Transcranial Direct-Current Stimulation and Functional Training: A Novel Neurorehabilitation Technique

By

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Transcranial Direct-Current Stimulation and Functional Training: A Novel Neurorehabilitation Technique

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List of Abbreviations

AMT: active motor threshold

a-tDCS: anodal transcranial direct-current stimulation

BDNF: brain derived neurotrophic factor

CNS: central nervous system

c-tDCS: cathodal transcranial direct-current stimulation

D-wave: direct wave

ECR: extensor carpi radialis

fMRI: functional magnetic resonance imaging

GABA: gamma-aminobutyric acid

ICF: intracortical facilitation

IHI: interhemispheric inhibition

ISI: inter-stimulus interval

I-wave: indirect wave

LI: laterality index

LICI: long-interval intracortical inhibition

LTP: long-term potentiation

M1: primary motor cortex
MAS: Motor Assessment Scale

MEP: motor evoked potential

MMSE: Mini-Mental State Examination

MT: motor threshold

MVIC: maximal voluntary isometric contraction

M-wave: maximal compound wave

NIBS: non-invasive brain stimulation

NMDA: $N$-methyl-D-aspartate

$rms_{EMG}$: root mean square electromyography

RMT: resting motor threshold

ROM: range of motion

$rTMS$: repetitive transcranial magnetic stimulation

sEMG: surface electromyography

SICI: short-interval intracortical inhibition

tDCS: transcranial direct-current stimulation

TES: transcranial electrical stimulation

TMS: transcranial magnetic stimulation

VAS: visual analogue scale
ABSTRACT

The process of natural ageing accompanies degeneration of neuronal tissue more prominently in the non-dominant primary motor cortex (M1), which may lead to a functional decline in motor skills. Comparably, the incident of a stroke causes neuronal death which disrupts the output from the M1, resulting in impaired motor function. Repetitive motor training promotes use-dependent plasticity and performance gains in a healthy population, however these responses may be reduced in older adults and chronic stroke patients. Transcranial direct-current stimulation (tDCS) is a non-invasive brain stimulation (NIBS) technique that modulates corticospinal plasticity and improves motor performance through mechanisms analogous to motor learning. The overarching aim of this thesis was to determine the neurophysiological and behavioural effects of tDCS as an adjunct to motor training and rehabilitation in older adults and chronic stroke patients.

Study one (chapter three) investigated the benefits of a single session of anodal-tDCS (a-tDCS) or sham-tDCS during unilateral training, on the cross-limb transfer of motor skills in older compared with younger adults. Unilateral visuomotor tracking (VT) training was performed whilst a-tDCS was applied to the M1 ipsilateral to the trained limb. Transcranial magnetic stimulation (TMS) was applied to the M1 corresponding to the trained and untrained extensor carpi radialis (ECR) muscle to elicit motor evoked potentials (MEPs) and short-interval intracortical inhibition (SICI). The trained limb exhibited improvements in VT error, facilitated motor evoked potentials (MEPs) and a release in short-interval intracortical inhibition (SICI) in both age groups regardless of the tDCS condition (all P < 0.05). In the untrained limb, VT error improved in young adults for both sham-tDCS (19.8%, P < 0.001) and a-tDCS (27.9%, P < 0.001)
P < 0.001) conditions, but only following a-tDCS in the older adults (18.9%, P < 0.001) with no change in the untrained limb for older adults receiving sham-tDCS (1.8%, P = 0.66). MEPs increased in all conditions (young a-tDCS, 34.2%, P = 0.003; young sham-tDCS, 27.6%, P = 0.01; old a-tDCS 27.3% P = 0.03), except the older adult’s receiving sham-tDCS (9.6%, P = 0.46). SICI was released in both conditions for young (sham-tDCS 24.1%, a-tDCS 22.2%) and older (sham-tDCS 13.7%, P = 0.01; a-tDCS 33.1%) adults (all P < 0.001). Overall, study one demonstrated that the addition of a-tDCS with unilateral training improved cross-limb transfer in older adults, to a similar magnitude as their younger counterparts.

Study two (chapter four) investigated whether alternate tDCS electrode montages potentiated any differences in indices of corticospinal plasticity or motor performance of the non-dominant limb in older adults immediately following and at 30 minutes post tDCS. This study involved a single session of unilateral a-tDCS, bilateral-tDCS and sham-tDCS combined with VT training of the non-dominant wrist. The findings showed that unilateral-tDCS and bilateral-tDCS improved VT error immediately post (unilateral-tDCS, 12.9%, P = 0.01; bilateral-tDCS 21.6%, P < 0.001) and at 30 minutes (unilateral-tDCS 11.9%, P = 0.01; bilateral-tDCS 21.7%, P < 0.001); with the sham-tDCS condition decreasing tracking error at 30 minutes only (10.0%, P = 0.02). In the non-dominant M1, MEPs were facilitated immediately post (unilateral-tDCS, 37.8%, P = 0.02; bilateral-tDCS 53.1%, P < 0.001) and at 30 minutes (unilateral-tDCS 49.0%, P = 0.01; bilateral-tDCS, 54.5%, P = 0.003) relative to the sham-tDCS condition. Similarly, SICI was released immediately post (unilateral-tDCS, 29.2%, P = 0.01; bilateral-tDCS, 36.3%, P = 0.003) and at 30 minutes (unilateral-tDCS, 21.2%, P = 0.03; bilateral-tDCS, 30.2%, P = 0.01) for both the unilateral-tDCS and bilateral-tDCS conditions relative to the sham-tDCS. Interestingly, no significant differences for any
dependant variable between unilateral-tDCS and bilateral-tDCS conditions were observed (all $P > 0.05$). These findings provide preliminary evidence that tDCS can improve the age-related reduction in use-dependent plasticity and motor function within the non-dominant limb, irrespective of the electrode montage.

Subsequently, study three (chapter five) applied bilateral-tDCS to a three week upper limb rehabilitation program in chronic stroke patients, with an additional three week follow-up (week six). Both real-tDCS and sham-tDCS groups improved Motor Assessment Scale (MAS) scores following the intervention (real-tDCS, 62.3%, sham-tDCS 42.7%, both $P < 0.001$), with no between-group differences. At week six, improvement on the MAS showed a significantly greater retention in the real-tDCS (64.0%) compared to the sham-tDCS group (20.9%, $P = 0.002$). For the ipsilesional M1, MEP amplitudes increased for the real-tDCS group at three (46.4%, $P = 0.001$) and six weeks (38.1%, $P = 0.03$), whereas no significant change in MEP amplitudes were observed for the sham-tDCS group at either time point. No changes in SICI were observed in the ipsilesional M1. In the contralesional M1, SPD increased by 32.8% following the intervention for the real-tDCS compared with the sham-tDCS group ($P = 0.04$), and these changes were maintained at follow-up (24.0%, $P = 0.04$). At six weeks, SICI increased by 27.1% only for the real-tDCS group compared with the sham-tDCS ($P = 0.04$). There were no significant changes in MEP amplitudes in the contralesional M1 for either group (all $P > 0.05$). Study three concluded that simultaneous bilateral-tDCS and upper limb rehabilitation improved indices of corticospinal plasticity and retention of motor function in chronic stroke patients.

Collectively, the findings support the potential for a-tDCS and bilateral-tDCS as an adjunctive therapy to motor training and rehabilitation. Importantly, both unilateral a-tDCS and bilateral-tDCS are equally as effective in modulating corticospinal plasticity...
and subsequently improving motor performance in older adults. The concurrent application of tDCS and motor training may promote corticospinal plasticity and improve motor function in an ageing population and in chronic stroke patients. However the clinical efficacy of tDCS is limited due to large variability in individual responses to stimulation protocols.
CHAPTER ONE: INTRODUCTION
The modifiable nature of the central nervous system (CNS) is termed ‘plasticity’, and can lead to functionally beneficial or in some cases, pathophysiological (maladaptive) alterations within the CNS (Joseph 2013). Such measures of plasticity within the corticospinal pathway (CSP) can be indexed by changes in corticospinal excitability and intracortical inhibition, and quantified through transcranial magnetic stimulation (TMS). The induction of corticospinal plasticity can be purposefully modulated following use-dependent protocols such as motor training (Butefisch et al. 2000), or experimentally-induced through the application of non-invasive brain stimulation (NIBS) (Ziemann et al. 2008).

Natural ageing is accompanied by progressive neurodegeneration within the CNS, which is often more prominent in the non-dominant hemisphere (Bonilha et al. 2009; Sale & Semmler 2005). Moreover, the incident of a stroke commonly results in reduced or loss of neuromuscular function on one side of the body. Although ageing and stroke differ in the degree of neuromuscular decline, unilateral degeneration and/or neurological injury has been suggested to generate asymmetries in corticospinal excitability and inhibition between the two primary motor cortices (M1s) (Farias da Guarda et al. 2010; Murase et al. 2004; Talelli et al. 2008). Consequently, significant interhemispheric imbalances may manifest as a maladaptive process and reduce motor function in the non-dominant and paretic upper limb in older adults and stroke patients respectively. A model of interhemispheric imbalance, towards improving neuromuscular function in the non-dominant and paretic limb in older adults and chronic stroke patients, forms the rationale for the studies within this thesis.

It has recently been suggested that age-related neurodegeneration may reduce the formation of use-dependent plasticity (Fujiyama et al. 2012a; Rogasch et al. 2009) and motor learning, particularly within the non-dominant limb (Hinder, Carroll &
Summers 2013). Moreover, reduced motor learning in older adults is evident following unilateral training (Hinder et al. 2011). In younger adults, unilateral training yields a cross-transfer of performance gains in the untrained limb, which appears to be less evident in the non-dominant limb for older adults (Hinder et al. 2011). The phenomena of cross-limb transfer is suggested to be mediated through the CNS, and therefore, the absence of cross-limb transfer in older adults is likely related to altered neurophysiological adaptation.

In a similar context, physical exercise is currently the benchmark for motor recovery following a stroke. However, stroke remains one of the leading causes of disability in developed countries (Mozaffarian et al. 2015), with approximately two thirds of survivors continuing to be dependent in activities of daily living (ADLs) (Kwakkel et al. 2003; Nakayama et al. 1994; Sturm et al. 2002). Although motor recovery following a stroke is multifaceted, it is suggested that interhemispheric competition between the contra- and ipsilesional M1s may interfere with regaining motor function (Murase et al. 2004). Therefore, there is a need to investigate techniques in which corticospinal plasticity can be purposefully modulated in older adults and stroke patients, in pursuance of improving motor function in the non-dominant and paretic limb respectively.

Transcranial direct-current stimulation (tDCS) is a non-invasive brain stimulation (NIBS) technique that is capable of modulating behavioural and neurophysiological activity (Nitsche & Paulus 2011). The effects of tDCS are polarity specific, with TMS studies reporting transient increases in corticospinal excitability following anodal-tDCS (a-tDCS) and decreases following cathodal-tDCS (c-tDCS), lasting for up to approximately 90 minutes (Fricke et al. 2011; Nitsche & Paulus 2000). In healthy individuals, the simultaneous application of a-tDCS and c-tDCS (i.e. bilateral-tDCS)
over both M1s has been reported to increase excitability in one hemisphere whilst suppressing it in the contralateral hemisphere (Mordillo-Mateos et al. 2012). Based on evidence from pharmacological interventions (Liebetanz et al. 2002; Nitsche et al. 2003a), tDCS appears to generate an ionic shift in the neuronal resting membrane potential, which is believed to potentiate synaptic efficacy through mechanisms involved in long-term potentiation (LTP). In addition, there is good evidence that these mechanisms may lead to facilitated motor learning in healthy adults (Antal et al. 2004; Nitsche et al. 2003b; Reis & Frisch 2011).

Given that LTP-like adaptations appear to be a common feature underpinning the after-effects of tDCS (Monte-Silva et al. 2013; Ranieri et al. 2012) and motor learning (Rioult-Pedotti, Friedman & Donoghue 2000), research has begun to investigate its application to improve motor performance and corticospinal plasticity in older adults (Fujiyama et al. 2014; Heise et al. 2014; Hummel et al. 2010; Parikh & Cole 2014; Zimerman et al. 2013). Two previous studies in older adults have shown enhanced skill acquisition following a single session of a-tDCS (Hummel et al. 2010; Zimerman et al. 2013). Moreover the concurrent application of a-tDCS with motor training in older adults has been demonstrated to retain the performance gains achieved through motor training (Parikh & Cole 2014). However, there is limited evidence for the underlying neurophysiological mechanisms by which a-tDCS may facilitate motor learning in older adults. One study demonstrated that older adults retain the ability to develop corticospinal plasticity following a-tDCS similar to magnitude reported in young adults (Fujiyama et al. 2014). However the neurophysiological responses to plasticity-inducing protocols in older adults may be delayed (Fujiyama et al. 2014) and are likely to be dependent on the integrity of the CSP (Heise et al. 2014). Given the interhemispheric asymmetries in corticospinal excitability and inhibition in older
adults (Davidson & Tremblay 2013; Fling et al. 2011), appropriate manipulation of the electrode montage may strengthen the induction of corticospinal plasticity in the non-dominant M1. One study comparing unilateral a-tDCS and bilateral-tDCS reported that bilateral-tDCS preferentially modulated transcallosal pathways in older adults (Lindenberg et al. 2013). However whether modulating these pathways with bilateral-tDCS is beneficial for motor function remains unknown. Importantly, the current evidence for tDCS in older adults has only examined the dominant limb and corresponding M1. Therefore, whether the effects are similar for the non-dominant M1 which may be subject to greater degeneration and loss of motor control, are unknown.

Accordingly, the overall aims of the first two experimental studies (chapters three and four) in this thesis were to examine techniques that would improve motor performance and indices of corticospinal plasticity within the non-dominant limb in older adults. Specifically, study one (chapter three) examined the application of a-tDCS over the ipsilateral M1 during unilateral training of the dominant limb and whether this was able to improve cross-limb transfer from the dominant to the non-dominant limb. As study one only investigated the efficacy of unilateral a-tDCS, study two (chapter four) used a theoretical model of interhemispheric imbalance to examine the immediate and time-course effects of unilateral a-tDCS and bilateral-tDCS on motor performance and corticospinal plasticity in the non-dominant limb of older adults. The findings from these studies informed the rationale for tDCS to be utilised in conditions with reduced neuromuscular function and interhemispheric asymmetries such as stroke (study three).

Given that chronic stroke may promote interhemispheric competition, there is a strong rationale for the application of bilateral-tDCS to improve functional recovery through restoring interhemispheric balance (Nowak et al. 2009; Takeuchi & Izumi 2012). The
benefits of simultaneous tDCS and physical rehabilitation are well supported (Page et al. 2015), whereby improvements in motor function following a combined intervention appear greater than physical therapy alone (Bolognini et al. 2011; Lefebvre et al. 2014; Lindenberg et al. 2010). Moreover, the application of tDCS during motor training rather than prior to training, appears to preferentially improve performance (Stagg et al. 2011). However, much of the previous evidence for the adjunctive use of tDCS and rehabilitation has been based on a single session or following five consecutive sessions of stimulation (Lefebvre et al. 2012; Lefebvre et al. 2014; Lindenberg et al. 2010). Only one previous study combined 10 sessions of constraint-induced movement therapy (CIMT) with bilateral-tDCS, demonstrating improvements in upper limb function for up to one month following the intervention (Bolognini et al. 2011). The improvements in upper limb function were attributed to a release of interhemispheric inhibition (IHI) onto the ipsilesional M1 and facilitated corticospinal excitability within the ipsilesional M1 (Bolognini et al. 2011). However, a limitation of this study was that these neurophysiological mechanisms were not quantified at follow-up. Therefore, study three (chapter five) investigated the neurophysiological and behavioural adaptations following three weeks of upper limb rehabilitation concurrent with bilateral-tDCS in chronic stroke patients. A novel aspect to study three was the quantification of neurophysiological changes underpinning the retention of gains in motor function. This study was conducted to provide preliminary evidence for the clinical efficacy of bilateral-tDCS as an adjunct to physical rehabilitation in the chronic phase of stroke.
1.1 Primary aim of this thesis

To quantify the neurophysiological and behavioural responses following concurrent motor training and transcranial direct-current stimulation (tDCS) in older adults and chronic stroke patients.

1.2 Specific aims of this thesis

1. To examine the effect of anodal-tDCS (a-tDCS) applied over the ipsilateral primary motor cortex (M1) during unilateral training, on the cross-transfer of motor performance to the non-dominant upper limb in older adults (study one).

2. To compare unilateral a-tDCS and bilateral-tDCS electrode montages on acute and time-course changes in corticospinal excitability, intracortical inhibition and motor function of the non-dominant upper limb in older adults (study two).

3. To establish the effects of bilateral-tDCS concurrent with upper limb rehabilitation on corticospinal excitability and inhibition of the ipsi- and contralesional M1 in chronic stroke patients, and any subsequent improvements in upper limb motor function (study three).

4. To establish any retention in upper limb motor function, corticospinal excitability and inhibition following three weeks of upper limb rehabilitation and bilateral-tDCS in chronic stroke patients (study three).
1.3 Primary hypothesis

It was hypothesised that the addition of tDCS would modulate indices of corticospinal plasticity (corticospinal excitability and inhibition), and that these physiological changes would lead to improvements in motor function in the non-dominant and paretic upper limb in older adults and chronic stroke patients.

1.4 Specific hypotheses

1. The application of a-tDCS to the ipsilateral M1 during unilateral training would increase corticospinal excitability and reduce intracortical inhibition in the ipsilateral M1, which would improve the cross-transfer of motor skills to the untrained upper limb in older adults (study one).

2. The application of bilateral-tDCS would modulate corticospinal excitability and intracortical inhibition in both M1s, leading to greater improvements in motor function immediately after and 30 minutes following stimulation, compared to unilateral a-tDCS and sham-tDCS conditions (study two).

3. Bilateral-tDCS concurrent with upper limb rehabilitation would modulate corticospinal excitability, inhibition and improve motor function in the paretic upper limb in chronic stroke patients, when compared to a sham-tDCS (rehabilitation alone) group (study three).

4. Bilateral-tDCS would retain changes in corticospinal excitability, inhibition and subsequent improvements in upper limb function in chronic stroke patients, compared to the sham-tDCS group (study three).
CHAPTER TWO: REVIEW OF THE LITERATURE
2.1 Overview

The efficient movement produced by the upper limb is fundamental for carrying out activities of daily living (ADLs). Tasks such as eating, writing and general self-care can be compromised as a result of neurodegeneration associated with normal ageing or neurological injury, such as a stroke. This chapter will provide a comprehensive review of the literature regarding specific techniques that modulate corticospinal plasticity and improve motor function in both older adults and chronic stroke patients, providing a clear rationale for the research questions addressed in this thesis.

This chapter will begin by outlining the anatomical structures of the central nervous system (CNS), highlighting its involvement in motor control of the upper limb. The chapter will then review techniques in which activity of the CNS can be quantified, followed by a discussion of the structural and functional changes associated with natural ageing and stroke. The evidence for corticospinal plasticity will then be discussed, followed by a thorough review of how this can be purposefully modulated through both motor skill training and non-invasive brain stimulation (NIBS) such as transcranial direct-current stimulation (tDCS). Finally, this chapter will discuss implications for the combination of motor skill training and tDCS, in regards to the formation of corticospinal plasticity and improving motor function of the upper limbs in both older adults and chronic stroke patients.
2.2 The human motor cortex

2.2.1 Organisation of the motor cortex

The human motor cortex can be identified as a region of the cerebral cortex located in the frontal agranular area. The motor cortex receives inputs from both sensory pathways and other motor control regions and is ultimately responsible for planning, initiating and executing voluntary movement (Donoghue & Sanes 1994; Haines 2006). The motor cortex can be subdivided into a number of interconnected regions that are thought to play a more specific role in the control of complex movement patterns (Nolte 2002). The primary motor cortex (M1), lies within the precentral gyrus (Brodmann’s area 4) and gives rise to many large output (pyramidal) cells, that synapse with motoneurons in the ventral horn of the spinal cord that are responsible for evoking muscular contractions. The M1 receives neural inputs from a number of cortical and subcortical regions involved in the planning and preparation of movement sequences, which include the basal ganglia, cerebellum, pre motor cortex (PMC), supplementary motor area (SMA) and posterior cingulate cortex (Nolte 2002; Rothwell 1994).

Through the use of electrical stimulation, neurosurgeon Wilder Penfield was the first to propose a topographical organisation of the M1, with distributions of corticospinal neurons representing and controlling a specific skeletal muscle (Penfield & Rasmussen 1950). The area devoted to a particular skeletal muscle is proportional to the number of motor units and the amount of precision and fine motor control required by that particular muscle (Nolte 2002), however these limb (and muscle) representations are not entirely separate, rather, they are divergent and convergent neuronal networks (Porter & Roger 1993). This is of particular importance as the structural layout of
neuronal networks provide an opportunity for reorganisation should brain injury occur (Sanes & Donoghue 1997, 2000; Schieber 2001).

The largest area of the M1 (neocortex) contains two types of neurons known as stellate and pyramidal cells, with their axons structurally organised into six horizontal layers (Figure 2.1, p. 13) (Mountcastle 1997; Porter & Roger 1993; Rothwell 1994). Stellate cells act as interneurons within the M1, with their axons confined to the cortex, for which the most predominant are basket cells that synapse with pyramidal neurons using the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (Jones 1993). Pyramidal neurons have axons that leave the cortex, descend through the medullary pyramid and are primarily involved in motor output (Mountcastle 1997; Nolte 2002; Rothwell 1994). Pyramidal neurons typically use the excitatory neurotransmitter glutamate in order to execute voluntary movement. The largest pyramidal neurons (Betz cells) are found in layer V of the M1 (Figure 2.1, p. 13), where they descend to synapse directly with alpha motoneurons in the spinal motoneuron pool, innervating specific muscle groups (Mountcastle 1997). This structural arrangement of the M1 provides an opportunity for enhanced synaptic efficacy between stellate and pyramidal neurons, and may be an important mechanism for the purposeful modulation of corticospinal plasticity in ageing and clinical populations.
Figure 2.1 Visual representation of the structural organisation of the M1

(Rothwell 1994).

(i) Molecular layer: Comprised of axons and dendrites with only a few cell bodies.

(ii) External granular layer: Contains densely packed small cells including a large number of small pyramidal and stellate cells.

(iii) External pyramidal layer: Contains mainly medium and large pyramidal cells.

(iv) Internal granular layer: Contains densely packed pyramidal and stellate cells.

(v) Ganglionic Layer: Contains large pyramidal (Betz) cells responsible for innervating the motoneuron pool for a target muscle.

(vi) Multiform layer: Innermost layer, which is relatively thin and composed of densely packed spindle-shaped cells.
2.2.2 The corticospinal pathway

A number of descending pathways that originate above the medulla influence the net movement produced by skeletal muscle. Pyramidal neurons arise from the M1, where they synapse with lower motoneurons in the spinal cord forming the corticospinal pathway (CSP) (Nathan & Smith 1955). The majority of these fibres originate in layer V of the M1, and approximately 80-90% of their axons decussate at the medulla oblongata, forming the lateral CSP (Nathan & Smith 1955; Rothwell 1994). Due to this decussation, corticospinal neurons in the M1 activate skeletal muscles on the contralateral side of the body (Rothwell 1994). The remaining uncrossed axons continue down the ipsilateral side forming the anterior CSP, terminating at the thoracic spinal cord and synapsing with lower motoneurons to control large postural muscles, predominately in the trunk (Porter 1985; Rothwell 1994). Although there are other motor control areas within the cortex that help shape voluntary movement, it is thought that the functional coordination of the wrist, hand and fingers are controlled primarily by the lateral CSP (Schieber & Santello 2004). Therefore, damage and degeneration of the neurons involved in this pathway often impairs the ability for the distal musculature of the upper limb to produce coordinated and efficient movement.

2.3 Techniques to assess the corticospinal pathway

Past techniques employed to measure functional activity of the CNS have included functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), where changes in cortical and subcortical blood flow related to neuronal activation have been examined in response to the execution of a motor task (Hallett 2000). Although useful in demonstrating spatial resolution of cortical changes, a major
limitation is their inadequacy to measure temporal synaptic activity within the M1 during and following movement (Hallett 2000). Alternatively, transcranial magnetic stimulation (TMS) is used to measure excitatory and inhibitory activity within the M1 and the CSP, which provides an opportunity to assess synaptic efficacy of this pathway following a given intervention.

2.3.1 Transcranial magnetic stimulation

Traditionally, transcranial electrical stimulation (TES) was used to assess neurological function, however due to the rapid, high-intensity stimulation during TES; much of the electrical stimuli travels along the surface of the scalp, bypassing the neurons within the M1. This causes large muscular contractions of the scalp resulting in discomfort (Hallett 2000; Rothwell 2003). Subsequently, Barker and colleagues (1985) developed TMS which today is a widely used, alternative method of quantifying excitability and inhibition within the CSP (Barker, Jalinous & Freeston 1985).

When applied over the M1 representation of the target muscle, TMS stimulates the underlying cortical tissue via a brief magnetic pulse, eliciting a muscle contraction (Hallett 2000). The response to TMS varies depending upon the type of coil used, the direction of the electrical current and the type and intensity of stimulation (Carroll, Riek & Carson 2001b; Kobayashi & Pascual-Leone 2003). A figure of eight coil produces a focal electrical field and is most commonly used for the assessment of corticospinal neurons corresponding to upper limb musculature (Hallett 2000; Hallett 2007). When a suprathreshold TMS stimulus is passed through the M1, underlying excitatory interneurons are depolarised resulting in a number of descending volleys
(direct [D-waves] and indirect [I-waves]) that synapse with motoneurons in the ventral horn of the spinal cord (Chen 2000; Di Lazzaro et al. 2003; Kobayashi & Pascual-Leone 2003). The result of these multiple, high-frequency volleys is an electromyographic (EMG) twitch response in the target muscle which is termed a motor evoked potential (MEP) (Di Lazzaro et al. 2004; Hallett 2000). At high TMS intensities, corticospinal neurons are depolarised directly producing an initial D-wave. At lower single-pulse TMS intensities, the activation of corticospinal neurons are generated tran-synaptically (i.e. indirectly) via excitatory interneurons, producing a series of I-waves (Di Lazzaro et al. 2012b; Reis et al. 2008). The first volley of I-waves (i.e. I_1) are most commonly observed following single-pulse TMS, due to activation of monophasic cortical interneurons projecting onto corticospinal neurons, which are then followed by a series of later I-waves (Di Lazzaro et al. 2003; Di Lazzaro et al. 2012b).

2.3.1.1 Single-pulse transcranial magnetic stimulation

Measurable components of a single-pulse TMS elicited muscle response are displayed in Figure 2.2 (p. 17). These physiological recordings can be analysed to ascertain information about the integrity and function of the CSP, which helps to provide useful insight regarding the CNS in both healthy and diseased populations.
Figure 2.2 Visual representation of the properties of a motor evoked potential (MEP) recorded through surface electromyography (sEMG) from the biceps brachii muscle during an active state. (A) Onset of TMS stimulation; (B) Latency period or corticospinal conduction time; (C) Peak-peak amplitude of the MEP; (D) Silent period duration (SPD); (E) Return of normal EMG activity (Pearce & Kidgell 2011).
The peak-to-peak amplitude of the MEP represents the balance of multiple excitatory and inhibitory neuronal inputs onto the CSP (Chen 2000; Kobayashi & Pascual-Leone 2003; Weber & Eisen 2002). Although not a direct component of the MEP per se, motor threshold (MT) is the minimum amount of stimulation required to depolarise corticospinal neurons and produce an MEP amplitude greater than 50 $\mu$V or 200 $\mu$V for at least three out of five successful trials, while the muscle is rested or lightly contracted respectively (Carroll, Riek & Carson 2001b; Rossini et al. 1994; Rossini & Rossi 2007; Rothwell et al. 1999). Motor threshold is thought to represent membrane excitability of corticospinal neurons in both the M1 and spinal cord (Kobayashi & Pascual-Leone 2003). The silent period duration (SPD) following the MEP response is a period of EMG suppression. It is believed that the initial suppression of EMG represents spinal mechanisms such as the neural refractory period (i.e. after-hyperpolarisation) and recurrent inhibition (Wilson et al. 1993; Ziemann et al. 1993). Alternatively, the latter part of the SPD (i.e. > 50 milliseconds) is suggested to reflect supraspinal inhibition (Inghilleri et al. 1993; Wilson et al. 1993) which provides information about GABA$\beta$-mediated inhibitory activity (Lang et al. 2006; McDonnell, Orekhov & Ziemann 2006).

Contraction of a target muscle increases excitability of the CSP and the corresponding motoneuron pool, therefore, the stimulus intensity required to elicit an MEP is often lower than in a resting muscle (Hallett 2007). When factors such as background muscle force (Weber & Eisen 2002), time of day (Sale & Semmler 2005) and coil orientation (Rothwell et al. 1999) are tightly controlled for, MEPs have been shown to be a reliable intra-participant measure of corticospinal excitability in healthy individuals (Kamen 2004; Livingston & Ingersoll 2008; O'Leary et al. 2015) and chronic stroke patients (Liu & Au-Yeung 2014).
2.3.1.2 Paired-pulse transcranial magnetic stimulation

The technique of paired-pulse TMS provides insight into the intracortical inhibitory or excitatory synaptic activity onto corticospinal neurons, that single-pulse TMS cannot examine alone (Chen 2004; Kujirai et al. 1993; Shirota et al. 2010). When a conditioning stimulus (subthreshold) precedes a test stimulus (suprathreshold), cortical interneurons are depolarized, allowing activity of the neurons within the M1 to be investigated through either a suppression or facilitation of the MEP amplitude (Kossev et al. 2003; Kujirai et al. 1993).

The inter-stimulus interval (ISI), which represents the time between the conditioning and test stimuli, as well as the TMS coil orientation (i.e. direction of electrical current) can be used to determine the activity of different interneuronal networks, which projects either a facilitatory or inhibitory effect on corticospinal neurons. Short-interval intracortical inhibition (SICI) has been demonstrated with shorter ISIs between 1-6 milliseconds, and conditioning stimuli of 70-80% of MT and is thought to reflect GABAA-mediated inhibition (Di Lazzaro et al. 2006; Kossev et al. 2003; Kujirai et al. 1993; Ziemann, Rothwell & Ridding 1996). Additionally, long-interval intracortical inhibition (LICI) is quantified using ISIs of approximately 50-200 milliseconds, and reflects involvement of GABAB receptors. There is also evidence to suggest that LICI interacts with, and may reduce excitability of GABAA (SICI) receptors through pre-synaptic inhibition (Chen, Lozano & Ashby 1999; Nakamura et al. 1997; Valls-Sole et al. 1992). The involvement of GABA-mediated SICI is signified by suppressed I3 waves, and are most readily observed with an anterior-posterior coil orientation (Zoghi, Pearce & Nordstrom 2003). D-waves and I1 (earlier) waves are rarely affected by the conditioning stimulus and are only inhibited with ISIs of ~1 millisecond (Di Lazzaro et al. 1998; Hanajima et al. 1998) Alternatively,
intracortical facilitation (ICF) can be measured with ISIs between 6-20 milliseconds and measures the influence of excitatory interneurons projecting onto corticospinal neurons (Kossev et al. 2003; Ziemann, Rothwell & Ridding 1996).

