Physiological studies investigating the effect of homeostatic plasticity of the motor cortex on the expression of muscle strength.

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Abstract

The effect of anodal transcranial direct current stimulation (tDCS) on corticospinal excitability and inhibition was studied throughout this thesis. The primary aim was to investigate the induction of homeostatic plasticity and its effect on muscle strength and to determine the influence of the brain-derived neurotrophic factor (*BDNF)* polymorphism on the induction of homeostatic plasticity and muscle strength.

Study 1 investigated the effect of a single session of anodal tDCS on corticospinal excitability and inhibition of both the stimulated and non-stimulated primary motor cortex (M1) and whether the induction of homeostatic plasticity was influenced by the *BDNF* polymorphism. Corticospinal excitability increased for both the stimulated and non-stimulated hemisphere, irrespective of whether the dominant or non-dominant M1 was stimulated, showing functional connectivity between the motor cortices. Interestingly, there was a shift in lateralisation of corticospinal inhibition towards the right (non-dominant) M1 irrespective of which M1 was stimulated. The corticospinal responses following anodal tDCS were influenced by the *BDNF* polymorphism, with greater responses from *Val/Val* participants.

Given that the induction of corticospinal plasticity is thought to be important for motor function, Study 2 examined the effect of four consecutive sessions of anodal tDCS applied over the left M1 on corticospinal excitability/inhibition and transcranial magnetic stimulation (TMS) voluntary activation and whether these responses were influenced by the *BDNF* polymorphism. Following four consecutive days of anodal tDCS, corticospinal excitability and TMS voluntary activation (VA_{TMS}) of the wrist flexors increased, whilst corticospinal inhibition decreased, which led to an increase in voluntary isometric force

production. There were no changes in short-interval intracortical inhibition (SICI), however, the magnitude of the corticospinal responses was influenced by the *BDNF* polymorphism, with the *Val/Val* showing greater responses.

As emerging evidence suggests that the M1 may be an important neural structure that underpins the rapid gain in muscle strength following short-term strength training, Study 3 examined the effect of homeostatic plasticity induced by 20 minutes of anodal tDCS over the left M1 prior to a single bout of heavy-load strength training on the corticospinal responses and whether the magnitude of these responses was differentially modulated by the presence of the *BDNF* polymorphism. Similar to Study 1 and 2, homeostatic plasticity of the M1, induced by 20 minutes of anodal tDCS over the left M1, led to an increase in corticospinal excitability, a decrease in corticospinal inhibition, but intriguingly, the *BDNF* polymorphism had no effect on the magnitude of these responses.

Given that Study 1 confirmed that anodal tDCS applied over the M1 induced bilateral increases in corticospinal excitability, Study 4 examined the influence of homeostatic plasticity which was induced by 20 minutes of anodal tDCS applied over the ipsilateral M1 (iM1) on the cross-transfer of strength. Anodal tDCS of the iM1, prior to a single bout of heavy-load strength training, increased strength of the untrained left arm by 12%, which was accompanied by an increase in corticospinal excitability, but there were no differences in corticospinal inhibition. Interestingly, the corticospinal responses were greater for the *Val/Met* participants following anodal tDCS and strength training, however, there was no difference in the magnitude of strength transfer between genotypes.

It was concluded that the induction of homeostatic plasticity plays an important role in the development of muscle strength. Importantly, anodal tDCS does not appear to act focally upon the site of stimulation, but rather has a global effect on distant non-stimulated regions, demonstrating functional connectivity. Critically, the presence of the *BDNF* polymorphism only influences the corticospinal responses to anodal tDCS (i.e., corticospinal excitability) but does not play a role in any functional measures such as the expression of maximum voluntary strength.

Declaration

"I, Ashlyn K. Frazer declare that the PhD thesis entitled "***Physiological studies investigating the effect of homeostatic plasticity of the motor cortex on the expression of muscle strength"*** is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

Signature: [redacted] **Date:** 29/11/16

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Lastly, I dedicate this thesis to Hayley and Georgia. As my parents and partner did for me, I hope that one day this acts as an inspiration to not only dream the ‘impossible’ but also to achieve the ‘impossible’.

Publications and Abstracts

Publications arising from thesis

Chapter 4

Frazer, A, Williams, J, Spittle, M, Rantalainen, T & Kidgell, D 2016, 'Anodal Transcranial Direct Current Stimulation of the Motor Cortex Increases Cortical Voluntary Activation and Neural Plasticity', *Muscle and Nerve*, vol. 54, no. 5, pp. 903-913.

Chapter 6

Frazer, A, Williams, J, Spittle, M, & Kidgell, D 2016, 'Cross-education of muscular strength is facilitated by homeostatic plasticity', *European Journal of Applied Physiology.* (Accepted January 5th 2017).

Abstract arising from thesis

Chapter 4

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

1RM: One-repetition maximum

AMT: Active motor threshold

AMT SI: Active motor threshold stimulus intensity

AUC: Area under the curve

***BDNF*:** Brain-derived neurotrophic factor

Ca^{2+}: Calcium

CMEPS: Cervicomedullary motor–evoked potentials

CNS: Central nervous system

CST: Corticospinal tract

DH atDCS: Anodal tDCS applied over the dominant M1

ECR: Extensor carpi radialis

EEG: Electroencephalography

EMG: Electromyography

FCR: Flexor carpi radialis

FDI: First dorsal interosseus

fMRI: Functional magnetic resonance imaging

GABA: Gamma-Aminobutyric acid

H-reflex: Hoffman reflex

ICF: Intracortical facilitation

IHI: Interhemispheric inhibition

iM1: Ipsilateral primary motor cortex

ISI: Interstimulus interval

iTMS: I-wave periodicity TMS

JTT: Jebsen-Taylor Hand Function Test

LI: Laterality index

LICI: Long-interval intracortical inhibition

LQ: Laterality quotient

LTD: Long-term depression

LTP: Long-term potentiation

MEG: Magnetoencephalography

Mg^{2+}: Magnesium

M1: Primary motor cortex

MEP: Motor evoked potential

M_{MAX}: Maximum compound wave

MT: Motor threshold

MVIC: Maximal voluntary isometric contraction

N-DH atDCS: Anodal tDCS applied over the non-dominant M1

NIBS: Non-invasive brain stimulation

NIRS: Near-infrared spectroscopy

NMDA receptor: N-methyl-D-aspartate receptor

PAS: Paired associative stimulation

PET: Positron emission tomography

PP: Paired-pulse

PTP: Post-tetanic potentiation

***rms*EMG:** Root mean square of the surface EMG

RMT: Resting motor threshold

rTMS: Repetitive transcranial magnetic stimulation

sEMG: Surface electromyography

SICF: short-interval intracortical facilitation

SICI: Short-interval intracortical inhibition

SP: Single-pulse

ST: Strength training

STP: Short-term potentiation

TBS: Theta burst stimulation

tDCS: Transcranial direct current stimulation

TMS: Transcranial magnetic stimulation

VAS: Visual analogue scale

VA_{TMS}: Voluntary activation measured using TMS

Chapter 1：Introduction

The human primary motor cortex (M1) is highly modifiable and plays a critical role in the acquisition of motor behaviours (Pascual-Leone et al. 1995, Classen et al. 1998, Rioult-Pedotti et al. 1998, Butefisch et al. 2000, Muellbacher et al. 2002). The modifiable nature of the M1 is termed plasticity and involves changes in synaptic efficacy of pyramidal neuron connections, involving the processes of long-term potentiation (LTP) and long-term depression (LTD) (Hess & Donoghue 1996, Sanes & Donoghue 2000). Improvements in synaptic efficacy are mediated by the N-methyl-D-aspartate (NMDA) receptor which operates as a channel responsible for the induction of LTP and LTD (Castro-Alamancos et al. 1995). However, it should be noted that the M1 contains a large number of pyramidal tract neurons which descend and synapse monosynaptically onto the ventral horn of the spinal cord forming the corticospinal tract (CST) (Nathan et al. 1990). Therefore, in the context of this thesis, modifications of the intrinsic circuitry of the M1 (i.e., increases in corticospinal excitability and decreases in inhibition), which improve neural transmission along the CST, will be termed *corticospinal plasticity*.

The induction of corticospinal plasticity has been shown to occur following both experimentally-induced plasticity using non-invasive brain stimulation (NIBS) protocols (e.g. transcranial direct current stimulation [tDCS]) (Nitsche & Paulus 2000, Nitsche & Paulus 2001, Nitsche et al. 2005, Sale et al. 2007, Kidgell et al. 2013) and use-dependent activities that involve repetitive activity (i.e., strength training and skill training) targeted at improving motor performance (Hortobagyi et al. 2011, Selvanayagam et al. 2011, Leung et al. 2015, Nuzzo et al. 2016).

tDCS utilizes weak direct currents to induce prolonged modulation of corticospinal excitability within the human M1 (Nitsche & Paulus 2000, Nitsche & Paulus 2001). Anodal tDCS has been shown to increase corticospinal excitability for up 90 min,

whilst cathodal tDCS decreases corticospinal excitability (Nitsche & Paulus 2000, Nitsche & Paulus 2001). This increased/decreased level of excitability following a low/high level of synaptic activity is termed *homeostatic plasticity*. Despite the substantial evidence establishing the capacity for tDCS to modulate the excitability of neurons within the M1, it has recently been shown to induce effects in distant brain areas caused by activity of interconnected brain zones (Sale et al. 2015). This concept is termed *functional connectivity* and is based upon the working hypothesis that changes in localised brain activity can influence distant, but functionally related areas, which is an essential function of the healthy brain (Sale et al. 2015). Thus, it has been suggested that anodal tDCS may be a plausible method to induce homeostatic plasticity of distant interconnected structures [i.e., the ipsilateral hemisphere] (Pavlova et al. 2014). Therefore, this concept was examined in Chapter 3 (Study 1) by investigating the bilateral effects of uni-hemisphere anodal tDCS.

The temporary modification of corticospinal plasticity (i.e., increases in corticospinal excitability) following single and repeated sessions of anodal tDCS has been reported to correspond with transient improvements in motor performance (Boggio et al. 2006, Vines et al. 2006, Cogiamanian et al. 2007, Reis et al. 2009, Tanaka et al. 2009, Tanaka et al. 2011, Kidgell et al. 2013). However, the exact sites (i.e., cortical or spinal) of adaptation underpinning the improvement in motor performance are inconsistent and the relationship between corticospinal plasticity and strength development remains unclear. Considering this, Chapter 4 (Study 2) employed the use of transcranial magnetic stimulation (TMS) to measure the net motor output from the M1, by using TMS to assess voluntary activation and the indices of corticospinal plasticity following repeated sessions of anodal tDCS. The impetus for Study 2 was to examine the induction of experimentally-

induced plasticity and its effect on muscle strength to explore the role of plasticity in regulating the expression of muscle strength.

To date, there has been significant attention surrounding the application of tDCS before and/or during motor training (known as motor priming), which is based upon the assumption that enhanced neural activity within the M1 will facilitate the mechanisms associated with LTP or LTD (Ziemann & Siebner 2008). Two sub-divisions of motor priming include *gating* and *homeostatic plasticity* which differ according to the timing of when tDCS is applied (i.e., during or before motor training) (Siebner 2010). The theory of *gating* occurs instantaneously and describes the influx of calcium ions into the targeted corticospinal neurons resulting in the disinhibition of intracortical inhibitory circuits (Ziemann & Siebner 2008, Siebner 2010). *Gating* is attained concurrently with motor training and has been shown to facilitate motor performance (Nitsche et al. 2003d, Boggio et al. 2006, Galea & Celnik 2009, Hunter et al. 2009, Reis et al. 2009, Stagg et al. 2011, Hendy & Kidgell 2014). Of relevance to this thesis is the principle of *homeostatic plasticity* whereby the resting state of corticospinal neurons are altered (increased/decreased level of excitability following a low/high level of synaptic activity) due to changes in postsynaptic glutamate receptor activity (Ziemann & Siebner 2008, Siebner 2010) prior to motor training.

Historically, there has been an emphasis placed on motor priming protocols involving the combination of tDCS (experimentally-induced corticospinal plasticity) and motor learning tasks (use-dependent induced corticospinal plasticity) to enhance motor performance (Sriraman et al. 2014, Christova et al. 2015). However, of interest, strength training has also been shown to modulate similar neural substrates to motor learning (i.e., corticospinal excitability and inhibition). For example, the rapid development of strength,

commonly seen following acute (single session) and short-term (i.e., < 3 weeks) strength training, has been attributed to changes in corticospinal plasticity in the absence of muscle hypertrophy (Beck et al. 2007, Griffin & Cafarelli 2007, Kidgell & Pearce 2010, Kidgell et al. 2010b, Hortobagyi et al. 2011, Leung et al. 2015, Nuzzo et al. 2016). A fundamental example of corticospinal plasticity underpinning improvements in strength is the cross-education phenomenon, whereby strength training of one limb results in an increase in strength of the opposite untrained limb (Munn et al. 2004, Carroll et al. 2006). Given that anodal tDCS has been shown to modulate NMDA receptor activity, and subsequently produce a shift in the resting membrane potential (Nitsche & Paulus 2000), it would be likely that anodal tDCS could be used to increase synaptic activity within the M1 prior to strength training. Whilst Chapter 5 (Study 3) investigated the effect of priming the M1 using anodal tDCS prior to a single bout of strength training on corticospinal responses, Chapter 6 (Study 4) determined whether priming the ipsilateral M1 was a plausible method to enhance the cross-transfer of strength.

A caveat to the current TMS strength training literature is that there is conflicting evidence regarding the loci of adaptation underpinning the improvement in strength and, critically, the relationship between the induction of corticospinal plasticity and strength development remains elusive. Several potential confounders have been suggested to contribute towards the variability in results for both experimentally-induced and use-dependent plasticity protocols including: gender, time of testing, prior level of physical activity and genetic factors (Sale et al. 2007, Ridding & Ziemann 2010, Li Voti et al. 2011). Brain-derived neurotrophic factor (*BDNF*) has been shown to influence mechanisms involved in the induction of corticospinal plasticity (Kleim et al. 2006, Cheeran et al. 2008, Antal et al. 2010, Hwang et al. 2015) and the interaction between

BDNF secretion and LTP/LTD processes suggests the importance of this factor in regulating corticospinal plasticity (Cheeran et al. 2008, Li Voti et al. 2011). To date, no studies have examined whether the *BDNF* polymorphism differentially regulates the induction of corticospinal plasticity and the acquisition of strength following anodal tDCS and strength training. Therefore, a collective secondary aim of this thesis (Studies 1-4) was to investigate the role of the *BDNF* polymorphism as a potential regulator of the efficacy of experimentally-induced and use-dependent plasticity protocols used to modulate the expression of muscle strength in healthy adults.

Overall, the purpose of this thesis was to systematically examine the corticospinal responses to various homeostatic-inducing protocols (anodal tDCS alone and in combination with strength training) to investigate the corticospinal responses that may contribute to the expression of strength.

1.1 Primary aim of the research

1. To systematically investigate the induction of homeostatic plasticity and its effect on muscle strength.

1.2 Secondary aim of the research

1. To investigate the influence of the *BDNF* polymorphism on indices of corticospinal plasticity and strength following experimentally-induced and use-dependent plasticity protocols.

1.3 Specific aims of the research

1. To examine the effect of a single session of anodal tDCS on indices of corticospinal excitability and inhibition of both the stimulated and non-stimulated hemisphere (Study 1).

2. To examine the effect of repeated sessions of anodal tDCS on muscle strength, VA_{TMS}, and indices of corticospinal plasticity (Study 2).

3. To examine the effect of inducing homeostatic plasticity of the M1 using anodal tDCS prior to a single bout of strength training on corticospinal excitability and inhibition (Study 3).

4. To examine the effect of inducing homeostatic plasticity of the ipsilateral M1 using anodal tDCS prior to a single bout of strength training on the cross-transfer of strength and corticospinal excitability/inhibition of the ipsilateral M1 (Study 4).

5. To investigate the role of the *BDNF* polymorphism as a potential regulator of the efficacy of experimentally-induced and use-dependent plasticity protocols used to the expression of muscle strength (Studies 1-4).

1.4 Primary hypothesis of the research

1. It was hypothesised that the induction of homeostatic plasticity (i.e., increased corticospinal excitability, decreased corticospinal inhibition, increased VA_{TMS}) would contribute to the expression of muscle strength.

2. It was hypothesised that the presence of the *BDNF* polymorphism would influence the corticospinal responses to anodal tDCS, but would not affect VA_{TMS} and the expression of muscle strength.

Chapter 2：Review of Literature

The human primary motor cortex (M1) is located within the precentral gyrus of the frontal lobe which lies anterior to the central sulcus consisting primarily of stellate and pyramidal cells (Rothwell 1994). The M1 is structurally organised into several layers which comprise of horizontal and vertical bands (Rothwell 1994, Mountcastle 1997). The horizontal bands, specifically layers three and five of the M1, provide the functional basis for plasticity through changes in synaptic efficacy (Sanes & Donoghue 2000). A large number of pyramidal tract neurons descend from layer 5, forming the corticospinal tract (CST) which is organised to project to motor neurons that control specific muscle groups (Sanes & Donoghue 2000). Modifications of the intrinsic circuitry within the M1 (i.e., increases in corticospinal excitability and decreases in inhibition), which improve neural transmission along the CST, can be termed *corticospinal plasticity*. Corticospinal plasticity has been shown to be induced both experimentally, through the use of non-invasive brain stimulation (NIBS) protocols (i.e., transcranial direct current stimulation [tDCS]) (Nitsche & Paulus 2000, Nitsche & Paulus 2001, Nitsche et al. 2005, Sale et al. 2007, Kidgell et al. 2013), and following use-dependent activities that involve repetitive activity (i.e., strength training) targeted at improving motor performance (Hortobagyi et al. 2011, Selvanayagam et al. 2011, Leung et al. 2015, Nuzzo et al. 2016). Various techniques have been employed to assess changes in corticospinal plasticity which include positron emission tomography (PET), functional magnetic resonance imaging (fMRI), near-infrared spectroscopy (NIRS), magnetoencephalography (MEG), electroencephalography (EEG) and transcranial magnetic stimulation (TMS) (Schaechter 2004). The use of TMS has emerged as a popular NIBS tool due to the safety and extensive ability to explore underlying mechanisms involved in corticospinal plasticity (Siebner & Rothwell 2003, Chipchase et al. 2012). Importantly, with the use of TMS, the

M1 and CST have been identified as integral functional structures of experimentally-induced and use-dependent corticospinal plasticity and, therefore, will be reviewed in further detail.

2.1 Organisation of primary motor cortex

The pyramidal and stellate cellular arrangement of the M1 forms a laminar appearance when viewed vertically (Rothwell 1994). Pyramidal tract cells are found within several layers within the M1, with the largest known as Betz cells located in the internal pyramidal layer (layer V). The origins of the pyramidal tract cells are grouped and distributed intermittently forming the horizontal component of layer V (Mountcastle 1997). These cells are among the largest in the central nervous system (CNS) and have long axons which descend and synapse monosynaptically onto the ventral horn of the spinal cord (Mountcastle 1997).

The dendrites of the pyramidal cells extend into layer I, giving the M1 strong perpendicular connections (Nathan et al. 1990, Rothwell 1994). In addition, the axons of the pyramidal cells extend horizontally providing the anatomical substrate necessary for dynamic activity-dependent changes within the M1 (Jacobs & Donoghue 1991, Huntley 1997). Given the distinct horizontal connections of the M1, it provides the opportunity for long-term potentiation (LTP) via synaptic plasticity (Aroniadou & Keller 1995, Hess & Donoghue 1996). The relationship between pyramidal cells and interneurons stimulates the formation of synaptic connections via the production and growth of axons and dendrites (Mountcastle 1997). Pyramidal tract cells are responsible for the changes in synaptic efficacy reflecting the ability for structural and functional reorganisation within the M1 (Sanes & Donoghue 2000). Stellate cells are the ‘true cerebral interneurons’ as

they generally have shorter axons which do not leave the cortex and are responsible for making inhibitory synapses (Rothwell 1994).

The M1 is structurally organised into six layers which consist of horizontally and vertically orientated bands. As shown in Figure 2.1, the layers of the M1 are:

(I) *Molecular layer*- this is the most superficial layer containing axons and dendrites.

(II) *External granular layer*- consists mainly of stellate cells and small pyramidal cells giving this layer a granular stripped appearance.

(III) *External pyramidal layer*- composed mostly of medium and large pyramidal cells which are the main source of corticocortical fibres.

(IV) *Internal granular layer*- consists predominately of stellate cells with some pyramidal cells present.

(V) *Internal pyramidal layer*- thickest area containing the giant pyramidal cells called Betz cells which are the source of efferent (output) fibres.

(VI) *Multiform layer*- deepest layer consisting of fusiform and pyramidal cells and interneurons.

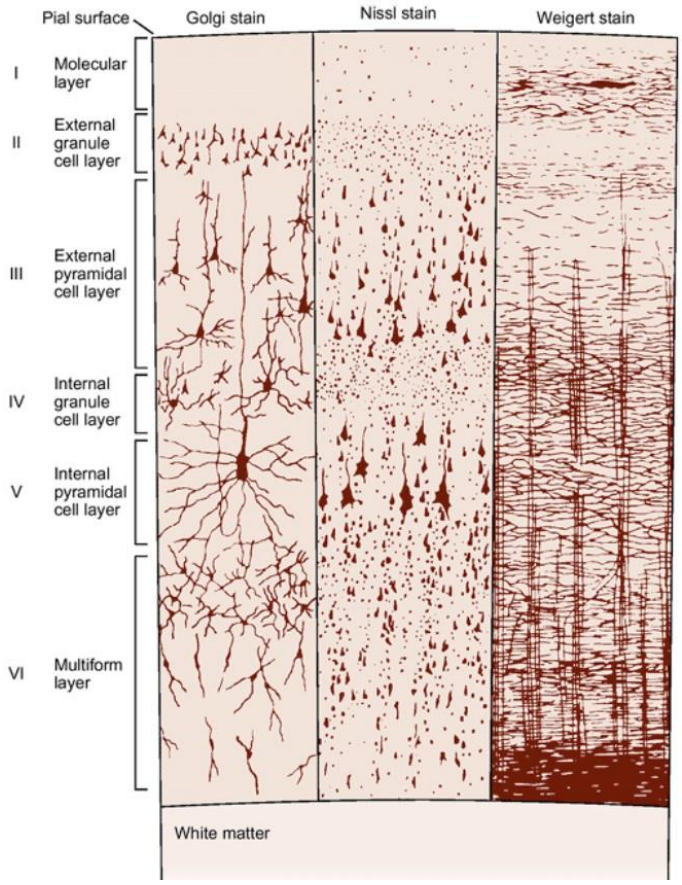

Figure 2.1: Cytoarchitecture of the primary motor cortex (Kandel et al. 2000, pp. 265).

2.2 Corticospinal tract

The pyramidal cell bodies of the internal pyramidal layer (layer V) of the M1 constitute the main output neurons of the descending motor pathway. Nearly two-thirds of the CST fibres arise from the pyramidal cell layer of the M1, whilst the remaining third originate from the parietal cortex. The pyramidal tract cells that originate from the M1 descend through the internal capsule, the midbrain, the medulla and then onto the spinal cord. The axons of the pyramidal cells, referred to as upper motor neurons, synapse in the spinal cord grey matter with interneurons, alpha and gamma motor neurons. Approximately 85-90% of the CST axons decussate at the medulla forming the pyramidal decussation and control precise movements of the distal muscles of the limbs. The

remaining 10%, that do not cross, form the anterior CST which controls movements of the trunk. Most of these fibres eventually cross just before terminating in the ventral horn of the spinal cord. The majority of axons enter the ventral horn and terminate in the intermediate and ventral areas on interneurons and motor neurons (Rothwell 1994). Changes within the M1 and along the CST can be quantified using several non-invasive techniques targeting direct and indirect activation of pyramidal tract cells.

2.2.1 Techniques used to measure motor cortical function

Several non-invasive human brain mapping techniques including TMS, fMRI, PET, NIRS, MEG and EEG have been used to investigate functional and structural reorganization within the M1 (Siebner & Rothwell 2003, Schaechter 2004, Imfeld et al. 2009). PET and fMRI techniques examine the brain's hemodynamic response to a task by assessing changes in regional cerebral blood flow indicating changes in neuron activation (Greenberg et al. 1979, Schaechter 2004). Both these neuroimaging methods enable non-invasive time course feedback of spatial and temporal dynamics of cortical activation, providing evidence of representational plasticity in the human brain (Hallett 2000, Siebner & Rothwell 2003). NIRS is a similar technique but examines changes in the absorption of near-infrared light by haemoglobin-containing elements in the cerebral blood to measure brain activation; however it has limited use due to poor spatial resolution (Schaechter 2004).

MEG and EEG record the electrical activity of the brain through electromagnetic fields and the induction of electrical currents. In contrast to fMRI and PET methods, MEG and EEG directly measure neural activity signals, resulting in faster and higher resolution images (Schaechter 2004). Although all these methods have advantages, TMS is a powerful tool for understanding the neural basis of corticospinal plasticity.

2.2.2 Transcranial magnetic stimulation

Over the past several decades, TMS has emerged as a robust, non-invasive tool used to examine physiological and pathophysiological functions of the CNS. Exploration into excitatory and inhibitory circuits within the M1 has provided valuable objective insight into the possible mechanisms of corticospinal plasticity (Rossini & Rossi 2007). TMS stimulates underlying cortical tissue via a brief electrical current that is passed through a coil which is placed over the individual's scalp (Hallett 2000). The magnetic field created by the circulating electrical current depolarises neurons or their axons (Hallett 2000) and causes a muscle response of the target muscle (Merton 1980). Single- and paired-pulse TMS can be used to measure various parameters of corticospinal plasticity involving changes confined to the M1 and changes along the CST and is currently used within clinical and research settings (Hallett 2000).

2.2.2.1 Single-pulse TMS

Stimulation of the M1 using single-pulse TMS enables the measurement of several important physiological variables including motor evoked potential (MEP) amplitude, motor threshold (MT), latency and silent period (Hallett 2000, Kidgell & Pearce 2011) which can be seen in Figure 2.2.

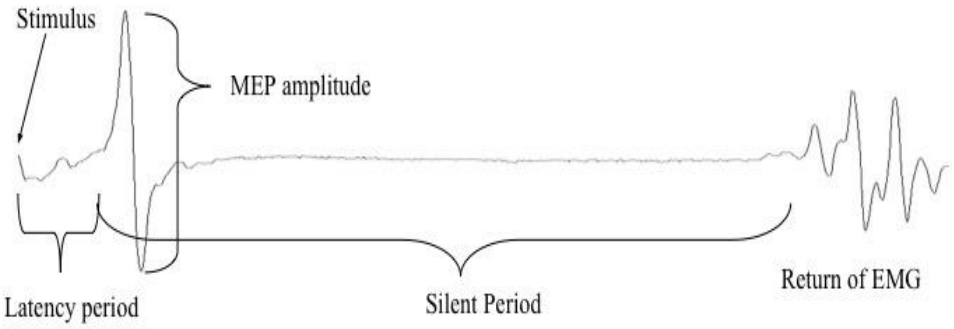

Figure 2.2: The components of an MEP recorded by surface electromyography (sEMG) from single-pulse TMS (Pearce & Kidgell 2011).

Peak-to-peak amplitude of the MEP represents corticospinal excitability which can be influenced by direct and indirect inhibitory and excitatory processes at the time of stimulation (Kidgell & Pearce 2011, Di Lazzaro et al. 2012b). Single-pulse TMS of the M1 elicits a series of descending volleys (waves) in the CST. Initially, these volleys are a result of direct activation of pyramidal cells (D-waves) that are subsequently followed by indirect activation of pyramidal cells via cortical interneurons (I-waves) (Sakai et al. 1997, Hallett 2000, Schaechter 2004, Di Lazzaro et al. 2012b). This brief high current pulse produces a relatively synchronous muscle response, known as a MEP, which is recorded by electromyography (EMG) (Hallett 2000). A recruitment curve is another quantifiable measure of corticospinal excitability which describes the relationship between MEP amplitude and stimulation intensity (Devanne et al. 1997, Hallett 2000). This provides a measure of the physiological strength of corticospinal projections onto the motor neuron pool and reflects the balance between inhibitory and excitatory inputs in the M1 and motor neuron pool (Devanne et al. 1997, Carroll et al. 2001a, Carroll et al. 2002). When controlled for torque, the MEP is a reliable intra-participant measure (Kamen 2004, van Hedel et al. 2007) allowing for confident interpretation of changes in corticospinal excitability following interventions. In addition, the total area under the

curve (AUC) can be calculated using the method of trapezoidal integration during the construction of corticospinal excitability recruitment curves (Talelli et al. 2008, Carson et al. 2013). AUC has high validity and provides additional insight into the input-output properties of the CST (Carroll et al. 2001a, Carson et al. 2013).

Motor threshold (MT) is the minimum amount of stimulation needed to produce an MEP (Hallett 2000). Threshold can be examined at rest (resting motor threshold [RMT]) or during a tonic voluntary contraction (active motor threshold [AMT]) (Kidgell et al. 2010b) and has previously been shown to change following motor training and NIBS (Pascual-Leone et al. 1995, Khedr et al. 2010). Contraction of the muscle reduces the threshold required to elicit an MEP due to the increased level of excitability of neurons at both a cortical and spinal level (Di Lazzaro et al. 2003, Di Lazzaro et al. 2004). Latency is measured from time of stimulation to the onset of the MEP, reflecting the conduction time from the M1 to the motor neuron pool in the spinal cord (Kidgell & Pearce 2011). During tonic voluntary contraction, latency is reduced by 2-3 ms due to facilitation of the spinal motor neurons (Rossini et al. 1994). Silent period is defined from the onset of the MEP to the return of EMG, characterised by a flat lining or non-activity of sEMG (Cantello et al. 1992, Wilson et al. 1993, Chen et al. 1999, Kidgell & Pearce 2011). Initially, the silent period is due to spinal cord refractoriness, however, the latter part is a result of cortical inhibition (Wilson et al. 1993, Chen et al. 1999, Hallett 2000). This measure is regulated by neurons that use Gamma-Aminobutyric acid (GABA), in particular $GABA_B$ (Chen et al. 1999, Werhahn et al. 2007) and represents the overall strength of inhibition in the corticospinal pathway (Werhahn et al. 2007). However, a limitation of single-pulse TMS is that it activates corticospinal neurons transynaptically,

which does not measure cortico-cortical inputs. To quantify changes specifically within the M1, paired-pulse TMS can be used.

2.2.2.2 Paired-pulse TMS

Paired-pulse TMS enables the examination of inhibitory and excitatory mechanisms specific to the M1 (Kujirai et al. 1993, Fisher et al. 2002, Rothwell et al. 2009, Di Lazzaro et al. 2012b). Short-interval intracortical inhibition (SICI), as described by Kujirai et al. (1993), involves a sub-threshold conditioning stimulus followed by a supra-threshold test stimulus, separated by an interstimulus interval (ISI) of 1-5 ms and is used to study intracortical inhibition (Chen 2004). This results in the suppression of I-waves (indirect activation of pyramidal cells) evoked by the test stimulus, providing a measure of the intrinsic cortical connections mediated by $GABA_A$ receptors (Kujirai et al. 1993, Chen et al. 1999, Fisher et al. 2002). To measure the facilitatory circuits of the M1, intracortical facilitation (ICF) and short-interval intracortical facilitation (SICF) can be employed (Chen 2004, Di Lazzaro et al. 2012b). Similar to the protocol of SICI, ICF is elicited by a subthreshold conditioning stimulus followed by supra-threshold test stimulus, however a ISI of 8–30 ms is used (Chen 2004). On the other hand, SICF (also known as facilitatory I-wave interaction) involves a supra-threshold stimulus followed by a subthreshold stimulus using short ISIs (Tokimura et al. 1996, Ziemann et al. 1998b). MEP facilitation has been shown at three distinct phases of ISIs including 1.1–1.5 ms, 2.3–3.0 ms, and 4.1–5.0 ms (Di Lazzaro et al. 2012b). Due to the versatile nature of TMS, motor output from the M1 can also be quantified, providing further insight into mechanisms regulating corticospinal plasticity.

2.2.2.3 TMS voluntary activation (VA_{TMS})

In addition to single and paired-pulse TMS techniques that examine the excitatory and inhibitory pathways within the M1 (Chen et al. 1999, Siebner & Rothwell 2003), motor cortical drive can also be assessed (Todd et al. 2003, Lee et al. 2008, Lee et al. 2009). This measure provides information regarding the net motor output from the M1 (i.e., VA_{TMS}), identifying potential sites of neural drive impairment (Todd et al. 2003, Todd et al. 2004). To quantify sub-maximal M1 output, the level of neural drive to the muscle is determined by the presence of a superimposed twitch force that is produced by single-pulse TMS during a maximal voluntary isometric contraction (MVIC) (Lee et al. 2008). The superimposed twitch represents the single-pulse TMS eliciting extra force from the muscle during a MVIC due to sub-maximal motor output from the M1, while the absence of a superimposed twitch suggests maximal output from the M1 (i.e., maximal neural drive) (Todd et al. 2003, Todd et al. 2004, Lee et al. 2008, Goodall et al. 2009). TMS has been shown to be a reliable and valid measurement of voluntary activation in the human wrist extensor, knee extensor and elbow flexor muscle (Todd et al. 2003, Todd et al. 2004, Lee et al. 2008, Goodall et al. 2009, Sidhu et al. 2009). This technique provides an additional measure of corticospinal efficiency, demonstrating changes in motor cortical output via the recruitment of motor units used in force generation.

2.3 Corticospinal plasticity

It is well-established that the M1 can modify its function in response to experimentally-induced plasticity protocols and use-dependent activities aimed at improving motor performance (Rioult-Pedotti et al. 1998, Sanes & Donoghue 2000, Ridding & Ziemann 2010). In this thesis, this response has been termed *corticospinal*

plasticity and involves reorganisation of neural assemblies that control movement at both a cortical and spinal level. Although the mechanisms involved in the induction of plasticity have been well described (Classen et al. 1998, Ziemann et al. 1998a, Carroll et al. 2001c, Kleim et al. 2002), less is understood about the potential role of the brain-derived neurotrophic factor (*BDNF*) polymorphism modulating corticospinal plasticity and its link to muscle function. This is of interest as individual responses to plasticity-inducing protocols are highly variable, making it difficult to draw conclusions regarding the efficacy of various protocols. In the following sections, the mechanisms of corticospinal plasticity will be reviewed before considering the potential modulatory role of the *BDNF* polymorphism.

2.3.1 Mechanisms of corticospinal plasticity

Rapid changes within the M1 associated with use-dependent behaviour and NIBS techniques are likely to be a result of enhanced efficiency of existing pyramidal tract neurons, unmasking of latent synapses and synaptogenesis (Ziemann et al. 1998a, Carroll et al. 2001c, Kleim et al. 2002). Unmasking of pre-existing connections involves the removal of GABA related local inhibition and has been shown to be a mediating step in shaping corticospinal plasticity (Jacobs & Donoghue 1991, Ziemann et al. 1998a). LTP and LTD have been identified as important underlying mechanisms involved in the modification of synaptic efficacy within corticocortical connections (Ziemann et al. 2004). Activation of the NMDA receptor has been proposed to be the primary mechanism responsible for the induction of experimentally and use-dependent corticospinal plasticity (Ziemann et al. 1998a, Butefisch et al. 2000). Considering this, the following will review LTP as a mechanism for corticospinal plasticity.

2.3.1.1 Long-term potentiation

Enhancement of synaptic connections can involve short-term potentiation (STP), which lasts 5-20 minutes, and LTP, which can last from hours to days (Bliss & Collingridge 1993). LTP is an activity-dependent process resulting in the long-lasting enhancement of synaptic transmission that provides the basis for information storage within the brain (Bear & Malenka 1994, Hess et al. 1996).

LTP is characterised by three distinct properties including cooperativity, associativity and input-sensitivity (Bliss & Collingridge 1993). Cooperativity refers to the range of threshold intensities required for the induction of activity-dependent potentiation. The threshold necessary for the induction of LTP is dependent on the interaction between intensity and pattern of tetanic stimulation. Unless stimulation is 'strong', LTP will not be triggered, resulting in STP and post-tetanic potentiation (PTP) being induced (McNaughton et al. 1978, Malenka 1991). Importantly, LTP is associative, meaning a weak input can only be potentiated if it is active at the same time as a strong input (McNaughton et al. 1978, Collingridge & Bliss 1987). These three properties are mediated by the NMDA receptor which is located on the post-synaptic dendrites of excitatory synapses (Collingridge & Bliss 1987).

2.3.1.2 NMDA receptor

NMDA is an essential molecule for regulating corticospinal plasticity in humans and operates as the channel responsible for LTP (Castro-Alamancos et al. 1995). To trigger the induction of LTP, two processes must occur involving the NMDA receptor channel complex (Bliss & Collingridge 1993). First, post-synaptic depolarization releases glutamate, resulting in the activation of post-synaptic NMDA receptors. This event reduces the voltage-dependent block of the NMDA receptor channel by magnesium

(Mg^{2+}), allowing the influx of calcium (Ca^{2+}) into the post-synaptic dendritic spine (Bear & Malenka 1994). The level of depolarization will consequently determine whether the cooperativity threshold will be enough to induce LTP. Failure to induce LTP is a result of inadequate reduction of the Mg^{2+} block, rather than the insufficient release of glutamate to activate the NMDA receptors (Bliss & Collingridge 1993).

Bliss and Collingridge (1993) demonstrated the necessity of NMDA receptor activation for the induction of LTP. This finding prompted investigation into the potential relationship between NMDA receptor activation and the induction of experimental and use-dependent corticospinal plasticity (Liebetanz et al. 2002, Nitsche et al. 2003a). Using pharmacological agents, Butefisch (2000) demonstrated that use-dependent plasticity of the hand area of the M1 following motor training was significantly reduced when the NMDA receptor was blocked. Similarly, the necessity of NMDA receptor activation for the induction of experimentally-induced plasticity was confirmed when the administration of the NMDA receptor antagonist, dextromethorphan was found to inhibit the long-lasting effects of tDCS (Liebetanz et al. 2002, Nitsche et al. 2003a). Collectively, these findings show that the NMDA receptor is an important operating mechanism in the formation of use-dependent and experimentally-induced corticospinal plasticity via activating LTP processes. In addition, other cellular mechanisms involving neurotrophic factors (i.e., neurotrophins, glial cell-line derived neurotrophic factor family ligands, and neuropoietic cytokines) that interact with the NMDA receptor and the induction of LTP have also been recognized in shaping corticospinal plasticity.

2.3.2 Brain-derived neurotrophic factor and corticospinal plasticity

Although the mechanisms that underpin corticospinal plasticity have been described within the literature, the extent of plasticity appears to be influenced by genetic factors.

BDNF is a neurotrophin which is involved in a variety of CNS functions including but not limited to cell survival, proliferation and synaptic growth (Antal et al. 2010). In humans, a naturally occurring single nucleotide polymorphism results in the substitution of valine to methionine at codon 66 (*val66met*), which has been associated with reduced episodic memory and increased risk of neuropsychiatric disorders (Egan et al. 2003, Pezawas et al. 2004, Bath & Lee 2006). The distribution of the polymorphism varies widely between regions and ethnicities, with approximately 30-50% of people worldwide identified as either heterozygous (*Val/Met*) or homozygous (*Met/Met*) for the Met substitution. Expression of the *Met* allele is more commonly found among Asian (51% in Japan) compared to Caucasian populations (30% in America) (Shimizu et al. 2004) with evidence suggesting those of Caucasian descent have larger associated cognitive and behavioural consequences (Bath & Lee 2006). Abnormal cortical morphology is a shared characteristic among carriers of the Met variant form of *BDNF* (Bath & Lee 2006). Smaller hippocampal volumes and poorer performance on memory tasks have been revealed, determining the anatomical and functional consequences of the *BDNF* polymorphism (Egan et al. 2003, Pezawas et al. 2004).

More recently, neurotrophic factors, particularly *BDNF* have been identified as critical molecules involved in the regulation of corticospinal plasticity in the human brain (Bath & Lee 2006). Evidence from hippocampal in vitro studies has demonstrated the modulatory role of *BDNF* on NMDA receptor-dependent LTP and LTD (Figurov et al., 1996, Woo et al. 2005). The facilitation of LTP because of *BDNF* secretion suggests the importance of *BDNF* in regulating experimentally-induced and use-dependent corticospinal plasticity (Schinder & Poo 2000, Gottmann et al. 2009). However, the interaction between *BDNF* and LTP processes has yet to be investigated beyond a

theoretical model. In addition, it is unclear what modulatory effect the *BDNF* polymorphism may have on experimentally-induced and use-dependent corticospinal plasticity. Therefore, a secondary aim of this thesis was to examine the potential impact of the *BDNF* polymorphism on the efficacy of tDCS and acute bouts of strength training to induce corticospinal plasticity.

2.4 Experimentally-induced corticospinal plasticity

Several NIBS methods have been used to assess the potential underlying mechanisms and regulators of corticospinal plasticity, including tDCS, theta burst stimulation (TBS), paired associative stimulation (PAS), I-wave periodicity TMS (iTMS) and repetitive transcranial magnetic stimulation (rTMS) (Nitsche et al. 2003b, Siebner & Rothwell 2003, Sale et al. 2007, Thickbroom 2007, Kidgell et al. 2016). Such NIBS techniques have been shown to modify levels of corticospinal excitability and inhibition which have been attributed to LTP and LTD (Thickbroom et al. 2006). More recently, tDCS has emerged as a common NIBS technique used to modulate corticospinal excitability/inhibition with the aim of modifying motor behaviour in both healthy and clinical populations (Ridding & Ziemann 2010, Nitsche & Paulus 2011). More specifically, tDCS has been used in combination with motor training, which has evolved into a popular paradigm known as *'motor priming'*. Motor priming is thought to facilitate motor learning and involves the application of tDCS either prior or during motor learning (Stoykov & Madhavan 2015). Two established priming theories have been proposed which include *gating* and *homeostatic plasticity* (Siebner 2010). *Gating* occurs concurrently with motor training (i.e., tDCS while training), while *homeostatic plasticity* involves modulating the resting state of neurons prior to training (i.e., tDCS applied before motor training).

Despite extensive research examining the indices of corticospinal plasticity of the stimulated M1 following anodal tDCS (Nitsche & Paulus 2000, Nitsche & Paulus 2001, Bastani & Jaberzadeh 2012, Kidgell et al. 2013, Pellicciari et al. 2013), little is understood about the bilateral effects (i.e., non-stimulated M1) of uni-hemisphere stimulation. Given that other NIBS techniques have been shown to modulate not only the intended stimulated tissue but also distal areas of the brain including the contralateral hemisphere (Gilio et al. 2003), it would appear evident that the bilateral effects of anodal tDCS must be explored to ensure the feasibility of tDCS as a priming method for inducing homeostatic plasticity prior to motor training. Furthermore, individual corticospinal responses to anodal tDCS are highly variable and the expression of the *BDNF* polymorphism has been identified as a potential contributing factor (Cheeran et al. 2008, Antal et al. 2010). Differential modulation of corticospinal plasticity between different *BDNF* genotype carriers is of interest when examining the induction of LTP, which is an essential physiological process involved in corticospinal plasticity and motor learning (Cheeran et al. 2008, Cirillo et al. 2012). Therefore, the following discussion will examine the induction of homeostatic plasticity following tDCS, the use of tDCS in the absence of motor training and prior to motor training (motor priming) to enhance motor performance, and the potential regulatory role of the *BDNF* polymorphism.

2.4.1 Transcranial direct current stimulation

In contrast to other NIBS techniques, tDCS does not rely on rapid depolarisation resulting in the induction of action potentials to stimulate corticospinal plasticity (Nitsche et al. 2008). Rather, this method is considered to be a ‘neuromodulator’, whereby a weak electrical current is passed through electrodes placed on the scalp resulting in polarity specific changes of the M1 (Nitsche & Paulus 2000, Nitsche & Paulus 2001). A number

of parameters have been shown to influence the efficacy of tDCS including current strength, electrode size, stimulation duration and orientation of the electrode field (Nitsche et al. 2008). Orientation includes the position and polarity of electrodes, which determines the direction of modulation (increase/decrease corticospinal excitability). Anodal stimulation (positively charged electrode) results in neuronal depolarisation and an increase in corticospinal excitability. Cathodal stimulation has the opposite effect whereby hyperpolarization of neurons occurs leading to decreased corticospinal excitability (Nitsche & Paulus 2000).

Two common electrode arrangements used to modulate corticospinal plasticity in healthy and clinical populations are uni-hemisphere and dual-hemisphere tDCS. Uni-hemisphere tDCS, where the anode is placed over the M1 of interest, has been shown to increase corticospinal excitability for up to 90 min post stimulation (Nitsche & Paulus 2001). In contrast, dual-hemisphere tDCS involves simultaneously applying anodal tDCS to one hemisphere and cathodal tDCS to the other. This arrangement leads to inhibitory effects in one hemisphere and increased excitability in the opposite (Nitsche et al. 2003c, Di Lazzaro et al. 2012a). Interestingly, the immediate and time-course effects of tDCS appear to be mediated by different mechanisms. Initially, tDCS is thought to modify corticospinal excitability primarily through altering the resting membrane potential (Nitsche & Paulus 2000, Nitsche et al. 2008). However, the longer lasting effects of tDCS appear to be dependent upon NMDA receptor function, indicating that changes in corticospinal excitability are likely due to LTP-like mechanisms (Liebetanz et al. 2002, Nitsche et al. 2004a, Nitsche et al. 2004b, Ridding & Ziemann 2010). At present, the consensus is that anodal tDCS induces focal changes in corticospinal excitability and inhibition of the M1 (Nitsche & Paulus 2000, Nitsche et al. 2008). However, it has

recently been shown that NIBS techniques, including tDCS, not only exerts a neuro-modulatory effect over the stimulated region, but also distal areas connected to the region of stimulation (Gilio et al. 2003, Lang et al. 2004).

