The following chapter has not been submitted. Chapter 7 evaluates the effects of application of single-session a-tDCS over M1, DLPFC or PPC on force-*target-by-force target based during SVIPT*. In this chapter, Tables and Figures have been inserted into the manuscript for ease of reading.

Aim

To investigate the site-specific effects of a single session a-tDCS of three FPN superficial sites on the temporospatial variables (response time, reaction time and force deviations) in SVIPT for both the trained or untrained hand.

Chapter 7: The effects of single-session anodal tDCS over the frontoparietal superfacial sites on motor sequence learning based on target force measurements.

The effects of single-session anodal tDCS over the frontoparietal superfacial sites on motor sequence learning based on target force measurements

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Abstract

Objectives: The aims of this study were to investigate the effects of single-session anodal transcranial direct current stimulation (a-tDCS) over the fronto-parietal network (FPN) sites such as dorsolateral prefrontal cortex (DLPFC), primary motor cortex (M1) or posterior parietal cortex (PPC) on target force measurements during a sequential visual isometric pinch task (SVIPT) in both the trained and untrained hands.

Methods: A total of 48 right-handed healthy participants were randomly assigned to one of the four stimulation groups: 1) a-tDCS of left M1, 2) left DLPFC, 3) left PPC and 4) sham. A-tDCS was applied during SVIPT in which participants precisely control their forces to reach different target forces presenting on a computer screen. Each training block consisted of eight trials and each trial included seven different target forces from 10 to 40% of maximum voluntary contraction (MVC). Force-by–force target measurement including

response time, reaction time and force deviations were assessed at three time points including baseline, post 15 min and post 24 hours after intervention in both the trained and untrained hands.

Results: We found no significant differences between sham and a-tDCS groups on measured temporal and spatial variables. There were significant differences between a-tDCS groups on response time and force deviations at some target forces. M1 and PPC a-tDCS groups showed significant differences on response time (target force of 10% MVC) with PPC times being faster ($F(3, 44) = 3.17$, $p = .033$). Whereas, the DLPFC a-tDCS group compared to the PPC group, revealed more improvement in force deviations for the target force of 30% MVC (F 3, 44) = 3.6, $p = .02$). Transfer of learning into the untrained hand was also observed for response time and force deviations, but not for reaction time, in all four stimulation groups.

Conclusions: Our findings suggest that different areas of the FPN sites (DLPFC, M1 or PPC) were differentially affected with regard to temporal and spatial processing in SVIPT. It seems the PPC is more involved in temporal processing compared to the DLPFC, which is more engaged in spatial processing, however, these effects were also found for some target forces, further research is needed to better understand fundamental aspects of these areas on sequence learning in a precision control task such as SVIPT.

Key words: Temporal processing, spatial processing, motor sequence learning, primary motor cortex, dorsolateral prefrontal cortex and posterior parietal cortex.

Introduction

In sequential visual isometric pinch task (SVIPT) studies, which have been recently used in neuroscience research, changes in performance following application of anodal transcranial direct current stimulation (a-tDCS) have been reported based on *trial-based* measurements (Reis et al., 2009a; Saucedo Marquez et al., 2013; Schambra et al., 2011). In SVIPT, a series of trials (consisting of different force targets) are clustered into blocks and participants are required to precisely squeeze a force transducer to reach different target forces as quickly and accurately as possible.

In *trial-based* measurement, behavioural outcomes (movement time, error rate and skill) are measured as the average of trials. For example, movement time is calculated as the average time for completion of each trial. Error rate refers to the ratio of trials with at least one error. In this kind of measurement, skill is considered as an index to represent the whole shift in the speed-accuracy trade-off, which is obtained from both movement time and error rate. Even though, *trial-based* measurements can provide information about changes in performance during sequence learning within the trial, this measurement is not able to determine the slight changes that occurred at each force target.

Movement time, which is the sum of response and reaction time for all target forces in each trial, is not able to differentiate changes produced in response and reaction times within each force target. In other words, movement time provides information about the time for completion of each trial so this variable as the only temporal variable is not able to detect small changes which may occur in other temporal variables such as "*reaction time,* which is defined as the interval from appearing stimuli until the moment any move was taken above resting range, or *"response time"*, which is referred to the interval from presentation of the target force to reach the maximum level of force target.

In addition, in trial-based measurement, error rate, which is obtained from proportion of errors in per block, is not not able to show the amount of absolute deviations occurs after training for each force separately. Therefore, the trial-based measurements make impossible to detect small changes at single events (forces) during SVIPT. To the best of authors knowledge, there is no published study to determine the specific role of three different sites of the FPN (DLPFC, M1 or PPC) on the temporospatial variables (response time, reaction time and force deviations) in SVIPT with both the trained and untrained hands.

The null results obtained on movement time, error rate and skill as conventional behavioural outcomes of SVIPT following a-tDCS over three different areas of frontoparietal network (FPN) sites including dorsolateral prefrontal cortex (DLPFC), M1 and the posterior parietal cortex (PPC) in both the trained and untrained hands.

In this study, *event-based* assessment of outcome measures used as an alternative way to obtain data during SVIPT. in this method, response time, reaction time and force deviations measured for each event (target force) within each trial. Therefore, the primary aim of this study was to investigate whether a-tDCS over three different sites of the FPN (DLPFC, M1 or PPC) could differentially affect the mentioned variables. The secondary aim of this study was to determine whether these effects are transferred into the untrained hand. We hypothesised that the application of a single session of a-tDCS over DLPFC or PPC, which are highly activated during early stages of motor learning, could induce more improvement in temporospatial variables compared to M1 a-tDCS and this site-dependency effects are transferred into the untrained hand.

Methods

Participants and study design

48 healthy right handed participants (34 females, 14 males; age 25.83 ± 6.174 years) took part in this study, which was a parallel randomized single-blind sham controlled study. Each participant was randomly assigned to one of the four stimulation groups: 1) a-tDCS of left M1, 2) a-tDCS of left DLPFC, 3) a-tDCS of left PPC, 4) sham a-tDCS. All participants signed a consent form before taking part in our experiment. The experimental procedure which was used in this study was same as the one used in study 6 (Hashemirad et al., 2017) except for data handling. All participants were naive to the purpose of the experiments. All tests were conducted between 8 am and 4 pm. To control for the effect of female hormonal fluctuation on the size of MEPs, the experimental sessions were carried out between the 7th and 23th day of women's menstrual cycles. The study was approved by the Human Ethics Committee at Monash University.

Experimental procedure

As described in one of the published papers in this thesis (Study 6) (Hashemirad et al., 2017), a force transducer (AD Instrument MLT004/ST, NSW, Australia) used for SVIPT. Participants were instructed to match their force production on the force transducer as precisely and quickly as possible to reach each target force, which appeared on a computer screen. Each participant completed eight blocks of sequence forces, with their dominant hand, except for the block 6, which was in a random order (Figure 1). The sequence of force target was (10, 35, 20, 40, 25, 15, and 30% of Maximum voluntary contraction (MVC)

Transcranial direct current stimulation (tDCS)

A commercial stimulator (Intelect Advanced Therapy System, Chattanooga, TN, USA) was used to deliver a direct current with intensity of 0.3 mA for 20 min during training. An active electrode ($1.5 \times 2 = 3$ cm^2) was placed over the left M1, DLPFC, or PPC and a return

electrode (12 cm^2) was placed over the contralateral supraorbital region. The two electrodes were covered by saline-soaked sponges and strapped in place by two bands (Koechlin et al., 1999). The location of the M1 area was identified using transcranial magnet stimulation (TMS) and cantered over the representational field of the right first interosseous muscles (FDI), which plays a dominant role for SVIPT (Liang et al., 2007). The location of DLPFC and PPC were determined using the international 10-20 system. Participants reported any feelings under the electrodes such as itching, tingling, burning sensations, headache and pain and any other side effects during stimulation (Poreisz et al., 2007). If participants reported pain or any other side effects, such as itching or burning under the electrodes, we injected some normal saline into the sponges using a syringe to keep them wet throughout the experiment (Koechlin et al., 1999).

for each participant (Figure 1), the same procedure was followed: 1) baseline measurement, 2) training concurrently with anodal/sham tDCS stimulation 3) assessment 15 min post intervention post and 4) 24- hour after intervention.

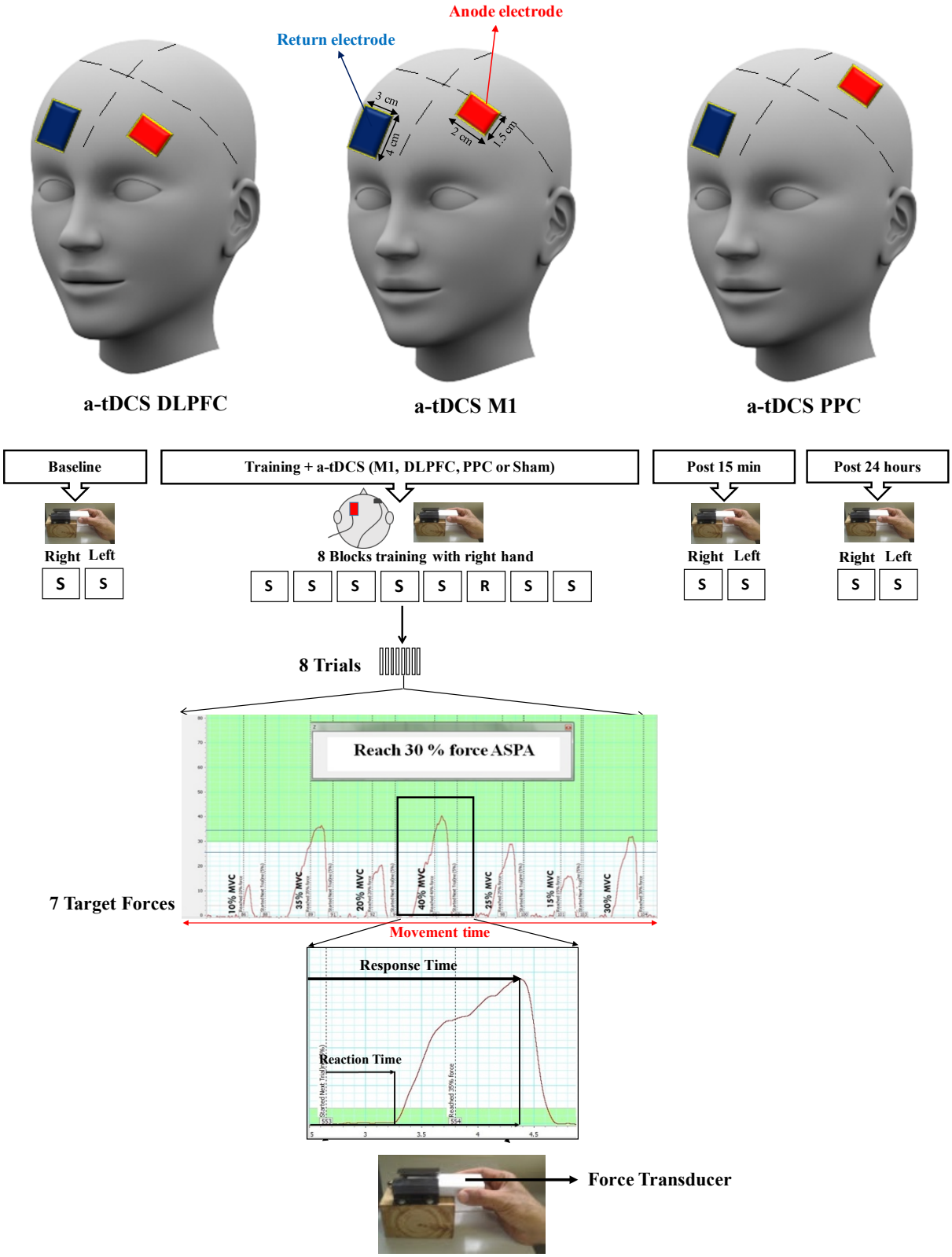

Figure 1: Experimental set up. Participants were instructed to squeeze a force transducer as precisely as possible to reach each target force that appeared on the computer screen. Each sequence block consisted of eight trials, which included seven different target forces from 10 to 40 % of their MVC. They were asked to complete each block as quickly and accurately as possible. Response time and reaction time were measured as temporal variables for each

target force. SVIPT: Sequential visual isometric pinch task, A-tDCS: Anodal transcranial direct current stimulation, M1: Primary motor cortex, DLPFC: Dorsolateral prefrontal cortex, PPC: Posterior parietal cortex, S: Sequence block, R: Random block.