In addition to intracortical inhibition, interhemispheric inhibition (IHI) across the corpus callosum can be measured with the application of a conditioning stimulus preceding a test stimulus to the opposite hemisphere, with an ISI of approximately 10 milliseconds (Ferbert et al. 1992). This technique measures the level of excitatory-inhibitory influence of one hemisphere over the other. Interhemispheric inhibition has been observed in some proximal muscles, but is more pronounced in distal muscles that perform precision and dexterous movements (Harris-Love et al. 2007). Both intracortical inhibition and IHI are believed to be important for the suppression of extraneous muscle activity during a motor task (Sohn & Hallett 2004; Sohn, Wiltz & Hallett 2002; Stinear & Byblow 2003). Therefore lack of interhemispheric inhibitory control, may impair motor function of the upper extremities in older individuals and stroke patients (Boudrias et al. 2012; Murase et al. 2004; Talelli et al. 2008).

2.4 Corticospinal plasticity

The process of ‘plasticity’ refers to the capacity of the human CNS to undergo morphological and functional reorganisation across the lifespan in response to experience and learning, as well as following neurological injury [for reviews see (Pascual-Leone et al. 2005; Tyc & Boyadjian 2006; Ward 2005)]. The broadly defined term ‘plasticity’ can be either functionally beneficial (i.e. adaptive) or can be pathophysiological, which is often referred to as maladaptive plasticity. Functional reorganisation is frequently observed following motor skill training which manifests
as motor learning, whereas maladaptive plasticity may occur spontaneously as a result of degeneration or neurological injury (Joseph 2013). Importantly, the formation of corticospinal plasticity can be induced through use-dependent (i.e. motor training or neurorehabilitation) (Adkins et al. 2006; Ward 2005) and experimental (Nitsche & Paulus 2000; Ziemann et al. 2008) protocols. This adaptive nature of the CNS provides an opportunity to improve motor function in a range of populations including older adults and chronic stroke patients. The following section will review the mechanisms driving corticospinal plasticity, whilst the evidence for corticospinal plasticity to be purposefully modulated through motor training and NIBS will be comprehensively discussed in sections 2.7.1 (pp. 40-43) and 2.7.2 (pp. 46-56).

2.4.1 Mechanisms of corticospinal plasticity

Evidence of structural and functional modifications within the M1 have been demonstrated in response to motor training (Ackerley, Stinear & Byblow 2011; Carroll, Rick & Carson 2001a; Tyc, Boyadjian & Devanne 2005) and various NIBS protocols (Fritsch et al. 2010; Ridding & Ziemann 2010). A commonly used measure of corticospinal plasticity is cortical mapping, which explores the area, volume and centre of gravity on the M1 targeting specific muscle groups (Kleim et al. 2006). An increase in the area (Kleim, Barbay & Nudo 1998; Pascual-Leone et al. 1995; Thickbroom et al. 2004; Tyc & Boyadjian 2011), volume and centre of gravity (Sawaki et al. 2014) of motor representation maps are thought to be associated with improvements in functional performance of the corresponding limb. Reorganisation of M1 output maps have been shown following limb immobilisation (Liepert, Tegenthoff & Malin 1995), amputations (Brenneis et al. 2005; Wu & Kaas 1999), stroke (Byrnes
et al. 1999; Sawaki et al. 2014; Thickbroom et al. 2004) and motor learning (Pascual-Leone, Grafman & Hallett 1994; Pascual-Leone et al. 1995). Over time, the map of the target or surrounding muscles can become topographically larger, allowing greater anatomical connectivity between neurons in the representational zone (Pascual-Leone, Grafman & Hallett 1994). Additionally, changes in the equilibrium between intrinsic excitatory and inhibitory projections onto pyramidal cells may be closely linked to enhancements in M1 maps (Ridding & Rothwell 1997; Tyc & Boyadjian 2011). An example of this concept is the increase in the slope of the TMS evoked recruitment curve, correlating with an increase in the motor representation of the target muscle, which is indicative of neuroplasticity within the M1 (Ridding & Rothwell 1997).

Modifications in the intrinsic circuitry of the M1 are also thought to occur through changes in synaptic efficacy (Ridding & Rothwell 1997). Such mechanisms potentially include unmasking of horizontal ‘latent’ connections, growth of new synaptic connections (i.e. collateral sprouting) and modifications in the strength of existing synapses through long-term potentiation (LTP) and depression (LTD) [for reviews see (Hess & Donoghue 1996; Sanes & Donoghue 1997, 2000)]. LTP has been described as the functional strengthening of synaptic transmission, through increasing activation of the glutamatergic system (i.e. \(N\)-methyl-D-aspartate \([NMDA]\) and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic \([AMPA]\) during post-synaptic depolarisation (Figure 2.3, p. 24) (Malenka & Bear 2004). When the post-synaptic neuron is depolarised, magnesium moves away and unblocks the NMDA receptor channels, allowing an influx of sodium and calcium, which is critical for initiating LTP (Malenka & Nicoll 1999; Malenka & Bear 2004). In turn, this increases both the number and sensitivity of these post-synaptic receptors, improving overall synaptic transmission (Keller 1993; Malenka & Nicoll 1999). These processes were first
described as the foundation for memory retention and learning (Bliss & Collingridge 1993; Bliss & Lomo 1973), although it is now understood that modulation of NMDA receptor activity is a fundamental mechanism for the formation of use-dependent plasticity (Malenka & Nicoll 1999). Alternatively, LTD refers to the suppression of post-synaptic excitation (Malenka & Bear 2004), and may be an important mechanism modulating the balance of corticospinal excitability and inhibition in populations such as ageing and stroke. An important property of unmasking latent synapses and enhancing NMDA receptor activity is the removal of local GABA-mediated inhibition (Hess & Donoghue 1994, 1996; Jacobs & Donoghue 1991). Therefore, it appears that the horizontal layers of intracortical neuronal networks form a substrate for reorganisation of the M1 (Chen, Cohen & Hallett 2002; Jacobs & Donoghue 1991), and may be an important aspect of LTP-like plasticity.
Figure 2.3 Schematic representation of the glutamatergic system and the basic mechanisms underpinning long-term potentiation (LTP). Glutamate release from the pre-synaptic neuron and NMDA and AMPA receptors (NMDA-R and AMPA-R) during normal synaptic transmission (A). Sufficient depolarisation of the post-synaptic neuron (B) causes magnesium (Mg^{2+}) to unblock the NMDA receptor channel, allowing an influx of sodium (NA^+) and calcium (CA^{2+}), inducing LTP [adapted from (Malenka & Nicoll 1999)].

2.5 Neurophysiology of ageing

The normal ageing process is accompanied by a gradual, but progressive reduction in neuromuscular function at a number of structures responsible for motor control. The mechanisms contributing to the age-related reduction in motor control and strength are multifactorial [for a comprehensive review see (Aagaard et al. 2010; Doherty 2003)], however the earlier onset of motor decline appears to be due to supraspinal mechanisms (Pitcher, Ogston & Miles 2003). Therefore, the focus of this section will be primarily directed to changes within the CSP and M1. Given that the ability to
perform every day movement relies predominantly on the CSP to control force output, specifically to the upper limb, degeneration within this system can have negative consequences on motor function.

2.5.1 Age-related morphological changes within the cerebral cortex

Ageing is associated with a decline in neuromuscular health which inevitably contributes to variability and reduced efficacy in which the CNS produces and controls movement (Galganski, Fuglevand & Enoka 1993; Laidlaw, Bilodeau & Enoka 2000; Ranganathan et al. 2001; Tracy & Enoka 2002). It is estimated that from the sixth decade of life there is a progressive reduction (approx. 0.5% annually) in cerebral volume, contributing to an approximate 14% reduction across the lifespan (Jernigan et al. 2001; Kennedy & Raz 2005). This decline in cerebral volume can be attributed to a loss in both grey and white matter (Bartzokis et al. 2001). The progressive loss of grey matter is thought to begin following adolescence, whilst the loss of white matter occurs later in life and continues at an accelerated rate (Bartzokis et al. 2001; Jernigan et al. 2001).

Compared with younger adults, fMRI studies have shown reductions in grey matter and cortical thinning specifically within the M1 and PMC of middle aged older adults (Good et al. 2001; Salat et al. 2004). There is further evidence to suggest that this volumetric loss in grey matter may be more pronounced in the non-dominant hemisphere (Bonilha et al. 2009). The implications for these reductions in grey and white matter contribute to declines in motor performance across a range of tasks (Aagaard et al. 2010; Enoka et al. 2003; Kennedy & Raz 2005; Sullivan, Rohlfing & Pfefferbaum 2010; Zahr et al. 2009).
2.5.2 Age-related functional changes within the corticospinal pathway

Age-related reductions in the number of corticospinal neurons synapsing with motoneurons in the spinal cord may reduce the efficacy of transmission between the descending motor commands and the spinal motoneuron pool (Kido, Tanaka & Stein 2004; Pitcher, Ogston & Miles 2003), which may limit both muscle strength and control of motor skills. By approximately the fifth decade, there is an estimated 35% loss in the number of functioning corticospinal neurons (Eisen, Entezari-Taher & Stewart 1996), which would likely contribute to the age-related reduction in corticospinal excitability (Oliviero et al. 2006). Furthermore, animal models have shown age-related changes in the glutamatergic system, specifically with regard to the sensitivity of the NMDA receptor, which may impede motor learning (Disterhoft et al. 1996; Thibault, Hadley & Landfield 2001). These changes are evidenced by a number of TMS studies in humans, demonstrating lower MEP amplitudes (Fujiyama et al. 2009; Oliviero et al. 2006; Rossini, Desiato & Caramia 1992; Sale & Semmler 2005), and higher MTs (Rossini, Desiato & Caramia 1992) in older compared with younger adults. In addition, these reductions appear to be more prominent within the non-dominant hemisphere (Figure 2.4, p. 27) (Sale & Semmler 2005).
Figure 2.4 Example of motor evoked potential (MEP) recordings in one young (A) and one older (B) right hand dominant adult, during an index finger abduction task and a scissor grip. The data reveals a significantly suppressed MEP amplitude in the older compared with the younger adult (B), more evident in the non-dominant left hand (Sale & Semmler 2005).

A plausible explanation for the net reduction in corticospinal excitability may be changes in GABA-mediated intracortical and interhemispheric inhibitory networks. A reduction in SPD has been consistently reported in older compared with younger adults (Eisen, Entezari-Taher & Stewart 1996; Oliviero et al. 2006; Sale & Semmler 2005). However, there are mixed findings with regard to intracortical inhibition, and this appears to differ between muscle conditions (i.e. resting versus an active state) for which the MEP was measured. In a resting muscle, studies have reported reductions (Heise et al. 2013; Marneweck, Loftus & Hammond 2011; Peinemann et al. 2001), increases (Kossev et al. 2002; McGinley et al. 2010) as well as no changes in SICI (Cirillo, Rogasch & Semmler 2010; Cirillo, Todd & Semmler 2011; Oliviero et al. 2006; Opie & Semmler 2014; Smith et al. 2009), with some evidence that the age-
related differences in SICI may be eliminated under active conditions (McGinley et al. 2010). Although variations in methodological design would partially explain these differences, it is possible that the reported reductions in SICI may compensate for the age-related degeneration of corticospinal neurons, whereas increases in SICI may result from interhemispheric imbalances during a movement. For example, changes in the amount of IHI during a task have been reported in older adults (Talelli et al. 2008). Taken together, it appears that the ageing process is accompanied by an imbalance of GABAergic interneuron activity, that may contribute to the reduced motor function observed in older adults (Heise et al. 2013; McGinley et al. 2010; Smith et al. 1999).

### 2.5.3 Age-related interhemispheric asymmetries in corticospinal excitability and inhibition

The inability to accurately modulate intracortical and IHI may contribute to an overflow of activity to the contralateral M1 and surrounding cortical regions during unilateral movements, which is commonly observed with advancing age (Hoy et al. 2004; Talelli et al. 2008; Ward 2006; Ward & Frackowiak 2003). Possible mechanisms contributing to age-related asymmetries in corticospinal excitability and inhibition may be a combination of transcallosal white matter degeneration (Hou & Pakkenberg 2012; Zahr et al. 2009), as well the preferential disuse of the non-dominant limb during skilled tasks (Bonilha et al. 2009; Sale & Semmler 2005). In older adults pronounced degeneration within the non-dominant M1 can generate disinhibition within the dominant M1 (Coppi et al. 2014), which has been suggested to be maladaptive rather than compensatory in regards to the effects on motor function (Bernard & Seidler 2012). Consequently, this dissociation between M1s is suggested to be partially
responsible for the widespread activation and motor overflow, which occurs during unilateral movement (Mattay et al. 2002; Sailer, Dichgans & Gerloff 2000; Ward, Swayne & Newton 2008).

As the balance of excitatory and inhibitory output is fundamental for the production and control of movement (Ashby et al. 1999; Chen 2004), disruption to these networks are likely to manifest as reduced motor function. For example Marneweck et al. (2011) showed that increased levels of intracortical facilitation, rather than reductions in SICI, were correlated with reduced hand function, which was more prominent in the non-dominant M1 (Marneweck, Loftus & Hammond 2011). It has also been reported that older adults experience no task-dependent differences in inhibitory responses (SPD and SICI) when performing complex versus simple limb coordination tasks (Fujiyama et al. 2009; Fujiyama et al. 2012a), as well as single joint versus synergistic muscle contractions (Opie, Ridding & Semmler 2015). These studies reflect an age-related reduction in the ability for the CNS to control the magnitude of corticospinal inhibition across different task demands, which is likely to contribute to reduced motor control. Similarly, the dominant M1 has been shown to exhibit greater potential for use-dependent changes in corticospinal excitability and inhibition compared to the non-dominant limb (Christie & Kamen 2014; Hinder et al. 2011; Sale & Semmler 2005).

Based on these studies, it is likely that maladaptive changes within the ageing nervous system may prevent use-dependent plasticity, particularly in the non-dominant hemisphere, with negative outcomes on motor function.

Additionally, imbalances in corticospinal excitability and inhibition may also be related to increased co-activation of the antagonist muscle, which is commonly observed during ageing (Hakkinen et al. 2000; Hakkinen et al. 1998). Both cortical and spinal mediated reciprocal inhibition (i.e. inhibition of the antagonist muscle) are
shown to be reduced with age (Hortobágyi, del Olmo & Rothwell 2006; Kido, Tanaka & Stein 2004), which may further be associated with declines in movement dexterity and motor function, leading to difficulties performing many common daily activities.

2.5.4 Behavioural changes associated with age-related neurodegeneration

Age-related neurophysiological decline may lead to increased risk of injury, loss of independence and ability to carry out ADLs. In the upper limbs, slower execution of gross motor tasks (Aagaard et al. 2010; Bennett & Castiello 1994), increased error when performing movements (Christou & Enoka 2011; Mattay et al. 2002; Smith et al. 1999), force variability (Christou & Carlton 2001; Marmon et al. 2011) and reduced visuomotor processing are common motor deficits associated with ageing (Guan & Wade 2000). This is evident in clinical tests that reflect everyday movement patterns, such as repetitive finger tapping (Shimoyama, Ninchoji & Uemura 1990), visually-guided hand movements, grip strength, pinch force and precision tasks (Cole, Rotella & Harper 1999; Hackel et al. 1992). Studies have demonstrated that the rate of finger tapping and hand movements decrease with advancing age, particularly in the non-dominant limb (Aoki & Fukuoka 2010; Sale & Semmler 2005; Shimoyama, Ninchoji & Uemura 1990; Smith et al. 1999). A decline in maximal grip strength, pinch force and finger abduction force are also observed in older compared with younger adults (Kallman, Plato & Tobin 1990; Marmon et al. 2011; Ranganathan et al. 2001). In relation to accuracy of movement, Parikh and colleagues (2012) observed misaligned finger placements as well as more errors and slower execution of a precision key-hole task in older adults (Parikh & Cole 2012).
Although the process of natural ageing is inevitable, there is a need to identify techniques that may restore interhemispheric balance and improve or preserve motor function, specifically within the non-dominant limb. Previously, motor skill training has been shown to improve neuromuscular function in the elderly (Enoka 1997; Hurley & Roth 2000; Raw et al. 2012), however, it is currently unknown as to whether the concurrent effects of motor skill training and NIBS can act as a countermeasure to the natural age-related regression in neuromuscular health and motor function. The application of NIBS to augment corticospinal plasticity and motor function in an ageing population will be discussed in greater detail in section 2.7.2.4 (pp. 53-56).

2.6 Consequences of motor cortex damage following a stroke

A stroke, also known as a cerebrovascular incident, occurs as a result of a lack of blood supply to both cortical and subcortical areas of the brain. Loss of blood supply to specific regions disrupts both the functional and structural integrity of the neural tissue (Schallert, Leasure & Kolb 2000). Damage to the neural tissue within the M1 following a stroke results in either complete hemiplegia (total paralysis) or hemiparesis (weakness) on the contralateral side of the body (Nowak et al. 2007), with the most severe functional impairments often observed in the distal upper limb musculature (Colebatch & Gandevia 1989).

Following a stroke, restoration of motor function is often incomplete, and relies on both structural and functional modification within the cerebral cortex (Hallett 2001). The application of TMS provides a non-invasive method of predicting functional recovery following a stroke, which may be useful in developing and tailoring rehabilitation protocols for motor recovery (Stinear 2010; Stinear et al. 2007;
Thickbroom et al. 2004). TMS studies typically observe higher thresholds to elicit an MEP, reduced MEP amplitudes and a prolonged MEP latency period in the acute stages after a stroke (Byrnes et al. 1999; Heald et al. 1993; Macdonell, Donnan & Bladin 1989; Rossini & Dal Forno 2004). In some cases, absence of an MEP response is often associated with reduced functional integrity of the CSP and may be a predictor of poor motor recovery (Heald et al. 1993; Stinear et al. 2007). TMS mapping studies have also observed a reduction in the motor map area within the ipsilesional M1, which is likely to reflect reduced motor output (Cicinelli, Traversa & Rossini 1997; Traversa et al. 1997).

The SPD following the MEP has also been used as a prognostic tool assessing integrity of the CSP (van Kuijk et al. 2005), though due to differences in location of the lesion and the phase in which it has been measured, there have been inconsistent findings (Ahonen et al. 1998; Catano et al. 1996; Classen et al. 1997; Cruz Martinez, Munoz & Palacios 1998). However, the majority of studies assessing the acute phase after stroke have observed a prolonged SPD in the ipsilesional hemisphere (Ahonen et al. 1998; Braune & Fritz 1995; Catano et al. 1996; Classen et al. 1997; Liepert et al. 2000b; Nardone & Tezzon 2002). Consequently, a gradual reduction in the SPD has been associated with clinical improvement in motor function (Classen et al. 1997; Traversa et al. 2000), with normal SPDs reported in well recovered patients (Byrnes et al. 2001). However in the chronic phase of a stroke, individuals with severe spasticity often display a significantly shorter SPD (Catano et al. 1996; Cruz Martinez, Munoz & Palacios 1998). Alternatively, the SPD within the contralesional M1 has been reported to remain within a normal physiological range (Braune & Fritz 1995; Byrnes et al. 2001; Cicinelli, Traversa & Rossini 1997; Liepert et al. 2000b).
2.6.1 Interhemispheric competition following a stroke

Evidence from TMS studies have suggested that the observed motor deficits in the paretic limb may be associated with interhemispheric asymmetries of corticospinal excitability and inhibition between the contra- and ipsilesional M1 (Figure 2.5) (Cicinelli et al. 2003; Trompetto et al. 2000). In patients with cortical lesions, studies have demonstrated disinhibition within the contralesional M1 (Liepert, Hamzei & Weiller 2000; Shimizu et al. 2002) which is likely a compensatory adaptation for the reduced motor output from the ipsilesional M1.

![Figure 2.5](image)

**Figure 2.5** Example motor evoked potentials (MEPs) upon transcranial magnetic stimulation (TMS) of the contralateral hemisphere, recorded from the non-affected (normal side) and paretic thenar muscles, in a representative stroke patient from one study (Trompetto et al. 2000).
Disinhibition of corticospinal neurons within the contralesional M1 may generate an increase in IHI towards the ipsilesional M1, resulting in maladaptive reorganisation and a further reduction of neuronal activity within the ipsilesional M1 (Butefisch et al. 2003; Manganotti et al. 2002; Murase et al. 2004; Shimizu et al. 2002). Although these adaptations are likely to have negative consequences on motor recovery of the paretic limb, the direct relationship to motor function remains unclear (Shimizu et al. 2002). In animal models, disinhibition of the contralesional M1 is associated with impairments in NMDA receptor binding along with GABA-mediated inhibition (Que et al. 1999; Reinecke et al. 1999), which in humans can be quantified through the measurement of SICI. Longitudinal studies have demonstrated that the restoration of SICI to normal levels within the contralesional M1 is associated with improved functional recovery, whereas individuals with greater disability display abnormally low levels of SICI within the contralesional M1 (Liepert, Hamzei & Weiller 2000; Manganotti et al. 2008; Manganotti et al. 2002). Moreover, high levels of SICI within the ipsilesional M1 have been observed during movement initiation of the paretic limb (Hummel et al. 2009), which may represent maladaptive inhibitory processes occurring between the contra- and ipsilesional M1.

Given that reduced motor output in the paretic limb appears to be due to asymmetries in GABAergic inhibition between the hemispheres, there is a requirement for rehabilitation interventions to focus on restoring interhemispheric balance. The use of NIBS, combined with regular sessions of motor skill training, may provide an opportunity to augment the adaptations induced from conventional rehabilitation by modulating corticospinal excitability and inhibition in the contra- and ipsilesional M1; however to date this has not been comprehensively investigated.
2.6.2 Motor impairments following a stroke

Stroke is one of the leading causes of disability throughout developed countries (Mozaffarian et al. 2015). Functional recovery following both ischemic and haemorrhagic stroke is poor, with many patients experiencing chronic disability, particularly related to the upper limb and hand (Kwakkel et al. 2003; Kwakkel, Kollen & Wagenaar 2002; Nakayama et al. 1994; Platz, Bock & Prass 2001). Following rehabilitation, as many as 42% of stroke patients continue to require assistance with functional daily tasks, even up to six years following the stroke (Feigin et al. 2008).

Impairments in object grasping and precision grip have been correlated with reduced performance on the Action Research Arm Test (ARAT), highlighting the importance of these movements in ADLs (McDonnell et al. 2006). For example, with impaired precision grip, the ability to pick up a cup and place it down without any spillage is reduced (McDonnell et al. 2006). During object grasping and lifting tasks using a precision grip, stroke patients exhibit slower force generation and a more uncoordinated grip (Nowak et al. 2007; Nowak, Hermsdorfer & Topka 2003). Reach-to-grasp movements are performed slower and with frequent errors, suggesting difficulty in controlling the position of the wrist prior to grasping an object (Lang et al. 2005; Wenzelburger et al. 2005). Specifically, one study observed excessively high grip forces to move and transport an object (despite low maximal grip strength) in stroke patients, when compared to healthy controls (Hermsdorfer et al. 2003). This suggests that force output and efficacy of movement may be impaired by a number of processes such as an inability to control excitatory-inhibitory output for different task demands, as well as possible sensory and visuomotor deficits (Hermsdorfer et al. 2003).
To date, the majority of stroke literature has assessed fine motor control of the digits, with less focus on muscles that manipulate the position of the hand and wrist. Many tendons from the forearm muscles that control and position the digits cross over the wrist, inserting onto the hand (Oatis 2004). Weakness of the wrist extensor muscles limits flexion of the digits, impairing grasp, pinch force and overall dexterity of the hand (Oatis 2004). One study observed a relationship between reduced range of motion (ROM) at the wrist and difficulties in performing tasks associated with personal hygiene (Ryu et al. 1991), supporting the synergistic relationship between the distal upper limb muscles. Further, the ability for stroke patients to regain wrist and finger extension is suggested to improve the prognosis of overall hand recovery (Taub et al. 1993). It is conceivable that strength, control and manipulation of the wrist will influence the ability of the hand and digits to produce efficient movement. However currently there is limited evidence investigating NIBS to modulate neuronal activity of the wrist extensors and its effects on motor function in chronic stroke patients.

2.6.2.1 Clinical assessment of upper limb function following a stroke

The examination of upper limb function should typically include a range of assessments targeting passive and active functional movement, quality of movement, as well as consideration of factors that may limit functional movement such as ROM and spasticity. A variety of tools assessing upper limb function following stroke are available to both researchers and clinicians (Lang et al. 2013). The most commonly utilised, non-self-report items to assess functional movement of the upper limb include the Chedoke Arm and Hand Inventory (Barreca et al. 2005), ARAT (Van der Lee et al. 2001), Fugl-Meyer Assessment (Fugl-Meyer et al. 1975), Jebsen-Taylor Test of
Hand Function (Jebsen et al. 1969) and the Motor Assessment Scale (MAS) (Carr et al. 1985). The MAS, developed by Carr et al. (1985), consists of three upper limb and hand items focused on ADLs and functional mobility. The assessment takes approximately 15-30 minutes to administer by a trained professional and both reliability (test-retest, r = 0.87-1.0; inter-rater, r = 0.95) and validity (concurrent with Fugl-Meyer assessment, r = 0.96) have been well established for its use in stroke patients of various phases (Carr et al. 1985; Dean & Mackey 1992; Malouin et al. 1994; Tyson & DeSouza 2004). Other measures that have been used to quantify movement quality in post-stroke individuals include the 9-hole pegboard, Box and Block and finger tapping test (Chanubol et al. 2012; Heller et al. 1987). Additionally, grip strength has been used as an objective measure of muscle integrity and can easily be administered to stroke patients with a range of functional abilities (Heller et al. 1987; Sunderland et al. 1989). Impairments in maximal grip strength have been shown to correlate with performance on clinical assessments including the Fugl-Meyer, Box and Block test, finger-to-nose and the ARAT (Boissy et al. 1999; McDonnell et al. 2006), highlighting the importance of the distal upper limb strength in performing common ADLs.

In stroke affected individuals, ADLs can be severely limited by impairments in joint ROM and spasticity. Traditionally, the ‘gold standard’ measure for spasticity in patients with a range of cerebral injuries was the Modified Ashworth Scale (Bohannon & Smith 1987), however, a major limitation of this tool is its inability to detect the ‘velocity’ component of spasticity (Pandyan et al. 1999). Alternatively, the Modified Tardieu Scale, adapted by Boyd and Graham (1999), addresses the passive response of the muscle at both slow and fast ROM, making it a more sensitive measure of spasticity (Boyd & Graham 1999). The Modified Tardieu Scale was found to have
excellent goniometry test-retest (ICC, 0.86) and good inter-rater (ICC, 0.66) reliability in stroke patients (Paulis et al. 2011), with the ability to differentiate between spasticity and contracture (Patrick & Ada 2006).

### 2.6.2.2 Exercise prescription for upper limb rehabilitation following a stroke

Conventional upper limb rehabilitation trends have emphasised the recovery of the proximal upper limb, with less emphasis on intensive training of the distal musculature (Butefisch et al. 1995). Although there is no ‘gold standard’ method of exercise for improving arm and hand function, a number of critical components have been put forward including the intensity (Kwakkel et al. 1997; Kwakkel et al. 1999; Parry, Lincoln & Appleyard 1999; Sunderland et al. 1992), meaningfulness of the task prescribed (Hubbard et al. 2009; van Vliet et al. 1995; Wu et al. 2000) as well as the volume (i.e. repetition) of each movement task (Langhorne, Coupar & Pollock 2009; Pollock et al. 2014).

Intensity of the rehabilitation can be enhanced through a number of elements, including individualised guidance and feedback from a physical therapist. For example, guided control of motor training has been shown to increase dendritic sprouting in rats, suggesting this may be an important factor for corticospinal plasticity (González-Burgos et al. 2011). Further, functional improvement in the upper limb has been shown to occur in a dose-response manner, with increasing intensity of rehabilitation leading to greater improvements on the Wolf Motor Function test, finger tapping speed, reaction time and grip and pinch strength (Byl, Pitsch & Abrams 2008). An important consideration when increasing the intensity of the rehabilitation, is that it is suggested to only be beneficial in the presence of meaningful task-related and
object-related exercises (Lincoln, Parry & Vass 1999). A large body of literature supports the efficacy of repetitive, task-specific training for both the induction of corticospinal plasticity and upper limb functional improvement (Barreca et al. 2003; Butefisch et al. 1995; Langhorne, Coupar & Pollock 2009; Pollock et al. 2014; Woldag & Hummelsheim 2002). Specifically, prescription of exercises that mimic common ADLs, rather than prescribing abstract tasks with no real-world functional objective, appear to improve the effectiveness of upper limb rehabilitation (Arya et al. 2012; Michaelsen, Dannenbaum & Levin 2006; van Vliet et al. 1995). For example, one study reported superior movement kinematics when reaching to drink from a cup filled with water, than when the same movement pattern was prescribed without the use of a cup (van Vliet et al. 1995).

Much of the impairment, specifically in chronic stroke patients, is thought to originate from a progressive disuse of the paretic limb (Franz, Scheetz & Wilson 1915). Moreover, as outlined in section 2.6.1 (pp. 33-34) asymmetries in excitatory and inhibitory transmission from the contra- to ipsilesional M1 may interfere with functional recovery of the paretic limb. It is conceivable that the efficacy of functional recovery may require focusing on the use of the paretic limb, with the subsequent induction of corticospinal plasticity in the ipsilesional hemisphere. The enforced use of the paretic limb has been extensively examined through constraint induced movement therapy (CIMT) (Taub, Uswatte & Pidikiti 1999). Studies restricting the use of the non-affected limb have shown both functional improvement, as well as corticospinal plasticity within the ipsilesional hemisphere (Liepert et al. 2000a; Taub et al. 2006). However, given that the non-affected limb is completely restricted during CIMT, this technique may only be feasible for patients with mild impairments. Therefore further investigation is warranted for alternate rehabilitation techniques that
may increase neuronal activity within the ipsilesional M1 and improve interhemispheric balance, with the overall objective to improve motor function in the paretic limb.

2.7 Techniques to induce corticospinal plasticity

The induction of corticospinal plasticity has been shown to produce functionally relevant changes in both older adults and stroke patients (Chen, Cohen & Hallett 2002; Jenkins & Merzenich 1987; Ward & Frackowiak 2003; Ward, Swayne & Newton 2008). In sub-acute stroke patients, recovery of motor function has been strongly correlated with the magnitude of corticospinal plasticity (Thickbroom et al. 2004). Therefore, it is clinically important to understand interventions in which corticospinal plasticity can be purposefully modulated. Evidence for use-dependent and experimentally-induced plasticity will be reviewed in the following sections. Moreover, the opportunity to combine use-dependent and experimental protocols to strengthen corticospinal plasticity in older adults and chronic stroke patients will be discussed.