2.4.2 NIBS and functional connectivity

Emerging evidence from TMS studies has revealed that NIBS techniques modulate not only the intended stimulated tissue but also distal connecting tissue and structures, as well as the opposite non-stimulated hemisphere (Gilio et al. 2003, Lang et al. 2004). This concept is termed "functional connectivity" and is based upon the working hypothesis that changes in localised brain activity can influence distant, but functionally related, areas which is an essential function of the healthy brain (Sale et al. 2015). Functional connectivity has evolved from the parallel use of neuroimaging techniques (i.e., fMRI) and NIBS methods (i.e., TMS, tDCS etc.) with the aim of understanding the interaction between distant neural structures caused by activity of interconnected brain zones (Sale et al. 2015). Previously, tDCS of the motor association cortex was shown to induce inhibitory effects in the M1 (Kirimoto et al. 2011) and stimulation of the premotor cortex facilitated the M1 by reducing SICI (Boros et al. 2008). Critically, the limited number of TMS studies examining the bilateral effect of uni-hemisphere stimulation have shown highly diverse findings regarding the direction of excitability of the non-stimulated hemisphere following various NIBS techniques (Gilio et al. 2003, Lang et al. 2004, Di Lazzaro et al. 2008, Suppa et al. 2008, Di Lazzaro et al. 2011). For example, various protocols using iTBS have shown increases in corticospinal excitability of the stimulated hemisphere and a decrease in corticospinal excitability of the non-stimulated hemisphere (Di Lazzaro et al. 2008, Suppa et al. 2008, Di Lazzaro et al. 2011). rTMS and PAS have been shown to increase excitability of both the stimulated and non-stimulated M1 (Gilio

et al. 2003, Schambra et al. 2003, Shin & Sohn 2011) and decrease interhemispheric inhibition (IHI) between the left and right M1 (Gilio et al. 2003). Likewise, Lang et al. (2004) found that 10 min of anodal and cathodal tDCS at 1 mA modulated transcallosal inhibition. Interestingly, this finding was not accompanied by a bilateral increase in M1 excitability, with only an increase in MEP amplitude seen in the stimulated M1. Importantly, it should be highlighted that a key methodological component of the studies investigating NIBS techniques and the concept of functional connectivity is that many used a dominant M1 arrangement whereby the stimulated hemisphere was the dominant M1 (left) and non-stimulated hemisphere was the non-dominant M1 (right). Notably, it has previously been shown that a hemispheric imbalance exists (dominant vs non-dominant) as demonstrated by the non-dominant hemisphere having a lower motor threshold, higher MEPs (De Gennaro et al. 2004) and shorter corticospinal silent period durations (Priori et al. 1999). A potential difference in hemispheric baseline characteristics poses an interesting question as to whether the magnitude of bilateral corticospinal plasticity is affected by the direction of stimulation (dominant vs non-dominant M1 stimulated), and if there is a greater scope for the induction of corticospinal plasticity of the non-dominant hemisphere.

2.4.3 The induction of homeostatic plasticity and its effect on motor performance

Historically, tDCS has been used as a NIBS technique to modulate corticospinal plasticity and modify motor behaviour (Ridding & Ziemann 2010). However, in an effort to further explore the efficacy of tDCS to enhance motor performance, the technique has evolved into a popular paradigm of motor priming, which is believed to facilitate motor learning (Stoykov & Madhavan 2015). Motor priming involves the application of tDCS

before or during motor training, with the working hypothesis that enhanced neural activity within the M1 will facilitate the mechanisms associated with LTP or LTD (Ziemann & Siebner 2008). Two theories have been proposed to underlie the response of corticospinal output neurons following priming protocols including gating and homeostatic plasticity (Siebner 2010). The theory of gating occurs instantaneously and describes the influx of calcium ions to the targeted corticospinal neurons resulting in the disinhibition of intracortical inhibitory circuits (Ziemann & Siebner 2008, Siebner 2010). Gating is attained concurrently with motor training and has been shown to facilitate motor performance tasks such as hand function using the Jebsen-Taylor Hand Function Test (JTT), maximal strength, movement speed, reaction time and speed-accuracy trade-off (Nitsche et al. 2003d, Boggio et al. 2006, Galea & Celnik 2009, Hunter et al. 2009, Reis et al. 2009, Stagg et al. 2011, Hendy & Kidgell 2014). For example, Christova et al. (2015) showed a significant reduction in SICI following the application of anodal tDCS during grooved pegboard training. However, it appears that the efficacy of priming during training may be limited to fine motor skill training tasks (i.e., pegboard). In support of this notion, Hendy et al. (2013) investigated the use of anodal tDCS applied to the active M1 during training to enhance maximal voluntary strength. Interestingly, there was no difference in strength gain between conditions, suggesting that strength training appeared to have a powerful effect on modulating mechanisms associated with LTP, therefore, potentially limiting further corticospinal responses induced by anodal tDCS.

More relevant to the thesis is the principle of homeostatic plasticity whereby the resting state of corticospinal neurons is altered prior to training (increased/decreased level of excitability following a low/high level of synaptic activity) due to changes in postsynaptic glutamate receptor activity (Ziemann & Siebner 2008, Siebner 2010).

Importantly, the lack of interactions observed between conditions by Hendy et al. (2013, 2014), suggests that a critical consideration to maximise the effectiveness of anodal tDCS as a M1 priming technique is the timing of application (i.e., during or prior the training). Given that anodal tDCS has been shown to modulate NMDA receptors, and subsequently produce a shift in the resting membrane potential (Nitsche & Paulus 2000), it would be conceivable that anodal tDCS is a promising priming tool to increase synaptic activity prior to a single bout of strength training to further augment the acute corticospinal responses to strength training (Leung et al. 2015). In addition, the application of anodal tDCS to the ipsilateral M1 prior to unilateral strength training may result in a shift of the resting membrane potential and increase synaptic activity of the ipsilateral M1, which may in turn further promote bilateral activation of both motor cortices and enhance the cross-transfer of strength; however, no studies have examined this. For further discussion of the corticospinal responses to strength training and the cross-transfer of strength, please see Section 2.5.

2.4.4 Improvements in motor performance following tDCS in the absence of training

Currently, there is promising evidence that the induction of homeostatic plasticity following a single session of anodal tDCS (i.e., increase in corticospinal excitability and inhibition) in the absence of training can also facilitate fine motor performance and increase muscle strength (Boggio et al. 2006, Vines et al. 2006, Cogiamanian et al. 2007, Tanaka et al. 2009, Tanaka et al. 2011, Kidgell et al. 2013, Frazer et al. 2016). For example, following a single session of tDCS (in the absence of motor training), improved motor performance in tasks such as the JTT, maximal strength of the elbow flexors and knee extensors, the Purdue pegboard test, maximal pinch force, reaction time, and tests

of motor sequencing tasks have all been reported (Boggio et al. 2006, Vines et al. 2006, Cogiamanian et al. 2007, Tanaka et al. 2009, Tanaka et al. 2011, Kidgell et al. 2013).

In healthy adults, accumulated bouts of anodal tDCS have been shown to improve motor performance with retention lasting up to three months following stimulation (Boggio et al. 2007, Reis et al. 2009). Although the underlying physiological changes were not examined, the induction of LTP has been suggested to underlie the improvement in motor performance (Reis et al. 2009). Previously, changes in corticospinal excitability have been examined over a five day period whereby participants were exposed to daily anodal tDCS stimulation (Alonzo et al. 2012). Corticospinal excitability was shown to significantly increase but, unfortunately, no motor performance outcome was used to assess any functional effects of the tDCS intervention. A recent study investigating the effect of repeated sessions of anodal tDCS demonstrated an increase in corticospinal excitability accompanied by an increase in muscle strength (Frazer et al. 2016). Interestingly, there was no change in SICI, however a reduction in corticospinal silent period was reported suggesting that accumulated bouts of anodal tDCS appear to modulate $GABA_B$ rather than $GABA_A$ neurons (Frazer et al. 2016). Because the corticospinal silent period that follows the excitatory MEP is caused by activation of long-lasting $GABA_B$ mediated inhibition and reflects the temporary suppression in motor cortical output (Werhahn et al. 2007), it appears that cumulative bouts of anodal tDCS specifically target neural circuits that use $GABA_B$ as their neurotransmitter (Frazer et al. 2016), resulting in the release of pyramidal tract neurons from inhibition (Floeter & Rothwell 1999). Therefore, a reduction in the temporary suppression of motor cortical output may be a putative neural mechanism underlying the changes in strength.

To date, the TMS literature has primarily focused on the acute effects of tDCS modulating corticospinal excitability and the subsequent change in motor performance. Although these studies have provided valuable insight into possible acute physiological mechanisms, motor output from the M1 can also be quantified via VA_{TMS} which provides further insight into the mechanisms regulating corticospinal plasticity and the expression of strength. The level of neural drive to a muscle during exercise is commonly termed 'voluntary activation' (Gandevia et al. 1995) and can be estimated by interpolation of a single supramaximal electrical stimulus to the motor nerve during an isometric voluntary contraction (Merton 1954). Although twitch interpolation assesses neural drive to a muscle during exercise, it cannot provide insight into the precise location of any neural drive impairment (cortical or sub-cortical) (Lee et al. 2009, Carroll et al. 2011). In light of this, TMS has been employed to measure net motor output from the M1 (i.e., VA_{TMS}) identifying potential sites of neural drive impairment (Todd et al. 2003, Todd et al. 2004). This technique can provide additional information regarding corticospinal efficiency following anodal tDCS by demonstrating changes in motor cortical output via the recruitment of motor units used in force generation.

At present, studies have concentrated on the reliability and validity of TMS to measure VA_{TMS} in various muscle groups (Todd et al. 2003, Todd et al. 2004, Lee et al. 2008, Sidhu et al. 2009). However, translation of this technique into applied research settings, such as assessing changes in corticospinal plasticity following accumulated bouts of anodal tDCS, has been only examined once (Frazer et al. 2016). Interestingly, an increase in VA_{TMS} and strength was observed, suggesting that accumulated bouts of anodal tDCS modulates synaptic efficacy, which improves the net descending drive (i.e., increased motor cortical drive) to the motor neuron pool, representing as an increase in

muscle strength (Frazer et al. 2016). Although, the use of tDCS to induce homeostatic plasticity (i.e., modify corticospinal excitability) and acutely improve motor performance is well established (Nitsche et al. 2008, Vines et al. 2008, Kidgell et al. 2013, Frazer et al. 2016), the efficacy of tDCS may also be influenced by individual genetic variations such as the *BDNF* polymorphism (Antal et al. 2010, Puri et al. 2015, Frazer et al. 2016). Therefore, it appears vital to identify individual variants that may impact on the effectiveness of tDCS protocols.

2.4.5 *BDNF* and the induction of experimentally-induced corticospinal plasticity

Corticospinal responses to various NIBS techniques have been shown to differ significantly between individuals (Cheeran et al. 2008, Chang et al. 2014, Hwang et al. 2015). Genetic factors, including the role of *BDNF*, have been reported as potential contributors to the variability of results observed within the literature. Evidence using TMS to evaluate the efficacy of NIBS techniques generally suggest that the presence of the *BDNF* polymorphism significantly impacts corticospinal plasticity and motor performance. This is highlighted by the response of *Met* allele carriers being different to *Val66Val* individuals following several NIBS protocols (Cheeran et al. 2008, Cirillo et al. 2012, Chang et al. 2014, Hwang et al. 2015). For example, Cheeran et al. (2008) found individuals that expressed the *BDNF* polymorphism demonstrated altered corticospinal responses to continuous and intermittent TBS, PAS and cathodal tDCS followed by rTMS compared to those without the *BDNF* polymorphism. Using a larger sample size and classification of three genotypes (*Val/Val, Val/Met, Met/Met*) Cirillo et al. (2012) confirmed the important role that *BDNF* plays in PAS-induced plasticity. Similarly, the influence of the *BDNF* polymorphism on the induction of homeostatic plasticity following rTMS has been further demonstrated in both healthy and clinical populations

(Chang et al. 2014, Hwang et al. 2015). However, the influence of *BDNF* on NIBS protocols is not always consistent with some studies showing no difference in corticospinal plasticity between *Val66Val* and *Val66Met* carriers following rTMS and iTBS (Li Voti et al. 2011, Nakamura et al. 2011). Importantly, it should be noted that the protocol duration used in these studies may not have been sufficient to activate cellular processes of activity-dependent *BDNF* secretion (Li Voti et al. 2011, Nakamura et al. 2011).

Interestingly, only a limited number of studies have investigated the impact of the *BDNF* polymorphism on corticospinal plasticity induced by anodal tDCS in young and old adults (Antal et al. 2010, Puri et al. 2015, Frazer et al. 2016). One study found that carriers of the *BDNF Met* allele (*Val/Met*) displayed enhanced corticospinal responses to a single session of anodal tDCS compared to the *Val/Val* genotype (Antal et al. 2010). Antal and colleagues (2010) concluded that this finding was due to tDCS modifying the transmembrane neuronal potential compared to the other NIBS techniques which act upon LTP mechanisms. However, given that long lasting changes in motor behaviour associated with repeated tDCS stimulation is likely to occur as a result of LTP-like mechanisms (Liebetanz et al. 2002, Nitsche et al. 2004a, Nitsche et al. 2004b), it was not unexpected that a recent study found that carriers of the *BDNF Met* allele displayed reduced corticospinal responses to accumulated bouts of anodal tDCS (Frazer et al. 2016). Given the evidence that the corticospinal responses to NIBS techniques are largely due to LTP mechanisms and the interaction between *BDNF* secretion and LTP/LTD processes, it is highly likely that *BDNF* is involved in the regulation of corticospinal plasticity and, potentially, subsequent changes in motor performance. However, further study is required to establish the impact that the *BDNF* polymorphism may have in mediating different

forms of experimentally-induced plasticity and specifically what mechanisms are involved. Furthermore, given the *BDNF* polymorphism has been shown to shape an individual's responsiveness to both experimentally-induced (i.e., tDCS) and use-dependent (i.e., motor skill training) plasticity protocols (Kleim et al. 2006, Cheeran et al. 2008, Antal et al. 2010), it would be critical to identify whether this genetic factor may also influence the effectiveness of using tDCS as a priming protocol prior to motor training to augment the corticospinal responses to a single bout of strength training.

2.5 Use-dependent corticospinal plasticity

It is well established that the modifiable nature of the M1 plays an important role in motor learning and performance (Pascual-Leone et al. 1995, Classen et al. 1998, Rioult-Pedotti et al. 1998, Butefisch et al. 2000, Muellbacher et al. 2002). Corticospinal plasticity has been shown to occur because of repetitive activity and is involved in the improvement in motor behaviour. TMS and fMRI imaging studies have established the M1's involvement during the early phase of skill acquisition following several different motor skill training paradigms including repetitive ballistic training, visuo-motor tracking and peg board training (Garry et al. 2004, Adkins et al. 2006, Carroll et al. 2008, Selvanayagam et al. 2011). Emerging evidence suggests that the induction of use-dependent corticospinal plasticity may differ according to a variation in the *BDNF* gene *(val66met).* Certainly, the influence of the *BDNF* polymorphism on the induction of corticospinal plasticity has been observed following use-dependent paradigms such as motor skill training (Kleim et al. 2006, Cirillo et al. 2012).

Evidence of use-dependent plasticity associated with ballistic motor skill training may provide valuable insight into the potential mechanisms responsible for the rapid development of strength (Muellbacher et al. 2001, Selvanayagam et al. 2011). Ballistic

motor skill tasks share similar characteristics to strength training as both require the repeated generation of high force production (Carroll et al. 2008, Hinder et al. 2013). Furthermore, the inherent requirements of a strength task (muscle recruitment, timing of muscle activation between agonists and antagonists, joint positioning) indicate that a level of skill and learning is necessary for the successful completion of the movement (Carroll et al. 2001b). Due to the similarity between training paradigms, the notion that motor performance gains are mediated by corticospinal plasticity may also be a likely explanation underlying rapid expression of strength (Carroll et al. 2001b, Leung et al. 2015). Although the evidence for corticospinal plasticity following a single session of strength training remains unresolved (Gabriel et al. 2006, Carroll et al. 2011, Hortobagyi et al. 2011, Leung et al. 2015), changes in synaptic efficacy within neural pathways that control specific muscles are likely to influence muscle activation and consequently improve torque production in the desired direction (Carroll et al. 2001b). Another form of strength training that has received significant attention is 'cross-education', which describes the phenomenon whereby strength training of one limb results in an increase in strength of the opposite untrained limb (Munn et al. 2004, Carroll et al. 2006). Although several theoretical frameworks have been suggested to underpin the cross-education effect, there is strong evidence from TMS and fMRI studies supporting the significant involvement of the ipsilateral M1 (*cross-activation* hypothesis) (Kobayashi & Pascual-Leone 2003, Hortobágyi et al. 2003b, Zijdewind et al. 2006, Perez & Cohen 2008, van Duinen et al. 2008, Howatson et al. 2011). Cross-activation is thought to lead to adaptations in the neural circuits that project to the muscles of the untrained contralateral limb (ipsilateral primary motor cortex [iM1]), manifesting as an improvement in motor performance of the untrained limb (Ruddy & Carson 2013). Therefore, the following

discussion will examine the corticospinal responses after a single strength training session, the phenomena of cross-education and the potential influence of the *BDNF* polymorphism on the induction of use-dependent corticospinal plasticity.

2.5.1 Corticospinal plasticity following a single session of strength training

Strength gains in the absence of muscle hypertrophy following a period of training has often been attributed to changes within the CNS (Carroll et al. 2001b, Carroll et al. 2011). Although underlying neural mechanisms inevitably play a role in the development of strength, it is unclear which specific pathways are involved and how they mediate the expression of muscle strength following a single session of strength training. Currently, studies are inconclusive regarding the role corticospinal plasticity plays in the development of strength and expression of muscle strength (following a single session), and whether the *BDNF* polymorphism contributes to this variability. Therefore, the following will discuss the corticospinal responses to a single session of strength training, as measured by TMS, and the potential regulatory role of the *BDNF* polymorphism.

2.5.5.1 Corticospinal adaptations to strength training and TMS

Although there is a consensus that changes in the efficacy of neural transmission along the corticospinal tract mediate strength development following a strength training intervention, emerging evidence from TMS studies has revealed highly mixed findings regarding specific sites of adaptation (Carroll et al. 2011, Taube 2011). Several studies have shown increases in corticospinal excitability following a single session of strength training (Selvanayagam et al. 2011, Leung et al. 2015, Nuzzo et al. 2016) suggesting the involvement of mechanisms associated with LTP underpinning the consolidation of strength. However, in direct contrast, Hortobagyi et al. (2011) showed no changes in corticospinal excitability following 1000 submaximal voluntary contractions of the right

first dorsal interosseus (FDI) at 80% MVC. To address these conflicting results, Nuzzo et al. (2016) used TMS and cervicomedullary motor–evoked potentials (CMEPs) to examine changes in synatpic efficacy and motor neuron output following a single bout of ballistic strength training involving high force and high rate of force development contractions of the elbow flexor muscles. The results showed an increase in motor-evoked potentials (MEPs) and CMEPs 15 min post training and increased CMEP and MEP twitch forces (Nuzzo et al. 2016). Given the combination of the robust techniques used in this study, it would appear that changes in synaptic efficacy within neural pathways that control specific muscles are likely to influence muscle activation and consequently improve force production (Carroll et al. 2001b). However, the influence of the *BDNF* polymorphism on the induction of corticospinal plasticity following a single session of strength training has yet to be examined and may in fact be a potential variable contributing to these mixed findings.

To date, many studies have primarily used single-pulse TMS to assess changes in corticospinal excitability following a single session of strength training. Although this measure provides valuable insight into the overall excitability of the corticospinal tract, changes in cortical inhibition have been proposed to attenuate M1 output via GABA receptor mediated interneuron transmission (McCormick 1989). Importantly, reductions in SICI, as mediated by $GABA_A$ receptors have also been shown to decrease following an acute bout of externally-paced strength training suggesting that alterations in cortical inhibition may underlie the development of strength (Stinear & Byblow 2003). Interestingly, investigators have also shown that the changes observed following externally-paced strength training are similar to those of skill training (visuo-motor tracking) suggesting that the corticospinal responses to skill and strength training may be

similar (Leung et al. 2015). Interestingly, corticospinal silent period duration has been shown to decrease following short-term strength training of the upper and lower limb (Kidgell & Pearce 2010, Latella et al. 2012, Hendy & Kidgell 2013) however, this is yet to be examined following a single session of strength training. Furthermore, the established role of *BDNF* mediating learning and memory related processes (Egan et al. 2003, Hariri et al. 2003) suggests that the presence of the polymorphism may also influence the induction of use-dependent plasticity following skill or strength training.

2.5.5.2 BDNF and the induction of use-dependent corticospinal plasticity

Emerging evidence suggests that the generation of corticospinal plasticity may differ according to a variation in the *BDNF* gene (*val66met*) which may, in part, further explain the magnitude of variability within the acute strength training literature (Hortobagyi et al. 2011, Selvanayagam et al. 2011, Leung et al. 2015, Nuzzo et al. 2016). Certainly, the influence of the *BDNF* polymorphism on the induction of corticospinal plasticity has been observed following use-dependent paradigms such as motor skill training (Kleim et al. 2006, Cirillo et al. 2012). For example, Cirillo et al. (2012) found those with the *BDNF* polymorphism (*Val/Met* and *Met/Met*) displayed no change in MEP amplitude or reduction in MEP amplitude following complex motor skill training. This was in direct contrast to those without the *BDNF* polymorphism (*Val/Val*) who demonstrated a significant increase in motor evoked amplitude following the training suggesting that the modulation of corticospinal excitability is strongly influenced by the variation of the *BDNF* gene (*val66met*). However, there are no studies to date that have investigated the potential influence of the *BDNF* polymorphism on the corticospinal responses to an acute bout of strength training. The notion that motor learning and strength training may share similar corticospinal responses (Leung et al. 2015) gives rise

to the idea that the *BDNF* polymorphism may also influence the corticospinal responses to strength training.

2.5.2 Cross-education

A fascinating phenomenon that provides profound evidence for the involvement of the CNS in the rapid development of strength following short-term strength training is cross-education. Cross-education describes the process whereby strength training of one limb results in an increase in strength of the opposite untrained limb (Munn et al. 2004, Carroll et al. 2006). Although this phenomenon has been well documented following short-term (i.e., three weeks) and moderate-term (i.e., eight weeks) unilateral strength training (Farthing et al. 2005, Lee et al. 2009, Goodwill et al. 2012, Latella et al. 2012), there are few studies that have examined the acute effects of cross-education (single session) (Hendy & Kidgell 2014, Leung et al. 2015). The exact physiological mechanisms that underlie the cross-education of strength is unknown, however the ipsilateral M1 to the trained limb has been suggested to play a significant role (Kobayashi & Pascual-Leone 2003, Hortobágyi et al. 2003b, Zijdewind et al. 2006, Perez & Cohen 2008, van Duinen et al. 2008, Howatson et al. 2011). For example, when rTMS is applied to the ipsilateral M1, the cross-transfer effect is abolished (Lee et al. 2010) but, when anodal tDCS has been applied to the ipsilateral M1 during strength training, the cross-transfer effect is augmented (Hendy & Kidgell 2014). Also, there have been no studies that have examined the induction of homeostatic plasticity of the ipsilateral M1 and the effect it may have on the cross-education of muscle strength. Again, given the importance of the *BDNF* polymorphism role in shaping the induction of corticospinal plasticity and the recent evidence showing the important role that tDCS in combination with strength training enhancing the cross-education of muscle strength (Hendy & Kidgell 2014), understanding

the regulatory mechanisms (corticospinal response and *BDNF* polymorphism) underpinning the cross-education effect is necessary to maximise clinical applications of the cross-education phenomenon.

2.5.2.1 Evidence of cross-education

Since the mid-19th century, there has been extensive evidence that strength training of one limb results in an increase in strength of the opposite untrained limb (Scripture et al. 1894, Carroll et al. 2006). Cross-education has been demonstrated following a variety of strength training protocols including isometric, dynamic and imaginary contractions (Yue & Cole 1992, Hortobagyi et al. 1997, Munn et al. 2005b, Lee et al. 2009, Kidgell et al. 2011, Latella et al. 2012). The magnitude of cross-education to the untrained limb has been shown to be proportional to the strength gain of the trained limb (Zhou 2000, Munn et al. 2005b). A recent meta-analysis reported an average of 7.6% increase in strength of the contralateral untrained limb which corresponds to the 52% of strength gained in the trained limb (Carroll et al. 2006). Although the degree of strength transfer reported is relatively small, this could in part be reflective of the variability of results. Indeed, one study reported a 20% gain in strength of the untrained contralateral limb (Latella et al. 2012), while others have reported a decrease in strength of the untrained contralateral limb following unilateral strength training (Farthing et al. 2005, Munn et al. 2005b). A number of methodological considerations including contraction type, dominant versus non-dominant limb and the type of muscle trained have all been examined to explain the variability in the cross-education of strength (Carroll et al. 2006). However, to understand the full clinical potential of the cross-education phenomenon, the contribution of regulatory mechanisms such as the *BDNF* polymorphism needs to be investigated.

2.5.2.2 Mechanisms of cross-education and the potential use of tDCS

Currently, the physiological mechanisms that underlie the cross-education phenomenon remain unclear (Carroll et al. 2006). Investigation into possible muscle and spinal mechanisms mediating cross-education have suggested that neural adaptations of the untrained limb primarily occur at a cortical level (Carroll et al. 2006, Ruddy & Carson 2013). It has been suggested that complex hemispheric interactions allow the untrained side to access the corticospinal adaptations of the trained side. Undoubtedly, the extensive neural network between hemispheres provides a platform to share neuromuscular adaptations obtained by the trained side (Carroll et al. 2006, Hendy et al. 2012) and can be seen in Figure 2.3. However, recent evidence from TMS studies has also proposed that unilateral strength training may cause a 'spill-over' of neural drive from the trained side to the untrained side resulting in corticospinal adaptations of the untrained limb. The spill-over hypothesis is supported by findings of increased corticospinal excitability and decreased SICI and interhemispheric inhibition (IHI) of both motor cortices following unilateral strength training (Hortobagyi et al. 2011, Kidgell et al. 2011, Goodwill et al. 2012, Latella et al. 2012).

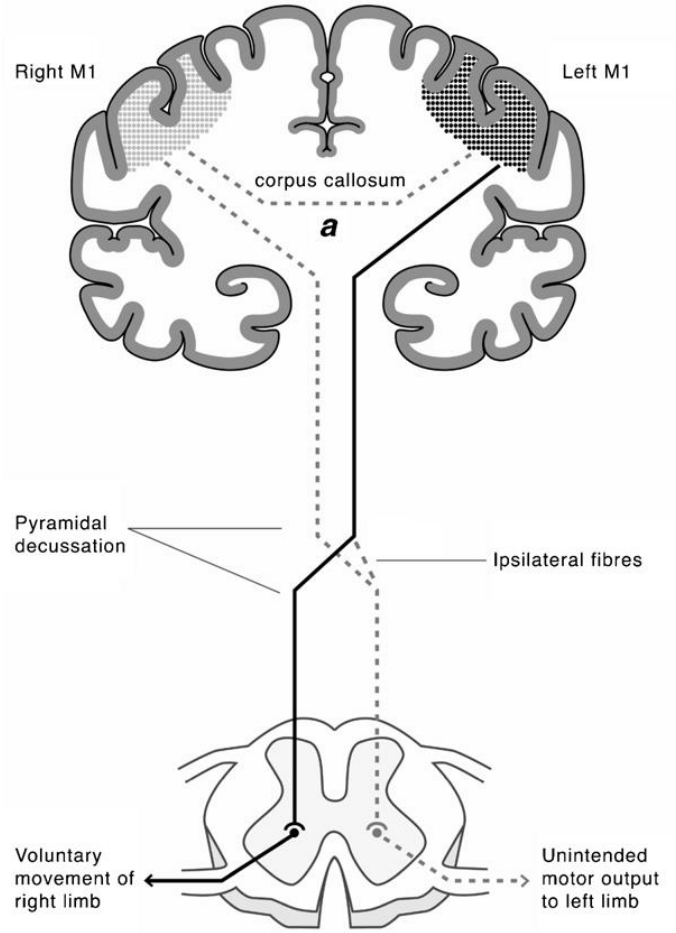

Figure 2.3: Example of interhemispheric communication between the right and left M1 via the corpus callosum (Hendy et al. 2012).

Activation of the untrained ipsilateral M1, in addition to the trained M1, appears to contribute to the strength gain of the untrained contralateral limb. This would suggest an opportunity to promote further activation of the ipsilateral cortex via external means and potentially enhance cross-education of muscle strength. Given that tDCS is known to induce homeostatic plasticity (Liebetanz et al. 2002, Nitsche et al. 2004a, Nitsche et al. 2004b, Ridding & Ziemann 2010), this NIBS tool may be useful to exploit the 'spill-over' effect. Indeed, the application of anodal tDCS to the ipsilateral M1 prior to unilateral strength training may result in a shift of the resting membrane potential and increase synaptic activity of the ipsilateral M1, which may in turn further promote bilateral activation of both motor cortices and enhance the cross-education of muscle strength. However, this hypothesis remains to be tested and forms a central point of this thesis.

Given the emerging evidence that suggests the induction of homeostatic plasticity may differ according to a variation in the *BDNF* gene *(val66met)* (Kleim et al. 2006, Cirillo et al. 2012, Frazer et al. 2016), and the notion that tasks involving force generation (i.e., strength training) may share similar underlying neural substrates to motor skill training (Carroll et al. 2001b, Leung et al. 2015), underscores the importance of future targeted research. It is possible that the *BDNF* polymorphism may influence the magnitude of bilateral activation (increase in corticospinal excitability) of both motor cortices, as described by the *cross-activation* hypothesis, which may in turn affect the level of strength transfer to the untrained limb. Certainly, if individuals with the *BDNF* polymorphism have reduced corticospinal responses to unilateral strength training, it may counteract the cross-transfer effect by either reducing the capacity of the strength gained in the training arm or the adaptations within the ipsilateral M1. However, to this end, no one has yet investigated the potential influence of the *BDNF* polymorphism on the cross-

education of strength and the ipsilateral corticospinal responses to a single session of strength training. Understanding the potential underlying regulatory factors is necessary to maximise the clinical applications of cross-education.

2.6 Conclusion

A considerable amount of research has been dedicated to exploring how the CNS mediates motor performance and whether this can be enhanced in an experimental and/or use-dependent manner. Experimentally-induced and use-dependent plasticity-inducing paradigms have both shown promising results regarding the enhancement of motor performance in healthy and clinical populations (Ridding & Ziemann 2010, Carroll et al. 2011). Consequently, the focus of investigation has shifted towards the combination of both plasticity-inducing protocols, known as motor priming, to further facilitate motor performance. Although preliminary data demonstrate potential functional benefits of motor priming, little is understood regarding the bilateral effects of NIBS (i.e., tDCS) and how this paradigm may be translated into cross-education models. Furthermore, individual responses have been shown to be highly variable, creating uncertainty regarding the therapeutic value of using plasticity-inducing protocols to improve motor performance. The *BDNF* polymorphism has been identified as a potential regulator involved in the induction of corticospinal plasticity (Cheeran et al. 2008, Antal et al. 2010), but little is known as to what extent this factor may impact on the efficacy of experimentally-induced and use-dependent plasticity protocols used to improve motor performance.

Therefore, the studies in this dissertation will systematically examine the corticospinal responses to various homeostatic-inducing protocols (anodal tDCS alone and in combination with strength training) to investigate the corticospinal responses that

may contribute to the expression of muscle strength. This thesis will also explore the influence of the *BDNF* polymorphism on indices of corticospinal plasticity and strength following experimentally-induced and use-dependent plasticity protocols.

Chapter 3 : Anodal tDCS increases bilateral cortical excitability irrespective of hemispheric dominance

3.1 Introduction

Altering the excitability of cortical neurons using electrical stimulation has been of particular interest for scientific and medical communities for over a decade (Roy et al. 2014). tDCS has emerged as a popular NIBS technique which involves the application of weak direct currents to the scalp. Please refer to Section 2.4 for a comprehensive review of tDCS.

The effects of anodal tDCS are known to induce corticospinal plasticity of the stimulated M1 via changes in corticospinal excitability (Nitsche & Paulus 2000, Nitsche & Paulus 2001, Bastani & Jaberzadeh 2012, Kidgell et al. 2013, Pellicciari et al. 2013). However, emerging evidence suggests that other NIBS techniques modulate not only the intended stimulated tissue but also distal connecting tissues and structures, as well as the opposite non-stimulated hemisphere (Gilio et al. 2003, Suppa et al. 2008, Di Lazzaro et al. 2011, Shin & Sohn 2011). Critically, evidence from TMS studies show diverse findings regarding the direction of excitability (increase/decrease) of the non-stimulated hemisphere following various NIBS techniques (Gilio et al. 2003, Lang et al. 2004, Di Lazzaro et al. 2008, Suppa et al. 2008, Di Lazzaro et al. 2011). Specifically, when applied separately, rTMS at 1 Hz and PAS has been shown to increase excitability of both the stimulated and non-stimulated M1 (Gilio et al. 2003, Schambra et al. 2003, Shin & Sohn 2011) and decrease interhemispheric inhibition (IHI) between the left and right M1 (Gilio et al. 2003). Similarly, Lang et al. (2004) found that 10 min of anodal and cathodal tDCS at 1 mA modulated transcallosal inhibition. However, this finding was not accompanied by a bilateral increase in M1 excitability, with only an increase in MEP amplitude seen in the stimulated M1. In contrast, iTBS has shown to increase corticospinal excitability of the stimulated hemisphere and decrease corticospinal excitability of the non-stimulated

hemisphere (Di Lazzaro et al. 2008, Suppa et al. 2008, Di Lazzaro et al. 2011). Importantly, many of these studies have used a dominant M1 arrangement whereby the stimulated hemisphere was the dominant M1 (left) and the non-stimulated hemisphere was the non-dominant M1 (right). It has been shown that the non-dominant hemisphere has lower motor thresholds, higher MEPs (De Gennaro et al. 2004) and shorter corticospinal silent period durations (Priori et al. 1999), suggesting a hemispheric difference in baseline characteristics. An interesting question to address is whether the magnitude of bilateral corticospinal plasticity is affected by the direction of stimulation (dominant vs non-dominant M1 stimulated), and if there is a greater scope for the induction of corticospinal plasticity of the non-dominant hemisphere.

Several studies have reported that modulating corticospinal excitability and inhibition with NIBS techniques leads to improvements in motor performance (Boggio et al. 2006, Tanaka et al. 2009, Kidgell et al. 2013), and the cross-transfer of motor skills (Vines et al. 2006). Recently, Hendy et al. (2014) reported an increase in maximal strength and cross-activation to the contralateral untrained limb (left hand) following a single session of anodal tDCS applied to the ipsilateral right M1 during strength training of the right hand. Given that the cross-transfer of strength following training is thought to be due to an increase in excitability of the ipsilateral M1 (Ruddy & Carson 2013), it would be apparent that the bilateral effects of anodal tDCS need to be clearly understood. If anodal tDCS increases excitability in both the stimulated and non-stimulated hemispheres, this NIBS technique may be vital to further exploit the cross-education phenomenon in clinical settings. Conversely, if anodal tDCS decreases excitability of the non-stimulated hemisphere, it may counteract the cross-education effect by either

reducing the capacity of the strength gained in the training arm or the corticospinal responses within the ipsilateral M1.

As discussed in Section 2.4.5, it appears that individual corticospinal responses to tDCS are highly variable and the *BDNF* polymorphism has been identified as a potential contributing factor (Hwang et al. 2015, Puri et al. 2015, Frazer et al. 2016). Critically, there are no studies of whether the *BDNF* polymorphism influences the induction of corticospinal plasticity to the non-stimulated hemisphere, and if the change in corticospinal excitability is proportional to the stimulated hemisphere. Therefore, the aim of this study was to examine the effect of a single session of anodal tDCS on the indices of corticospinal excitability and inhibition of both the stimulated and non-stimulated hemisphere. A secondary aim of this chapter was to examine corticospinal excitability/inhibition and the influence on these responses of the *BDNF* polymorphism. It was hypothesised that the induction of experimentally-induced corticospinal plasticity (increased cortical excitability and reduced cortical inhibition) would be evident in both the stimulated and non-stimulated M1 regardless of which hemisphere was stimulated (dominant vs non-dominant), but the magnitude of these responses would be influenced by the *BDNF* polymorphism.

3.2 Methods

3.2.1 Participants

Sixteen participants (8 women, 8 men aged 18-35 years) volunteered to participate. All volunteers provided written informed consent prior to participation in the study, which was approved by the Human Research Ethics Committee in accordance with the standards by the Declaration of Helsinki. All participants were right-hand dominant as determined by the Edinburgh Handedness Inventory (Oldfield 1971) with an laterality quotient (LQ) score of 86 ± 5 and were free from any known history of peripheral or neurological impairment. Prior to the experiment, all participants completed the adult safety screening questionnaire to determine their suitability for TMS and tDCS (Keel et al. 2001).

3.2.2 Experimental approach

Figure 3.1 outlines the organization of the study. After obtaining consent, participants completed a familiarization session one week prior to the study and were exposed to single-pulse TMS. In a double-blinded cross-over design, all participants received to 20 min of anodal tDCS over the dominant (anode over the left M1; Figure 3.2i) and non-dominant (anode over right M1; Figure 3.2ii) M1, and 20 min of sham tDCS (half the participants using the dominant M1 arrangement, the other half using the non-dominant M1 arrangement). The order of the conditions was counterbalanced and randomized between participants, with a wash-out period of one week between each condition (Vines et al. 2008). Both tDCS conditions followed the identical testing protocol as shown in Figure 3.1 for the right and left biceps brachii muscles. Similarly, the order of muscle testing (right and left biceps brachii muscles) was counterbalanced and randomized between participants. All participants underwent TMS prior to and

following the tDCS intervention. Participants were required to attend three separate sessions where they were exposed to 20 min of anodal (dominant M1 and non-dominant M1 arrangements) and sham tDCS applied at 2 mA with a current density of 0.08 mA/cm^2.

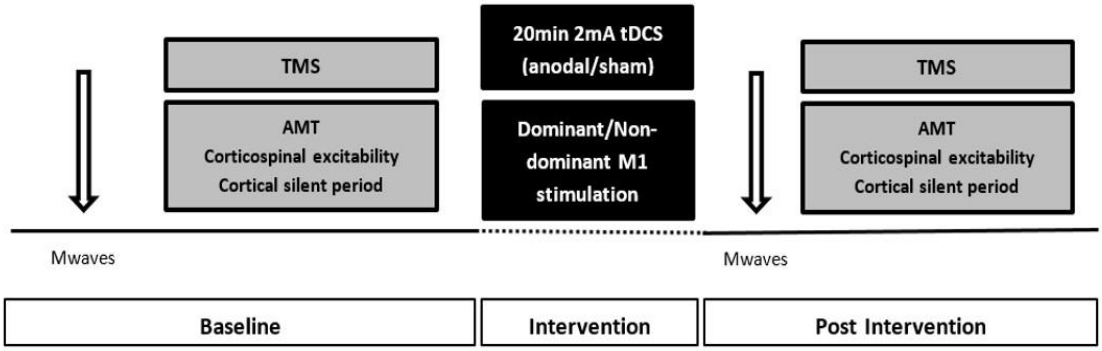

Figure 3.1: Schematic representation of the experimental design with measures obtained prior to and following sham and anodal tDCS (dominant and non-dominant M1 stimulation). Pre-and post-measures included the assessment of peripheral muscle excitability (M-waves), corticospinal excitability and corticospinal inhibition of the stimulated and non-stimulated hemispheres.

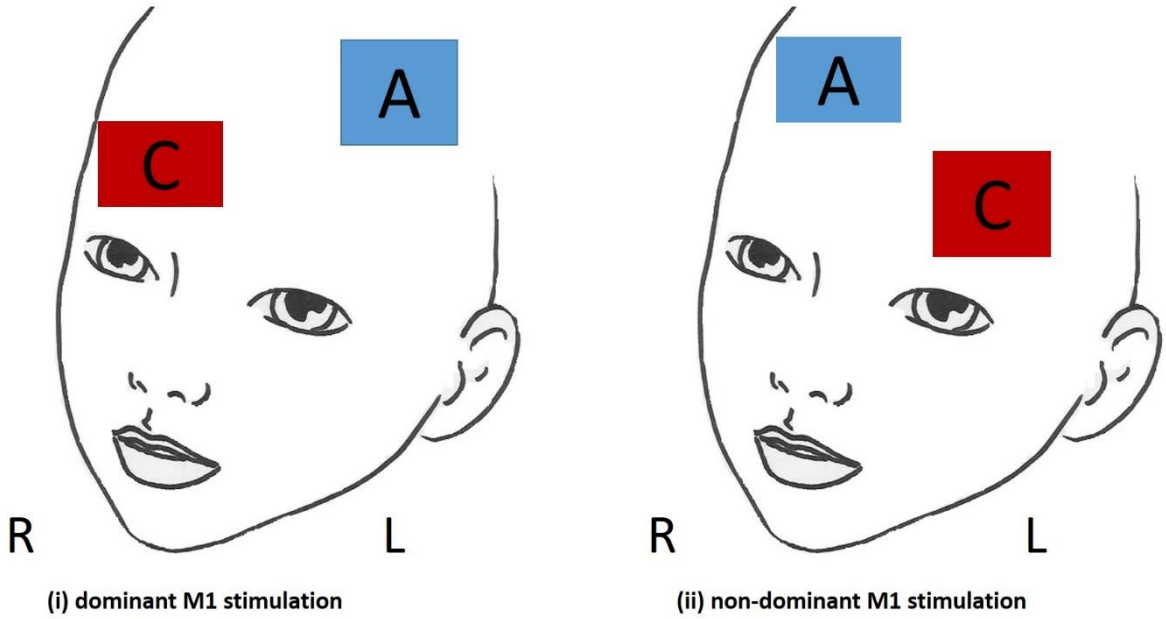

Figure 3.2: Schematic representation of the two tDCS electrode arrangements used. **(i)** dominant M1 stimulation whereby the anode (A) was fixed over the optimal cortical representation of the right biceps brachii muscle and the cathode (C) was placed over the right contralateral supra orbital area **(ii)** non-dominant M1 stimulation whereby the anode (A) was fixed over the optimal cortical representation of the left biceps brachii muscle and the cathode (C) was placed over the left contralateral supra orbital area.

3.2.3 Root mean square electromyography and maximal voluntary isometric contraction

To determine the maximal *rms*EMG of both the right and left biceps brachii muscles, participants were seated in a chair, shoulders relaxed with their elbow flexed at 90 degrees. With the hand supinated and the force transducer (Futek Force Transducer LSB302, Melbourne) positioned over the middle aspect of the palmar surface of the hand, the participant was instructed to push up against the transducer as forcefully as possible for 3 sec. Three trials were performed; each trial was 3 sec in duration, separated by 3 min rest to minimize fatigue. The *rms*EMG during MVIC was calculated from a 500 ms segment occurring during the peak asymptote of MVIC force (Griffin & Cafarelli 2007). The greatest force output and corresponding surface electromyography (sEMG) served as the MVIC and maximal *rms*EMG.

3.2.4 Surface electromyography

The area of electrode placement was shaven to remove fine hair, rubbed with an abrasive skin gel to remove dead skin, and then cleaned with 70% isopropyl alcohol. sEMG was recorded from the right and left biceps brachii muscles using bipolar Ag-AgCl electrodes (ADInstruments, Bella Vista Australia). The site of measurement was determined by marking the skin two thirds of the distance between the acromion and the lateral epicondyle, while the participant stood relaxed in the anatomical position (Pearce et al. 2013). This mark was then extended to the most anterior point of the muscle bulk and, as described by Wilson et al. (1993), the electrodes were placed 2 cm apart over the mid-belly of the biceps brachii, with a ground electrode secured on the lateral epicondyle of the humerus. sEMG signals were amplified (x1000), band pass filtered (high pass at

13 Hz, low pass at 1000 Hz), digitized online at 2 kHz, recorded (1 sec), and analyzed using Power Lab 4/35 (AD Instruments, Bella Vista, Australia).

3.2.5 Transcranial magnetic stimulation

TMS was delivered using a Magstim 200^2 stimulator (Magstim Co, Dyfed, UK) and a single figure-of-eight coil (external diameter of each loop 70 mm). The motor hotspot for the right and left biceps brachii muscles (with posterior-to anterior-induced current flow in the cortex) was determined, and AMT was established as the intensity at which at least five of ten stimuli produced MEP amplitudes of greater than 200 μV in the right and left biceps brachii muscles, respectively. Following the tDCS intervention, AMT was retested and adjusted if required. To ensure all stimuli were delivered to the optimal motor hotspot throughout testing, participants wore a tight-fitting cap that was marked with a latitude-longitude matrix, positioned with reference to the nasion-inion and interaural lines.

During a low-level isometric contraction of the right and left biceps brachii muscles (4 ± 1% of maximal *rms*EMG), ten single-pulse stimuli were delivered at 150% and 170% AMT (Hendy et al. 2015). Participants were required to maintain an elbow joint angle of 90^{O} elbow flexion. Joint angle was measured with an electromagnetic goniometer (ADInstruments, Bella Vista, Australia), with visual feedback provided on a screen visible to both the participant and the researcher (Hendy et al. 2015). This joint position equated to 4 ± 1% of maximal *rms*EMG, with consistent muscle activation confirmed by recording pre-stimulus *rms*EMG for the 100-ms epoch before the delivery of each stimulus (Table 3-1).

3.2.6 Maximum compound muscle action potential

Direct muscle responses were obtained from the right and left biceps brachii muscles by supramaximal electrical stimulation (pulse width, 200 μs) of the brachial plexus at Erbs point (MLADDF30 Stimulating Bar Electrode via a DS7A; Digitimer, Hertfordshire, United Kingdom). The stimuli were delivered while the participant sat in an upright position, with the elbow at 90^{O} elbow flexion holding 4 ± 1% of maximal *rms*EMG. This low level of muscle activity was used to match the conditions under which TMS was delivered. An increase in current strength was applied to Erbs point until there was no further increase observed in the amplitude of the sEMG response (M_{MAX}). To ensure maximal responses, the current was increased an additional 20% and the average M_{MAX} was obtained from five stimuli, with a period of 6–9 sec separating each stimulus. M_{MAX} was recorded at baseline and following the tDCS intervention to control for possible changes in peripheral muscle excitability that could influence MEP amplitude.

3.2.7 Transcranial direct current stimulation

In all tDCS conditions, participants received 20 min of tDCS (2 mA) delivered by a battery-driven constant current transcranial direct current stimulator (NeuroConn, Ilmenau, Germany). Stimulation was delivered by a pair of conductive rubber electrodes (anode 25 cm^2; cathode 35 cm^2; current density 0.08 mA/cm^2) each soaked in saline solution (0.9% NaCl) and secured on the head with a rubber strap (Nitsche et al. 2007). For the dominant M1 arrangement, the anode was fixed over the optimal cortical representation of the right biceps brachii muscle, as identified by TMS over the left cortex, and the cathode was placed over the right contralateral supra orbital area. For the non-dominant M1 arrangement, the anode was fixed over the optimal cortical

representation of the left biceps brachii muscle, as identified by TMS over the right cortex, and the cathode was placed over the left contralateral supra orbital area (Figure 3.2). To ensure consistency of the site of stimulation, the participant's head was marked with a latitude-longitude matrix, positioned with reference to the nasion-inion and interaural lines. Both the experimenter and participant were blinded to the tDCS condition (i.e., sham versus anodal tDCS) using codes on the tDCS machine. Using the protocol suggested by the international consensus paper on NIBS techniques (Ziemann et al. 2008), the sham protocol had the identical arrangement to the anodal tDCS condition, using both the dominant and non-dominant M1 arrangements (50% each), but the stimulation terminated after approximately 20 sec. This resulted in the participant experiencing the initial sensation of tDCS, however no experimental effects occurred. To obtain the participant's perception of discomfort throughout all tDCS conditions, discomfort (which included pain, itching, and tingling sensations) was assessed using a visual analogue scale (VAS) during the first 3 minutes of stimulation. The VAS ranged from 0 to 10 as visually described in cm units: 0 cm indicates "no discomfort" and 10 cm means "extremely uncomfortable".