Three outcomes, response time and reaction time (as temporal variables) and force deviations (as spatial variable) were measured for each target force in all assessment blocks.

Temporal variables:

Response time

Response time was defined as the interval between the appearance of each stimulus and the moment the force response reached its maximum level at each force target. Mean response times were calculated for all eight repetitions of the same target force across a block. Each target force appeared eight times per block (once per trial).

Reaction time

As shown in Figure 1, reaction time was the interval from appearance of a stimulus until the moment any response rose above resting range. The eight repetitions of the same target force across a block were averaged and taken as the reaction time for the given target in that block.

Spatial variable:

Force deviations

Force deviations were defined as the difference between the force produced by the participant and the given target force. For example, force deviations for force 10% of MVC in a set of

eight trials in a given block was calculated from the following formula, where a = force production, subscribe number from 1 to n are according to number of trials, and n = 8.

$$\sqrt{\frac{(a_1 - 10)^2 + (a_2 - 10)^2 + ... + (a_n - 10)^2)}{n}}$$

Data Analysis

One-way ANOVA was conducted to assess where there was any significant difference in measured variables among the four groups at baseline. The normality of data was assessed using a Kolmogorov-Smirnov (K-S) test. For normal distributed variables, a mixed-design ANOVA (Repeated-measure with the factor Time (baseline, post 15 min after and 24 hours after intervention) as a within-subjects factor, and factor Group (a-tDCS of M1, DLPFC, PPC and sham) as the between-subjects factor was used to assess the effects of a-tDCS on temporal and spatial variables among the four groups over time. This analysis was separately applied for the trained and untrained hands. If the assumption of Mauchly`s test of sphericity was violated, a Greenhouse-Geisser was used in order to correct non-sphericity. Post-hoc tests with Bonferroni correction were performed, as appropriate, to determine where differences occurred. For non-normally distributed data, log transformation was used to equalize variances and perform parametric statistical analyses. After the transformation, the skewness of the log data was close to zero, confirming the proximity to the Normal distribution of the data.

SPSS (version 20) and MATLAB (R2014a) were used to analyse data in this study. Statistical significance was set at p = .05.

Results

There was no significant difference in participants' characteristics and outcome measures, including temporal and spatial variables in all target forces at the baseline ($p > .05$).

A mixed-design ANOVA was separately applied on logarithmically transformed data of temporal and spatial variables for each target force from 10 to 40 % of MVC for both the trained and untrained hands (Tables 1 & 2).

Response times

Right trained hand

The results of a mixed-design ANOVA on response time of each target force from 10 to 40% of MVC is represent in Table 3. Response time significantly decreased after intervention over time (Figure 2). No significant effects were observed in factor Group and interaction between Group and Time. Therefore, intervention of a-tDCS during training resulted in improved response times in all four groups over all three time points.

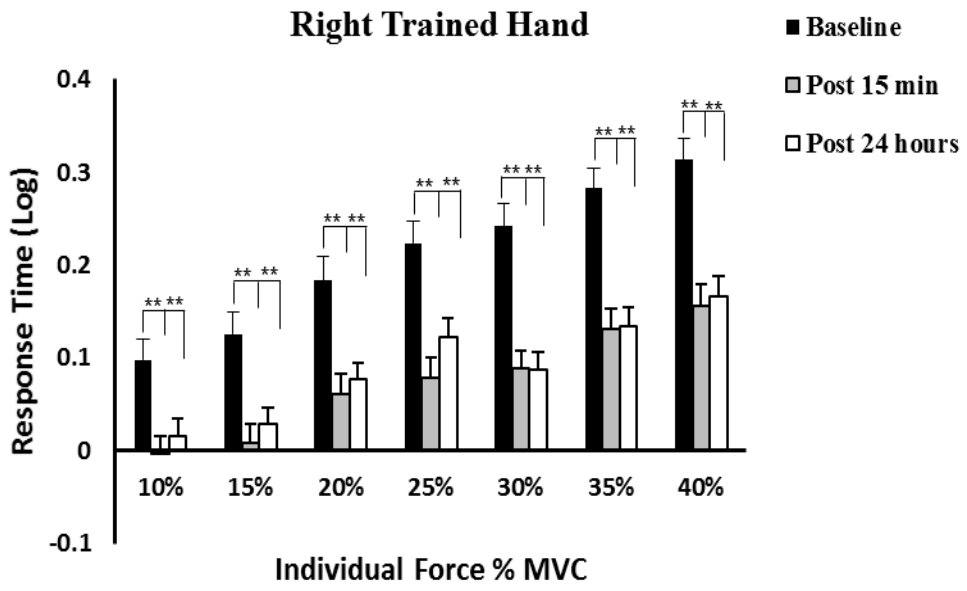

Figure 2: Changes in response time for each target force from 10 to 40% MVC after intervention in the trained hand over time. The Post-hoc results are marked by asterisks. Values are presented as average ± SEM, $p < .05$ *, $p < .01$ **

Left untrained hand

As shown in Figure 3, significant improvements in response times were seen in the untrained hand over time for all four groups.

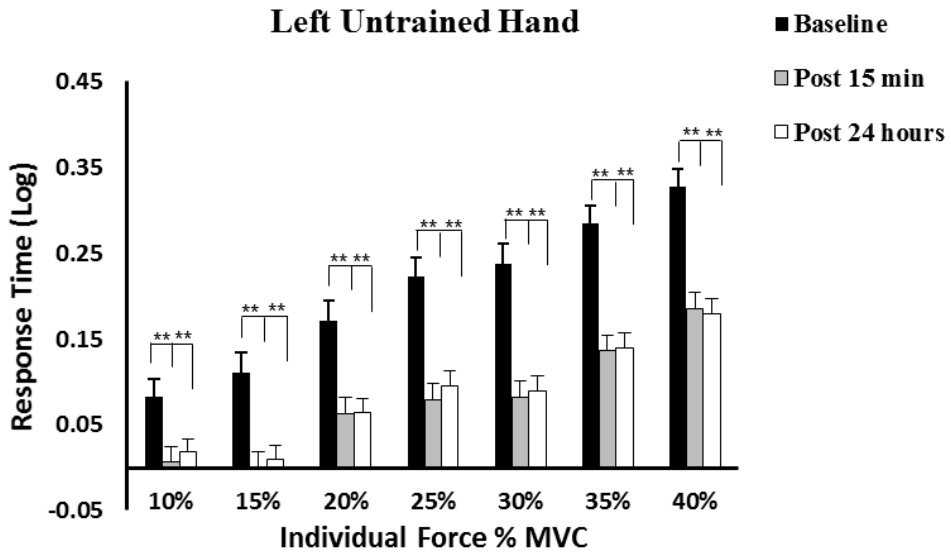

Figure 3: Changes in average of response time for each target force in the untrained hand over three time points. The Post-hoc results are marked by asterisks. Values are presented as average ± SEM. $p < .05$ *, $p < .01$ **

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No significant effects were found in factor Group or interaction between Group and Time in any target force from 10 to 40 % of MVC. The only significant between-group effects was observed for force 10% MVC ($F(3, 44) = 3.17$, $p = .033$). Pairwise comparison showed a significant reduction of response time in the PPC a-tDCS group compared to the M1 a-tDCS group at post 15 min ($p = .049$) (Figure 4).

Figure 4: Changes in mean response time for force 10% of MVC among the four groups at three time points. Significant differences were observed in main effects Group for force 10% of MVC. The PPC group showed more reduction in response time for force 10% MVC compared to the M1 group at post 15 min. The Post-hoc results are marked by asterisks. Values are presented as average ± SEM. $p < .05$ *, $p < .01$ **

Table 1: Results of a mix-design ANOVA on lognormal response time in both trained and untrained hands over times.

Response time/ Log	**Block**	**Time**		**Interaction**		**Group**	
		F value	**P**	**F value**	**P**	**F value**	**P**
10% MVC	Trained	F (1.57, 62.2) = 12.6	**<.001**	F (4.72, 62.2) = .12	.99	F (3,44) = 1.8	.16
	Untrained	F (1.74, 76.9) = 7.8	**.001**	F (5.24, 76.9) = 0.37	.87	F (3,44) = 3.17	**.033**
15 % MVC	Trained	F (1.78, 78.5) = 25.5	**<.001**	F (5.35, 78.5) = 0.35	.89	F (3,44) = 1.26	.29
	Untrained	F (1.65, 72.9) = 23.9	**<.001**	F (4.2, 62.5) = 1.34	.26	F (3,44) = 1.63	.19
20 % MVC	Trained	F (1.63, 72) = 21.7	**<.001**	F (4.91, 72) = .39	0.87	F (3,44) = 0.75	.52
	Untrained	F (1.47, 64.8) = 22.3	**<.001**	F (4.42, 64.8) = 0.62	.66	F (3,44) = 1.11	.35
25 % MVC	Trained	F (1.68, 74.3) = 30.9	**<.001**	F (5.06, 74.3) = .48	0.79	F (3,44) = 1.27	.29
	Untrained	F (1.62, 71.2) = 42.2	**<.001**	F (4.86, 71.2) = 1.15	.34	F (3,44) = 1.29	.29
30 % MVC	Trained	F (1.4, 62) = 27.4	**<.001**	F (4.22, 62) = 0.11	.97	F (3,44) = 1.1	.35
	Untrained	F (1.35, 59.8) = 43.1	**<.001**	F (4.07, 59.8) = 0.41	.86	F (3,44) = 1.59	.2
35 % MVC	Trained	F (1.2, 56.9) = 27.9	**<.001**	F (3.8, 56.94) = 0.44	.77	F (3,44) = 1.004	.4
	Untrained	F (1.64, 72.2) = 41.3	**<.001**	F (4.92, 72.2) = 0.82	.53	F (3,44) = 1.3	.28
40 % MVC	Trained	F (1.53, 67.5) = 39.4	**<.001**	F (7.6, 67.5) = 1.07	.41	F (3,44) = 0.92	.43
	Untrained	F (1.65, 72.9) = 32.1	**<.001**	F (4.97, 72.9) = 0.59	.7	F (3,44) = 1.52	.22

Reaction times

Right trained hand

The results of main effects of Time showed no significant improvement in reaction time for any force target:10% (1.74,76.7) = 0.61, p = .52), 15% (1.57, 67.5) = 3.14, p = .061), 20% (F (1.3,57.4) = 0.27, p = .66), 25% (1.56, 68.7) = 1.82, p = .17), 30% (F (1.26,55.4) = 1.53, p = .22), 35% (F (2,88) = 1.23, p = .29) and 40% of MVC (F (1.22, 54) = .39, p = .57). No significant main effects Group were found for any target force (p > .05). Interaction Group and Time were not significant for any target forces, except 15% of MVC (F (4.45, 67.5) = 2.55, p = .039). The results of the interaction test revealed reaction times significantly decreased from baseline to post 15 min for the PPC (p = .027) and sham (p = .039) groups, but not for the two other groups.

Left untrained hand

Similar to the right hand, there were no significant differences in reaction time for any force target from 10 to 40% of MVC (p > .05) with the left, untrained, hand over three time points. However, significant interaction effects were found for three target forces: 15% (F (4.46, 65.4) = 3.18, p = .015), 20% (F (3.45, 50.7) = 2.71, p = .047) and 30% MVC (F (4.93, 72.3) = 3.51, p = .007). The results of the interaction test by Bonferroni correction showed no significant differences for force 15% MVC in any group at any time point. For two other target forces 20 % and 30 % MVC, there was a significant increase in reaction time for the M1group. In the M1 group, participants showed increases in reaction time for force 20% of MVC from baseline to post 15 min (p = .044) and from baseline to post 24 hours (p = .038). A significant increase in reaction time was also observed from baseline to post 15 min for force 30% of MVC (p = .027).

Force deviations

Right trained hand

The results of a mixed design ANOVA on force deviations showed that factor Time significantly differed for all target forces except target force 30% of MVC ($F(1.63, 76.5) = 5.65$, $p = .73$) (Figure 5). Factor Group was only significant for force 30% of MVC ($F 3, 44) = 3.6$, $p = .02$). The results of pairwise comparison by Bonferroni correction revealed a significant difference between the DLPFC and PPC groups at post 24 hours ($p = .02$) force deviations were reduced more for force 30% of MVC in the DLPFC group compared to the PPC group one day after intervention (Figure 6). No interaction effects were found for any target force (Table 2).