2.7.1 Use-dependent plasticity

2.7.1.1 Motor skill training

Activity-dependent reorganisation of neocortical networks have been thoroughly demonstrated in both animal and human M1s following repetitive practice of simple motor tasks (Classen et al. 1998; Hayashi, Hasegawa & Kasai 2002; Hayashi, Shimura & Kasai 2005; Ziemann et al. 2001), as well as tasks promoting the acquisition of a
movement pattern (Karni et al. 1995; Muellbacher et al. 2001; Nudo et al. 1996; Pascual-Leone et al. 1995). Given the hypothesis that use-dependent plasticity and motor learning share similar LTP-like mechanisms, it is conceivable that an element of skill or novelty related to the motor task is advantageous for the longer-term induction of plasticity. This concept has been observed in animal models, where increases in the number and strength of synapses have been reported following skilled compared to ‘unskilled’ tasks (Kleim, Barbay & Nudo 1998; Kleim et al. 1996; Rioult-Pedotti et al. 1998). In adult monkeys, even repetitive motor training alone wasn’t sufficient to induce cortical reorganisation (Plautz, Milliken & Nudo 2000), highlighting the importance of incorporating novel skilled tasks into plasticity-inducing protocols. This theory has additionally been supported in the human M1, whereby TMS studies have shown marked increases in corticospinal excitability following motor skill training (Cirillo, Todd & Semmler 2011; Liepert, Terborg & Weiller 1999; Pascual-Leone et al. 1995; Perez et al. 2004). Specifically, following novel visuomotor tracking (VT) tasks, facilitated MEPs coupled with reductions in SICI have been observed for both the ankle and hand muscles in young and old adults (Cirillo, Todd & Semmler 2011; Perez et al. 2004).

Despite the evidence for motor training to induce corticospinal plasticity in healthy adults (Cirillo, Todd & Semmler 2011; Classen et al. 1998; Perez, Lundbye-Jensen & Nielsen 2006; Perez et al. 2004; Tyc & Boyadjian 2011), less is understood about the formation of use-dependent plasticity in populations with reduced/or impaired neurological function such as ageing and stroke. Rehabilitation of motor function following a stroke may be conceptualised as a form of motor learning. In both primates and humans, motor skill training has been shown to improve brain activation patterns in the ipsilesional hemisphere following damage to the M1 (Bosnell et al. 2011; Boyd,
Vidoni & Wessel 2010; Nudo 1997). Boyd et al. (2010) observed that in chronic stroke patients, task-specific training compared with non-meaningful movements improved motor learning, and shifted the M1 laterality index (LI) towards reduced contralesional activation (Boyd, Vidoni & Wessel 2010). Therefore, task-specific training may induce corticospinal plasticity within the ipsilesional M1, through similar mechanisms described following motor skill training in healthy adults. However, the neurophysiological mechanisms, and whether these parallel improvements in motor function in chronic stroke patients remain unclear.

Given that ageing is paralleled by changes in GABAergic transmission (Ziemann 2004a; Ziemann et al. 2001), it has been put forward that older adults may have a reduced ability to acquire new motor skills, possibly due to altered corticospinal plasticity (Boyke et al. 2008; Rogasch et al. 2009; Seidler et al. 2010). Certainly, studies in older adults have demonstrated a reduced or non-significant formation of corticospinal plasticity following motor training (Rogasch et al. 2009; Sawaki et al. 2003) as well as following NIBS techniques such as paired associative stimulation (PAS) (Fathi et al. 2010; Kishore et al. 2014; Tecchio et al. 2008), repetitive TMS (rTMS) (Todd et al. 2010) and theta burst stimulation (TBS) (Freitas et al. 2011). Conversely, other findings have shown no differences in use-dependent plasticity following motor training (Cirillo, Rogasch & Semmler 2010; Cirillo, Todd & Semmler 2011; Hinder et al. 2011) or NIBS protocols (Dickins, Sale & Kamke 2015b) between young and older adults. These different findings across the literature may be attributed to the complexity of the motor task (Cirillo, Todd & Semmler 2011), attentional focus (Kamke et al. 2012; Kamke et al. 2014; McNevin, Wulf & Carlson 2000) emotional state (Tormos et al. 1997) as well as the physical activity levels (Cirillo et al. 2009; Cotman & Berchtold 2002) of the older adult populations used within these studies.
Moreover, the limb used to quantify motor performance improvements is an important consideration when interpreting skill acquisition in older adults. Recent evidence has suggested that the non-dominant limb may be more susceptible to motor learning deficits (Hinder, Carroll & Summers 2013), which may be attributed to more pronounced degeneration within the non-dominant M1. Based on the above findings, it is feasible that augmenting the response to motor learning, specifically within the non-dominant limb in older adults, may be an important factor in preserving functional independence with increasing age.

As ADLs involve a combination of force production and coordination, optimal prescription of motor training is important for rehabilitation following stroke and the preservation of neural control in the elderly. Furthermore, given that motor learning appears to be driven by the induction of use-dependent corticospinal plasticity, augmenting the mechanisms that reinforce motor learning may be a viable technique to improve motor function in older adults and chronic stroke patients.

2.7.1.2 Cross-limb transfer following unilateral motor training

One phenomenon that is indicative of use-dependent plasticity is cross-limb transfer, whereby unilateral practice yields performance improvements in both the trained and contralateral homologous limb (Scripture, Smith & Brown 1894). For the cross-transfer of motor skills, the degree of transfer is suggested to be related to the training parameters, such as sensorimotor integration and the novelty and complexity of the learned training task (Farthing, Chilibeck & Binsted 2005; Farthing 2009; Wang & Sainburg 2006). The mechanisms mediating cross-limb transfer have been suggested to be cortical in origin, although spinal and peripheral mechanisms cannot be
completely ruled out (Hortobágyi et al. 2011; Lee et al. 2010). Two central theories underpin this phenomenon, and although not mutually exclusive, appear to be somewhat task-dependent. The ‘cross-activation’ hypothesis suggests a bilateral increase in neuronal activity during unilateral contractions (Lee & Carroll 2007; Parlow & Kinsbourne 1989; Ruddy & Carson 2013). Additionally, the ‘bilateral access’ hypothesis implies that training-induced motor engrams of muscle activation patterns become accessible by the pathways controlling the contralateral limb, most likely through transcallosal pathways (Lee & Carroll 2007; Ruddy & Carson 2013). Although the latter view may be more representative of motor skill and learning transfer, both hypotheses support the role of the M1 ipsilateral to the trained limb in mediating cross-limb transfer.

TMS studies have observed MEP increases within the ipsilateral M1 following unilateral ballistic contractions, alongside improvements in performance of the untrained limb (Carroll et al. 2008; Carroll, Poh & de Rugy 2014; Lee et al. 2010). Moreover, both intracortical and IHI have recently been accentuated as mechanisms underpinning cross-limb transfer of motor skills (Hinder et al. 2010a; Hortobágyi et al. 2011; Perez et al. 2007b). A release of SICI in the ipsilateral M1 is more commonly observed following forceful and ballistic unilateral contractions (Hinder et al. 2011; Liang et al. 2008; Perez & Cohen 2008), however has also recently been observed following slower paced mirror-viewing wrist and finger movements (Reissig et al. 2014; Zult et al. 2015). This suggests that in addition to force production, visual feedback during a movement may modulate intracortical inhibitory networks in the ipsilateral M1 (Reissig et al. 2014; Zult et al. 2015). Further, Perez et al. (2007b) demonstrated reduced SICI within the ipsilateral M1 as well as a reduction in IHI from the contralateral to ipsilateral M1, following training on a serial reaction time task.
(Perez et al. 2007b). This same study reported a correlation between the reduction in IHI and the performance improvement in the untrained limb, highlighting the importance of intracortical and interhemispheric inhibitory processes in mediating the cross-transfer of motor skills (Perez et al. 2007b).

The clinical application of cross-limb transfer has been highlighted in studies whereby neuromuscular strength appears to be maintained throughout periods of unilateral disuse or immobilisation (Farthing, Krentz & Magnus 2009; Magnus et al. 2013; Magnus et al. 2010; Magnus et al. 2014; Pearce et al. 2013). This may be particularly relevant for older adults, where immobilisation due to unilateral injury, post-surgery, or the incident of a stroke is increasingly common. The evidence for cross-limb transfer in ageing is limited, however recent work from Hinder and colleagues (2011 & 2013) has suggested that cross-limb transfer in older adults is absent only from the dominant to non-dominant limb (Hinder, Carroll & Summers 2013; Hinder et al. 2011). Although the mechanisms as to why this occurs are not clear, differences in interhemispheric activity between young and older adults may be involved. The findings from Hinder and colleagues (2011 & 2013), suggest that in older adults the efficacy of cross-limb transfer is limb-dependent. In this respect, the clinical applicability of this training technique during periods of unilateral disuse may be limited. Accordingly, there is a demand to investigate potential methods in which cross-limb transfer can be purposefully enhanced in older adults, particularly within the non-dominantly limb. The application of NIBS may be a viable technique to upregulate the cross-limb transfer of performance to the non-dominant limb, and will be discussed in the following section.
2.7.2 Experimentally-induced plasticity

In addition to use-dependent protocols, the formation of corticospinal plasticity can occur following experimental NIBS. The most common NIBS techniques include TBS, rTMS and tDCS [for review see (Sandrini & Cohen 2013)]. Both TBS and rTMS involve electromagnetic currents delivered via a coil over the target area on the scalp, which discharge action potentials along cortical neurons (Ziemann 2004b). TBS is typically delivered at a frequency of 50 Hz in either intermittent or continuous pulses (Huang et al. 2005), whilst rTMS involves repetitive pulses at low (≤ 1 Hz) or high (≥ 10 Hz) frequencies (Muller et al. 2014; Pascual-Leone et al. 1998). In addition to TBS and rTMS, tDCS involves a painless, low level direct electrical current delivered through surface electrodes placed over the target area on the scalp. tDCS does not typically discharge action potentials, but rather modulates the resting membrane potential of underlying neuronal tissue (Nitsche et al. 2003a). While the mode of delivery differs between these NIBS techniques, there appears to be a common effect on corticospinal excitability and inhibition, which is thought to involve LTP and LTD-like mechanisms (Chen & Seitz 2001; Fritsch et al. 2010; Liebetanz et al. 2002; Thickbroom 2007; Ziemann et al. 2008). In addition to adaptations in corticospinal excitability and inhibition, there is some evidence of behavioural improvements following NIBS combined with motor training (Bolognini et al. 2011; Khedr et al. 2005; Lindenberg et al. 2010; Parikh & Cole 2014; Talelli, Greenwood & Rothwell 2007), which has important implications for motor learning in populations such as ageing and stroke.

Although TBS and rTMS allow for greater spatial resolution compared with tDCS (Priori, Hallett & Rothwell 2009), the technical experience and cost of administration makes these techniques more difficult to translate into clinical practice. Due to the
portable, simplistic and cost-effective nature of tDCS, this method of NIBS has significant clinical relevance and thus has emerged as a popular focus of recent literature. The following sections will review the mechanisms and application of tDCS and the implications for its use with older adults and chronic stroke patients.

2.7.2.1 Transcranial direct-current stimulation

tDCS is a neuro-modulating technique, which has emerged as a potential therapeutic tool to improve motor function in the elderly and following a stroke (Hummel & Cohen 2006; Hummel et al. 2010). tDCS involves a painless, non-invasive low level electrical current applied to the motor area of the brain. The direct current is delivered through two dampened surface electrodes, placed on the target muscle representation over the M1 (Nitsche et al. 2008). The effects of tDCS can be selectively controlled by its parameters; including the polarity and montage of the electrodes, current density and the duration of stimulation (Nitsche et al. 2008; Sohn, Kim & Song 2012). The electrode montage influences the polarity of the underlying neural tissue. Anodal-tDCS (a-tDCS) involves placing the anode over the M1 representation of a specific muscle, whilst the cathode is placed over the contralateral supraorbital area, resulting in increased corticospinal excitability (Bastani & Jaberzadeh 2012). Inversely, cathodal-tDCS (c-tDCS) involves placing the cathode over the M1 representation of a target muscle, whilst the anode is placed over the supraorbital area, resulting in a reduction of corticospinal excitability (Bastani & Jaberzadeh 2012; Nitsche et al. 2008; Nitsche et al. 2005). The configuration of electrodes during bilateral-tDCS involves stimulation of both left and right hemispheres simultaneously, with the anode and cathode placed over both M1s (Mordillo-Mateos et al. 2012). Previous TMS studies
have shown polarity specific changes in corticospinal excitability with stimulation intensities between 1-2 mA for approximately five to 20 minutes, with changes remaining above baseline for up to an hour following a single session (Bastani & Jaberzadeh 2012; Nitsche et al. 2008; Nitsche & Paulus 2000).

2.7.2.2 Neurophysiological mechanisms of tDCS

In healthy younger adults, older adults and stroke patients, studies implementing a single session of tDCS have demonstrated improved hand and arm function on tasks including the Jebsen-Taylor Hand Function Test (Boggio et al. 2006; Fregni et al. 2006; Hummel et al. 2010) simple and choice reaction time (Fregni et al. 2006; Hummel et al. 2006), pegboard (Fregni et al. 2006) and pinch force (Hummel et al. 2006). The functional gains induced by tDCS are accepted to be modulated by a combination of neurophysiological mechanisms, which appear to be analogous to motor learning (Fritsch et al. 2010; Liebetanz et al. 2002; Ziemann & Siebner 2008). During the application of tDCS, there is a shift in the polarity of the resting membrane potential, whereas the prolonged adaptations appear to be modulated by strengthening or weakening of synaptic activity (Creutzfeldt, Fromm & Kapp 1962; Nitsche et al. 2003a; Nitsche et al. 2004c).

Early animal studies demonstrated that the application of a-tDCS depolarises underlying corticospinal neurons whilst c-tDCS produces the inverse effect (Bindman, Lippold & Redfearn 1964; Bishop & O'Leary 1950; Creutzfeldt, Fromm & Kapp 1962). In line with these findings, TMS studies in humans have demonstrated increases in MEP amplitude immediately following a-tDCS, and decreases in MEP amplitude following c-tDCS (Nitsche & Paulus 2000). Additionally, sodium blockers
(carbamazepine) and calcium channel blockers (flunarizine) have been shown to abolish the effects of a-tDCS (Nitsche et al. 2003a), providing evidence that the immediate effects of tDCS are mediated through ionic shifts of sodium and calcium across the neuronal membrane.

The mechanisms responsible for the after-effects of tDCS have been less extensively examined. It is suggested that the after-effects of tDCS appear to be comparable to use-dependent plasticity, in particular, altering synaptic activity at the post-synaptic membrane (Di Lazzaro et al. 2012a; Hummel & Cohen 2005; Monte-Silva et al. 2013). The first line of evidence demonstrating improved synaptic transmission following electrical stimulation of the prefrontal cortex was demonstrated in rats in 1973 (Bliss & Lomo 1973). In more recent animal models, the induction of LTP-like plasticity appeared to be dependent on the activation of the NMDA receptor (Fritsch et al. 2010). Pharmacological interventions have provided evidence for NMDA-dependent synaptic activity following both a-tDCS and c-tDCS (Liebetanz et al. 2002; Malenka & Bear 2004; Monte-Silva et al. 2013; Nitsche et al. 2003a; Nitsche et al. 2004a; Nitsche et al. 2004b). Dextromethorphan, an antagonist of the NMDA receptor, has been shown to suppress the time-course effects of both a-tDCS and c-tDCS, without influencing corticospinal excitability during the stimulation period (Liebetanz et al. 2002; Malenka & Bear 2004; Nitsche et al. 2003a) Similarly, NMDA agonist drugs (D-Cycloserine and amphetamine) prolonged the increases in corticospinal excitability induced by a-tDCS (Nitsche et al. 2004a; Nitsche et al. 2004b). Therefore, it appears that a-tDCS enhances NMDA receptor activity whilst c-tDCS weakens NMDA receptor activity at the post-synaptic membrane (Dudek & Bear 1992; Ranieri et al. 2012). This provides evidence that the modulation of NMDA receptor activity is important for the induction of LTP and LTD-like plasticity following tDCS protocols.
Collectively, the tDCS-induced adaptations appear to be due to an initial shift in the resting membrane potential, which is thought to promote changes in NMDA receptor sensitivity, improving the net synaptic efficacy along the CSP (Liebetanz et al. 2002). Based on the evidence that NMDA receptor activity is involved in both the after-effects of tDCS as well as the process of motor learning, tDCS applied concurrently with motor training may be a beneficial tool to modulate indices of corticospinal plasticity and improve motor performance in populations with altered neurological function, such as older adults and stroke patients.

2.7.2.3 tDCS electrode montage

The polarity specific nature of tDCS allows an opportunity to modulate underlying cortical tissue to achieve physiologically beneficial changes across a range of different populations. Given that older adults and chronic stroke patients are believed to share a pattern of interhemispheric imbalance, appropriate manipulation of the tDCS electrode montage may provide an opportunity to restore the asymmetries in corticospinal excitability and inhibition. The application of a-tDCS increases excitability of the stimulated M1 (Antal et al. 2011; Jeffery et al. 2007; Lang et al. 2004) which has implications for motor performance [For review see (Reis & Fritsch 2011)]. In healthy adults, multiple TMS studies have reported an increase in MEP amplitude in both upper and lower limbs following a single session of a-tDCS (Lang et al. 2004; Nitsche & Paulus 2000; Nitsche & Paulus 2001). The increase in corticospinal excitability following a-tDCS has also been accompanied by a release of SICI (Edwards et al. 2009; Hummel et al. 2005), suggesting that a-tDCS modulates GABAergic neuronal inhibitory circuits.
c-tDCS and its ability to improve motor performance has not been as widely explored. In healthy adults, c-tDCS over the dominant M1 has been thought to supress IHI to the homologous region of the opposite M1 and along the ipsilateral CSP, which indirectly modulates corticospinal excitability (Bradnam, Stinear & Byblow 2011; McCambridge et al. 2011). These proposed mechanisms may underpin the improvements in motor performance of both the contralateral and ipsilateral upper limb (McCambridge et al. 2011; Vines, Nair & Schlaug 2008; Vines, Nair & Schlaug 2006). Similar findings have also been found with stroke patients, whereby c-tDCS over the contralesional M1 indirectly produced improvements in motor function of the paretic limb (Au-Yeung et al. 2014; Fregni et al. 2005). Taken together, tDCS appears to target the activity of intracortical neurons (Lang et al. 2004), which subsequently influence the net excitability along the CSP. Therefore modulating the balance of corticospinal excitability and inhibition through tDCS appears to have positive implications for motor function.

There is much discussion as to the preferential effects of bilateral-tDCS in populations with movement pathology (Bolognini, Pascual-Leone & Fregni 2009; Zimerman & Hummel 2010), however there are limited studies examining the time-course neurophysiological mechanisms. In healthy young individuals, the application of bilateral-tDCS has been shown to simultaneously facilitate neuronal activity within one hemisphere and suppress it in the other (Mordillo-Mateos et al. 2012), with preferential improvements in motor performance compared to unilateral a-tDCS (Vines, Cerruti & Schlaug 2008). Moreover, the reduction in IHI following bilateral-tDCS appears to be an important mechanism mediating improvements in motor function of the non-dominant limb (Williams, Pascual-Leone & Fregni 2010). In older adults, only one study to date has demonstrated the preferential use of bilateral-tDCS
compared to unilateral a-tDCS to modulate activity in both M1s as well as associated motor areas of the neocortex (Lindenberg et al. 2013), but whether or not this was beneficial for motor function was not quantified. Although the available evidence demonstrates the potential for tDCS to augment corticospinal plasticity in an ageing population, the optimal electrode montage and its effects on motor performance are unknown.

The benefits of bilateral-tDCS compared with unilateral electrode montages are mixed; two studies in stroke patients and healthy adults suggested that bilateral-tDCS may be less effective for the formation of corticospinal plasticity and improvements in reaction time and the 9-hole pegboard test (Fusco et al. 2013; O'Shea et al. 2014). However, in the study by Fusco et al. (2013) they examined patients after hospital admission in the very acute phase of stroke, whereby suppressing the contralesional hemisphere may be disadvantageous for motor function of the paretic limb (Bradnam, Stinear & Byblow 2013). Further, in the study by O’Shea et al. (2014), the authors included a population of healthy adults, who may not show a preferential effect from bilateral-tDCS due to the lack of interhemispheric asymmetry, which is supported by previous data (Kidgell et al. 2013). Based on the current evidence, the application of bilateral-tDCS may be advantageous for motor performance where interhemispheric differences in corticospinal excitability and inhibition are observed, such as in older adults and chronic stroke patients.
2.7.2.4 Corticospinal and behavioural responses to tDCS in older adults and stroke patients

The application of tDCS has been presented as a stand-alone therapeutic approach, as well as an add-on technique either before (priming) or during (augmenting) motor training. Although the optimal timing of tDCS delivery remains unclear, there is emerging evidence to suggest that tDCS applied concurrently with motor training produces larger performance gains compared with tDCS applied independently or prior to motor training (Fusco et al. 2014; Parikh & Cole 2014; Reis et al. 2009; Stagg et al. 2011).

In older adults, applying a single session of a-tDCS has been shown to elicit improvements in motor performance and cognition (Hummel et al. 2010; Park et al. 2014; Zimerman et al. 2013), but whether the neurophysiological correlates underpinning behaviour are similar to young adults is unclear. Few studies to date have attempted to quantify the age-related neurological response to a-tDCS (Fujiyama et al. 2014; Heise et al. 2014; Puri et al. 2015). These studies demonstrated that in older adults the response to a-tDCS may be delayed (Fujiyama et al. 2014) and vary considerably with regard to the integrity of the CSP (Heise et al. 2014) and the BDNF polymorphism (Puri et al. 2015). Therefore it is conceivable that applying tDCS concurrent with motor training may augment corticospinal plasticity induced from either motor training or tDCS alone, and improve motor performance in an ageing population. Parikh and Cole (2014), demonstrated a preferential effect of a-tDCS concurrent with motor training on retention of performance (Parikh & Cole 2014), and although neurophysiological mechanisms were not measured, this may suggest that the combination of tDCS and motor training leads to an induction of corticospinal plasticity that consolidates motor learning in older adults.
In stroke patients, a large body of evidence has emerged to support the efficacy of combined tDCS with a range of physical therapies (Bolognini et al. 2011; Edwards et al. 2009; Kim et al. 2014; Lee & Chun 2014; Lefebvre et al. 2012; Madhavan, Weber & Stinear 2011; Ochi et al. 2013). Two recent studies have shown no priming effects of either a-tDCS or bilateral-tDCS applied prior to upper limb rehabilitation (Ang et al. 2015; Fusco et al. 2014). In sub-acute stroke patients, a-tDCS applied prior to a single upper limb rehabilitation session had no additional benefit on hand dexterity (Fusco et al. 2014). Similarly, Ang et al. (2015) reported no additional benefit of bilateral-tDCS applied prior to upper limb rehabilitation with robotic feedback, following a two-week intervention (Ang et al. 2015). The potential use of combined therapy in chronic stroke is further exemplified by the recent findings from Goh et al. (2015), demonstrating that although corticospinal excitability of the ipsilesional M1 was enhanced for up to an hour following a-tDCS, in the absence of concurrent motor training, no effect on motor function of the paretic limb was observed (Goh, Chan & Abdul-Latif 2015). Taken together, these studies suggest that the functional benefits of tDCS may be more pronounced when applied as a supplement to clinical rehabilitation and may augment corticospinal plasticity in older adults and chronic stroke patients, although future longitudinal studies examining the neurological mechanisms are needed.

Following bilateral-tDCS in stroke patients, several studies have reported improved motor function (Bolognini et al. 2011; Lefebvre et al. 2015; Lefebvre et al. 2012; Lefebvre et al. 2014; Lindenberg et al. 2010) as well as corticospinal plasticity (Bolognini et al. 2011; Di Lazzaro et al. 2014; Lefebvre et al. 2015; Lindenberg et al. 2010). However there is a lack of multi-session interventions examining the functional and neurophysiological adaptations of concurrent bilateral-tDCS and rehabilitation.
Evidence from both healthy adults and stroke patients have demonstrated a cumulative
effect of repeated a-tDCS stimulation sessions for improvement and retention of motor
performance (Boggio et al. 2007; Reis et al. 2009). However, neither of these studies
measured the neurophysiological changes associated with the maintenance of these
performance improvements.

Recent findings by Di Lazzaro et al. (2014) demonstrated that in acute stroke patients,
IHI was released following five consecutive bilateral-tDCS and CIMT sessions,
however this did not correspond to any preferential clinical improvement on the Fugl-
Meyer assessment. The lack of tDCS-induced functional improvement may in part be
explained by a ceiling effect that would be likely to occur in the acute phase following
a stroke (Di Lazzaro et al. 2014). In support of this notion, a previous study
demonstrated that five stimulation sessions was insufficient to achieve additional a-
tDCS-induced clinical outcomes (Rossi et al. 2013), and therefore larger doses of tDCS
should be explored. In chronic stroke patients, Bolognini and colleagues (2011)
combined 10 sessions of bilateral-tDCS with CIMT, demonstrating a positive
correlation between functional improvement and reduced IHI from the contra- to
ipsilesional M1 (Bolognini et al. 2011). The authors demonstrated functional
improvements that outlasted the stimulation period up to four weeks, however they did
not measure any corticospinal adaptations at follow-up. Similarly, Lindenberg et al.
(2010) observed improvements in hand function as well as increased cortical activation
of the ipsilesional M1 in chronic stroke patients, outlasting the stimulation period for
one week, however the precise mechanisms involved were not quantified (Lindenberg
et al. 2010). Although there is good evidence for the application of bilateral-tDCS
concurrent with motor training, for improving the retention of motor function
(Bolognini et al. 2011; Lefebvre et al. 2012; Lefebvre et al. 2014), there is still a need
for studies to examine the neurophysiological correlates underpinning these long lasting functional improvements. Furthermore, there is limited evidence to suggest that bilateral-tDCS concurrent with multiple motor training sessions may be beneficial for restoring interhemispheric balance of corticospinal excitability and inhibition.

The application of tDCS has been shown to induce positive behavioural outcomes, through modulating neuronal activity within the M1. Using a model of interhemispheric imbalance in older adults and chronic stroke patients, it is conceivable that bilateral-tDCS may have the potential to restore the balance of corticospinal excitability and inhibition. Further, using tDCS as a supplement to motor training may augment the formation of use-dependent corticospinal plasticity, with favourable outcomes on motor function of the non-dominant and paretic upper limb in older adults and chronic stroke patients respectively.

2.8 Summary

Natural ageing accompanies interhemispheric imbalances in corticospinal excitability and inhibition, most likely due to progressive disuse of the non-dominant limb. Similarly, interhemispheric asymmetries are observed in individuals who have suffered a stroke, with undesirable consequences on motor function of the upper limb. Despite intensive rehabilitation, restoration of motor control following a stroke is incomplete, leaving individuals with impaired motor function and reduced quality of life. The concept of interhemispheric imbalance appears mutual in both an ageing and stroke-affected population. Therefore this thesis investigated whether the application of bilateral-tDCS may have the potential to restore interhemispheric balance between the dominant/contralesional and non-dominant/ipsilesional M1s in these populations.
Further, given that ageing reduces the response to motor training techniques such as cross-limb transfer, it is feasible that the addition of a-tDCS to the ipsilateral M1 may augment the response to this use-dependent plasticity protocol. Taken together, this thesis hypothesised that concurrent tDCS with motor training protocols may enhance the formation of corticospinal plasticity, resulting in favourable outcomes on motor function of the non-dominant and paretic limb. To my knowledge, no studies have quantified the mechanisms involved in the time-course effects and retention of corticospinal plasticity and gains in motor function following bilateral-tDCS and motor training in older adults and chronic stroke patients.
CHAPTER THREE: STUDY ONE

The Effects of Anodal-tDCS on Cross-limb Transfer in Older Adults

Adapted from: Goodwill, AM, Daly, RM & Kidgell, DJ 2015, 'The effects of anodal-tDCS on cross-limb transfer in older adults', Clinical Neurophysiology, vol. 126, no 11, pp. 2189-97.
3.1 Introduction

Bilateral performance improvements can be attained following unilateral training, termed cross-limb transfer. This training technique provides an opportunity to preserve neuromuscular and motor function during periods of unilateral disuse. Although not fully understood, the mechanisms mediating cross-limb transfer involve activation of the ipsilateral primary motor cortex (M1) during unilateral movement, which can be attributed partially to a release of gamma-aminobutyric (GABA)-mediated interhemispheric (Hinder et al. 2010b; Hortobágyi et al. 2011; Perez & Cohen 2008) and/or intracortical inhibition (Goodwill, Pearce & Kidgell 2012; Hinder et al. 2011; Perez & Cohen 2008).

In young adults, cross-limb transfer has been demonstrated following strength (Lee, Gandevia & Carroll 2009), ballistic (Carroll et al. 2008; Lee et al. 2010) and motor skill training such as visuomotor tracking (VT) (Imamizu & Shimojo 1995; Perez et al. 2007a; Sainburg & Wang 2002; Schulze, Luders & Janke 2002). However, evidence in older adults suggests that cross-limb transfer may be unidirectional (Hinder, Carroll & Summers 2013; Hinder et al. 2011), which raises some concern in regards to its clinical application following unilateral injury or immobilisation. Hinder and colleagues (2010 & 2013) showed that following unilateral ballistic training, cross-limb transfer in older adults was present when the non-dominant limb was trained (Hinder, Carroll & Summers 2013) but absent following training of the dominant limb (Hinder et al. 2011). The authors speculated that due to age-related degeneration of interhemispheric networks, cross-limb transfer might not be mediated by cross-activation, which is a viable mechanism mediating this phenomena in young adults (Ruddy & Carson 2013). In addition, the magnitude of cross-limb transfer may be task-dependent, with skilled, novel tasks exerting a greater learning effect in the
untrained limb (Dickins, Sale & Kamke 2015a; Parikh & Cole 2013). Given that older adults and the elderly have an increased risk of unilateral disuse, due to musculoskeletal and neurological injury, it is important to investigate techniques in which cross-limb transfer can be purposefully maximised in the non-dominant limb. Further, the neurophysiological mechanisms contributing to cross-limb transfer in older adults need to be thoroughly investigated.

When performing a unilateral task, there is an age-related increase in electromyography (EMG) activity (i.e. motor overflow) to the contralateral limb, which is thought to reflect enhanced activation in the ipsilateral M1 (Bodwell et al. 2003; Hinder et al. 2011; Mattay et al. 2002; Sailer, Dichgans & Gerloff 2000). However, this appears to have no preferential effect on cross-limb transfer in older adults (Hinder et al. 2011), which implies that the underlying mechanisms of cross-limb transfer reside from a cortical level, and may differ from those involved in motor overflow. It is conceivable that the reduced ability for older adults to modulate intracortical and interhemispheric inhibition (IHI) (Fujiyama et al. 2012b; Sale & Semmler 2005; Talelli et al. 2008) may be a contributing factor to the absence of cross-limb performance transfer observed in this population (Hinder et al. 2011).

Transcranial direct-current stimulation (tDCS) has shown to improve motor performance and corticospinal plasticity in older adults (Heise et al. 2014; Hummel et al. 2010; Zimerman et al. 2013). Given that tDCS has been shown to alter excitability of intracortical neurons (Lang et al. 2011), it conceivable that its application may be beneficial to augment cross-limb transfer in a population with weakened inhibitory transmission, such as older adults. In accordance with this concept, recent data has demonstrated the additive benefits of anodal-tDCS (a-tDCS) modulating short-interval intracortical inhibition (SICI) in older adults (Goodwill et al. 2013; Heise et al. 2014).
Taken together, a-tDCS may be an effective tool to modulate intracortical inhibition within the ipsilateral M1 and mediate the cross-transfer of performance in older adults, but this has not been quantified. Therefore, this study investigated the neurophysiological effects of a-tDCS over the ipsilateral M1 during unilateral VT training, on the cross-limb transfer of motor performance in older compared with younger adults. It was hypothesised that a-tDCS would increase corticospinal excitability and release SICI in the ipsilateral M1 and facilitate cross-limb transfer of motor performance in older adults, to a similar degree as their younger counterparts.