3.2.8 *BDNF* genotyping

As described by Frazer et al. (2016), blood samples were obtained and participants were genotyped for the *BDNF Val66Met* polymorphism. Whole blood was obtained in EDTA tubes, and DNA was extracted using the QiaAmp DNA Mini Kit (Qiagen, N.V.) according to the manufacturer's protocol. Briefly, 200 µl of whole blood was added to 20 µl of protease, followed by addition of 200 µl lysis buffer (Buffer AL). Samples were pulse-vortexed for 15 sec, briefly centrifuged (4000 rpm, 15 sec), then incubated at 56°C for 10 min. Following incubation, 200 µl of absolute ethanol was added, the samples were

again pulse-vortexed for 15 sec, and centrifuged (4000 rpm, 15 sec). The samples were then transferred to a QIAamp mini-column and centrifuged at 8000 rpm for 1 min. The QIAamp mini-column was then placed in a clean 2 ml collection tube, and the used collection tube containing filtrate was discarded (this process was completed following each wash). Following this, 500 µl of wash buffer 1 (Buffer AW1) was added to the samples and centrifuged at 8000 rpm for 1 min. This process was repeated with wash buffer 2 on two occasions (Buffer AW2), and then the columns were transferred to a 2 ml collection tube and centrifuged at 14,000 rpm for 1 min to completely dry the membrane. To elute the DNA from the spin column, 150 µl of nuclease-free water (Life Technologies, Mulgrave, VIC) was added to the membrane and incubated at room temperature for 5 min, followed by centrifugation at 8000 rpm for 1 min. The DNA concentration was determined using the NanoDrop 2000 (NanoDrop products, Wilmington, DE), and samples were diluted to 2.5 ng/µl and stored at -80°C until further analysis (Frazer et al. 2016).

The *Val66Met* single nucleotide polymorphism in the *BDNF* gene was typed by a polymerase chain reaction (PCR) in a total of 25 µl containing 125 ng of DNA, 10 × buffer (Life Technologies), 1.5 mM magnesium chloride ($MgCl_2$) (Sigma-Aldrich, St Louis, MO), 200 µM deoxyribonucleotide triphosphate (dNTP) (Life Technologies), 400 µM of each primer and 1 U Taq polymerase (Life Technologies) using a thermal cycler (Takara Bio, Shiga, Japan). In accordance with Neves-Pereira et al. (2002), primer sequences included ACTCTGGAGAGCGTGAATGG / AGAAGAGGAGGCTCCAAAGG. PCR started with an initial denaturation at 95°C for 5 min, followed by 94°C for 30 s, 60°C for 30 s, and 72°C for 30s for 30 cycles, with a final extension at 72°C for 5min. The PCR product was then digested with the restriction

enzyme FastDigest PmlI (Eco72I) (Thermo Scientific, Massachusetts, USA). Briefly, 10 µl of the PCR sample was added to 17 µl of nuclease-free water (Life Technologies), 2 µl of 10X FastDigest Buffer and 1 µl of the FastDigest enzyme (Thermo Scientific). Samples were pulse-vortexed for 15 sec, briefly centrifuged (4000 rpm, 15 sec), then incubated at 37°C for 5 mins. Using the 2100 Bioanalyzer, together with the DNA 1000 LabChip Kit (Agilent Technologies, Böblingen, Germany), participants were classified as *Val/Val*, *Val/Met* or *Met/Met*. The samples were classified based on the observed banding pattern. The uncut product size was 113 bp (*Met/Met*), and *Val/Val* comprised the cut bands of 78 and 35 bp (Neves-Pereira et al. 2002, Frazer et al. 2016).

3.2.9 Data analysis

Pre-stimulus *rms*EMG activity was determined in the right and left biceps brachii muscles 100 ms prior to each TMS stimulus during pre- and post-testing. Any trial in which pre-stimulus *rms*EMG exceeded 4 ± 1 % of maximal *rms*EMG was discarded, and the trial was repeated. The peak-to-peak amplitude of MEPs evoked because of stimulation was measured in the right and left biceps brachii muscles contralateral to the cortex being stimulated in the period 10-50 ms after stimulation. MEP amplitudes were analyzed (LabChart 8 software, ADInstruments, Bella Vista, NSW, Australia) after each stimulus was automatically flagged with a cursor, providing peak-to-peak values in µV, averaged and normalized to the M_{MAX}, and multiplied by 100.

Corticospinal silent period durations were obtained from single-pulse stimuli delivered at 150% and 170% AMT during a light contraction (4% ± 1 of maximal *rms*EMG of the right and left biceps brachii muscles). The duration between the onset of the MEP and the resolution of background sEMG was visually inspected and manually

cursored, with the experimenter blinded to each condition. The average from ten stimuli was used for corticospinal silent period duration (Wilson et al. 1993).

In addition, the laterality index (LI) for interhemispheric asymmetries in corticospinal excitability and inhibition was calculated based on the mean difference in MEP amplitudes between the two hemispheres and the mean difference in corticospinal silent period duration between the two hemispheres, respectively. In accordance with Cramer et al. (1997) and Langan et al. (2010), LI was calculated for each condition defined as (L-R)/(L+R), where L = left hemisphere and R = right hemisphere. A score of 1 reflects complete lateralization to the left side. Conversely, a score of -1 indicates complete lateralization to the right side. In this experiment, a positive score indicates greater excitability of the dominant M1 (left hemisphere, right arm).

3.2.10 Statistical analysis

All data were screened with the Shapiro-Wilk test and found to be normally distributed (all $P > 0.05$) and, thus, the assumptions of the ANOVA were not violated. Subsequently, for the primary analysis, a 3 (conditions) × 2 (hemisphere) × 2 (time) repeated measures ANOVA was used to determine any difference between conditions, hemispheres and time for the dependent variables, *rms*EMG, M_{MAX}, corticospinal excitability and corticospinal silent period duration. If significant main effects were found, a Bonferroni correction was used for *post-hoc* testing to compare the interaction of condition (sham and anodal tDCS) by hemisphere (stimulated and non-stimulated) and time (pre, post) for each dependent variable.

For the secondary analysis, a 2-way ANOVA of genotype (*Val/Val*, *Val/Met*) and time (pre-testing and post-testing) was used to examine the effect of genotype on multiple

dependent variables (corticospinal excitability and corticospinal silent period duration) following anodal tDCS (dominant and non-dominant M1 stimulation arrangements). In addition, paired *t*-tests were performed on VAS scales and LI score variables. IBM SPSS Statistics 23.0 (United States) was used for all statistical analyses with the level of significance set as $P < 0.05$ for all testing. All data are presented as mean ± SE.

3.3 Results

The *BDNF* genotype analysis for the ten participants for whom genetic data was available revealed that seven were homozygous for the *Val* allele (*Val66Val*), while three were genotyped as *Val66Met*. The remaining six participants declined to have a blood sample taken for genetic analysis.

3.3.1 Pre-stimulus *rms*EMG, active motor threshold stimulus intensity, maximal compound wave, and visual analogue scale

Table 3-1 presents the mean (± SE) for AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG for the stimulated and non-stimulated hemispheres prior to and following sham and anodal tDCS (dominant and non-dominant M1 stimulation). Pre-stimulus *rms*EMG, AMT stimulus intensity and M_{MAX} were similar between sham and anodal tDCS (dominant and non-dominant M1 stimulation) conditions at baseline for each hemisphere (stimulated and non-stimulated; $P > 0.05$). Pre-stimulus *rms*EMG did not vary between single-pulse trials, and there were no TIME, TIME × CONDITION or TIME × CONDITION × HEMISPHERE interactions observed (all $P > 0.05$). Similarly, there were no TIME, TIME × CONDITION or TIME × CONDITION × HEMISPHERE interactions detected for AMT stimulus intensity (all $P > 0.05$). Furthermore, there were no TIME, TIME × CONDITION or TIME × CONDITION × HEMISPHERE interactions detected for M_{MAX} (all $P > 0.05$). VAS data were collected for each condition and there was no difference in participants' perception of discomfort between anodal tDCS (dominant and non-dominant M1 stimulation) and sham tDCS conditions (3.3 ± 0.5, 3.2 ± 0.5, 2.8 ± 0.7, respectively; $P = 0.48$).

Table 3-1: Mean (± SE) for AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG for the stimulated and non-stimulated M1 prior to and following sham and anodal tDCS (dominant and non-dominant M1 stimulation).

		Sham tDCS		**DH atDCS**		**N-DH atDCS**		
		Pre	Post	Pre	Post	Pre	Post	***P* value**
AMT SI (%)	Stimulated M1	42 ± 3	43 ± 3	44 ± 2	45 ± 2	41 ± 2	42 ± 2	0.28
	Non-Stimulated M1	45 ± 3	45 ± 3	42 ± 2	42 ± 2	44 ± 2	43 ± 2	0.98
Mwave (mV)	Stimulated M1	9.41 ± 1.31	9.53 ± 1.42	8.92 ± 0.79	8.96 ± 0.79	9.46 ± 0.93	9.42 ± 0.92	0.30
	Non-Stimulated M1	10.67 ± 1.68	10.81 ± 1.73	11.05 ± 1.43	11.13 ± 1.53	11.56 ± 1.61	11.59 ± 1.65	0.36
SP *rms*EMG (%MVIC$_{MAX}$)	Stimulated M1	4.26 ± 0.59	4.65 ± 0.78	3.78 ± 0.63	4.48 ± 0.52	3.50 ± 0.60	3.69 ± 0.47	0.68
	Non-Stimulated M1	3.72 ± 0.47	3.53 ± 0.59	3.84 ± 0.59	3.76 ± 0.66	3.41 ± 0.43	3.26 ± 0.43	0.99

DH atDCS: anodal tDCS applied over the dominant M1; N-DH atDCS: anodal tDCS applied over the non-dominant M1; AMT SI: active motor threshold stimulus intensity. Single-pulse (SP) *rms*EMG was pooled across both intensities (150% and 170% AMT). *P* values represent the 3 (conditions) × 2 (hemisphere) × 2 (time) repeated measures ANOVA used to determine any differences between conditions, hemispheres and time for the dependent variables AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG.

3.3.2 Corticospinal excitability

Figure 3.3A-B shows the mean MEP amplitude normalized as a percentage of M_{MAX} for the stimulated and non-stimulated hemispheres prior to and following sham and anodal tDCS (dominant and non-dominant M1 stimulation) at 150% and 170% of AMT. MEP amplitudes were similar between sham and anodal tDCS (dominant and non-dominant M1 stimulation) conditions at baseline for each hemisphere (stimulated and non-stimulated) and stimulus intensities ($P > 0.05$).

At 150% AMT, there was a main effect for TIME *(*$P < 0.001$), CONDITION ($P = 0.001$) and a TIME × CONDITION interaction detected (Figure 3.3A; $P = 0.001$). Importantly, there was no main effect for HEMISPHERE ($P = 0.816$), or TIME × CONDITION × HEMISPHERE interaction ($P = 0.993$) denoting a bilateral increase in MEP amplitude irrespective of which hemisphere was stimulated. *Post hoc* analysis revealed that MEP amplitude increased following anodal tDCS applied over the dominant and non-dominant M1 which was significantly different to sham tDCS ($P = 0.022$; $P = 0.002$, respectively), however this magnitude of change was not different between the dominant and non-dominant M1 ($P = 0.663$). Corticospinal excitability increased by 24% for the stimulated left M1 and increased by 21% for the non-stimulated right M1 which was significantly different to sham tDCS ($P = 0.022$). Corticospinal excitability increased by 30% for the stimulated right M1 and increased by 29% for the non-stimulated left M1 which was significantly different to sham tDCS ($P = 0.002$).

Interestingly, the GENOTYPE × TIME ANOVA revealed only a TIME effect at 150% AMT for the *Val/Val* group following anodal tDCS applied over the non-dominant M1 only. Corticospinal excitability increased by 35% for the stimulated right M1 and increased by 40% for the non-stimulated left M1 ($P < 0.03$; $P = 0.04$, respectively). This

was compared to a 20% increase and 21% decrease in MEP amplitude in those with the *Val/Met* polymorphism for the stimulated and non-stimulated hemispheres following anodal tDCS of the non-dominant M1. However, *post hoc* analysis revealed that the magnitude of change in MEP amplitude was not statistically significant between genotypes ($P > 0.05$).

At 170% AMT, there was a main effect for TIME ($P < 0.001$), CONDITION ($P = 0.009$) and a TIME × CONDITION interaction detected (Figure 3.3B; $P = 0.009$). Importantly, there was no main effect for HEMISPHERE ($P = 0.215$), or TIME × CONDITION × HEMISPHERE interaction ($P = 0.062$) again denoting a bilateral increase in MEP amplitude irrespective of which hemisphere was stimulated. *Post hoc* analysis revealed that MEP amplitude increased following anodal tDCS applied over the dominant and non-dominant M1 which was significantly different to sham tDCS ($P = 0.019$; $P = 0.010$, respectively), however, this magnitude of change was not different between the dominant and non-dominant M1 ($P = 0.825$). Corticospinal excitability increased by 24% for the stimulated left M1 and increased by 16% for the non-stimulated right M1 which was significantly different to sham tDCS ($P = 0.022$). Corticospinal excitability increased by 45% for the stimulated right M1 and increased by 11% for the non-stimulated left M1 which was significantly different to sham tDCS ($P = 0.002$).

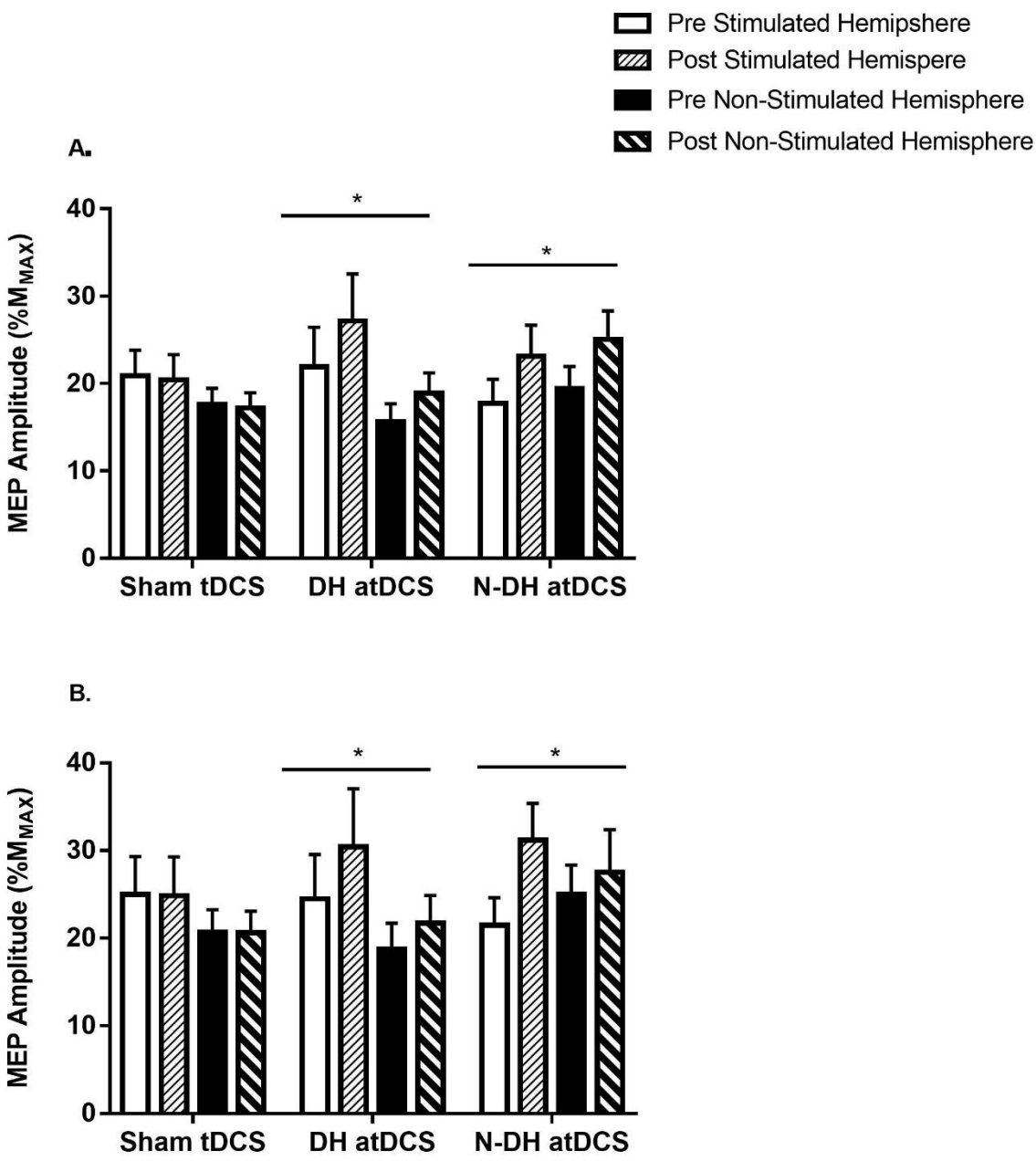

Figure 3.3A-B: Mean (± SE) changes in MEP amplitude for the stimulated and non-stimulated hemispheres projecting to the biceps brachii prior to and following sham, anodal tDCS over the dominant M1 (DH atDCS) and anodal tDCS over the non-dominant M1 (N-DH atDCS) at **(A)** 150% and **(B)** 170% AMT. * denotes significant to sham tDCS.

Interestingly, the GENOTYPE × TIME ANOVA revealed only a TIME effect at 170% AMT for the *Val/Val* group following anodal tDCS applied over the dominant and non-dominant M1 for the stimulated hemisphere (Figure 3.4A-B; 31%, $P = 0.03$; 50%, $P = 0.001$, respectively). This was compared to a 13% and 31% increase in MEP amplitude in those with the *Val/Met* polymorphism for the stimulated hemisphere following anodal tDCS of the dominant and non-dominant M1. *Post hoc* analysis, however, revealed that the magnitude of change in MEP amplitude was not statistically significant between genotypes ($P > 0.05$).

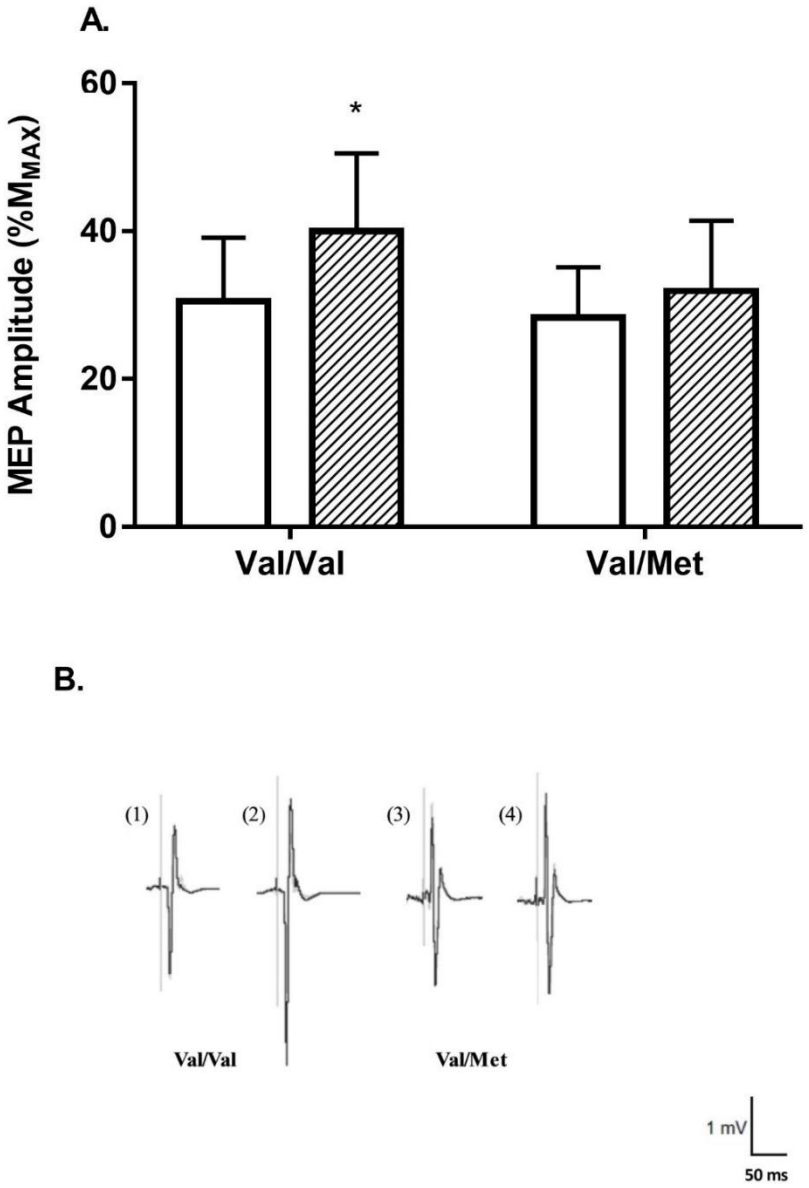

Figure 3.4A-B: (A) Mean (± SE) changes in MEP amplitude of the stimulated hemisphere projecting to the biceps brachii at 170% AMT following anodal tDCS of the dominant M1 with different *BDNF* genotypes. * denotes significant to baseline. **(B)** Raw EMG responses (MEPs) of the stimulated hemisphere projecting to the biceps brachii produced following anodal tDCS of the dominant M1 with different *BDNF* genotypes, whereby **(1)** and **(2)** depict pre-and post MEP sweeps for the *Val/Val* individuals, **(3)** and **(4)** depict pre-and post MEP sweeps for the *Val/Met* individuals.

3.3.3 Corticospinal inhibition

Figure 3.5A-B shows the mean corticospinal silent period duration for the stimulated and non-stimulated hemispheres prior to and following sham and anodal tDCS of the dominant and non-dominant M1 at 150% and 170% of AMT. Corticospinal silent period durations were similar between sham and anodal tDCS (dominant and non-dominant M1 stimulation) conditions at baseline for each hemisphere (stimulated and non-stimulated) and stimulus intensities ($P > 0.05$). At 150% AMT, there were no main effects for TIME, TIME × CONDITION or TIME × CONDITION × HEMISPHERE interactions detected following the intervention (*all* $P > 0.05$). At 170% AMT, there were a TIME and TIME × CONDITION interaction detected (all $P < 0.05$). *Post hoc* analysis revealed that corticospinal silent period decreased by 9% following anodal tDCS applied over the non-dominant M1 for the non-stimulated hemisphere which was significant to sham tDCS ($P = 0.049$); however, this magnitude of change was not different between the dominant and the non-dominant M1 ($P > 0.05$). Furthermore, there were no TIME ($P > 0.05$) or TIME × CONDITION ($P > 0.05$) interaction detected between genotypes following the intervention.

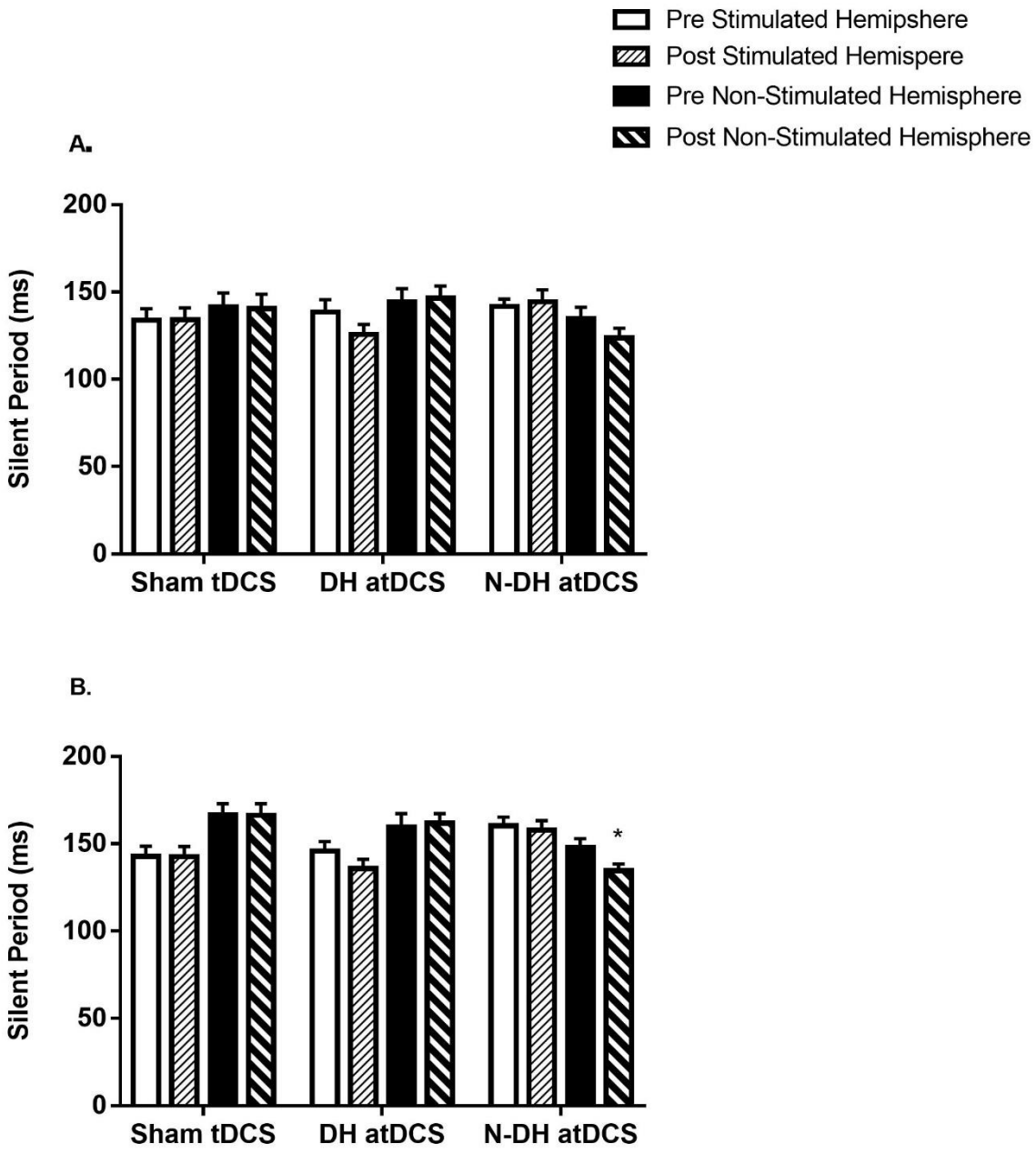

Figure 3.5A-B: Mean (± SE) changes in corticospinal silent period duration for the stimulated and non-stimulated hemispheres prior to and following sham, anodal tDCS over the dominant M1 (DH atDCS) and anodal tDCS over the non-dominant M1 (N-DH atDCS) at (A) 150% and (B) 170% AMT. * denotes significant to sham tDCS.

3.3.4 Laterality index

As shown in Table 3-2, laterality index scores were calculated for each condition for corticospinal excitability and inhibition.

There was no difference in LI detected for corticospinal excitability at 150% AMT for the sham or anodal tDCS conditions (dominant and non-dominant M1 stimulation; $P > 0.05$). There was a significant difference in LI for corticospinal excitability at 170% AMT following anodal tDCS of the non-dominant M1 towards the right (non-dominant) M1 ($P = 0.0047$). However, there was no difference in LI detected for the sham condition or following anodal tDCS of the dominant M1 ($P > 0.05$).

There was a significant difference in LI for corticospinal inhibition at 150% AMT following anodal tDCS of the dominant and non-dominant M1 towards the right (non-dominant) M1 ($P = 0.04$; $P = 0.036$, respectively). However, there was no difference in LI detected for the sham condition ($P > 0.05$). Similarly, there was a significant difference in LI for corticospinal inhibition at 170% AMT following anodal tDCS of the dominant and non-dominant M1 towards the right (non-dominant) M1 ($P = 0.02$; $P = 0.018$, respectively) and no difference in LI detected for the sham condition ($P > 0.05$).

Table 3-2: Mean (± SE) for laterality index prior to and following sham and anodal tDCS (dominant and non-dominant hemisphere stimulation arrangements).

	Sham tDCS		**DH atDCS**		**N-DH atDCS**	
	Pre	Post	Pre	Post	Pre	Post
MEP (150% AMT)	0.06 ± 0.10	0.06 ± 0.20	0.10 ± 0.05	0.10 ± 0.07	0.06 ± 0.07	0.06 ± 0.06
MEP (170% AMT)	0.06 ± 0.09	0.06 ± 0.10	0.09 ± 0.08	0.08 ± 0.08	0.07 ± 0.08	-0.08 ± 0.08*
SP (150% AMT)	-0.02 ± 0.04	-0.02 ± 0.03	-0.02 ± 0.03	-0.08 ± 0.03*	-0.03 ± 0.03	-0.07 ± 0.03*
SP (170% AMT)	-0.08 ± 0.04	-0.08 ± 0.02	-0.04 ± 0.03	-0.09 ± 0.03*	-0.04 ± 0.03	-0.08 ± 0.02*

DH atDCS: anodal tDCS over the dominant hemisphere; N-DH atDCS: anodal tDCS over the non-dominant hemisphere; MEP: motor evoked potential; SP: silent period; AMT: active motor threshold. * denotes significant to baseline.

3.4 Discussion

This study investigated the effect of a single session of anodal tDCS on corticospinal excitability and inhibition of both the stimulated and non-stimulated hemisphere following anodal tDCS. This chapter also examined corticospinal excitability/inhibition and the influence of these responses by the *BDNF* polymorphism. The main finding of this chapter was that a single session of anodal tDCS resulted in a bilateral increase in corticospinal excitability irrespective of which hemisphere (dominant vs non-dominant) was stimulated. In addition, a shift in lateralization of inhibition towards the right (non-dominant) M1 irrespective of which hemisphere was stimulated was observed. Contrary to the hypothesis, only a change in corticospinal inhibition in the non-stimulated hemisphere following anodal tDCS applied over the non-dominant M1 was observed. Although no significant genotype by time interaction were found, the within effects for increased corticospinal excitability in the *Val/Val* individuals following anodal tDCS is an important finding that warrants some discussion.

Only a limited number of studies have examined the bilateral effect of NIBS techniques on both the stimulated and non-stimulated hemisphere (Gilio et al. 2003, Schambra et al. 2003, Lang et al. 2004, Di Lazzaro et al. 2008, Shin & Sohn 2011). Interestingly, the finding of increased bilateral corticospinal excitability is not consistent with previous work from Lang et al. (2004) who only observed an increase in MEP amplitude in the stimulated hemisphere following anodal tDCS. The difference may lie in different methodology used as Lang et al. (2004) used a protocol of anodal tDCS for 10 min at 1 mA, which may have been insufficient to elicit changes in the contralateral hemisphere (non-stimulated). The findings of the present chapter are consistent with studies using other NIBS techniques such as rTMS and PAS which also showed bilateral

increases in corticospinal excitability (Gilio et al. 2003, Schambra et al. 2003, Shin & Sohn 2011). The present chapter, however, extends these findings by showing that the magnitude of corticospinal excitability of both the stimulated and non-stimulated hemisphere occurs irrespective of hemispheric dominance. This finding is noteworthy given the non-dominant hemisphere has previously been shown to have lower motor thresholds, higher MEPs (De Gennaro et al. 2004) and shorter corticospinal silent period durations (Priori et al. 1999), suggesting a greater allowance for the rapid induction of corticospinal plasticity of the non-dominant hemisphere following anodal tDCS. Although the current chapter findings suggest that there were no hemispheric differences per se, a preferential shift of inhibition towards the non-dominant M1 was shown. This supports the notion that the non-dominant M1 may be more responsive to anodal tDCS, manifesting as a reduction in the synaptic efficacy between intracortical inhibitory neurons and corticospinal neurons.

Interestingly, a reduction in corticospinal silent period duration in the non-stimulated hemisphere following the application of anodal tDCS over the non-dominant M1 was also observed. This finding is notable given that anodal tDCS applied over the non-dominant M1 had no effect on corticospinal silent period duration in the stimulated hemisphere (right), yet a reduction in corticospinal silent period duration in the non-stimulated hemisphere (left) was observed. Further, there was a similar increase in MEP amplitude between hemispheres (i.e., bilateral increases) which adds further confusion. Although MEP amplitude and corticospinal silent period duration are independent of each other, changes in corticospinal inhibition have been proposed to attenuate M1 output via GABA receptor mediated interneuron transmission (Kojima et al. 2013, McCormick 1989). At a minimum, the reduction in corticospinal silent period duration of the non-

stimulated left hemisphere, following anodal tDCS of the right stimulated hemisphere, shows that there was a reduction of inhibitory input to the motor neuron pool. Although, it was hypothesised that anodal tDCS of the stimulated hemisphere (irrespective of dominant/non-dominant) would reduce the corticospinal silent period duration the result that anodal tDCS of the right hemisphere had no effect on the corticospinal silent period, was surprising. Whilst the cortical projections to the biceps brachii are less divergent than other upper limb muscle groups (Palmer & Ashby 1992), this suggests that the potential to undergo plasticity following anodal tDCS may have been limited. However, a caveat to this interpretation, is confounded by the fact that there was only a reduction in corticospinal silent period duration for the non-stimulated hemisphere, following stimulation of the right M1. Despite this, given the bilateral increases in corticospinal excitability demonstrated, a single session of anodal tDCS appears to modulate mechanisms associated with LTP rather than neurons that use $GABA_B$ as their neurotransmitters.

The after-effects of tDCS are well established, with the consensus that the mechanisms underlying corticospinal plasticity are due to changes in synaptic strength due to modulation of the NMDA receptor (Nitsche et al. 2005, Boggio et al. 2006). Therefore, this chapter provides evidence that anodal tDCS not only improves synaptic efficacy of the stimulated hemisphere, but also modulates corticospinal connections of the non-stimulated hemisphere. One possible explanation is that anodal tDCS of the stimulated hemisphere leads to a reduction in IHI of the non-stimulated hemisphere and consequently increases corticospinal excitability of the non-stimulated hemisphere (Gilio et al. 2003). A caveat to this interpretation is that IHI was not measured and, thus, no definitive conclusion regarding potential underlying mechanisms can be made. However,

given that fMRI studies in humans have shown anodal tDCS to activate extended neural networks, via functional connectivity (Krishnamurthy et al. 2015) and reduced transcallosal inhibition using TMS (Lang et al. 2004), it is possible that anodal tDCS acts on both excitatory and inhibitory synaptic inputs which may shift the balance in excitability between hemispheres. Furthermore, a small but significant shift in lateralization of inhibition towards the right (non-dominant) M1, irrespective of which hemisphere was stimulated, was observed. Although it is unclear as to why this has occurred, and how this may affect motor performance, it may have relevance to rehabilitation following pathology to the right M1. Given that the underlying mechanisms causing this shift in lateralization are unclear, it can only be speculated as to the potential implications of this finding and further experiments would be required to resolve this point of discussion. Nonetheless, uni-hemisphere anodal tDCS applied to either the dominant or non-dominant hemisphere, results in a uniform increase in corticospinal excitability, supporting the notion of tDCS a priming method that may augment the corticospinal response to strength training and cross-education.

Although the number of participants that consented to provide a blood sample for *BDNF* analysis was small, the current chapter still shows that the *BDNF* polymorphism shaped the induction of corticospinal plasticity following a single session of anodal tDCS. Recent data have shown that carriers of the *BDNF Met* allele (*Val/Met*) display reduced corticospinal responses following repeated bouts of anodal tDCS compared to the *Val/Val* genotype (Frazer et al. 2016). Similarly, it was found that when individuals were sub-grouped into genotype, and individual data was examined, the *Val/Val* individuals showed a greater increase in MEP amplitude compared to *Val/Met* individuals. This trend was evident for both the dominant and non-dominant M1, irrespective of which

hemisphere was stimulated; however, this magnitude was not statistically significant due to the limited sample size of the *Val/Met* group. Importantly, these data provide preliminary insight into the important role that the *BDNF* polymorphism plays in the induction of experimentally-induced plasticity, and that this effect may be evident from as little as 20 min of anodal tDCS.

Overall, these findings from the chapter show that a single session of anodal tDCS induces bilateral effects in corticospinal excitability, irrespective of which hemisphere is stimulated (dominant vs non-dominant). Albeit, in a small sample size, the induction of corticospinal plasticity appears to be influenced by the *BDNF* polymorphism; however, this notion should be interpreted with caution given the small sample size, indicating the need for future investigation.

Chapter 4 : Anodal transcranial direct current stimulation of the motor cortex increases TMS voluntary activation and corticospinal plasticity

4.1 Introduction

The excitability of cortical neurons in the M1 can be readily modified by the application of weak transcranial direct currents, which leads to the induction of corticospinal plasticity. Specifically, tDCS of the M1 elicits changes in corticospinal excitability in a polarity-specific manner when measured by TMS (Nitsche & Paulus 2000, Nitsche & Paulus 2001, Bastani & Jaberzadeh 2012, Kidgell et al. 2013, Pellicciari et al. 2013). For a detailed review of tDCS please refer to Section 2.4. The temporary modification in corticospinal plasticity following anodal tDCS has been reported to correspond with transient improvements in motor performance (Boggio et al. 2006, Vines et al. 2006, Cogiamanian et al. 2007, Tanaka et al. 2009, Tanaka et al. 2011, Kidgell et al. 2013). For example, following a single session of tDCS (in the absence of motor training), improved motor performance in tasks such as the Jebsen-Taylor hand function test (JTT), maximal strength of the elbow flexors and knee extensors, the Purdue pegboard test, maximal pinch force, reaction time, and tests of motor sequencing tasks have all been reported (Boggio et al. 2006, Vines et al. 2006, Cogiamanian et al. 2007, Tanaka et al. 2009, Tanaka et al. 2011, Kidgell et al. 2013). In a similar context, in healthy adults, repeated sessions of tDCS have also been shown to improve motor performance (JTT and sequential visual isometric pinch task), with retention lasting up to 3 months following stimulation (Boggio et al. 2007, Reis et al. 2009). Despite this evidence, a recent systematic review and meta-analysis reported that the effects of a single session of anodal tDCS did not show any statistically significant difference for motor function in healthy participants or stroke patients (Bastani & Jaberzadeh 2012).

Following central nervous system injury, such as stroke, there is reduced neural drive to the affected muscle which produces reduced voluntary activation (Merton 1954).

Deficits in voluntary activation have traditionally been assessed with the interpolated twitch technique. Briefly, twitch interpolation involves the application of a single supramaximal electrical stimulus to the corresponding motor nerve during a MVIC. If the supramaximal electrical stimulus fails to produce additional force during the MVIC, it has been suggested that the muscle force produced voluntarily is maximal, and voluntary activation is complete (Herbert & Gandevia 1996, Allen 1998). On the other hand, if extra force is evoked during supramaximal stimulation, voluntary activation may be incomplete. Voluntary activation is determined by comparing the size of the evoked twitch force (superimposed twitch force) with the force that is produced by the same stimulus intensity at rest (resting twitch force).

Several studies have shown that incomplete voluntary activation (using twitch interpolation) manifests as a reduction in the 'voluntary' force generating capacity of the muscle due to reduced neural drive at or above the site of stimulation of the motor nerve (Herbert & Gandevia 1996, Allen 1998). A potential limitation of twitch interpolation is that it fails to define the site of neural drive impairment (Todd et al. 2003). Thus, more recently, TMS has been used to assess 'cortical' voluntary activation (Lee et al. 2008) or more appropriately VA_{TMS}. However, unlike twitch interpolation, the presence of a superimposed twitch force produced by a supra-threshold TMS pulse during an MVIC indicates a failure in neural drive at the level of the M1 (Todd et al. 2003). Of interest, although previous studies have shown that anodal tDCS applied over the leg motor cortex improves force production (Tanaka et al. 2009, Tanaka et al. 2011), no studies have examined the effects of repeated sessions of anodal tDCS on muscle strength and VA_{TMS}.

Furthermore, recovery from neuromuscular injury often requires induction of corticospinal plasticity within the M1 (Sanes & Donoghue 2000); however, in humans

there is a single nucleotide polymorphism of the *BDNF* gene (*BDNF Val66Met*) that results in reduced *BDNF* release in cortical neurons (Egan et al. 2003). As discussed in Section 2.4.5, the induction of corticospinal plasticity, assessed with TMS, is reduced in both experimentally induced (i.e., rTMS, tDCS) and use-dependent M1 plasticity (e.g., motor learning) in participants with the *BDNF* polymorphism (Antal et al. 2010, Hwang et al. 2015). Therefore, the aim of this study was to examine the effect of repeated sessions of anodal tDCS on the expression of muscle strength, VA_{TMS}, and indices of corticospinal plasticity. This study also examined corticospinal excitability/inhibition and the influence on these responses by the *BDNF* polymorphism. It was hypothesised that induction of experimentally induced corticospinal plasticity (increased cortical excitability and reduced cortical inhibition) would improve muscle strength and VA_{TMS}, but the magnitude of these responses would be influenced by the *BDNF* polymorphism.

4.2 Methods

For methods that are mainly replications of Chapters 3, the reader will be directed to the appropriate section for a complete description of the methods employed.

4.2.1 Participants

Fourteen participants (8 women, 6 men; aged 18–35 years) with an LQ score of 83 ± 5 (right-hand dominant) volunteered to participate. Refer to Section 3.2.1 for a comprehensive description of exclusion/inclusion criterias and safety screening procedures.

4.2.2 Experimental approach

Figure 4.1 outlines the organization of the study. After obtaining consent, participants completed a familiarization session one week before the study that involved performing five isometric contractions of the right wrist flexors and extensors and were exposed to single-pulse TMS to reduce any potential learning effect. In a double-blinded cross-over design, all participants were exposed to four days of anodal and sham tDCS. The order of the conditions was counterbalanced and randomized between participants, with a washout period of one week between each condition (Vines et al. 2008). Both tDCS conditions followed the identical testing protocol as shown in Figure 4.1. All participants underwent TMS and isometric strength testing (MVIC) of the right wrist flexors and extensors before and following the tDCS intervention. Participants were also required to attend four sessions on consecutive days where they were exposed to 20 min of anodal or sham tDCS applied at 2 mA. Post-testing was carried out 24 hours after the final tDCS session.

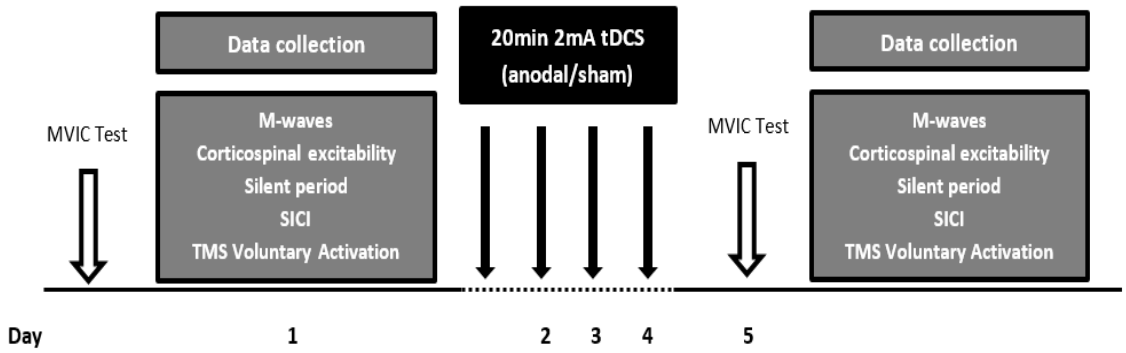

Figure 4.1: Schematic representation of the experimental design with measures obtained prior to and following 4 consecutive sessions of sham and anodal tDCS. Pre- and post-measures included assessment of peripheral muscle excitability (M-waves), corticospinal excitability and inhibition recruitment curves, VA_{TMS}, SICI, and MVIC strength test of the right wrist flexors and extensors.

4.2.3 Voluntary strength testing

MVIC of the right wrist flexors and extensors was determined on a custom-made force transducer (Futek Force Transducer LSB302, Melbourne). For the wrist flexor MVIC, participants were seated in a chair, shoulders relaxed with their elbows flexed at 110^{O}. With the hand supinated and the force transducer positioned over the middle aspect of the palmar surface of the hand, the participant was instructed to push up against the transducer as forcefully as possible for 3 sec. For wrist extensors MVIC, the forearm was pronated, and the participant was instructed to extend the dorsum of their hand as forcefully as possible against the force-transducer. Three trials were performed; each trial was 3 sec in duration, separated by 3 min rest to minimize fatigue. The greatest recorded output was recorded as the participant's MVIC for the wrist flexors and extensors.

4.2.4 Surface electromyography

sEMG activity was recorded from both the left and right flexor carpi radialis (FCR) and right extensor carpi radialis (ECR) muscles. As described by Selvanayagam et al. (2012), the electrodes for the FCR were positioned 9 cm from the medial epicondyle of the humerus with an inter-electrode distance (centre to centre) of 2 cm. The ECR electrodes were positioned at 45% of the distance from the medial epicondyle of the humerus to the radial styloid process with an interelectrode distance of 2 cm. A grounding strap was placed around the wrist as the common reference point for all electrodes. Refer to Section 3.2.4 for a more detailed description of the methods employed.

4.2.5 Transcranial magnetic stimulation and TMS voluntary activation (VA_{TMS})

As described in Chapter 3 (Section 3.2.5), TMS was delivered using Magstim 200^2 stimulator (Magstim Co, Dyfed, UK) connected by means of a Bistim unit and a single figure-of-eight coil (external diameter of each loop 70 mm). A modified stimulus response curve was constructed by stimulating at a range of intensities including 110, 130, 150, 170 190 and 210% of AMT.

All stimuli were delivered during a low level isometric contraction of the wrist flexors, which were performed on the custom-made force transducer and involved supinating the hand and maintaining 110^O of elbow flexion. Using a horizontal line on the computer screen as visual feedback, participants were instructed to hold 2% of the wrist flexors MVIC which equated to 0.97 ± 0.09% of the maximal *rms*EMG, which was obtained during MVIC testing. Consistent muscle activation was confirmed by recording pre-stimulus *rms*EMG for the 100 ms epoch before the delivery of each stimulus (see Table 4-1). To control for background sEMG before TMS stimulation, all MEPs obtained during isometric contractions post intervention were obtained at the pre-force level.

To quantify SICI, five single-pulse stimuli and five short-interval paired-pulse stimuli were delivered in a random order (Rantalainen et al. 2013). The stimulator output intensity was set at 120% AMT, which was determined during familiarization and adjusted if there was a change following tDCS. The conditioning stimulus for paired-pulse stimulation was set at 80% AMT, the inter-stimulus interval was 3 ms, and subsequent posterior to anterior current flow was used (Kujirai et al. 1993, Lackmy & Marchand-Pauvert 2010).

In accordance with Lee et al. (2009), VA_{TMS} was calculated using an average of 3 trials. Each trial consisted of 3 isometric wrist contractions (3 sec) with a 2 min rest between trials. Participants were instructed to match a required force (50, 75, and 100% of wrist flexors MVIC) using a horizontal line on the computer screen as visual feedback. TMS was delivered over the contralateral M1 to evoke superimposed twitches during voluntary contractions. The TMS stimulus intensity for each subject was determined by MEP_{MAX} which was identified from the stimulus response curve. This stimulus intensity corresponded to at least 50% of the M_{MAX} of the wrist flexors and a relatively small MEP (< 10% M_{MAX}) of the wrist extensors.