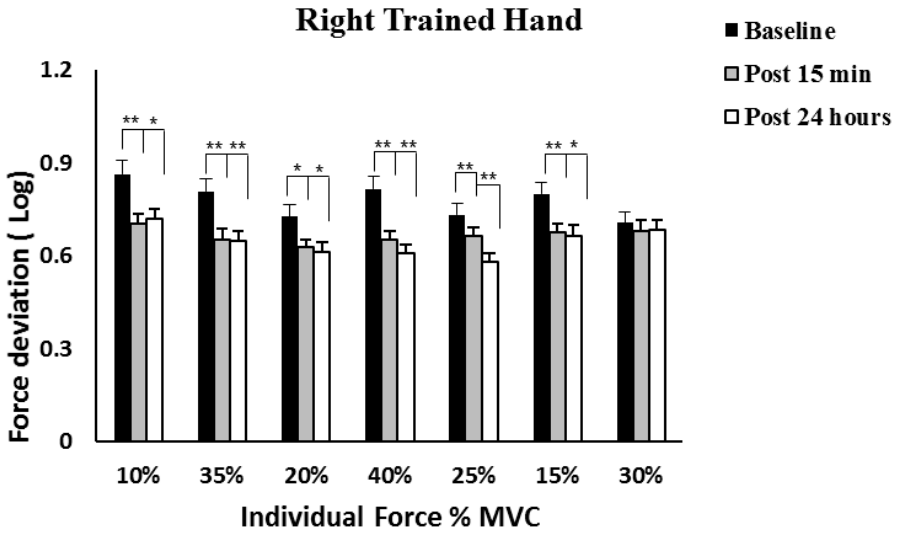

Figure 5: Changes in average of force deviations for each target force over three time points. No significant differences were found for force 30 % of MVC, which was the last target force in the sequence order. The Post-hoc results are marked by asterisks. Values are presented as average ± SEM. $p < .05$ *, $p < .01$ **

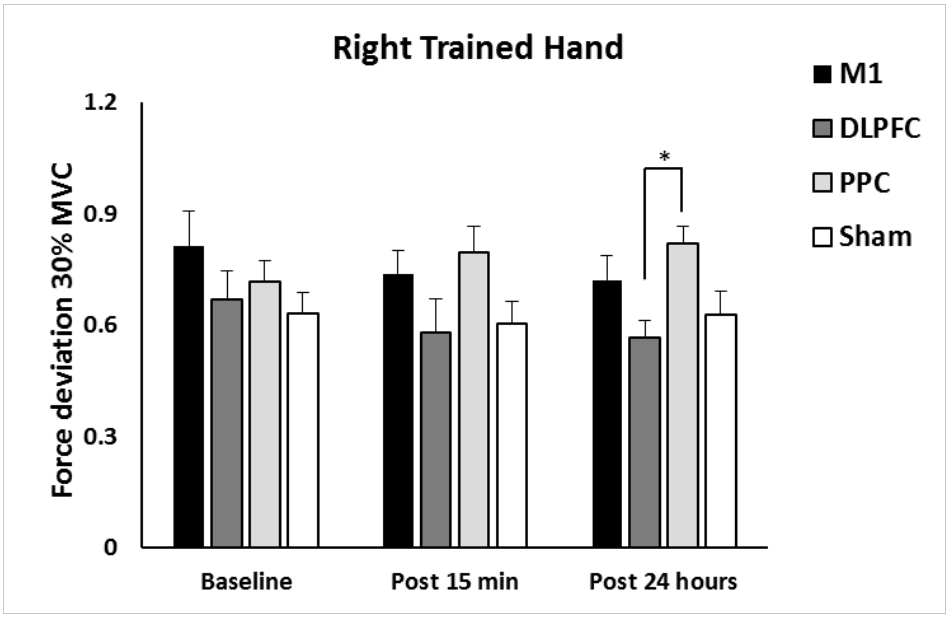

Figure 6: Changes in average force deviations for force target 30% of MVC over three time points among the four groups. Significant differences were found between the DLPFC group and PPC groups for force 30% of MVC at 24 hours after intervention. The Post-hoc results are marked by asterisks. Values are presented as average ± SEM. $p < .05$ *, $p < .01$ **

Left untrained hand

The results of a mixed design ANOVA showed deviations for all target forces significantly reduced over time (Figure 7). The main effects of Group or interaction were not significant for either target force (Table 2). Therefore, our results indicated that spatial processing can be transferred into the untrained hand for all force targets from 10 to 40% of MVC after training regardless of stimulation conditions.

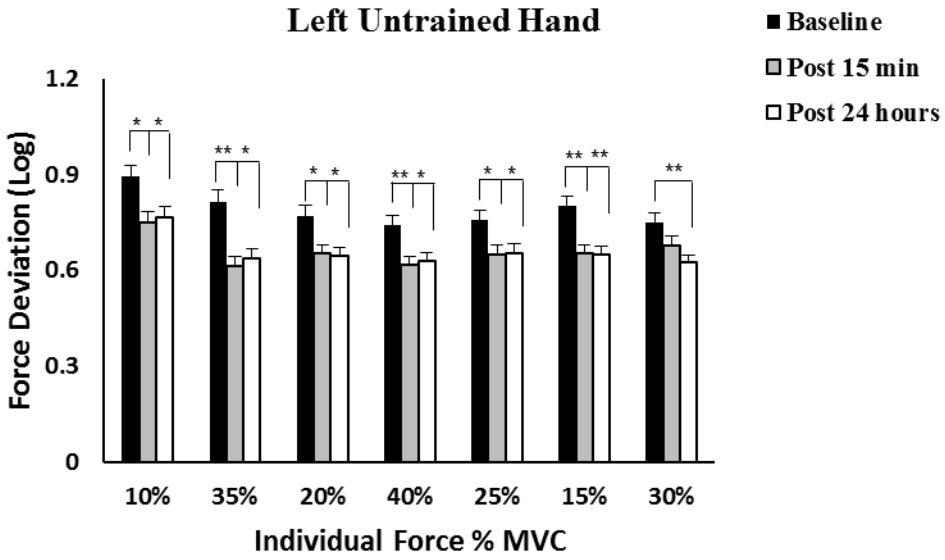

Figure 7: Changes in force deviations for each target force over three time points in the untrained hand. The post hoc results are marked by asterisks. Values are presented as average ± SEM. $p < .05$ *, $p < .01$ **

Table 2: Results of a mix-design ANOVA on force deviations in both the right trained hand over times.

Force deviations / Log	Block	Time		Interaction		Group	
		F value	**P**	**F value**	**P**	**F value**	**P**
10% MVC	Trained	F (1.7, 74.8) = 8.3	**.001**	F (5.1, 74.8) = 0.203	.96	F (3,44) = 1.69	.18
	Untrained	F (2,88) = 6.65	**.002**	F (6,88) = 1.06	.38	F (3,44) = 2.08	.11
15 % MVC	Trained	F (2,88) = 7.67	**.001**	F (6,8) = 0.45	.83	F (3,44) = 0.809	.49
	Untrained	F (2,88) = 10.3	**<.001**	F (6,88) = 1.21	.31	F (3,44) = 2.63	.061
20 % MVC	Trained	F (1.73,76.5) = 5.65	**.007**	F (5.21,76.5) = 1.05	.39	F (3,44) = 0.89	.46
	Untrained	F (1.39, 61.2) = 7.01	**.001**	F (4.14,61.2) = 0.63	.64	F (3,44) = 0.24	.86
25 % MVC	Trained	F (1.57, 69.2) = 8.13	**.002**	F (4.72, 69.2) = 0.34	.87	F (3,44) = 2.49	.072
	Untrained	F (2,88) = 5.39	**.006**	F 6,88) = 0.57	.75	F (3,44) = 0.27	.84
30 % MVC	Trained	F (1.63, 72) = 0.25	.73	F (4.91, 72) = 0.76	.57	F (3,44) = 3.6[1]	**.021**
	Untrained	F (2,88) = 5.6	**.005**	F (6,88) = 0.33	.91	F (3,44) = 1.78	.16
35 % MVC	Trained	F (1.9,83.9) = 9.23	**<.001**	F (5.72,83.9) = 1.29	.26	F (3,44) = 1.38	.26
	Untrained	F (2,88) = 13.3	**<.001**	F (6,88) = 1.21	.307	F (3,44) = 0.28	.83
40 % MVC	Trained	F (1.75, 77.3) = 14.6	**<.001**	F 5.27,77.3) = 0..46	.808	F (3,44) = 2.05	.12
	Untrained	F (1.7,75.1) = 7.08	**.001**	F (5.12,75.1) = 0.67	.65	F (3,44) = 0.89	.45

Discussion

The primary aim of this study was to investigate whether there are any site-dependency effects of a-tDCS over the FPN (DLPFC, M1, PPC or sham) on reaction times, response times or force deviations within SVIPT. The secondary aim was to determine whether the effects of training and a-tDCS on measured variables can be transferred into the untrained hand. Our findings showed no significant differences between a-tDCS groups and sham. However, there were significant differences in temporospatial improvements for some target forces. We also found the effects of interventions on measured variables were transferred into the untrained hand.

In the previous study (Hashemirad et al., 2017), no site-specific effects of a-tDCS over the FPN were observed when we measured behavioural outcomes, including movement time, error rate and skill, according to the *trial-based* measurements. Site-dependency effects of a-tDCS for some target forces are determined for temporospatial processing in SVIPT when variables are measured based on *force-by-force target measurements (event-based measurement)*.

Temporal variables

One limitation of SVIPT studies is that, in these studies, only one temporal variable has been used, movement time. This variable is defined as the whole time for completion of each trial. Even though this trial-based time variable provides information about how fast a participant completes a trial, it fails to show changes at different force target events within a trial. In other words, if there is any difference between the pattern of changes in reaction time or response time for different target forces, they are not determined by the movement time or trial-based monitoring.

In the previous study (Chapter 6), we observed no main effects of group on "movement time", while measuring "response time" as a temporal variable in this study showed a significant difference between groups. According to our result, more reduction was found in response time in left PPC stimulation compared to left M1 stimulation for 10% of MVC. This result suggests that the left PPC is more relevant for temporal processing with the left hand, rather than left M1, especially for the target force which appeared at the beginning of a sequence. The relevance of the left PPC as an anticipatory motor control for precise sensorimotor timing has been identified in the study by Krause et al. (2012). They showed that activity in the PPC is essential for precise sensorimotor tasks, especially when quick and flexible adjustment of movements with respect to external changes is required (Krause et al., 2012). The PPC is assumed to fulfil two main functions: integration of multisensory information and anticipatory motor control (R. A. Andersen & C. A. Buneo, 2002; Andersen & Cui, 2009; Blakemore & Sirigu, 2003; Creem-Regehr, 2009; Culham et al., 2006; Culham & Valyear, 2006). The observed improvements in response times after training were transferred into the untrained hand for all target forces. Therefore, participants were able to reach each target force more quickly in both the trained and untrained hand.

Unlike response times that significantly improved in both the trained and untrained hands after training, reaction time showed no significant reduction with either hand. This finding can be related to the fact that single-session training is not sufficient to improve the variable of reaction time. In contrast to our results, Waters-Metenier et al. (2014) showed enhancement in both response time (which they called execution time) and reaction time following a 4-day application of bihemespheric M1tDCS during a piano-like key task. As shown in Figure 8, the observed improvements in response time after one single training session is probably related to improvement in moving time, which is defined as the time of initiation of any movement by participants to reach the target. This finding, that response

time and reaction time are not similarly affected by training, is consistent with studies that have shown that reaction time and moving time can be separate entities (McMorris et al., 2011; Schall, 2002; Tversky & Kahneman, 1985).