3.2 Materials and methods

Many of the methodological procedures outlined in this chapter are either identical or similar to the methods outlined in the following experimental chapters four and five. Where there is repetition of methodology in the succeeding chapters, the reader will be redirected to sections in this chapter for comprehensive details.

3.2.1 Participants

Twelve healthy older (mean ± SD, 66 ± 1 years; male, n = 6; female, n = 6) and twelve healthy young adults (mean ± SD, 26 ± 1 years; male n = 6; female n = 6) were recruited to participate in this study. All participants were recruited from within the local community in Melbourne, Australia. Participants were excluded from the study if they reported a history of neurological impairment or musculoskeletal injury of the upper limb in the last 12 months or were taking medication known to influence the CNS. One participant reported mild arthritis, however this was not in the wrist. All
participants were tested for handedness according to the 10 item version of the Edinburgh Handedness Inventory (Oldfield 1971) (mean ± SD laterality quotient, 93.0 ± 3.2). Two participants were left handed (mean ± SD laterality quotient -75.0 ± 5.0) and were not excluded from the analyses, rather, their dominant limb was trained. All participants completed an Adult Safety Screening Questionnaire to determine their suitability for transcranial magnetic stimulation (TMS) and tDCS application (Keel et al. 2001). Participants were free of any cognitive impairment as assessed by the Mini-Mental State Examination (MMSE; mean ± SD, young 29.0 ± 0.3; old 29.0 ± 0.5). All participants completed the long version of the International Physical Activity Questionnaire (IPAQ), consisting of 31 items relating to levels of physical activity, specifically; aerobic exercise (i.e. walking, lifting, running, cycling and swimming) in a range of areas such as leisure, work, active transport, and household activities (mean ± SD MET-min/week older adults 3722 ± 1014, younger adults 4430 ± 1143) (Fogelholm et al. 2006). No participants reported playing a long-term musical instrument. All participants provided written informed consent prior to participation in the study, which was approved by the Deakin University Human Research Ethics Committee (2012-081). All procedures were conducted according to the standards established by the Declaration of Helsinki. Copies of the participant information, handedness, MMSE, IPAQ and TMS safety screening questionnaires can be found in the appendices’ (Appendix A-E, pp. 253-262).

3.2.2 Experimental design

The study was a randomised, double-blinded cross-over trial, whereby all participants were exposed to a single session of real a-tDCS and sham-tDCS concurrent with
unilateral training of the dominant limb. Both the researcher and participants were blinded as to whether they were receiving real or sham stimulation. The order of conditions were counterbalanced across participants and separated by a one week wash-out period, which has been recommended to eliminate any carry-over effects of tDCS (Nitsche et al. 2008; Nitsche & Paulus 2001). One week prior to their first experimental session, participants received familiarisation practice trials with the VT task. All participants were exposed to two experimental sessions involving motor training of their dominant limb, with either sham-tDCS or a-tDCS projecting to the M1 ipsilateral to the training limb. Participants were assessed for baseline measures of corticospinal excitability and intracortical inhibition for both M1s, with the order of testing randomised across participants. Following baseline testing, participants were asked to perform 15 10 second bouts of VT of their dominant limb (wrist extensors and flexors). Following VT training, measurements of motor performance, corticospinal excitability and intracortical inhibition were obtained for both limbs, following the same protocols as the baseline measurements. A rest period of five minutes was taken following the training block, to eliminate the potential influence of fatigue on corticospinal excitability and inhibition (Carroll, Riek & Carson 2001a). Experimental procedures are outlined in Figure 3.1 (p. 64) and were identical for both young and older adults and sham-tDCS and a-tDCS conditions.
Figure 3.1 Schematic representation of the experimental protocol. Neurophysiological measures of corticospinal excitability, intracortical inhibition and motor performance were quantified before and five minutes following the cessation of the intervention. AMT, active motor threshold; M_{MAX}, maximum M-wave; MEPs, motor evoked potentials; RMT, resting motor threshold; VT, visuomotor tracking.
3.2.3 Assessment of motor performance

Participants were seated in an office armchair, upright in a neutral position. The elbow was flexed at 90°; shoulder abducted at 45° and the wrist rested on a chair in the neutral anatomical position. This position allowed free movement of the wrist. Participants were fitted with a sensor icon (i.e. actual limb) driven by a single axis goniometer (3DM-GX2, Williston, VT, USA). Participants were instructed to perform voluntary wrist extension and replicate the movement of a target limb displayed on a PC monitor in front of them, as accurately as possible (Figure 3.2, p. 67). The position of the participant’s wrist joint was displayed as a mirrored anatomical representation of their upper limb, which was positioned parallel to the target limb on the screen. The moving target consisted of three, 30 second unique frames that moved automatically in a vertical manner (i.e. wrist extension and flexion) across the screen with varied frequencies (1.1, 1.3 & 1.5 Hz). The presentation of the frequencies was randomised and blinded from the participant.

3.2.4 Transcranial direct-current stimulation protocol

tDCS was applied over the ipsilateral M1 for 15 minutes with a fade-in-fade out of five seconds, to avoid alternating currents causing transient neuronal firing. Two 25 cm² electrodes, soaked in a saline solution (0.9% NaCl), were placed over the cortical representation of the non-dominant extensor carpi radialis (ECR) muscle, as explored and determined with transcranial magnetic stimulation (TMS), and secured with a rubber strap. In all conditions, the anode was placed over the ipsilateral M1 to the trained limb, in the area corresponding with the participant’s non-dominant “ECR optimal site”, and the cathode over the contralateral supraorbital area. Stimulation was
delivered at 1 mA (current density 0.040 mA/cm²) through a DC-stimulator (NeuroConn DC stimulator, Ilmenau, Germany). Both the primary researcher and participants were blinded to whether they received real or sham stimulation. This was achieved as the tDCS machine used was coded to allow for real or pseudo (sham) stimulation. In the sham-tDCS condition, stimulation ceased after approximately 20 seconds providing a pseudo-stimulation effect (Gandiga, Hummel & Cohen 2006). In order to obtain the participants perception of discomfort across both tDCS conditions, a visual analogue scale (VAS) was used. During the first minute of stimulation participants were asked to rate their perceived sensation on a 10 point scale with 0 relating to no sensation or discomfort and 10 representing extreme sensation and discomfort.

3.2.5 Motor training protocol

During exposure to tDCS, participants were required to perform 15 10 second trials of VT of the wrist, with 30 seconds rest between each trial (Figure 3.2, p. 67). Frequencies of 1.1, 1.3 and 1.5 Hz were presented randomly, so that the participant was exposed to each frequency five times throughout the training block. Surface EMG (sEMG) was recorded from the untrained ECR muscle throughout the training block to quantify the presence or absence of motor overflow.
Figure 3.2 Visual representation of the motor training protocol, with a-tDCS electrode placement and surface electromyography (sEMG) on the contralateral untrained limb.
3.2.6 Recording of surface electromyography

sEMG was recorded from the ECR muscle in both limbs using bipolar Ag-AgCl electrodes. Two electrodes were placed 2 cm apart on the mid belly of the ECR, with a ground strap placed around the wrist as a common reference for all electrodes. All cables were fastened with tape to prevent movement artefact. The skin was prepared (i.e. shaved and swabbed with alcohol) prior to electrode placement to ensure a clear signal was obtained. sEMG signals were amplified (x1000), bandpass filtered (high pass at 13 Hz, low pass at 1000 Hz), digitized online at 2 kHz for 500 milliseconds, recorded and analysed using PowerLab 4/35 (ADInstruments, Bella Vista, Australia).

3.2.7 Transcranial magnetic stimulation and maximal compound waves

Single and paired-pulse TMS were delivered over the cortical representation of the ECR, using a figure-of-eight coil (external wing diameter 90 mm) attached via a BiStim unit, to two Magstim 200\(^2\) stimulators (Magstim, Dyfed, UK). The coil was positioned over the M1 so that the current flowed in a posterior-anterior direction. Sites near the estimated centre of the ECR were explored to obtain the largest MEP amplitude (i.e. optimal site), and this area was marked by a small “X”. Participants maintained this mark throughout the intervention to ensure consistency and reliability of coil placement within and between sessions.

Measures of resting motor threshold (RMT), active motor threshold (AMT), motor evoked potential (MEP) amplitudes at 130% AMT and SICI were recorded in order to quantify corticospinal excitability and intracortical inhibition. A five minute rest period following the cessation of the intervention was allowed to minimise any effects of fatigue. RMT and AMT were defined as the stimulator intensity at which at least
five out of ten stimuli produced MEP amplitudes of greater than 50 µV and 200 µV respectively (Rossini et al. 1994). MEP amplitudes were evaluated by producing 10 stimuli at a test-intensity of 130% AMT. All MEPs were recorded during weak voluntary contraction whereby participants positioned their hand in line with their wrist (i.e. anatomically neutral). To maintain a constant level of background muscle activity, participants performed three maximal isometric contractions and the largest maximal root mean square EMG (maximal \textit{rmsEMG}) recording was obtained. Visual feedback of muscle \textit{rmsEMG} was displayed on an oscilloscope (HAMEG, Mainhausen, Germany) and participants were asked to maintain a light contraction no greater than 5% ± 2 of maximal \textit{rmsEMG}. Pre stimulus \textit{rmsEMG} of the ECR was obtained 100 milliseconds prior to each TMS stimulus. SICI was obtained by first delivering a conditioning stimulus at 80% of AMT (subthreshold) followed by a test stimulus at 120% AMT (suprathreshold), separated by a three millisecond inter-stimulus interval (ISI) (Zoghi, Pearce & Nordstrom 2003; Garry & Thomson 2009). Specifically, 10 test stimuli and 10 conditioned stimuli were delivered with the order of presentation randomised throughout the sessions. A rest period of 30 seconds was provided between stimuli sets to avoid muscular fatigue. For the paired-pulse paradigm, both the test and conditioning stimulator intensities were adjusted if any changes in AMT were observed, so that the MEP amplitudes were always equivalent to the true percentage of AMT.

Maximal compound waves (M-waves) were obtained from the ECR muscle by direct supramaximal electrical stimulation (pulse duration one millisecond) of the radial nerve under resting conditions. A high-voltage constant current stimulator (DS7, Digitimer®, Hertfordshire, UK) delivered each electrical pulse. Stimulation was delivered by positioning bipolar electrodes over the radial nerve on the distal, lateral
shaft of the humerus. An increase in current strength was applied until there was no further increase in sEMG amplitude ($M_{MAX}$). To ensure maximal responses, the current intensity was increased an additional 20% and the average $M_{MAX}$ obtained from five stimuli was delivered and recorded at 0.2 Hz.

All TMS and M-wave procedures were performed for both limbs at each time point and the order of limb testing was randomised across participants and conditions.

3.2.8 Data analysis

VT error was assessed in 10 second epochs, and calculated by normalising the root mean square error/deviation by using the actual data’s range (maximum minus minimum) and then converted to a percentage.

Any MEPs with pre stimulus $rms$EMG that exceeded $5\% \pm 2$ maximal $rms$EMG were discarded and repeated at the appropriate intensity (Sale & Semmler 2005). MEP amplitudes were analysed using LabChart 8 software (ADInstruments, Bella Vista, Australia), which provided peak-to-peak values in mV and were then expressed as a ratio of $M_{MAX}$ for each individual.

In order to quantify SICI, the raw average conditioned MEP was divided by the raw average single-pulse (i.e. test response) MEP and then multiplied by 100 (Kujirai et al. 1993). Based on this calculation, an increase in the SICI ratio depicts a release of intracortical inhibition and a decrease in the ratio indicates the inverse.

sEMG of the contralateral limb during training was measured as the average peak amplitude of the $rms$EMG from the onset of VT through to the offset of motor training.
3.2.9 Statistical analysis

The number of participants required was based on power calculations for the expected changes in mean VT error and change in corticospinal excitability of the untrained limb, using data from a previous study examining cross-limb transfer in older adults (Hinder et al. 2011). It was estimated that 24 participants would provide at least 80% power to detect a 20% difference (effect size of .35) in VT error and MEP amplitudes assuming a SD of 10-15% and 12-20% respectively, between conditions at P < 0.05 (two-tailed).

All data was screened for normal distribution using the Shapiro-Wilks test, with the data being judged as normally distributed (P > 0.05). Independent t-tests were used to compare baseline differences in continuous variables between young and old and between sham-tDCS and a-tDCS for the same age, sEMG motor overflow in the contralateral wrist during training between young and old and VAS scores between sham-tDCS and a-tDCS. Motor performance (VT error), corticospinal excitability (RMT, AMT, MEPs at 130% AMT) and SICI were assessed using linear mixed-models. The model included time (baseline and post), age group (young and old) and condition (a-tDCS and sham-tDCS) as fixed main effects, and an interaction between age, condition and time, with participant as a random effect. Where significant time main effects were detected, paired samples t-tests were used to examine pre and post-intervention changes for each condition by age group. Analysis was performed using IBM SPSS Statistics 21 with an alpha cut off < 0.05 deemed significant. All data are presented as Mean ± SEM unless stated otherwise.
3.3 Results

3.3.1 Baseline characteristics

There were no differences between the sham-tDCS and a-tDCS conditions for each age group at baseline. Baseline differences between young and old revealed that older adults displayed a higher VT error score across both a-tDCS and sham-tDCS conditions, compared with younger adults (P < 0.05). There were no differences in RMT or AMT, MEP amplitudes at 130% AMT (% M\textsubscript{MAX}) or SICI for either hemisphere or limb between young and older adults at baseline (Tables 3.1 and 3.2, p. 73 and 81). There were no differences in VAS ratings between the sham-tDCS and a-tDCS conditions during the first minute of stimulation (P = 0.19).
Table 3.1 Mean (±SEM) baseline TMS data.

<table>
<thead>
<tr>
<th>Condition</th>
<th>RMT (%SO)</th>
<th>AMT (%SO)</th>
<th>MMAX (mV)</th>
<th>Pre stimulus</th>
<th>Conditioning</th>
<th>Test Stimuli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a-tDCS</td>
<td>Sham</td>
<td>a-tDCS</td>
<td>Sham</td>
<td>a-tDCS</td>
<td>Sham</td>
</tr>
<tr>
<td>Dominant M1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>44.8 ± 3.1</td>
<td>44.8 ± 3.0</td>
<td>36.5 ± 3.1</td>
<td>36.0 ± 2.9</td>
<td>5.1 ± 0.4</td>
<td>4.9 ± 0.6</td>
</tr>
<tr>
<td>Young</td>
<td>45.6 ± 3.4</td>
<td>45.4 ± 3.4</td>
<td>36.7 ± 2.4</td>
<td>36.8 ± 2.4</td>
<td>7.4 ± 0.7</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>Non-dominant M1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>43.3 ± 2.7</td>
<td>43.9 ± 2.2</td>
<td>35.4 ± 3.1</td>
<td>35.7 ± 3.1</td>
<td>3.9 ± 0.4</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>Young</td>
<td>44.8 ± 3.7</td>
<td>44.9 ± 3.6</td>
<td>36.8 ± 2.5</td>
<td>37.5 ± 2.9</td>
<td>5.3 ± 0.5</td>
<td>5.2 ± 0.5</td>
</tr>
</tbody>
</table>

AMT, active motor threshold; MEP, motor evoked potential; MMAX, maximum M-wave; RMT, resting motor threshold; SICI, short-interval intracortical inhibition; SO, stimulator output; VT, visuomotor tracking.
3.3.2 Surface electromyography of the untrained limb

Values for sEMG of the untrained limb during unilateral training (% of maximal \( r_{ms}EMG \)) were 0.7%, 0.5%, 2.5%, 3.6% for young sham-tDCS, young a-tDCS, old sham-tDCS and old a-tDCS respectively. Older adults displayed a significantly larger degree of motor overflow than the young adults (\( P < 0.001 \)). Older adults receiving a-tDCS also displayed a significantly larger degree of motor overflow compared with the sham-tDCS (\( P = 0.04 \)) (Figure 3.3).

**Figure 3.3** Mean (±SEM) surface electromyography (sEMG) recording from the untrained limb during unilateral training. Results are presented for young and older adults in both the sham-tDCS and a-tDCS conditions. * denotes \( P < 0.05 \).
3.3.3 Motor performance

3.3.3.1 Trained limb

For the trained dominant limb, there were no significant differences in the magnitude of VT improvement between older and young adults or sham-tDCS and a-tDCS (time-condition-age interaction; F = 0.6, P = 0.65). On average, VT error improved from baseline by 17.6% ± 3.0 for the older sham-tDCS (P < 0.001), 26.8% ± 3.2 for the older a-tDCS (P < 0.001), 18.1% ± 5.4 for the young sham-tDCS (P = 0.02) and 28.0% ± 4.8 for the young a-tDCS (P = 0.003) (Figure 3.4 A, p. 76).

3.3.3.2 Untrained limb

For the untrained non-dominant limb, older adults receiving sham-tDCS did not improve VT error (1.8% ± 4.0, P = 0.66), whereas the older a-tDCS, young sham-tDCS and young a-tDCS conditions improved VT error from baseline by 18.9% ± 2.5, 19.8% ± 2.5 and 27.9% ± 3.7 respectively (all P < 0.001) (Figure 3.4 B, p. 76). However, there was no time-condition-age interaction for the change in VT error (F = 0.6, P = 0.66).
Figure 3.4 Mean (±SEM) percentage change values for visuomotor tracking (VT) error for the trained, dominant (A) and untrained, non-dominant (B) limbs in young and older adults, for the sham-tDCS and a-tDCS conditions. ^ denotes P < 0.05 within-condition change from baseline.
3.3.4 Corticospinal excitability

There were no main effects for time, or time-condition-age interactions in the trained or untrained limb for MMAX, AMT, RMT or pre stimulus rmsEMG (all P > 0.05).

3.3.4.1 Trained primary motor cortex

For the trained (dominant) M1, the magnitude of MEP facilitation at 130% AMT following unilateral training was similar across both sham-tDCS and a-tDCS conditions for young and older adults (time-condition-age interaction; F = 0.5, P = 0.75). On average, MEP facilitation improved by 37.7% ± 8.0 in older sham-tDCS (P = 0.003), 36.3% ± 13.4 in older a-tDCS (P = 0.02); 47.7% ± 14.7 in young sham-tDCS (P = 0.003) and 34.9% ± 13.8 in young a-tDCS (P = 0.002) (Figure 3.5 A, p. 78).

3.3.4.2 Ipsilateral (untrained) primary motor cortex

For the ipsilateral (non-dominant) M1, there was no significant time-condition-age interaction (F = 0.2, P = 0.95) for the change in MEP amplitude in the ipsilateral M1 following training (Figure 3.5 B, p. 78). However, within-condition analysis revealed that there was significant facilitation of MEP amplitude for the older a-tDCS (27.3% ± 8.0, P = 0.03), young sham-tDCS (27.6% ± 6.1, P = 0.01) and young a-tDCS (34.2% ± 8.8, P = 0.003), but not for the older adults receiving sham-tDCS (9.6% ± 8.5, P = 0.46).
Figure 3.5 Mean (±SEM) percentage change values for motor evoked potential (MEP) amplitudes at 130% active motor threshold (AMT) for the trained, dominant (A) and untrained, non-dominant (B) M1s in young and older adults, for the sham-tDCS and a-tDCS conditions. ^ denotes $P < 0.05$ within-condition change from baseline.
Figure 3.6 Overlayed motor evoked potential (MEP) recordings from the ipsilateral, untrained M1. MEPs recorded at 130% active motor threshold (AMT) in one participant from the older adults at baseline (i) and post intervention (ii), for the sham-tDCS (A) and a-tDCS (B) conditions.
3.3.5 Short-interval intracortical inhibition

3.3.5.1 Trained primary motor cortex

For the trained (dominant) M1, there was no time-condition-age interaction for SICI (F = 0.2, P = 0.91), but there was a significant release of SICI following unilateral motor training in both tDCS conditions for young and older adults (older sham-tDCS 32.5% ± 9.8 and old a-tDCS 30.7% ± 4.4, both P < 0.001; young sham-tDCS 16.9% ± 4.9 and young a-tDCS 20.3% ± 6.6, both P = 0.002; Figure 3.7 A, p. 82).

3.3.5.2 Ipsilateral (untrained) primary motor cortex

In the ipsilateral (non-dominant) M1, there were no differences in the magnitude of SICI release in the ipsilateral M1 following unilateral training (time-condition-age interaction; F = 0.3, P = 0.89). However, within-condition analysis revealed a significant release in SICI across both tDCS conditions for young and older adults (older sham-tDCS 13.7% ± 5.0, P = 0.01; old a-tDCS 33.1% ± 8.2, young sham-tDCS 24.1% ± 5.3, young a-tDCS 22.2% ± 2.2, all P < 0.001; Figure 3.7 B, p. 82).
Table 3.2  Mean (±SEM) raw values for motor performance and neurophysiological variables.

<table>
<thead>
<tr>
<th>Group</th>
<th>VT error (%)</th>
<th>MEP amplitudes (%M\textsubscript{MAX})</th>
<th>SICI ratio (% of test response)</th>
<th>VT error (%)</th>
<th>MEP amplitudes (%M\textsubscript{MAX})</th>
<th>SICI ratio (% of test response)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
<td>Baseline</td>
<td>Post</td>
<td>Baseline</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
<td>Baseline</td>
<td>Post</td>
<td>Baseline</td>
<td>Post</td>
</tr>
<tr>
<td><strong>Trained (dominant)</strong></td>
<td><strong>Sham-tDCS</strong></td>
<td>34.7 ± 2.6 * 28.6 ± 2.4 ^</td>
<td>23.6 ± 4.8</td>
<td>32.5 ± 5.6 ^</td>
<td>46.4 ± 4.0</td>
<td>61.5 ± 3.6 ^</td>
</tr>
<tr>
<td></td>
<td><strong>Real-tDCS</strong></td>
<td>37.9 ± 3.2</td>
<td>27.8 ± 1.9 ^</td>
<td>18.3 ± 2.2</td>
<td>46.3 ± 3.6</td>
<td>60.4 ± 3.7 ^</td>
</tr>
<tr>
<td><strong>Untrained (non-dominant)</strong></td>
<td><strong>Sham-tDCS</strong></td>
<td>32.1 ± 2.3</td>
<td>31.4 ± 2.7</td>
<td>48.1 ± 5.0</td>
<td>48.1 ± 5.0</td>
<td>54.8 ± 5.7 ^</td>
</tr>
<tr>
<td></td>
<td><strong>Real-tDCS</strong></td>
<td>34.7 ± 2.4</td>
<td>28.1 ± 2.1 ^</td>
<td>28.2 ± 4.6</td>
<td>46.3 ± 4.8</td>
<td>61.6 ± 4.1 ^</td>
</tr>
</tbody>
</table>

Shaded boxes indicate significant variables. * denotes $P < 0.05$ baseline difference in older compared with younger adults for the same variable. ^ denotes $P < 0.05$ within-condition change from baseline. MEP, motor evoked potential; M\textsubscript{MAX}, maximum M-wave; SICI, short-interval intracortical inhibition; VT, visuomotor tracking.
Figure 3.7 Mean (±SEM) percentage change values for the release of short-interval intracortical inhibition (SICI) in the trained, dominant (A) and untrained, non-dominant (B) M1s in young and older adults, for sham-tDCS and a-tDCS conditions. ^ denotes P < 0.05 within-condition change from baseline.
3.4 Discussion

The purpose of this study was to examine whether the application of a-tDCS applied over the ipsilateral M1 would facilitate corticospinal plasticity and cross-limb transfer following motor skill training in young and older adults. There were several important new findings from this study that add to the clinical efficacy of tDCS in modulating motor performance in older adults. First, the extent of motor performance improvement in the trained limb did not differ between young and old adults. However, when a-tDCS was applied to the ipsilateral M1, older adults exhibited cross-limb transfer to the untrained non-dominant limb, which was absent in the sham-tDCS condition. These findings suggest that a-tDCS may improve the cross-transfer of motor skills in older adults. Second, corticospinal plasticity was not significantly different between young and older adults when a-tDCS was applied to the ipsilateral M1. This demonstrates that motor skill training in the absence of a-tDCS is still effective in forming use-dependent plasticity in both young and older adults. Based upon these findings, the present data indicate that young and older adults demonstrate similar levels of use-dependent plasticity, which is not significantly influenced by the application of a-tDCS.

3.4.1 Motor performance in the trained and untrained limb following unilateral training

In the trained limb, motor training improved VT error in both conditions for older and young adults by 18 to 28% with no significant differences in the magnitude of improvement between the age groups. While some studies have reported diminished performance improvements and corticospinal excitability/inhibition following motor
training in older adults (Goodwill et al. 2013; Rogasch et al. 2009; Sawaki et al. 2003), the current findings are consistent with reports that there are no significant age-related differences in the ability to improve performance following motor training, regardless of differences in baseline performance (Cirillo, Rogasch & Semmler 2010; Cirillo, Todd & Semmler 2011). A number of factors may explain these contrast findings. Certainly, reductions in baseline performance between older and younger adults are not always consistent, with some evidence demonstrating the ability for older adults to retain a high capacity to learn and perform new motor skills (Wu & Hallett 2005). Further, physical activity may be a prominent factor influencing the induction of corticospinal plasticity and acquisition of motor skills. It is well-established that regular physical activity upregulates the expression of neurotrophins in the cortex, in particular, brain derived neurotrophic factor (BDNF) (Neeper et al. 1995). BDNF is as a key mediator for motor learning, synaptic efficacy and use-dependent plasticity (McAllister, Katz & Lo 1999; Schinder & Poo 2000), and although not explicitly quantified, may in part have contributed to the induction of use-dependent plasticity in the physically active (~3000 MET-mins/week) older adults in this study.

In the untrained limb, unilateral training produced cross-limb transfer of performance for young adults, regardless of the tDCS condition, but only for the older adults receiving a-tDCS. These findings are in agreement with previous studies suggesting cross-limb transfer is absent in the non-dominant limb following ballistic tasks (Hinder et al. 2011; Parikh & Cole 2013), but are inconsistent with recent evidence suggesting intermanual transfer is maintained in the non-dominant limb following motor skill training (Dickins, Sale & Kamke 2015a). One key difference that may have contributed to the discrepancies of these findings may be the motor tasks used to quantify performance. Previous studies used the first dorsal interosseous (FDI) and
abductor pollicis brevis (APB) muscles, whereas the current study used the ECR muscle. Despite the lack of interaction between young and older adults, the within-effects over time for improved cross-transfer of motor performance with a-tDCS in the older adults warrant further discussion. When a-tDCS was applied in conjunction with motor training, the 19% transfer of performance improvement to the untrained limb in older adults was comparable to the 20 to 28% improvement observed in younger adults following unilateral training.

To the best of my knowledge, this is the first study to demonstrate that a-tDCS applied to the ipsilateral M1 can induce a similar magnitude of cross-limb performance transfer in older adults when compared with that of their younger counterparts. There are several possibilities as to why the cross-transfer of performance was facilitated following a-tDCS in the older adults but not in the sham-tDCS condition. The magnitude of motor overflow was significantly lower in the older adults receiving sham-tDCS, whereby no improvement in performance was observed. When a-tDCS was combined with unilateral training, a greater amount of motor overflow and a significant improvement in performance was observed in the untrained limb. The findings from Bodwell and colleagues (2003) revealed that an increase in motor overflow manifests as a result of increased bi-hemispheric activity of both the contralateral and ipsilateral M1 (Bodwell et al. 2003). Therefore, it is conceivable that increasing bilateral corticospinal excitability via the application of a-tDCS, may play an important role in the induction of motor learning of the untrained limb (Hendy, Spittle & Kidgell 2012; Ruddy & Carson 2013). Certainly, theories of bi-hemispheric activity such as cross-activation, which suggests a spill-over of neural drive from the active to the inactive hemisphere via transcallosal pathways, may regulate the cross-transfer of motor performance (Carroll et al. 2006; Hortobágyi et al. 2011; Ruddy &
Carson 2013). The increase in corticospinal excitability of the ipsilateral M1 in the current study supports such a theory, which may have contributed to the improvements in motor learning of the non-dominant untrained limb in older adults. Importantly, the absence of cross-transfer of performance following sham-tDCS supports previous findings that reported no cross-limb transfer to the non-dominant limb following a ballistic motor task (Hinder et al. 2011). Whilst further large-scale studies are needed, the current findings for the application of a-tDCS applied to the ipsilateral M1 could be of clinical importance during periods of unilateral injury.

3.4.2 Bilateral corticospinal excitability following unilateral training

It is well established that there is an induction of corticospinal plasticity that occurs following motor skill training and tDCS (Ziemann et al. 2004; Ziemann et al. 2001; Zimerman & Hummel 2010). In the present study, the magnitude of corticospinal excitability following motor training was not different between conditions, demonstrating that young and older adults can form a similar degree of use-dependent corticospinal adaptations following motor training of the dominant limb. Importantly, the magnitude of change in corticospinal excitability in the older adults was consistent with previous studies in young adults (Carroll et al. 2008; Cirillo, Todd & Semmler 2011; Lee et al. 2010; Muellbacher et al. 2000). On this basis, the results of this study show that VT training facilitates corticospinal plasticity of the trained M1 in older adults, which is in contrast to the findings from several previous studies (Goodwill et al. 2013; Rogasch et al. 2009; Sawaki et al. 2003).

Although there was no significant time-condition-age interaction found in the present study, an important finding was the within-condition effects for increased MEP
amplitudes in the ipsilateral M1 of older adults only following a-tDCS. Interestingly, the magnitude of MEP facilitation following a-tDCS to the ipsilateral M1 was similar to the magnitude of motor learning improvement of the untrained limb. Therefore, it is possible that the application of a-tDCS to the ipsilateral M1 may have facilitated the cross-transfer of performance to some degree. Certainly, the involvement of the ipsilateral M1 regulating the cross-limb transfer has been well documented in young adults (Carroll et al. 2008; Chen, Cohen & Hallett 1997; Lee et al. 2010; Verstynen et al. 2005), but is absent in the non-dominant limb for older adults (Hinder et al. 2011). Despite the lack of an interaction found in this study, older adults exhibited increased corticospinal excitability in the ipsilateral M1 only following a-tDCS, which suggests that its application may be beneficial in facilitating the induction of corticospinal plasticity and improving the cross-transfer of motor performance. However, these findings need to be confirmed in large-scale trials.

3.4.3 Bilateral intracortical inhibition following unilateral training

It is well accepted that modulation of intracortical inhibitory pathways help the acquisition of skilled movements (Stinear & Byblow 2003; Zoghi, Pearce & Nordstrom 2003) and the release of intracortical inhibition plays a crucial role in mediating use-dependent plasticity (Ziemann et al. 2001). In relation to the age-related changes in SICI, mixed findings have been reported across the literature, with some studies reporting an increase (Kossev et al. 2002; McGinley et al. 2010), a decrease (Peinemann et al. 2001), or no differences (Oliviero et al. 2006) in cortical inhibition in older compared to younger adults. In this study, no differences in SICI between young and older adults were observed at baseline for either hemisphere. The
aforementioned differences in baseline SICI between young and older adults may in part be a result of the background muscle activity in which testing was conducted.