4.2.6 Maximum compound muscle action potential

Direct muscle responses were obtained from the right FCR and ECR muscles by supramaximal electrical stimulation (pulse width 200 μs) of the median and radial nerves, respectively, while holding 2% of the wrist flexors and extensors MVIC, which equated to 0.97 ± 0.09% of the maximal *rms*EMG (DS7A, Digitimer, UK). This low level of muscle activity was used to match the conditions under which TMS was delivered. The stimulation site that produced the largest M-wave was located by positioning the bipolar electrodes in the cubital fossa (median nerve) and on the lateral aspect of the arm above

the elbow (radial nerve). An increase in current strength was applied to the median and radial nerves until there was no further increase observed in the amplitude of the sEMG response (M_{MAX}). To ensure maximal responses, the current was increased an additional 20%, and the average M_{MAX} was obtained from five stimuli, with a period of 6–9 sec separating each stimulus. M_{MAX} was recorded at baseline and following the tDCS intervention to control for possible changes in peripheral muscle excitability that could influence MEP amplitude.

4.2.7 Transcranial direct current stimulation

The tDCS protocol is generally similar to Chapter 3 (Section 3.2.7). In this case, all participants received 20 min of tDCS for four consecutive days with the anode fixed over the optimal cortical representation of the right FCR muscle, as identified by TMS over the left cortex, and the cathode was placed over the right contralateral supra orbital area. Refer to Section 3.2.7 for a detailed description of the anodal/sham protocol used and the collection of VAS data.

4.2.8 *BDNF* analysis

As described in Chapter 3, blood samples were obtained and participants were genotyped for the *BDNF val66met* polymorphism (see Section 3.2.8). Figure 4.2 shows an example of a PCR gel analysis. Like Chapter 3, 13 participants consented to a blood sample, whilst one participant did not consent.

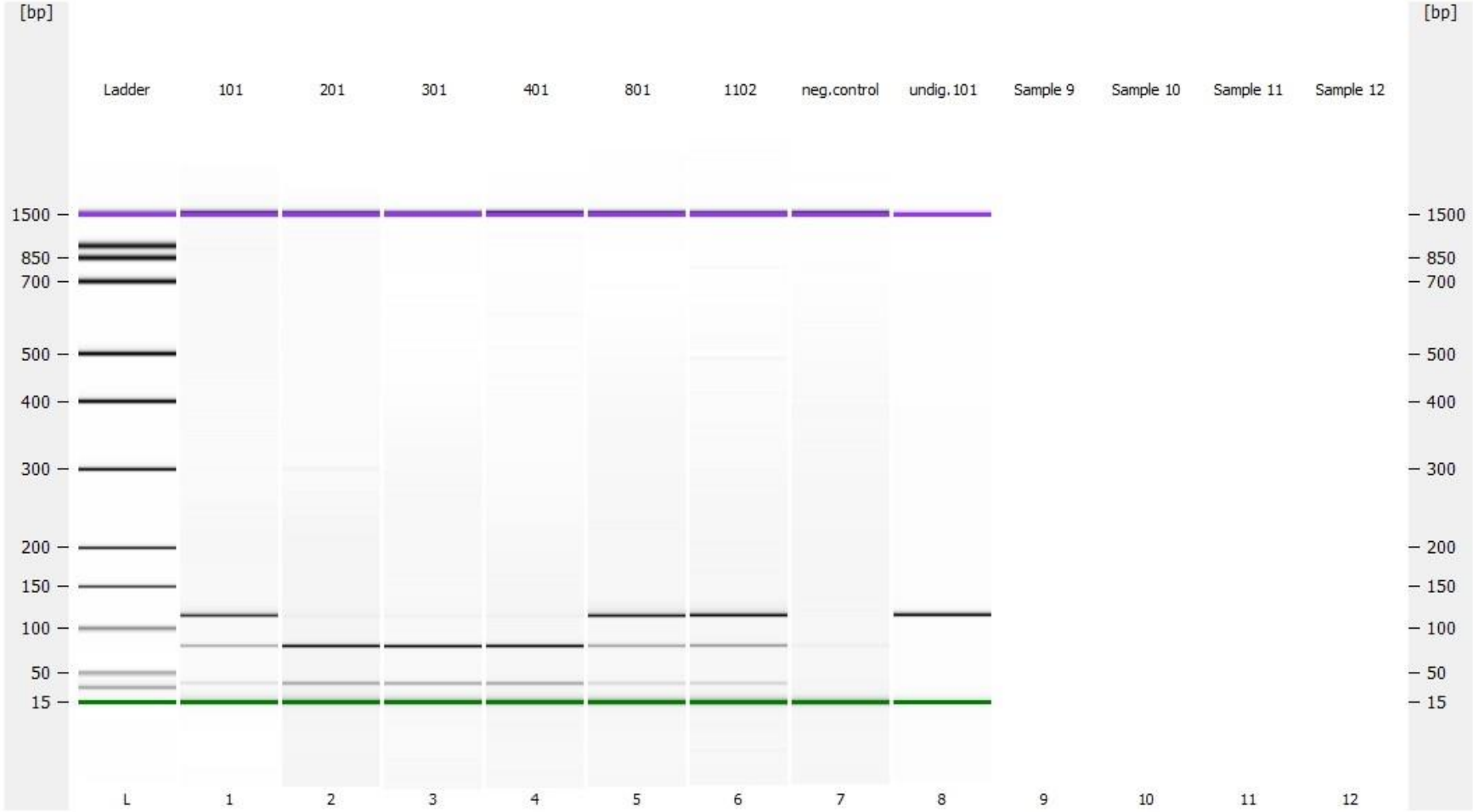

Figure 4.2: Example of PCR analysis for five unidentified subjects.
Subjects 1, 5 - 6 = *Val/Met* (Cut bands at 113, 78 and 35 bp);
Subjects 2 - 4 = *Val/Val* (Cut bands appear at 78 and 35 bp).

4.2.9 Data analysis

Similar to Chapter 3 (Section 3.2.9), pre-stimulus *rms*EMG activity was determined in the right wrist flexors 100 ms before each TMS stimulus during pre- and post-testing. Any trial in which pre-stimulus *rms*EMG exceeded 1 ± 0.5% of maximal *rms*EMG was discarded, and the trial was repeated.

The conditioned MEP amplitude was expressed as a percentage of the unconditioned test MEP amplitude to calculate the level of SICI. Corticospinal silent period durations were obtained from single-pulse stimuli delivered at 110–210% AMT (increments of 20%) during a light contraction (2% of the wrist flexors MVIC which equated to 0.97 ± 0.09% of the maximal *rms*EMG). All post-measures were obtained at the pre-force level as increases in background sEMG because of the tDCS intervention could confound MEP amplitudes. The duration between the onset of the MEP and the resolution of background sEMG was visually inspected and manually cursored, with the experimenter blinded to each condition. The average from eight stimuli was used for corticospinal silent period duration (Wilson et al. 1993).

To calculate VA_{TMS}, a linear regression of the amplitude of the superimposed twitch was plotted against voluntary force levels of 50, 75 and 100% of the wrist flexors MVIC for each participant to determine the level of VA_{TMS}. VA_{TMS} was calculated using a linear regression between the superimposed twitches and the voluntary maximal force, whereby the y-intercept was taken as the estimated resting twitch. VA_{TMS} was calculated using the following equation:

$$VA_{TMS}\ \% = (1\text{-superimposed twitch force/estimated resting twitch force}) \times 100$$

(Todd et al. 2003, Lee et al. 2008, Lee et al. 2009).

4.2.10 Statistical analysis

All data were screened with the Shapiro-Wilk test and found to be normally distributed (all $P > 0.05$) and, thus, the assumptions of the ANOVA were not violated. Subsequently, for the primary analysis, a split-plot in time, repeated measure ANOVA was used to compare the effects of anodal and sham tDCS conditions on multiple dependent variables (MVIC force, pre-stimulus EMG, corticospinal excitability, SICI, corticospinal silent period, and VA_{TMS}) over two time points (pre-testing and post-testing). For the secondary analysis, a 2-way ANOVA of genotype (*Val/Val*, *Val/Met*) and time (pre-testing and post-testing) was used to examine the effect of genotype on multiple dependent variables (MVIC force, corticospinal excitability, SICI, corticospinal silent period, and VA_{TMS}) following anodal tDCS. Bonferroni correction for multiple comparisons was applied for each dependent variable where significant multivariate effects were found. Prism 6 for Windows (Graphpad Software Inc, California) was used for all statistical analyses with the level of significance set as $P < 0.05$ for all testing. All data are presented as mean ± SE.

4.3 Results

The *BDNF* genotype analysis for the 13 participants for whom genetic data was available revealed that ten were homozygous for the *Val* allele (*Val66Val*), while three were genotyped as *Val66Met*.

4.3.1 Pre-stimulus *rms*EMG, maximal compound wave, and visual analogue scale

Pre-stimulus *rms*EMG did not vary between single- and paired-pulse trials, and there were no TIME ($P > 0.05$) or TIME × CONDITION ($P > 0.05$) interaction observed. Similarly, no TIME ($P > 0.05$) or TIME × CONDITION ($P > 0.05$) interaction were detected for M_{MAX} (Table 4-1). VAS data were pooled across four sessions for each condition, and there was no difference in participants' perception of discomfort between sham and anodal tDCS conditions (2.9 ± 0.3, 2.9 ± 0.2, respectively; $P = 0.93$).

Table 4-1: Mean **(**± SE) for M_{MAX}, single-pulse TMS pre-stimulus *rms*EMG at 130% AMT, paired-pulse TMS pre-stimulus at 80% and 120% AMT (CS, TS respectively) and SICI for the flexor carpi radialis prior to and following four consecutive sessions of sham and anodal tDCS.

	Sham tDCS		**Anodal tDCS**		
	Pre	Post	Pre	Post	***P* value**
M_{MAX} (mV)	7.21 ± 0.69	7.32 ± 0.61	7.63 ± 0.82	7.25 ± 0.78	> 0.9999
SP *rms*EMG (% *rms*EMG$_{MAX}$)	1.01 ± 0.40	1.12 ± 0.40	0.83 ± 0.16	0.90 ± 0.15	0.1191
PP *rms*EMG (% *rms*EMG$_{MAX}$)	0.86 ± 0.23	0.91 ± 0.18	0.63 ± 0.10	0.82 ± 0.12	0.6960
SICI	36.64 ± 4.93	38.06 ± 6.45	42.03 ± 6.06	38.06 ± 6.60	> 0.9999

M_{MAX}: maximum compound wave; SICI: short-interval intracortical inhibition; SP: single-pulse; PP: paired-pulse; TMS: transcranial magnetic stimulation.

4.3.2 Maximal voluntary isometric contraction

Isometric strength was assessed for the right wrist flexors and extensors before and after 4 consecutive sessions of sham and anodal tDCS. Figure 4.3 shows the mean change in isometric strength for the right wrist flexors. There were no differences in isometric strength at baseline for the wrist flexors and extensors between sham and anodal conditions ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P = 0.01$) and a TIME × CONDITION interaction ($P = 0.02$) for an increase in isometric wrist flexor strength. *Post hoc* analysis showed that anodal tDCS stimulation resulted in an 8% increase in isometric wrist flexor strength compared with 3% following sham tDCS. However, there was no difference in isometric wrist flexors strength between genotypes ($P > 0.05$). Furthermore, no TIME ($P > 0.05$) or TIME × CONDITION ($P > 0.05$) interaction were detected for isometric wrist extensor strength following the intervention.

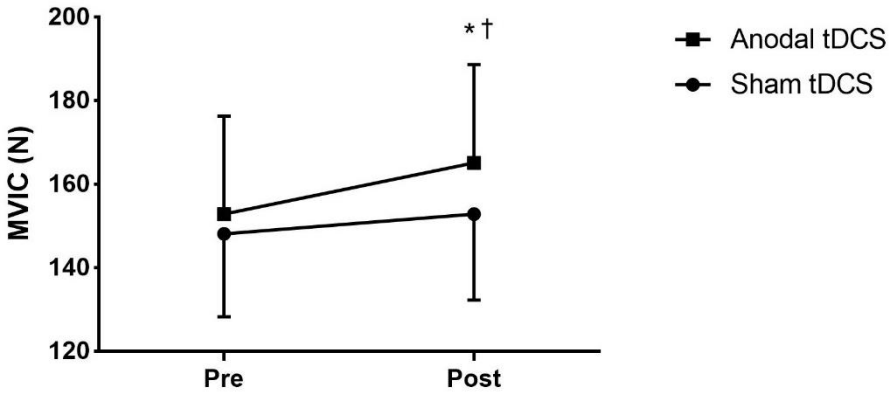

Figure 4.3: Mean (± SE) changes in MVIC strength of the right wrist flexors following four consecutive sessions of sham and anodal tDCS. * significant to sham tDCS; † significant to baseline. Anodal tDCS stimulation resulted in an 8% increase in isometric wrist flexor strength compared to 3% following sham tDCS.

4.3.3 Corticospinal excitability

Figure 4.4A-B shows the mean MEP amplitude normalized as a percentage of M_{MAX} for anodal and sham tDCS conditions at 110–210% of AMT (increments of 20%) of the wrist flexors. MEP amplitudes were similar between sham and anodal tDCS conditions at baseline across all stimulus intensities ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P < 0.05$) and a TIME × CONDITION interaction ($P < 0.05$) at all stimulus intensities (110– 210% AMT). *Post hoc* analysis showed that anodal tDCS stimulation resulted in a 32–67% increase in MEP amplitude across 110–210% of AMT compared with an average 1–9% change in the sham tDCS condition (Table 4-2).

Table 4-2: Mean **(**± SE) for MEP amplitudes expressed at percentage of M_{MAX} at 110-210% AMT (increments of 20%) for the flexor carpi radialis prior to and following four consecutive sessions of sham and anodal tDCS.

MEP Amplitude (%M_{MAX})	**Sham tDCS**		**Anodal tDCS**		
	Pre	Post	Pre	Post	***P* value**
110% AMT	6.33 ± 0.84	6.42 ± 0.85	6.60 ± 0.97	11.04 ± 1.66*†	0.0004
130% AMT	12.64 ± 1.69	13.60 ± 2.03	11.70 ± 1.75	19.29 ± 2.56*†	0.0104
150% AMT	17.03 ± 2.20	17.14 ± 2.06	15.54 ± 2.25	23.53 ± 3.45*†	0.0332
170% AMT	18.91 ± 2.45	19.87 ± 2.70	18.83 ± 2.47	28.41 ± 4.35*†	0.0040
190% AMT	21.52 ± 2.59	21.45 ± 1.77	21.67 ± 2.79	28.74 ± 4.68*†	0.0162
210% AMT	24.22 ± 3.46	21.92 ± 1.94	21.55 ± 2.65	29.66 ± 4.95*†	0.0323

* significant to sham tDCS condition ($P > 0.05$); † significant to baseline ($P > 0.05$).

Of interest, the GENOTYPE × TIME ANOVA revealed only a TIME effect for the *Val/Val* group at 110%, 130% and 150% AMT ($P < 0.05$; Figure 4.4C). At 110% AMT, MEP amplitude increased by 60% compared with a 48% increase in those with the *Val/Met* polymorphism. At 130% AMT, *Val/Val* individuals increased their mean MEP amplitude by 68% compared with a 26% increase in those with the *Val/Met* polymorphism. A similar effect was observed at 150% AMT, with *Val/Val* individuals increasing mean MEP amplitude by 55% compared with a 4% increase in those with the *Val/Met* polymorphism. However, *post hoc* analysis revealed that the magnitude of change in MEP amplitude was not statistically significant between genotypes ($P > 0.05$).

There were no differences in MEP amplitudes across all stimulus intensities (110–210% AMT; increments of 20%) of the wrist extensors between groups at baseline ($P > 0.05$). There were no main effects for TIME ($P > 0.05$) or CONDITION × TIME ($P > 0.05$) interaction detected following the intervention. Furthermore, there were no TIME ($P > 0.05$) or TIME × CONDITION ($P > 0.05$) interaction detected between genotypes following the intervention.

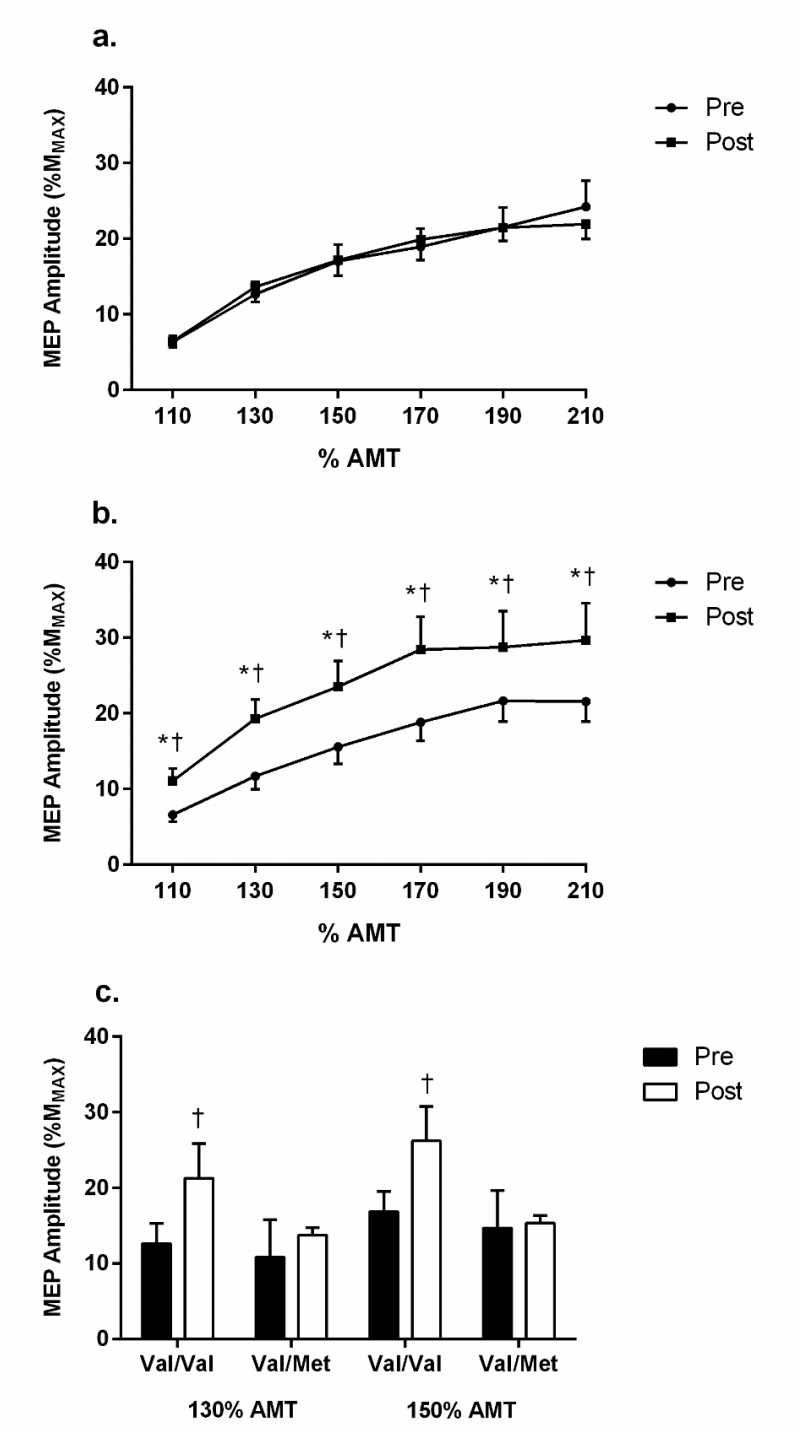

Figure 4.4A-C: Mean (± SE) changes in MEP amplitude following four consecutive sessions of **(A)** sham tDCS and **(B)** anodal tDCS. **(C)** changes in MEP amplitude before and after four consecutive sessions of anodal tDCS in healthy subjects with different *BDNF* genotypes. *significant to sham tDCS; † significant to baseline.

4.3.4 Corticospinal inhibition

As shown in Figure 4.5A-B, corticospinal inhibition was assessed with the duration of the corticospinal silent period obtained at several stimulus intensities above AMT (110–210% AMT; increments of 20%). Corticospinal silent period durations were similar between sham and anodal tDCS conditions at baseline across all stimulus intensities ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P < 0.05$) and a CONDITION × TIME interaction ($P < 0.05$) at 130–210% of AMT. *Post hoc* analysis showed that anodal tDCS resulted in a 8–12% decrease in corticospinal silent period duration compared with an average of 1% decrease in the sham tDCS condition. Of interest, the GENOTYPE × TIME ANOVA revealed only a TIME effect for the *Val/Val* group at 130% and 150% of AMT ($P < 0.05$; Figure 4.5C). At 130% of AMT, corticospinal silent period duration reduced by 14% following anodal tDCS in the *Val/Val* group compared with a 3% reduction in those with the *Val/Met* polymorphism. A similar effect was observed at 150% AMT, with *Val/Val* individuals reducing corticospinal silent period duration by 17% compared with a 6% reduction in those with the *Val/Met* polymorphism. However, *post hoc* analysis revealed that the magnitude of change in corticospinal silent period duration was not statistically significant between genotypes ($P > 0.05$).

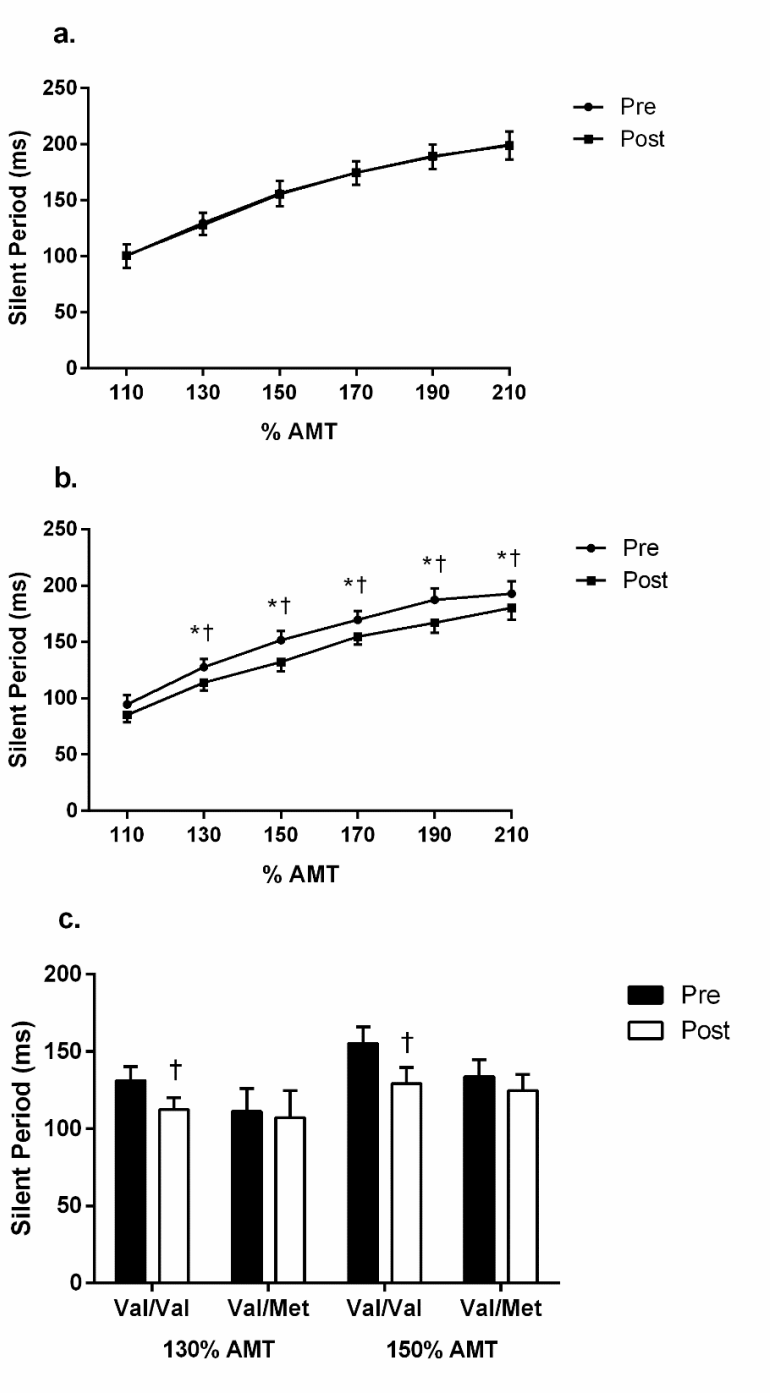

Figure 4.5A-C: Mean (± SE) changes in corticospinal silent period duration following four consecutive sessions of **(A)** sham tDCS and **(B)** anodal tDCS. **(C)** changes in corticospinal silent period duration before and after four consecutive sessions of anodal tDCS in healthy subjects with different *BDNF* genotypes. *significant to sham tDCS; †significant to baseline.

4.3.5 SICI

There were no differences in SICI between groups at baseline ($P > 0.05$). There were no main effects for TIME ($P = 0.55$) or CONDITION × TIME ($P = 0.78$) interaction detected following the intervention (Table 4-1). Furthermore, there were no TIME ($P > 0.05$) or TIME × CONDITION ($P > 0.05$) interaction detected between genotypes following the intervention.

4.3.6 TMS voluntary activation (VA_{TMS})

Figure 4.6 shows the three levels of wrist flexor force which a subject produced in a typical trial. TMS was delivered over the left M1 during the plateau of each contraction to evoke a superimposed twitch as shown in Figure 4.6B. As expected, the amplitude of the evoked twitches was greatest during the 50% MVIC and smallest during 100% MVIC. Figure 4.7 shows the change in VA_{TMS} following four consecutive sessions of sham and anodal tDCS. VA_{TMS} levels were similar between sham and anodal tDCS conditions at baseline ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P = 0.0015$) and a CONDITION × TIME interaction ($P = 0.0003$). *Post hoc* analysis showed that following four sessions of anodal tDCS, VA_{TMS} increased from 88.14 ± 1.60% to 91.33 ± 1.24% compared with sham tDCS (88.54 ± 1.57% to 87.48 ± 1.85%). There were no TIME ($P > 0.05$) or TIME × CONDITION ($P > 0.05$) interaction detected between genotypes following the intervention. Table 4-3 demonstrates the r^2 values for the estimated resting twitch for both conditions (sham and anodal) and time points (pre-and post) for each subject.

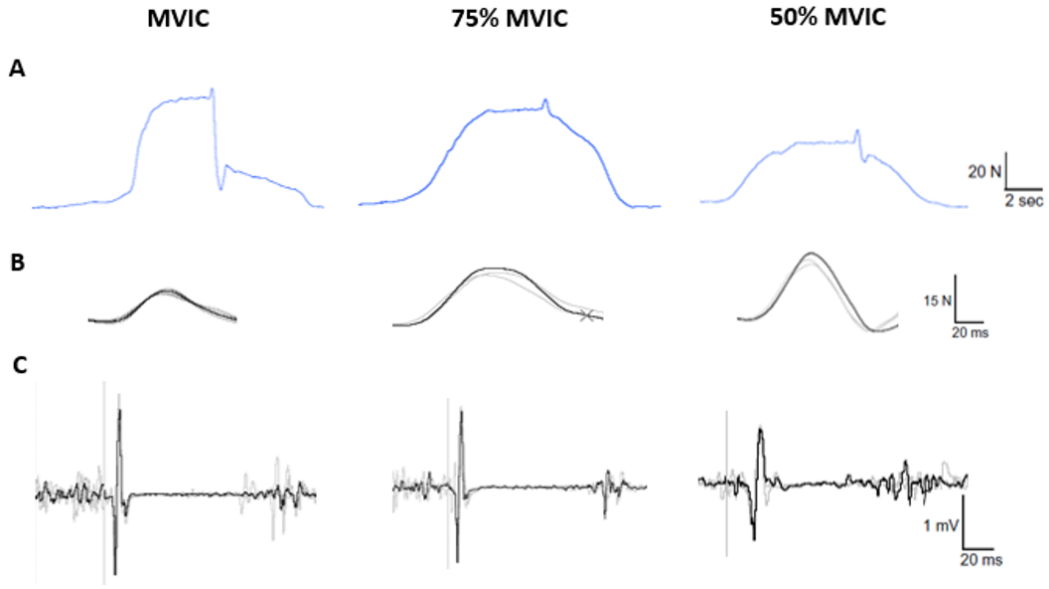

Figure 4.6A-C: (A) Raw force traces for three levels of wrist flexor voluntary contraction force taken from a representative subject in a typical testing trial. TMS was delivered over the contralateral motor cortex during 100%, 75% and 50% MVIC. **(B)** Raw traces of the superimposed twitches produced by cortical stimulation during 100%, 75% and 50% MVIC. **(C)** Raw EMG responses (MEPs) produced by cortical stimulation during 100%, 75% and 50% MVIC.

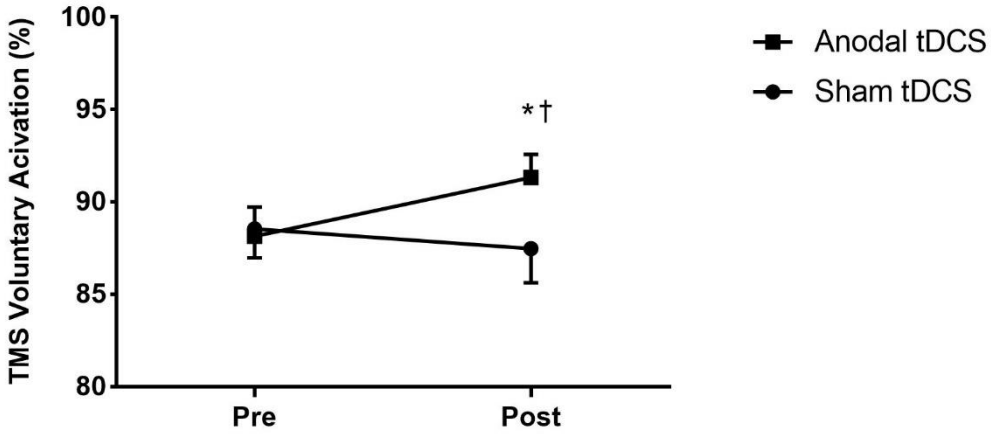

Figure 4.7: Mean (± SE) changes in VA_{TMS} following four consecutive sessions of sham and anodal tDCS. * significant to sham tDCS; † significant to baseline.

Table 4-3: r^2 values for the estimated resting twitch for both conditions (sham and anodal) and time points (pre-and post) for each subject.

	Sham tDCS		Anodal tDCS	
Subject Number	**Pre**	**Post**	**Pre**	**Post**
1	0.8337	0.8926	0.8337	0.9547
2	0.881	0.9175	0.9718	0.9685
3	0.9523	0.9785	0.9831	0.8443
4	0.9494	0.8984	0.8782	0.9793
5	0.9492	0.9222	0.9492	0.9377
6	0.9497	0.9228	0.8786	0.9286
7	0.8702	0.9167	0.9167	0.9488
8	0.7977	0.9227	0.9490	0.9491
9	0.9234	0.9569	0.9785	0.9244
10	0.8546	0.9794	0.9619	0.9230
11	0.9774	0.8046	0.9098	0.8804
12	0.8918	0.8950	0.946	0.9561
13	0.9883	0.9866	0.9385	0.9719
14	0.8649	0.9312	0.8335	0.9196
AVERAGE	**0.9060**	**0.9232**	**0.9235**	**0.9347**

4.4 Discussion

This study investigated the cumulative effect of four consecutive bouts of anodal tDCS on muscle strength and indices of corticospinal plasticity, VA_{TMS} and the influence of the *BDNF* polymorphism on these responses. The main finding from this chapter was that repeated sessions of anodal tDCS increased VA_{TMS} and isometric wrist flexor strength compared with repeated sessions of sham tDCS in the absence of motor training. Corticospinal excitability increased and corticospinal silent period duration decreased following anodal tDCS, demonstrating the induction of corticospinal plasticity. Although no significant genotype by time interaction were found, the within main time effects for increased corticospinal excitability and decreased corticospinal silent period duration in the *Val/Val* individuals following anodal tDCS, are important new findings that warrant some discussion, albeit in a small sample size.

4.4.1 Repeated sessions of anodal tDCS increased isometric strength and TMS voluntary activation (VA_{TMS})

Only a limited number of studies have examined the cumulative effect of anodal tDCS on motor performance (Boggio et al. 2007, Reis et al. 2009). The increase in isometric wrist muscle strength is similar to other studies that have reported an improvement in fine motor control of the hand following repeated sessions of tDCS (anodal and cathodal) (Boggio et al. 2007, Reis et al. 2009); in this case an increase in VA_{TMS} was reported, which is a new finding. Although the mechanisms of force gradation are well-described (Duchateau et al. 2006), it has not been established whether improved force production following tDCS is associated with increased VA_{TMS} or corticospinal plasticity. To this end, as VA_{TMS} is a measure of the level of neural drive to a muscle and reflects motor cortical drive, the finding of increased VA_{TMS} following anodal tDCS

illustrates that NIBS increases the net motor output (i.e., neural drive) from the M1 to the wrist flexors only. Therefore, accumulated bouts of anodal tDCS improve voluntary drive at the level of the M1, which presents as an increase in wrist flexor muscle strength. The improvement in VA_{TMS} is likely a result of tDCS modulating synaptic efficacy, which has improved the net descending drive (i.e., increased motor cortical drive) to the motor neuron pool.

It is unclear why anodal tDCS had no effect on wrist extensor strength or corticospinal plasticity. Although it is well established that the M1 can undergo both rapid, reversible, and long-term plastic changes, and that shifts in body representations provide an insight into how various body parts can reorganize relative to one another (Giraux et al. 2001), such plastic changes do not inform us whether all muscles in a given body part reorganize in a similar manner and to the same extent. Based on these findings, tDCS over the wrist flexor region had no effect on muscle strength or indices of plasticity of wrist extensor muscles. These findings show that the wrist flexors differ in their potential to undergo plasticity following anodal tDCS compared with the wrist extensors, despite how anatomically close these muscles are on M1 (Palmer & Ashby 1992, de Noordhout et al. 1999).

4.4.2 Corticospinal excitability and inhibition following accumulated bouts of anodal tDCS in different *BDNF* genotypes

The finding that corticospinal excitability increased following multiple sessions of anodal tDCS is consistent with the results from a previous study which also reported increased MEP amplitudes following five consecutive sessions of anodal tDCS (Alonzo et al. 2012). However, the present chapter extends these findings by demonstrating that anodal tDCS produces general enhancement of corticospinal excitability by changing the

gain in the stimulus-response curve. The increase in MEP amplitude of the target muscle following anodal tDCS reflects elements of corticospinal plasticity by means of mechanisms associated with LTP (Liebetanz et al. 2002, Nitsche et al. 2004b). The mechanisms mediating the after-effects of tDCS are well described; the consensus is that the after-effects are associated with a change in synaptic strength due to modulation of the NMDA receptor (Nitsche et al. 2005, Boggio et al. 2006). Involvement of the NMDA receptor is highlighted by pharmacological studies in which the after-effects of anodal tDCS are suppressed following the use of the NMDA receptor antagonist, dextromethorphan (Liebetanz et al. 2002). The increased MEP amplitude evoked by TMS in this experiment provides evidence that cumulative bouts of anodal tDCS have specifically modulated corticospinal connections (i.e., improved synaptic efficacy) that potentially favour production of force and are likely reinforced as a result of mechanisms associated with LTP.

The role of the *BDNF* polymorphism in modulating corticospinal plasticity in humans is less established compared with animal models, however, the findings of this chapter are consistent with previous studies that have shown that corticospinal plasticity is differentially modulated following experimentally induced plasticity (Cirillo et al. 2012). For example, it has been reported that there is an 18% to 30% reduction in activity-dependent secretion of *BDNF* in *Val/Met* allele carriers (Egan et al. 2003). In this Study, the *BDNF* polymorphism shaped the induction of corticospinal plasticity following repeated sessions of anodal tDCS. The significant increase in MEP amplitude observed following anodal tDCS was a result of *Val/Val* and *Val/Met* individuals being pooled. However, when the pooled individuals were sub-grouped into genotype and the individual data examined, the *Val/Val* individuals showed a greater increase in MEP amplitude

compared with *Val/Met* individuals. However, due to the small sample size of the *Val/Met* group, this difference was not statistically significant. Although there were no genotype interactions, the within-time effects warrant some discussion. For example, at 150% AMT, the mean MEP amplitude in *Val/Val* individuals increased by 55% compared with a 4% increase in those with the *Val/Met* polymorphism. It is likely that the significant increase in corticospinal excitability observed is a product of the larger representative sample of *Val/Val* individuals. However, in contrast to these experimental findings, it has been found that carriers of the *BDNF Met* allele (*Val/Met*) display enhanced corticospinal responses to both anodal and cathodal tDCS protocols compared with the *Val/Val* genotype (Antal et al. 2010, Puri et al. 2015). The differences may lie in the experimental methodology as a single session of anodal tDCS may only be sufficient to modify the transmembrane neuronal potential (Antal et al. 2010, Puri et al. 2015).

TMS and neuroimaging studies have reported a profound effect of the *BDNF* polymorphism on cortical morphology (Pezawas et al. 2004) and synaptic activity (LTP, efficacy of neural transmission, brain activation volumes) (Garry et al. 2004, Kleim et al. 2006, McHughen et al. 2010) underlying plasticity. Based on this, the time effect found for inducing corticospinal plasticity in the *Val/Val* participants alone following anodal tDCS supports the important role that the *BDNF* polymorphism plays in shaping experimentally induced corticospinal plasticity.

Understanding the effects of anodal tDCS on intracortical inhibition is important as modulation of SICI is crucial for motor performance. Of interest, no changes in SICI following four consecutive sessions of anodal tDCS were observed. Although this was an unexpected finding, it suggests that accumulated bouts of anodal tDCS appear to modulate $GABA_B$ rather than $GABA_A$ neurons; however, the reason is unclear. However,

a reduction in corticospinal silent period duration was reported. Because the corticospinal silent period that follows the excitatory MEP is caused by activation of long-lasting $GABA_B$ mediated inhibition and reflects temporary suppression in motor cortical output (Werhahn et al. 2007), it appears that cumulative bouts of anodal tDCS specifically target neural circuits that use $GABA_B$ as their neurotransmitter, resulting in the release of pyramidal tract neurons from inhibition. Therefore, a reduction in the temporary suppression of motor cortical output may be a putative neural mechanism underlying the changes in VA_{TMS}.

Of interest, the *BDNF* polymorphism did not differentially affect muscle strength or VA_{TMS}. Instead, the *BDNF* polymorphism appears to influence indices of corticospinal plasticity. Given the small sample size and lack of representation from *Met/Met* individuals, this is speculative. Indeed, a larger sample size of each *BDNF* genotype would allow for correlational analyses of changes in neurophysiological parameters and genotype, which could further strengthen the potential influence of the *BDNF* polymorphism on motor function and plasticity. However, these preliminary data highlight the importance of investigating the role of the *BDNF* polymorphism in the induction of corticospinal plasticity and whether this may manifest as a difference in motor performance. Another limitation to this chapter is that measures at a segmental level, particularly cervicomedullary MEPs and Hoffman Reflex (H-reflex), were not recorded. These would have provided additional information as to the site of adaptation within the corticospinal tract following stimulation.

Collectively, the findings of this chapter showed that repeated session of anodal tDCS induced corticospinal plasticity and increased VA_{TMS} which manifests as an improvement in isometric muscle strength. The induction of corticospinal plasticity

appears to be influenced by the *BDNF* polymorphism; however, these data should be interpreted with caution given the limited sample size, and further investigation is warranted. At a minimum, the clinical implications of these findings is that accumulated bouts of anodal tDCS could be used in participants who have deficiencies in muscle strength, as the *BDNF* polymorphism only appears to affect the induction of plasticity and not strength development.

Chapter 5：Augmenting the corticospinal responses to strength training through homeostatic plasticity

5.1 Introduction

The underlying neural adaptations that accompany the acquisition of new motor skills have been studied extensively using a range of neurophysiological techniques (Pascual-Leone et al. 1995, Perez et al. 2007, Dayan & Cohen 2011). TMS and functional magnetic resonance imaging (fMRI) imaging have established the modifiable nature of the M1 and its extensive involvement during the early phase of skill acquisition (Selvanayagam et al. 2011). This is highlighted by an observed increase in corticospinal excitability following a single session of ballistic and visuo-motor tracking tasks and isometric contractions of the FDI at low force levels (Muellbacher et al. 2001, Ziemann & Hallett 2001, Zoghi et al. 2003, Perez et al. 2004, Rogasch et al. 2009, Hinder et al. 2010, Lee et al. 2010, Pearce & Kidgell 2010, Smyth et al. 2010, Cirillo et al. 2011, Schmidt et al. 2011, Kouchtir-Devanne et al. 2012). Collectively, these studies highlight the underlying neural substrates that contribute to the early retention and performance of motor skills (Lee et al. 2010). Interestingly, it has been proposed that tasks involving the generation of force (i.e., strength training) may also share similar underlying corticospinal responses (Carroll et al. 2001b). Indeed, given the inherent requirements of a strength training task (i.e., muscle recruitment, timing of muscle activation between agonists and antagonists, joint positioning) this would indicate that a level of skill and learning is necessary for the successful completion of the movement under load (Carroll et al. 2001b).

In regards to the strength training literature, there are mixed findings from studies that have investigated the potential underlying corticospinal responses following a single session of strength training (Hortobagyi et al. 2011, Selvanayagam et al. 2011, Leung et al. 2015, Nuzzo et al. 2016). For example, Selvanayagam et al. (2011) showed an increase

in corticospinal excitability and a shift in the TMS-induced twitch force vector toward the training direction. This finding was further supported by Leung et al. (2015) who demonstrated an increase in cortiospinal excitability and a decrease in SICI following an acute bout of externally-paced strength training. Interestingly, the investigators also showed that the changes observed in the externally-paced strength training group were similar to those who completed skill training (visuo-motor tracking), suggesting that the corticospinal responses to skill and strength training may be similar (Leung et al. 2015). However, in direct contrast, Hortobagyi et al. (2011) showed no changes in corticospinal excitability or SICI. To address these conflicting results, Nuzzo et al. (2016) used TMS and cervicomedullary motor–evoked potentials (CMEPs) to examine changes in synatpic efficacy and motor neuron output following a single bout of ballistic strength training involving high force and high rate of force development contractions of the elbow flexor muscles. The results showed an increase in MEPs and CMEPs 15 min post training and increased CMEP and MEP twitch forces (Nuzzo et al. 2016). Given the combination of the robust techniques used in this study, it would appear that changes in synaptic efficacy within neural pathways that control specific muscles are likely to influence muscle activation and consequently improve force production (Carroll et al. 2001b).

As discussed in Chapters 3 and 4, tDCS utilizes weak direct currents that induce prolonged modulation of corticospinal excitability within the M1. Anodal tDCS has been shown to increase corticospinal excitability for up 90 min, whilst cathodal tDCS decreases corticospinal excitability (Nitsche & Paulus 2000, Nitsche & Paulus 2001). Primarily, tDCS has been used as a NIBS technique to modulate corticospinal excitability/inhibition and modify motor behaviour (Ridding & Ziemann 2010). However, more recently, the NIBS technique of tDCS has evolved into a popular

paradigm of motor priming, which is believed to facilitate motor learning (Stoykov & Madhavan 2015). The greater part of the literature has examined the application of tDCS before or during motor training, with the working hypothesis that enhanced neural activity within the M1 will facilitate the mechanisms associated with LTP or LTD (Ziemann & Siebner 2008). Two mechanisms have been proposed to underlie the response of corticospinal output neurons to priming protocols including *gating* and *homeostatic plasticity* (Siebner 2010). The theory of *gating* occurs instantaneously and describes the influx of calcium ions to the targeted corticospinal neurons resulting in the disinhibition of intracortical inhibitory circuits (Ziemann & Siebner 2008, Siebner 2010). *Gating* is attained concurrently with motor training and has been shown to facilitate motor performance (Nitsche et al. 2003d, Boggio et al. 2006, Galea & Celnik 2009, Hunter et al. 2009, Reis et al. 2009, Stagg et al. 2011, Hendy & Kidgell 2014). For example, Christova et al. (2015) showed a significant reduction in SICI following the application of anodal tDCS during grooved pegboard training. More relevant to the aim of this chapter, is the principle of *homeostatic plasticity* whereby the resting state of corticospinal neurons is altered (increased/decreased level of excitability following a low/high level of synaptic activity) due to changes in postsynaptic glutamate receptor activity (Ziemann & Siebner 2008, Siebner 2010). Given that anodal tDCS has been shown to modulate NMDA receptors, and subsequently produce a shift in the resting membrane potential (Nitsche & Paulus 2000), it would be conceivable that anodal tDCS is a promising priming tool to increase synaptic activity prior to a single bout of strength training to further augment the acute corticospinal responses to strength training (Leung et al. 2015). Critically, changes in corticospinal excitability and inhibition as a result of homeostatic plasticity may be an important component for the expression of muscle

strength; however, no studies have yet examined this. Nevertheless, Frazer et al. (2016) demonstrated that accumulative bouts of anodal tDCS increased VA_{TMS} which manifested as an improvement in voluntary strength. This finding suggests that the corticospinal responses underlying the early development of strength (Selvanayagam et al. 2011) and the expression of strength are likely to involve the modulation of synaptic efficacy, resulting in the increase in motor cortical drive to the intended motor neuron pool. Although speculative, the manipulation of homeostatic plasticity could indeed result in greater changes in synaptic efficacy (i.e., increased neural drive) which could ultimately augment the corticospinal responses to a single bout of strength training.

As previously discussed (Section 2.4.5), it has been recognised that individual responses to anodal tDCS and motor skill training, in both young and older adults, may differ according to a variation in the *BDNF* gene *(val66met)* (Kleim et al. 2006, Cirillo et al. 2012, Puri et al. 2015, Frazer et al. 2016). However, no one has yet investigated the potential influence of the *BDNF* polymorphism on the corticospinal responses to an acute bout of strength training. The notion that motor learning and strength training may share similar corticospinal responses (Leung et al. 2015), gives rise to the idea that the *BDNF* polymorphism may also influence the corticospinal responses to strength training. Considering the *BDNF* polymorphism has been shown to shape an individual's responsiveness to both experimentally-induced (i.e., tDCS) and use-dependent (i.e., motor skill training) plasticity protocols (Kleim et al. 2006, Antal et al. 2010, Cirillo et al. 2012, Puri et al. 2015, Frazer et al. 2016), it would be critical to identify whether this genetic factor may also influence the effectiveness of using a priming protocol prior to motor training to augment the corticospinal responses to a single bout of strength training.

Therefore, the aim of this study was to examine the effect of priming the M1 using anodal tDCS prior to a single bout of strength training on corticospinal excitability and inhibition. Furthermore, it was also investigated whether any of these outcome measures were influenced by the *BDNF* polymorphism. It was hypothesised that the application of anodal tDCS prior to a single bout of strength training would facilitate the induction of corticospinal plasticity (increased corticospinal excitability and reduced corticospinal inhibition), and that the magnitude of these responses would be influenced by the *BDNF* polymorphism.