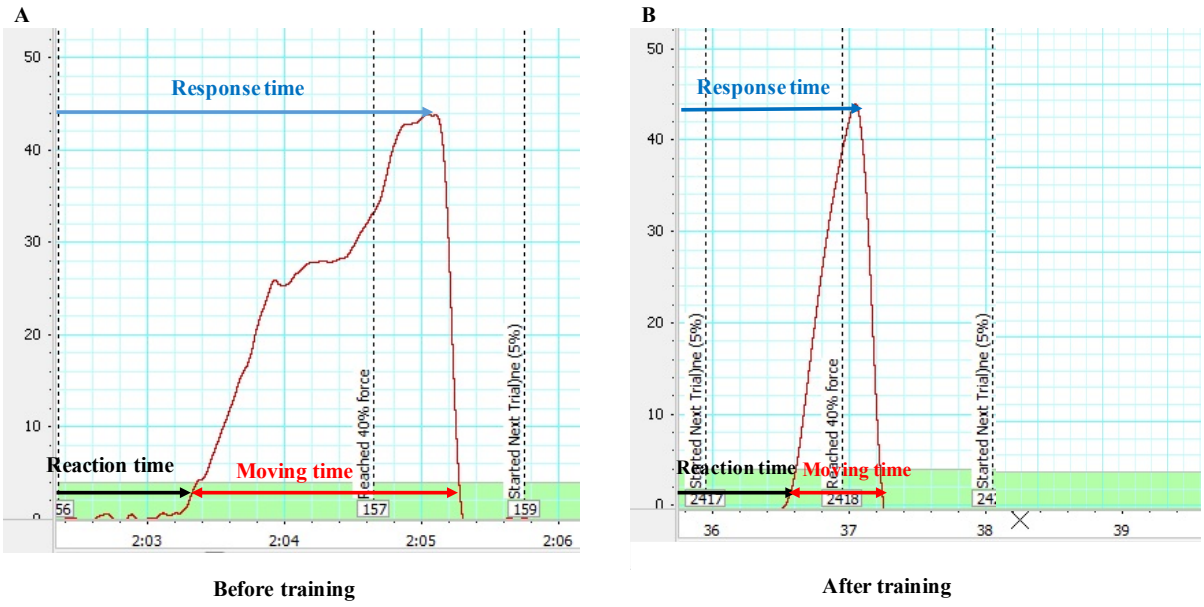

Figure 8: Represented data from one participant that shows three temporal factors including response time, reaction time and moving time before (A) and after training using SVIPT (B).

Reaction times for target forces of 15% and 40 % of MVC showed significant improvements in the left PPC and sham groups, compared to the other two groups (DLPFC and M1) at post 15 min. These results are in line with studies suggesting that the PPC area has an essential role in sensory-motor transformations and temporal processing for eye and hand movements such as SVIPT.

It should be noted that participants who received left M1 stimulation showed a significant increase, instead of reduction, in reaction times for two target forces of 20% and 30% of MVC with the left hand. In the other words, a negative impact of a-tDCS was found on reaction times with the left hand in participants who received left M1 stimulation.

Spatial variable

In SVIPT studies, error rate is usually measured as a trial-based measurement in which there is no chance to determine absolute force deviations. In the current study, we measured individual force deviations for each target force across assessment blocks. Our results showed there are between-group effects (DLPFC and PPC a-tDCS groups) on force deviations for the 30% of MVC target force, which was the final target force in the sequence. Participants who received left DLPFC a-tDCS showed more reduction in force deviations compared to the left PPC group with the right trained hand after 24 hours. The PPC area appears to be involved in the integration of sensory and motor activities (Krause et al., 2012), however our results demonstrated that left DLPFC seems to be more associated in spatial processing for the final target force in SVIPT. Consistent with our results some studies have shown that the left DLPFC can improve accuracy in in different kinds of tasks (Foerster et al., 2013; Fregni et al., 2005; Kincses et al., 2004; Kuo & Nitsche, 2015; Zaehle et al., 2011).

In the previous study (Chapter 6), in which the error rates were measured *trial-based*, no significant difference was found among three different areas of the FPN. We also found no transfer learning into the untrained hand for the error rate, while in the current study, improvement in force deviations were transferred into the untrained hand. Further basic research is needed to confirm our results on temporal and spatial processing in SVIPT.

Limitation

There are some limitations in this study. We included healthy young participants, so we cannot generalize our results to elderly populations or patients with neurological disorders. We did not consider the effects of gender differences on measured variables. Long-term outcome measures were not evaluated in this study.

Conclusion

Our findings suggest that temporal and spatial processing in SVIPT were differentially affected by a-tDCS over left PPC and DLPFC brain regions for some target forces. Our results demonstrated that left PPC is more involved in temporal processing, while the DLPFC seems to be associated in spatial processing for a visually guided movement task such as SVIPT. We also observed transfer learning into the untrained hand for response time and force deviations variables, but not for reaction time.

Acknowledgements

The authors gratefully thank Sabastian White for his help to create macros in LabChart software to measure reaction time and automation analysis of data obtained from the force transducer. We also appreciate Dr Allie Ford for her proofreading on this manuscript. The authors are grateful to all participants who participated in this study. We also appreciate the financial support of Monash University which made this project possible.

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Chapter 8: Summary and Concluding Remarks

In this thesis, the intention was to bring together research on the effects of a-tDCS over three areas of the FPN (DLPFC, M1 or PPC) on fast stage of motor sequence learning. In addition, we assessed the effects of these sites on transfer of learning into the untrained hand in healthy young individuals (Chapters 6 &7). To the best of our knowledge there have been no other studies investigating the effects of single-session a-tDCS over the FPN to modulate sequence learning using SVIPT as well as transfer of learning into the untrained hand. To achieve these aims, a number of preliminary studies were necessary (Chapters 2, 3, and 5) as well as development of a number of macros in LabChart^{TM} and MATLAB software for automation of data handling (Chapter 4). The remainder of this chapter provide an overview of our main findings and conclusions from the various studies as well as identifying the most significant limitations, and presenting commendations for future research. Findings arising from the series of studies described in this thesis are summarized in Table 1-8.

Table 1-8: Summary of findings and implications of studies 1-5.

Study /Chapter	Findings	Implications & suggestions
Study 1/Chapter 2	The effects of M1 a-tDCS on motor sequence learning may depend on the stages of motor sequence learning and the type of acquired task. Application of multiple sessions of a-tDCS over M1, compared to single session a-tDCS, induces significant changes in behavioural outcomes in both SRTT and SVIPT learning tasks at post intervention time. The effects of a-tDCS over M1 on retention time might be task specific because significant improvement was only observed in the SEQTAP/SRTT task but not for SVIPT.	The results of this meta-analysis suggest that attention must be directed to the optimization of stimulation sites especially for fast stage of learning. Exploring of optimum stimulation sites for increasing the efficacy of single session of a-tDCS on sequential learning were considered as main aims of this thesis.
Study 2/Chapter 3	Increase the number of trials from 10 to 20 MEPs led to increased ICC values in both	Our results suggest that compared to

	techniques including 120% RMT and 1mV were found in blocks with variable number of MEPs. Removal of the first three or five MEPs in blocks of 10, 15 and 20 trials resulted in no further increase in reliability of MEP responses.	removal of initial MEPs, it is better to increase the number of MEP responses to get higher reliability scores. Therefore, in clinical studies that TMS is used as an assessment tool, the number of elicited MEPs should be increased to achieve an excellent reliability score.
Study 3/ Chapter 5	Separating sequenced movements with intervals ranging from 1 to 4 sec does not affect implicit learning of SVIPT. Our findings indicated implicit learning of SVIPT is independent of inter-trial interval within this range in healthy individuals and inserting of intervals in such visually-guided movements could not create any additional attentional demand in order to improve sequential organization.	Regarding the growing interest in using SVIPT as a visuomotor sequence task in research, understanding important influences, such as temporal and spatial factors on acquired learning in this task would be of great value. Further research is needed to investigate the effects of different temporal and spatial factors on implicit or explicit learning during SVIPT.
Study 4/ Chapter 6	A single session of a-tDCS over DLPFC, M1 or PPC combined with training has no differential effects on implicit motor sequence learning in the trained hand based on conventional data handling. Transfer learning into the untrained hand was seen only for movement time but not for error rate or skill after the training using SVIPT. Concurrent a-tDCS and training using SVIPT did not induce any significant changes on CSE, SICI or ICF in M1 area.	Application of a single session of a-tDCS over FPN superficial sites did not induce any specific effects on movement time, error rate or skill of SVIPT in both the trained and untrained hands. Transfer learning into the untrained hand was only observed for movement time but not for error rate and skill.
Study 5/ Chapter 7	Our findings demonstrated that temporal and spatial processing in SVIPT differentially affected by a-tDCS over left PPC and DLPFC for some level of target forces 10, 15, 20 and 30 % of MVC. No significant differences were found between sham and a-tDCS groups. No significant improvements were found in reaction time either in trained and untrained hand. Transfer learning into the untrained hand was seen for variables of response time and force deviations but not for reaction time.	Left PPC seems to be more involved in temporal processing while DLPFC is probably more associated in spatial processing for some target forces in a visually guided movement task such as the SVIPT. Unlike, response time and force deviations, no improvement was observed in reaction time. It seems that reaction time is a variable that needs more practice to be improved in SVIPT. Transfer learning into the untrained hand were observed in both variables of response time and force deviations. .

The first step in this thesis was to review the current literature to verify whether previous studies support the view that a-tDCS can increase motor sequence learning in healthy individuals. From the findings of this systematic review and meta-analysis (Chapter 2) it was concluded that multiple-sessions of M1 a-tDCS (linked to the slow stage of learning) can increase motor sequence learning, while single-session M1 a-tDCS (fast stage of learning) does not lead to any improved motor sequence learning for either SRTT or SVIPT tasks. In other words, despite a number of studies (Reis et al., 2015; Reis et al., 2009a; Saucedo Marquez et al., 2013; Schambra et al., 2011) has determined the beneficial effects of multiple session a-tDCS on improvement sequence learning, not much is known about efficacy of single session of a-tDCS. Therefore, we focussed to discover optimal stimulation sites, for the fast stage of sequence learning in this thesis.

There is no doubt that time is crucial for both patients and health care providers. Being able to increase motor sequence learning by using a single session of a-tDCS can be helpful for the rehabilitation systems due to saving time as well as money. Therefore, it is worthwhile to find optimum parameters for an effective single session a-tDCS to enhance motor learning.

Due to the growing interest in use of SVIPT as a visuomotor sequence task in research (Reis et al., 2015; Reis et al., 2009a; Saucedo Marquez et al., 2013; Schambra et al., 2011), the fine-motor SVIPT task used in this thesis which requires strong coordination between visual and motor systems. This fine-pinch control task relies on the activation of a widespread cortical network and involves more neuronal resources than gross movements of the whole-hand. Precise pinch force is essential for carrying out most activities of daily life, and loss of this ability is often present after brain lesions. Our results therefor could have implications for

some patients who struggle with force production or fine motor tasks, such as those with stroke or Parkinson's disease.

One limitation to SVIPT studies is that, in these studies, only one kind of data management has been used which is *trial-based*. In this method, behavioural outcomes (movement time, error rate and skill) are measured in the span of a trials. While we believe that this method of data handling is gross and does not able to detect detailed changes which might occurred in each target force at early stage of learning during SVIPT. Therefore, we considered a new data handling technique for data obtained of SVIPT as explained in Chapter 7.

The *trial-based* method used in study 4 for assessment of behavioural outcomes, which is similar to the literature. In study 5, response time, reaction time and force deviations were measured within the span of an individual force.

In study 4, the effects of single-session a-tDCS over three superficial areas of the FPN, M1, DLPFC and PPC on cortical and conventional behavioral outcomes of SVIPT were investigated and we hypothesized that improved performance would observe in both the trained and untrained hands after DLPFC or PPC a-tDCS compared to M1a-tDCS due to higher activation of DLPFC and PPC at early stage of learning.

The results of study 4 showed significant effects of learning in SVIPT for all three regions. No main effects of group (DLPFC, M1, PPC and sham a-tDCS) were observed on *trial-based* behavioural outcomes (movement time, error rate and skill). We also found no significant effects on neurophysiologic outcomes (CSE, SICI and ICF of M1) following single-session a-tDCS over any of the FPN sites. In addition, transfer of learning into the untrained hand was observed for movement time but not for the error rate or skill in this study, as explained in Chapter 6.

In study 5, response time, reaction time and force deviations were measured within the span of an individual force. Our findings indicated that different areas of the FPN (DLPFC, M1 and PPC) were differentially affected in terms of temporal and spatial processing at some target forces, as discussed in Chapter 7. No significant effects were observed between a-tDCS and the sham groups. Improvement in *response times* and *force deviations* in the right trained hand were transferred into the left untrained hand, while, *reaction times* showed no improvement after a single-session training combined with a-tDCS in either right or left hand.