Following unilateral training, a bilateral reduction in SICI was shown in both young and older adults, irrespective of the a-tDCS or sham-tDCS condition. This suggests that the use of a VT task was able to modulate inhibitory circuits in both the trained and ipsilateral M1 across both age groups, which is consistent with findings in young (Perez et al. 2004) and older adults (Cirillo, Todd & Semmler 2011) following VT training. In both young and older adults, tDCS over the dominant M1 has been shown to modulate SICI (Goodwill et al. 2013; Kidgell et al. 2013; Nitsche et al. 2005), but to my knowledge no studies to date have quantified the effect of a-tDCS over the ipsilateral M1 during unilateral training. In the older adults, SICI was reduced in the ipsilateral M1 following both sham-tDCS and a-tDCS. Interestingly, the magnitude of SICI release between hemispheres for both young and old adults was not different. On this basis, the complexity of a VT task may have reduced the synaptic efficacy of GABA\(_A\)-mediated neurons forming cortico-cortical networks, releasing pyramidal neurons from inhibition (Floeter & Rothwell 1999; Kujirai et al. 1993). In support of this notion, recent work from Zult et al. (2015) demonstrated that mirror feedback of the moving hand modulated inhibitory networks in the M1 ipsilateral to the moving hand (Zult et al. 2015). Therefore, it is conceivable that the visual feedback of the moving limb obtained from performing a VT task modulated SICI within the ipsilateral M1, and contributed to the cross-limb transfer of performance. Taken together, these results suggest that there is a bilateral use-dependent modulation of SICI following unilateral training of a VT task, which is not influenced by the addition of a-tDCS.
3.4.4 Limitations

Although the mechanisms of a-tDCS alone have been well documented, a potential limitation of the current study was the lack of a control condition that received a-tDCS in the absence of motor training. Thus, it is possible that the performance improvements in the untrained limb may have been a direct result of the a-tDCS rather than the up-regulation of the mechanisms within the ipsilateral M1 produced by unilateral training. A further limitation was the inability to quantify IHI, which has recently been identified to be reduced following tDCS (Williams, Pascual-Leone & Fregni 2010) as well as following unilateral training (Hortobágyi et al. 2011). As the current study observed a release of SICI in both the trained and ipsilateral M1, the contribution of IHI contributing to changes in SICI following a-tDCS and unilateral training cannot be overlooked, and need to be quantified in future work. Further, the establishment of SICI in a resting muscle may have provided additional insight into the intracortical mechanisms mediating the differences in motor performance of the non-dominant limb between young and older adults. It is important to note that the physical activity levels between the young and older adults in this cohort did not significantly vary. Therefore it should be considered that a-tDCS may have had a more profound effect on sedentary older adults, which may be subject to greater degeneration.

3.4.5 Conclusions and future directions

Cross-limb transfer has important implications for the maintenance of motor function during immobilisation following surgery as well as neurological injury such as stroke. Given the emerging evidence that this phenomenon may be absent with advancing age,
it is of importance that techniques to augment cross-limb transfer are identified. Although this study found no interactions between the young and older adults for tDCS conditions, the improvements observed in the older adults following a-tDCS provide preliminary evidence for the potential role of the ipsilateral stimulation contributing to the cross-transfer of performance. Furthermore, the efficacy of cross-limb transfer and ipsilateral a-tDCS applied as a clinical add-on technique during rehabilitative programs needs to be addressed by future studies. This study provides preliminary evidence that unilateral a-tDCS is a promising technique to improve motor learning in older adults, to a similar magnitude as their younger counterparts. These findings provide a rationale to explore alternate tDCS electrode montages aiming to maximise performance gains in an ageing population, which forms the basis for the research questions addressed in the following chapter.
CHAPTER FOUR: STUDY TWO

Formation of Corticospinal Plasticity in Older Adults Following tDCS and Motor Training

Adapted from: Goodwill, AM, Reynolds, J, Daly, RM & Kidgell, DJ 2013, 'Formation of cortical plasticity in older adults following tDCS and motor training', Frontiers in Aging Neuroscience, vol. 5, p. 87.
4.1 Introduction

The natural age-related decline in neuromuscular control may be more prominent within the non-dominant upper limb (Coppi et al. 2014; Sale & Semmler 2005; Ward & Frackowiak 2003). With the projected rise in life expectancy, there is a demand to identify strategies to preserve neuromuscular function with advancing age. In young adults, single sessions of motor training modulates corticospinal plasticity in a task-dependent manner (Adkins et al. 2006; Cirillo, Todd & Semmler 2011; Perez, Lundbye-Jensen & Nielsen 2006; Perez et al. 2004) with evidence of improved motor performance (Garry, Kamen & Nordstrom 2004; Ziemann et al. 2001). However, ageing may impair the ability to modulate gamma-aminobutyric acid (GABA)-mediated inhibition (Opie, Ridding & Semmler 2015; Talelli et al. 2008), which has been suggested to disrupt the formation of use-dependent plasticity following motor training (Rogasch et al. 2009; Sawaki et al. 2003).

Transcranial direct-current stimulation (tDCS) is a promising technique to preserve motor function in older adults (Hummel et al. 2010; Parikh & Cole 2014; Zimerman et al. 2013). The electrode montage is an important element, shaping the neurophysiological responses to tDCS. Unilateral anodal-tDCS (a-tDCS) produces transient increases, whilst cathodal-tDCS (c-tDCS) exerts momentary decreases in corticospinal excitability (Bastani & Jaberzadeh 2012; Nitsche et al. 2008). Additionally, bilateral-tDCS (simultaneously increasing excitability in one hemisphere whilst suppressing it in the other) has been shown to modulate intracortical and interhemispheric excitability and inhibition in healthy adults (Mordillo-Mateos et al. 2012; Williams, Pascual-Leone & Fregni 2010) and stroke patients (Bolognini et al. 2011; Lefebvre et al. 2012; Lindenberg et al. 2010). However, limited studies have
examined the time-course neurophysiological mechanisms following bilateral-tDCS in older adults.

Given that the formation of use-dependent plasticity may be reduced in older adults (Rogasch et al. 2009; Sawaki et al. 2003), concurrently applying tDCS with motor training may consolidate the mechanisms that are observed following motor training in young adults. In older adults, a-tDCS combined with a single session of motor training has shown to improve performance, lasting up to 24 hours after the stimulation (Parikh & Cole 2014; Zimerman et al. 2013). However, as both these studies only measured changes in motor performance, the neurophysiological after-effects of tDCS mediating the retention of motor skills are unclear.

There is limited evidence as to whether the physiological effects of tDCS are similar in older and younger adults. One study applying a single session of a-tDCS, observed a delay in the formation of corticospinal plasticity, however the overall magnitude was similar to younger adults (Fujiyama et al. 2014). Moreover, the formation of corticospinal plasticity may be dependent on the functional connectivity of the corticospinal pathway (CSP) (Heise et al. 2014). On this basis, tDCS has the potential to induce corticospinal plasticity in healthy older adults, however the optimal electrode montage to improve motor function in older adults remains unknown.

In younger adults (aged 22-40), experimental evidence comparing the effects of bilateral-tDCS and unilateral-tDCS on motor performance and corticospinal excitability have produced mixed findings. Vines et al. (2008) observed greater improvements in motor performance of the non-dominant hand following bilateral-tDCS compared to unilateral-tDCS and sham-tDCS (Vines, Cerruti & Schlaug 2008). Moreover, task-concurrent bilateral-tDCS may have a preferential effect on motor
learning, through effective modulation of corticospinal excitability (Karok & Witney 2013). In contrast, Mordillo-Mateos et al. (2012) reported no difference in corticospinal excitability between unilateral-tDCS and bilateral-tDCS (Mordillo-Mateos et al. 2012), however, changes in GABA-mediated inhibition have not been quantified in these studies. Only one study has compared unilateral a-tDCS and bilateral-tDCS in older adults using functional magnetic resonance imaging (fMRI) (Lindenberg et al. 2013). During both active and resting-state, bilateral-tDCS produced stronger bi-hemispheric activation of motor areas compared to the unilateral a-tDCS (Lindenberg et al. 2013). However, the after-effects of different electrode montages on GABA-mediated inhibition and motor performance are unknown.

Given asymmetries in corticospinal excitability and inhibition have been reported in older adults (Coppi et al. 2014), the combination of bilateral-tDCS and motor training may enhance motor performance through improving interhemispheric balance, however this has not been examined. Therefore, this study compared sham-tDCS, unilateral-tDCS and bilateral-tDCS combined with motor training on corticospinal excitability, short-interval intracortical inhibition (SICI) and motor performance of the non-dominant upper limb in healthy older adults. It was hypothesised that bilateral-tDCS would improve motor performance of the non-dominant limb immediately and 30 minutes following stimulation compared with unilateral-tDCS and sham-tDCS.
4.2 Materials and methods

Many of the methods employed in the current study are comprehensively outlined in chapter three. The following methods are an abridged version of the sections specific to this chapter.

4.2.1 Participants

Eleven healthy older adults (5 female, 6 male; mean ± SD, 63 ± 2 years; range 55-80) with no history of neurological or musculoskeletal impairment participated in the study. No medications taken by participants influenced central nervous system (CNS) conduction (acimax-1; zometa-1; allopurinol-1; Glucosamine-1; Fish Oil Capsules-1; Minipress-1; Aspirin-1; Karvezide-1; Nexium-1; Oruvail-1). Two participants reported mild arthritis, however this was not confined to the wrist. All participants were tested for handedness to quantify their non-dominant limb, according to the 10 item version of the Edinburgh Handedness Inventory (mean ± SD laterality quotient, 89.0 ± 5.2) (Oldfield 1971). One participant was left handed (mean laterality quotient -70.0) and was not excluded from the analyses, rather, this participant’s non-dominant limb was tested. All participants completed an Adult Safety Screening Questionnaire to determine their suitability for transcranial magnetic stimulation (TMS) and tDCS application (Keel, Smith & Wassermann 2001). Participants were free of any cognitive impairment as assessed by the Mini-Mental State Examination (MMSE, mean ± SD 29 ± 0.8). All participants completed the long version of the International Physical Activity Questionnaire (IPAQ), consisting of 31 items relating to levels of physical activity, specifically, aerobic exercise (i.e. walking, lifting, running, cycling and swimming) in a range of areas such as leisure, work, active transport, and household
activities (Craig et al. 2003; Fogelholm et al. 2006). No participants reported playing a long term musical instrument. All participants provided written informed consent prior to participation in the study, which was approved by the Deakin University Human Research Ethics Committee (2012-081). All procedures were conducted according to the standards established by the Declaration of Helsinki.

4.2.2 Experimental design

This study was a double-blinded, cross-over sham controlled trial, whereby all participants were exposed to three single session tDCS conditions combined with motor training, with a one week wash-out period between each condition. Active tDCS conditions included both a unilateral and bilateral electrode montage, whereas the third condition was a sham-tDCS. The delivery of each condition was randomised across participants and followed identical testing protocols. Participants were required to complete a familiarisation session one week prior to the commencement of the study to reduce the effect of learning the motor performance task. All participants were tested for baseline measures of corticospinal excitability and intracortical inhibition, as well as motor performance. Following baseline testing, participants were exposed to a total of 15 minutes of tDCS. After the first five minutes of stimulation, participants performed five minutes of visuomotor tracking (VT) training. Time-course measures of corticospinal excitability, SICI and VT error were taken immediately after and 30 minutes following the stimulation (Figure 4.1, p. 97).
Figure 4.1 Schematic representation of the experimental protocol. Neurophysiological measures of corticospinal excitability, intracortical inhibition and motor performance were quantified at baseline, immediately following and 30 minutes following the intervention. AMT, active motor threshold; $M_{\text{MAX}}$, maximum M-wave; MEPs, motor evoked potentials; VT, visuomotor tracking.
4.2.3 Assessment of motor performance

The moving target consisted of three, 30 second unique frames that moved automatically in a vertical manner (i.e. wrist extension and flexion) across the screen with varied frequencies. Each frame was repeated twice, with a total testing time of three minutes, and the presentation of the frequencies were randomised.

Refer to section 3.2.3. (p. 65) for a detailed description of the VT protocol.

4.2.4 Transcranial direct-current stimulation protocol

In all conditions, the anode was placed over the non-dominant primary motor cortex (M1) in the area corresponding with the participant’s non-dominant extensor carpi radialis (ECR) “optimal site”. In the unilateral-tDCS condition, the cathode was placed over the contralateral supraorbital area. During the bilateral-tDCS condition, the cathode was placed over the dominant M1 corresponding to the “ECR optimal site”. For the sham-tDCS condition, participants randomly received either the unilateral or bilateral electrode montage (i.e. 50% of participants were allocated to each montage) following the electrode placements described above.

Refer to section 3.2.4 (pp. 65-66) for a detailed description of the tDCS protocols and delivery of stimulation.
4.2.5 Motor training protocol

After the first five minutes of tDCS, all participants performed five minutes of VT with their non-dominant wrist (Figure 4.2). Five 30 second frames were used with alternating movement frequencies, where each frame was repeated twice.

Figure 4.2 Visual representation of the motor training protocol during the bilateral-tDCS condition.
4.2.6 Recording of surface electromyography

Recording of surface electromyography (sEMG) followed the same procedures described in Section 3.2.6 (p. 68).

4.2.7 Transcranial magnetic stimulation and maximal compound waves

Refer to Section 3.2.7 (pp. 68-70) for a detailed description of the single and paired-pulse TMS and M-wave protocols. Variations to the protocols in chapter three are described below.

For the paired-pulse paradigm only, the test stimulus intensity used was the stimulator output required to produce motor evoked potentials (MEPs) of ~1 mV. The test stimulus intensity was adjusted if necessary, so that the test MEP amplitudes were always equivalent to ~1 mV (Cirillo, Todd & Semmler 2011). SICI was obtained by delivering a conditioning stimulus at 80% of AMT (subthreshold) followed by a test stimulus (~1 mV; suprathreshold), separated by a three millisecond inter-stimulus interval (ISI).

4.2.8 Data analysis

Refer to Section 3.2.8 (p. 70) for the description of analyses regarding the VT error, MEP amplitudes and SICI.

Additionally, a laterality index (LI) for interhemispheric asymmetries in corticospinal excitability was calculated on the basis of the mean difference in MEP amplitudes between the two hemispheres. These methods have been widely described using both
MEP amplitudes and fMRI in stroke patients and older adults (Cramer et al. 1997; Di Lazzaro et al. 2014; Langan et al. 2010). Based on these studies, LI ranges from −1 to +1 with a greater distance from 0 representing a higher interhemispheric imbalance. In older adults, positive values denote greater excitability of the dominant M1. In healthy individuals, LI tends to 0, with slight differences due to hemispheric (i.e. limb) dominance.

4.2.9 Statistical analysis

The number of participants required was based on power calculations for the expected mean change motor performance (i.e. VT error). Using data from a previous cross-over study using tDCS and motor training in older adults (Fujiyama et al. 2012a; Hummel et al. 2010), it was estimated that 10 participants would provide at least 80% power to detect a 20% difference in VT error and corticospinal plasticity, assuming a SD of 10-20% between conditions at P < 0.05 (two-tailed).

A split-plot in time, repeated measures analysis of variance (ANOVA) was used to determine the effects of motor training over time (baseline, immediately post [post 0] and 30 minutes post [post 30]) and conditions (sham-tDCS, unilateral-tDCS and bilateral-tDCS) on all outcome variables (motor performance, corticospinal excitability, SICI). One-way ANOVA was used to assess VAS scores and baseline LI across each condition. Paired t-tests were conducted to quantify any differences in AMT, MEP amplitudes and SICI between hemispheres. The Greenhouse-Geisser epsilon correction was applied to the degrees of freedom associated with F-tests and t-tests when Box’s test indicated a departure from the assumption of sphericity and the epsilon was < 0.8. Consequently, some F-ratios are reported with non-integer degrees.
of freedom. Diagnostic plots of residuals were used to check the assumptions of homogeneity of variance and normality. When significant main effects or interactions were present, Fisher’s LSD was used to compare means. All analyses including calculation of means ± SEMs were performed with GenStat statistical software (Release 14.2) using a 5% significance level (P < 0.05).

4.3 Results

All participants were comfortable with both TMS and tDCS procedures and reported no adverse side effects. Visual analogue scale (VAS) recordings during the first three minutes of tDCS revealed no differences between the perception of the stimulation between conditions (F2, 20 = 0.6, P = 0.57; mean 2.4 ± 0.4).

4.3.1 Baseline characteristics

Table 4.1 (p. 103) displays the mean baseline values for TMS measures of corticospinal excitability and inhibition. No differences in rmsEMG, MMAX, AMT, MEP amplitudes and SICI ratios at baseline were observed across conditions (all P > 0.05). There were no differences between the dominant and non-dominant M1 for baseline AMT (t32 = -1.9, P = 0.07), MEP amplitude (t32 = -1.2, P = 0.26), and SICI ratio (t32 = 1.5, P = 0.13). The LI for interhemispheric excitability averaged across all conditions was 0.04 ± 0.05, indicating no asymmetries in corticospinal excitability between the dominant and non-dominant M1. There was no difference in baseline LI across conditions (F2, 32, = 0.2, P = 0.85).
### Table 4.1 Mean (±SEM) baseline TMS data. Results are averaged for all conditions.

<table>
<thead>
<tr>
<th>M1</th>
<th>AMT (%SO)</th>
<th>MMAX (mV)</th>
<th>Test stimuli (%SO) ~1 mV</th>
<th>Conditioning stimuli (%SO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND</td>
<td>30.8 ± 1.4</td>
<td>11.5 ± 0.8</td>
<td>41.4 ± 1.4</td>
<td>24.6 ± 1.2</td>
</tr>
<tr>
<td>D</td>
<td>29.1 ± 1.1</td>
<td>12.6 ± 0.7</td>
<td>38.3 ± 1.2</td>
<td>23.3 ± 0.9</td>
</tr>
</tbody>
</table>

AMT, active motor threshold; D, dominant M1; MMAX, maximal M-wave; ND, non-dominant M1; SO, stimulator output.

### 4.3.2 Pre stimulus rmsEMG

The mean rmsEMG (mV) prior to single and paired-pulse recordings for the non-dominant M1 was 0.05 ± 0.004 and 0.05 ± 0.003 respectively. There were no condition-by-time interactions for pre stimulus rmsEMG for single-pulse (F_{2.41,36.18} = 1.4, P = 0.26), or paired-pulse (F_{4,60} = 0.4, P = 0.84) signals. For the dominant M1, the mean pre stimulus rmsEMG for single-pulse MEPs were 0.06 ± 0.004 and 0.05 ± 0.004 for paired-pulse MEPs. There was no condition-by-time interaction for rmsEMG for single-pulse (F_{4,58} = 1.9, P = 0.13) or paired-pulse (F_{4,60} = 1.7, P = 0.15) signals.

### 4.3.3 Motor performance

Figure 4.3 (p. 104) and Table 4.2 (p. 110) display the average VT errors for each condition across time. There was a significant reduction in the proportion of VT error...
over time (F_{1.38,40.13} = 19.0, P < 0.001) and a significant main effect for condition (F_{2,19} = 4.0, P = 0.03), but the condition-by-time interaction was not significant (F_{2.77,40.13} = 2.0, P = 0.13). Immediately following tDCS, only the unilateral-tDCS and bilateral-tDCS conditions improved motor performance by 12.9% ± 2.2 (P = 0.01), and 21.6% ± 4.0 (P < 0.001) respectively. At 30 minutes all conditions appeared to improve relative to baseline (sham-tDCS 10.0% ± 4.5, P = 0.02; unilateral-tDCS 11.9% ± 3.0, P = 0.01; bilateral-tDCS 21.7% ± 4.0, P < 0.001). There were no significant differences between unilateral-tDCS or bilateral-tDCS for either time point (all P > 0.05).

**Figure 4.3** Mean (±SEM) values for visuomotor tracking (VT) error for the sham-tDCS, unilateral-tDCS and bilateral-tDCS conditions at baseline, immediately post (post 0) and 30 minutes following the cessation of stimulation (post 30). * denotes P < 0.05 within-condition change relative to baseline.
4.3.4 Corticospinal excitability

4.3.4.1 Non-dominant primary motor cortex

There was no condition-by-time interaction for $M_{\text{MAX}}$ in the non-dominant ($F_{2.63, 39.46} = 0.8, P = 0.50$) or dominant M1 ($F_{4, 60} = 0.2, P = 0.95$). Figure 4.4 (p. 107) and Table 4.2 (p. 110) display the average MEP amplitudes for the non-dominant and dominant M1. There was a significant condition-by-time interaction ($F_{4, 60} = 4.3, P = 0.004$). The change from baseline to immediately post stimulation for both the unilateral-tDCS and bilateral-tDCS conditions (37.8% ± 4.8, $P = 0.02$ and 53.1% ± 6.7, $P < 0.001$, respectively) were significantly greater than the change for the sham-tDCS condition, and this was sustained at 30 minutes (unilateral-tDCS 49.0% ± 9.9, $P = 0.01$, and bilateral-tDCS, 54.5% ± 6.2, $P = 0.003$). There were no significant differences in MEP amplitude between unilateral-tDCS and bilateral-tDCS conditions at either time point (all $P > 0.05$).

4.3.4.2 Dominant primary motor cortex

For the dominant M1, there was a significant condition-by-time interaction ($F_{4, 60} = 3.7, P = 0.01$). There were no significant changes over time in either the sham-tDCS or the unilateral-tDCS condition ($P > 0.05$) but in the bilateral-tDCS condition the decreases over time from baseline to the immediate and 30 minutes post time points were significant (13.8% ± 3.7, $P = 0.01$ and 14.7% ± 3.7, $P = 0.003$, respectively).
4.3.4.3 Laterality index

Based on the change in MEP amplitudes within the dominant and non-dominant M1, there was a significant condition-by-time interaction for LI ($F_{4, 60} = 11.9$, $P < 0.001$). Immediately following stimulation bilateral-tDCS resulted in a shift in the LI towards a more negative value (bilateral-tDCS, $27.3\% \pm 3.5$), which was greater than the change following the unilateral-tDCS ($14.3\% \pm 3.7$; $P = 0.003$) and sham-tDCS (0.9$\% \pm 3.4$; $P < 0.001$) conditions, and was maintained at 30 minutes post stimulation (both $P < 0.05$).
Figure 4.4 Mean (±SEM) motor evoked potential (MEP) amplitudes (%M_max) recorded at 130% active motor threshold (AMT) for the sham-tDCS, unilateral-tDCS and bilateral-tDCS conditions at baseline, immediately post (post 0) and 30 minutes (post 30): non-dominant M1 (A) and dominant M1 (B). * denotes P < 0.05 within-condition change relative to baseline. † denotes P < 0.05 compared with the sham-tDCS condition.
Figure 4.5 Overlayed motor evoked potential (MEP) recordings at 130% active motor threshold (AMT) from the non-dominant M1 for one participant at baseline (i), immediately post (ii) and 30 minutes following the cessation of stimulation (iii) for the sham-tDCS (A), unilateral-tDCS (B) and bilateral-tDCS (C) conditions.
4.3.5 Short-interval intracortical inhibition

4.3.5.1 Non-dominant primary motor cortex

For the mean SICI ratios, there was a significant condition-by-time interaction ($F_{4, 60} = 3.4$, $P = 0.01$) (Figure 4.6, p. 111; Table 4.2, p. 110). The change from baseline to immediately post stimulation and 30 minutes post stimulation in the unilateral-tDCS (post $29.2\% \pm 6.4$, $P = 0.01$; 30 minutes $21.2\% \pm 7.8$, $P = 0.03$) and bilateral-tDCS (post $36.3\% \pm 6.3$, $P = 0.003$; 30 minutes $30.2\% \pm 7.1$, $P = 0.01$) conditions was significantly greater than for the change in the sham-tDCS condition ($4.1\% \pm 4.1$ and $0.4\% \pm 5.7$). There were no significant differences in SICI ratios between the unilateral-tDCS and bilateral-tDCS conditions at any time point (all $P > 0.05$).

4.3.5.2 Dominant primary motor cortex

For the dominant hemisphere, there was no effect for time ($F_{2, 60} = 1.1$, $P = 0.36$) or condition-by-time interaction ($F_{4, 60} = 1.4$, $P = 0.24$) for SICI.
Table 4.2 Mean (±SEM) raw values for motor performance and neurophysiological variables.

<table>
<thead>
<tr>
<th>Group</th>
<th>VT error</th>
<th>MEP amplitudes (%M_{MAX}) at 130% ATM</th>
<th>SICI ratio (% of test response)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline Post 0 Post 30</td>
<td>Baseline Post 0 Post 30</td>
</tr>
<tr>
<td>Non-dominant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>24.5 ± 1.6 23.5 ± 1.5 22.1 ± 1.0 *</td>
<td>13.7 ± 2.1 14.7 ± 2.3 15.9 ± 2.7</td>
<td>32.0 ± 2.3 33.3 ± 2.9 31.8 ± 3.1</td>
</tr>
<tr>
<td>Unilateral</td>
<td>23.1 ± 0.6 20.1 ± 0.8 20.3 ± 0.9 * *</td>
<td>14.0 ± 2.0 19.2 ± 2.8 20.8 ± 3.1</td>
<td>33.5 ± 2.4 43.2 ± 3.1 40.6 ± 3.1</td>
</tr>
<tr>
<td>Bilateral</td>
<td>23.2 ± 1.4 18.3 ± 0.9 18.2 ± 0.9 * *</td>
<td>14.5 ± 2.1 22.1 ± 3.3 22.3 ± 3.3</td>
<td>31.9 ± 2.3 43.5 ± 4.1 41.6 ± 3.3</td>
</tr>
<tr>
<td>Dominant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td></td>
<td>12.9 ± 2.2 13.6 ± 2.2 13.7 ± 2.5</td>
<td>32.8 ± 2.7 33.1 ± 2.6 32.9 ± 2.3</td>
</tr>
<tr>
<td>Unilateral</td>
<td></td>
<td>13.5 ± 2.4 14.0 ± 2.4 13.9 ± 2.2</td>
<td>35.1 ± 2.7 35.1 ± 2.6 35.5 ± 2.3</td>
</tr>
<tr>
<td>Bilateral</td>
<td></td>
<td>12.5 ± 2.1 10.8 ± 2.2 10.7 ± 2.0 * *</td>
<td>36.0 ± 3.3 32.0 ± 2.1 32.6 ± 2.5</td>
</tr>
</tbody>
</table>

Shaded boxes indicate significant variables. * denotes P < 0.05 within-condition change from baseline. † denotes P < 0.05 difference compared with the sham-tDCS condition. AMT, active motor threshold; MEP, motor evoked potential; M_{MAX}, maximum M-wave; NA, non-applicable (not measured); SICI, short-interval intracortical inhibition.
Figure 4.6 Mean (±SEM) short-interval intracortical inhibition (SICI) ratios (% of the test response) at baseline, immediately post (post 0) and 30 minutes following the cessation of stimulation (post 30), for the sham-tDCS, unilateral-tDCS and bilateral-tDCS conditions: non-dominant (A) and dominant M1 (B). * denotes P < 0.05 within-condition change relative to baseline. † denotes P < 0.05 compared with the sham-tDCS condition.
4.4 Discussion

The main finding from this study was that motor training combined with unilateral-tDCS or bilateral-tDCS induced corticospinal plasticity and improved motor performance that persisted for up to 30 minutes post stimulation, whereas performance improvements following sham-tDCS were observed only by 30 minutes. No differences in the indices of corticospinal plasticity or motor performance were observed between unilateral-tDCS and bilateral-tDCS conditions. Collectively, these findings suggest that combining either unilateral-tDCS or bilateral-tDCS with motor training represents an effective strategy to induce corticospinal plasticity and expedite motor learning in older adults.

4.4.1 Motor performance following tDCS

The present findings suggest that the tDCS electrode montage does not differentially modulate motor performance in older adults. Although motor improvements in older adults have been shown to be sustained following unilateral a-tDCS (Hummel et al. 2010; Parikh & Cole 2014; Zimerman et al. 2013), a novel aspect to this study was that bilateral-tDCS produced similar improvements in older adults. Previous findings have suggested a preferential increase in motor performance following bilateral-tDCS, compared to unilateral-tDCS and sham-tDCS (Vines, Cerruti & Schlaug 2008), however this was not evident in this study. The current findings suggest that the addition of motor training of the non-dominant limb may have augmented the excitatory response from the anode (in both conditions), modulating corticospinal excitability of the non-dominant M1 in a similar manner, contributing to the non-significant differences between unilateral-tDCS and bilateral-tDCS.
In this study, the 13 to 21% improvement in motor performance following active tDCS (unilateral-tDCS and bilateral-tDCS) is slightly larger than a previous investigation in older adults that observed a 2-10% improvement following a single session session of a-tDCS applied in isolation (i.e. no motor training) (Hummel et al. 2010). The greater performance gains in the current study could be explained by the fact that tDCS was applied not only in conjunction with, but prior to and following the VT task. It is established that the acute effects of tDCS induce spontaneous neuronal firing which primes the M1 and may therefore consolidate the effects of motor training (Nitsche & Paulus 2000; Nitsche & Paulus 2001; Vines, Nair & Schlaug 2006). Although not quantified, the use of a VT task may have activated other cortical and subcortical motor areas that are known to contribute to motor performance and learning of a motor task (Bolam et al. 2000). In support of this, the magnitude of performance improvement observed in this study is consistent with the performance improvements induced by VT in young healthy adults, without the addition of tDCS (Cirillo, Todd & Semmler 2011; Perez, Lundbye-Jensen & Nielsen 2006). Given the evidence supporting the reduced effectiveness of motor training alone in older compared with young adults (Rogasch et al. 2009; Zimerman et al. 2013), it appears the addition of tDCS has acted to accelerate motor learning in an older population, which has been previously shown in healthy younger adults (Parasuraman & McKinley 2014). This finding suggests that the improved corticospinal activity following tDCS may be beneficial to facilitate motor performance. However, it should be noted that there was some improvement in motor performance (~10%) at 30 minutes following motor training alone, suggesting a practice effect of the training in which older adults are still able to improve motor performance. In light of this, the findings support the concept that tDCS can accelerate motor learning in this population, though, older adults do still appear to benefit from motor training alone.
4.4.2 Corticospinal excitability following tDCS

Motor performance improvements have been observed following unilateral a-tDCS in older adults (Hummel et al. 2010; Zimerman et al. 2013); however the mechanisms underpinning these after-effects have not been previously quantified. Further, no study has examined the effects of bilateral-tDCS on modulating corticospinal excitability and motor performance in older adults. The current findings demonstrate no significant differences in the indices of corticospinal plasticity between unilateral-tDCS and bilateral-tDCS. These results are in agreement with Mordillo-Mateos et al. (2012) who also observed that corticospinal excitability in young adults was not differentially modulated by unilateral-tDCS and bilateral-tDCS (Mordillo-Mateos et al. 2012). Based upon these findings, it can be speculated that the alternate tDCS electrode montages induce similar physiological mechanisms, underlying the improvements in motor performance.