5.2 Methods

For methods that are mainly replications of Chapters 3 and 4, the reader will be directed to the appropriate section for a complete description of the methods employed in this chapter.

5.2.1 Participants

Fifteen participants (7 women, 8 men aged 18-35 years) with an LQ score of 87 ± 3 (right-hand dominant) volunteered to participate. Refer to Section 3.2.1 for a comprehensive description of exclusion/inclusion criterias and safety screening procedures.

5.2.2 Experimental approach

All participants completed two experiments as outlined in Figure 5.1A-B. After obtaining consent, participants completed a familiarization session one week prior to the study that involved performing a one-repetition maximum (1RM) strength test of the right bicep brachii (to establish training load) and were exposed to single-pulse TMS. In a double-blinded cross-over design, all participants were exposed to two conditions in Experiment 1. Each participant was exposed to 20 min of anodal and sham tDCS followed by a single strength training session of the right biceps brachii (anodal tDCS + ST and sham tDCS + ST, respectively). The order of the conditions was counterbalanced and randomized between participants, with a wash-out period of one week between each condition (Vines et al. 2008). All participants underwent TMS and isometric strength testing (MVIC) of the right biceps brachii prior to and following the tDCS and strength training intervention. Participants were required to attend two separate sessions where they were exposed to 20 min of anodal or sham tDCS applied at 2 mA with a current density of 0.08 mA/cm^2 and completed a strength training session (see Figure 5.1A).

To determine the effects of anodal tDCS without strength training on indices of corticospinal plasticity (corticospinal excitability and inhibition), participants also completed Experiment 2. Each participant was exposed to 20 min of anodal and sham tDCS applied at 2 mA with a current density of 0.08 mA/cm^2 with a wash-out period of one week between each condition (Vines et al. 2008). Prior to and following the tDCS intervention, 20 single-pulse TMS stimuli were collected at 150% and 170% AMT (see Figure 5.1B).

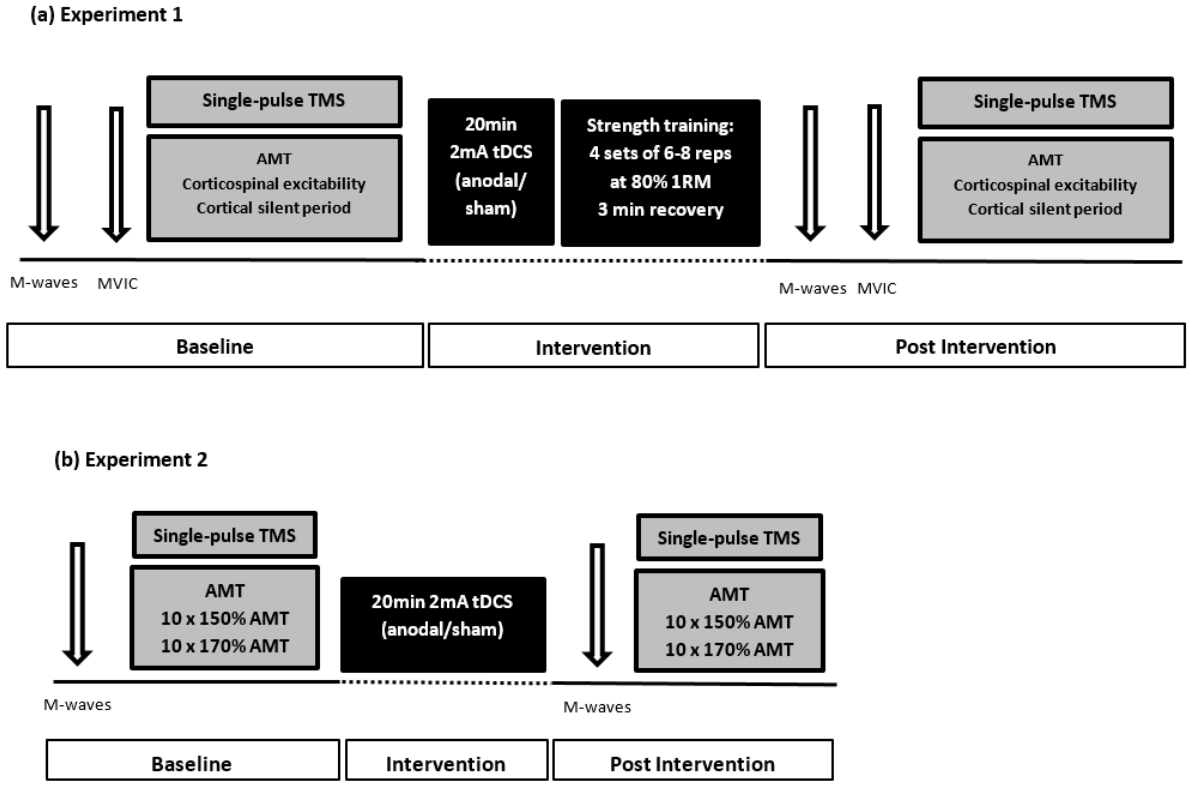

Figure 5.1A-B: (A) Schematic representation of the design of Experiment 1 with measures obtained prior to and following 20 min anodal and sham tDCS and strength training. Pre- and post-measures included assessment of peripheral muscle excitability (M_{MAX}), corticospinal excitability and inhibition recruitment curves and MVIC strength test of the right biceps brachii muscle. **(B)** Schematic representation of the design of

Experiment 2 with measures obtained prior to and following 20 min anodal and sham tDCS. Pre- and post- measures included assessment of peripheral muscle excitability (M_{MAX}), corticospinal excitability and inhibition at 150% and 170% AMT.

5.2.3 Voluntary strength testing

As described in Chapter 3 (Section 3.2.3), MVIC was determined of the right biceps brachii muscle only. To determine maximal voluntary dynamic force, participants completed a 1RM test of the right biceps brachii muscle. As described by Munn et al. (2005a) participants stood against a wall with the dumbbell held in their right hand and their left arm placed behind their back to prevent excessive body movement. The starting position involved the participant holding the weight in their right hand with their elbow in full extension and forearm supinated. The participant was then instructed to flex their arm and lift the dumbbell. If the lift was successful, the weight was increased until the participant could no longer perform one repetition. Between each trial, 3 min rest was given to minimise muscular fatigue. The last successful trial was recorded as their 1RM strength and was used to determine individual training load (Kidgell et al. 2010a).

5.2.4 Strength training protocol

Participants completed a supervised strength training session following the anodal and sham tDCS intervention (Experiment 1). Using the same set-up as the 1RM, participants completed flexion-extension movements of the elbow with the forearm supinated (biceps curl) of the right arm. Participants completed 4 sets of 6-8 repetitions at 80% 1RM with 3 min recovery between sets (Munn et al. 2005a, Kidgell et al. 2010a). A repetition timing of 3 sec concentric and 4 sec for the eccentric phase was maintained using an electronic metronome (Kidgell et al. 2010a). The use of an automated timing device was selected as previous research has shown that controlled velocity strength

training facilitates greater neural adaptations compared to self-paced velocity (Kidgell et al. 2010a, Leung et al. 2015).

5.2.5 Surface electromyography

As described in Chapter 3, sEMG activity was recorded from the right biceps brachii muscle (see Section 3.2.4).

5.2.6 Transcranial magnetic stimulation

As described in Chapter 3 (Section 3.2.5), TMS was delivered using a Magstim 200^2 stimulator (Magstim Co, Dyfed, UK) and a single figure-of-eight coil (external diameter of each loop 70 mm) over the motor hotspot for the right biceps brachii muscle.

All stimuli were delivered during a low-level isometric contraction of the right biceps brachii muscle. Participants were required to maintain an elbow joint angle of 90^{o} elbow flexion. Joint angle was measured with an electromagnetic goniometer (ADInstruments, Bella Vista, Australia), with visual feedback provided on a screen visible to both the participant and the researcher (Hendy et al. 2015). This joint position equated to 4 ± 1% of maximal *rms*EMG, with consistent muscle activation confirmed by recording pre-stimulus *rms*EMG for the 100-ms epoch before the delivery of each stimulus (Table 5-1).

5.2.7 Maximum compound muscle action potential

As described in Section 3.2.6, direct muscle responses were obtained from the right biceps brachii muscle by supramaximal electrical stimulation of the brachial plexus at Erbs point.

5.2.8 Transcranial direct current stimulation

The tDCS protocol used in this chapter is identical to Chapter 3 (Section 3.2.7). All participants received anodal tDCS involving 20 min at 2 mA stimulation intensity with the anode fixed over the optimal cortical representation of the right biceps brachii muscle, as identified by TMS over the left cortex, and the cathode was placed over the right contralateral supra orbital area. Refer to Sections 3.2.7 and 4.2.7 for a detailed description of the anodal/sham protocol used and the collection of VAS data.

5.2.9 *BDNF* genotyping

As described in Chapter 3, blood samples were obtained and participants were genotyped for the *BDNF val66met* polymorphism (see Section 3.2.8). Of the 15 participants recruited, only ten consented to providing a blood sample for genetic analysis.

5.2.10 Data analysis

Experimental data for corticospinal excitability, inhibition and *rms*EMG was analysed per the procedures described in Section 3.2.9. However, the total area under the curve (AUC) was calculated for Experiment 1 via the method of trapezoidal integration using the actual data collected during the construction of corticospinal excitability and inhibition recruitment curves (Talelli et al. 2008, Carson et al. 2013).

5.2.11 Statistical analysis

All data were screened with the Shapiro-Wilk test and found to be normally distributed (all $P > 0.05$) and, thus, the assumptions of the ANOVA were not violated. Subsequently, for Experiment 1, a split-plot in time, repeated measure ANOVA was used to compare the effects of anodal tDCS + ST and sham tDCS + ST conditions on multiple dependent variables (MVIC force, pre-stimulus EMG, corticospinal excitability,

corticospinal silent period duration and AUC) over two time points (pre-testing and post-testing). For the secondary analysis, a 2-way ANOVA of genotype (*Val/Val*, *Val/Met*) and time (pre-testing and post-testing) was used to examine the effect of genotype on multiple dependent variables (MVIC force, corticospinal excitability and corticospinal silent period duration) following anodal tDCS + ST and sham tDCS + ST.

A sub-analysis was also conducted for Experiment 2 to determine if anodal tDCS without strength training had an effect on indices of corticospinal excitability and inhibition. Again, a split-plot in time, repeated measure ANOVA was used to compare the effects of anodal tDCS and sham tDCS conditions on multiple dependent variables (corticospinal excitability and corticospinal silent period duration at 150% and 170% AMT) over two time points (pre-testing and post-testing). In addition, paired *t*-tests were performed on VAS scales. Bonferroni correction for multiple comparisons was applied for each dependent variable where significant multivariate effects were found. Prism 7 for Windows (Graphpad Software Inc, CA, USA) was used for all statistical analyses with the level of significance set as $P < 0.05$ for all testing. All data are presented as mean ± SE.

5.3 Results

The *BDNF* genotype analysis for the ten participants for whom genetic data was available revealed that six were homozygous for the *Val* allele (*Val66Val*), while four were genotyped as *Val66Met*.

5.3.1 Pre-stimulus *rms*EMG, maximal compound wave, and visual analogue scale

Table 5-1 presents the mean (± SE) for AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG prior to and following anodal tDCS + ST and sham tDCS + ST. Pre-stimulus *rms*EMG, AMT stimulus intensity and M_{MAX} were similar between the two conditions at baseline ($P > 0.05$). Pre-stimulus *rms*EMG did not vary between single-pulse trials, and there were no TIME or TIME × CONDITION interaction observed (all $P > 0.05$). Similarly, there was no TIME or TIME × CONDITION interaction detected for AMT stimulus intensity (all $P > 0.05$). Furthermore, there was no TIME or TIME × CONDITION interaction detected for M_{MAX} (all $P > 0.05$). VAS data were collected for each condition and there was no difference in the participants perception of discomfort between anodal tDCS + ST and sham tDCS + ST conditions (3.3 ± 0.5, 3.2 ± 0.5, 2.8 ± 0.7, respectively; $P = 0.48$).

Table 5-1: Mean (± SE) for AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG for the biceps brachii prior to and following sham tDCS + ST and anodal tDCS + ST.

	Sham tDCS + ST		**Anodal tDCS + ST**		
	Pre	Post	Pre	Post	***P* value**
AMT SI (%)	43 ± 2	42 ± 2	44 ± 2	43 ± 2	0.78
M_{MAX} (mV)	9.41 ± 1.31	9.53 ± 1.42	8.92 ± 0.79	8.96 ± 0.79	0.40
SP *rms*EMG (% *rms*EMG_{MAX})	4.26 ± 0.59	4.65 ± 0.78	3.78 ± 0.63	4.48 ± 0.52	0.64

AMT SI: active motor threshold stimulus intensity. Single-pulse (SP) *rms*EMG was pooled across stimulus intensities. *P* values represent the 2 (conditions) × 2 (time) repeated measures ANOVA used to determine any differences between conditions and time for the dependent variables AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG.

5.3.2 Maximal voluntary isometric contraction force

Isometric strength was assessed for the right biceps brachii muscle prior to and following the anodal tDCS + ST and sham tDCS + ST intervention. Figure 5.2 shows the mean change in isometric strength for the right biceps brachii muscle. There were no differences in isometric strength at baseline between anodal tDCS + ST and sham tDCS + ST conditions ($P > 0.05$). Following the intervention, the ANOVA revealed only a TIME effect for both the anodal tDCS + ST and sham tDCS + ST conditions (both $P < 0.001$); no TIME × CONDITION interaction was detected ($P > 0.05$). Isometric biceps brachii strength decreased by 11% following anodal tDCS + ST and, similarly, by 12% following sham tDCS + ST. However, there was no difference in biceps brachii muscle strength between genotypes ($P > 0.05$).

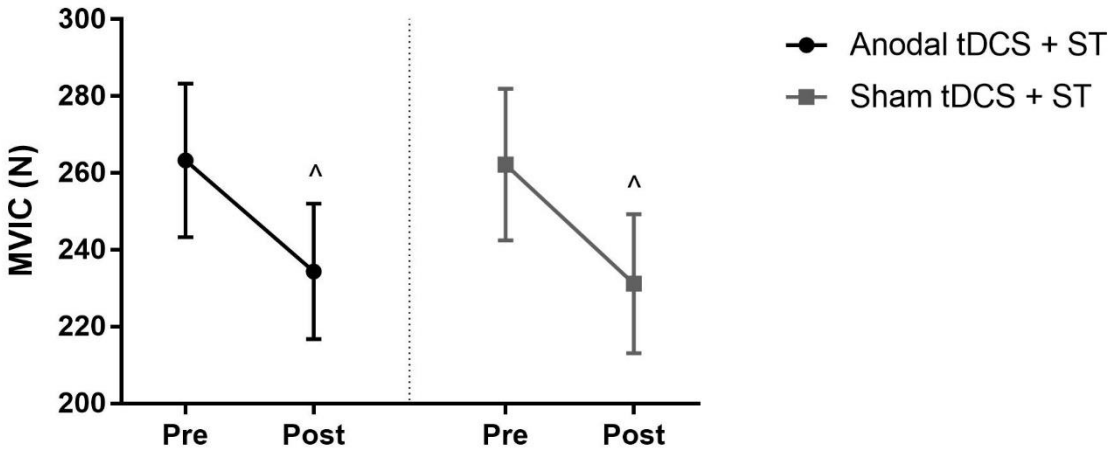

Figure 5.2: Mean (± SE) changes in MVIC strength of the right biceps brachii muscle following anodal tDCS + ST and sham tDCS + ST. ^ indicates significant to baseline.

5.3.3 Corticospinal excitability

Experiment 1

Figure 5.3A-B shows the mean MEP amplitude normalized as a percentage of M_{MAX} for anodal tDCS + ST and sham tDCS + ST conditions at 110-190% of AMT (increments of 20%) for the right biceps brachii. MEP amplitudes were similar between conditions at baseline across all stimulus intensities ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P < 0.05$) at all stimulus intensities (110-190% AMT), however a TIME × CONDITION interaction was only detected at 190% AMT ($P < 0.05$). *Post hoc* analysis revealed that anodal tDCS + ST increased MEP amplitude by 26% compared to 7% following sham tDCS + ST ($P = 0.039$) at 190% AMT. Across the other stimulus intensities (110-170% AMT), anodal tDCS + ST resulted in a 15-45% increase in MEP amplitude compared to an average of 1-15% change in the sham tDCS + ST condition. However, *post hoc* analysis revealed that the magnitude of change in MEP amplitude was not statistically significant between conditions at 110-170% AMT ($P > 0.05$).

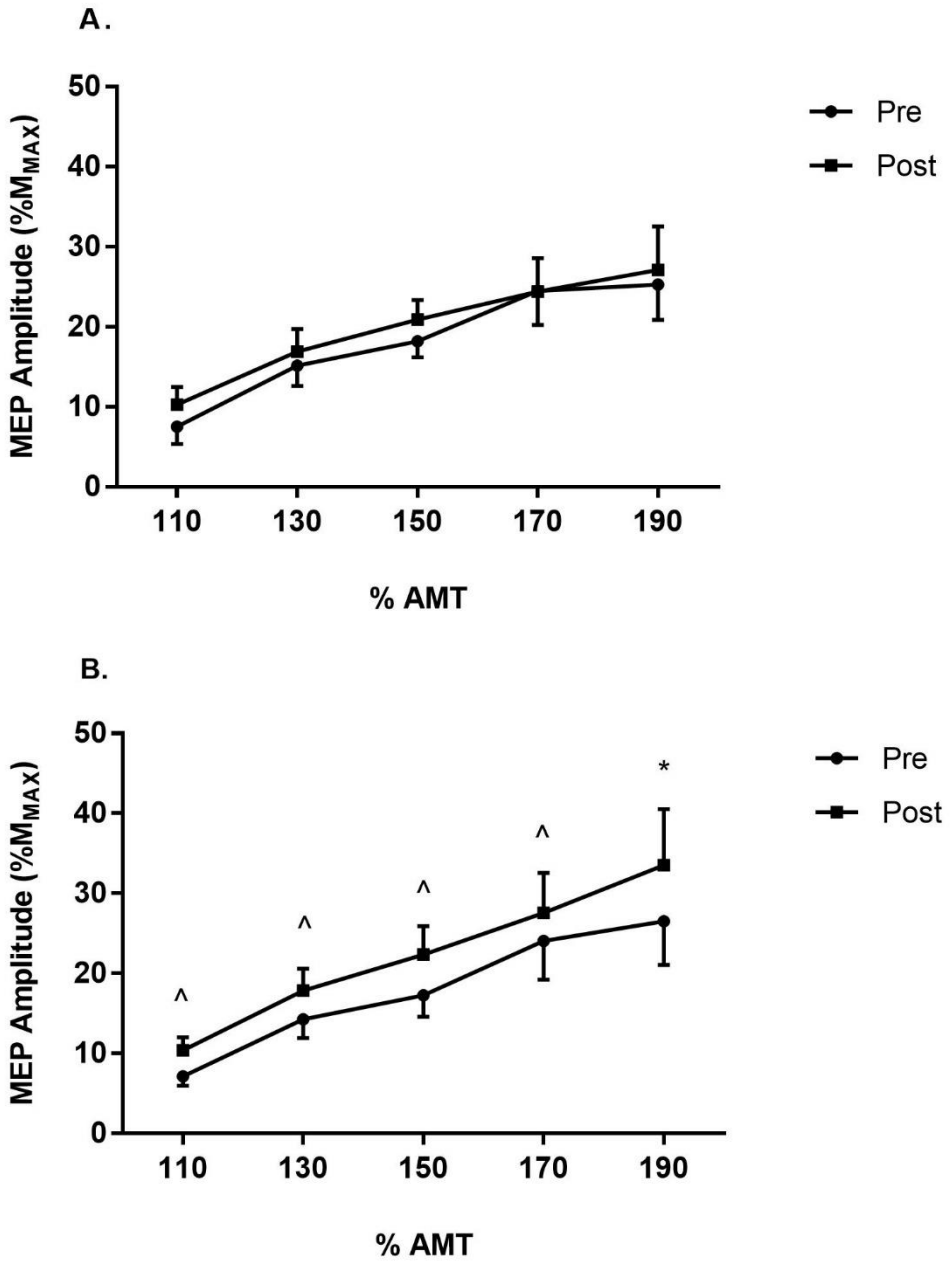

Figure 5.3A-B: Mean (± SE) changes in MEP amplitude following **(A)** sham tDCS + ST **(B)** anodal tDCS + ST. ^ indicates significant to baseline; * indicates significant to sham tDCS + ST.

Figure 5.4A shows the AUC obtained prior to and following the anodal tDCS + ST intervention. The total areas under the curves were similar between conditions at baseline across all stimulus intensities ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P = 0.0056$), but there was no TIME × CONDITION interaction detected ($P > 0.05$). Anodal tDCS + ST resulted in a 24% increase (pre 1512 ± 238 arb. units; post 1895 ± 289 arb. units) in the total area under the curve compared to a 9% increase (pre 1483 ± 193.4 arb. units; post 1604 ± 211 arb. units) in the sham tDCS + ST condition. However, *post hoc* analysis revealed that the magnitude of change in total area under the curve was not statistically significant between conditions ($P > 0.05$).

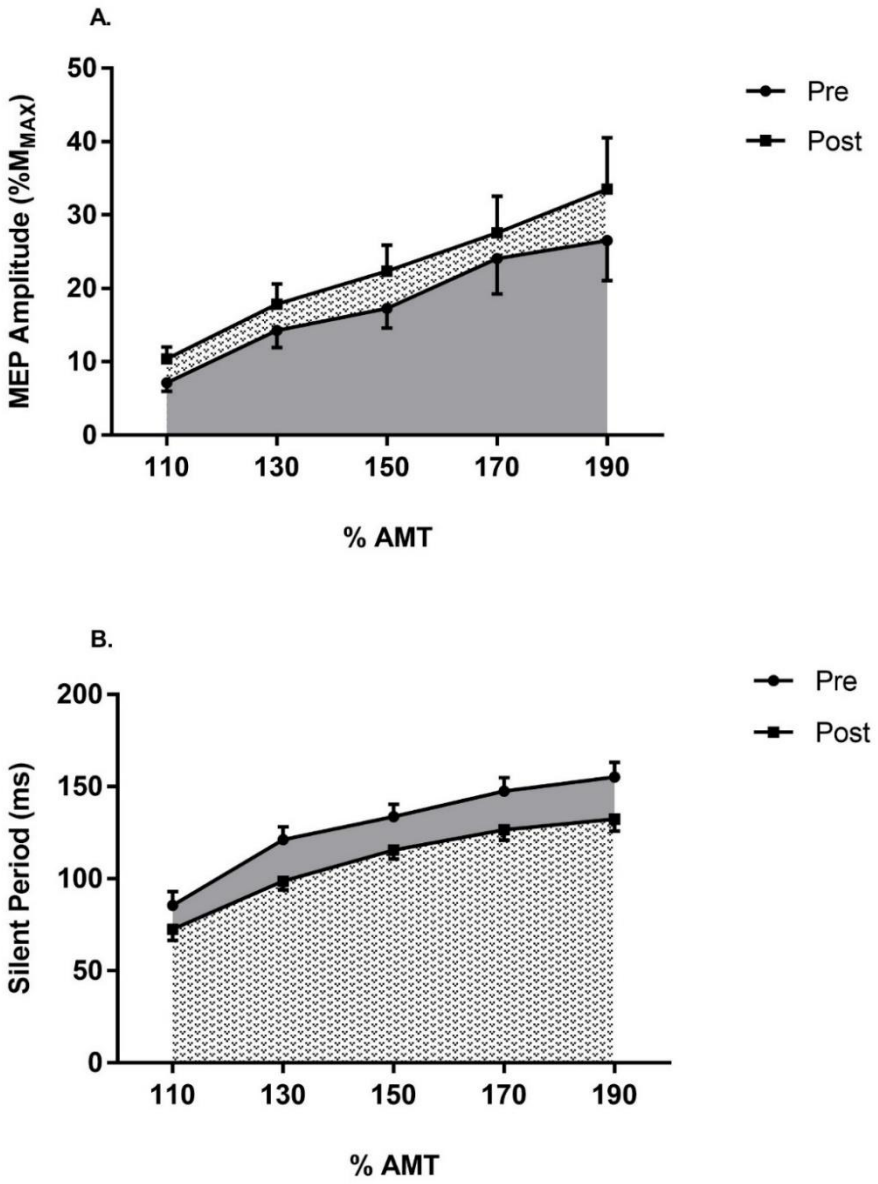

Figure 5.4A-B: The AUC was calculated using the method of trapezoidal integration. The AUC obtained prior to anodal tDCS + ST intervention is shaded in grey. The additional area enclosed by the recruitment curve obtained following anodal tDCS + ST intervention is patterned**. (A)** depicts the AUC calculated from corticospinal excitability recruitment curves whereby MEP amplitude was plotted against stimulus intensity. **(B)** depicts the AUC calculated from corticospinal inhibition curves whereby corticospinal silent period duration was plotted against stimulus intensity.

Experiment 2

For the secondary experiment, MEP amplitudes were similar between sham and anodal tDCS conditions at baseline for each stimulus intensity ($P > 0.05$). Following the anodal tDCS intervention, there was a main effect for TIME ($P < 0.05$) and a TIME × CONDITION interaction ($P < 0.05$) detected at 150% and 170% of AMT (see Figure 5.5). *Post hoc* analysis showed that anodal tDCS increased MEP amplitude by 24% compared to 1-2% following sham tDCS ($P = 0.002$; $P = 0.003$, respectively).

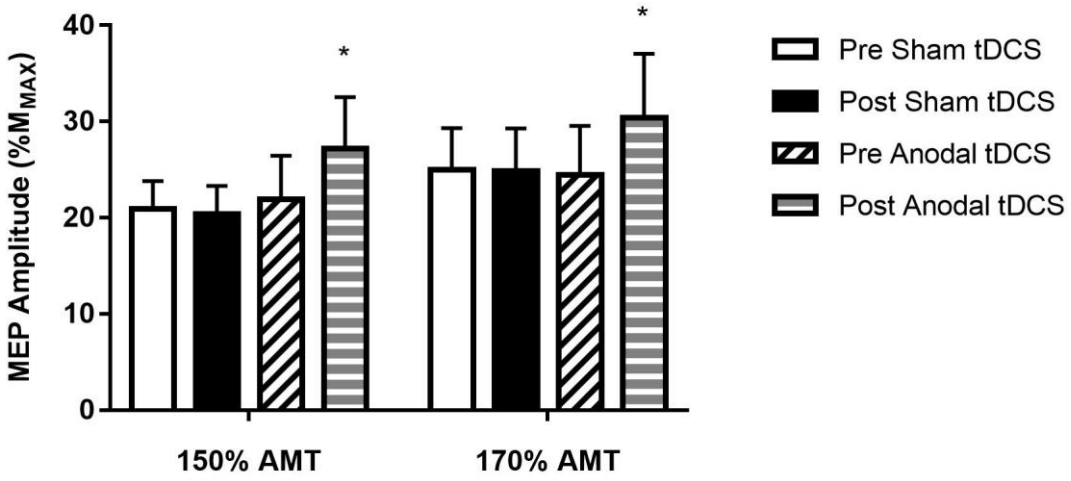

Figure 5.5: Mean (± SE) changes in MEP amplitude at 150% and 170% AMT before and after 20 min of anodal and sham tDCS (Experiment 2). * indicates significant to sham tDCS.

5.3.4 Corticospinal inhibition

Experiment 1

As shown in Figure 5.6A-B, corticospinal inhibition was assessed with the duration of corticospinal silent period obtained at several stimulus intensities above AMT (110-190% AMT; increments of 20%). Corticospinal silent period durations were similar between conditions at baseline across all stimulus intensities ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P < 0.05$) and a TIME × CONDITION interaction ($P < 0.05$) at a 130-170% of AMT. *Post hoc* analysis showed that anodal tDCS + ST resulted in a 14-18% decrease in corticospinal silent period duration compared to an average of 2-6% decrease in the sham tDCS + ST condition (all $P < 0.05$). However, *post hoc* analysis revealed that the magnitude of change in corticospinal silent period duration was not statistically significant between the two conditions ($P > 0.05$).

Figure 5.4B shows the AUC obtained prior to and following the anodal tDCS + ST intervention. The total areas under the curves were similar between conditions at baseline across all stimulus intensities ($P > 0.05$). Following the intervention, there was a main effect for TIME and a TIME × CONDITION interaction detected ($P < 0.05$). *Post hoc* analysis showed that anodal tDCS + ST decreased the total area under the curve by 14% (pre 10461 ± 512 arb. units; post 88712 ± 3312 arb. units) compared to 5% following sham tDCS + ST (pre 10481 ± 377 arb. units; post 9970 ± 375 arb. units; $P = 0.0037$).

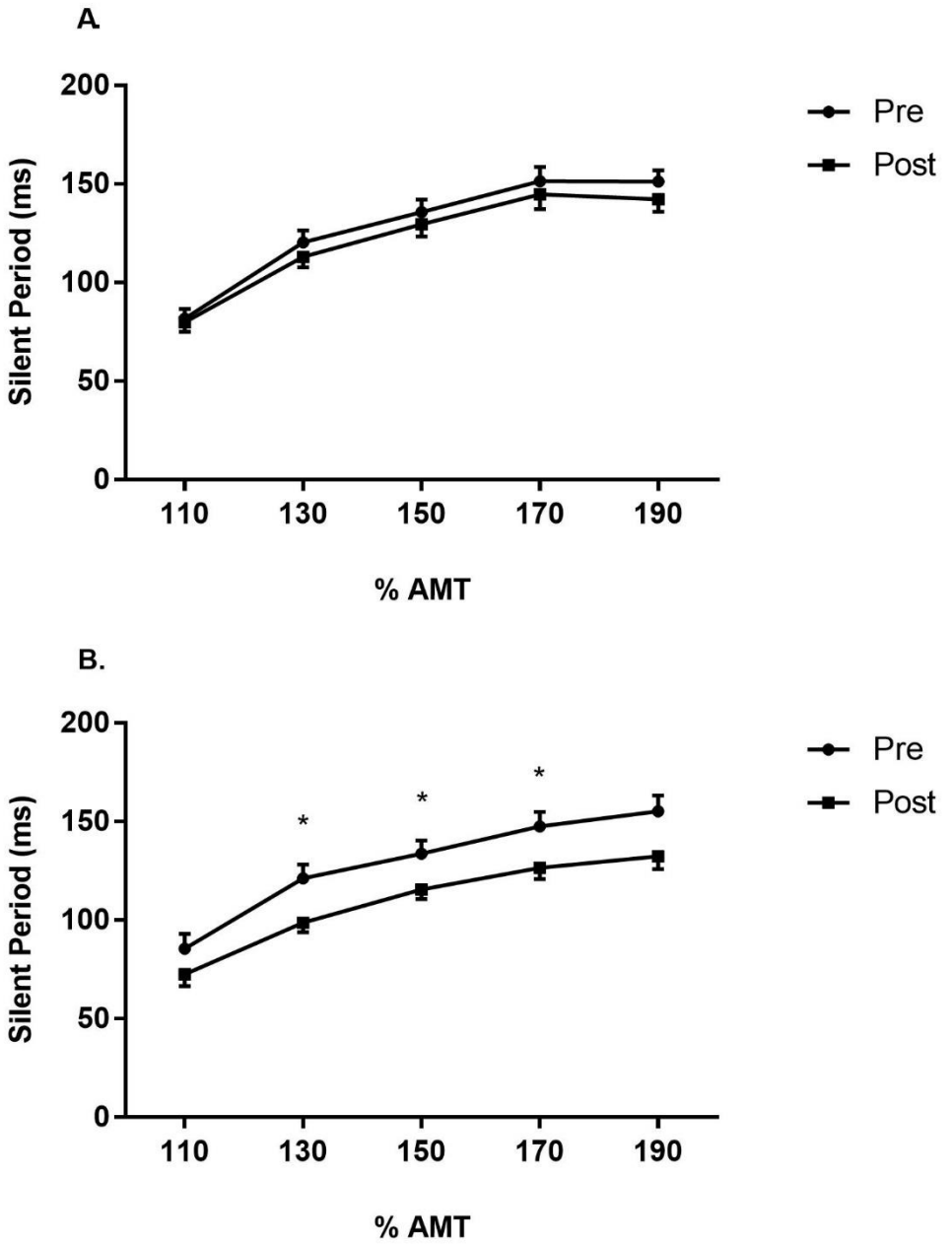

Figure 5.6A-B: Mean (± SE) changes in corticospinal silent period duration following **(A)** sham tDCS + ST **(B)** anodal tDCS + ST. * indicates significant to sham tDCS + ST.

Experiment 2

For the secondary experiment, corticospinal silent period durations were similar between sham and anodal tDCS conditions at baseline for each stimulus intensity ($P > 0.05$). Following the tDCS intervention, there was a main effect for TIME ($P < 0.05$) and a TIME × CONDITION interaction ($P < 0.05$) detected at 150% and 170% of AMT (see Figure 5.7). *Post hoc* analysis showed that anodal tDCS decreased corticospinal silent period duration by 7-9% compared to an average of 1% following sham tDCS ($P = 0.002$; $P = 0.007$, respectively).

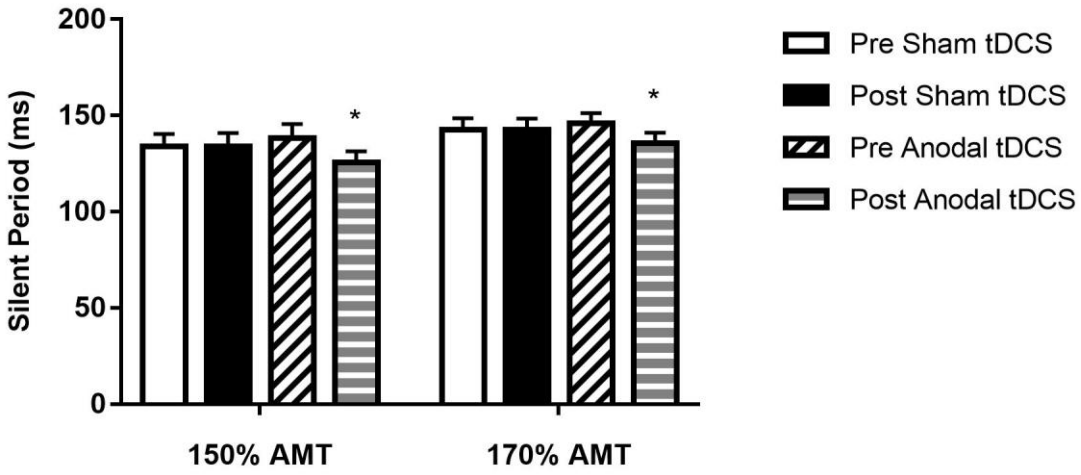

Figure 5.7: Mean (± SE) changes in corticospinal silent period duration at 150% and 170% AMT before and after 20 min of anodal and sham tDCS (Experiment 2). * indicates significant to sham tDCS.

5.3.5 Corticospinal excitability and inhibition in different *BDNF* genotypes

Experiment 1

For the primary experiment, there were no TIME ($P > 0.05$) or TIME × GENOTYPE ($P > 0.05$) interaction detected following the intervention (anodal tDCS + ST and sham tDCS + ST) for MEP amplitude across all stimulus intensities (110-190% AMT). Similarly, there were no TIME ($P > 0.05$) or TIME × GENOTYPE ($P > 0.05$) interaction detected following the intervention (anodal tDCS + ST and sham tDCS + ST) for corticospinal silent period duration across all stimulus intensities (110-190% AMT).

Experiment 2

For the secondary experiment, the GENOTYPE × TIME ANOVA revealed only a TIME effect for corticospinal excitability at 170% AMT for the *Val/Val* group following anodal tDCS (Figure 5.8; $P = 0.03$). Corticospinal excitability increased by 31% in the *Val/Val* group compared to 13% increase in those with the Val/Met polymorphism. *Post hoc* analysis, however, revealed that the magnitude of change in MEP amplitude was not statistically significant between genotypes ($P > 0.05$). Furthermore, there were no TIME ($P > 0.05$) or TIME × GENOTYPE ($P > 0.05$) interaction detected following the intervention for corticospinal silent period durations (150% and 170% AMT).

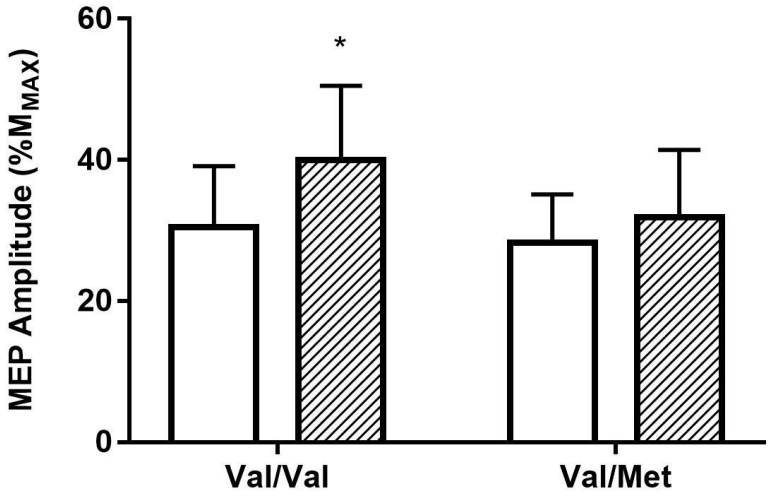

Figure 5.8: Mean (± SE) changes in MEP amplitude of the stimulated hemisphere at 170% AMT before and after 20 min of anodal and sham tDCS (Experiment 2). * indicates significant to sham tDCS.

5.4 Discussion

The purpose of this study was to investigate the effect of priming the M1 using anodal tDCS prior to a single bout of strength training to augment the potential corticospinal responses to strength training. This chapter also examined corticospinal excitability/inhibition and the influence on these responses of the *BDNF* polymorphism. Importantly, it should be highlighted that the current study did not examine fatigue and the associated corticospinal responses, and this will be discussed below. The main findings from the ***Experiment 1*** were: (i) As expected, MVIC of the biceps brachii declined in both groups (sham tDCS + ST and anodal tDCS + ST) to a similar magnitude following a single bout of strength training; (ii) The application of anodal tDCS prior to a single bout of strength training (anodal tDCS + ST) facilitated the induction of corticospinal plasticity, which was evident by the increase in corticospinal excitability, reduction in corticospinal silent period duration and changes in the total area under the curve (AUC); and (iii) Contrary to the chapter hypothesis, the induction of corticospinal plasticity (corticospinal excitability and cortical inhibition) following anodal tDCS and strength training (anodal tDCS + ST) was not influenced by the *BDNF* polymorphism. The main findings for ***Experiment 2*** were: (i) The application of anodal tDCS increased corticospinal excitability and decreased corticospinal silent period duration; and (ii) Interestingly, a within time effect for increased corticospinal excitability in the *Val/Val* individuals following anodal tDCS was observed which is an important finding that warrants some discussion.

5.4.1 Anodal tDCS induces homeostatic plasticity (Experiment 2)

The first key finding of this chapter was the observed increase in corticospinal excitability and decreased corticospinal silent period duration following the application

of anodal tDCS only (experiment 2). Anodal tDCS has been shown previously to increase corticospinal excitability for up to 90 min post stimulation (Nitsche & Paulus 2000, Nitsche & Paulus 2001, Bastani & Jaberzadeh 2012, Kidgell et al. 2013, Pellicciari et al. 2013) and decrease inhibition (Hummel et al. 2005, Nitsche et al. 2005, Kidgell et al. 2013), with the changes in synaptic strength attributed to modulation of the NMDA receptor (Nitsche et al. 2005, Boggio et al. 2006). Pharmacological interventions have further highlighted the importance of the NMDA receptor by using a NMDA receptor antagonist (i.e., dextromethorphan) to block the after-effects of tDCS (Liebetanz et al. 2002, Nitsche et al. 2003a). Importantly, the results from Experiment 2 provide the theoretical basis for using anodal tDCS as a priming method to the M1 prior to a single bout of strength training to potentially further enhance the corticospinal responses to strength training. Given that the underlying principal of inducing homeostatic plasticity is to enhance neural activity (Ziemann & Siebner 2008, Siebner 2010), it has been sufficiently demonstrated that anodal tDCS by itself (Experiment 2), is a low-cost, clinically applicable technique to prime the M1 resulting in homeostatic plasticity.

5.4.2 Anodal tDCS prior to strength training increases corticospinal excitability and decreases intracortical inhibition

At present, there are conflicting results regarding the effect of using anodal tDCS to prime the M1 prior to a motor training task (Antal 2008, Sriraman et al. 2014). Visuo-motor tracking performance has been shown to improve following 10-15 min of anodal tDCS at 1 mA prior to training (Antal 2008, Sriraman et al. 2014), with retention lasting up to 24 hours (Sriraman et al. 2014). In direct contrast, Stagg et al. (2011) found that anodal tDCS applied to the M1 prior to a reaction time task had a negative effect on motor learning. Currently, no study has investigated the effect of priming the M1 using anodal

tDCS prior to a single bout of strength training and the effects of this on corticospinal excitability and inhibition.

Although a novel finding, the increase in corticospinal excitability following anodal tDCS + ST has been similarly observed following anodal tDCS and ankle motor skill training (Sriraman et al. 2014). Even though the increase in MEP amplitude reported by Sriraman et al. (2014) was a trend and did not reach statistical significance, it suggests that both interventions (motor training and strength training) shared similar underlying corticospinal responses. The increase in corticospinal excitability at multiple points along the stimulus-response curve is further reflected by the increase in the total AUC. Recently, AUC has been shown to have high validity providing additional insight into the input-output properties of the corticospinal pathway (Carroll et al. 2001a, Carson et al. 2013). Indeed, the facilitated responses following anodal tDCS + ST (increased corticospinal excitability and total AUC) may be due to anodal tDCS increasing the resting levels of synaptic activity, and thereby, pre-activating synapses. In turn, the synaptic activation threshold is lowered, allowing for the effective stimuli of strength training to more readily strengthen corticocortical connections, increase synaptic efficacy and enhance the net neural output from the M1 (Antal 2008, Frazer et al. 2016).

The findings of the present chapter extends the working hypothesis that anodal tDCS + ST modulates corticospinal connections (i.e., improved synaptic efficacy) by exhibiting a decrease in the duration of the corticospinal silent period. Initially, the duration of the corticospinal silent period is thought to be due to spinal cord refractoriness, however, the latter part is a result of cortical inhibition (Wilson et al. 1993, Chen et al. 1999, Hallett 2000) which represents the overall strength of inhibition within the corticospinal tract (Werhahn et al. 2007). It appears that the combination of anodal tDCS

+ ST specifically targets neural circuits that use Gamma-Aminobutyric acid ($GABA_B$) as their neurotransmitter, resulting in the release of corticospinal neurons from inhibition. Importantly, it should be noted that Ruotsalainen et al. (2014) showed an increase in corticospinal silent period duration following a single session of strength training of the elbow flexors. Ruotsalainen and colleagues (2014) examined muscle fatigue and the associated corticospinal responses of the elbow flexors. Therefore, they used a strength training mode (hypertrophic resistance training) designed to induce central and peripheral fatigue, which was demonstrated by a 46% reduction in strength (Ruotsalainen et al. 2014). In contrast, the findings of this chapter were only a 11-12% decrease in MVIC strength, thus not inducing central fatigue and this most likely accounts for the difference in findings between the studies.

With respect to the input-output relationship between stimulus intensity and corticospinal silent period duration, a decrease in total AUC was shown. This finding highlights that priming the M1 using anodal tDCS prior to strength training assists in the unmasking of latent synapses involving the removal of GABA mediated inhibitory projections, which likely results in enhanced synaptic efficacy. Furthermore, the decrease in corticospinal silent period duration and total AUC is of particular interest, as the modulation of intracortical inhibition has been shown to be critical for motor performance (Stinear & Byblow 2003). However, a caveat to the current study is that SICI, which is indicative of inhibitory mechanisms specific to the M1 (Kujirai et al. 1993, Fisher et al. 2002, Rothwell et al. 2009), was not measured and, thus, no comment can be made regarding the potential role of $GABA_A$ neurotransmission. Nevertheless, a reduction in corticospinal silent period duration may form an important neural adaptation to strength training (Kidgell & Pearce 2010, Christie & Kamen 2013, Coombs et al. 2016). The

present chapter finding, suggest that priming the M1 prior to a bout of strength training augments the reduction in cortical inhibition.

5.4.3 Corticospinal excitability and intracortical inhibition is not differentially modulated following strength training in different *BDNF* genotypes

Contrary to the chapter hypothesis, the corticospinal response to a single session of anodal tDCS + ST was not differently modulated by the *BDNF* polymorphism. There are several reasons which might potentially explain this, namely: (i) the relative small sample size; and (ii) the combination of anodal tDCS (experimentally-induced plasticity) and strength training (use-dependent plasticity) protocols. It should be noted that in the Experiment 2 (tDCS only), *Val/Val* individuals showed a greater increase in MEP amplitude compared to *Val/Met* individuals following 20 min of anodal tDCS. However, due to the small sample size of the *Val/Met* individuals, this was not statistically significant between genotypes (*Val/Val, Val/Met*). Carriers of the *BDNF Met* allele have previously shown reduced corticospinal responses to several NIBS paradigms, including anodal tDCS and rTMS (Chang et al. 2014, Hwang et al. 2015, Puri et al. 2015, Frazer et al. 2016). Although these preliminary findings should be viewed with caution, they give rise to an interesting discussion point. It could be suggested that the combination of anodal tDCS + ST reduced the genotype effect that has previously been reported following motor skill training (Kleim et al. 2006, Cirillo et al. 2012). Indeed, it has previously been shown that intense training of the FDI muscle eliminated the effect of the val66met *BDNF* on short-term plasticity (McHughen et al. 2011). Therefore, it could be proposed that individuals with the *BDNF Met* allele may require the addition of tDCS as a priming technique to maximise use-dependent plasticity protocols. However, future work would be required to establish the effect of the *BDNF* polymorphism on strength training and

priming techniques, given the low representative of the *Val/Met* participants in this chapter.

Overall, the current chapter indicate that priming the M1 by using anodal tDCS prior to a single bout of strength training, increases corticospinal excitability, reduces the corticospinal silent period duration and changed the total AUC. The induction of corticospinal plasticity following anodal tDCS and strength training was not influenced by the *BDNF* polymorphism; however, as a minimum, it shapes the corticospinal responses to anodal tDCS only.