Overall, we found no significant differences between a-tDCS groups (DLPFC, M1 or PPC) and the sham group for either kind of measurement; *trial based or* event-*based (force based)*. However, some small site-specific effects were found between a-tDCS groups (DLPFC and PPC) and (M1 and PPC) for event-*based (Chapter 7)*.

Due to activation of extensive cortical areas of the brain such as premotor, supplementary motor areas and cerebellum in a visually guided sequence task such as SVIPT, further research is needed to explore the specific role of other areas of brain during SVIPT using two method of data handling. Refer to the findings from study 5, reaction time showed no improvement after DLPFC, PPC or M1 a-tDCS. This result suggests that detection of the best cortical area for improving reaction time during SVIPT might be an appropriate solution to increase the efficacy of single-session a-tDCS at fast stage of learning.

The null results obtained between a-tDCS groups and the sham group could be explained based on a number of reasons which are discussed in Chapters 6 & 7.

One explanation can be related to the a-tDCS characteristics used in this thesis. Because we aimed to selectively stimulate M1, not nearby motor areas (such as pre motor, SMA or primary sensory area), we used a small electrode size (3 cm^2) and low intensity stimulation of

0.3 mA that produced a current density of 0.1 mA/cm^2. We expected a-tDCS with these characteristics could affect neurophysiological changes due to some studies that showed a significant increase in M1 excitability following application of a-tDCS with this intensity of stimulation of 0.3 mA *at rest state* (Bastani & Jaberzadeh, 2013; Vaseghi et al., 2015). However, we found no significant changes in CSE, SICI or ICF following a-tDCS during training with SVIPT. It is likely a-tDCS with these characteristics do not impact on neurophysiology outcomes, when applied *during training*. It should be noted that, a systematic review by Hovarth et al. (2014) showed that tDCS generates little-to-no neurophysiological effects on CSE, SICI or ICF, which is consistent with our results. Another reason that can explain our null results is that ceiling effects may be present in healthy and young participants. Older people or patients with brain disorders such as Parkinson or stroke, which ceiling effects is not usually occurred during a single session training, might be benefited from FPN a-tDCS compared to healthy individuals. In other words, we examined this novel intervention on motor sequence learning in healthy individuals as the first step. Further research is needed to investigate the effects of a-tDCS on the aforementioned superficial sites of FPN on elderly people or patients with neurological disorders in order to shed more light on the mechanisms underlying improvement in different conditions.

Inter-variability between subjects might be another reason for negative results obtained in the studies within this thesis (Lopez-Alonso et al., 2014). The lack of any behavioral effects following tDCS can be related to the concept of inter-individual variability introduced by Lopez-Alonsos in 2014. This substantial issue should be tested before using any NIBS techniques to make sure subjects, who participated in this kind of experiments, are responders or non-responders.

Thesis limitations

There are some limitations in each study; these have been provided within each chapter in this thesis. To avoid repetition, only the limitations in the framework of multiple studies are presented here. Participants in this series of studies were selected from healthy young individuals. Therefore, our findings cannot be extrapolated to elderly populations or patients with neurological conditions. Additionally, participants from both sexes participated in all studies; the gender differences were not explored. Inter-individual variability of participants in terms of responder or non-responder were not investigated in tDCS studies. Recruitment of more participants in each condition can increase the power of a study to find significant difference between groups if any exist. Finally, all studies in this thesis are single-blinded, where participants were not aware of type of stimulation while the researcher was not blinded to the condition of each group.

Recommendations for future research

Regarding our findings, we suggest some recommendations for future studies to shed light on this area of research:

a) Exploring different areas of the FPN

Stimulation of other areas of the brain, such as premotor cortex, SMA or cerebellum is required to systematically identify optimal stimulation sites for enhancement of motor sequence learning especially for *the fast stage of motor learning*. According to the results of a study by Nitcheh et al. (2003), significant improvements were found in reaction time of SRTT after stimulation of *single-session M1 a-tDCS* with 35 cm^2 electrode size. Therefore,

this raises the question that stimulation of all three motor areas (premotor, SMA and M1) might be more effective, compared to the stimulation of a single motor area (M1). There are currently no studies that have investigated the effects of stimulation of SMA, premotor cortex or cerebellum during SVIPT, so it is worthwhile examining stimulation of these areas in order to identify their role in this fine-pinch control task.

b) Exploring different characteristics of a-tDCS

Regarding the fact that the a-tDCS method used in this thesis showed no significant effects on CSE, further research is needed to investigate the impact of different characteristics of a-tDCS in terms of electrode size or intensity on sequence learning. In addition, application of *multiple-sessions* a-tDCS over the FPN can help us to discover differential effects of these areas in the two different stages of motor sequence learning

c) Exploring different types and structure of SVIPT

In this study, we examined the effects of single-session a-tDCS of the FPN on implicit motor sequence learning. It is likely that application of this technique to explicit learning during SVIPT might produce different results. In addition, changing the sequenced order of target forces can give us more information about the role of each area on temporospatial processing. In this thesis, forces of 10 to 40 % of MVC were investigated; different target forces need to be investigated to shed light on the effects of each area across different force production.

d) Using different intervention tools

Other NIBS technique such as tACS, tRNS, rTMS or TBS should be also investigated to improve temporospatial variables during early stage of learning SVIPT as a fine motor control task in both healthy and patients' population. It is likely that the fast stage of learning benefited from other NIBS protocols more than tDCS.

e) Using different assessment tools

To explore physiological mechanisms of a-tDCS on motor learning, pharmacological experiments using GABAergic and/or Glutamergic agonists or antagonists are recommended. In addition, computational modelling studies or fMRI may be also helpful in gaining a realistic picture from the patterns of a-tDCS over functionally connected cortical sites of the brain. Diffusion tensor imaging (DTI) can be helpful for examining the anatomical basis for non-invasive brain stimulation effects. Combining fMRI with TMS, including DTI and functional connectivity analysis, could help to explain the involvement of specific pathways to respective a-tDCS effects in future studies.

f) Exploring a-tDCS of FPN sites in patients with neurological disorder

Knowledge about plasticity induced by non-invasive brain stimulation during motor learning tasks in healthy individuals might have implications for motor rehabilitation processes. In this study, we examined the effects a-tDCS on young healthy participants, it is worthwhile to investigate these effects in elderly populations, as well as patients with neurological disorders, such as stroke or Parkinson's disease.

There is hope that the findings of the present thesis might ultimately assist in development of non-invasive brain stimulation protocols as a safe and non-invasive technique for improvement of motor sequence learning.

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Appendices

Appendix 1. Human Ethics Certificate of Approval

MONASH University

Monash University Human Research Ethics Committee (MUHREC)
Research Office

Human Ethics Certificate of Approval

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

Project Number:	**CF13/3302 - 2013001720**
Project Title:	**Does transcranial direct current stimulation (tDCS) of movement related functional areas of the brain affect fast stage of motor skill learning?**
Chief Investigator:	**Dr Shapour Jaberzadeh**
Approved:	**From: 2 January 2014** **To: 2 January 2019**

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

1. The Chief investigator is responsible for ensuring that permission letters are obtained, if relevant, before any data collection can occur at the specified organisation.
2. Approval is only valid whilst you hold a position at Monash University.
3. It is 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 include your project number.
6. **Amendments to the approved project (including changes in personnel):** Require 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 Nip Thomson
Chair, MUHREC

cc: Dr Maryam Zoghi, Prof Paul Fitzgerald, Ms Fahimeh Hashemirad

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Email muhrec@monash.edu http://www.monash.edu.au/researchoffice/human/
ABN 12 377 614 012 CRICOS Provider #00008C

Appendix 2. tDCS stimulation procedure (Koechlin et al., 1999)

1) Subjects are initially screened for skin diseases and use of any skin treatments which could potentially cause irritation.
(2) Before every tDCS session, skin under the electrodes is checked for cuts, lesions, and skin disease. tDCS is not given if there is any skin damage, rash or other skin lesion under the electrode sites.
(3) Skin is lightly cleaned with a swab, taking care not to abrade the skin.
(4) Disinfected rubber electrodes with clean, singleuse sponges dampened with normal saline are placed over the stimulation sites and held against the head with wide rubber bands which cover the entire surface of the electrodes. Care is taken to ensure contact with the skin is firm and even over the entire surface of the electrode.
5) The static impedance measurement is checked stimulation does not proceed unless levels are within limits recommended by the tDCS device manufacturer.
(6) Stimulation is commenced and subjects are advised to report immediately if the stimulation feels painful, or anything other than itchy or tingling, at any time during the period of stimulation.
(7) After the first 2 min, the subject is questioned about pain at the electrode sites. If the stimulation is painful, a small amount of additional saline (approximately 4 ml) is added to the sponge, taking care to avoid wetting adjacent hair and thereby increasing the electrode area, and the tightness and placement of the band are checked. If pain persists, the stimulation is stopped and the electrode sites checked. This procedure is repeated every 10 min during tDCS with a small amount of saline solution being routinely applied at these times.
(8) At the end of stimulation, the electrode site is checked for redness or skin damage.
(9) Rubber electrodes and headbands are cleaned with a disinfectant solution.

Appendix 3. 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 A study is considered to have used random allocation if the report states that allocation was random.
2. Subjects were randomly allocated to groups (in a crossover study, subjects were randomly allocated an order in which treatments were received)	The precise method of randomisation need not be specified. Procedures such as coin-tossing and dice-rolling should be considered random. Quasi-randomisation allocation procedures such as allocation by hospital record number or birth date, or alternation, do not satisfy this criterion
3. Allocation was concealed	Concealed allocation means that the person who determined if a subject was eligible for inclusion in the trial was unaware, when this decision was made, of which group the subject would be allocated to. A point is awarded for this criteria, even if it is not stated that allocation was concealed, when the report states that allocation was by sealed opaque envelopes or that allocation involved contacting the holder of the allocation schedule who was "off-sit
4. The groups were similar at baseline regarding the most important prognostic indicators	At a minimum, in studies of therapeutic interventions, the report must describe at least one measure of the severity of the condition being treated and at least one (different) key outcome measure at baseline. The rater must be satisfied that the groups' outcomes would not be expected to differ, on the basis of baseline differences in prognostic variables alone, by a clinically significant amount. This criterion is satisfied even if only baseline data of study completers are presented.
5. There was blinding of all subjects 6. There was blinding of all therapists who administered the therapy 7. There was blinding of all assessors who measured at least one key outcome	*Blinding* means the person in question (subject, therapist or assessor) did not know which group the subject had been allocated to. In addition, subjects and therapists are only considered to be "blind" if it could be expected that they would have been unable to distinguish between the treatments applied to different groups. In trials in which key outcomes are self-reported (eg, visual analogue scale, pain diary), the assessor is considered to be blind if the subject

	was blind. 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.
8. Measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups	
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 4. 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 analyze 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 5. Plot Digitizer

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

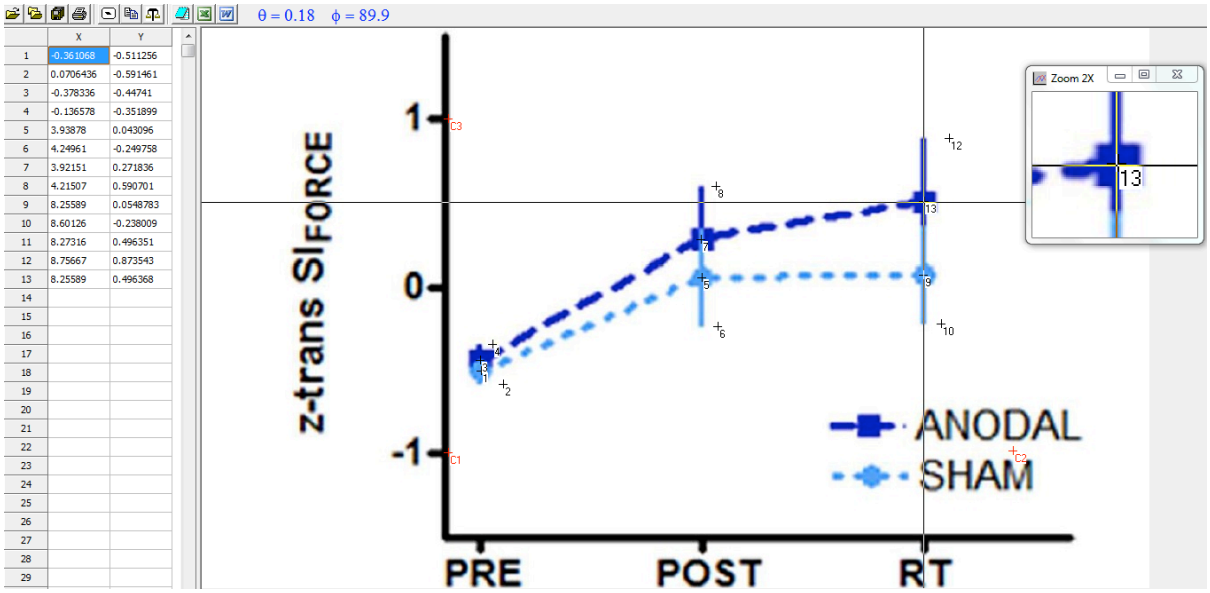
This program will allow you to take a scanned image of a plot (in JPEG or Bitmap) and quickly digitize values off the plot just by clicking the mouse on each data point after calibration. Any 3 non-collinear points can be used for calibration and calibration points **do not** need to be on the axes.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

Usage Notes

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

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

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



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

Appendix 6. TMS safety Questionnaire

MONASH University
Medicine, Nursing and Health Sciences

Project Title: ……..