The current findings show that in older adults, both unilateral-tDCS and bilateral-tDCS facilitated MEPs both immediately after and 30 minutes following stimulation. These findings are in agreement with studies performed in young adults whereby MEPs were increased following an acute bout of either unilateral-tDCS or bilateral-tDCS (Lang et al. 2004; Mordillo-Mateos et al. 2012; Nitsche & Paulus 2000; Nitsche & Paulus 2001; Williams, Pascual-Leone & Fregni 2010). However, contrary to a previous study conducted in older adults (Fujiyama et al. 2014), the current study did not observe any delay in the onset of facilitated corticospinal excitability. It is feasible that the concurrent effects of the VT training with tDCS applied in this study, augmented and potentially promoted the earlier onset of corticospinal plasticity induction.
In the dominant M1, MEP amplitudes were suppressed for the bilateral-tDCS condition only, which supports previous literature in young adults (Williams, Pascual-Leone & Fregni 2010). Reduced corticospinal excitability within the dominant M1 suggests that the cathode may have suppressed motor overflow of neuronal activity in the M1 ipsilateral to the trained limb (dominant M1), however this did not appear to differentially affect motor performance in the bilateral-tDCS compared with unilateral-tDCS condition. Further, the shift in the LI towards excitability in the non-dominant M1 following bilateral-tDCS depicts the simultaneous increase and decrease in excitability of the non-dominant and dominant M1s respectively. However, as baseline LI values were almost symmetrical (i.e. close to 0), this would have been unlikely to have a profound effect on motor performance. As there were no differences in corticospinal excitability or motor performance of the non-dominant limb following either unilateral-tDCS or bilateral-tDCS, the effect of the cathode over the dominant M1 may have been minimal. Although a 15% reduction in corticospinal excitability of the dominant M1 was observed in this study, previous data has suggested that individual variability in the response to tDCS, in particular cathodal stimulation, may be due to genetic factors such as the polymorphism of brain derived neurotrophic factor (BDNF) (Antal et al. 2010). Although not quantified in this study, the potential for these factors to contribute to the non-significant differences between the responses to unilateral-tDCS or bilateral-tDCS cannot be overlooked. Therefore, it should be considered that there may be no optimal electrode montage for inducing corticospinal plasticity and improving motor performance in older adults.

The finding that tDCS induced corticospinal excitability which outlasted the stimulation period, appears reflective of LTP-like mechanisms that have been observed following motor learning in rats (Rioult-Pedotti, Friedman & Donoghue...
2000; Sanes & Donoghue 2000), and has more recently been suggested to occur in humans (Ziemann et al. 2004). Previous pharmacological investigations have applied dextromethorphan (an NMDA receptor antagonist) to alter the after-effects of tDCS (Liebetanz et al. 2002; Nitsche et al. 2003a), therefore it can be proposed that the after-effects of tDCS are indicative of modifications in NMDA receptor-dependent neurotransmission. The small and non-significant increase in corticospinal excitability following motor training with sham-tDCS supports the reduced ability for older adults to form use-dependent plasticity following motor training alone, at least from a short bout of motor training (Rogasch et al. 2009; Sawaki et al. 2003).

The current results indicate that during natural ageing, there may be a limited response to use-dependent plasticity inducing protocols that reflect the involvement of LTP-like processes. In vivo studies have certainly shown that in an ageing rat model, the interaction between dopamine, GABA and glutamate in the basal ganglia is decreased, which may be reflective of decreased activity in glutamate receptor binding sites (i.e. NMDA receptor) (Mora, Segovia & Del Arco 2008; Segovia & Mora 2005). Additionally, in aged human brain tissue, a reduction in dopamine uptake and NMDA receptor activity has been observed (Villares & Stavale 2001). Given that these neurotransmitters located in the basal ganglia are important for the acquisition and performance of motor patterns, degeneration of these structures may contribute to the delayed onset of motor performance improvement observed in this study. Collectively, the current evidence suggests the additive combination of tDCS and motor training may have improved sensitivity and unmasking of excitatory synapses at the post-synaptic membrane, improving synaptic efficacy and neural transmission along the corticospinal pathway (Nielsen & Cohen 2008).
4.4.3 Intracortical inhibition following tDCS

It is established that GABA-mediated neurotransmission plays an important role in shaping excitatory output, and is partially modulated by NMDA receptor activity (Ziemann et al. 1998; Ziemann et al. 2001; Zoghi, Pearce & Nordstrom 2003). Therefore, the balance of intracortical inhibition is vital for efficient and coordinated movement (Marneweck, Loftus & Hammond 2011; Rothwell et al. 2009). Previously, the effect of tDCS on intrinsic inhibitory circuits has not been investigated in older adults. The current study demonstrated a release of SICI in the non-dominant M1 following both unilateral-tDCS and bilateral-tDCS relative to sham-tDCS, but importantly, there were no differences between the two different electrode montages.

The current finding that tDCS reduced SICI by 21-36% is comparable to studies in young adults and stroke patients (Batsikadze et al. 2013; Edwards et al. 2009; Hummel et al. 2005; Nitsche et al. 2005), which demonstrates that improvement in corticospinal excitability may be modulated by a release of intracortical inhibition. Contrary to the findings from this study, Williams et al. (2010) combined bilateral-tDCS with motor training in healthy young adults and found no effect on SICI (Williams, Pascual-Leone & Fregni 2010). Although comparisons to this study should be viewed with caution due to different tDCS parameters used, the age of the cohort used in the current study may have also contributed to the tDCS induced changes in SICI. Certainly, there is evidence for age-related deficits in SICI circuitry (Fujiyama et al. 2009; Fujiyama et al. 2012b; Sale & Semmler 2005), and therefore it is possible that older adults may respond more favourably to modulation of inhibitory circuits via the application of tDCS.
Based upon the current findings it can be speculated that a release in GABAergic inhibition has improved the synaptic efficacy between intracortical and corticospinal neurons (Nitsche et al. 2003a). In support of this, previous data in rats suggests that synaptic plasticity is enhanced by a release of intracortical inhibition (Hess, Aizenman & Donoghue 1996). In this study, there was a non-significant change in SICI in the dominant M1, possibly contributing to the non-significant differences between the release of SICI following unilateral-tDCS and bilateral-tDCS. Although a recent study in older adults observed differences in spatial activation between bilateral-tDCS and unilateral-tDCS (Lindenberg et al. 2013), it is conceivable that contribution from other motor control pathways such as interhemispheric networks, basal ganglia circuits and the posterior cingulate cortex involving GABAergic synapses, may contribute to corticospinal excitability and motor performance improvements following tDCS (Baudewig et al. 2001; Bolam et al. 2000; Lindenberg et al. 2013; Williams, Pascual-Leone & Fregni 2010). Irrespective of this, these results support the contribution of released GABA-related inhibitory activity in the M1 on the overall net excitatory output and improved motor performance of the non-dominant limb in older adults.

4.4.4 Limitations

Several limitations of the study need to be considered. The hypothesis that bilateral-tDCS may induce greater motor performance gains was based around interhemispheric differences in older adults. Although there was a trend towards a larger percentage improvement for motor performance following bilateral-tDCS, the non-significant differences between unilateral-tDCS and bilateral-tDCS may be due to a lack of interhemispheric asymmetry (i.e. LI close to 0) at baseline in the cohort of healthy
older adults recruited into this study. It is possible that more deconditioned older adults and other clinical populations with greater interhemispheric imbalances, may demonstrate a greater responsiveness to bilateral-tDCS compared with other montages. Further, this study used a conditioning stimulus of 80% AMT which has been shown to induce SICI mediated by GABA_A receptors (Smyth, Summers & Garry 2010; Zoghi & Nordstrom 2007), however, the interaction between intracortical inhibitory and facilitatory circuits contributing to the reduction in SICI should be considered when interpreting these findings (Shirota et al. 2010). Lastly, although the sample size was similar to previous studies in older adults, larger trials are needed to detect whether any clinically meaningful differences are present between unilateral and bilateral electrode montages.

4.4.5 Conclusions and future directions

This study indicates that tDCS induced corticospinal plasticity and expedited the improvement in motor performance in older adults, irrespective of the unilateral or bilateral electrode montage. These findings underscore the prospective use of tDCS to improve the activity of neurons within the M1 and motor performance in the elderly. Future investigations need to employ larger sample sizes and longer trials assessing retention, to evaluate whether the tDCS electrode montage differentially improves motor performance in an ageing population. Given that tDCS concurrent with motor training has shown to improve motor performance in an ageing model, investigation into the efficacy of this technique in clinical populations such as stroke is warranted. Furthermore, as repeated bouts of tDCS have been suggested to have a cumulative effect (Boggio et al. 2007; Reis et al. 2009), the longer term outcomes of a multi-
session intervention in chronic stroke patients will be explored in the subsequent chapter (study three).
CHAPTER FIVE: STUDY THREE

Concurrent Bilateral-tDCS and Upper Limb Rehabilitation

Improves Retention of Motor Function in Chronic Stroke
5.1 Introduction

Recovery of upper limb function following a stroke is thought to be limited by a combination of factors, including the extent of damage within the corticospinal pathway (CSP) and abnormal interactions between the ipsi- and contralesional primary motor cortex (M1) (Boroojerdi, Diefenbach & Ferbert 1996; Stinear et al. 2007). In particular, disinhibition of the contralesional M1 has been shown to exert an inhibitory effect onto the ipsilesional M1, which may be a maladaptive characteristic impeding motor recovery the paretic limb (Liepert, Hamzei & Weiller 2000; Murase et al. 2004).

In healthy adults, bilateral transcranial direct-current stimulation (bilateral-tDCS) has been shown to increase corticospinal excitability in one hemisphere whilst simultaneously suppressing excitability in the contralateral hemisphere (Mordillo-Mateos et al. 2012). In this regard, it is plausible that bilateral-tDCS may serve to normalise excitatory and inhibitory corticospinal networks within the ipsi- and contralesional M1 in stroke patients and improve motor function in the paretic limb (Feng, Bowden & Kautz 2013; Nowak et al. 2009).

Preliminary evidence in acute and chronic stroke patients have reported increased activation in the ipsilesional M1 and reduced interhemispheric inhibition (IHI) from the contra- to ipsilesional M1 following bilateral-tDCS (Bolognini et al. 2011; Di Lazzaro et al. 2014; Lefebvre et al. 2015; Lindenberg et al. 2010), with evidence of improved motor function (Bolognini et al. 2011; Lefebvre et al. 2015; Lindenberg et al. 2010). However limited studies have assessed the effects of bilateral-tDCS on intracortical inhibition, which is thought to play a significant role in motor recovery following a stroke (Clarkson et al. 2010; Hummel et al. 2009; Schiene et al. 1996). Given that bilateral-tDCS has shown to have a neuromodulatory effect on cortical
neurons (Lang et al. 2011; Lang et al. 2004), it is reasonable to suggest that modulation of inhibitory interneurons within the ipsi- and contralesional M1 simultaneously will improve motor function within the paretic limb.

Both type A and B gamma-aminobutyric acid (GABA<sub>A</sub> and GABA<sub>B</sub>) mediated inhibition can be quantified through short-interval intracortical inhibition (SICI) and the silent period duration (SPD) respectively (Byrnes et al. 2001; Siebner et al. 1998; Ziemann et al. 1993). Previous studies in chronic stroke have observed no change in SPD following a single session of both anodal-tDCS (a-tDCS) and cathodal-tDCS (c-tDCS) (Suzuki et al. 2012; Tremblay et al. 2013). Furthermore, although there is strong evidence indicating a release of inhibition within the ipsilesional M1 is an important processes for functional recovery (Clarkson et al. 2010; Hummel et al. 2005; Swayne et al. 2008), the effects of bilateral-tDCS on SICI and SPD within the ipsi- and contralesional M1 in chronic stroke have not been thoroughly examined.

The available evidence supports the addition of bilateral-tDCS to improve motor function, however the optimal timing of stimulation delivery is still unclear. Recent studies applying both a-tDCS (Fusco et al. 2014) and bilateral-tDCS (Ang et al. 2015) prior to rehabilitation have not observed any additive effect, which suggests the stimulatory benefits may be more pronounced when applied as an adjunct to rehabilitation (Bolognini, Pascual-Leone & Fregni 2009). Studies implementing single and multiple sessions of bilateral-tDCS concurrent with various upper limb physical therapy protocols, have demonstrated improvements in motor function greater than the therapy alone (Bolognini et al. 2011; Lefebvre et al. 2015; Lindenberg et al. 2010). More importantly, these reported behavioural improvements outlasted the stimulation period for up to one month (Bolognini et al. 2011; Lindenberg et al. 2010). Only one study has demonstrated a relationship between retention of behavioural improvements
with increased ipsilesional activation using functional magnetic resonance imaging (fMRI) (Lindenberg et al. 2010). However to date, the transcranial magnetic stimulation (TMS) measures of corticospinal excitability and inhibition, underpinning sustained behavioural improvements have not been examined.

Therefore, this study investigated the immediate and lasting effects of three weeks of bilateral-tDCS concurrent with upper limb rehabilitation on motor function, corticospinal excitability and inhibition within the ipsi- and contralesional M1. It was hypothesised that the concurrent application of bilateral-tDCS and upper limb rehabilitation compared with rehabilitation and sham-tDCS would yield greater improvements in motor function that would outlast the stimulation period. It was further hypothesised that bilateral-tDCS concurrent with upper limb rehabilitation would modulate corticospinal excitability and inhibition within both the ipsi- and contralesional M1 and that these changes would outlast the stimulation period compared with sham-tDCS and rehabilitation.

5.2 Materials and methods

Many of the methods employed in this study are comprehensively outlined in chapters three and four. The following methods are an abridged version of the sections specific to this chapter.
5.2.1 Participants

Sixteen participants aged 34-80 years with a single, unilateral hemispheric ischemic or haemorrhagic stroke (> six months clinically diagnosed and imaging confirmed) were recruited into the study. Information noting the side of hemiparesis, stroke subtype and year of stroke was obtained through a screening questionnaire (Table 5.2, p. 138). Participants were excluded on the following: 1) a score of < 2 or > 15 out of 18 on the combined upper limb items of the Motor Assessment Scale (MAS); 2) pre-stroke upper limb disability; 3) other known neurological disorder; 4) excessive upper limb pain (including glenohumeral joint subluxation); 5) Botulinum Toxin (BOTOX) injections < six months; 6) medications known to directly influence central nervous system (CNS) conduction; 7) severe mental health condition or cognitive impairment [Mini-Mental State Examination (MMSE)] score < 18] and 8) contraindications to TMS/tDCS. The study was approved by the Deakin University Human Research Ethics Committee (2012-081). All procedures were conducted according to the standards established by the Declaration of Helsinki.

5.2.2 Experimental design and study flow

This was a double-blinded randomised controlled trial consisting of a three week intervention and follow-up at week six (six weeks post-intervention) (Figure 5.1, p. 127). Baseline measures of corticospinal excitability, grip strength, spasticity, and motor function were assessed. Thereafter, participants were systematically matched for MAS scores and randomly allocated to a real-tDCS or sham-tDCS group with upper limb rehabilitation. Randomisation was performed by a researcher independent to the study, using a computer-generated random numbers table in Excel. Both
participants and the researcher were blinded to the group allocation. All participants then undertook nine (three sessions per week), 40 minute, individually supervised upper limb training sessions, with real or sham bilateral-tDCS applied during the first 20 minutes. The rehabilitation was designed in conjunction with an experienced neurophysiotherapist and delivered by a trained exercise scientist. Post and follow-up assessments of motor function, spasticity and corticospinal excitability and inhibition were administered 48 hours and three weeks following the final intervention session. Participants were asked to maintain their current physical activity levels throughout the timeline of the study.
**Figure 5.1** Consort diagram of study flow from recruitment to data analyses.
5.2.3 Assessment of motor function

5.2.3.1 Motor Assessment Scale

To obtain measurements of motor function the amended, upper limb items of the MAS (Carr & Shepherd 1989) were administered. This method of assessing motor function in stroke patients has shown to have high reliability (test-retest, $r = 0.87-1.0$; inter-rater, $r = 0.95$) and validity (concurrent with Fugl-Meyer assessment, $r = 0.96$) (Carr & Shepherd 1989; Dean & Mackey 1992; Malouin et al. 1994). The upper limb MAS assessment comprised of 18 tasks, split into three items corresponding to ‘upper limb’, ‘hand’ and ‘advanced hand’ activities (see Appendix F p. 268). Each sub-section was scored out of six and summed to provide a total score out of 18.

5.2.3.2 Grip strength

A maximal isometric contraction (MVIC) using a pre-calibrated strain gauge isometric dynamometer with a linear response in the 0–800 N range (ADInstruments, Bella Vista, Sydney, Australia) was used to quantify maximal grip strength (N). Participants were seated in an armchair with the elbow flexed at 90 degrees and forearm rested in pronation. The wrist was in an anatomically neutral position and the participant squeezed the transducer as maximally as possible for three seconds, while maximal root mean square electromyography ($rm{sEMG}$) was recorded for a 100 millisecond epoch during the asymptote of the MVIC. Three MVIC trials were performed and the highest results (N) was recorded.
5.2.3.3 *Tardieu Scale*

The Tardieu Scale (Boyd & Graham 1999) was performed to quantify upper limb spasticity at the beginning of each assessment session (baseline, week three and six), after the participant had rested supine on a comfortable massage table for five minutes to avoid any muscular fatigue contributing to spasticity measurements. For the elbow and wrist extensors, an assessor initially guided the patients arm through a maximal passive range of motion (ROM) (R2) as slowly as possible (V1). Following V1, the assessor passively moved the patients arm through the same joint movement as quickly as possible (V3). The quality of this muscle reaction at V3 was recorded as an ‘X’ value with scores ranging from 0-5; whereby 0 = no resistance throughout passive movement, 1 = slight resistance throughout, 2 = clear catch at precise angle, 3 = fatigable clonus < 10 sec, 4 = unfatigued clonus > 10 sec and 5 = joint immobile (see Appendix G, p. 272). This method of quantifying spasticity in stroke patients has excellent goniometry test-retest (ICC, 0.86) and good inter-rater (ICC, 0.66) reliability in stroke patients (Paulis et al. 2011).

5.2.4 Upper limb rehabilitation intervention

Following a five minute warm-up consisting of active upper limb ROM activities, participants completed four individually tailored exercises for a total duration of approximately six minutes each. This consisted of three sets for each exercise and as many controlled repetitions as possible within two minutes. A rest period of 30 seconds was provided between sets and two to three minutes between exercises. All exercises were standardised to target the distal upper limb musculature and training for participants was matched for volume, intensity and rest. Exercises promoted
sensorimotor integration, functional muscle synergies and were task-dependent, reflecting common ADLs including: reaching, grasp and release, rotation and object manipulation as described by a previously published motor relearning program (Carr & Shepherd 2003). A list of exercises used are described in Table 5.1 (p. 131). Exercises were rated on a three-point difficulty scale (1 = performed with ease; 2 = performed with some difficulty; 3 = cannot perform) to ensure appropriate progressive overload and prevent ceiling effects of training.
**Table 5.1** Examples of the exercises prescribed for the upper limb rehabilitation intervention.

<table>
<thead>
<tr>
<th>Difficulty</th>
<th>Exercise task</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Rolling out arm on a ball keeping wrist in neutral/extension</td>
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<tr>
<td></td>
<td>Alternating pronation/supination (with/without weight) keeping wrist neutral</td>
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<tr>
<td></td>
<td>Gripping thera putty without allowing wrist flexion (progress to grip and release)</td>
</tr>
<tr>
<td></td>
<td>Flattening/kneading out thera putty with palm of hand (using finger and wrist extension)</td>
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<tr>
<td></td>
<td>Lifting and lowering an object using wrist extension and flexion (beanbag or tennis ball) over the edge of a table</td>
</tr>
<tr>
<td></td>
<td>Feeling for and/or picking up embedded objects in sand box</td>
</tr>
<tr>
<td>Moderate-high</td>
<td>Punching or reaching for targets ensuring elbow and wrist extension</td>
</tr>
<tr>
<td></td>
<td>Turning magazine pages</td>
</tr>
<tr>
<td></td>
<td>Tracing shapes / alphabetic letters</td>
</tr>
<tr>
<td></td>
<td>9-hole pegboard</td>
</tr>
<tr>
<td></td>
<td>Grasping and lifting different weight and shaped cups</td>
</tr>
<tr>
<td></td>
<td>Grasping, lifting, releasing stacking blocks (circular)</td>
</tr>
<tr>
<td></td>
<td>Opening different sized jar lids</td>
</tr>
<tr>
<td></td>
<td>Pouring water from saucepan to cup</td>
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</table>
**Figure 5.2** One participant undertaking a selection of the above rehabilitation exercises during the application of bilateral-tDCS. Exercises shown include: grasping task using stacking blocks (A); turning pages of a scrapbook (B); 9-hole pegboard (C); grasping saucepan and pouring water into a cup (D); opening hand using finger and wrist extension to grasp and pick up cup (E); feeling for blocks in sand and picking them up (F). The picture on the left depicts the beginning of the task whilst the right shows the end range of the task.
5.2.5 Transcranial direct-current stimulation protocol

Bilateral-tDCS was applied over both M1s for the initial 20 minutes of the training session. In all conditions, the anode was placed over the ipsilesional M1 and the cathode over the contralesional M1, in the area corresponding with the participant’s extensor carpi radialis (ECR) “optimal hotspot”. For both conditions, stimulation intensity was delivered at 1.5 mA (current density 0.06 mA/cm²).

Refer to section 3.2.4 (pp. 65-66) for comprehensive details of the tDCS apparatus and protocols.

5.2.6 Recording of surface electromyography

Recording of sEMG followed the same procedures described in section 3.2.6 (p. 68).

5.2.7 Transcranial magnetic stimulation and maximal compound waves

Refer to section 3.2.7 (pp. 68-70) for a detailed description of the single and paired-pulse TMS and M-wave protocols. Variations to the protocols described in chapter three are described below.

Measures of corticospinal excitability and inhibition were taken in both a resting and active muscle state. Measurements included resting motor threshold (RMT), active motor threshold (AMT), motor evoked potential (MEP) amplitudes and SPD at 150% AMT and MEPs at 120% AMT & RMT, as well as resting and active SICI.
MEPs and SPD recorded during weak voluntary contraction involved participants positioning their hand in line with their wrist (i.e. anatomically neutral). MEPs were additionally recorded at a test stimulus of 120% RMT, whereby the hand was unsupported and relaxed with the forearm supported in pronation. For both single and paired-pulse MEPs, five stimuli at each intensity were obtained with the order of the presentation randomised.

5.2.8 Data analysis

Refer to section 3.2.8 (p. 70) for analyses of single and paired-pulse MEP amplitudes and M-waves ($M_{MAX}$).

Resting MEPs were only elicited in four out of 15 participants. Therefore only active MEPs, SPD and SICI were included in the data and statistical analysis. SPD was recorded as the distance from the end of the MEP amplitude to the return of normal EMG activity (Christie & Kamen 2014). An example of where the cursers were placed for SPD measurement is shown in Figure 5.3 (p. 135). As there was no clear suppression of EMG activity in the ipsilesional M1 for the majority of participants, SPDs were only included in analysis for the contralesional M1. An absence of SPD in the ipsilesional M1 has previously been reported in stroke patients (Schnitzler & Benecke 1994).

Refer to section 4.2.8 (pp. 100-101) for details regarding analysis of the laterality index (LI). In stroke patients, commonly reported positive values denote greater excitability of the contralesional M1, with well recovered patients scoring closer to 0 (Di Lazzaro et al. 2014).
Figure 5.3 Cursor placement for the analysis of silent period duration (SPD) in the contralesional M1. SPD was measured from the onset of the motor evoked potential (MEP, cursor 1) to the return of EMG (cursor 2) (Kidgell et al. 2015).
5.2.9 Statistical analysis

Based on previous data examining motor function and corticospinal excitability in stroke patients (Bolognini et al. 2011), a priori power calculations revealed that 14 participants were needed to detect a 20% difference between-groups for these outcomes assuming a SD of 15-25% with 80% power (two-tailed, \( P < 0.05 \)).

Statistical analyses were conducted using Stata statistical software (StataSE version 13). Data was screened with Shapiro-Wilk and due to skewness, all variables except for \( M_{MAX} \), grip strength, Tardieu scores and LI were log-transformed before analysis. Independent t-tests were used to compare groups at baseline. For change in spasticity (0-5), a Chi-Square test was used to determine between-group differences in the number of participants that had no change, increased or decreased spasticity scores over time. Generalised linear mixed-models were used to assess within-group changes (time) and group-by-time interactions for all other dependent variables. Within-group changes after three and six weeks are presented as percentage change from baseline. The percentage change in the log-transformed measures represent the absolute difference from baseline in log-transformed data multiplied by 100. Between-group differences were calculated by subtracting within-group changes from baseline for the real-tDCS group from within-group changes for the sham-tDCS group. \( P < 0.05 \) determined statistical significance and data are presented as mean ± SEM.
5.3 Results

5.3.1 Participant characteristics

Of the eight participants randomised to both the sham-tDCS and real-tDCS groups, one participant withdrew from the sham-tDCS group before the intervention. Intervention compliance was 100% for all participants and no adverse events were reported. There were no between-group differences in age (P = 0.83), height (P = 0.86), weight (P = 0.07), MMSE (P = 0.46) scores and years/months since stroke (P = 0.06) (Table 5.2, p. 138).
Table 5.2  Clinical and demographic details of participants.

<table>
<thead>
<tr>
<th>Group</th>
<th>ID</th>
<th>Sex</th>
<th>Age</th>
<th>Weight(kg)</th>
<th>Height(cm)</th>
<th>Years</th>
<th>Hemisphere</th>
<th>Location</th>
<th>Type</th>
<th>Vascular Territory</th>
<th>MMSE</th>
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<tr>
<td>Sham-tDCS</td>
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<td>F</td>
<td>46</td>
<td>86</td>
<td>167</td>
<td>3</td>
<td>L</td>
<td>Sc</td>
<td>H</td>
<td>IC</td>
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<td>53</td>
<td>149</td>
<td>5</td>
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<td>3</td>
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<td>Real-tDCS</td>
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</tbody>
</table>

M, male; F, female; L, left; R, right; C, cortical, Sc, subcortical; I, ischemic; H, haemorrhagic; ICA, internal carotid artery; IC, internal capsule; MCA, middle cerebral artery; MMSE, Mini-Mental State Examination; PP, paramedian pontine; SC, striatocapsular.
5.3.2 Visual analogue scale

Mean VAS scores for real-tDCS, 2.0 ± 0.5; sham-tDCS, 1.9 ± 0.8. No differences in VAS scores were reported between groups (t = -0.2, P = 0.56).

5.3.3 Motor function

5.3.3.1 Motor Assessment Scale

Baseline MAS scores were not different between groups (t = 0.2, P = 0.43). After the three week intervention MAS scores improved relative to baseline in both the sham-tDCS (42.7% ± 9.2) and real-tDCS (62.3% ± 9.5) groups (P < 0.001), with no significant difference between groups (group-by-time interaction, P = 0.08) (Table 5.5, p. 153; Figure 5.4, p. 140). After six-weeks, only the real-tDCS group showed a retention in the MAS improvements (64.0%, P < 0.001) whereas the gains in the sham-tDCS group began to return to baseline (21.9%, P = 0.08) which led to a group-by-time interaction (P = 0.002).
**Figure 5.4** Mean (±SEM) log Motor Assessment Scale (MAS) scores. Results are displayed for post intervention (week 3) and follow-up (week 6) as percentage changes from baseline (week 0). * denotes $P < 0.05$ within-group change relative to baseline. † denotes $P < 0.05$ between-groups.
5.3.3.2 Maximal grip strength

No baseline differences in grip strength (N) of the paretic limb were observed between groups (t = -1.1, P = 0.86). Following the intervention there were no changes in grip strength of the paretic limb for either group relative to baseline (sham-tDCS, P = 0.80, real-tDCS, P = 0.39), and no group-by-time interaction (P = 0.42). Similarly, at week six there were no within-group changes (sham-tDCS, P = 0.37, real-tDCS P = 0.89) or between-group differences (group-by-time interaction, P = 0.50).

There were no baseline differences in $\text{rmsEMG}$ during grip strength (t = -1.31, P = 0.89). Following the three week intervention, there were no changes in $\text{rmsEMG}$ during the grip task for either group relative to baseline (sham-tDCS, P = 0.83, real-tDCS, P = 0.10) and no group-by-time interaction (P = 0.16). There were also no within-group changes (sham P = 0.07, real P = 0.18) or between-group differences over time (group-by-time interaction, P = 0.94) at six weeks.

5.3.3.3 Tardieu Scale

At baseline, no participants scored higher than ‘2’ on the Tardieu Scale, and the sham-tDCS group had a slightly higher proportion of participants scoring ‘2’ for the wrist ($\chi^2 = 6.6, P = 0.04$) with no group differences at the elbow ($\chi^2 = 2.6, P = 0.10$). Tardieu scores did not significantly change over time for either group (Table 5.3, p. 142).
Table 5.3  Tardieu scores. Number of participants scoring a 0, 1 or 2 at V3 for the wrist and elbow extensors.

Results are reported for both sham-tDCS and real-tDCS groups at baseline, post and follow-up.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Number of participants</th>
<th>Wrist</th>
<th>Elbow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 (No</td>
<td>1 (slight</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>catch)</td>
<td>resistance)</td>
</tr>
<tr>
<td>Sham-tDCS</td>
<td>Baseline</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>Post</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Real-tDCS</td>
<td>Baseline</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>Post</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Follow-up</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
5.3.4 Maximal compound waves and pre stimulus $r_{ms}EMG$

There were no between-group differences for $M_{MAX}$ and pre stimulus $r_{ms}EMG$ observed at baseline for either limb (all $P > 0.05$, Table 5.4, p. 144). Additionally, following the intervention there were no within-group changes or group-by-time interactions for $M_{MAX}$ and pre stimulus $r_{ms}EMG$ for either limb after three or six weeks (all $P > 0.05$).

5.3.5 Corticospinal excitability

5.3.5.1 Ipsi- and contralesional active motor threshold

There were no between-group differences for AMT at baseline for either limb (all $P > 0.05$, Table 5.4, p. 144). Following the intervention there were no within-group changes or group-by-time interactions for AMT for either limb at week three or six (all $P > 0.05$).
Table 5.4  Mean (±SEM) baseline TMS data.

<table>
<thead>
<tr>
<th></th>
<th>Sham-tDCS</th>
<th>Real-tDCS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ipsilesional</td>
<td>Contralesional</td>
</tr>
<tr>
<td>MMAX (mV)</td>
<td>2.6 ± 0.5</td>
<td>4.6 ± 0.7</td>
</tr>
<tr>
<td>AMT (%SO)</td>
<td>67.3 ± 6.1</td>
<td>37.1 ± 3.4</td>
</tr>
<tr>
<td>Test stimuli (%SO)</td>
<td>80.8 ± 7.4</td>
<td>39.0 ± 7.1</td>
</tr>
<tr>
<td>Conditioning stimuli (%SO)</td>
<td>53.9 ± 4.9</td>
<td>26.0 ± 4.7</td>
</tr>
<tr>
<td>rmsEMG (%max)</td>
<td>11.9 ± 2.2</td>
<td>4.9 ± 0.9</td>
</tr>
</tbody>
</table>

AMT, active motor threshold; MMAX, maximum M-wave; SO, stimulator output.