Chapter 6：Cross-education of muscle strength is facilitated by homeostatic plasticity

6.1 Introduction

Cross-education is the phenomenon whereby strength training of one limb results in an increase in strength of the opposite untrained limb (Munn et al. 2004, Carroll et al. 2006). Although several theoretical frameworks have been suggested to underpin the cross-education effect, there is strong evidence from TMS studies supporting the *cross-activation* hypothesis (Hortobágyi et al. 2003b, Zijdewind et al. 2006, Perez & Cohen 2008, Howatson et al. 2011). The theory of *cross-activation* is based upon the well-documented observation that unilateral contractions results in a bilateral increase in corticospinal excitability (Dettmers et al. 1995, Muellbacher et al. 2000, Hortobágyi et al. 2003a, Carson et al. 2013, Ruddy & Carson 2013). Cross-activation is thought to lead to adaptations in the neural circuits that project to the muscles of the untrained contralateral limb (ipsilateral M1), manifesting as an improvement in motor performance of the untrained limb (Ruddy & Carson 2013). In support of this, a number of TMS studies have revealed increased corticospinal excitability (Hortobágyi et al. 2003b, Zijdewind et al. 2006, Perez & Cohen 2008), decreased SICI (Perez & Cohen 2008) and decreased IHI of the ipsilateral M1 following unilateral contractions (Hortobagyi et al. 2011, Howatson et al. 2011). This has been further highlighted by fMRI studies representing an increase in activity of the ipsilateral M1 during unilateral voluntary isometric contractions (Kobayashi & Pascual-Leone 2003, van Duinen et al. 2008).

The magnitude of cross-education has been shown to be proportional to the strength gain of the trained limb (Zhou 2000, Munn et al. 2005b, Zult et al. 2014). Meta-analyses have reported an average ~8% increase in strength of the contralateral untrained limb (Munn et al. 2004, Carroll et al. 2006, Zult et al. 2014). Although the degree of cross-education reported is relatively small, this could in part be reflective of the

variability of results due to the different types of training employed (i.e., eccentric, concentric, isometric) (Kidgell et al. 2015). Indeed, studies have reported contralateral strength increases ranging from as little as 3% (Garfinkel & Cafarelli 1992) to as large as 77% (Hortobagyi et al. 1997), while others have reported a decrease in strength of the untrained contralateral limb following unilateral strength training (Farthing et al. 2005, Munn et al. 2005b). This may be due to several methodological considerations including contraction type, dominant versus non-dominant limb and the type of muscle trained (Farthing & Chilibeck 2003, Farthing et al. 2005, Carroll et al. 2006, Farthing & Zehr 2014, Magnus et al. 2014, Kidgell et al. 2015, Coombs et al. 2016). Importantly, it should be noted that these methodological considerations may also shape the induction of corticospinal plasticity (i.e., increased corticospinal excitability of the ipsilateral M1) which may ultimately underpin the magnitude of cross-education.

As discussed in Section 2.4.5, the influence of the *BDNF* polymorphism on the induction of corticospinal plasticity has been observed following use-dependent paradigms such as motor skill training (Kleim et al. 2006, Cirillo et al. 2012). Of particular importance, it has been suggested that tasks involving force generation (i.e., strength training) share similar underlying neural substrates to motor skill training due to the learning and skill required to complete a specific movement under load (Carroll et al. 2001b). It is, therefore, conceivable that the *BDNF* polymorphism may influence the magnitude of bilateral activation (increase in corticospinal excitability) of both motor cortices, as described by the *cross-activation* hypothesis, which may in turn effect the level of strength transfer to the untrained limb. Certainly, if individuals with the *BDNF* polymorphism have reduced corticospinal responses to unilateral strength training, it may counteract the cross-transfer effect by either reducing the capacity of the strength gained

in the training arm or the adaptations within the ipsilateral M1. However, no one has yet investigated the potential influence of the *BDNF* polymorphism on the cross-transfer of strength and the ipsilateral corticospinal responses to a single session of strength training. Understanding the potential underlying regulatory factors, such as the *BDNF* polymorphism, is necessary to maximise the clinical applications of cross-education.

Recently, a novel study by Hendy et al. (2014) examined the application of anodal tDCS over the ipsilateral M1 while completing unilateral strength training of the wrist extensors. Intriguingly, Hendy and colleagues (2014) found that anodal tDCS applied to the ipsilateral M1 (right hemisphere), concurrently with unilateral strength training, resulted in an increase in maximal strength and cross-activation to the contralateral untrained limb as a result of manipulating the priming principal of *gating. Gating*, which describes the influx of calcium ions to the targeted corticospinal neurons resulting in the disinhibition of intracortical inhibitory circuits (Ziemann & Siebner 2008, Siebner 2010), has previously been shown to improve motor performance such as hand function using the JTT, maximal strength, movement speed, reaction time and speed-accuracy trade-off (Boggio et al. 2006, Hunter et al. 2009, Reis et al. 2009, Stagg et al. 2011, Hendy & Kidgell 2014). On the other hand, *homeostatic plasticity*, another established priming theory, describes the process whereby the resting state of corticospinal neurons is altered (increased/decreased level of excitability following a low/high level of synaptic activity) due to changes in postsynaptic glutamate receptor activity (Ziemann & Siebner 2008, Siebner 2010). The central difference between *gating* and *homeostatic plasticity* is the timing of the priming protocol. *Gating* usually occurs concurrently with motor training (i.e., tDCS while training), while *homeostatic plasticity* involves modulating the resting state of neurons prior to training (i.e., tDCS applied before training). Given the lack of

interaction between conditions observed in the study by Hendy et al. (2014), it is unclear as to whether the anode over the ipsilateral M1 induced *gating*, or if the stimuli of the strength training negated the ability of anodal tDCS to sufficiently act as a M1 priming method. Importantly, the study by Hendy et al. (2014) gives rise to the concept of using anodal tDCS as a M1 'priming' technique in anticipation of augmenting the cross-transfer of strength and ipsilateral corticospinal responses via homeostatic plasticity. However, to maximise the effectiveness of anodal tDCS as a M1 priming technique, it appears that a critical consideration is the timing of application (i.e., during or prior the training). Given that tDCS utilizes weak direct currents to induce prolonged modulation of corticospinal excitability (Nitsche & Paulus 2000, Nitsche & Paulus 2001) due to LTP and LTD mechanisms, this chapter considers that anodal tDCS is a plausible technique to induce *homeostatic plasticity* of the ipsilateral M1. Further, the application of anodal tDCS to the ipsilateral M1 prior to unilateral strength training may result in a shift of the resting membrane potential and increase synaptic activity of the ipsilateral M1, which may in turn further promote bilateral activation of both motor cortices and enhance the cross-education effect.

Therefore, the primary aim of this study was to examine the effect of priming the ipsilateral M1 by inducing *homeostatic plasticity* by using anodal tDCS, prior to a single bout of strength training, on the cross-transfer of strength and corticospinal excitability/inhibition of the ipsilateral M1. Given that anodal tDCS induces polarity specific effects in corticospinal excitability/inhibition (Nitsche & Paulus 2001, Nitsche et al. 2005), a secondary aim was to confirm that anodal tDCS applied to the ipsilateral M1 in the absence of strength training modifies corticospinal excitability and inhibition. This was required to confirm that *homeostatic plasticity* was induced by tDCS, thus creating

support for Experiment 1. Furthermore, this study also examined whether any of these outcome measures were influenced by the *BDNF* polymorphism. It was hypothesised that the application of ipsilateral anodal tDCS prior to a single bout of unilateral strength training would augment the cross-transfer of strength and facilitate the induction of corticospinal plasticity (increased corticospinal excitability and reduced corticospinal inhibition) of the ipsilateral M1. A secondary hypothesis was that the magnitude of these responses (strength and corticospinal responses) would be influenced by the *BDNF* polymorphism.

6.2 Methods

For methods that are mainly replications of Chapters 3-5, the reader will be directed to the appropriate section for full comprehensive description of the methods employed in this chapter.

6.2.1 Participants

Thirteen participants (5 women, 8 men aged 18-35 years) with a LQ score of 81 ± 3 (right-hand dominant) volunteered to participate. Refer to Section 3.2.1 for a comprehensive description of exclusion/inclusion criterias and safety screening procedures.

6.2.2 Experimental approach

All participants completed two experiments, as outlined in Figure 6.1A-B. After obtaining consent, participants completed a familiarization session one week prior to the study that involved performing a 1RM strength test of the right (to establish training load) and left biceps brachii, and were exposed to single-pulse TMS. For Experiment 1, in a double-blinded cross-over design, all participants were exposed to two conditions. Each participant was exposed to 20 min of ipsilateral anodal and sham tDCS followed by a single strength training session of the right biceps brachii (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST, respectively). For the ipsilateral anodal and sham tDCS conditions, the anode was fixed over the optimal cortical representation of the left biceps brachii muscle (right hemisphere; ipsilateral to the training limb), and the cathode was placed over the left contralateral supra orbital area (left hemisphere). The order of the conditions was counterbalanced and randomized between participants, with a wash-out period of one week between each condition (Vines et al. 2008). All participants underwent TMS and dynamic muscle strength testing (1RM) of the left biceps brachii prior to and

following the tDCS and strength training intervention. Participants were required to attend two separate sessions where they were exposed to 20 min of ipsilateral anodal or sham tDCS applied at 2 mA with a current density of 0.08 mA/cm^2 and completed a strength training session (see Figure 6.1A).

To determine the effects of ipsilateral anodal tDCS without strength training on indices of ipsilateral corticospinal plasticity (corticospinal excitability and inhibition), participants also completed Experiment 2. Each participant was exposed to 20 min of ipsilateral anodal and sham tDCS applied at 2 mA with a current density of 0.08 mA/cm^2 with a wash-out period of one week between each condition (Vines et al. 2008). Prior to and following the tDCS intervention, 20 single-pulse TMS stimuli were collected at 150% and 170% AMT (see Figure 6.1B).

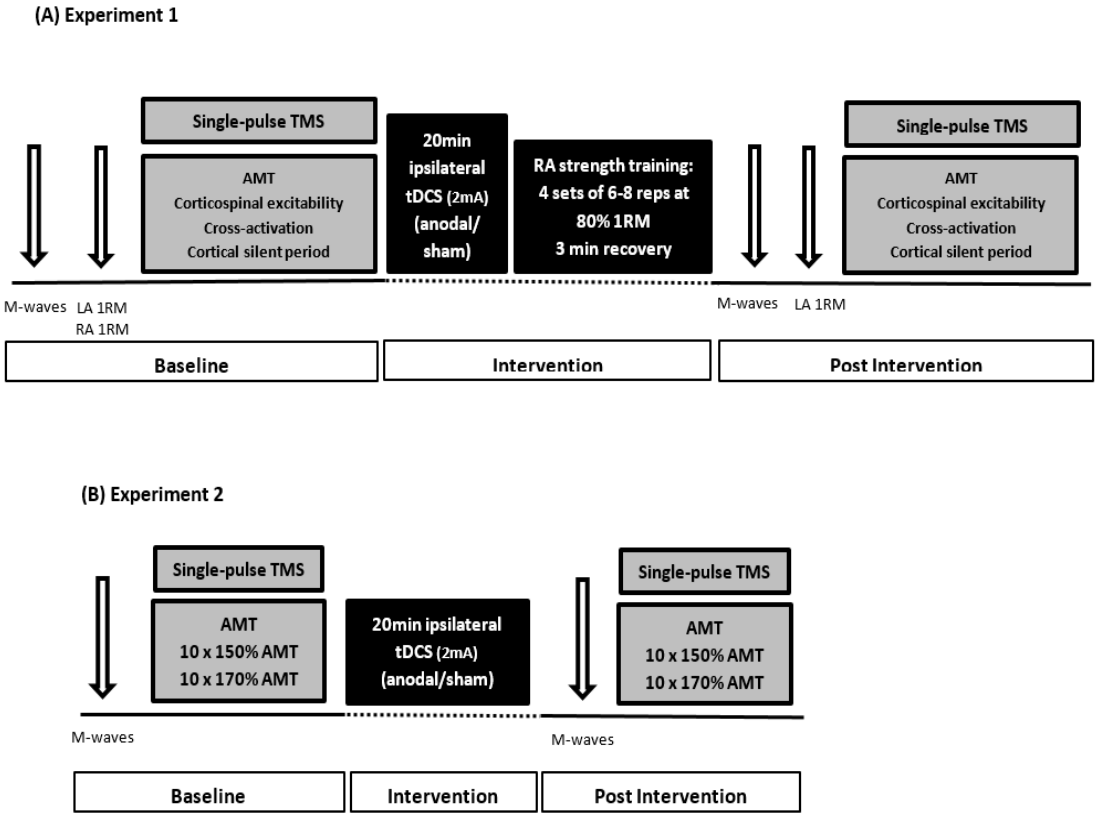

Figure 6.1A-B: (A) Schematic representation of Experiment 1 with measures obtained prior to and following 20 min ipsilateral anodal and sham tDCS and strength training of the right biceps brachii. Pre- and post-measures included assessment of peripheral muscle excitability (M_{MAX}), cross-activation, corticospinal excitability and inhibition recruitment curves and maximal dynamic strength testing (1RM) of the left (untrained) biceps brachii muscle. **(B)** Schematic representation of Experiment 2 with measures obtained prior to and following 20 min ipsilateral anodal and sham tDCS. Pre- and post- measures included assessment of peripheral muscle excitability (M_{MAX}), corticospinal excitability and inhibition at 150% and 170% AMT.

6.2.3 Voluntary strength testing

To determine maximal voluntary dynamic force, participants completed a 1RM test of the left and right biceps brachii muscle as described in Chapters 3 and 5. For a detailed explanation of the strength testing procedure refer to Section 5.2.3.

6.2.4 Strength training protocol

Participants completed a supervised strength training session following the anodal and sham tDCS intervention (ipsilateral anodal and sham tDCS). Using the same set-up as the 1RM, participants completed flexion-extension movements of the elbow with the forearm supinated (biceps curl) of the right arm. For a detailed explanation of the strength training protocol, refer to Section 5.2.4.

6.2.5 Surface electromyography

As described in Chapters 3 and 5, sEMG was recorded from the left biceps brachii muscle (see Sections 3.2.4 and 5.2.5).

6.2.6 Transcranial magnetic stimulation

As detailed in Chapter 3 (Section 3.2.5), TMS was delivered using a Magstim 200^2 stimulator (Magstim Co, Dyfed, UK) and a single figure-of-eight coil (external diameter of each loop 70 mm) was placed over the motor hotspot for the left biceps brachii muscle.

All stimuli were delivered during a low-level isometric contraction of the left biceps brachii muscle. Participants were required to maintain an elbow joint angle of 90^{O} elbow flexion. Joint angle was measured with an electromagnetic goniometer (ADInstruments, Bella Vista, Australia), with visual feedback provided on a screen visible to both the participant and the researcher (Hendy et al. 2015). This joint position

equated to 3.5 ± 1% of maximal *rms*EMG, with consistent muscle activation confirmed by recording pre-stimulus *rms*EMG for the 100-ms epoch before the delivery of each stimulus (Table 6-1).

6.2.7 Maximum compound muscle action potential

As described in Section 3.2.6, direct muscle responses were obtained from the left biceps brachii muscle by supramaximal electrical stimulation of the brachial plexus at Erbs point. The stimuli were delivered while the participant sat in an upright position, with the elbow at 90 degrees elbow flexion holding 3.48 ± 1% of maximal *rms*EMG.

6.2.8 Transcranial direct current stimulation

The tDCS protocol was identical to Chapter 5 (Section 5.2.8). In this case, the anode was fixed over the optimal cortical representation of the left biceps brachii muscle, as identified by TMS over the right cortex, and the cathode was placed over the left contralateral supra orbital area.

6.2.9 *BDNF* genotyping

As detailed in Chapter 3, blood samples were obtained and participants were genotyped for the *BDNF val66met* polymorphism (see Section 3.2.8).

6.2.10 Data analysis

Data analysis was as described in Chapter 5 (Section 5.2.10), however all measures were taken from the left biceps brachii muscle.

6.2.11 Statistical analysis

All data were screened with the Shapiro-Wilk test and found to be normally distributed (all $P > 0.05$) and, thus, the assumptions of the ANOVA were not violated. Subsequently, for Experiment 1, a split-plot in time, repeated measure ANOVA was used

to compare the effects of ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST conditions on multiple dependent variables (1RM of the left biceps brachii [cross-transfer of strength], pre-stimulus EMG, corticospinal excitability, corticospinal silent period duration and cross-activation) over two time points (pre-testing and post-testing). For the secondary analysis, a 2-way ANOVA of genotype (*Val/Val*, *Val/Met*) and time (pre-testing and post-testing) was used to examine the effect of genotype on multiple dependent variables (1RM of the left biceps brachii [cross transfer of strength], corticospinal excitability, corticospinal silent period duration and cross-activation) following ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST.

A sub-analysis was also conducted for Experiment 2 to determine if ipsilateral anodal tDCS without strength training influenced the indices of corticospinal excitability and inhibition. A split-plot in time, repeated measure ANOVA was used to compare the effects of ipsilateral anodal and sham tDCS conditions on multiple dependent variables (ipsilateral corticospinal excitability and corticospinal silent period duration at 150% and 170% AMT) over two time points (pre-testing and post-testing). In addition, paired *t*-tests were performed on the VAS scales. Bonferroni correction for multiple comparisons was applied for each dependent variable where significant multivariate effects were found. Prism 7 for Windows (Graphpad Software Inc, CA, USA) was used for all statistical analyses with the level of significance set as $P < 0.05$ for all testing. All data are presented as mean ± SE.

6.3 Results

The *BDNF* genotype analysis for the ten participants for whom genetic data was available revealed that seven were homozygous for the *Val* allele (*Val66Val*), while three were genotyped as *Val66Met*.

6.3.1 Pre-stimulus *rms*EMG, maximal compound wave, and visual analogue scale

Table 6-1 presents the mean (± SE) for AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG prior to and following ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST. Pre-stimulus *rms*EMG, AMT stimulus intensity and M_{MAX} were similar between the two conditions at baseline ($P > 0.05$). Pre-stimulus *rms*EMG did not vary between single-pulse trials, and there were no TIME or TIME × CONDITION interaction observed (all $P > 0.05$). Similarly, there was no TIME or TIME × CONDITION interaction detected for AMT stimulus intensity (all $P > 0.05$). Furthermore, there was no TIME or TIME × CONDITION interaction detected for M_{MAX} (all $P > 0.05$). VAS data was collected for each condition and there was no difference in participants' perception of discomfort between ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST conditions (3.2 ± 0.8, 3.3 ± 0.8, respectively; $P > 0.05$).

Table 6-1: Mean (± SE) for AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG for the biceps brachii prior to and following ipsilateral sham tDCS + ST and ipsilateral anodal tDCS + ST.

	Ipsilateral Sham tDCS + ST		**Ipsilateral Anodal tDCS + ST**		
	Pre	Post	Pre	Post	***P* value**
AMT SI (%)	43 ± 2	43 ± 2	41 ± 2	41 ± 2	0.41
M_{MAX} (mV)	10.67 ± 1.68	10.81 ± 1.73	11.55 ± 1.61	11.59 ± 1.65	0.34
SP *rms*EMG (% *rms*EMG_{MAX})	3.72 ± 0.47	3.53 ± 0.59	3.41 ± 0.43	3.26 ± 0.43	0.27

AMT SI: active motor threshold stimulus intensity. Single-pulse (SP) *rms*EMG was pooled across stimulus intensities. *P* values represent the 2 (conditions) × 2 (time) repeated measures ANOVA used to determine any differences between conditions and time for the dependent variables AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG.

6.3.2 Dynamic strength of the untrained limb

Dynamic muscle strength was assessed for the left biceps brachii muscle prior to and following the ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST intervention. Figure 6.2 shows the mean change in 1RM strength for the left biceps brachii muscle. There were no differences in 1RM strength at baseline between ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST conditions ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P < 0.0001$) and a TIME × CONDITION interaction ($P = 0.0018$). *Post hoc* analysis showed that ipsilateral anodal tDCS + ST resulted in a 12% increase in dynamic muscle strength compared to a 3% increase in the ipsilateral sham tDCS + ST condition ($P = 0.0057$).

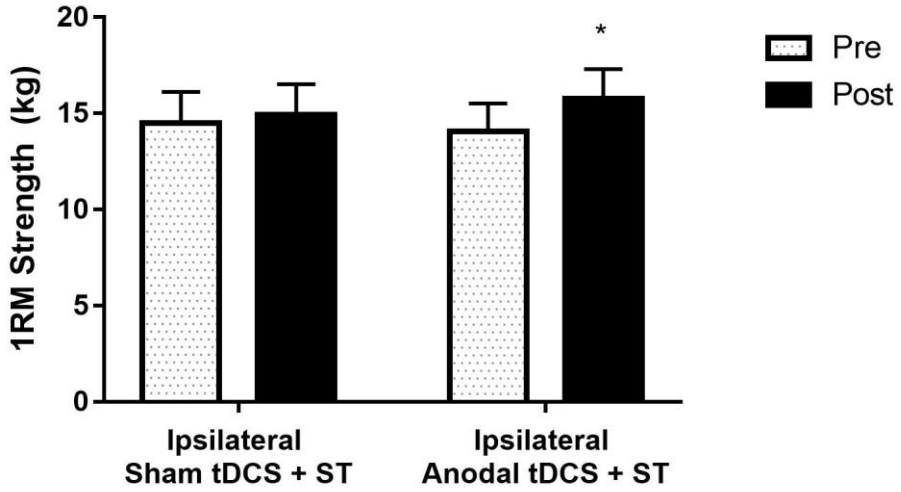

Figure 6.2: Mean (± SE) changes in 1RM strength of the left biceps brachii muscle following ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST. * indicates significant to ipsilateral sham tDCS + ST.

6.3.3 Corticospinal excitability

Experiment 1

Figure 6.3A-B shows the mean MEP amplitude normalized as a percentage of M_{MAX} for ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST conditions at 110-190% of AMT (increments of 20%) for the left biceps brachii. MEP amplitudes were similar between conditions at baseline across all stimulus intensities ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P < 0.05$) at stimulus intensities of 110-170% AMT for both conditions (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST), however no TIME × CONDITION interaction was detected. Across stimulus intensities of 110-170% AMT, ipsilateral anodal tDCS + ST resulted in a 12-33% increase in MEP amplitude and ipsilateral sham tDCS + ST resulted in an average increase in MEP amplitude of 2-32%.

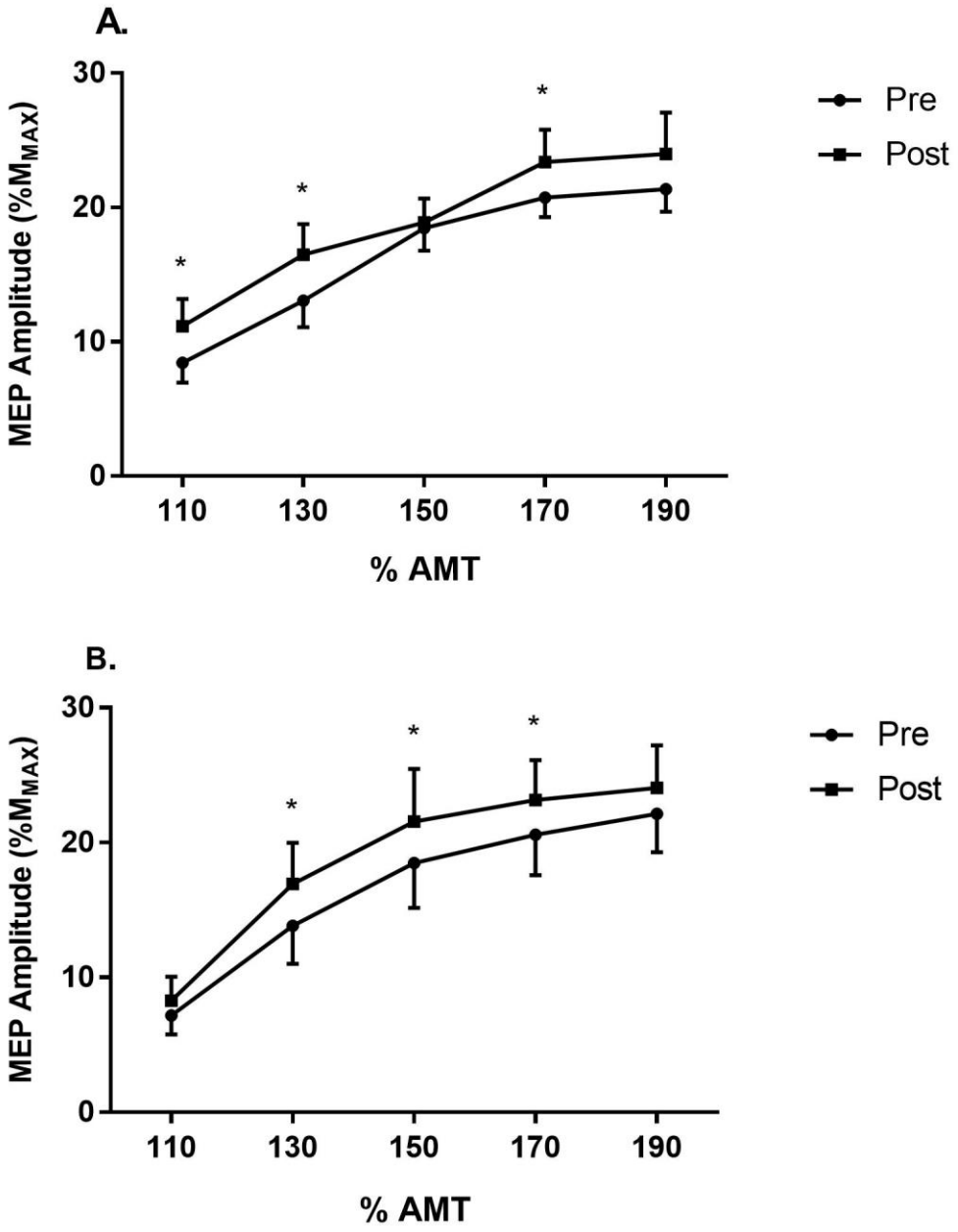

Figure 6.3A-B: Mean (± SE) changes in MEP amplitude following **(A)** ipsilateral sham tDCS + ST **(B)** ipsilateral anodal tDCS + ST. * indicates significant to baseline.

Experiment 2

For the Experiment 2, MEP amplitudes were similar between ipsilateral sham and anodal tDCS without strength training conditions at baseline for each stimulus intensity ($P > 0.05$). Following the ipsilateral anodal tDCS intervention, there was a main effect for TIME ($P < 0.05$) and a TIME × CONDITION interaction ($P < 0.05$) detected at 150% and 170% of AMT (see Figure 6.4). *Post hoc* analysis showed that ipsilateral anodal tDCS increased MEP amplitude by 30-45% compared to a 1-2% change following ipsilateral sham tDCS ($P = 0.002$; $P = 0.003$, respectively).

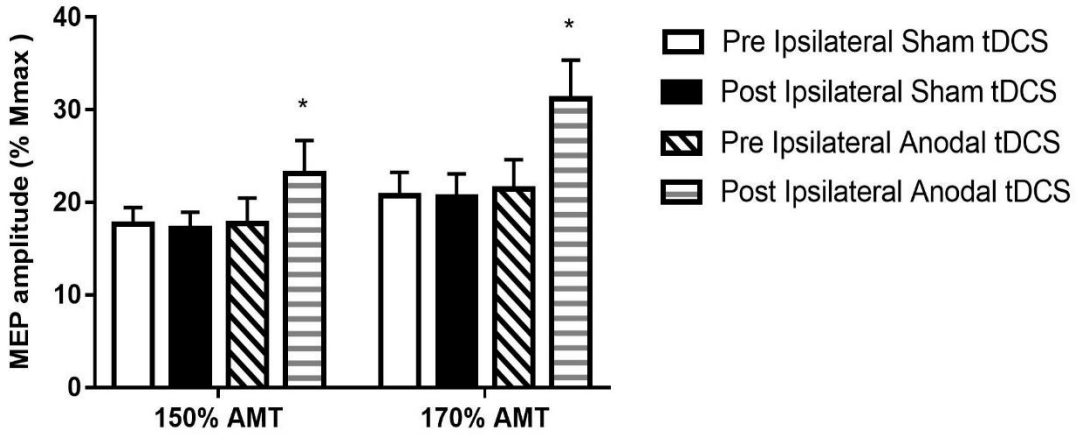

Figure 6.4: Mean (± SE) changes in MEP amplitude at 150% and 170% AMT before and after 20 min of ipsilateral anodal and sham tDCS (Experiment 2) * indicates significant to sham tDCS.

6.3.4 Corticospinal inhibition

Experiment 1

As shown in Figure 6.5A-B, corticospinal inhibition was assessed with the duration of corticospinal silent period obtained at several stimulus intensities above AMT (110-190% AMT; increments of 20%). Corticospinal silent period durations were similar between conditions at baseline across all stimulus intensities ($P > 0.05$). There were no TIME ($P > 0.05$) or TIME × CONDITION ($P > 0.05$) interaction detected following the intervention (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST) for corticospinal silent period durations across all stimulus intensities (110-190% AMT).

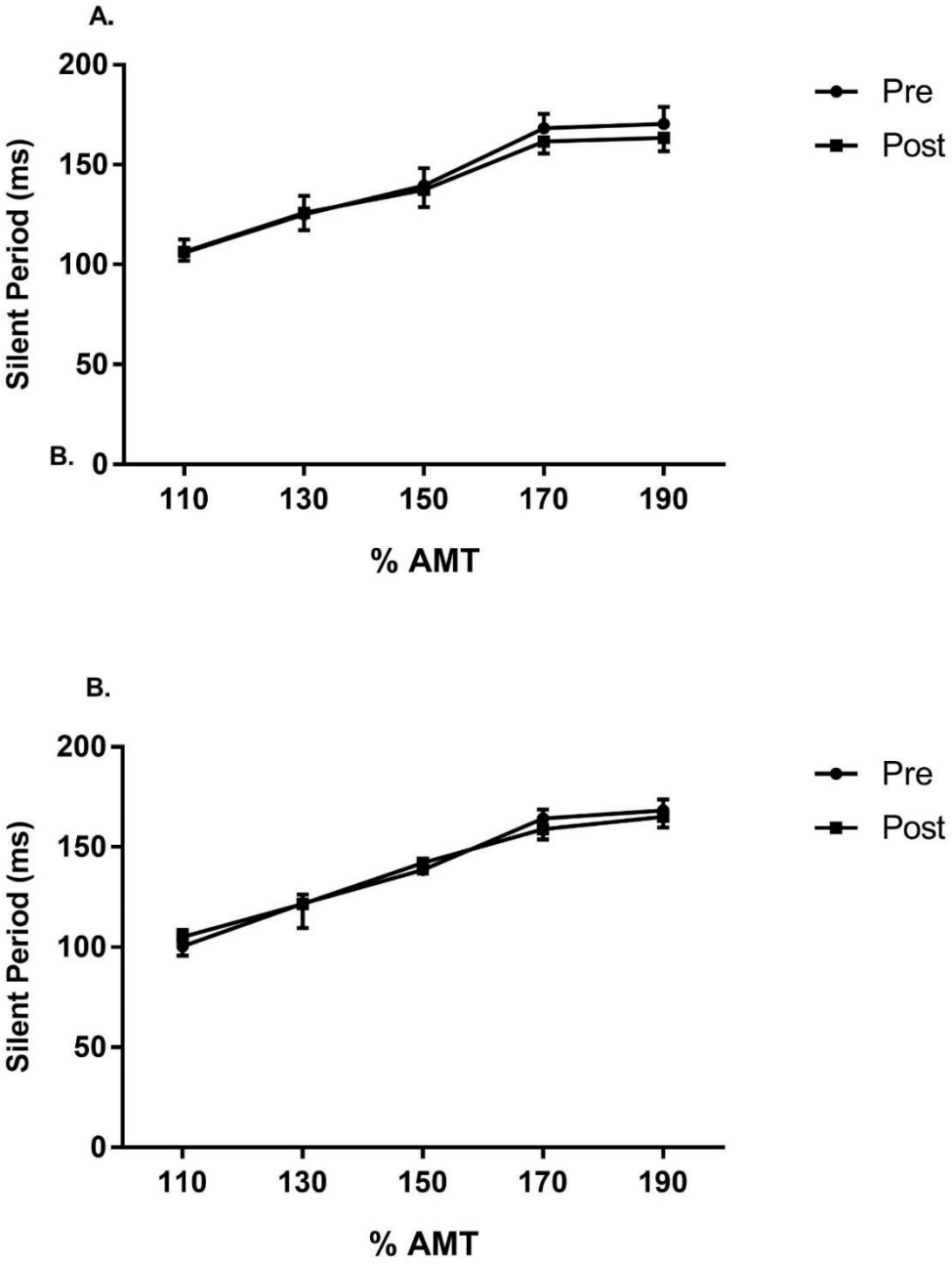

Figure 6.5A-B: Mean (± SE) changes in corticospinal silent period duration following **(A)** ipsilateral sham tDCS + ST **(B)** ipsilateral anodal tDCS + ST.

Experiment 2

For Experiment 2, corticospinal silent period durations were similar between ipsilateral sham and anodal tDCS conditions at baseline for each stimulus intensity (Figure 6.6; $P > 0.05$). Following the tDCS intervention, there were no TIME ($P < 0.05$) or TIME × CONDITION interaction ($P > 0.05$) detected at 150% and 170% of AMT.

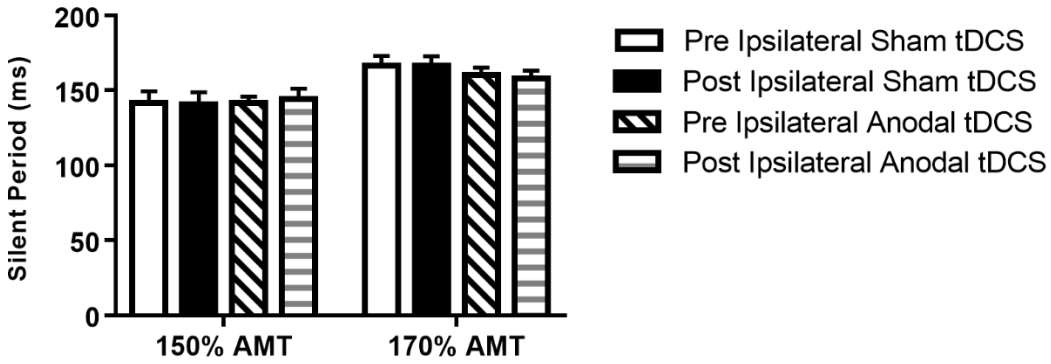

Figure 6.6: Mean (± SE) changes in corticospinal silent period duration at 150% and 170% AMT before and after 20 min of ipsilateral anodal and sham tDCS (Experiment 2).

6.3.5 Cross-activation

MEPs were elicited during maximal contraction of the right (trained) biceps brachii, to determine the effect of activity in the left M1 on corticospinal excitability of the right M1. Figure 6.7 shows the mean change in cross-activation following both conditions (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST). MEP amplitudes for the left biceps brachii during contralateral MVIC were similar between conditions at baseline ($P > 0.05$). Following the anodal tDCS + ST intervention, there was a main effect for TIME ($P = 0.02$); however, no TIME × CONDITION interaction was detected ($P > 0.05$). Cross-activation increased by 25% in the ipsilateral anodal tDCS + ST condition compared to a 7% increase in the ipsilateral sham tDCS + ST condition. However, *post hoc* analysis revealed that the magnitude of change in MEP amplitude was not statistically significant between conditions *(P* > 0.025).

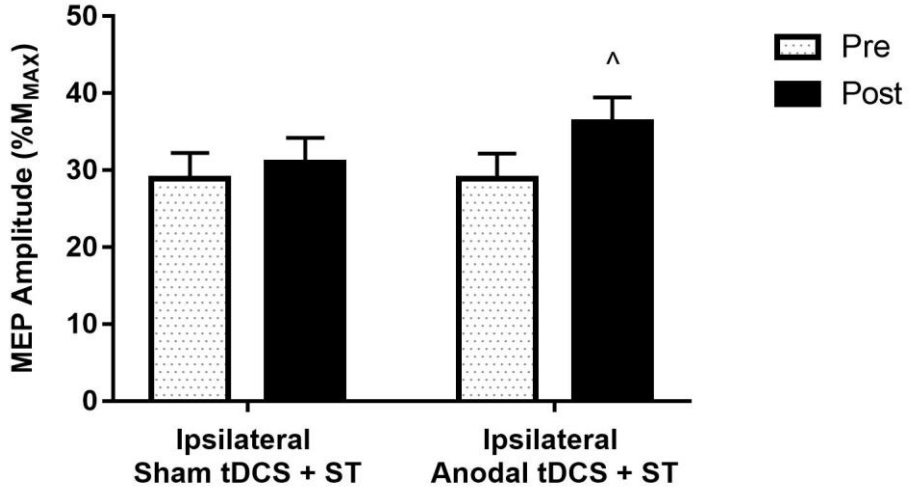

Figure 6.7: Mean (± SE) changes in MEP amplitude at 130% AMT during a contralateral MVIC following ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST. ^ indicates significant to baseline.

6.3.6 Dynamic strength of the untrained limb, cross-activation, corticospinal excitability and inhibition in different *BDNF* genotypes

Experiment 1

For dynamic strength of the left biceps brachii (untrained limb), the GENOTYPE × TIME ANOVA revealed only a TIME effect for both the *Val/Val* and *Val/Met* groups following anodal tDCS ($P = 0.0145$; $P = 0.0327$, respectively). Dynamic muscle strength of the left biceps brachii increased by 11% in the *Val/Val* group and 15% in those with the *Val/Met* polymorphism. *Post hoc* analysis, however, revealed that the magnitude of change in dynamic muscle strength was not statistically significant between genotypes ($P > 0.025$).

For cross-activation, the GENOTYPE × TIME ANOVA revealed no TIME ($P > 0.05$) or TIME × GENOTYPE ($P > 0.05$) interaction following the intervention (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST).

For MEP amplitude, the GENOTYPE × TIME ANOVA revealed only a TIME effect for corticospinal excitability at 130%, 150% and 190% AMT for the *Val/Met* group following ipsilateral anodal tDCS + ST ($P = 0.043$; $P = 0.030$, $P = 0.017$, respectively). At 130% AMT, corticospinal excitability increased by 41% in the *Val/Met* group compared to a 11% increase in the *Val/Val* group. Similarly, at 150% AMT, corticospinal excitability increased by 26% in the *Val/Met* group compared to a 16% increase in the *Val/Val* group. Furthermore, at 190% AMT, corticospinal excitability increased by 17% in the *Val/Met* group compared to 4% increase in the *Val/Val* group. *Post hoc* analysis, however, revealed that the magnitude of change in

MEP amplitude was not statistically significant between genotypes ($P > 0.025$; see Figure 6.8).

For corticospinal silent period duration across all stimulus intensities (110-190% AMT) there were no TIME ($P > 0.05$) or TIME × GENOTYPE ($P > 0.05$) interaction detected following the intervention (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST).

Experiment 2

For MEP amplitude across all stimulus intensities (110-190% AMT) there were no TIME ($P > 0.05$) or TIME × GENOTYPE ($P > 0.05$) interaction detected following the intervention (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST). Similarly, for corticospinal silent period duration across all stimulus intensities (110-190% AMT), there were no TIME ($P > 0.05$) or TIME × GENOTYPE ($P > 0.05$) interaction detected following the intervention (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST).

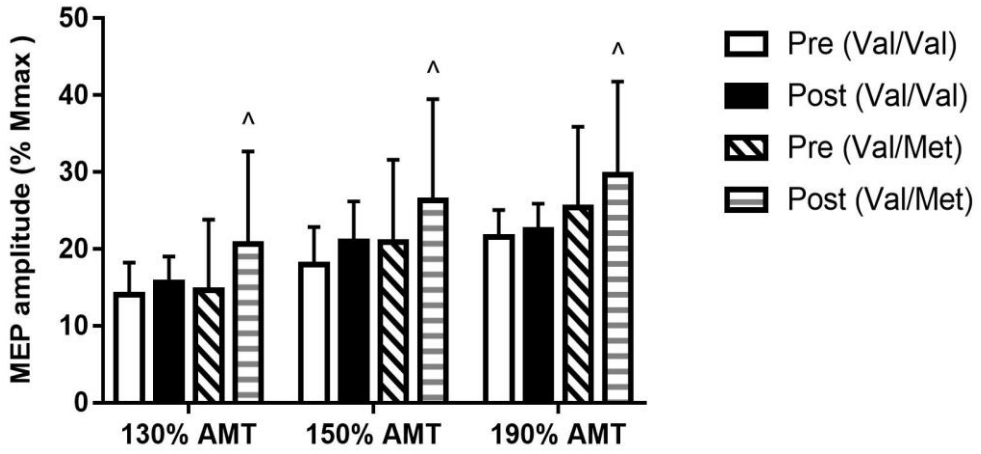

Figure 6.8: Mean (± SE) changes in MEP amplitude at 130%, 170% and 190% AMT (Experiment 1) with different genotypes. ^ indicates significant to baseline.

6.4 Discussion

The aim of this study was to investigate the effect of priming the ipsilateral M1 using anodal tDCS prior to a single bout of strength training on the cross-transfer of strength and to examine the corticospinal responses of the ipsilateral M1. As a secondary investigation, the influence on these responses by the *BDNF* polymorphism were examined. The main findings of ***Experiment 1*** were: (i) 1RM strength of the left biceps brachii (untrained) increased only in the ipsilateral anodal tDCS + ST condition; (ii) Contrary to the hypothesis, both conditions (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST) increased corticospinal excitability to a similar magnitude; and (iii) Corticospinal silent period duration did not change; (iv) The application of ipsilateral anodal tDCS prior to a single bout of strength training (ipsilateral anodal tDCS + ST) facilitated the induction of corticospinal plasticity, which was evident by the increase in cross-activation; and (v) Interestingly, a within time effect for increased corticospinal excitability in the *Val/Met* individuals following ipsilateral anodal tDCS + ST was observed; this is an important finding that warrants some discussion. The main findings for ***Experiment 2*** were: (i) The application of ipsilateral anodal tDCS increased corticospinal excitability; (ii) had no effect on corticospinal silent period duration; and (iii) Contrary to the chapter hypothesis, the induction of corticospinal plasticity (corticospinal excitability and cortical inhibition) following ipsilateral anodal tDCS was not influenced by the *BDNF* polymorphism.

6.4.1 Anodal tDCS applied to the ipsilateral M1 induces homeostatic plasticity

The first important finding of this chapter was the observed increase in corticospinal excitability following the application of ipsilateral anodal tDCS only (Experiment 2). It has been well established that anodal tDCS can induce long-lasting

increases in corticospinal excitability for up to 90 min post stimulation (Nitsche & Paulus 2000, Nitsche & Paulus 2001, Bastani & Jaberzadeh 2012, Kidgell et al. 2013, Pellicciari et al. 2013) with the changes in synaptic strength attributed to modulation of the NMDA receptor (Nitsche et al. 2005, Boggio et al. 2006). Interestingly, no change in corticospinal silent period duration following the application of anodal tDCS to the right M1 (ipsilateral to the trained arm) was observed which may suggest that anodal tDCS does not appear to modulate $GABA_B$ neurons. This finding is similar to a number of studies that have reported no change in corticospinal silent period duration following the application of anodal tDCS (Suzuki et al. 2012, Batsikadze et al. 2013). Of critical importance to Experiment 1, the results of Experiment 2 provide the hypothetical foundation for using anodal tDCS as an ipsilateral M1 priming method prior to a single bout of strength training to augment the cross-education effect and facilitate the induction of corticospinal plasticity of the ipsilateral M1 via the induction of homeostatic plasticity. The increase in corticospinal excitability observed demonstrates that ipsilateral anodal tDCS enhances neural activity of the right M1 (ipsilateral to the training side), thereby effectively inducing homeostatic plasticity.

6.4.2 Anodal tDCS applied to the ipsilateral M1 prior to strength training augments the cross-transfer of strength

Consistent with the chapter hypothesis, a substantial increase in 1RM strength of the left biceps brachii (untrained) was observed when anodal tDCS was applied to the ipsilateral M1 (right hemisphere) prior to a single bout of strength training of the right arm. This finding suggests that the induction of homeostatic plasticity to the ipsilateral M1 prior to the single bout of strength training augmented the cross-transfer of strength. The enhanced cross-education effect observed in the current chapter has occurred by

manipulating the principal of *homeostatic plasticity* and this is new and novel finding. Of interest, Hendy et al. (2014) similarly demonstrated an increase in strength of the untrained forearm when anodal tDCS was applied to the ipsilateral M1 during the strength training task (i.e., gating). However, in contrast to the current chapter, the proportion of strength gained in the untrained arm reported was relatively small (5% vs 12%). Irrespective of what muscle groups were trained (wrist extensors vs biceps brachii), if the fundamental purpose of strength training is to increase strength, then the M1 must adjust by increasing the activation of the motor neuron pool that contributes to strength development (Lee et al. 2009). Therefore, it could be suggested that the timing of the priming technique may be the critical point of difference (not the muscles trained), and thus underpins the larger magnitude of cross-education seen in this chapter.

6.4.3 Corticospinal responses of the ipsilateral M1 following ipsilateral tDCS and cross-education

Several lines of evidence support the view that an increase in corticospinal excitability of both the contralateral and ipsilateral M1 underpins the cross-transfer of motor performance (Ziemann & Hallett 2001, Carroll et al. 2008, Hinder et al. 2010, Hortobagyi et al. 2011, Ruddy & Carson 2013, Leung et al. 2015). Interestingly, an increase in MEP amplitude at several points along the stimulus-response curve following both intervention conditions, (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST) was demonstrated highlighting the potency of unilateral strength training as an effective technique to induce use-dependent corticospinal plasticity. In agreement with this, Leung et al. (2015) recently showed an increase in corticospinal excitability of the ipsilateral M1 following a single session of externally paced biceps brachii strength training. However, in the current chapter, it was unpredicted that the addition of ipsilateral

anodal tDCS prior to the strength training session would not result in greater changes in corticospinal excitability compared to the ipsilateral sham tDCS + ST condition. The lack of interaction between the two conditions (ipsilateral anodal tDCS + ST and ipsilateral anodal tDCS + ST) may be due to a ceiling effect. The application of anodal tDCS may have increased resting levels of synaptic activity prior to the strength training session. However, the intensity of the strength training bout may have overcome the baseline imbalance between conditions, resulting in similar overall increases in corticospinal excitability. Nonetheless, a within effect for cross-activation for those that received anodal tDCS to the ipsilateral M1 prior to the single bout of strength training was observed, which is similar to the findings of Hendy et al. (2014). Cross-activation is indicative of the level of activity of the left M1 on modulating corticospinal excitability of the right M1 (Hendy & Kidgell 2014). This suggests that priming the ipsilateral M1 did augment the corticospinal responses of the ipsilateral M1 under maximal conditions of the contralateral M1, manifesting as an increase in strength of the untrained arm. It is, therefore, likely that anodal tDCS applied to the ipsilateral M1 increased the resting levels of synaptic activity and thereby lowered the synaptic activation threshold of corticospinal neurons of the ipsilateral M1 (Antal 2008). Subsequently, strength training further enhanced corticospinal excitability of the ipsilateral M1, resulting in greater modulation in the neural circuits that project to the untrained limb. This, in turn, may have underpinned the strength gained in the untrained limb observed only when anodal tDCS was applied to the ipsilateral M1 prior to training. Although anodal tDCS + ST increased cross-activation, there was not a significant increase in MEP amplitude between conditions. A possible explanation for this may be due to the timing of TMS measurements post-training. Certainly, there is good evidence that anodal tDCS induces

long-lasting modifications in the membrane potential of neurons which are thought to evolve over a time period of up to 90 min (Nitsche & Paulus 2001). Given the TMS measurements were only taken following the intervention, and no additional time-course measures were obtained, it may be that additional modifications in corticospinal excitability were not detected.