Screening questions for initial telephone contact

Inclusion criteria: Participant

☐ Is an adult aged 18 years or older?

☐ Is right handed?

☐ Is able to speak, read and write English comprehension

Exclusion criteria:

Please circle your response. Have you ever:

1. Had an adverse reaction to Transcranial Magnetic Stimulation (TMS)? Yes/No
2. Had a seizure or epileptic fit? Yes/No
3. Had an Electroencephalogram (EEG)? Yes/No
4. Had a stroke? Yes/No
5. Had a head injury or neurosurgery? Yes/No
6. Do you have any metal in your head (outside of the mouth,) such as shrapnel, surgical clips, or fragments from welding or metalwork? Yes/No
7. Do you have any implanted devices such as cardiac pacemakers, medical pumps, or intracardiac lines? Yes/No
8. Do you suffer from frequent or severe headaches? Yes/No
9. Have you ever had any other brain-related condition? Yes/No
10. Have you ever had any illness that caused brain injury? Yes/No
11. Are you taking any medications? Yes/No

Please specify:

12. If you are a woman, are you pregnant or is it possible that you may be pregnant? Yes/No
13. Does anyone in your family have epilepsy? Yes/No
14. Do you need further explanation of Transcranial Magnetic Stimulation and its associated risks? Yes/No

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

……
……

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

Participant's name: ……
Participant's signature: ……
Date: ……..

Appendix 7. Consent Form

CONSENT FORM

Project title: ……

NOTE: This consent form will remain with the Monash University researcher for their records

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

☐I agree to participate in two phases of testing

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

a. **TranscranialDirectCurrentStimulation (tDCS)**
b. **TranscranialMagneticbrain Stimulation (TMS)**
c. **Recording of muscle activity using surface electrodes**

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

I understand that my participation is voluntary, that I can choose not to participate in part or all of the project, and that I can withdraw at any stage of the project without being penalised or disadvantaged in any way.
I understand that any information I provide is confidential, and that no information that could lead to the identification of any individual will be disclosed in any reports on the project, or to any other party.
I understand that data from this study will be kept in a secure storage and accessible to the research team. I also understand that the data will be destroyed after a 5-year period.
I understand that any data that the researcher uses from the study reports or in published findings will not, under any circumstances, contain names or identifying characteristics.

Participant's name (please print): _____

Signature: _____Date: _____

Researcher's name (please print: _____

Signature: _____Date: _____

Appendix 8. Edinburgh Handedness Questionnaire

Subject's Code:

Please indicate with a check (✓) your preference in using your left or right hand in the following tasks.
Where the preference is so strong you would never use the other hand, unless absolutely forced to, put two checks (✓✓).
If you are indifferent, put one check in each column (✓| ✓).
Some of the activities require both hands. In these cases, the part of the task or object for which hand preference is wanted is indicated in parentheses.

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

Appendix 9. Macro A

```
Sub Calibrate

Call Doc.SetGuidelinesInfo (0, 1, False, False, 4521478)

for i = 5 to 1 step -1
		Call Doc.SetDataPadValue(1, 1, 26, "Give a Maximal contraction in " & i &
"secs")
		Call WaitFor (0, 0, 1)
	next
	Call Doc.StartSampling (10, False, kSMUserStop)
	for i = 5 to 1 step -1
		Call Doc.SetDataPadValue(1, 1, 26, "SQEEEEZE for " & i & "secs")
		Call WaitFor (0, 0, 1)
	next
	Call Doc.StopSampling ()

	' Begin SetSelection
	Set selobj = CreateObject("ADIChart.Selection")
	Call selobj.SetSelectionRange (15, 5850, 15, 5850)
	Call selobj.SetChannelRange (0, 1, -1)
	Doc.SelectionObject = selobj
	' End SetSelection


	' Begin Find
	ChannelIndex = kCurrentChannel
	SetAction = kSetActivePoint
	SelectMode = kSelectAround
	SelectTime = 1
	DataDisplayMode = kViewDataVisible
	SelectAll = False
	Direction = kSearchForward
	FindType = "End of file"
	FindData = ""
	Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime, DataDisplayMode,
SelectAll, Direction, FindType, FindData)
	' End Find

	' Begin Find
	ChannelIndex = kCurrentChannel
	SetAction = kSetToPreviousPoint
	SelectMode = kSelectAround
	SelectTime = 1
	DataDisplayMode = kViewDataVisible
	SelectAll = False
	Direction = kSearchForward
	FindType = "Move backward"
	FindData = "AmountToMove=3;"
	Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime, DataDisplayMode,
SelectAll, Direction, FindType, FindData)
	' End Find

	Call WaitFor (0, 0, 1)

	maxforce = Doc.GetDataPadValue (1, 1, 25)

	msgbox("To complete calibration do the following:" & Chr(13) & "1. Close this
dialogue box." & Chr(13) & "2. Open Units Conversion Dialogue (In the channel drop
down)." & Chr(13) & "3. Ensure the unit dropdown is set to %." & Chr(13) & "4. Enter 0V
=> 0% in the top 2 boxes." & Chr(13) & "5. Select the region of maximal contraction in
the data visible." & Chr(13) & "6. Click the arrow icon next to point 2." & Chr(13) & "7.
Put 100 in the box to the right." & Chr(13) & "8. Click OK to close the unit conversion
dialogue box." & Chr(13) & "9. When you are done run the Grip Protocol macro.")

	Call Doc.SetDataPadValue(1, 1, 26, "When you have finished with calibration run
GripProtocol macro")

End Sub
```

Grip protocol

```
'%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
'Change Parameters in Here

'Target Parameters
baseline = 5 'Enter baseline % to return to after each contraction
undershoot = 7 'Enter amount of room to leave for possible undershoot (%)

'Parameters about each trial
notargets = 7 'Enter number of trails you wish to run
orderedtargets = Array(10,35,20,40,25,15,30) 'List of targets in order, length must be
the same as notargets

'Paramaters about reapeating each of the above trials
notrials = 8 ' How many times to repeat the above mentioned trials
trialtypes = array(1,1,1,1,1,1,1,1) 'Which trials to randomise, 1 means randomise, zero
means ordered) length must be the same as notrials

'%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

totaltrials = notargets*notrials
dim mastertargets(200) 'Must be longer than notargets times notrials

z=0
for x = 0 to notrials - 1
       if trialtypes(x) = 1 then
              temptargets = orderedtargets
              call ShuffleArray(temptargets)
       else
              temptargets = orderedtargets
       end if

       for y = 0 to notargets - 1
              mastertargets(z) = temptargets(y)
              z = z + 1
       next
next



'First, Clear all current guidelines.
Call Doc.SetGuidelinesInfo (0, 1, False, False, 4521478)

'Run a countdown
call runcountdown(trialtypes(0))


'initialise counter
counter = 0
onwayup = 1
fahime=0
' Go to end of current block before starting
Call Doc.Find (kCurrentChannel, kSetActivePoint, kSelectAround, 1, kViewDataVisible,
False, kSearchForward, "End of this block", "")

'Set First Guideline
target = mastertargets(0)
Call ChangeGuideline(target, 1)


'Event Handler Code
Call Script.RegisterScriptEvent (3, "ch1", "OnGuidelineCrossed_ch1")

'This runs every time the guideline is crossed
Sub OnGuidelineCrossed_ch1 (ChannelNumber, GuidelineNumber, IsRising, Position,
GuidelineValue, SignalValue)
```

```
if GuidelineNumber = 2 then
	if counter < totaltrials then
		if IsRising="True" and onwayup = 1 then
			onwayup = 0
			counter = counter + 1
			fahime=fahime+1

			if fahime=7 then
			fahime=0
			Call Doc.SetDataPadValue(1, 1, 26, "-------------")
			Call WaitFor (0, 0, 0)

			end if

			Call Doc.AddCommentAtEnd (0, "Reached " & target & "% force")
			call ChangeGuideline(baseline, 2)
		end if

		if IsRising = "False" and onwayup = 0 then
			onwayup = 1
			target = mastertargets(counter)
			Call Doc.AddCommentAtEnd (0, "Returned to Baseline (" & baseline &
"%)")
			if totaltrials mod notargets = 0 then
				Call Doc.AddCommentAtEnd (0, "Started Next Trial)")
			end if

			call ChangeGuideline(target, 1)
		end if
	else
		Call Doc.AddCommentAtEnd (0, "Returned to Baseline (" & baseline & "%)")
		Call Doc.StopSampling ()
		Call Doc.SetGuidelinesInfo (0, 1, False, False, 4521478)
		Call Doc.SetDataPadValue(1, 1, 26, "Stopped, run a macro to start")
		Call Services.StopMacroExecution

	end if
end if

End Sub

function runcountdown (israndom)
	'Stop Sampling
	Call Doc.StopSampling ()
	'Run a countdown
	for i = 5 to 1 step -1
		Call Doc.SetDataPadValue(1, 1, 26, "Get ready to start next trial in " & i
& "sec")
		Call WaitFor (0, 0, 1)
	next
	Call Doc.StartSampling (10, False, kSMUserStop)
end function


function ChangeGuideline (percentage, upordown)

	' Turn On Guidelines
	Call Doc.SetGuidelinesInfo (0, 1, True, True, 4521478)
	Call Doc.SetGuidelinesInfo (0, 2, True, True, 12500670)

	if upordown = 1 then
		Call Doc.SetGuidelineValue (0, 2, percentage - undershoot, "%", "")
		Call Doc.SetGuidelineRegionInfo (0, True, 11140787, False, 12975793, False,
12500670)
		Call Doc.SetGuidelineValue (0, 1, percentage, "%", "")
		Call Doc.SetDataPadValue(1, 1, 26, "Reach " & percentage & "% force ASAP")
	else
		Call Doc.SetGuidelineValue (0, 2, percentage - 1, "%", "")
		Call WaitFor (0, 0, 1)
```

```
             Call Doc.SetGuidelineRegionInfo (0, False, 11140787, False, 12975793, True,
11140787)
              Call Doc.SetGuidelineValue (0, 1, percentage, "%", "")
              Call Doc.SetDataPadValue(1, 1, 26, "Release Grip")
       end if

       Call WaitFor (hours, minutes, seconds)
end function


Function ShuffleArray(MyArray())
    Dim I, J, Temp

    Randomize
    For I = LBound(MyArray) To UBound(MyArray)
        J = CLng(((UBound(MyArray) - I) * Rnd) + I)
        If I <> J Then
            Temp = MyArray(I)
            MyArray(I) = MyArray(J)
            MyArray(J) = Temp
        End If
    Next
End Function




Sub Analyse ()

       ' Begin Find
       ChannelIndex = kCurrentChannel
       SetAction = kSetActivePoint
       SelectMode = kSelectAround
       SelectTime = 1
       DataDisplayMode = kViewDataVisible
       SelectAll = False
       Direction = kSearchForward
       FindType = "Start of this block"
       FindData = ""
       Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime, DataDisplayMode,
SelectAll, Direction, FindType, FindData)
       ' End Find

       ' Begin Find
       ChannelIndex = kCurrentChannel
       SetAction = kSetToPreviousPoint
       SelectMode = kSelectAround
       SelectTime = 1
       DataDisplayMode = kViewDataVisible
       SelectAll = False
       Direction = kSearchForward
       FindType = "Search for comment"
       FindData = "JustThisChannel=0;WhatToLookFor=Returned;"
       Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime, DataDisplayMode,
SelectAll, Direction, FindType, FindData)
       ' End Find

       ' Begin Find
       ChannelIndex = kCurrentChannel
       SetAction = kSetEndOfSelection
       SelectMode = kSelectAround
       SelectTime = 1
       DataDisplayMode = kViewDataVisible
       SelectAll = False
       Direction = kSearchForward
       FindType = "Move backward"
       FindData = "AmountToMove=0.1;"
       Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime, DataDisplayMode,
SelectAll, Direction, FindType, FindData)
```

```
        ' End Find

        Call Doc.AddToDataPad ()
        startRec = Doc.SelectionStartRecord

        Do While (IsCurrentSelectionWithin (startRec, 0, startRec, kRecordEndOffset) =
True)

                ' Begin Find
                ChannelIndex = kCurrentChannel
                SetAction = kSetActivePoint
                SelectMode = kSelectAround
                SelectTime = 1
                DataDisplayMode = kViewDataVisible
                SelectAll = False
                Direction = kSearchForward
                FindType = "Search for comment"
                FindData = "JustThisChannel=0;WhatToLookFor=Returned;"
                Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime,
DataDisplayMode, SelectAll, Direction, FindType, FindData)
                ' End Find




                ' The function below will return true if the last operation failed, which
will cause the current loop to exit
                If (Services.ShouldExitCurrentRepeat()) Then Exit Do
                If Not (IsCurrentSelectionWithin (startRec, 0, startRec, kRecordEndOffset)
= True) Then Exit Do


                ' Begin Find
                ChannelIndex = kCurrentChannel
                SetAction = kSetEndOfSelection
                SelectMode = kSelectAround
                SelectTime = 1
                DataDisplayMode = kViewDataVisible
                SelectAll = False
                Direction = kSearchForward
                FindType = "Search for comment"
                FindData = "JustThisChannel=0;WhatToLookFor=Returned;"
                Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime,
DataDisplayMode, SelectAll, Direction, FindType, FindData)
                ' End Find



                ' The function below will return true if the last operation failed, which
will cause the current loop to exit
                If (Services.ShouldExitCurrentRepeat()) Then Exit Do
                If Not (IsCurrentSelectionWithin (startRec, 0, startRec, kRecordEndOffset)
= True) Then Exit Do


                ' Begin Find
                ChannelIndex = kCurrentChannel
                SetAction = kSetEndOfSelection
                SelectMode = kSelectAround
                SelectTime = 1
                DataDisplayMode = kViewDataVisible
                SelectAll = False
                Direction = kSearchForward
                FindType = "Move backward"
                FindData = "AmountToMove=0.1;"
                Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime,
DataDisplayMode, SelectAll, Direction, FindType, FindData)
                ' End Find
```

```
              Call Doc.AddToDataPad ()
              If Not (IsCurrentSelectionWithin (startRec, 0, startRec, kRecordEndOffset)
= True) Then Exit Do

       Loop

       If Not (startRec = kEndRecordIndex) Then
              Call Doc.SetSelectionRange (startRec, 2147483647, startRec, 2147483647)
       End If
       Call Doc.SetRightXPos (1016388, "Chart View")

End Sub
```