5.3.5.2 Ipsi- and contralesional motor evoked potentials

For the ipsilesional M1, baseline MEP amplitudes were greater in the sham-tDCS group (P = 0.01) and thus analysis was adjusted for baseline values. After the three week intervention, no group-by-time interaction was observed (P = 0.12), but within-group analysis revealed facilitated MEPs for the real-tDCS group relative to baseline (46.4% ± 8.1, P < 0.001), with no change the sham-tDCS (12.1% ± 6.3, P = 0.36). After six weeks, the real-tDCS group maintained larger MEP amplitudes relative to baseline (38.1% ± 7.8, P < 0.001) with no change in the sham-tDCS group (9.4% ± 14.7, P = 0.57), but there was no between-group difference (group-by-time interaction, P = 0.09, Table 5.5, p. 153; Figure 5.5, p. 146).
For contralesional MEPs there were no between-group differences at baseline ($t = 0.7$, $P = 0.26$). Following the three week intervention there were no changes over time for either sham-tDCS ($P = 0.43$) or real-tDCS ($P = 0.12$) groups and there was no group-by-time interaction ($P = 0.58$). Similarly, at week six no changes were observed for either group relative to baseline (sham-tDCS $P = 0.52$, real-tDCS $P = 0.73$), and there was no group-by-time interaction ($P = 0.83$).

5.3.5.3 Laterality index

Baseline LI for both groups combined, tended towards greater excitability of the contralesional M1 (LI = $0.5 \pm 0.1$; Figure 5.6, p. 147) but there were no significant group differences at baseline for LI ($P = 0.94$). Following the three week intervention, there was a significant shift in LI for real-tDCS ($P < 0.001$), with a trend for a shift in the sham-tDCS group ($P = 0.06$) but the between-group differences over time were not significant (group-by-time interaction, $P = 0.40$). After six weeks, the shift in LI for the real-tDCS was maintained relative to baseline ($P = 0.03$), but there remained no group-by-time interaction ($P = 0.97$).
Figure 5.5 Mean (±SEM) log motor evoked potential (MEP) amplitudes (%M_{\text{MAX}}) recorded at 150% AMT, for the ipsilesional (A) and contralesional (B) M1. Results are displayed for post intervention (week 3) and follow-up (week 6) as percentage changes from baseline (week 0). * denotes P < 0.05 within-group change relative to baseline.
Figure 5.6 Mean (±SEM) raw values for laterality index (LI) at baseline (week 0), post intervention (week 3) and follow-up (week 6). * denotes P < 0.05 within-group relative to baseline.
Figure 5.7 Overlayed motor evoked potential (MEP) recordings taken at 150% AMT from one participant in the sham-tDCS group (A) and another in the real-tDCS group (B) at week 0 (i), week 3 (ii) and week 6 (iii).
5.3.6 Corticospinal inhibition

5.3.6.1 Contralesional silent period duration

There were no baseline differences in the SPD between groups (P = 0.49). At three weeks, there was a 32.8% ± 12.0 increase in SPD relative to baseline (P = 0.01) in the real-tDCS group, with no marked change in the sham-tDCS group (4.9% ± 3.1, P = 0.32) which led to a significant group-by-time interaction (P = 0.04, Table 5.5, p. 153; Figure 5.8, p. 150). After six weeks, the increase in the real-tDCS group was maintained relative to baseline (24.0% ± 13.6, P = 0.04) with no change in the sham-tDCS (7.1% ± 1.8, P = 0.16) and no group-by-time interaction (P = 0.22).
Figure 5.8 Mean (±SEM) log silent period duration (SPD) for the contralesional M1. Results are displayed for post intervention (week 3) and follow-up (week 6) as percentage changes from baseline (week 0). * denotes $P < 0.05$ within-group change relative to baseline. † denotes $P < 0.05$ between-groups.
5.3.6.2 *Ipsi- and contralesional short-interval intracortical inhibition*

There were no differences between the groups at baseline for ipsilesional (t = 0.8, P = 0.23) or contralesional (t = -0.9, P = 0.82) SICI. In addition, ipsilesional SICI did not change over time or differ between groups after three or six weeks (all P > 0.05).

Similarly, there was no within-group changes (sham-tDCS, P = 0.64; real-tDCS, P = 0.20) or between group differences (group-by-time interaction, P = 0.22) in contralesional SICI after three weeks. After six weeks, there was a 27.1% ± 12.6 increase in SICI in the real-tDCS compared with sham-tDCS (group-by-time interaction (P = 0.04) (Table 5.5, p. 153; Figure 5.9, p. 152).
Figure 5.9 Mean (±SEM) log short-interval intracortical inhibition (SICI) for the ipsilesional (A) and contralesional (B) M1. Results are displayed for post intervention (week 3) and follow-up (week 6) as percentage changes from baseline (week 0). * denotes P < 0.05 within-group change relative to baseline. † denotes P < 0.05 between-groups.
Table 5.5  Mean (±SEM) raw values for motor function and neurophysiological variables.

<table>
<thead>
<tr>
<th>Group</th>
<th>MAS score</th>
<th>MEP amplitude at 150% AMT (%M_MAX)</th>
<th>SICI (% of test response)</th>
<th>SPD (milliseconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
<td>Fu</td>
<td>Baseline</td>
</tr>
<tr>
<td>Paretic limb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-tDCS</td>
<td>6.3 ± 1.9</td>
<td>9.0 ± 2.3 *</td>
<td>8.0 ± 2.2</td>
<td>14.6 ± 2.3</td>
</tr>
<tr>
<td>Real-tDCS</td>
<td>5.9 ± 1.3</td>
<td>10.0 ± 2.8 *</td>
<td>10.3 ± 2.1 *</td>
<td>6.3 ± 1.4</td>
</tr>
<tr>
<td>Non-affected limb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham-tDCS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Real-tDCS</td>
<td>28.0 ± 7.6</td>
<td>20.4 ± 5.5</td>
<td>26.1 ± 5.5</td>
<td>64.0 ± 6.3</td>
</tr>
</tbody>
</table>

Shaded boxes indicate significant variables. * P < 0.05 within-group relative to baseline, † P < 0.05 between-groups. AMT, active motor threshold; Fu, follow-up; MAS, Motor Assessment Scale; MEP, motor evoked potential; NA, not applicable (not measured); SICI, short-interval intracortical inhibition; SPD, silent period duration.
5.4 Discussion

This study investigated the functional and neurophysiological adaptations following upper limb rehabilitation concurrent with bilateral-tDCS in chronic stroke patients. The main findings were that there was no marked effect of real-tDCS compared with sham-tDCS on the immediate improvements in motor function, however the real-tDCS promoted significantly larger retention of newly gained improvement on the MAS. Additional important findings demonstrate increased corticospinal excitability and intracortical inhibition (SPD and SICI), within the ipsi- and contralesional M1 respectively, that outlasted the intervention up to three weeks. It was concluded that bilateral-tDCS as an adjunct to upper limb rehabilitation modulated indices of corticospinal plasticity within both the ipsi- and contralesional M1, which was important for retaining improvements in upper limb motor function in chronic stroke patients.

5.4.1 Motor function following bilateral-tDCS and upper limb rehabilitation

Improved motor function was observed in both groups immediately following the intervention, however was maintained to a greater degree in the real-tDCS compared with the sham-tDCS group for up to three weeks following the intervention. A large body of evidence has demonstrated a preferential improvement in motor function following bilateral-tDCS with physical therapy, compared with physical therapy alone (Bolognini et al. 2011; Lefebvre et al. 2015; Lefebvre et al. 2012; Lefebvre et al. 2014; Lindenberg et al. 2010). Although comparisons with previous studies are difficult, due to different modes and intensities of both the stimulation and physical therapy
protocols, one key difference between previous literature and this study is the length of the intervention. Most previous studies have prescribed single or five consecutive intervention sessions (Lefebvre et al. 2015; Lefebvre et al. 2012; Lefebvre et al. 2014; Lindenberg et al. 2010), whereby the longer, multi-session intervention prescribed in this study likely promoted a practice effect in the sham-tDCS and rehabilitation group, contributing to improved motor function immediately following the intervention.

Importantly, bilateral-tDCS promoted retention of functional gains, which is in line with previous findings following single and repeated bilateral-tDCS sessions (Bolognini et al. 2011; Lefebvre et al. 2015; Lefebvre et al. 2014; Lindenberg et al. 2010). The novelty of the current study was that the larger retention observed following bilateral-tDCS, occurred irrespective of similar improvements between-groups immediately following the intervention. These findings suggest that bilateral-tDCS may be markedly important for preserving newly regained motor skills in chronic stroke patients. A recent study prescribing a similar multi-session intervention (10 sessions for two weeks) demonstrated that bilateral-tDCS with CIMT modulated corticospinal excitability and inhibition between both the ipsi- and contralesional M1, which is critical for recovery of motor function (Bolognini et al. 2011). As the authors did not measure the retention of the neurophysiological adaptations, this study is the first to demonstrate the preferential effect of bilateral-tDCS on maintaining improvements in motor function, through lasting modulation of corticospinal networks within the ipsi-and contralesional M1s.

As expected, there were no changes in spasticity or grip strength concurrent with an improvement in MAS scores. These findings confirm previous reports in moderate to severely affected stroke patients, demonstrating non-significant changes in grip
strength despite improvements in hand reaction times (Stagg et al. 2012). Moreover, there is limited research to suggest tDCS may have a positive effect on spasticity in stroke patients. Some preliminary findings have observed improvements following both c-tDCS and bilateral-tDCS (Vandermeeren et al. 2013; Wu et al. 2013), possibly through a reduction in hyper-excitability (Wu et al. 2013). In this study, the improvements in MAS scores independent of changes in spasticity confirms previous literature (Ada, O'Dwyer & O'Neill 2006), indicating that the mechanisms modulating improvements in motor function and spasticity may be mutually exclusive, and were not likely affected by bilateral-tDCS.

5.4.2 Corticospinal excitability and inhibition following bilateral-tDCS and upper limb rehabilitation

The neurophysiological mechanisms involved in recovery of motor function are complex, and involve restoration of functional connectivity between a number of subcortical and cortical regions (Cheng et al. 2014; Richards et al. 2008; Seitz et al. 1998). Nevertheless, it is well documented that functional recovery involves strengthening of synapses amongst residual motor networks, in particular the M1 and CSP (Byrnes et al. 1999; Stinear et al. 2007; Ward et al. 2006). The current findings demonstrate that an increase in SPD and SICI within the contralesional M1 following bilateral-tDCS and upper limb rehabilitation may augment the excitatory response in the ipsilesional M1 and improve interhemispheric balance. These mechanisms likely improved motor learning, leading to a greater retention of motor function improvements following bilateral-tDCS.
The combination of bilateral-tDCS and rehabilitation resulted in an increase in corticospinal excitability within the ipsilesional M1, which was maintained up to three weeks following the intervention. Although no significant between-group interactions for MEP amplitudes in the ipsilesional M1 were found, it is worth noting that the improvement in the real-tDCS group following the intervention was nearly four-fold greater than the gain in the sham-tDCS group. These findings are consistent with previous fMRI (Lefebvre et al. 2015; Lindenberg et al. 2010) and TMS (Bolognini et al. 2011; Di Lazzaro et al. 2014) studies, demonstrating enhanced cortical activation and corticospinal excitability within the ipsilesional M1 following bilateral-tDCS.

Contrary to the hypothesis and previous findings applying a-tDCS in stroke (Edwards et al. 2009; Hummel et al. 2005), this study observed no change in SICI in the ipsilesional M1 for either group. There are a number of possibilities as to why no significant changes in SICI within the ipsilesional M1 were observed. Firstly, it has previously been suggested that the induction of long-term potentiation (LTP) following tDCS depends on the baseline excitability state of the M1 (Nitsche et al. 2007b; Siebner et al. 2004; Ziemann et al. 2004). Therefore, the individual variability regarding the level of SICI following bilateral-tDCS may have been largely dependent on the initial level of CSP damage and thus, the individual excitability state of the CSP prior to the intervention. Furthermore, it is well accepted that functional connectivity of cortical and subcortical regions remote to the lesion site are disrupted following stroke (Chen, Cohen & Hallett 2002), and that restoration of these connections may facilitate motor recovery (Feydy et al. 2002; Westlake & Nagarajan 2011). Evidence from fMRI has demonstrated ipsilateral and interhemispheric connectivity between the ipsilesional M1 and the supplementary motor area (SMA) (Grefkes & Fink 2014; Park et al. 2011; Rosso et al. 2013), PMC (Seitz et al. 1998; Sharma, Baron & Rowe 2009)
cerebellum (Rosso et al. 2013) and thalamus (Park et al. 2011; Young et al. 2014). During bilateral-tDCS the current flow (M1-M1 arrangement) is likely to be different to unilateral-tDCS montages (M1-supraorbital arrangement), and may generate electrical activity onto adjacent interconnected regions (Schlaug, Renga & Nair 2008). Moreover, the relatively large and non-focal nature of the tDCS electrodes may have additionally targeted activity of remote neuronal tissue (DaSilva et al. 2011; Nitsche et al. 2007a). Therefore, it is feasible that improved synaptic efficacy from surrounding motor areas may have additionally contributed to the net excitability of the ipsilesional M1.

In contrast to the hypothesis, no significant change in MEP amplitudes within the contralesional M1 were observed. In line with these findings, a previous study in sub-acute stroke patients reported large variability in the direction of excitability changes within the contralesional M1 receiving the cathode (O'Shea et al. 2014). However, the addition of bilateral-tDCS still shifted the LI towards an improvement in interhemispheric balance, driven primarily by an increase in ipsilesional corticospinal excitability, which is consistent with previous findings (Di Lazzaro et al. 2014). Moreover, although IHI was not measured in this study, there is some evidence to suggest a relationship between intracortical inhibition and IHI in patients with cortical lesions (Butefisch et al. 2008). Based on this, it is reasonable to suggest that normalising intracortical inhibitory networks in the contralesional M1 through bilateral-tDCS may have augmented corticospinal excitability in the ipsilesional M1 via transcallosal pathways (Bolognini et al. 2011; Di Lazzaro et al. 2014).

In contrast to previous studies observing no change in SPD following a single session of both a-tDCS and c-tDCS (Suzuki et al. 2012; Tremblay et al. 2013), this study
demonstrated that following multiple sessions of bilateral-tDCS and upper limb rehabilitation, the SPD was increased and maintained at follow-up. The increase in SPD occurred in the absence of any significant suppression in the contralesional corticospinal excitability, which supports previous work in healthy individuals (Wilson et al. 1993) suggesting these parameters are not directly correlated and may be modulated through different neuronal circuits. Indeed, the direct current from bilateral-tDCS may have a stronger influence on the activity of intracortical inhibitory interneurons (Lang et al. 2011; Lang et al. 2004), which are likely to exert an effect on excitability of the ipsilesional M1, through transcallosal pathways.

Interestingly, SICI within the contralesional M1 was only significantly increased three weeks following the intervention, which suggests bilateral-tDCS may have strengthened motor memory consolidation following the intervention. Indeed, in healthy individuals offline tDCS effects have been suggested to consolidate synaptic plasticity and LTP-like processes (Fritsch et al. 2010; Galea & Celnik 2009; Reis et al. 2009), which are important for improving and retaining motor learning. Therefore, it is plausible that bilateral-tDCS during upper limb rehabilitation may have augmented the excitatory response in the ipsilesional M1 from the rehabilitation itself, through up-regulating inhibition within the contralesional M1.

Animal models have established that disinhibition of the contralesional M1 is due in part to down-regulation of GABA-mediated inhibitory activity (Que et al. 1999; Reinecke et al. 1999), but it remains unclear as to how this may influence motor recovery of the paretic limb in humans. Evidence for compensatory mechanisms within the contralesional M1 highlight the influence of the ipsilateral corticospinal and cortico-reticulo-proprioospinal pathways on motor function of the paretic limb.
(Bradnam et al. 2012; Bradnam, Stinear & Byblow 2013). In this light, it is reasonable to suggest that disinhibition within the contralesional M1 may interfere with motor function of the paretic limb, through an inability to inhibit antagonist muscle activation and control muscle synergies (Bradnam et al. 2010; Schwerin et al. 2008). In healthy adults, c-tDCS improved selective muscle activation through inhibition of the antagonist muscle in the proximal upper limb (McCambridge et al. 2011; Uehara, Coxon & Byblow 2015). In stroke patients, these processes are dependent on the degree of spasticity (Bradnam et al. 2012) and CSP damage (Ward et al. 2007), which may contribute to variability in the responsiveness to bilateral-tDCS. Although these previous studies assessed proximal muscles, it can be speculated that the increase in inhibition of the contralesional M1 in this study may have contributed to improved motor control of the paretic limb, via the ipsilateral uncrossed CSP.

Taken together, the current findings demonstrate excitatory balance between the ipsi- and contralesional M1 appears to be due at least in part to increased SPD and SICI within the contralesional M1. Many previous studies have attributed performance gains following bilateral-tDCS to a reduction in IHI from the contralesional to ipsilesional M1. Although measures of IHI were not obtained in this study, it is plausible that increased inhibition within the contralesional M1 may have amplified the excitatory effects of the anode, improving interhemispheric balance and motor learning in this cohort of chronic stroke patients.
5.4.3 Limitations

A number of limitations should be considered when interpreting these findings. Firstly, IHI was not able to be quantified, which has been shown to be a key pathway involved in improving interhemispheric plasticity following bilateral-tDCS (Bolognini et al. 2011; Di Lazzaro et al. 2014). Secondly, due to the clinical applicability of tDCS, participants were not excluded based on the type (ischemic/haemorrhagic) location (cortical/subcortical) or severity (presence of MEP amplitudes) of the stroke, which may influence the responsiveness to bilateral-tDCS and contribute to inter-participant variability. Evidence from bilateral-tDCS and repetitive TMS (rTMS) have reported that the benefits of stimulatory protocols are only present if there are spared synapses within the ipsilesional CSP (Ameli et al. 2009; Lindenberg et al. 2010). Therefore, the severity of damage and location of the lesion may influence the magnitude of responsiveness from bilateral-tDCS. Finally, although the sample size was calculated to give sufficient power based on a-priori analysis from previous studies, the sample size remained quite small. Considering the heterogeneous nature of a stroke as well as the variability in responders to bilateral-tDCS, larger, multicentre clinical trials are needed to determine the clinical efficacy of bilateral-tDCS in stroke rehabilitation.

5.4.4 Conclusions and future directions

The current findings indicate the potential for bilateral-tDCS to improve retention of upper limb motor function in chronic stroke patients. Restoring interhemispheric balance appears to be due in part to increasing inhibition within the contralesional M1, which may subsequently have amplified the excitatory effects of the anode and
improved retention of motor function in this cohort of stroke survivors. Larger clinical trials are needed to identify the variables influencing individual responsiveness to bilateral-tDCS, individually tailoring stimulation parameters based on these cofounders. Moreover, the optimal intensity of concurrent rehabilitation provided needs to be identified in order to improve efficacy and generalisability across a broad range of stroke-affected individuals.
CHAPTER SIX: GENERAL DISCUSSION
The studies described in this thesis have systematically investigated the efficacy of transcranial direct-current stimulation (tDCS) to improve motor and neurophysiological function of the non-dominant and paretic limb in older adults and individuals with chronic stroke. This chapter will provide an integrated discussion regarding how the findings from this thesis contribute to the field of non-invasive brain stimulation (NIBS), motor training and neurorehabilitation. Specifically, this discussion will pay attention to some of the overarching themes arising from the experimental studies. To conclude, this chapter will provide an overview of some key questions that remain unanswered, and that should be the focus of future research in order to progress towards the therapeutic application of tDCS in older adults and stroke patients.

The experimental studies presented throughout this thesis contribute novel findings to the literature regarding the application of tDCS in ageing and chronic stroke. In study one (chapter three) it was demonstrated that following ipsilateral anodal-tDCS (a-tDCS), cross-limb transfer could be achieved in the non-dominant limb of older adults, which was otherwise absent from unilateral motor training alone. Furthermore, an increase in corticospinal excitability similar to the magnitude in younger adults, was observed in the ipsilateral primary motor cortex (M1) only following the application of a-tDCS. Interestingly, intracortical inhibition was released in the ipsilateral M1 regardless of sham-tDCS or a-tDCS. These findings provide preliminary support for the use of a-tDCS as an adjunct to unilateral training in older adults and the elderly during periods of unilateral disuse, such as immobilisation from a fracture or following a stroke.
As study one only employed a unilateral a-tDCS electrode montage, study two (chapter four) compared the efficacy of unilateral a-tDCS and bilateral-tDCS on corticospinal excitability, intracortical inhibition and motor function of the non-dominant limb in older adults. Study two showed that the combination of a-tDCS and bilateral-tDCS with motor training of the non-dominant limb preferentially modulated corticospinal excitability and inhibition in the non-dominant M1, compared with motor training alone. This resulted in an accelerated improvement in motor function compared with motor training and sham-tDCS, regardless of a unilateral or bilateral electrode montage. Additionally, the findings presented in study two show a retention in neurophysiological and motor improvements up to 30 minutes following a single session of unilateral a-tDCS or bilateral-tDCS. Collectively, studies one and two support the benefits of a-tDCS and bilateral-tDCS as an adjunct to motor training to improve motor function of the non-dominant limb in an ageing population. These findings provide a robust framework for the concurrent application of tDCS and rehabilitation in chronic stroke patients. In study three (chapter five), it was demonstrated that a multi-session intervention of bilateral-tDCS with concurrent upper limb rehabilitation preferentially improved the retention of newly regained motor function in chronic stroke patients. These sustained improvements in motor function appear to be due in part to an increase in corticospinal excitability of the ipsilesional M1, and increased inhibition within the contralesional M1 following bilateral-tDCS. Additionally, these neurophysiological adaptations generated a shift in the laterality index (LI) towards a more balanced excitation-inhibition profile between the ipsi- and contralesional M1, which is believed to be important for functional recovery following a stroke. The findings from study three provide preliminary evidence that bilateral-
tDCS concurrent with upper limb rehabilitation may act to consolidate the functional gains achieved from traditional rehabilitation alone.

Overall, the application of tDCS and motor training is beneficial for improving motor function within the non-dominant and paretic limb in older adults and chronic stroke patients respectively. The findings from this thesis suggest that modulating intracortical inhibition is an important mechanism for improving synaptic efficacy along the corticospinal pathway (CSP), which leads to subsequent improvements in motor function. Further, the findings from all three studies provide evidence for the use of tDCS in preserving age-related neuromuscular degeneration and improving rehabilitation outcomes in chronic stroke patients.

6.1 Motor function following tDCS and motor training in older adults and chronic stroke patients

Previous studies suggest that older adults may have a reduced response to plasticity inducing protocols (Rogasch et al. 2009; Sawaki et al. 2003; Todd et al. 2010), including cross-limb transfer (Hinder et al. 2011) which may be more pronounced in the non-dominant limb. The findings from studies one and two support these previous studies (Hinder et al. 2011; Rogasch et al. 2009; Sawaki et al. 2003; Todd et al. 2010), whereby in the absence of tDCS, older adults displayed reduced cross-limb transfer and non-significant improvements in visuomotor tracking (VT) within the non-dominant limb. Furthermore, reductions in motor performance (VT error) appeared to be due to reduced use-dependent adaptations in corticospinal excitability and intracortical inhibition within the non-dominant M1. However, in both studies one and
two, the addition of either a-tDCS or bilateral tDCS applied to the non-dominant M1 augmented cross-limb transfer and improved motor performance in older adults, which supports previous research (Parikh & Cole 2014; Zimerman & Hummel 2010; Zimerman et al. 2013). More importantly, whilst study two demonstrated that tDCS elicited earlier gains in motor performance compared to the sham-tDCS condition, these improvements were not dependent on the unilateral or bilateral electrode montage.

Study one demonstrated that the addition of a-tDCS improved the cross-transfer of motor skills to the untrained (non-dominant) limb, which was not achieved following unilateral motor training with sham-tDCS. Cross-limb transfer was accompanied by increased corticospinal excitability within the ipsilateral M1 and motor overflow to the untrained limb. Interestingly, a-tDCS had no differential effect on the release of short-interval intracortical inhibition (SICI) within the ipsilateral M1, which suggests that other motor regions such as the pre motor cortex (PMC) and supplementary motor area (SMA) may play a part in the cross-transfer of motor skills, particularly in older adults. For example, Hinder and colleagues (2011) observed an absence of cross-limb transfer in older adults, irrespective of increased motor overflow and ipsilateral corticospinal excitability in the untrained limb. This finding further highlights the involvement of adjacent motor areas modulating cross-limb transfer in older adults (Hinder et al. 2011). Certainly, there is evidence for an increased reliance on surrounding interconnected motor areas such as the PMC and SMA when older adults learn new motor skills (Seidler et al. 2010). Therefore, it is possible that the addition of a-tDCS in study one targeted areas adjacent to the M1, contributing to cross-limb transfer of performance in older adults. The findings from study one illustrate that a-tDCS can improve cross-limb transfer from the dominant to the non-dominant limb in older
adults, which may otherwise be absent (Hinder et al. 2011). This finding has important clinical implications for preserving motor function in older adults and the elderly throughout periods of unilateral motor impairment (i.e. fractures, post-surgery limb immobilisation and stroke).

An important finding in study two was that both a-tDCS and bilateral-tDCS elicited earlier improvements in motor learning, which has been previously shown following a-tDCS in younger adults (Stagg et al. 2011). However by 30 minutes the overall magnitude of performance improvement was not different. Previous studies in older adults have suggested that the induction of experimentally-induced plasticity (Fujiyama et al. 2014) and motor skill acquisition (Daselaar et al. 2003) may be significantly delayed, however the overall magnitude of improvement remains comparable to younger adults. Accordingly, study two showed that the concurrent application of motor training with a-tDCS or bilateral-tDCS expedited motor learning in older adults, which was accompanied by facilitated corticospinal excitability and a release of SICI in the non-dominant M1.

The findings from studies two and three demonstrated that gains in motor function can still be achieved in older adults and chronic stroke patients, independent of a-tDCS or bilateral-tDCS. This finding is somewhat contrary to previous studies in both older adults (Parikh & Cole 2014) and chronic stroke patients (Bolognini et al. 2011; Lindenberg et al. 2010), which have shown preferential improvements in motor function following tDCS compared with motor training alone. Some possible differences between the studies in this thesis and previous work may relate to the training task administered, as well as the length of the intervention. In regards to motor learning, the novelty and complexity of the training task has been associated with
increased task performance (Hlustik et al. 2004) as well as facilitated corticospinal plasticity (Pascual-Leone et al. 1995; Perez et al. 2004). Therefore differences between the findings in study two and previous findings in older adults (Parikh & Cole 2014) may be reside in the use of a skilled, novel VT task compared with an isometric force task. Moreover, in study three, the administration of multiple supervised rehabilitation sessions likely induced a short-term practice effect, leading to significant gains from the rehabilitation and sham-tDCS group immediately following the intervention.

Studies two and three showed that the addition of tDCS improved short (30 minutes) and long term (three week) retention of motor function and indices of corticospinal plasticity. These findings are in agreement with studies in healthy older adults (Parikh & Cole 2014) and stroke patients (Bolognini et al. 2011; Lefebvre et al. 2012), demonstrating that the simultaneous application of motor training and either a-tDCS or bilateral-tDCS improved retention of motor skills. An important finding from study three was that bilateral-tDCS preferentially retained motor function compared with rehabilitation alone, despite a similar magnitude of improvement immediately post-intervention between the bilateral-tDCS and sham-tDCS groups. While rehabilitation with sham-tDCS was able to improve motor function immediately following the intervention, bilateral-tDCS with rehabilitation led to greater retention of newly regained motor function up to three weeks post-intervention. Based on these findings, bilateral-tDCS may have upregulated long-term potentiation (LTP)-like processes involved with motor learning, which has valuable clinical implications for generating longer term improvements in upper limb recovery following a stroke.

There are a number of explanations as to why the addition of tDCS may be beneficial for the retention of motor learning in older adults and stroke patients. Firstly, afferent
feedback from exercise, coupled with enhanced corticospinal excitability from the anode, may induce a summative effect that enhances corticospinal plasticity (Monte-Silva et al. 2013). In support of this, previous studies have demonstrated advantageous effects on hand function following simultaneous a-tDCS and peripheral nerve stimulation in chronic stroke patients (Celnik et al. 2009; Sattler et al. 2015). Furthermore, consolidation of motor learning has been shown to occur during the offline period after training (Janacsek & Nemeth 2012), which suggests a ‘window’ of opportunity after an intervention where LTP-like adaptations, that are optimal for motor learning, can be maximised. Indeed, successive daily sessions of a-tDCS or c-tDCS have been shown to enhance the retention of motor function, which was not achieved when stimulation sessions were separated by a one week wash-out period (Boggio et al. 2007). Given that the after-effects of stimulation share common features to motor learning (Stagg et al. 2011), it is plausible that the persistent excitatory effects following a-tDCS and bilateral-tDCS may interact with the offline adaptations of the motor training itself, strengthening synaptic efficacy of the corticospinal pathway (CSP) and improving retention of motor skills in the older adults and stroke patients within this thesis.

6.2 Neurophysiological adaptations following motor training and tDCS in older adults and chronic stroke patients

There is some ambiguity as to whether older adults retain the ability to form use-dependent plasticity. Previous studies have reported reduced use-dependent plasticity in the dominant M1 following motor skill training (Rogasch et al. 2009; Sawaki et al. 2003), whilst Cirillo and colleagues (2010) reported that use-dependent plasticity was
greater in the non-dominant M1 following ballistic exercise (Cirillo, Rogasch & Semmler 2010). Interestingly, the findings from this thesis support an age-related reduction in use-dependent plasticity, which was more notable in the non-dominant M1. Despite using the same VT training task, study one found that training the dominant limb increased corticospinal excitability and released SICI within the dominant M1, whilst study two showed no effect on the same neurophysiological measures following VT of the non-dominant limb. Possible reasons for these disparate findings may be attributed to variations in participant physical activity levels (Cirillo et al. 2009), attentional focus (McNevin, Wulf & Carlson 2000) and genetic influences such as brain derived neurotrophic factor (BDNF) polymorphism (Cheeran et al. 2008; Kleim et al. 2006).

Although differences were observed in regards to age-related use-dependent plasticity following motor training alone, studies one and two demonstrated that the addition of both a-tDCS and bilateral-tDCS augmented corticospinal excitability within the non-dominant M1. The neurophysiological mechanisms by which a-tDCS and bilateral-tDCS improves motor function in older adults and stroke patients are likely to be multifactorial and not completely understood. However the findings from this thesis support that purposefully modulating excitatory and inhibitory circuitry within both the non-dominant/ipsilesional M1 and the dominant/contralesional M1 can lead to improvements in motor function. In study one, the addition of a-tDCS facilitated corticospinal excitability within the ipsilateral M1 concomitant with motor performance gains in the untrained non-dominant limb. Importantly, these responses were absent following unilateral training with sham-tDCS. A further observation was the release of SICI in both M1s following a-tDCS and sham-tDCS. It may be that the skilled nature of a VT task would require the suppression of extraneous movement,
and therefore modulated SICI in the ipsilateral and contralateral M1. In addition, visual feedback during task observation has been shown to modulate SICI in a similar manner to task execution, and may involve cortico-cortical projections from the PMA to the M1 (Strafella & Paus 2000).