Surprisingly, no change in corticospinal silent period duration following both conditions (ipsilateral anodal tDCS + ST and ipsilateral sham tDCS + ST) was observed. It could be suggested that the combination of anodal tDCS and strength training and strength training alone does not appear to modulate $GABA_B$ neurons, however, we are unclear as to why. Certainly, the duration of the corticospinal silent period has been shown to be unchanged following anodal tDCS alone (Suzuki et al. 2012, Batsikadze et al. 2013); however, a single bout of strength training has previously been shown to decrease SICI of the ipsilateral M1 (Howatson et al. 2011, Leung et al. 2015). Critically, very few strength training or cross-education studies have examined the corticospinal silent period duration (Kidgell & Pearce 2010, Coombs et al. 2016). It may be that a single session of strength training is insufficient to remove local inhibition. A limitation to the current study is that SICI was not measured and, thus, no comment can be made regarding whether the combination of ipsilateral anodal tDCS and strength training may have modulated $GABA_A$ neurons which is indicative of inhibitory mechanisms specific to the M1 (Kujirai et al. 1993, Fisher et al. 2002, Rothwell et al. 2009). Although changes in corticospinal excitability and inhibition for the trained limb (contralateral M1) were not assessed, it remains unresolved as to whether the putative mechanisms that underlie corticospinal changes in the trained limb (contralateral hemisphere) are transferred symmetrically to the ipsilateral hemisphere (Coombs et al. 2016). Regardless of this

limitation, it has been demonstrated that strength improvements in the untrained limb were accompanied by an increase in cross-activation and, as a minimum, it provides evidence that improved ipsilateral corticospinal excitability following anodal tDCS + ST contributes to the cross-education of muscle strength.

6.4.4 Corticospinal excitability and intracortical inhibition is differentially modulated following ipsilateral anodal tDCS and strength training in different BDNF genotypes

Recent data have shown that carriers of the *BDNF Met* allele (*Val/Met*) display reduced corticospinal responses following use-dependent (Kleim et al. 2006, Cirillo et al. 2012) and experimentally-induced plasticity paradigms (Chang et al. 2014, Hwang et al. 2015, Puri et al. 2015, Frazer et al. 2016). However, when individuals were sub-grouped into genotype and individual data examined, the *Val/Met* individuals showed a greater increase in MEP amplitude compared to *Val/Val* individuals. Given the small sample size and lack of research investigating the potential role of the *BDNF* polymorphism within the paradigms of strength training and cross-education, it is difficult to speculate as to why. Although the neurophysiological mechanisms thought to underlie the cross-education effect are believed to involve rapid changes in bilateral cortical excitability that originates at a synaptic level via LTP processes (Ruddy & Carson 2013), it should be highlighted that both groups (*Val/Val* and *Val/Met*) exhibited similar increases in strength of the untrained arm (11% and 15%, respectively). Albeit speculative, this suggests that the *BDNF* polymorphism does not influence the behavioural outcome (i.e., magnitude of strength gained in the untrained arm) in young healthy subjects. Whether this polymorphism plays an important role in long-term training paradigms (i.e., training

study) or in subjects with neurodegenerative diseases (i.e., Parkinson's disease) or following neuro-trauma (i.e., brain lesions, stroke) needs to be clarified in future studies.

Overall, the findings from his chapter show that priming the ipsilateral M1 using anodal tDCS prior to a single bout of strength training augmented the cross-transfer of strength which was accompanied by an increase in corticospinal excitability and cross-activation. The induction of corticospinal plasticity following ipsilateral anodal tDCS and strength training appeared to be influenced by the *BDNF* polymorphism, but interestingly the *Val/Met* individuals demonstrated facilitated responses compared to the *Val/Val* individuals; however, it did not modulate the magnitude of strength gained in the untrained arm.

Chapter 7：General

Discussion and Conclusion

The primary objective of this thesis was to systematically investigate the induction of homeostatic plasticity and its effect on the expression of muscle strength. The secondary objective of this thesis was to investigate the influence of the *BDNF* polymorphism on indices of corticospinal plasticity and strength following experimentally-induced and use-dependent plasticity protocols. This chapter presents an integrated discussion outlining how the major findings of each study described within the thesis contribute to the overarching research questions. Specifically, the discussion will focus on three key themes which include, the induction of homeostatic plasticity, the functional consequences of homeostatic plasticity and the influence of the *BDNF* polymorphism on corticospinal responses and the expression of muscle strength. The discussion concludes with a section that recommends the future direction of research in this area.

7.1 The induction of homeostatic plasticity

tDCS has been used as a NIBS technique to modulate corticospinal excitability and inhibition with the aim of modifying motor behaviour (Ridding & Ziemann 2010). The mechanism proposed to underlie the response of corticospinal output neurons to NIBS protocols including tDCS (Siebner 2010) is the principal of *homeostatic plasticity*. This mechanism describes the process whereby the resting state of corticospinal neurons are altered (increased/decreased level of excitability following a low/high level of synaptic activity) due to changes in postsynaptic glutamate receptor activity (Ziemann & Siebner 2008, Siebner 2010). It is well established that anodal tDCS induces facilitatory effects on MEPs, therefore inducing homeostatic plasticity of the stimulated M1 (Nitsche & Paulus 2000, Nitsche & Paulus 2001). This has led to a rapid influx of studies assessing the feasibility of using anodal tDCS in rehabilitation settings (e.g., stroke) (Hummel et al.

2005, Boggio et al. 2007, Tanaka et al. 2011) and in combination with training interventions to enhance motor function outcomes (Hendy & Kidgell 2014, Sriraman et al. 2014, Christova et al. 2015). However, this accelerated progression of studies would appear to be premature, given that very few studies have examined the bilateral effects of anodal tDCS, and more importantly, whether anodal tDCS modulates adjacent cortical regions. In the context of this thesis, understanding the bilateral effects of anodal tDCS is critical, particularly when using anodal tDCS in a contralateral M1-supraorbital arrangement combined with strength training or the cross-education of strength (Hendy & Kidgell 2014). For example, if anodal tDCS increases corticospinal excitability of the stimulated hemisphere but reduces the excitability of the non-stimulated hemisphere, this may counteract the potential mechanism that are associated with the cross-education of muscle strength. Previously, it has been suggested that the ipsilateral M1 may underpin the cross-education of strength (Lee et al. 2010, Hendy & Kidgell 2014, Hendy et al. 2015). However, given that the magnitude of strength transfer is proportional to the amount of strength gained in the trained limb, it possible that anodal tDCS applied to the ipsilateral M1 may reduce the excitability of the contralateral non-stimulated M1 and, thus, reduce the capacity for neural adaptations and strength development of the trained limb. Thus, based upon this concept, Chapter 3 (Study 1) was an exploratory study that examined the effect of a single session of anodal tDCS on corticospinal excitability and inhibition of both the stimulated and non-stimulated hemisphere. The result of Chapter 3 (Study 1) were significant as they confirmed that anodal tDCS not only improves synaptic efficacy of the stimulated hemisphere, but also modulates corticospinal connections of the non-stimulated hemisphere, giving rise to the notion of *functional connectivity* (Sale et al. 2015). Given that the primary aim of Chapter 3 (Study 1) was to determine if anodal

tDCS resulted in any negative inhibitory effects to the unstimulated M1, it was surprising to observe changes in *functional connectivity*. However, this was an essential finding that shaped Chapter 5 (Study 3) and Chapter 6 (Study 4).

Functional connectivity describes the concept whereby distributed brain regions transiently interact to perform a particular neural function (Shafi et al. 2012). Functional connectivity has evolved from the parallel use of neuroimaging techniques (i.e., fMRI) and brain stimulation methods (e.g., TMS, tDCS etc.) within healthy and clinical populations (Sale et al. 2015). Importantly, understanding the interaction between different specialised cortical neural areas and how changes in localised brain activity can influence distant, but functionally related, areas is critical to maintaining healthy brain function (Sale et al. 2015). Previously, tDCS of the motor association cortex has been shown to induce inhibitory effects in the M1 (Kirimoto et al. 2011); also, stimulation of the premotor cortex facilitated the M1 by reducing short-interval intracortical inhibition (SICI) (Boros et al. 2008). The bilateral increase in corticospinal excitability observed in Chapter 3 (Study 1) further extends the current knowledge of tDCS activating widespread neural structures by showing the application of anodal tDCS to the contralateral M1 increases corticospinal excitability of the ipsilateral MI. Given that tDCS is easy to use and affordable, Chapter 3 (Study 1) provides a theoretical framework for using anodal tDCS to induce ipsilateral effects. These will form a foundation for future targeted interventions using tDCS in people with neurological disorders, particularly where there is reduced capacity to use a single limb. Furthermore, there has been a considerable amount of research examining the focal properties of tDCS in regards to the shape and size of electrodes (Nitsche et al. 2007, Bastani & Jaberzadeh 2013). To increase the efficacy of anodal tDCS interventions by improving the focality of stimulation, it has

been proposed that future tDCS studies reduce the stimulation electrode size to one third of the conventional size (Bastani & Jaberzadeh 2013). However, if tDCS is being used within a functional connectivity model, whereby the aim is to stimulate nearby functionally connected cortical areas, the use of large rectangular-pad electrode configurations is likely to be crucial. The translation of anodal tDCS into a functional connectivity model may be an effective rehabilitation tool to maintain functional connectivity within the healthy ageing brain and in re-establish optimal neural activity within clinical populations.

The collective results of Chapters 3 (Study 1) and 4 (Study 2) show that anodal tDCS improves synaptic efficacy by means of increased corticospinal excitability of the stimulated and non-stimulated hemisphere, and importantly, increases the net descending drive (VA_{TMS}) to the motor neuron pool (Chapter 4, Study 2). It has been well established that anodal tDCS can induce long-lasting increases in corticospinal excitability of the stimulated hemisphere for up to 90 minutes post stimulation (Nitsche & Paulus 2000, Nitsche & Paulus 2001, Bastani & Jaberzadeh 2012, Kidgell et al. 2013, Pellicciari et al. 2013), with the changes in synaptic strength attributed to modulation of the NMDA receptor (Nitsche et al. 2005, Boggio et al. 2006). Additionally, Chapter 3 (Study 1) added to our methodological understanding by exploring where the magnitude of bilateral corticospinal plasticity was affected by the direction of stimulation (dominant vs non-dominant M1 stimulated), and if there was a greater scope for the induction of corticospinal plasticity of the non-dominant hemisphere. Chapter 3 (Study 1) showed that the magnitude of corticospinal excitability of both the stimulated and non-stimulated hemisphere was not directional and was irrespective of hemispheric dominance. This finding was unexpected as the non-dominant hemisphere has previously been shown to

have lower motor threshold, higher MEPs (De Gennaro et al. 2004) and shorter corticospinal silent period durations (Priori et al. 1999), which suggests that the non-dominant hemisphere would have had a greater capacity for the rapid induction of corticospinal plasticity following anodal tDCS. However, in Chapter 3 (Study 1) there were no baseline differences in corticospinal excitability or inhibition measurements which may account for this finding. Interestingly, this finding has important clinical implications for Studies 3 and 4 which focussed on the effect of anodal tDCS on the corticospinal responses to strength training and the effect of ipsilateral anodal stimulation on the cross-education of muscle strength. At a minimum, Chapter 3 (Study 1) proposes that ipsilateral anodal tDCS could be applied to either hemisphere to induce corticospinal plasticity, suggesting that either the left or right limb may be strength trained to improve the cross-education of strength for either limb. This notion is supported by recent cross-education and tDCS studies, showing the importance of the ipsilateral M1 (Hendy & Kidgell 2014, Hendy et al. 2015, Coombs et al. 2016).

At present, the greater part of the tDCS literature has focused heavily on the modulation of corticospinal excitability and subsequent improvements in motor performance (Boggio et al. 2006, Vines et al. 2006, Cogiamanian et al. 2007, Tanaka et al. 2009, Tanaka et al. 2011, Kidgell et al. 2013). In contrast, there are very few studies that have examined the effect of anodal tDCS on corticospinal inhibition. This is surprising considering the removal of local inhibition is thought to be critical for the activation of skeletal muscle and improvements in motor performance (Stinear & Byblow 2003). It is important to understand how anodal tDCS may modulate inhibitory pathways mediated by $GABA_A$ and $GABA_B$, as a reduction in the temporary suppression of motor cortical output may be a putative neural mechanism underlying changes in motor unit

recruitment (VA_{TMS}), therefore contributing to improvements in muscle strength. However, the effects of anodal tDCS on corticospinal inhibition, and the contribution of SICI and corticospinal silent period duration to the development and/or expression of muscle strength, remain unresolved.

Considering the above, Chapter 3 (Study 1) did not show a bilateral decrease in corticospinal silent period duration, rather it showed a shift in lateralization of inhibition to the right (non-dominant) M1, irrespective of which hemisphere was stimulated. Contrary to the hypothesis, only a change in corticospinal inhibition in the non-stimulated hemisphere was observed following anodal tDCS applied over the non-dominant M1. This finding was surprising given that Chapter 4 (Study 2) demonstrated a decrease in corticospinal silent period duration following four consecutive sessions of anodal tDCS. It may be that a single session of anodal tDCS is insufficient to remove local inhibition, and that modulation of $GABA_B$ receptors requires repeated stimulation (e.g., four consecutive bouts of anodal tDCS in Study 2). However, it should be noted that, unexpectedly, there was no change in SICI in Chapter 4 (Study 2) suggesting that accumulated bouts of anodal tDCS appear to preferentially modulate $GABA_B$ rather than $GABA_A$ neurons. The explanation for this finding remains unclear and, given that SICI was not measured in Chapter 3 (Study 1), it is difficult to speculate whether there is a difference in the response of inhibitory pathways between single session stimulation and repeated stimulation. Another plausible explanation could be, that the bilateral effects of anodal tDCS may simply be different to the unilateral effects. For example, the shift in hemispheric activity observed in Chapter 3 (Study 1) may be due to changes in transcallosal inhibition, which is thought to be mediated via projections confined to the contralateral M1 (Lang et al. 2004). This finding is similar to the conclusions of Lang et

al. (2004) who found that 10 min of anodal and cathodal tDCS at 1 mA modulated transcallosal inhibition. Of particular significance, it has previously been demonstrated that transcallosal pyramidal neurons appear to share similar inhibitory control as corticospinal pyramidal neurons (Trompetto et al. 2004). In light of this evidence, and the shift in hemispheric activity observed in Chapter 3 (Study 1), it appears that anodal tDCS acts upon transcallosal fibres which are thought to be excitatory and glutamatergic, which in turn synapse onto inhibitory interneurones within the opposite cortex to produce their inhibitory effect (Meyer et al. 1995). This provides additional evidence that anodal tDCS modulates functional connectivity, which strengthens the working hypothesis that anodal tDCS is a viable method to induce homeostatic plasticity of motor and associative areas, which in turn potentially contribute to the development and/or expression of muscle strength.

As Chapter 3 (Study 1) demonstrated that anodal tDCS was a viable technique to induce homeostatic plasticity, this supported the concept that neural substrates that are thought to underpin strength development could be augmented by the application of anodal tDCS. Chapter 4 (Study 2) confirmed this concept and assessed whether the temporary modification of corticospinal plasticity following anodal tDCS corresponded with transient improvements in motor performance (in the absence of motor training). This chapter found that four consecutive sessions of anodal tDCS increased corticospinal excitability but, more notably, increased VA_{TMS} which manifested as an improvement in voluntary strength. Several studies have shown that incomplete voluntary activation (using twitch interpolation) manifests as a reduction in the 'voluntary' force generating capacity of the muscle due to reduced neural drive at or above the site of stimulation of the motor nerve (Herbert & Gandevia 1996, Allen 1998). However, a limitation of the

twitch interpolation technique is that it fails to define the site of neural drive impairment (Todd et al. 2003). Thus, more recently, TMS has been used to assess 'cortical' voluntary activation (Lee et al. 2008). Unlike twitch interpolation, the presence of a superimposed twitch force produced by a suprathreshold TMS pulse during an MVIC indicates a failure in neural drive at the level of the M1 (Todd et al. 2003). The use of this robust measure in Study 2 suggests that the corticospinal responses underlying the early development of strength (Selvanayagam et al. 2011) likely involve the modulation of synaptic efficacy, resulting in an increase in motor cortical drive to the motor neuron pool of the target muscle (wrist flexors).

7.1.1 The influence of the *BDNF* polymorphism on the induction of homeostatic plasticity

A fundamental theme of the thesis was to identify whether the *BDNF* polymorphism influenced the induction of homeostatic plasticity to anodal tDCS. Although the sample size of each genotype (*Val/Val*, *Val/Met*) was relatively small, the within (time) effects observed in Studies 1 and 2 suggest that the presence of the *BDNF* polymorphism influences the corticospinal responses to anodal tDCS. This was demonstrated in Chapters 3 (Study 1) and 4 (Study 2) whereby carriers of the *BDNF Met* allele displayed reduced corticospinal responses following the application of a single session and repeated bouts of anodal tDCS. In Chapter 3 (Study 1) it was demonstrated that the *Val/Val* individuals showed a greater increase in MEP amplitude compared to *Val/Met* individuals following a single session of anodal tDCS, and this trend was evident for both the dominant and non-dominant M1, irrespective of which hemisphere was stimulated. This was similar to the findings in Chapter 4 (Study 2) where the *Val/Val* individuals again showed a greater increase in MEP amplitude compared with *Val/Met*

individuals following four consecutive sessions of anodal tDCS. Importantly, previous TMS and neuroimaging studies have reported a profound effect of the *BDNF* polymorphism on cortical morphology (Pezawas et al. 2004) and synaptic activity (LTP, efficacy of neural transmission, brain activation volumes) underlying corticospinal plasticity (Garry et al. 2004, Kleim et al. 2006, McHughen et al. 2010). Based upon this, the time effects observed for inducing corticospinal plasticity in only the *Val/Val* participants following anodal tDCS further confirms that the *BDNF* polymorphism plays an important role in shaping experimentally-induced corticospinal plasticity. The lack of *Met/Met* individuals recruited was not unexpected given the expression of the *Met* allele is more commonly found among Asian (51% in Japan) compared to Caucasian populations (30% in America) (Shimizu et al. 2004). Therefore, it remains undetermined whether there is a difference in corticospinal plasticity responses to anodal tDCS between the three genotypes (homocygote *Val/Val* and *Met/Met,* and heterocygote *Val/Met*).

Of interest, the *BDNF* polymorphism did not differentially modulate VA_{TMS} or muscle strength in Chapter 4 (Study 2). This finding is not necessarily unexpected given that VA_{TMS} describes changes in motor cortical output via the recruitment of motor units used in force generation (Todd et al. 2003, Todd et al. 2004), and a change in muscle strength is fundamentally represented by a change in motor unit behaviour. Despite the *BDNF* polymorphism being important for higher cognitive functions such as motor learning (Egan et al. 2003, Hariri et al. 2003, Chen et al. 2006), the results from Chapter 4 (Study 2) suggest that the *BDNF* polymorphism does not play a critical role in the voluntary activation of skeletal muscle. Furthermore, the exchange of valine to methionine (*val66met* polymorphism) also appears to be inconsequential for the cross-

transfer of strength (Chapter 6, Study 4). This is discussed in more detail below (Section 7.2.1).

7.2 The functional consequences of homeostatic plasticity

tDCS has emerged as a popular paradigm of motor priming, which is thought to facilitate motor learning (Stoykov & Madhavan 2015). Both *gating* and *homeostatic plasticity* have been proposed to underlie the corticospinal responses to priming protocols including tDCS (Siebner 2010). Briefly, *gating* is attained concurrently with motor training and has previously been shown to facilitate motor performance (Nitsche et al. 2003d, Boggio et al. 2006, Galea & Celnik 2009, Hunter et al. 2009, Reis et al. 2009, Stagg et al. 2011, Hendy & Kidgell 2014) due to the disinhibition of intracortical inhibitory circuits (Ziemann & Siebner 2008, Siebner 2010). However, in line with the overall aim of this thesis, the principal of *homeostatic plasticity* will be the focus of the general discussion. As previously stated (introduction), *homeostatic plasticity* describes the process whereby the resting state of corticospinal neurons is altered (increased/decreased level of excitability following a low/high level of synaptic activity) due to changes in postsynaptic glutamate receptor activity (Ziemann & Siebner 2008, Siebner 2010). Importantly, Chapter 4 (Study 2) demonstrated that accumulative bouts of anodal tDCS increased VA_{TMS}, which manifested as an improvement in voluntary strength. This provided the working hypothesis that the manipulation of homeostatic plasticity via anodal tDCS could result in greater changes in synaptic efficacy, which could ultimately augment the corticospinal responses to a single bout of strength training. Recently, strength training has been employed to induce use-dependent plasticity (Gabriel et al. 2006, Falvo et al. 2010, Carroll et al. 2011), however, there are mixed findings from studies that have investigated the potential underlying corticospinal responses following

a single session of strength training (Hortobagyi et al. 2011, Selvanayagam et al. 2011, Leung et al. 2015, Nuzzo et al. 2016). Nonetheless, the general consensus is that changes in synaptic efficacy within neural pathways that control specific muscles are likely to influence muscle activation and consequently improve force production (Carroll et al. 2001b).

Given the findings of Chapter 3 (Study 2), it was established that anodal tDCS alone (i.e., in the absence of training) induces homeostatic plasticity and, consequently, improves muscle strength. This provided the theoretical framework for anodal tDCS to be utilised as a M1 priming tool to increase synaptic activity prior to a single bout of strength training to further augment the acute corticospinal responses to strength training. The results of Chapter 5 (Study 3) confirmed that the manipulation of homeostatic plasticity resulted in the facilitation of corticospinal responses (i.e., increased corticospinal excitability and decreased corticospinal silent period duration) to a single session of strength training, thus, further extending the idea of anodal tDCS being useful as a M1 priming protocol. Despite the insufficient evidence correlating changes in corticospinal excitability and inhibition and strength development, Chapter 4 (Study 2) established that accumulative bouts of anodal tDCS increased VA_{TMS}, which manifested as an improvement in voluntary strength. This finding suggests that the corticospinal responses underlying the early development of strength (Selvanayagam et al. 2011) are likely to involve the modulation of synaptic efficacy, resulting in the increase in motor cortical drive to the intended motor neuron pool. Therefore, it may be speculated that the manipulation of homeostatic plasticity showed in Chapter 5 (Study 3), resulting in changes in synaptic efficacy (i.e., increased corticospinal responses), could have enhanced the development of strength for the trained arm (right Bicep Brachii). However,

given the nature of the heavy-load strength training protocol employed (80% 1RM, 4-6 sets of 6-8 repetitions) in this chapter, it was not feasible to re-measure dynamic strength of the right arm following the intervention (anodal tDCS and strength training). Importantly, this established the methodology for Chapter 6 (Study 4) whereby a cross-education model was used to investigate the working hypothesis of inducing homeostatic plasticity prior to a single bout of strength training to increase muscle strength of the untrained limb.

Given that Chapter 3 (Study 1) established that anodal tDCS did not have a negative effect on the non-stimulated hemisphere (i.e., does not decrease corticospinal excitability), it gave rise to the notion that anodal tDCS could be applied to the ipsilateral M1 in anticipation of increasing ipsilateral corticospinal responses to strength training and the strength of the untrained limb (Study 4). Chapter 6 (Study 4) revealed that anodal tDCS applied to the ipsilateral M1 (ipsilateral to the trained arm) increased the strength of the untrained arm. However, in Chapter 6 (Study 4), it was not predicted that the addition of ipsilateral anodal tDCS prior to the strength training session would not result in greater changes in corticospinal excitability compared to the ipsilateral sham tDCS and strength training condition. The lack of interaction between the two conditions (ipsilateral anodal tDCS and strength training/sham tDCS and strength training) may be due to a ceiling effect. The application of anodal tDCS may have increased resting levels of synaptic activity prior to the strength training session. However, the intensity of the strength training bout may have overcome the baseline imbalance between conditions, resulting in similar overall increases in corticospinal excitability. However, there was a within effect observed for cross-activation for those that received anodal tDCS to the ipsilateral M1 prior to the single bout of strength training. Given that cross-activation is

indicative of the level of activity of the left M1 on corticospinal excitability of the right M1 (Hendy & Kidgell 2014), this suggests that priming the ipsilateral M1 augmented the corticospinal responses of the ipsilateral M1 under maximal conditions of the contralateral M1, displaying as an increase in strength of the untrained arm. However, a limitation of Chapter 6 (Study 4) was that VA_{TMS} was not measured. This would have provided additional information regarding changes in motor cortical output via the recruitment of motor units used in force generation.

The effects of priming the M1 using anodal tDCS prior to a single session of strength training on cortical inhibition remain elusive. This was highlighted in Chapter 5 (Study 3) whereby a decrease in corticospinal silent period was only observed when anodal tDCS was combined with a single session of strength training. Surprisingly, a similar trend in the ipsilateral M1 was not observed (Chapter 6, Study 4) when anodal tDCS was applied to the ipsilateral M1 prior to a single session of strength training. A potential overarching explanation for the inconsistency of results for corticospinal silent period duration across all Studies (1-4) may also lie in the reliability of TMS. At present, there are limited studies examining the reliability of corticospinal silent period duration and there is no universal specified method of measurement (Fritz et al. 1997, Damron et al. 2008, Hermsen et al. 2016). One positive aspect, however, is that, during all studies, the experimenter was blinded to each condition and every signal was measured manually by the same individual. It has been previously shown that automated analysis of corticospinal silent period is no more reliable than visual inspection (Damron et al. 2008, Hermsen et al. 2016). Although speculative, the additional measure of SICI in Studies 3 and 4 may have also provided further insight as to whether the combination of strength training and anodal tDCS preferentially modulates $GABA_A$ or $GABA_B$ neurons, or if the

responses of the inhibitory pathways are dependent upon the hemisphere stimulated by anodal tDCS (contralateral or ipsilateral to the training arm).

7.2.1 The influence of the *BDNF* polymorphism on experimentally-induced and use-dependent plasticity

Chapter 3 (Study 1) provided the foundation that the presence of the *BDNF* polymorphism may influence indices of corticospinal plasticity (corticospinal excitability/inhibition) which are thought to underpin the development of strength and the cross-transfer of strength to the untrained limb following strength training. Contrary to this hypothesis, there were no effects of the *BDNF* polymorphism on corticospinal excitability or inhibition in Chapter 5 (Study 3) when anodal tDCS was applied prior to the single session of strength training. This finding was unexpected; however, it may be that the combination of anodal tDCS and strength training reduced the genotype effect that has previously been reported following motor skill training (Kleim et al. 2006, Cirillo et al. 2012). It has previously been shown that intense training of the FDI muscle eliminated the effect of the *val66met BDNF* on short-term plasticity (McHughen et al. 2011). However, given that those with the *BDNF* polymorphism have reduced responses to anodal tDCS, it could be that individuals with the *BDNF Met* allele require the addition of tDCS as a priming technique to maximise use-dependent plasticity protocols (i.e., strength training). Interestingly, in Chapter 6 (Study 4) the *Val/Met* individuals showed a greater increase in MEP amplitude compared to *Val/Val* individuals following ipsilateral anodal tDCS and strength training. Despite the differences in corticospinal responses between genotypes observed in Chapters 4 (Study 2) and 6 (Study 4), it should be highlighted that both *Val/Val* and *Val/Met* individuals exhibited similar increases in isometric and dynamic strength (Study 2: 10% and 9%; Study 4: 11% and 15%,

respectively). Considering this, it could be proposed that those without the *BDNF* polymorphism (*Val/Val)* respond more rapidly to anodal tDCS and, thus, there is less scope for further changes in corticospinal plasticity following strength training (i.e., ceiling effect). In contrast, carriers of the *BDNF Met* allele do not respond as rapidly to anodal tDCS and, therefore, require additional stimuli (i.e., strength training) to maximise the induction of corticospinal plasticity. Certainly, the results of Chapter 3 (Study 1) and Chapter 4 (Study 2) whereby carriers of the *BDNF Met* allele displayed reduced corticospinal responses to anodal tDCS compared to *Val/Val* individuals, support this working hypothesis.

Overall, the findings from this thesis collectively suggest that, although the *BDNF* polymorphism appears to influence indices of corticospinal plasticity, it does not affect the expression of strength in healthy populations. If the fundamental goal of motor training intervention is to increase muscle strength, whether it be by means of experimentally-induced plasticity (i.e., anodal tDCS), use-dependent plasticity (i.e., strength training) or a combination of the two paradigms, the presence of the *BDNF* polymorphism does not appear to impact upon functional outcomes. However, it should be distinguished that, within the rehabilitation setting, whereby the goal of an intervention is to maximise the induction of corticospinal plasticity via experimental means (i.e., tDCS) and/or use-dependent protocols (i.e., strength training), the *BDNF* polymorphism may be an important factor when planning individualised rehabilitation programs. Certainly, it may be that carriers of the *BDNF Met* allele require the combination of experimentally-induced (i.e., tDCS) and use-dependent plasticity protocols (i.e., strength training) to maximise rehabilitation outcomes.

Given that Chapters 3 (Study 2), 4 (Study 3) and 6 (Study 4) all demonstrated that the *BDNF* polymorphism influences indices of corticospinal plasticity (i.e., corticospinal excitability and inhibition), this gave rise to an interesting consideration of the variability of previous literature. As evident in Chapter 4 (Study 2) the significant increase in corticospinal excitability observed was a product of the larger representative sample of *Val/Val* individuals compared to *Val/Met* individuals. Conversely, if there was a larger sample of *Val/Met* individuals, there may have not been such dramatic changes in corticospinal excitability and inhibition and, therefore, it may have not been concluded that anodal tDCS improved synaptic efficacy by means of modulation of the NMDA receptor. At a minimum, it suggests that the literature may be unknowingly biased in reporting results of responders (*Val/Val* individuals) giving a skewed outlook of the prospective applications of the NIBS technique. However, as previously discussed, this may also be due to the *BDNF* polymorphism being expressed at lower levels among Caucasians within the general population (Shimizu et al. 2004).

7.3 Conclusion

The objective of this thesis was to systematically investigate the induction of homeostatic plasticity and its effect on muscle strength. A secondary objective was to investigate the influence of the *BDNF* polymorphism on indices of corticospinal plasticity and strength following experimentally-induced (anodal tDCS) and use-dependent plasticity (strength training) protocols. This thesis has shown that the induction of homeostatic plasticity plays an important role in the expression of muscle strength. Anodal tDCS does not appear to act focally upon the site of stimulation, but rather has a global effect on other neural structures (i.e., non-stimulated M1), demonstrating functional connectivity. The presence of the *BDNF* polymorphism influences

corticospinal responses to anodal tDCS (i.e., corticospinal excitability) but does not play a role in functional measures such as VA_{TMS} or the expression of muscle strength.

From the results of the thesis, several areas of future research have been identified. The addition of anodal tDCS time-course measures on both the stimulated and non-stimulated hemispheres in Study 1 would provide further information regarding the potential offline processes resulting from anodal tDCS, and whether this is different between hemispheres. Certainly, changes in inhibition have previously been shown to occur 30 min post stimulation (Hummel et al. 2005, Kidgell et al. 2013) which suggest that changes in cortical inhibition may have been missed. Given that functional connectivity was demonstrated between hemispheres in Study 1, this would indicate that there is a need for a series of studies to investigate the effect of stimulating neural structures other than the M1 (i.e., pre-motor cortex) on indices of corticospinal excitability of the M1 and the effect on motor function. Furthermore, to extend the understanding of the effects of priming the M1 using anodal tDCS prior to strength training on cortical inhibition, future studies should also measure SICI and long-interval intracortical inhibition (LICI). This would provide further insight into the modulation of inhibitory pathways which may contribute to the cross-transfer of strength.

An overall limitation to the thesis is that measures at a segmental level, particularly cervicomedullary MEPs and H-reflex, were not recorded. These would have provided additional information as to the site of adaptation within the corticospinal tract following experimentally-induced and use-dependent plasticity protocols. Furthermore, to strengthen the understanding of the influence of the *BDNF* polymorphism on corticospinal plasticity, a larger sample size of each *BDNF* genotype would allow for correlational analyses of changes in neurophysiological parameters and genotype.

Although this thesis did not observe any relationship between the *BDNF* polymorphism and the development of acute strength, future studies need to clarify whether this polymorphism plays an important role in long-term training paradigms (i.e., strength training study) or in subjects with neurodegenerative diseases (e.g., Parkinson's disease) or following neuro-trauma (e.g., brain lesions, stroke).

References

Adkins, DL, Boychuk, J, Remple, MS & Kleim, JA 2006, 'Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord', *Journal of Applied Physiology,* vol. 101**,** no. 6, pp. 1776-1782.

Allen, GM & Gandevia, SC 1998, 'Twitch interpolation of the elbow flexor muscles at high forces', *Muscle & Nerve,* vol. 2**,** no. 3, pp. 318-328.

Alonzo, A, Brassil, J, Taylor, JL, Martin, D & Loo, CK 2012, 'Daily transcranial direct current stimulation (tDCS) leads to greater increases in cortical excitability than second daily transcranial direct current stimulation', *Brain Stimulation,* vol. 5**,** no. 3, pp. 208-213.

Antal, A, Nitsche, MA & Paulus, W 2008, 'Prior state of cortical activity influences subsequent practicing of a visuomotor coordination task.', *Neuropsychologia,* vol. 46**,** no. 13, pp. 3157-3161.

Antal, A, Chaieb, L, Moliadze, V, Monte-Silva, K, Poreisz, C, Thirugnanasambandam, N, Nitsche, MA, Shoukier, M, Ludwig, H & Paulus, W 2010, 'Brain-derived neurotrophic factor (BDNF) gene polymorphisms shape cortical plasticity in humans', *Brain Stimulation,* vol. 3**,** no. 4, pp. 230-237.

Aroniadou, VA & Keller, A 1995, 'Mechanisms of LTP Induction in Rat Motor Cortex in vitro', *Cerebral Cortex,* vol. 5**,** no. 4, pp. 353-362.

Bastani, A & Jaberzadeh, S 2012, 'Does anodal transcranial direct current stimulation enhance excitability of the motor cortex and motor function in healthy individuals and subjects with stroke: A systematic review and meta-analysis', *Clinical Neurophysiology*, vol. 123**,** no. 4, pp. 644-657.

Bastani, A & Jaberzadeh, S 2013, 'a-tDCS Differential Modulation of Corticospinal Excitability: The Effects of Electrode Size', *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation,* vol. 6**,** no. 6, pp. 932-937.

Bath, K & Lee, F 2006, 'Variant BDNF (Val66Met) impact on brain structure and function', *Cognitive Affective Behavioral Neuroscience,* vol. 6**,** no. 1, pp. 79-85.

Batsikadze, G, Moliadze, V, Paulus, W, Kuo, MF & Nitsche, MA 2013, 'Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans', *Journal of Physiology,* vol. 591**,** no. 7, pp. 1987-2000.

Bear, MF & Malenka, RC 1994, 'Synaptic plasticity: LTP and LTD', *Current Opinion in Neurobiology,* vol. 4**,** no. 3, pp. 389-399.

Beck, S, Taube, W, Gruber, M, Amtage, F, Gollhofer, A & Schubert, M 2007, 'Task-specific changes in motor evoked potentials of lower limb muscles after different training interventions', *Brain Research,* vol. 1179**,** no. null, pp. 51-60.

Bliss, TV & Collingridge, GL 1993, 'A synaptic model of memory: long-term potentiation in the hippocampus', *Nature,* vol. 361**,** no. 6407, pp. 31-39.

Boggio, P, Castro, L, Savagim, E, Braite, R, Cruz, V, Rocha, R, Rigonatti, S, Silva, M & Fregni, F 2006, 'Enhancement of non-dominant hand motor function by anodal transcranial direct current stimulation', *Neuroscience Letters,* vol. 404**,** no. 1-2, pp. 232-236.

Boggio, PS, Nunes, A, Rigonatti, SP, Nitsche, MA, Pascual-Leone, A & Fregni, F 2007, 'Repeated sessions of noninvasive brain DC stimulation is associated with motor

function improvement in stroke patients', *Restorative Neurology and Neuroscience,* vol. 25**,** no. 2, pp. 123-129.

Boros, K, Poreisz, C, Münchau, A, Paulus, W & Nitsche, MA 2008, 'Premotor transcranial direct current stimulation (tDCS) affects primary motor excitability in humans', *European Journal of Neuroscience,* vol. 27**,** no. 5, pp. 1292-1300.

Butefisch, C, Davis, B, Wise, S, Sawaki, L, Kopylev, L, Classen, J & Cohen, L 2000, 'Mechanisms of use-dependent plasticity in the human motor cortex', *Proceedings of the National Academy of Sciences of the United States of America,* vol. 97**,** no. 7, pp. 3661-3665.

Cantello, R, Gianelli, M, Civardi, C & Mutani, R 1992, 'Magnetic brain stimulation: The silent period after the motor evoked potential', *Neurology,* vol. 42**,** no. 10, pp. 1951.

Carroll, R, Riek, S & Carson, R 2002, 'The sites of neural adaptation induced by resistance training in humans', *Journal of Physiology,* vol. 544**,** no. 2, pp. 641-652.

Carroll, T, Riek, S & Carson, R 2001a, 'Reliability of the input–output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation', *Journal of Neuroscience Methods,* vol. 112**,** no. 2, pp. 193-202.

Carroll, T, Riek, S & Carson, RG 2001b, 'Neural adaptations to resistance training', *Sports Medicine,* vol. 31**,** no. 12, pp. 829-840.

Carroll, TJ, Herbert, RD, Munn, J, Lee, M & Gandevia, SC 2006, 'Contralateral effects of unilateral strength training: evidence and possible mechanisms', *Journal of Applied Physiology,* vol. 101**,** no. 5, pp. 1514-1522.

Carroll, TJ, Lee, M, Hsu, M & Sayde, J 2008, 'Unilateral practice of a ballistic movement causes bilateral increases in performance and corticospinal excitability', *Journal of Applied Physiology*, vol. 104**,** no. 6, pp. 1656-1664.

Carroll, TJ, Riek, S & Carson, RG 2001c, 'Corticospinal responses to motor training revealed by transcranial magnetic stimulation', *Exercise and Sport Sciences Reviews,* vol. 29**,** no. 2, pp. 54-59.

Carroll, TJ, Selvanayagam, VS, Riek, S & Semmler, JG 2011, 'Neural adaptations to strength training: Moving beyond transcranial magnetic stimulation and reflex studies', *Acta Physiologica,* vol. 202**,** no. 2, pp. 119-140.

Carson, RG, Nelson, BD, Buick, AR, Carroll, TJ, Kennedy, NC & Cann, RM 2013, 'Characterizing Changes in the Excitability of Corticospinal Projections to Proximal Muscles of the Upper Limb', *Brain Stimulation,* vol. 6**,** no. 5, pp. 760-768.

Castro-Alamancos, M, Donoghue, J & Connors, B 1995, 'Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex', *Journal of Neuroscience,* vol. 15**,** no. 7, pp. 5324-5333.

Chang, WH, Bang, OY, Shin, Y-I, Lee, A, Pascual-Leone, A & Kim, YH 2014, 'BDNF Polymorphism and Differential rTMS Effects on Motor Recovery of Stroke Patients', *Brain Stimulation,* vol. 7**,** no. 4, pp. 553-558.

Cheeran, B, Talelli, P, Mori, F, Koch, G, Suppa, A, Edwards, M, Houlden, H, Bhatia, K, Greenwood, R & Rothwell, JC 2008, 'A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS', *Journal of Physiology,* vol. 586**,** no. 23, pp. 5717-5725.

Chen, R 2004, 'Interactions between inhibitory and excitatory circuits in the human motor cortex', *Experimental Brain Research,* vol. 154**,** no. 1, pp. 1-10.

Chen, R, Lozano, AM & Ashby, P 1999, 'Mechanism of the silent period following transcranial magnetic stimulation Evidence from epidural recordings', *Experimental Brain Research,* vol. 128**,** no. 4, pp. 539-542.

Chen, Z-Y, Jing, D, Bath, KG, Ieraci, A, Khan, T, Siao, C-J, Herrera, DG, Toth, M, Yang, C, Mcewen, BS, Hempstead, BL & Lee, FS 2006, 'Genetic Variant BDNF (Val66Met) Polymorphism Alters Anxiety-Related Behavior', *Science,* vol. 314**,** no. 5796, pp. 140-143.

Chipchase, L, Schabrun, S, Cohen, L, Hodges, P, Ridding, M, Rothwell, J, Taylor, J & Ziemann, U 2012, 'A checklist for assessing the methodological quality of studies using transcranial magnetic stimulation to study the motor system: An international consensus study', *Clinical Neurophysiology,* vol. 123**,** no. 9, pp. 1698-1704.

Christie, A & Kamen, G 2013, 'Cortical inhibition is reduced following short-term training in young and older adults', *AGE,* vol. 36**,** no. 2, pp. 1-10.

Christova, M, Rafolt, D & Gallasch, E 2015, 'Cumulative effects of anodal and priming cathodal tDCS on pegboard test performance and motor cortical excitability', *Behavioural Brain Research,* vol. 287**,** no. null, pp. 27-33.

Cirillo, J, Hughes, J, Ridding, M, Thomas, PQ & Semmler, JG 2012, 'Differential modulation of motor cortex excitability in BDNF Met allele carriers following experimentally induced and use-dependent plasticity', *European Journal of Neuroscience,* vol. 36**,** no. 5, pp. 2640-2649.

Cirillo, J, Todd, G & Semmler, JG 2011, 'Corticomotor excitability and plasticity following complex visuomotor training in young and old adults', *European Journal of Neuroscience,* vol. 34**,** no. 11, pp. 1847-1856.

Classen, J, Liepert, J, Wise, S, Hallett, M & Cohen, L 1998, 'Rapid plasticity of human cortical movement representation induced by practice', *Journal of Neurophysiology,* vol. 79**,** no. 2, pp. 1117-1123.

Cogiamanian, F, Marceglia, S, Ardolino, G, Barbieri, S & Priori, A 2007, 'Improved isometric force endurance after transcranial direct current stimulation over the human motor cortical areas', *European Journal of Neuroscience,* vol. 26**,** no. 1, pp. 242-249.

Collingridge, GL & Bliss, TVP 1987. 'NMDA receptors - their role in long-term potentiation', *Trends in Neurosciences,* vol. 10**,** no. 7, pp. 288-293.

Coombs, TA, Frazer, AK, Horvath, DM, Pearce, AJ, Howatson, G & Kidgell, DJ 2016, 'Cross-education of wrist extensor strength is not influenced by non-dominant training in right-handers', *European Journal of Applied Physiology,* vol. 116**,** no. 9, pp. 1757-1769.

Cramer, SC, Nelles, G, Benson, RR, Kaplan, JD, Parker, RA, Kwong, KK, Kennedy, DN, Finklestein, SP & Rosen, BR 1997, 'A Functional MRI Study of Subjects Recovered From Hemiparetic Stroke', *Stroke,* vol. 28**,** no. 12, pp. 2518-2527.

Damron, LA, Dearth, DJ, Hoffman, RL & Clark, BC 2008, 'Quantification of the corticospinal silent period evoked via transcranial magnetic stimulation', *Journal of Neuroscience Methods,* vol. 173**,** no. 1, pp. 121-128.

Dayan, E & Cohen, LG 2011, 'Neuroplasticity subserving motor skill learning', *Neuron,* vol. 72**,** no. 3, pp. 443-454.

De Gennaro, L, Cristiani, R, Bertini, M, Curcio, G, Ferrara, M, Fratello, F, Romei, V & Rossini, PM 2004, 'Handedness is mainly associated with an asymmetry of corticospinal excitability and not of transcallosal inhibition', *Clinical Neurophysiology*, vol. 115**,** no. 6, pp. 1305-1312.

De Noordhout, AM, Rapisarda, G, Bogacz, D, Gérard, P, De Pasqua, V, Pennisi, G & Delwaide, PJ 1999, 'Corticomotoneuronal synaptic connections in normal man', *An electrophysiological study*, vol. 122**,** no. 7, pp. 1327-1340.

Dettmers, C, Fink, GR, Lemon, RN, Stephan, KM, Passingham, RE, Silbersweig, D, Holmes, A, Ridding, MC, Brooks, DJ & Frackowiak, RS 1995, 'Relation between cerebral activity and force in the motor areas of the human brain', *Journal of Neurophysiology*, vol. 74**,** no. 2, pp. 802-815.

Devanne, H, Lavoie, BA & Capaday, C 1997, 'Input-output properties and gain changes in the human corticospinal pathway', *Experimental Brain Research*, vol. 114**,** no. 2, pp. 329-338.

Di Lazzaro, V, Dileone, M, Pilato, F, Capone, F, Musumeci, G, Ranieri, F, Ricci, V, Bria, P, Di Iorio, R, De Waure, C, Pasqualetti, P & Profice, P 2011, 'Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation', *Journal of Neurophysiology*, vol. 105**,** no. 5, pp. 2150-2156.

Di Lazzaro, V, Manganelli, F, Dileone, M, Notturno, F, Esposito, M, Capasso, M, Dubbioso, R, Pace, M, Ranieri, F, Minicuci, G, Santoro, L & Uncini, A 2012a, 'The effects of prolonged cathodal direct current stimulation on the excitatory and inhibitory circuits of the ipsilateral and contralateral motor cortex', *Journal of Neural Transmission*, vol. 119**,** no. 12, pp. 1499-1506.

Di Lazzaro, V, Oliviero, A, Pilato, F, Mazzone, P, Insola, A, Ranieri, F & Tonali, PA 2003, 'Corticospinal volleys evoked by transcranial stimulation of the brain in conscious humans', *Neurological Research,* vol. 25**,** no. 2, pp. 143-150.

Di Lazzaro, V, Oliviero, A, Pilato, F, Saturno, E, Dileone, M, Mazzone, P, Insola, A, Tonali, PA & Rothwell, JC 2004, 'The physiological basis of transcranial motor cortex stimulation in conscious humans', *Clinical Neurophysiology,* vol. 115**,** no. 2, pp. 255-266.

Di Lazzaro, V, Pilato, F, Dileone, M, Profice, P, Oliviero, A, Mazzone, P, Insola, A, Ranieri, F, Meglio, M, Tonali, PA & Rothwell, JC 2008, 'The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex', *Journal of Physiology,* vol. 586**,** no. 16, pp. 3871-3879.

Di Lazzaro, V, Profice, P, Ranieri, F, Capone, F, Dileone, M, Oliviero, A & Pilato, F 2012b, 'I-wave origin and modulation', *Brain Stimulation,* vol. 5**,** no. 4, pp. 512-525.

Duchateau, J, Semmler, J & Enoka, R 2006, 'Training adaptations in the behavior of human motor units', *Journal of Applied Physiology,* vol. 101**,** no. 6, pp. 1766 - 1775.

Egan, MF, Kojima, M, Callicott, JH, Goldberg, TE, Kolachana, BS, Bertolino, A, Zaitsev, E, Gold, B, Goldman, D, Dean, M, Lu, B & Weinberger, DR 2003, 'The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function', *Cell,* vol. 112**,** no. 2, pp. 257-269.