Appendix 10. Macro B

```
'%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
'Change Parameters in Here

'Target Parameters
baseline = 5 'Enter baseline % to return to after each contraction
undershoot = 7 'Enter amount of room to leave for possible undershoot (%)

'Parameters about each trial
notargets = 7 'Enter number of trails you wish to run
orderedtargets = Array(10,35,20,40,25,15,30) 'List of targets in order, length must be
the same as notargets

'Paramaters about reapeating each of the above trials
notrials = 8 ' How many times to repeat the above mentioned trials
trialtypes = array(0,0,0,0,0,0,0,0) 'Which trials to randomise, 1 means randomise, zero
means ordered) length must be the same as notrials

'%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

totaltrials = notargets*notrials
dim mastertargets(200) 'Must be longer than notargets times notrials

z=0
for x = 0 to notrials - 1
	if trialtypes(x) = 1 then
		temptargets = orderedtargets
		call ShuffleArray(temptargets)
	else
		temptargets = orderedtargets
	end if

	for y = 0 to notargets - 1
		mastertargets(z) = temptargets(y)
		z = z + 1
	next
next



'First, Clear all current guidelines.
Call Doc.SetGuidelinesInfo (0, 1, False, False, 4521478)

'Run a countdown
call runcountdown(trialtypes(0))


'initialise counter
counter = 0
onwayup = 1
fahime=0
' Go to end of current block before starting
Call Doc.Find (kCurrentChannel, kSetActivePoint, kSelectAround, 1, kViewDataVisible,
False, kSearchForward, "End of this block", "")

'Set First Guideline
target = mastertargets(0)
Call ChangeGuideline(target, 1)


'Event Handler Code
Call Script.RegisterScriptEvent (3, "ch1", "OnGuidelineCrossed_ch1")

'This runs every time the guideline is crossed
Sub OnGuidelineCrossed_ch1 (ChannelNumber, GuidelineNumber, IsRising, Position,
GuidelineValue, SignalValue)
```

```
if GuidelineNumber = 2 then
	if counter < totaltrials then
		if IsRising="True" and onwayup = 1 then
			onwayup = 0
			counter = counter + 1
			fahime=fahime+1

			if fahime=7 then
			fahime=0
			Call Doc.SetDataPadValue(1, 1, 26, "-------------")
			Call WaitFor (0, 0, 0)

			end if

			Call Doc.AddCommentAtEnd (0, "Reached " & target & "% force")
			call ChangeGuideline(baseline, 2)
		end if

		if IsRising = "False" and onwayup = 0 then
			onwayup = 1
			target = mastertargets(counter)
			Call Doc.AddCommentAtEnd (0, "Returned to Baseline (" & baseline &
"%)")
			if totaltrials mod notargets = 0 then
				Call Doc.AddCommentAtEnd (0, "Started Next Trial)")
			end if

			call ChangeGuideline(target, 1)
		end if
	else
		Call Doc.AddCommentAtEnd (0, "Returned to Baseline (" & baseline & "%)")
		Call Doc.StopSampling ()
		Call Doc.SetGuidelinesInfo (0, 1, False, False, 4521478)
		Call Doc.SetDataPadValue(1, 1, 26, "Stopped, run a macro to start")
		Call Services.StopMacroExecution

	end if
end if

End Sub

function runcountdown (israndom)
	'Stop Sampling
	Call Doc.StopSampling ()
	'Run a countdown
	for i = 5 to 1 step -1
		Call Doc.SetDataPadValue(1, 1, 26, "Get ready to start next trial in " & i
& "sec")
		Call WaitFor (0, 0, 1)
	next
	Call Doc.StartSampling (10, False, kSMUserStop)
end function


function ChangeGuideline (percentage, upordown)

	' Turn On Guidelines
	Call Doc.SetGuidelinesInfo (0, 1, True, True, 4521478)
	Call Doc.SetGuidelinesInfo (0, 2, True, True, 12500670)

	if upordown = 1 then
		Call Doc.SetGuidelineValue (0, 2, percentage - undershoot, "%", "")
		Call Doc.SetGuidelineRegionInfo (0, True, 11140787, False, 12975793, False,
12500670)
		Call Doc.SetGuidelineValue (0, 1, percentage, "%", "")
		Call Doc.SetDataPadValue(1, 1, 26, "Reach " & percentage & "% force ASAP")
	else
		Call Doc.SetGuidelineValue (0, 2, percentage - 1, "%", "")
		Call WaitFor (0, 0, 1)
		Call Doc.SetGuidelineRegionInfo (0, False, 11140787, False, 12975793, True,
11140787)
```

```
              Call Doc.SetGuidelineValue (0, 1, percentage, "%", "")
              Call Doc.SetDataPadValue(1, 1, 26, "Release Grip")
       end if

       Call WaitFor (hours, minutes, seconds)
end function


Function ShuffleArray(MyArray())
    Dim I, J, Temp

    Randomize
    For I = LBound(MyArray) To UBound(MyArray)
        J = CLng(((UBound(MyArray) - I) * Rnd) + I)
        If I <> J Then
            Temp = MyArray(I)
            MyArray(I) = MyArray(J)
            MyArray(J) = Temp
        End If
    Next
End Function







Sub Analyse ()

       ' Begin Find
       ChannelIndex = kCurrentChannel
       SetAction = kSetActivePoint
       SelectMode = kSelectAround
       SelectTime = 1
       DataDisplayMode = kViewDataVisible
       SelectAll = False
       Direction = kSearchForward
       FindType = "Start of this block"
       FindData = ""
       Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime, DataDisplayMode,
SelectAll, Direction, FindType, FindData)
       ' End Find

       ' Begin Find
       ChannelIndex = kCurrentChannel
       SetAction = kSetActivePoint
       SelectMode = kSelectAround
       SelectTime = 1
       DataDisplayMode = kViewDataVisible
       SelectAll = False
       Direction = kSearchForward
       FindType = "Move forward"
       FindData = "AmountToMove=0.3;"
       Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime, DataDisplayMode,
SelectAll, Direction, FindType, FindData)
       ' End Find


       ' Begin Find
       ChannelIndex = kCurrentChannel
       SetAction = kSetToPreviousPoint
       SelectMode = kSelectAround
       SelectTime = 1
       DataDisplayMode = kViewDataVisible
       SelectAll = False
       Direction = kSearchForward
       FindType = "Search for comment"
       FindData = "JustThisChannel=0;WhatToLookFor=Returned;"
```

```
	Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime, DataDisplayMode,
SelectAll, Direction, FindType, FindData)
	' End Find

	' Begin Find
	ChannelIndex = kCurrentChannel
	SetAction = kSetEndOfSelection
	SelectMode = kSelectAround
	SelectTime = 1
	DataDisplayMode = kViewDataVisible
	SelectAll = False
	Direction = kSearchForward
	FindType = "Move backward"
	FindData = "AmountToMove=0.1;"
	Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime, DataDisplayMode,
SelectAll, Direction, FindType, FindData)
	' End Find

	Call Doc.AddToDataPad ()
	startRec = Doc.SelectionStartRecord

	Do While (IsCurrentSelectionWithin (startRec, 0, startRec, kRecordEndOffset) =
True)

		' Begin Find
		ChannelIndex = kCurrentChannel
		SetAction = kSetActivePoint
		SelectMode = kSelectAround
		SelectTime = 1
		DataDisplayMode = kViewDataVisible
		SelectAll = False
		Direction = kSearchForward
		FindType = "Search for comment"
		FindData = "JustThisChannel=0;WhatToLookFor=Returned;"
		Call Doc.Find (ChannelIndex, SetAction, SelectMode, SelectTime,
DataDisplayMode, SelectAll, Direction, FindType, FindData)
		' End Find
```