Comparable to previous findings (Hinder et al. 2011), cross-limb transfer was absent in the untrained, non-dominant limb following unilateral training alone. However in contrast to study one, Hinder et al. (2011) observed an absence in cross-limb transfer in spite of a significant increase in corticospinal excitability within the ipsilateral M1. These differences may be partially attributed to the nature of the training task and the inclusion of a-tDCS over the ipsilateral M1. The use of a VT task is likely to integrate multiple sensorimotor areas due to its spatiotemporal demands (Perez, Lundbye-Jensen & Nielsen 2006; Perez et al. 2004), compared with ballistic contractions. Furthermore the addition of a-tDCS over the ipsilateral M1 may have upregulated bilateral corticospinal excitability during unilateral VT training. Therefore, it is plausible that the combination of a-tDCS with unilateral VT training strengthened corticospinal excitability within the ipsilateral M1, which manifested as improved motor performance in the untrained, non-dominant limb.

Given that study one demonstrated facilitated corticospinal excitability within the non-dominant M1 following a-tDCS, studies two and three employed a model of interhemispheric imbalance to investigate the efficacy of bilateral-tDCS on corticospinal plasticity within both M1s in older adults and chronic stroke patients. Although there is some evidence suggesting an advantageous improvement in motor function following bilateral-tDCS (Lindenberg et al. 2013; Vines, Cerruti & Schlaug 2008), study two observed no differences in motor function or the indices of
corticospinal plasticity between unilateral a-tDCS and bilateral-tDCS. These findings were partially attributed to the fact that the older adults in the study did not display significant interhemispheric asymmetries in corticospinal excitability, and therefore the addition of the cathode in the bilateral-tDCS montage may have become somewhat redundant. When applied unilaterally, cathodal-tDCS (c-tDCS) has been shown to suppress corticospinal excitability and improve motor function (Au-Yeung et al. 2014; Boggio et al. 2007; Fregni et al. 2005; Lee & Chun 2014; Nair et al. 2011), however the role of the cathode in a bilateral-tDCS montage is not completely understood. In healthy adults and stroke patients, it is suggested that bilateral-tDCS decreases interhemispheric inhibition (IHI) from the dominant/contralesional to the non-dominant/ipsilesional M1, which has been associated with improvements in motor function (Bolognini et al. 2011; Williams, Pascual-Leone & Fregni 2010). A limitation throughout this thesis was that IHI was unable to be quantified, and therefore the contribution of adaptations within transcallosal pathways mediating improvements in motor function in older adults and chronic stroke patients cannot be conclusively discussed.

In study two, there was no effect on SICI and only a small suppression of corticospinal excitability (15%) in the dominant M1 receiving the cathode electrode. In contrast, study three demonstrated an increase in SICI and silent period duration (SPD) within the contralateral M1 receiving the cathode, suggesting that bilateral-tDCS may modulate intracortical inhibitory neurons differently in healthy older adults compared with chronic stroke patients. Accordingly, when comparing the findings of studies two and three, the concept of homeostatic plasticity should also be considered (Fricke et al. 2011; Siebner et al. 2004; Stagg et al. 2011; Ziemann et al. 2004), whereby different excitability states have been suggested to influence the response to various NIBS
protocols (Bradnam et al. 2012; Li, Uehara & Hanakawa 2015; Siebner et al. 2004), and are likely to differ between healthy older adults and chronic stroke patients. For example, in stroke patients, the magnitude of intact cortical tissue appears to significantly affect the neurophysiological and behavioural responses from c-tDCS and bilateral-tDCS (Bradnam et al. 2012; Lindenberg et al. 2013; Lindenberg et al. 2010). Although speculative, it is feasible that in stroke patients, the degree of inhibitory imbalance between the ipsi- and contralesional M1 may influence the responsiveness to bilateral-tDCS. Moreover, in healthy adults, no differences in corticospinal excitability have been observed between unilateral-tDCS and bilateral-tDCS (Kidgell et al. 2013). Based on this, populations without severe interhemispheric imbalances may not respond to the addition of the cathode during the bilateral-tDCS electrode montage.

In study three, no suppression of corticospinal excitability was observed in the contralesional M1 (receiving the cathode electrode), which is contrary with previous studies examining the after-effects of both c-tDCS and bilateral-tDCS in healthy adults (Di Lazzaro et al. 2012a) and stroke patients (Bolognini et al. 2011; Zimerman et al. 2012). One hypothesis to explain these findings may be that in more severely affected stroke patients, activation of the ipsilateral CSP may be an adaptive, rather than maladaptive process to produce movement (Bradnam, Stinear & Byblow 2013; Bradnam et al. 2010). When performing training of the paretic limb, increased activation of the ipsilateral CSP may have counteracted the hyperpolarisation effect from the cathode in some of the more severely affected participants. As study three did not exclude participants based on the presence of a motor evoked potential (MEP), the role of the cathode during bilateral-tDCS in more severely impaired individuals may not have had an advantageous effect.
Although bilateral-tDCS did not suppress corticospinal excitability in the contralesional M1 in stroke patients, a novel finding from study three was that SPD was prolonged in the contralesional M1 following bilateral-tDCS, which was not quantified in the earlier studies. This finding is in contrast to previous studies using unilateral c-tDCS, whereby no changes in SPD were observed (Suzuki et al. 2012; Tremblay et al. 2013). A major difference between the findings in study three and previous studies is the prescription of a multi-session intervention, whereby the previous studies measuring SPD have only applied a single session of c-tDCS. As no change in contralesional M1 excitability was observed in study three, it is likely that bilateral-tDCS targeted intracortical inhibitory circuits, contributing to retention of motor improvement in the paretic limb, either via ipsilateral (Bradnam, Stinear & Byblow 2013) or transcallosal pathways (Tazoe et al. 2014; Volz et al. 2014). In study three, the increase in SICI at the follow-up, observed in conjunction with an increase in SPD, suggests that strengthening of gamma-aminobutyric acid (GABA)-mediated inhibitory synapses within the contralesional M1 may improve retention of newly regained motor skills in chronic stroke patients. Based on these findings and previous pharmacological evidence (Nitsche et al. 2004c), GABA-mediated intracortical inhibition may be a key mechanism involved in prolonging the after-effects of tDCS and therefore retaining improvements in motor function. Although SPD is indicative of GABA\textsubscript{B} mediated inhibition (Lang et al. 2006), the measurement of long-interval intracortical inhibition (LICI) to further understand the role of GABA\textsubscript{B} receptors modulating motor function in older adults and stroke patients would have also been beneficial, and is a limitation of this thesis.

Comparisons between the neurophysiological adaptations in studies two and three suggest that the excitatory and inhibitory effects of bilateral-tDCS are different in older
adults compared with chronic stroke patients. Based on these findings, it is evident that the optimal electrode montage for the induction of corticospinal plasticity is not a “one-size fits all” approach. These findings warrant future research to investigate individually tailoring the electrode montage, to reduce inter-participant variability as well as variance amongst the literature (Ridding & Ziemann 2010).

When considering the neurophysiological adaptations following motor training and tDCS, it is important to consider the limitations of transcranial magnetic stimulation (TMS) as a technique to quantify indices of corticospinal plasticity, particularly in degenerative populations and those with widespread neurological deficits. As TMS-evoked responses rely on activation of the CSP, the integrity of the CSP would likely influence the neurophysiological responses. As some of the participants in study three experienced improvement in motor function independent of significant increases in MEP amplitude, it is likely that TMS may overlook the contribution of additional motor areas responsible for functional recovery. Similarly, throughout the ageing process there is an increased demand on motor areas outside the M1 in order to produce efficient movement (Seidler et al. 2010). Certainly, studies using functional magnetic resonance imagining (fMRI) during and following both a-tDCS and bilateral-tDCS, have reported improved activation in the M1 and surrounding regions including; the hippocampus, SMA and posterior cingulate cortex (Antal et al. 2011; Lindenberg et al. 2013; Stagg et al. 2011). Therefore, although only speculative, it is plausible that the non-focal nature of a-tDCS and bilateral-tDCS (through the use of 25cm² electrodes) may have exerted a neuromodulatory effect on surrounding neural tissue. This would seemingly contribute to strengthening the formation of muscle synergies into effective sequences, and improve motor learning of the non-dominant and paretic limb in older adults and chronic stroke patients.
6.3 Future research directions

The findings from this thesis give rise to several key research questions that warrant future work, in order to progress the therapeutic application of tDCS in neurodegenerative and pathological conditions. A fundamental consideration moving towards the clinical translation of tDCS is the variability of ‘responders’ and ‘non-responders’ to stimulation protocols, as well as variability in intra-participant responses between sessions (Jacobson, Koslowsky & Lavidor 2012; Puri et al. 2015). Previous pilot trials have focused on recruiting homogenous samples to reduce inter-participant variability. Confounding factors have been identified to influence the responsiveness to tDCS including; genetic factors such as BDNF polymorphism (Antal et al. 2010; Puri et al. 2015), physical activity levels and gender differences (Ridding & Ziemann 2010). However in populations with reduced neuromuscular health such as ageing and stroke, confounding variables such as the integrity of the M1 (Heise et al. 2014; Stinear et al. 2007), lesion size and type (Bolognini et al. 2015), time since stroke (Marquez et al. 2013) as well as medications and cognitive factors (motivation, mood and attention) should be thoroughly examined.

Future research would also benefit from genetic sampling to identify BDNF polymorphism, as well as matching groups for the level of corticospinal excitability prior to the intervention. As tDCS has been shown to involve events at both the cellular and molecular level (Medeiros et al. 2012), more thorough research into the neurobiological mechanisms is warranted, as well as the combination of fMRI and TMS to probe a clearer picture as to the mechanisms and regions responsible for improvements in motor function. Furthermore, there is strong evidence that the neurophysiological effects of tDCS are highly dependent upon the excitatory state of
the M1 (Brunoni et al. 2012). In animal models, the direction of plasticity formation has been shown to be influenced by theta oscillations in the brain (Huerta & Lisman 1995). Therefore, EEG recording during combined tDCS and motor training could be beneficial for future research to gain a more in-depth understanding of the neurological processes involved during the application of tDCS in humans. This information should be the focus of future research in ageing and stroke affected populations, in order to inform optimal dosage and prescription guidelines for clinically meaningful adaptations.

While the studies in this thesis had adequate sample sizes predicted for sufficient power, larger-scale clinical trials, tailoring the prescription of stimulation parameters such as current density and frequency of treatment, are warranted. To date, only one multicentre trial applying a-tDCS or c-tDCS and robotic therapy in acute stroke has been reported (Hesse et al. 2011), and thus there is a need for more multicentre clinical trials in order to establish the efficacy of concurrent rehabilitation and tDCS. One impediment towards obtaining a larger sample size in study three was due to the individuals not meeting the inclusion criteria for TMS and/or tDCS, because of a high prevalence of post-stroke seizures as well as cranial metal implants as a result of craniotomies. Although bilateral-tDCS has produced encouraging results for the improvement of motor function, the prevalence of these contraindications raises some question on the feasibility of its use amongst chronic stroke patients.

A fundamental consideration for future research is the frequency of stimulation sessions and the optimal ‘window’ in which the application of tDCS is most beneficial. Given that offline effects of motor learning have been shown to be relevant in promoting retention, future research may consider administering tDCS on the rest days...
of a rehabilitation or training intervention. As the age-related functional and neurophysiological decline is degenerative, future research should also focus on investigating whether tDCS can be used as a preventative tool for neurological decline as opposed to rehabilitative. In stroke patients, given the effects of tDCS are affected by the physiological state of the CSP, different parameters and electrode montages should be explored in both the acute and chronic phases of rehabilitation. The length of treatment as well as the duration of each stimulation session also needs to be considered in regards to safety, as well as identifying potential ceiling effects. In addition to study three, to my knowledge only two other studies have prescribed over five sessions of bilateral-tDCS combined with physical therapy, with no reported side effects (Ang et al. 2015; Bolognini et al. 2011). Therefore, there is a need for future research to investigate longer intervention durations with multiple follow-ups to determine the optimal safety and prescription guidelines for clinicians and users.

It is worthwhile noting that the older adults and stroke patients within this thesis did observe some gains in motor function following motor training alone. This highlights the relevance of exploring different modes (i.e. motor skill, aerobic and resistance) and intensities of exercise that may preferentially up regulate use-dependent plasticity in older adults and chronic stroke patients. The expression of neurotrophic factors, in particular BDNF plays a significant role in modulating use-dependent corticospinal plasticity and motor learning in healthy adults (Achiron & Kalron 2008; Lu 2003; Ridding & Ziemann 2010). In humans, regular aerobic exercise has been shown to upregulate BDNF expression in both older (Coelho et al. 2013) and younger adults (Griffin et al. 2011). On this basis, it may be of interest for future research to explore the combination of aerobic physical activity prior to or between tDCS and motor
training/rehabilitation sessions, in order to investigate whether larger, clinically meaningful changes in motor function can be achieved.

6.4 Conclusion

Understanding techniques that can purposefully and beneficially modify the human CNS is of increasing importance as our population ages. Moreover, as there is currently no cure for stroke, improving rehabilitation outcomes for surviving patients remains the cornerstone of contemporary research. This thesis contributes to the existing literature in the field of exercise neurophysiology and NIBS, by demonstrating that the combination of tDCS with motor training is beneficial for improving motor function in the non-dominant/paretic limb of older adults and chronic stroke patients. Even more so, a multi-session intervention appears to induce long-lasting improvements in motor function which are indicative of corticospinal plasticity. Although the mechanisms mediating motor learning in ageing and stroke are multifaceted, changes in the balance of corticospinal excitability and inhibition within both M1s appear to have some significance towards improvements in motor function observed throughout the studies in this thesis. Collectively, the findings from this thesis provide novel insight into the advantages of concurrent tDCS and motor training on behavioural and neurophysiological function as well as retaining improvements in motor function in older adults and chronic stroke patients.
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APPENDICES

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Appendix A:  Transcranial Magnetic Stimulation† (TMS) Adult Safety Screen

**Name:**

**Date:**

**Age:**

Please answer the following:

Have you ever:

Had an adverse reaction to TMS?  □Yes  □No

Had a seizure?  □Yes  □No

Had an electroencephalogram (EEG)? □Yes □No

Had a serious head injury (include neurosurgery)? □Yes □No

Had any other brain-related condition? □Yes □No

Had any illness that caused brain injury? □Yes □No

Do you have any metal in your head (outside the mouth) such as shrapnel, surgical clips, or fragments from welding or metalwork? □Yes □No

Do you have any implanted devices such as cardiac pacemakers, medical pumps, or intracardiac lines? □Yes □No

Do you suffer from frequent or severe headaches/migraines? □Yes □No

Are you taking any medications? □Yes □No

Are you pregnant, or is it possible that you may be pregnant? □Yes □No

Does anyone in your family have epilepsy? □Yes □No

Do you need further explanation of TMS and its associated risks? □Yes □No

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

____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________

253
† For use with single-pulse TMS, paired-pulse TMS, or repetitive TMS.
SUBJECT INFORMATION

Subject Details

Subject Name:________________________________________________________

Address:_____________________________________________________________

Ph:___________________________

Sex:__________________________

DOB:_________________________

Occupation:__________________________________________________________

Ethnic Background______________

Background information

Do you suffer from any known neurological disorders? (Not including your stroke)

______________________________________________________________

Are you currently taking any medication which influences nerve conduction or blood clotting? If so, what?

______________________________________________________________

Do you regularly drink beverages containing caffeine? If so, how many cups per day?

______________________________________________________________

Which arm do you consider your dominant arm?

______________________________________________________________

Have you had previous musculoskeletal injuries (specifically in the last 12 months) relating to your arm/hand?

______________________________________________________________

Do you where glasses/contact lenses

______________________________________________________________
Appendix B: Medical Questionnaire

Responses to this questionnaire will be kept strictly confidential. The responses from this questionnaire will provide the investigators with appropriate information to establish suitability of your participation in this study. Anyone who currently has, or has had in the past, a serious musculoskeletal injury, epilepsy, are pregnant or have a cardiac pacemaker may be excluded from the study for health and safety reasons.

NAME: …………………………………………… AGE: ……. (yrs)
GENDER: ……
BODY MASS: …………. (kg) HEIGHT: ……….. (cm)

Are you currently undertaking any form of regular exercise/rehabilitation?
YES NO

If yes, briefly describe the type and amount (i.e frequency, duration, intensity, types of activities) of exercise you perform.
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________

1. Are you a smoker? YES NO (Please circle)

Has anyone ever told you that you:

2.1 Are overweight? YES NO UNKNOWN

2.2 Have high blood pressure? YES NO UNKNOWN

2.3 Have a heart condition or heart murmur? YES NO UNKNOWN

2.4 Have asthma or a respiratory condition? YES NO UNKNOWN

2.5 Have diabetes? YES NO UNKNOWN

2.6 Have a bleeding disorder (e.g. haemophilia)? YES NO UNKNOWN
Have you ever experienced:

3.1 Chest pain, chest discomfort, chest tightness or chest heaviness?
YES  NO  UNKNOWN

3.2 Shortness of breath out of proportion to exercise undertaken?
YES  NO  UNKNOWN

3.3 Heart palpitations (sensation of abnormally fast and/or irregular heart beat)?
YES  NO  UNKNOWN

3.4 Episodes of fainting, collapse or loss of consciousness?
YES  NO  UNKNOWN

3.5 Abnormal bleeding or bruising?  YES  NO  UNKNOWN

3.6 Gastrointestinal problems?  YES  NO  UNKNOWN

If you answer YES to any of the following, please elaborate in the space provided:

4. Do you have a family history of cardiovascular disease? (eg. heart attack, chest pain/angina, stroke)  YES  NO  UNKNOWN

_______________________________________________________________________
_______________________________________________________________________

5. Do you have a family history of diabetes?  YES  NO  UNKNOWN

_______________________________________________________________________
_______________________________________________________________________

6. Have you ever suffered any musculoskeletal injury?  YES  NO  UNKNOWN

_______________________________________________________________________
_______________________________________________________________________

7. Have you ever experienced difficulty swallowing or any other gastrointestinal problem?  YES  NO  UNKNOWN

_______________________________________________________________________
_______________________________________________________________________

8. Do you have any allergies? (Including to medications)  YES  NO  UNKNOWN

_______________________________________________________________________

257
9. Are you currently on any medication? (if yes please list)  YES  NO
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________

10. Are you currently taking anabolic steroids or any other performance-enhancing agents?  YES  NO
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________

11. Is there any other reason which you know of that would prevent you from undertaking the proposed exercise and other tests?  YES  NO
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________

Questions specific to stroke

1. Is this your first stroke?  YES  NO  UNKNOWN
_______________________________________________________________________

2. If unilateral / which side of your brain did your stroke occur in?
_______________________________________________________________________

3. What type of stroke did you have (i.e. Ischemic or Haemorrhagic)?
_______________________________________________________________________

4. Are you currently taking any medications to control spasticity?
YES  NO  UNKNOWN
_______________________________________________________________________

5. Where did your stroke occur (i.e. cortical, sub-cortical?)
_______________________________________________________________________
6. What year/month did your stroke occur?

_______________________________________________________________________
_______________________________________________________________________

7. Do you suffer from severe pain in your stroke affected limb?  YES NO
_______________________________________________________________________
_______________________________________________________________________

8. Do you suffer from severe a) aphasia, b) neglect or c) depression?  YES NO
_______________________________________________________________________
_______________________________________________________________________

9. How would you describe your stroke affected upper limb in relation to function (i.e. full function, moderate function, low function?)
_______________________________________________________________________
_______________________________________________________________________

10. What type of rehabilitation did you receive following your stroke (include examples of activities and duration of rehabilitation)
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________

11. Is there any other reason/information which you know of that would prevent you from undertaking the proposed exercise and other tests?  YES NO
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________
_______________________________________________________________________

I believe the information I have provided to be true and correct.

SIGNED: .................................................................DATE: ..........................
Appendix C: Edinburgh Handedness Inventory

You’re Initials: __________________

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.

<table>
<thead>
<tr>
<th>Task / Object</th>
<th>Left Hand</th>
<th>Right Hand</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Writing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Drawing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Throwing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Scissors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Toothbrush</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Knife (without fork)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Spoon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Broom (upper hand)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Striking a Match (match)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Opening a Box (lid)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total checks:</th>
<th>LH =</th>
<th>RH =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative Total</td>
<td>CT = LH + RH =</td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>D = RH – LH =</td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td>R = (D / CT) × 100 =</td>
<td></td>
</tr>
</tbody>
</table>

Interpretation:
(Left Handed: R < -40)
(Ambidextrous: -40 ≤ R ≤ +40)
(Right Handed: R > +40)
**Appendix D: Mini-Mental State Examination**

Patient name........................................Date of birth......................... Date of test..............................

<table>
<thead>
<tr>
<th>Section</th>
<th>Questions</th>
<th>Max points</th>
<th>Patient score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Orientation</td>
<td>a) Can you tell me today's (date)/(month)/(year)?</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Which (day of the week) is it today?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can you also tell me which (season) it is?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) What city/town are we in?</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>What is the (county)/(country)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>What (building) are we in and on what (floor)?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Registration</td>
<td>I should like to test your memory. (name 3 common objects: e.g. &quot;ball, car, man&quot;)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Can you repeat the words I said (score 1 point for each word)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(repeat up to 6 trials until all three are remembered)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>record number of trials needed here:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Attention and Calculation</td>
<td>a) From 100 keep subtracting 7 and give each answer:</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) Stop after 5 answers. (93 - 86 - 79 - 72 - 65 - ).</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alternatively</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>b) Spell the word 'WORLD' backwards, (D - L - R - O - W).</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>4 Recall</td>
<td>What were the three words I asked you to say earlier?</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Skip this test if all three objects were not remembered during registration test).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Language - Naming</td>
<td>Name these objects (show a watch) (show a pencil)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Repeating Repeat the following: &quot;no ifs, and or buts&quot;</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6 Reading</td>
<td>(show card or write &quot;CLOSE YOUR EYES&quot;)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Read this sentence and do what is says.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Writing Now can you write a short sentence for me?</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7 Language - Three stage command</td>
<td>(Present paper)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Take this paper in your left (or right) hand, fold it in half, and put it on the floor.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 Construction</td>
<td>Will you copy this drawing please?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Patient Score: /30

Notes
Appendix E: International Physical Activity Questionnaire

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT
FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous and moderate activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home? Yes No Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the last 7 days as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, heavy construction, or climbing up stairs as part of your work? Think about only those physical activities that you did for at least 10 minutes at a time.

___________ days per week
No vigorous job-related physical activity Skip to question 4

3. How much time did you usually spend on one of those days doing vigorous physical activities as part of your work?

___________ hours per day
4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads as part of your work? Please do not include walking.

__________ days per week
No moderate job-related physical activity  Skip to question 6

5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?

__________ hours per day
__________ minutes per day

6. During the last 7 days, on how many days did you walk for at least 10 minutes at a time as part of your work? Please do not count any walking you did to travel to or from work.

__________ days per week
No job-related walking  Skip to PART 2: TRANSPORTATION

7. How much time did you usually spend on one of those days walking as part of your work?

__________ hours per day
__________ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you travelled from place to place, including to places like work, stores, movies, and so on.

8. During the last 7 days, on how many days did you travel in a motor vehicle like a train, bus, car, or tram?

__________ days per week
No traveling in a motor vehicle  Skip to question 10
9. How much time did you usually spend on one of those days traveling in a train, bus, car, tram, or other kind of motor vehicle?

_______ hours per day

_______ minutes per day

Now think only about the bicycling and walking you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the last 7 days, on how many days did you bicycle for at least 10 minutes at a time to go from place to place?

_______ days per week

No bicycling from place to place    Skip to question 12

11. How much time did you usually spend on one of those days to bicycle from place to place?

_______ hours per day

_______ minutes per day

12. During the last 7 days, on how many days did you walk for at least 10 minutes at a time to go from place to place?

_______ days per week

No walking from place to place    Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

13. How much time did you usually spend on one of those days walking from place to place?

_______ hours per day

_______ minutes per day
PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the last 7 days in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, chopping wood, shoveling snow, or digging in the garden or yard?

__________ days per week

No vigorous activity in garden or yard Skip to question 16

15. How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?

__________ hours per day

__________ minutes per day

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, sweeping, washing windows, and raking in the garden or yard?

__________ days per week

No moderate activity in garden or yard Skip to question 18

17. How much time did you usually spend on one of those days doing moderate physical activities in the garden or yard?

__________ hours per day

__________ minutes per day

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, washing windows, scrubbing floors and sweeping inside your home?

__________ days per week
19. How much time did you usually spend on one of those days doing moderate physical activities inside your home?

__________ hours per day
__________ minutes per day

20. Not counting any walking you have already mentioned, during the last 7 days, on how many days did you walk for at least 10 minutes at a time in your leisure time?

__________ days per week

No walking in leisure time  Skip to question 22

21. How much time did you usually spend on one of those days walking in your leisure time?

__________ hours per day
__________ minutes per day

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like aerobics, running, fast bicycling, or fast swimming in your leisure time?

__________ days per week

No vigorous activity in leisure time  Skip to question 24

23. How much time did you usually spend on one of those days doing vigorous physical activities in your leisure time?

__________ hours per day
__________ minutes per day
24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis in your leisure time?

__________days per week

No moderate activity in leisure time  Skip to PART 5: TIME SPENT SITTING

25. How much time did you usually spend on one of those days doing moderate physical activities in your leisure time?

__________hours per day

__________minutes per day

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the last 7 days, how much time did you usually spend sitting on a weekday?

__________hours per day

__________minutes per day

27. During the last 7 days, how much time did you usually spend sitting on a weekend day?

__________hours per day

__________minutes per day

This is the end of the questionnaire, thank you for participating
Appendix F: Motor Assessment Scale (upper limb items)

6. Upper Arm Function

1. Supine, protract shoulder girdle with arm in 90 degrees of shoulder flexion. (Therapist places arm in position and supports elbow in extension).

2. Supine, hold arm in 90 degrees of shoulder flexion for 2 seconds. (Therapist places arm in position and patient must maintain position with some [45 degrees] external rotation. Elbow must be held within at least 20 degrees of full extension).

3. Supine, hold arm in 90 degrees of shoulder flexion, flex and extend elbow to take palm to forehead. (Therapist may assist supination of forearm).

4. Sitting, hold extended arm in forward flexion at 90 degrees to body for 2 seconds. (Therapist should place arm in position and patient maintains position. Patient must hold arm in mid-rotation [thumb pointing up]. Do not allow excess shoulder elevation).

5. Sitting, patient lifts arm to above position, holds it there for 10 seconds and then lowers it. (Patient must maintain position with some external rotation. Do not allow pronation).

6. Standing, hand against wall. Maintain hand position, while turning body toward wall. (Arm is abducted to 90 degrees with palm flat against the wall).
7. **Hand Movements**

1. Sitting, extension of wrist. (Patient sits at a table with forearm resting on the table. Therapist places cylindrical object in palm of patient's hand. Patient is asked to lift object off the table by extending the wrist. Do not allow elbow flexion).

2. Sitting, radial deviation of wrist. (Therapist places forearm in mid pronation-supination, i.e., resting on ulnar side, thumb in line with forearm and wrist in extension, fingers around a cylindrical object. Patient is asked to lift hand off table. Do not allow elbow flexion or pronation).

3. Sitting, elbow into side, pronation and supination. (Elbow unsupported and at a right angle. Three-quarter range is acceptable).

4. Sitting, reach forward, pick up large ball of 14 cm (5in) diameter with both hands and put it down. (Ball should be placed on table at a distance that requires elbow extension. Palms should be kept in contact with the ball).

5. Sitting, pick up a polystyrene cup from table and put it on table across other side of body (Do not allow alteration in shape of cup).

6. Sitting, continuous opposition of thumb and each finger more than 14 times in 10 seconds. (Each finger in turn taps the thumb, starting with index finger. Do not allow thumb to slide from one finger to the other, or to go backwards).
8. **Advanced Hand Activities**

1. Pick up the top of a pen and put it down again. (Patient reaches forward to arm's length, picks up pen top, and releases it on table close to body).

2. Pick up one jellybean from a cup and place it in another cup. (Teacup contains eight jellybeans. Both cups must be at arms’ length. Left hand takes jellybean from cup on right and releases it in cup on left.

3. Draw horizontal lines to stop at a vertical line 10 times in 20 seconds. (At least five lines must touch and stop at the vertical line. Lines should be approximately 10cm in length).

4. Hold a pen, make rapid consecutive dots on a sheet of paper. (Patient must do at least 2 dots a second for 5 seconds. Patient picks pen up and positions it without assistance. Pen must be held as for writing. Dots not dashes).

5. Take a dessert spoon of liquid to the mouth (Do not allow head to lower towards spoon. Liquid must not spill).

6. Hold a comb and comb hair at back of head (Shoulder must be externally rotated, abducted at least 90°. Head erect).
## MOTOR ASSESSMENT SCALE

**Name:**

<table>
<thead>
<tr>
<th>MOVEMENT SCORING SHEET</th>
<th>MOVEMENT SCORING SHEET</th>
</tr>
</thead>
<tbody>
<tr>
<td>DATE: ........................</td>
<td>DATE: ........................</td>
</tr>
<tr>
<td>1. Supine to side lying</td>
<td>1. Supine to side lying</td>
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<tr>
<td>2. Supine to sitting over side of bed</td>
<td>2. Supine to sitting over side of bed</td>
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<tr>
<td>4. Sitting to standing</td>
<td>4. Sitting to standing</td>
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<tr>
<td>5. Walking</td>
<td>5. Walking</td>
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<tr>
<td>6. Upper arm function</td>
<td>6. Upper arm function</td>
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<tr>
<td>8. Advanced hand activities</td>
<td>8. Advanced hand activities</td>
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</tbody>
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**COMMENTS (IF APPLICABLE)**

**J. Carr and R. Shepherd**  
School of Physiotherapy  
Faculty of Health Sciences  
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September 1994
Appendix G: Tardieu Scale

TARDIEU SCALE

This scale quantifies muscle spasticity by assessing the response of the muscle to stretch applied at specified velocities.

Grading is always performed at the same time of day, in a constant position of the body for a given limb. For each muscle group, reaction to stretch is rated at a specified stretch velocity with 2 parameters x and y.

Velocity to stretch (V)

| V1  | As slow as possible |
| V2  | Speed of the limb being falling |
| V3  | As fast as possible (> natural drop) |

V1 is used to measure the passive range of Motion (PROM). Only V2 and V3 are used to rate spasticity.

Quality of muscle reaction (X)

| 0   | No resistance throughout passive movement |
| 1   | Slight resistance throughout, with a clear catch at a precise angle |
| 2   | Clear catch at a precise angle, followed by release |
| 3   | Fatigable clonus (<10 secs) occurring at a precise angle |
| 4   | Unfatigable clonus (>10 secs) occurring at a precise angle |
| 5   | Joint Immobile |

Angle of muscle reaction (Y)

Measure relative to the position of maximal stretch of the muscle (corresponding at angle)

Spasticity Angle

R1 Angle of catch seen at Velocity V2 or V3
R2 Full range of motion achieved when muscle is at rest and tested at V1 velocity

Boyd, Graham 1999
- A large difference between R1 & R2 values in the outer to middle range of normal m. length indicates a large dynamic component
- A small difference in the R1 & R2 measurement in the middle to inner range indicates predominantly fixed contracture

<table>
<thead>
<tr>
<th>Date</th>
<th>Joint/Muscle</th>
<th>L/R</th>
<th>V</th>
<th>X</th>
<th>R1</th>
<th>R2</th>
<th>Active ROM</th>
<th>Power MRC</th>
<th>Ashworth Rating</th>
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