Falvo, M, Sirevaag, E, Rohrbaugh, J & Earhart, G 2010, 'Resistance training induces supraspinal adaptations: evidence from movement-related cortical potentials', *European Journal of Applied Physiology*, vol. 109**,** no. 5, pp. 923 - 933.

Farthing, JP & Chilibeck, PD 2003, 'The effect of eccentric training at different velocities on cross-education', *European Journal of Applied Physiology*, vol. 89**,** no. 6, pp. 570-577.

Farthing, JP, Chilibeck, PD & Binsted, G 2005, 'Cross-education of arm muscular strength is unidirectional in right-handed individuals', *Medicine & Science in Sports & Exercise*, vol. 37**,** no. 9, pp. 1594-1600.

Farthing, JP & Zehr, EP 2014, 'Restoring Symmetry: Clinical Applications of Cross-Education', *Exercise and Sport Sciences Reviews*, vol. 42**,** no. 2, pp. 70-75.

Figurov, A, Pozzo-Miller, LD, Olafsson, P, Wang, T & Lu, B 1996, 'Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus', *Nature*, vol. 381**,** no. 6584, pp. 706-709.

Fisher, RJ, Nakamura, Y, Bestmann, S, Rothwell, JC & Bostock, H 2002, 'Two phases of intracortical inhibition revealed by transcranial magnetic threshold tracking', *Experimental Brain Research*, vol. 143**,** no. 2, pp. 240-248.

Floeter, MK & Rothwell, JC 1999, 'Releasing the brakes before pressing the gas pedal', *Neurology*, vol. 53**,** no. 4, pp. 664.

Frazer, AK, Williams, J, Spittles, M, Rantalainen, T & Kidgell, DK 2016, 'Anodal Transcranial Direct Current Stimulation of the Motor Cortex Increases Cortical Voluntary Activation and Neural Plasticity', *Muscle & Nerve*, vol. 54**,** no. 5, pp. 903-913.

Fritz, C, Braune, HJ, Pylatiuk, C & Pohl, M 1997, 'Silent period following transcranial magnetic stimulation: a study of intra- and inter-examiner reliability', *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control,* vol. 105**,** no. 3, pp. 235-240.

Gabriel, D, Kamen, G & Frost, G 2006, 'Neural adaptations to resistive exercise', *Sports Medicine,* vol. 36**,** no. 2, pp. 133-149.

Galea, JM & Celnik, P 2009, 'Brain polarization enhances the formation and retention of motor memories', *Journal of Neurophysiology,* vol. 102**,** no. 1, pp. 294-301.

Gandevia, SC, Enoka, RM, Mccomas, AJ, Stuart, DG & Thomas, CK 1995, *Neurobiology of Muscle Fatigue*, Springer, United States of America.

Garfinkel, S & Cafarelli, E 1992, 'Relative changes in maximal force, EMG, and muscle cross-sectional area after isometric training', *Medicine & Science in Sports & Exercise,* vol. 24**,** no. 11, pp. 1220.

Garry, MI, Kamen, G & Nordstrom, MA 2004, 'Hemispheric Differences in the Relationship Between Corticomotor Excitability Changes Following a Fine-Motor Task and Motor Learning', *Journal of Neurophysiology,* vol. 91**,** no. 4, pp. 1570-1578.

Gilio, F, Rizzo, V, Siebner, HR & Rothwell, JC 2003, 'Effects on the right motor hand-area excitability produced by low-frequency rTMS over human contralateral homologous cortex', *Journal of Physiology,* vol. 551**,** no. 2, pp. 563-573.

Giraux, P, Sirigu, A, Schneider, F & Dubernard, JM 2001, 'Cortical reorganization in motor cortex after graft of both hands', *Nature Neuroscience,* vol. 4**,** no. 7, pp. 691-692.

Goodall, S, Romer, LM & Ross, EZ 2009, 'Voluntary activation of human knee extensors measured using transcranial magnetic stimulation', *Experimental Physiology*, vol. 94**,** no. 9, pp. 995-1004.

Goodwill, AM, Pearce, AJ & Kidgell, DJ 2012, 'Corticomotor plasticity following unilateral strength training', *Muscle & Nerve*, vol. 46**,** no. 3, pp. 384-393.

Gottmann, K, Mittmann, T & Lessmann, V 2009, 'BDNF signaling in the formation, maturation and plasticity of glutamatergic and GABAergic synapses', *Experimental Brain Research*, vol. 199**,** no. 3-4, pp. 203-234.

Greenberg, J, Hand, P, Sylvestro, A & Reivich, M 1979, 'Localized metabolic-flow couple during functional activity', *Acta Neurologica Scandinavica*, vol. 60**,** no. suppl 72, pp. 12-13.

Griffin, L & Cafarelli, E 2007, 'Transcranial magnetic stimulation during resistance training of the tibialis anterior muscle', *Journal of Electromyography and Kinesiology*, vol. 17**,** no. 4, pp. 446-452.

Hallett, M 2000, 'Transcranial magnetic stimulation and the human brain', *Nature*, vol. 406**,** no. 6792, pp. 147-150.

Hariri, AR, Goldberg, TE, Mattay, VS, Kolachana, BS, Callicott, JH, Egan, MF & Weinberger, DR 2003, 'Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance', *Journal of Neuroscience*, vol. 23**,** no. 17, pp. 6690-6694.

Hendy, A & Kidgell, D 2014, 'Anodal-tDCS applied during unilateral strength training increases strength and corticospinal excitability in the untrained homologous muscle', *Experimental Brain Research*, vol. 232**,** no. 10, pp. 3243-3252.

Hendy, AM & Kidgell, DJ 2013, 'Anodal tDCS applied during strength training enhances motor cortical plasticity', *Medicine & Science in Sports & Exercise,* vol. 45(9)**,** no. 9, pp. 1721-1729.

Hendy, AM, Spittle, M & Kidgell, DJ 2012, 'Cross education and immobilisation: Mechanisms and implications for injury rehabilitation', *Journal of Science and Medicine in Sport,* vol. 15**,** no. 2, pp. 94-101.

Hendy, AM, Teo, W & Kidgell, DJ 2015, 'Anodal tDCS Prolongs the Cross-education of Strength and Corticomotor Plasticity', *Medicine & Science in Sports & Exercise,* vol. 48**,** no. 9, pp. 1788-1797.

Herbert, RD & Gandevia, SC 1996, 'Muscle activation in unilateral and bilateral efforts assessed by motor nerve and cortical stimulation', *Journal of Applied Physiology,* vol. 80**,** no. 4, pp. 1351-1356.

Hermsen, AM, Haag, A, Duddek, C, Balkenhol, K, Bugiel, H, Bauer, S, Mylius, V, Menzler, K & Rosenow, F 2016, 'Retest reliability of single and paired pulse transcranial magnetic stimulation parameters in healthy subjects', *Journal of the Neurological Sciences,* vol. 362**,** no. null, pp. 209-216.

Hess, G, Aizenman, CD & Donoghue, JP 1996, 'Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex', *Journal of Neurophysiology,* vol. 75**,** no. 5, pp. 1765-1778.

Hess, G & Donoghue, J 1996, 'Long-term potentiation and long-term depression of horizontal connections in rat motor cortex', *Acta Neurobiologiae Experimentalis,* vol. 56**,** no. 1, pp. 397-405.

Hinder, M, Carroll, T & Summers, J 2013, 'Inter-limb transfer of ballistic motor skill following non-dominant limb training in young and older adults', *Experimental Brain Research,* vol. 227**,** no. 1, pp. 19-29.

Hinder, MR, Schmidt, MW, Garry, MI & Summers, JJ 2010, 'The effect of ballistic thumb contractions on the excitability of the ipsilateral motor cortex', *Experimental Brain Research,* vol. 201**,** no. 2, pp. 229-238.

Hortobagyi, T, Lambert, NJ & Hill, JP 1997, 'Greater cross education following training with muscle lengthening than shortening', *Medicine & Science in Sports & Exercise,* vol. 29**,** no. 1, pp. 107-112.

Hortobagyi, T, Richardson, SP, Lomarev, M, Shamim, E, Meunier, S, Russman, H, Dang, N & Hallett, MS 2011, 'Interhemispheric plasticity in human', *Medicine & Science in Sports & Exercise,* vol. 43**,** no. 7, pp. 1188-1199.

Hortobágyi, T, Taylor, JL, Petersen, NT, Russell, G & Gandevia, SC 2003a, 'Changes in Segmental and Motor Cortical Output With Contralateral Muscle Contractions and Altered Sensory Inputs in Humans', *Journal of Neurophysiology,* vol. 90**,** no. 4, pp. 2451-2459.

Hortobágyi, T, Taylor, JL, Petersen, NT, Russell, G & Gandevia, SC 2003b, 'Changes in segmental and motor cortical output with contralateral muscle contractions and altered sensory inputs in humans', *Journal of Neurophysiology,* vol. 90**,** no. 4, pp. 2451-2459.

Howatson, G, Taylor, MB, Rider, P, Motawar, BR, Mcnally, MP, Solnik, S, Devita, P & Hortobágyi, T 2011, 'Ipsilateral motor cortical responses to TMS during lengthening and shortening of the contralateral wrist flexors', *European Journal of Neuroscience,* vol. 33**,** no. 5, pp. 978-990.

Hummel, F, Celnik, P, Giraux, P, Floel, A, Wu, W, Gerloff, C & Cohen, L 2005, 'Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke', *Brain*, vol. 128**,** no. Pt 3, pp. 490-499.

Hunter, T, Sacco, P, Nitsche, MA & Turner, DL 2009, 'Modulation of internal model formation during force field-induced motor learning by anodal transcranial direct current stimulation of primary motor cortex', *Journal of Physiology*, vol. 587**,** no. 12, pp. 2949-2961.

Huntley, GW 1997, 'Correlation between patterns of horizontal connectivity and the extend of short-term representational plasticity in rat motor cortex', *Cerebral Cortex*, vol. 7**,** no. 2, pp. 143-156.

Hwang, JM, Kim, Y-H, Yoon, KJ, Uhm, KE & Chang, WH 2015, 'Different responses to facilitatory rTMS according to BDNF genotype', *Clinical Neurophysiology*, vol. 126**,** no. 7, pp. 1348-1353.

Imfeld, A, Oechslin, MS, Meyer, M, Loenneker, T & Jancke, L 2009, 'White matter plasticity in the corticospinal tract of musicians: A diffusion tensor imaging study', *NeuroImage*, vol. 46**,** no. 3, pp. 600-607.

Jacobs, KM & Donoghue, JP 1991, 'Reshaping the cortical motor map by unmasking latent intracortical connections', *Science*, vol. 251**,** no. 4996, pp. 944-947.

Kamen, G 2004, 'Reliability of motor-evoked potentials during resting and active contraction conditions', *Medicine & Science in Sports & Exercise*, vol. 36**,** no. 9, pp. 1574-1579.

Kandel, ER, Schwartz, JH, Jessell, TM, Siegelbaum, SA & Hudspeth, A 2000, *Principles of neural science*, McGraw-hill, New York, United States of America.

Keel, JC, Smith, MJ & Wassermann, EM 2001, 'A safety screening questionnaire for transcranial magnetic stimulation', *Clinical Neurophysiology,* vol. 112**,** no. 4, pp. 720.

Khedr, EM, Etraby, AE, Hemeda, M, Nasef, AM & Razek, AE 2010, 'Long-term effect of repetitive transcranial magnetic stimulation on motor function recovery after acute ischemic stroke', *Acta Neurologica Scandinavica,* vol. 121**,** no. 1, pp. 30-37.

Kidgell, DJ, Goodwill, A, Frazer, AK & Daly, R 2013, 'Induction of cortical plasticity and improved motor performance following unilateral and bilateral transcranial direct current stimulation of the primary motor cortex', *BMC Neuroscience,* vol. 14**,** no. 1, pp. 64.

Kidgell, DJ, Stokes, M, Castricum, T & Pearce, A 2010a, 'Neurophysiological responses after short-term strength training of the biceps brachii muscle', *Journal of Strength and Conditioning Research,* vol. 24**,** no. 11, pp. 3123-3132.

Kidgell, DJ, Frazer, A, Rantalainen, T, Ruotsalainen, I, Ahtiainen, J, Avela, J & Howatson, G 2015, 'Increased cross-education of muscle strength and reduced corticospinal inhibition following eccentric strength training', *Neuroscience,* vol. 300**,** no., pp. 566-575.

Kidgell, DJ, Mason, J, Frazer, AK & Pearce, AJ 2016, 'I-wave periodicity transcranial magnetic stimulation (iTMS) on corticospinal excitability. A systematic review of the literature', *Neuroscience,* vol. 322**,** no. null, pp. 262-272.

Kidgell, DJ & Pearce, AJ 2010, 'Corticospinal properties following short-term strength training of an intrinsic hand muscle', *Human Movement Science,* vol. 29**,** no. 5, pp. 631-641.

Kidgell, DJ & Pearce, AJ 2011, 'What has transcranial magnetic stimulation taught us about neural adaptations to strength training? A brief review', *Journal of Strength and Conditioning Research,* vol. 25**,** no. 11, pp. 3208-3217.

Kidgell, DJ, Stokes, MA, Castricum, TJ & Pearce, AJ 2010b, 'Neurophysiological responses after short-term strength training of the biceps brachii muscle', *Journal of Strength and Conditioning Research,* vol. 24**,** no. 11, pp. 3123-3132.

Kidgell, DJ, Stokes, MA & Pearce, AJ 2011, 'Strength training of one limb increases corticomotor excitability projecting to the contralateral homologous limb', *Motor Control,* vol. 15**,** no. 2, pp. 247-266.

Kirimoto, H, Ogata, K, Onishi, H, Oyama, M, Goto, Y & Tobimatsu, S 2011, 'Transcranial direct current stimulation over the motor association cortex induces plastic changes in ipsilateral primary motor and somatosensory cortices', *Clinical Neurophysiology,* vol. 122**,** no. 4, pp. 777-783.

Kleim, JA, Barbay, S, Cooper, NR, Hogg, TM, Reidel, CN, Remple, MS & Nudo, RJ 2002, 'Motor learning-dependent synaptogenesis is localized to functionally reorganized motor cortex', *Neurobiology of Learning and Memory,* vol. 77**,** no. 1, pp. 63-77.

Kleim, JA, Chan, S, Pringle, E, Schallert, K, Procaccio, V, Jimenez, R & Cramer, SC 2006, 'BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex', *Nature Neuroscience,* vol. 9**,** no. 6, pp. 735-737.

Kobayashi, M & Pascual-Leone, A 2003, 'Transcranial magnetic stimulation in neurology', *The Lancet Neurology,* vol. 2**,** no. 3, pp. 145-156.

Kojima, S, Onishi, H, Sugawara, K, Kirimoto, H, Suzuki, M & Tamaki H 2013, ‘Modulation of the cortical silent period elicited by single- and paired-pulse transcranial magnetic stimulation’, *BMC Neuroscience,* vol. 14, no. null, pp. 43.

Kouchtir-Devanne, N, Capaday, C, Cassim, F, Derambure, P & Devanne, H 2012, 'Task-dependent changes of motor cortical network excitability during precision grip compared to isolated finger contraction', *Journal of Neurophysiology,* vol. 107**,** no. 5, pp. 1522-1529.

Krishnamurthy, V, Gopinath, K, Brown, GS & Hampstead, BM 2015, 'Resting-state fMRI reveals enhanced functional connectivity in spatial navigation networks after transcranial direct current stimulation', *Neuroscience Letters,* vol. 604**,** no. null, pp. 80-85.

Kujirai, T, Caramia, MD, Rothwell, JC, Day, BL, Thompson, PD, Ferbert, A, Wroe, S, Asselman, P & Marsden, CD 1993, 'Corticocortical inhibition in human motor cortex', *Journal of Physiology,* vol. 471**,** no. 1, pp. 501-519.

Lackmy, A & Marchand-Pauvert, V 2010, 'The estimation of short intra-cortical inhibition depends on the proportion of spinal motoneurones activated by corticospinal inputs', *Clinical Neurophysiology,* vol. 121**,** no. 4, pp. 612-621.

Lang, N, Nitsche, MA, Paulus, W, Rothwell, JC & Lemon, RN 2004, 'Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability', *Experimental Brain Research,* vol. 156**,** no. 4, pp. 439-443.

Langan, J, Peltier, S, Bo, J, Fling, BW, Welsh, RC & Seidler, RD 2010, 'Functional implications of age differences in motor system connectivity', *Frontiers in Systems Neuroscience,* vol. 4**,** no. null, pp. 17.

Latella, C, Kidgell, D & Pearce, A 2012, 'Reduction in corticospinal inhibition in the trained and untrained limb following unilateral leg strength training', *European Journal of Applied Physiology*, vol. 112**,** no. 8, pp. 3097-3107.

Lee, M, Gandevia, SC & Carroll, TJ 2008, 'Cortical voluntary activation can be reliably measured in human wrist extensors using transcranial magnetic stimulation', *Clinical Neurophysiology*, vol. 119**,** no. 5, pp. 1130-1138.

Lee, M, Gandevia, SC & Carroll, TJ 2009, 'Unilateral strength training increases voluntary activation of the opposite untrained limb', *Clinical Neurophysiology*, vol. 120**,** no. 4, pp. 802-808.

Lee, M, Hinder, MR, Gandevia, SC & Carroll, TJ 2010, 'The ipsilateral motor cortex contributes to cross-limb transfer of performance gains after ballistic motor practice', *Journal of Physiology*, vol. 588**,** no. 1, pp. 201-212.

Leung, M, Rantalainen, T, Teo, WP & Kidgell, D 2015, 'Motor cortex excitability is not differentially modulated following skill and strength training', *Neuroscience*, vol. 305**,** no. null, pp. 99-108.

Li Voti, P, Conte, A, Suppa, A, Iezzi, E, Bologna, M, Aniello, MS, Defazio, G, Rothwell, JC & Berardelli, A 2011, 'Correlation between cortical plasticity, motor learning and BDNF genotype in healthy subjects', *Experimental Brain Research*, vol. 212**,** no. 1, pp. 91-99.

Liebetanz, D, Nitsche, MA, Tergau, F & Paulus, W 2002, 'Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability', *Brain*, vol. 125**,** no. 10, pp. 2238-2247.

Magnus, CRA, Boychuk, K, Kim, SY & Farthing, JP 2014, 'At-home resistance tubing strength training increases shoulder strength in the trained and untrained limb',

Scandinavian Journal of Medicine & Science in Sports, vol. 24**,** no. 3, pp. 586-593.

Malenka, RC 1991, 'The role of postsynaptic calcium in the induction of long-term potentiation', *Molecular Neurobiology,* vol. 5**,** no. 2-4, pp. 289-295.

Mccormick, DA 1989, 'GABA as an inhibitory neurotransmitter in human cerebral cortex', *Journal of Neurophysiology,* vol. 62**,** no. 5, pp. 1018-1027.

Mchughen, S, Pearson-Fuhrhop, K, Ngo, V & Cramer, S 2011, 'Intense training overcomes effects of the val66met BDNF polymorphism on short-term plasticity', *Experimental Brain Research,* vol. 213**,** no. 4, pp. 415-422.

Mchughen, SA, Rodriguez, PF, Kleim, JA, Kleim, ED, Crespo, LM, Procaccio, V & Cramer, SC 2010, 'BDNF Val66Met Polymorphism Influences Motor System Function in the Human Brain', *Cerebral Cortex,* vol. 20**,** no. 5, pp. 1254-1262.

Mcnaughton, BL, Douglas, RM & Goddard, GV 1978, 'Synaptic enhancement in fascia dentata: Cooperativity among coactive afferents', *Brain Research,* vol. 157**,** no. 2, pp. 277-293.

Merton, PA 1954, 'Voluntary strength and fatigue', *Journal of Physiology,* vol. 123**,** no. 3, pp. 553-564.

Merton, PA, Morton, H. B. 1980, 'Stimulation of the cerebral cortex in the intact human subject', *Nature,* vol. 285**,** no. 5762, pp. 227.

Meyer, B-U, Röricht, S, Von Einsiedel, HG, Kruggel, F & Weindl, A 1995, 'Inhibitory and excitatory interhemispheric transfers between motor cortical areas in normal humans and patients with abnormalities of the corpus callosum', *Brain,* vol. 118**,** no. 2, pp. 429-440.

Mountcastle, VB 1997, 'The columnar organization of the neocortex', *Brain,* vol. 120**,** no. 4, pp. 701-722.

Muellbacher, W, Facchini, S, Boroojerdi, B & Hallett, M 2000, 'Changes in motor cortex excitability during ipsilateral hand muscle activation in humans', *Clinical Neurophysiology,* vol. 111**,** no. 2, pp. 344-349.

Muellbacher, W, Ziemann, U, Boroojerdi, B, Cohen, L & Hallett, M 2001, 'Role of the human motor cortex in rapid motor learning', *Experimental Brain Research,* vol. 136**,** no. 4, pp. 431-438.

Muellbacher, W, Ziemann, U, Wissel, J, Dang, N, Kofler, M & Et Al. 2002, 'Early consolidation in human primary motor cortex', *Nature,* vol. 415**,** no. 6872, pp. 640-644.

Munn, J, Herbert, RD & Gandevia, SC 2004, 'Contralateral effects of unilateral resistance training: a meta-analysis', *Journal of Applied Physiology,* vol. 96**,** no. 5, pp. 1861-1866.

Munn, J, Herbert, RD, Hancock, MJ & Gandevia, SC 2005a, 'Resistance training for strength: Effect of number of sets and contraction speed', *Medicine & Science in Sports & Exercise,* vol. 37**,** no. 9, pp. 1622-1626.

Munn, J, Herbert, RD, Hancock, MJ & Gandevia, SC 2005b, 'Training with unilateral resistance exercise increases contralateral strength', *Journal of Applied Physiology,* vol. 99**,** no. 5, pp. 1880-1884.

Nakamura, K, Enomoto, H, Hanajima, R, Hamada, M, Shimizu, E, Kawamura, Y, Sasaki, T, Matsuzawa, D, Sutoh, C, Shirota, Y, Terao, Y & Ugawa, Y 2011, 'Quadri-pulse stimulation (QPS) induced LTP/LTD was not affected by

Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene', *Neuroscience Letters,* vol. 487**,** no. 3, pp. 264-267.

Nathan, PW, Smith, MC & Deacon, P 1990, 'The corticospinal tracts in man: course and location of fibres at difference segmental levels', *Brain,* vol. 113**,** no. 2, pp. 303-324.

Neves-Pereira, M, Mundo, E, Muglia, P, King, N, Macciardi, F & Kennedy, JL 2002, 'The Brain-Derived Neurotrophic Factor Gene Confers Susceptibility to Bipolar Disorder: Evidence from a Family-Based Association Study', *American Journal of Human Genetics,* vol. 71**,** no. 3, pp. 651-655.

Nitsche, MA, Cohen, LG, Wassermann, EM, Priori, A, Lang, N, Antal, A, Paulus, W, Hummel, F, Boggio, PS, Fregni, F & Pascual-Leone, A 2008, 'Transcranial direct current stimulation: State of the art 2008', *Brain Stimulation,* vol. 1**,** no. 3, pp. 206-223.

Nitsche, MA, Doemkes, S, Karaköse, T, Antal, A, Liebetanz, D, Lang, N, Tergau, F & Paulus, W 2007, 'Shaping the Effects of Transcranial Direct Current Stimulation of the Human Motor Cortex', *Journal of Neurophysiology,* vol. 97**,** no. 4, pp. 3109-3117.

Nitsche, MA, Fricke, K, Henschke, U, Schlitterlau, A, Liebetanz, D, Lang, N, Henning, S, Tergau, F & Paulus, W 2003a, 'Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans', *Journal of Physiology,* vol. 553**,** no. 1, pp. 293-301.

Nitsche, MA, Grundey, J, Liebetanz, D, Lang, N, Tergau, F & Paulus, W 2004a, 'Catecholaminergic consolidation of motor cortical neuroplasticity in humans', *Cerebral Cortex,* vol. 14**,** no. 11, pp. 1240-1245.

Nitsche, MA, Liebetanz, D, Lang, N, Antal, A, Tergau, F & Paulus, W 2003b, 'Safety criteria for transcranial direct current stimulation (tDCS) in humans', *Clinical Neurophysiology*, vol. 114**,** no. 11, pp. 2220-2222.

Nitsche, MA, Liebetanz, D, Schlitterlau, A, Henschke, U, Fricke, K, Frommann, K, Lang, N, Henning, S, Paulus, W & Tergau, F 2004b, 'GABAergic modulation of DC stimulation-induced motor cortex excitability shifts in humans', *European Journal of Neuroscience*, vol. 19**,** no. 10, pp. 2720-2726.

Nitsche, MA, Nitsche, MS, Klein, CC, Tergau, F, Rothwell, JC & Paulus, W 2003c, 'Level of action of cathodal DC polarisation induced inhibition of the human motor cortex', *Clinical Neurophysiology*, vol. 114**,** no. 4, pp. 600-604.

Nitsche, MA & Paulus, W 2000, 'Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation', *Journal of Physiology*, vol. 527**,** no. 3, pp. 633-639.

Nitsche, MA & Paulus, W 2001, 'Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans', *Neurology*, vol. 57**,** no. 10, pp. 1899-1901.

Nitsche, MA & Paulus, W 2011, 'Transcranial direct current stimulation – update 2011', *Restorative Neurology and Neuroscience*, vol. 29**,** no. 6, pp. 463-492.

Nitsche, MA, Schauenburg, A, Lang, N, Liebetanz, D, Exner, C, Paulus, W & Tergau, F 2003d, 'Facilitation of Implicit Motor Learning by Weak Transcranial Direct Current Stimulation of the Primary Motor Cortex in the Human', *Journal of Cognitive Neuroscience*, vol. 15**,** no. 4, pp. 619-626.

Nitsche, MA, Seeber, A, Frommann, K, Klein, CC, Rochford, C, Nitsche, MS, Fricke, K, Liebetanz, D, Lang, N, Antal, A, Paulus, W & Tergau, F 2005, 'Modulating

parameters of excitability during and after transcranial direct current stimulation of the human motor cortex', *Journal of Physiology*, vol. 568**,** no. 1, pp. 291-303.

Nuzzo, JL, Barry, BK, Gandevia, SC & Taylor, JL 2016, 'Acute Strength Training Increases Responses to Stimulation of Corticospinal Axons', *Medicine & Science in Sports & Exercise*, vol. 48**,** no. 1, pp. 139-150.

Oldfield, R 1971, 'The assessment and analysis of handedness: the Edinburgh inventory', *Neuropsychologia*, vol. 9**,** no. 1, pp. 97-113.

Palmer, E & Ashby, P 1992, 'Corticospinal projections to upper limb motoneurones in humans', *Journal of Physiology*, vol. 448**,** no. null, pp. 397-412.

Pascual-Leone, A, Nguyet, D, Cohen, L, Brasil-Neto, J, Cammarota, A & Hallet, M 1995, 'Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills', *Journal of Neurophysiology*, vol. 74**,** no. 3, pp. 1037 - 1045.

Pavlova, E, Kuo, M-F, Nitsche, MA & Borg, J 2014, 'Transcranial direct current stimulation of the premotor cortex: Effects on hand dexterity', *Brain Research*, vol. 1576**,** no. null, pp. 52-62.

Pearce, AJ, Hendy, A, Bowen, WA & Kidgell, DJ 2013, 'Corticospinal adaptations and strength maintenance in the immobilized arm following 3 weeks unilateral strength training', *Scandinavian Journal of Medicine and Science in Sports*, vol. 23**,** no. 6, pp. 740-748.

Pearce, AJ & Kidgell, DJ 2010, 'Comparison of corticomotor excitability during visuomotor dynamic and static tasks', *Journal of Science and Medicine in Sport*, vol. 13**,** no. 1, pp. 167-171.

Pearce, AJ & Kidgell, DJ 2011, *Neuroplasticity following skill and strength training,* Nova Biomedical, New York, USA.

Pellicciari, MC, Brignani, D & Miniussi, C 2013, 'Excitability Modulation of the Motor System Induced by Transcranial Direct Current Stimulation: A Multimodal Approach', *NeuroImage,* vol. 83**,** no., pp. 569-580.

Perez, M, Lungholt, B, Nyborg, K & Nielsen, J 2004, 'Motor skill training induces changes in the excitability of the leg cortical area in healthy humans', *Experimental Brain Research,* vol. 159**,** no. 2, pp. 197-205.

Perez, MA & Cohen, LG 2008, 'Mechanisms Underlying Functional Changes in the Primary Motor Cortex Ipsilateral to an Active Hand', *Journal of Neuroscience,* vol. 28**,** no. 22, pp. 5631-5640.

Perez, MA, Tanaka, S, Wise, SP, Sadato, N, Tanabe, HC, Willingham, DT & Cohen, LG 2007, 'Neural Substrates of Intermanual Transfer of a Newly Acquired Motor Skill', *Current Biology,* vol. 17**,** no. 21, pp. 1896-1902.

Pezawas, L, Verchinski, BA, Mattay, VS, Callicott, JH, Kolachana, BS, Straub, RE, Egan, MF, Meyer-Lindenberg, A & Weinberger, DR 2004, 'The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology', *Journal of Neuroscience,* vol. 24**,** no. 45, pp. 10099-10102.

Priori, A, Oliviero, A, Donati, E, Callea, L, Bertolasi, L & Rothwell, CJ 1999, 'Human handedness and asymmetry of the motor cortical silent period', *Experimental Brain Research,* vol. 128**,** no. 3, pp. 390-396.

Puri, R, Hinder, MR, Fujiyama, H, Gomez, R, Carson, RG & Summers, JJ 2015, 'Duration-dependent effects of the BDNF Val66Met polymorphism on anodal

tDCS induced motor cortex plasticity in older adults: a group and individual perspective', *Frontiers in Aging Neuroscience,* vol. 7**,** no. null, pp. 107.

Rantalainen, T, Weier, A, Leung, M, Brandner, C, Spittle, M & Kidgell, D 2013, 'Short-interval intracortical inhibition is not affected by varying visual feedback in an isometric task in biceps brachii muscle', *Frontiers in Human Neuroscience,* vol. 7**,** no. null, pp 68.

Reis, J, Schambra, HM, Cohen, LG, Buch, ER, Fritsch, B, Zarahn, E, Celnik, PA & Krakauer, JW 2009, 'Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation', *Proceedings of the National Academy of Sciences,* vol. 106**,** no. 5, pp. 1590-1595.

Ridding, MC & Ziemann, U 2010, 'Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects', *Journal of Physiology,* vol. 588**,** no. 13, pp. 2291-2304.

Rioult-Pedotti, MS, Friedman, D, Hess, G & Donoghue, JP 1998, 'Strengthening of horizontal cortical connections following skill learning', *Nature Neuroscience,* vol. 1**,** no. 3, pp. 230-234.

Rogasch, NC, Dartnall, TJ, Cirillo, J, Nordstrom, MA & Semmler, JG 2009, 'Corticomotor plasticity and learning of a ballistic thumb training task are diminished in older adults', *Journal of Applied Physiology,* vol. 107**,** no. 6, pp. 1874-1883.

Rossini, PM, Barker, AT, Berardelli, A, Caramia, MD, Caruso, G, Cracco, RQ, Dimitrijević, MR, Hallett, M, Katayama, Y, Lücking, CH, Maertens De Noordhout, AL, Marsden, CD, Murray, NMF, Rothwell, JC, Swash, M & Tomberg, C 1994, 'Non-invasive electrical and magnetic stimulation of the

brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee', *Electroencephalography and Clinical Neurophysiology,* vol. 91**,** no. 2, pp. 79-92.

Rossini, PM & Rossi, S 2007, 'Transcranial magnetic stimulation: Diagnostic, therapeutic, and research potential', *Neurology,* vol. 68**,** no. 7, pp. 484-488.

Rothwell, JC 199, *Control of Human Voluntary Movement*, 2nd ed. Chapman & Hall, London, England.

Rothwell, JC, Day, BL, Thompson, PD & Kujirai, T 2009, 'Short latency intracortical inhibition: one of the most popular tools in human motor neurophysiology', *Journal of Physiology,* vol. 587**,** no. 1, pp. 11-12.

Roy, A, Baxter, B & He, B 2014, 'High-Definition Transcranial Direct Current Stimulation Induces Both Acute and Persistent Changes in Broadband Cortical Synchronization: A Simultaneous tDCS-EEG Study', *IEEE Transactions on Biomedical Engineering,* vol. 61**,** no. 7, pp. 1967-1978.

Ruddy, KL & Carson, RG 2013, 'Neural pathways mediating cross education of motor function', *Frontiers in Human Neuroscience,* vol. 7**,** no. null, pp. 397.

Ruotsalainen, I, Ahtiainen, J, Kidgell, D & Avela, J 2014, 'Changes in corticospinal excitability during an acute bout of resistance exercise in the elbow flexors', *European Journal of Applied Physiology,* vol. 114**,** no. 7, pp. 1545-1553.

Sakai, K, Ugawa, Y, Terao, Y, Hanajima, R, Furubayashi, T & Kanazawa, I 1997, 'Preferential activation of different I waves by transcranial magnetic stimulation with a figure-of-eight-shaped coil', *Experimental Brain Research,* vol. 113**,** no. 1, pp. 24-32.

Sale, MV, Mattingley, JB, Zalesky, A & Cocchi, L 2015, 'Imaging human brain networks to improve the clinical efficacy of non-invasive brain stimulation', *Neuroscience and Biobehavioral Reviews,* vol. 57**,** no. null, pp. 187-198.

Sale, MV, Ridding, MC & Nordstrom, MA 2007, 'Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation', *Experimental Brain Research,* vol. 181**,** no. 4, pp. 615-626.

Sanes, JN & Donoghue, JP 2000, 'Plasticity and primary motor cortex', *Annual Review of Neuroscience,* vol. 23**,** no. 1, pp. 393.

Schaechter, JD 2004, 'Motor rehabilitation and brain plasticity after hemiparetic stroke', *Progress in Neurobiology,* vol. 73**,** no. 1, pp. 61-72.

Schambra, HM, Sawaki, L & Cohen, LG 2003, 'Modulation of excitability of human motor cortex (M1) by 1 Hz transcranial magnetic stimulation of the contralateral M1', *Clinical Neurophysiology,* vol. 114**,** no. 1, pp. 130-133.

Schinder, AF & Poo, M-M 2000, 'The neurotrophin hypothesis for synaptic plasticity', *Trends in Neurosciences,* vol. 23**,** no. 12, pp. 639-645.

Schmidt, MW, Hinder, MR, Summers, JJ & Garry, MI 2011, 'Long-Lasting Contralateral Motor Cortex Excitability Is Increased by Unilateral Hand Movement That Triggers Electrical Stimulation of Opposite Homologous Muscles', *Neurorehabilitation and Neural Repair,* vol. 25**,** no. 6, pp. 521-530.

Scripture, E, Smith, TL & Brown, EM 189, 'On the education of muscular control and power', *Stud Yale Psychol Lab,* vol. 2**,** no. null, pp. 114-119.

Selvanayagam, VS, Riek, S & Carroll, TJ 2011, 'Early neural responses to strength training', *Journal of Applied Physiology,* vol. 111**,** no. 2, pp. 367-375.

Selvanayagam, VS, Riek, S & Carroll, TJ 2012, 'A systematic method to quantify the presence of cross-talk in stimulus-evoked EMG responses: Implications for TMS studies', *Journal of Applied Physiology*, vol. 112, no. null, pp. 259–265.

Shafi, MM, Westover, MB, Fox, MD & Pascual-Leone, A 2012, 'Exploration and modulation of brain network interactions with noninvasive brain stimulation in combination with neuroimaging', *European Journal of Neuroscience*, vol. 35**,** no. 6, pp. 805-825.

Shimizu, E, Hashimoto, K & Iyo, M 2004, 'Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: The possibility to explain ethnic mental traits', *American Journal of Medical Genetics Part B*, vol. 126B**,** no. 1, pp. 122-123.

Shin, H-W & Sohn, YH 2011, 'Interhemispheric transfer of paired associative stimulation-induced plasticity in the human motor cortex', *NeuroReport*, vol. 22**,** no. 4, pp. 166-170.

Sidhu, SK, Bentley, DJ & Carroll, TJ 2009, 'Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation', *Muscle & Nerve*, vol. 39**,** no. 2, pp. 186-196.

Siebner, H & Rothwell, J 2003, 'Transcranial magnetic stimulation: new insights into representational cortical plasticity', *Experimental Brain Research*, vol. 148**,** no. 1, pp. 1-16.

Siebner, HR 2010, 'A primer on priming the human motor cortex', *Clinical Neurophysiology*, vol. 121**,** no. 4, pp. 461-463.

Smyth, C, Summers, JJ & Garry, MI 2010, 'Differences in motor learning success are associated with differences in M1 excitability', *Human Movement Science,* vol. 29**,** no. 5, pp. 618-630.

Sriraman, A, Oishi, T & Madhavan, S 2014, 'Timing-dependent priming effects of tDCS on ankle motor skill learning', *Brain Research,* vol. 1581**,** no. null, pp. 23-29.

Stagg, CJ, Jayaram, G, Pastor, D, Kincses, ZT, Matthews, PM & Johansen-Berg, H 2011, 'Polarity and timing-dependent effects of transcranial direct current stimulation in explicit motor learning', *Neuropsychologia,* vol. 49**,** no. 5, pp. 800-804.

Stinear, CM & Byblow, WD 2003, 'Role of Intracortical Inhibition in Selective Hand Muscle Activation', *Journal of Neurophysiology,* vol. 89**,** no. 4, pp. 2014-2020.

Stoykov, ME & Madhavan, S 2015, 'Motor Priming in Neurorehabilitation', *Journal of Neurologic Physical Therapy,* vol. 39**,** no. 1, pp. 33-42.

Suppa, A, Ortu, E, Zafar, N, Deriu, F, Paulus, W, Berardelli, A & Rothwell, JC 2008, 'Theta burst stimulation induces after-effects on contralateral primary motor cortex excitability in humans', *Journal of Physiology,* vol. 586**,** no. Pt 18, pp. 4489-4500.

Suzuki, K, Fujiwara, T, Tanaka, N, Tsuji, T, Masakado, Y, Hase, K, Kimura, A & Liu, M 2012, 'Comparison of the After-Effects of Transcranial Direct Current Stimulation Over the Motor Cortex in Patients With Stroke and Healthy Volunteers', *International Journal of Neuroscience,* vol. 122**,** no. 11, pp. 675-681.

Talelli, P, Waddingham, W, Ewas, A, Rothwell, JC & Ward, NS 2008, 'The effect of age on task-related modulation of interhemispheric balance', *Experimental Brain Research,* vol. 186**,** no. 1, pp. 59-66.

Tanaka, S, Hanakawa, T, Honda, M & Watanabe, K 2009, 'Enhancement of pinch force in the lower leg by anodal transcranial direct current stimulation', *Experimental Brain Research,* vol. 196**,** no. 3, pp. 459-465.

Tanaka, S, Takeda, K, Otaka, Y, Kita, K, Osu, R, Honda, M, Sadato, N, Hanakawa, T & Watanabe, K 2011, 'Single session of transcranial direct current stimulation transiently increases knee extensor force in patients with hemiparetic stroke', *Neurorehabilitation and Neural Repair,* vol. 25**,** no. 6, pp. 565-569.

Taube, W 2011, '"What trains together, gains together": strength training strengthens not only muscles but also neural networks', *Journal of Applied Physiology,* vol. 111**,** no. 2, pp. 347-348.

Thickbroom, G 2007, 'Transcranial magnetic stimulation and synaptic plasticity: experimental framework and human models', *Experimental Brain Research,* vol. 180**,** no. 4, pp. 583-593.

Thickbroom, GW, Byrnes, ML, Edwards, DJ & Mastaglia, FL 2006, 'Repetitive paired-pulse TMS at I-wave periodicity markedly increases corticospinal excitability: A new technique for modulating synaptic plasticity', *Clinical Neurophysiology,* vol. 117**,** no. 1, pp. 61-66.

Todd, G, Taylor, JL & Gandevia, SC 2003, 'Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation', *Journal of Physiology,* vol. 551**,** no. 2, pp. 661-671.

Todd, G, Taylor, JL & Gandevia, SC 2004, 'Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation', *Journal of Applied Physiology*, vol. 97**,** no. 1, pp. 236-242.

Tokimura, H, Ridding, MC, Tokimura, Y, Amassian, VE & Rothwell, JC 1996, 'Short latency facilitation between pairs of threshold magnetic stimuli applied to human motor cortex', *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control*, vol. 101**,** no. 4, pp. 263-272.

Trompetto, C, Bove, M, Marinelli, L, Avanzino, L, Buccolieri, A & Abbruzzese, G 2004, 'Suppression of the transcallosal motor output: a transcranial magnetic stimulation study in healthy subjects', *Experimental Brain Research*, vol. 158**,** no. 2, pp. 133-140.

Van Duinen, H, Renken, R, Maurits, NM & Zijdewind, I 2008, 'Relation between muscle and brain activity during isometric contractions of the first dorsal interosseus muscle', *Human Brain Mapping*, vol. 29**,** no. 3, pp. 281-299.

Van Hedel, HJA, Murer, C, Dietz, V & Curt, A 2007, 'The amplitude of lower leg motor evoked potentials is a reliable measure when controlled for torque and motor task', *Journal of Neurology*, vol. 254**,** no. 8, pp. 1089-1098.

Vines, B, Cerruti, C & Schlaug, G 2008, 'Dual-hemisphere tDCS facilitates greater improvements for healthy subjects' non-dominant hand compared to uni-hemisphere stimulation', *BMC Neuroscience*, vol. 9**,** no. 1, pp. 103.

Vines, BW, Nair, DG & Schlaug, G 2006, 'Contralateral and ipsilateral motor effects after transcranial direct current stimulation', *NeuroReport*, vol. 17**,** no. 6, pp. 671-674.

Werhahn, KJ, Behrang-Nia, M, Bott, MC & Klimpe, S 2007, 'Does the recruitment of excitation and inhibition in the motor cortex differ?', *Journal of Clinical Neurophysiology*, vol. 24**,** no. 5, pp. 419-423.

Wilson, S, Lockwood, R, Thickbroom, G & Mastaglia, F 1993, 'The muscle silent period following transcranial magnetic cortical stimulation', *Journal of the Neurological Sciences,* vol. 114**,** no. 2, pp. 216-222.

Woo, NH, Teng, HK, Siao, C-J, Chiaruttini, C, Pang, PT, Milner, TA, Hempstead, BL & Lu, B 2005, 'Activation of p75NTR by proBDNF facilitates hippocampal long-term depression', *Nature Neuroscience,* vol. 8**,** no. 8, pp. 1069-1077.

Yue, G & Cole, KJ 1992, 'Strength increases from the motor program: comparison of training with maximal voluntary and imagined muscle contractions', *Journal of Neurophysiology,* vol. 67**,** no. 5, pp. 1114-1123.

Zhou, S 2000, 'Chronic neural adaptations to unilateral exercise: Mechanisms of cross education', *Exercise and Sport Sciences Reviews,* vol. 28**,** no. 4, pp. 177-184.

Ziemann, U & Hallett, M 2001, 'Hemispheric asymmetry of ipsilateral motor cortex activation during unimanual motor tasks: further evidence for motor dominance', *Clinical Neurophysiology,* vol. 112**,** no. 1, pp. 107-113.

Ziemann, U, Hallett, M & Cohen, LG 1998a, 'Mechanisms of deafferentation-induced plasticity in human motor cortex', *Journal of Neuroscience,* vol. 18**,** no. 17, pp. 7000-7007.

Ziemann, U, Iliać, TV, Pauli, C, Meintzschel, F & Ruge, D 2004, 'Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex', *Journal of Neuroscience,* vol. 24**,** no. 7, pp. 1666-1672.

Ziemann, U, Paulus, W, Nitsche, MA, Pascual-Leone, A, Byblow, WD, Berardelli, A, Siebner, HR, Classen, J, Cohen, LG & Rothwell, JC 2008, 'Consensus: Motor cortex plasticity protocols', *Brain Stimulation,* vol. 1**,** no. 3, pp. 164-182.

Ziemann, U & Siebner, HR 2008, 'Modifying motor learning through gating and homeostatic metaplasticity', *Brain Stimulation,* vol. 1**,** no. 1, pp. 60-66.

Ziemann, U, Tergau, F, Wassermann, EM, Wischer, S, Hildebrandt, J & Paulus, W 1998b, 'Demonstration of facilitatory I wave interaction in the human motor cortex by paired transcranial magnetic stimulation', *Journal of Physiology,* vol. 511**,** no. 1, pp. 181-190.

Zijdewind, I, Butler, JE, Gandevia, SC & Taylor, JL 2006, 'The origin of activity in the biceps brachii muscle during voluntary contractions of the contralateral elbow flexor muscles', *Experimental Brain Research,* vol. 175**,** no. 3, pp. 526-535.

Zoghi, M, Pearce, SL & Nordstrom, MA 2003, 'Differential Modulation of Intracortical Inhibition in Human Motor Cortex during Selective Activation of an Intrinsic Hand Muscle', *Journal of Physiology,* vol. 550**,** no. 3, pp. 933-946.

Zult, T, Howatson, G, Kádár, E, Farthing, J & Hortobágyi, T 2014, 'Role of the Mirror-Neuron System in Cross-Education', *Sports Medicine,* vol. 44**,** no. 2, pp. 159-178.

Appendices

Appendix A: Edinburgh Handedness Inventory

Please indicate your preferences in the use of hands in the following activities by putting a check in the appropriate column.

If the preference is so strong that you would never try to use the other hand unless absolutely forced to, put two "√√". If in any case you are really indifferent, put a "√" in both columns.

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

Please try and answer all of the questions, and only leave a blank if you have no experience at all with the object or task.

Task	**Left Hand**	**Right Hand**
1. Writing	☐ ☐	☐ ☐
2. Drawing	☐ ☐	☐ ☐
3. Throwing	☐ ☐	☐ ☐
4. Scissors	☐ ☐	☐ ☐
5. Toothbrush	☐ ☐	☐ ☐
6. Knife (without fork)	☐ ☐	☐ ☐
7. Spoon	☐ ☐	☐ ☐
8. Broom (upper hand)	☐ ☐	☐ ☐
9. Striking match (match)	☐ ☐	☐ ☐
10. Opening box (lid)	☐ ☐	☐ ☐
Total (count √ in both columns)		
Difference	**Cumulative (total)**	**Result**

Scoring:

- Add up the total number of checks in the "Left hand" and "Right hand" columns and enter in the "Total" row for each column.
- Add the left total and the right total and enter in the "Cumulative (total)" cell.
- Subtract the left total from the right total and enter in the "Difference" cell.
- Divide the "Difference" cell by the "Cumulative (total)" cell (round to 2 digits if necessary) and multiply by 100; enter the result in the "Result" cell.

Interpretation (based on result):

- Below -40 = left-handed
- Between -40 and +40 = ambidextrous
- Above +40 = right-handed

Appendix B: 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 stroke?	❍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?	❍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):*

_____

_____

_____

_____

_____

† *For use with single-pulse TMS, paired-pulse TMS, or repetitive TMS.*