Appendix 11. Macro C

```
%%
clc
clear

%% telorance
tel=5;
z=7;
x=8;
v=z*x;
pau=0;
%%
% _n _

name='mooly result2';
outputname='result';
columntime='f';
columntimeCC='c';
columnerror='a';
columnerrorBB='b';
columnee='e';
columndd='d';
CT=[];
GT=[];
%%
%A=xlsread(name,'f1:f336');   %only for time
A=xlsread(name,[columntime  ':'  columntime]);   %only for time
C=xlsread(name,[columntimeCC  ':'  columntimeCC]);   %only for time

B=xlsread(name,[columnerror ':'  columnerror]);

BE=xlsread(name,[columnee   ':'  columnee]);
BD=xlsread(name,[columndd   ':'  columndd]);
%A
B=B(:,1);
%A=datestr(A,'HH:MM:SS');
A=datestr(A,'MM:SS.FFF');
AA=zeros(1,size(A,1));
for i=1:size(A,1)
    a=A(i,:);
    M=str2double(a(1,1:2));
    S=str2double(a(1,4:9));
    AA(i)=60*M+S;
end

FFF=AA(:);
BBB=xlsread(name,[columnerrorBB ':'  columnerrorBB]);
AAA=B;

DDD=BD;

EEE=BE;

C=datestr(C,'MM:SS.FFF');
CC=zeros(1,size(C,1));
for i=1:size(C,1)
    a=C(i,:);
```

```
    M=str2double(a(1,1:2));
    S=str2double(a(1,4:9));
    CC(i)=60*M+S;
end

CCC=CC(:);



rty=0;
for i=1:size(A,1)
   AA(i)=AA(i)-pau*rty;
    if rem(i,z)==0
        rty=rty+1;
    end

    if rem(i,z*x)==0
        rty=0;
    end
end




yui={'block' , 'number' , 'mean' ,' SD' ,'VV','meanVV','meanR_T'};
DE=[BD  BE];
a=length(A);
a=a/v;
oioi=1;
for j=1:a

BE1=BE((j-1)*v+1:j*v);

for i=1:z
    oioi=oioi+1;
    BE2=BE1(i  :z:end)    ;
    %plot(1:x,BE2');

    %plot(1:x,BD(i)*ones(1,x))


     wwww=sqrt(sum(( BE2-BD(i)).^2)/length(BE2));

     wwwwww=  sum(abs(BE2-BD(i)))/length(BE2);

    fprintf(['block ' num2str(j)  ', number ' num2str(BD(i)) ' mean is '
num2str(mean(BE2)) ' and standard is ' num2str(std(BE2))  ' and VV = '
num2str(wwww)    ' and meanVV = ' num2str(wwwwww)    '\n' ]  )

    yui{oioi,1}=j;
    yui{oioi,2}=BD(i);
   yui{oioi,3}=mean(BE2);
    yui{oioi,4}=std(BE2);
    yui{oioi,5}=wwww;
    yui{oioi,6}=wwwwww;
```

```
end
fprintf('\n')
end
```

```
A=AA;
YY=AA';
%%
a=length(A);
a=a/v;
C=cell(1,a);
A=A(:);
B=B(:);
for i=1:a
    C{1,i}=[B(1:v,1) A(1:v)];
    B(1:v)=[];
    A(1:v)=[];
end
```

```
%%
for i=1:a
   D=C{1,i};

    t=zeros(1,x);
    t(1)=D(z,2);
    for j=2:x
    t(j)=D(z*j,2)-D(z*(j-1),2);
    end

    c=zeros(1,x);
    for j=1:x

      y1=  sum( D((j-1)*z+1 : j*z  ,1) >tel);
      y2= sum(  D((j-1)*z+1 : j*z  ,1) <-tel);
      if y1+y2<=0
         c(j)=1;
      end

    s=['block ' num2str(i) ' under shoot = ' num2str(y2) ' and over shoot =
' num2str(y1) ];

    CCT=[i y2 y1];
    CT=[CT;CCT ];

    disp(s);
```

```
    end

    erorrate=(x-sum(c))/x;

    movementtime=mean(t);
    %movementtime=sum((~c).*t);

    E=erorrate;
    T=movementtime;

    %s=['block ' num2str(i) ' under shoot = ' num2str(y2) ' and over shoot
= ' num2str(y1) ];

    %disp(s);
    skill=(1-E)/(E*5.424*log(T));
    %skill=(1-E)/( log(T+1))^(.3849);
    GT=[GT;T    E  E*x  skill ];


    s=['block ' num2str(i) ' skill = ' num2str(skill) ' and Number of Error
= ' num2str(E*x)   ' and Error rate = ' num2str(E) ' and movement time = '
num2str(T) ' secound' ];
    fprintf('\n')
    disp(s)
    fprintf('\n##############################################\n\n')

end


%clc
%clear

d = {'Block','under shoot' ,'overshoot' ,'movement time'  ,'error
rtae' ,'Number of error rate'  , 'skill'};

[m n]=size(CT);
o=1;
for i=1:m
   for j=1:n
      d{i+1,j}=CT(i,j);
   end
   if rem(i,x)==0
      d{i+1,4}=GT(o,1);
      d{i+1,5}=GT(o,2);
      d{i+1,6}=GT(o,3);
      d{i+1,7}=GT(o,4);
      o=o+1;
   end
end


%d={'s','d';[1 12 12121]',[1 1223 121]'}
xlswrite('result.xls', d, 1, 'A1')
xlswrite('result.xls', yui, 2, 'A1')


z=yui(2:end,2);
```

```
z=cell2mat(z);

[q w]=sort(z);
for i=1:6
    r=yui(2:end,i);
    r=r(w);
   yui(2:end,i)=r;
end




%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%



a=length(AA);
a=a/v;


A=[AAA BBB CCC DDD EEE FFF];

y={'block' ,'trial', 'center error',' reaction time','time',
'number'  ,'5','6', 'mean' ,' SD' ,'VV','meanVV','mean Reaction time'};

o=1;
u=1;
for j=1:a
    p=1;
    B=A( (j-1)*v+1:j*v,: );
    a=B(:,4);
    [q w]=sort(a);
    C=B(w,:);
    for i=1:v
        o=o+1;
        y{o,1}=j;
        y{o,2}=p;
        p=p+1;
        if rem(p,9)==0
            p=1;

            y{o,9} = yui{u+1,3};
            y{o,10}=yui{u+1,4};
            y{o,11}=yui{u+1,5};
            y{o,12}=yui{u+1,6};
            y{o,13}=mean(C(  (i-x+1) :i     ,2));

            yui{u+1,7}=y{o,13};

            u=u+1;

        end
        y{o,3}=C(i,1);
        y{o,4}=C(i,2);
        y{o,5}=C(i,3);
        y{o,6}=C(i,4);
```

```
        y{o,7}=C(i,5);
        y{o,8}=C(i,6);

    end
end


xlswrite('result.xls', yui, 3, 'A1')


xlswrite('result.xls', y, 4, 'A1')
```

Appendix 12. Macro D

```
%%
clc
clear

%% telorance
tel=5;
z=7;
x=8;
v=z*x;

name='mooly'; %esme failet ro inja benevis
filename='result2.xls';

c=xlsread(name,'a1:f1232','sheet1');%ba tavajoh be tedade satrhaye file
excle adad 56 avaz mishavad
[m,n]=size(c);
CT=ones(m,n);




for i=1:7:m

    for j=i:(i+6)

       if c(j,4)==10
        CT(i,:)=c(j,:);
       end

       if c(j,4)==15
        CT(i+1,:)=c(j,:);
       end

       if c(j,4)==20
        CT(i+2,:)=c(j,:);
       end

       if c(j,4)==25
        CT(i+3,:)=c(j,:);
       end

       if c(j,4)==30
        CT(i+4,:)=c(j,:);
       end

       if c(j,4)==35
        CT(i+5,:)=c(j,:);
       end

       if c(j,4)==40
        CT(i+6,:)=c(j,:);
       end

    end
```

```
end
xlswrite('result2.xls',CT,'sheet1')
%% vv &center error & R-T

columnbb='b';

columnee='e';
columndd='d';
BE=xlsread(filename,[columnee   ':'  columnee]);
BD=xlsread(filename,[columndd   ':'  columndd]);
BB=xlsread(filename,[columnbb   ':'  columnbb]);
DE=[BD  BE];

for k=1:z:m

     BE2=BE(k:k+6);
        BD2=BD(k:k+6);

      CT(k+6,7)=sqrt(sum(( BE2 -BD2).^2)/z);%soton 7 varians

       CT(k+6,8)=  sum(abs (BE2-BD2))/z ;  %soton 8 mean vv


end
for k=1:z:m

     BB2=BB(k:k+6);


      CT(k+6,9)=(sum(BB2)/z);   %soton 9 R_T


end


xlswrite('result2.xls',CT,'sheet2')
%% ave vv & ave center error & ave R-T har belak
columngg ='g';
columnhh ='h';
columnii ='i';
BG = xlsread(filename,'sheet2',[columngg   ':'  columngg]);
BH = xlsread(filename,'sheet2',[columnhh   ':'  columnhh]);
BI = xlsread(filename,'sheet2',[columnii   ':'  columnii]);

for k=1:v:m

    BG2 = BG(k:k+55);


   CT(k+55,10)=(sum(BG2))/x ; %soton 10 mean varianshaye har belak


end
for k=1:v:m
```

```
    BH2 = BH(k:k+55);


   CT(k+55,11)=(sum(BH2))/x ; %soton11 mean center error har belak


end
for k=1:v:m

    BI2 = BI(k:k+55);


   CT(k+55,12)=(sum(BI2))/x ; %soton 12 meanR-T har belak


end

Z=zeros(m,2);
CT=[Z CT];

xlswrite('result2.xls',CT,'sheet2')
%% block & target
columnaa ='a';
columnbb ='b';
BA = xlsread(filename,'sheet2',[columnaa    ':'  columnaa]);
BB = xlsread(filename,'sheet2',[columnbb    ':'  columnbb]);

i=1;
o=1;
for i=i:v:m
     BA2 = BA(i:i+55);

        BA2(:,1)=o;


    CT(i:i+55,1)=BA2(:,1);
    o=o+1;

end
j=1;
 for j=j:z:m
     BB2 = BB(j:j+6);
     QQ=[1; 2 ;3; 4; 5; 6; 7];

        BB2(:,1)=QQ;


    CT(j:j+6,2)=BB2(:,1);


end

xlswrite('result2.xls',CT,'sheet2')

%% sheet3 average
columnaaa ='a';
```

```
columnlll ='l';
columnmmm ='m';
columnnnn ='n';
BA3 = xlsread(filename,'sheet2',[columnaaa   ':'  columnaaa]);
BL3 = xlsread(filename,'sheet2',[columnlll   ':'  columnlll]);
BM3 = xlsread(filename,'sheet2',[columnmmm   ':'  columnmmm]);
BN3 = xlsread(filename,'sheet2',[columnnnn   ':'  columnnnn]);

ALMN=[BA3 BL3 BM3 BN3];
[m,u]=size(ALMN);
p=length(ALMN);
p=p/v;
CY=ones(p,u);
j=1;
k=1;
for k=k:v:m

    CY(j,:)= ALMN(k+55,:);

    j=j+1;


end


CT=CY;


xlswrite('result2.xls',CT ,'sheet3')
```

Appendix 13. Explanatory statement

MONASH University
Medicine, Nursing and Health Sciences

Explanatory Statement

Dear Participant

Does transcranial direct current stimulation (tDCS) of movement related functional areas of the brain affect fast stage of motor skill learning?

Date:
This information sheet is for you to keep.

Student Research Project

My name is Fahimeh Hashemirad 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 areas of brain may affect fast stage of motor skill learning.

Possible benefits

There are no direct benefits for the participants from this study. We hope the study will benefit society by helping us to establish the best way for application of tDCS as a non-invasive brain modulation technique to improve motor skill learning.

What does the research involve?

A TMS Safety Screening questionnaire will be completed before taking part in the study. Then each participant will be tested before and after application of tDCS.

To explore the site-specific effects of single session of tDCS on the fast stage motor skill learning, we will apply tDCS over three different parts of your brain which may involve in initial stage of motor skill learning. To assess the effectiveness of tDCS, muscle responses induced by magnetic stimulation (TMS) of one part of the brain which controls hand movements during SVIPT task will be used for assessment of the changes before and after interventions. 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 hand 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 70 to 90 minutes and involves five sessions of data collection.

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. Fahimeh Hashemirad, Physiotherapist, PhD Candidate, Physiotherapy Department, School of Primary Health Care, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne – Peninsula Campus
T : 0415660921

or email: fahimeh.hashemirad@monash.edu

2. Dr Shapour Jaberzadeh, Senior Lecturer Physiotherapy, School of Primary Health Care, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne - Peninsula Campus
T: 9904 4827

F: 9904 4812 or email: shapour.jaberzadeh@monash.edu

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
Tel: +61 3 9905 2052 Fax: +61 3 9905 3831
Email: muhrec@adm.monash.edu.au

Thank you.

Appendix 14. TDCS questionnaire

MONASH University
Medicine, Nursing and Health Sciences

Name Date Group

		Numbness (1-10)	Itching (1-10)	Burning (1-10)	Pain (1-10)	Fatigue (1-10)	Nervousness (1-10)	Headache (1-10)	Other (1-10)
Active electrode (Overhead) 	1-2 min								
	9-10 min								
	14-15 min								
	19-20 min								

		Numbness (1-10)	Itching (1-10)	Burning (1-10)	Pain (1-10)	Fatigue (1-10)	Nervousness (1-10)	Headache (1-10)	Other (1-10)
Return electrode (Over forehead) 	1-2 min								
	9-10 min								
	14-15 min								
	19-20 min								

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

Appendix 15. Sample size calculation

Power analysis for the analysis of variance

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

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

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

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

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

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

Sample size needed for the ANOVA for α = 0.05

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