TOWARDS ESTABLISHING LONG-LASTING NEUROPLASTIC CHANGE IN THE HUMAN PRIMARY MOTOR CORTEX

A thesis submitted for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY of ADELAIDE

By

Mitchell Ryan Goldsworthy

B. Sc. (Biomedical Science), Hons.

The Robinson Institute

Discipline of Obstetrics and Gynaecology

School of Paediatrics and Reproductive Health

The University of Adelaide

May 2013

ABSTRACT

Neuroplasticity is critical for learning, memory, and recovery of lost function following neurological insult. Whilst non-invasive brain stimulation techniques capable of inducing these neuroplastic changes within the human cortex could be therapeutically beneficial for a range of neurological and psychiatric conditions, the short duration, instability, and variability of induced effects limits their therapeutic potential. This thesis has investigated approaches to enhance the duration, stability, and consistency of the neuroplastic response to non-invasive brain stimulation protocols applied to the human primary motor cortex.

The neuroplasticity-inducing paradigm employed throughout this thesis was continuous theta burst stimulation (cTBS), a repetitive transcranial magnetic stimulation (rTMS) paradigm shown to suppress human motor cortical excitability. Studies in animals have shown the repeated, spaced application of stimulation protocols to prolong the duration of experimentally-induced synaptic plasticity. Therefore, Chapter 2 examined whether the spaced application of repeated cTBS protocols enhanced the lifetime of induced neuroplastic effects within the human primary motor cortex. Whilst the neuroplastic response to a single cTBS protocol was minimal, paired cTBS protocols spaced 10 min apart induced a strong suppression of motor cortical excitability that lasted for at least 2 h. A further set of experiments were performed to determine the possible contribution of the inhibitory motor networks to this enhanced neuroplastic response (Chapter 3). Although paired cTBS reduced the excitability of $GABA_A$-mediated inhibitory motor networks, this effect was only modest. Also, paired cTBS had no effect on $GABA_B$-mediated inhibition. These findings suggest that the enhanced neuroplastic response to

paired cTBS was likely the result of greater suppression within excitatory motor networks rather than a facilitation of inhibitory motor networks.

In addition to prolonging the duration of experimentally-induced synaptic plasticity, the repeated application of stimulation protocols has also been shown to consolidate these plastic changes in animal models, making them resistant to reversal by subsequent behaviourally-relevant physiological activity. In Chapter 4, I investigated whether the neuroplastic response to paired cTBS was similarly resistant to reversal by behavioural engagement of the stimulated motor regions. Whilst a voluntary activation of the targeted hand muscles reversed the neuroplastic response to a single cTBS protocol, the long-lasting neuroplastic response to paired cTBS was resistant to the same reversal effects. These results suggest that, similar to animal models of synaptic plasticity, the neuroplasticity induced by cTBS may be consolidated when repeated protocols are applied in a spaced manner.

Although Chapters 2, 3, and 4 show a long-lasting and robust response to repeated cTBS protocols, the neuroplastic response to a single cTBS was highly variable between subjects. This may have been due, in part, to non-optimal stimulation characteristics. Therefore, the experiments described in Chapter 5 compared the efficacy of the standard cTBS paradigm ($cTBS_{std}$) to that of a slightly modified variant ($cTBS_{mod}$). Compared to $cTBS_{std}$, $cTBS_{mod}$-induced neuroplasticity was highly consistent between subjects, suggesting that this may be the more effective neuroplasticity-inducing paradigm.

This thesis demonstrates approaches for inducing long-lasting neuroplastic changes within the human primary motor cortex. These findings have important implications for the therapeutic application of rTMS.

DECLARATION

I, Mitchell Ryan Goldsworthy, certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Goldsworthy MR, Pitcher JB, Ridding MC (2012) The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. Eur J Neurosci 35:125-134.

Goldsworthy MR, Pitcher JB, Ridding MC (2012) A comparison of two different continuous theta burst stimulation paradigms applied to the human primary motor cortex. Clin Neurophysiol 123:2256-2263.

Goldsworthy MR, Pitcher JB, Ridding MC (2013) Neuroplastic modulation of inhibitory motor cortical networks by spaced theta burst stimulation protocols. Brain Stimul 6:340-345.

SIGNED…….. DATE……

ACKNOWLEDGEMENTS

First and foremost, I would like to sincerely thank my supervisors Associate Professor Michael Ridding and Dr Julia Pitcher. Their constant support and willingness to help at a moment's notice has been invaluable throughout this process, and I am extremely grateful to have had such excellent supervision. This thesis certainly would not have been possible without them.

I would like to thank all members of the Neuromotor Plasticity and Development (NeuroPAD) group, past and present, for their assistance over the years. Their friendship and encouragement have meant the world to me. I would also like to extend thanks to Professor Ulf Ziemann and Dr Florian Müller-Dahlhaus for their guidance and supervision during my research stay in Frankfurt, and also to the entire Motor Cortex Group from the Department of Neurology at the Goethe-University Frankfurt for their friendship and support.

I am incredibly grateful to my amazing family. I would like to thank my parents for their unconditional support over the years (and their willingness to feed me!), as well as my brother and sister for putting up with me! I would especially like to thank Mal for her patience and loving support.

Last, but not least, I would like to thank each and every subject who gave up their time to participate in my experiments. Without their help this thesis would not have been possible.

LIST OF FIGURES

LIST OF TABLES

AIMS & GENERAL INTRODUCTION

Neuronal networks within the human brain undergo constant reorganizational changes throughout life in response to different experiences, a phenomenon termed neuroplasticity. Neuroplasticity is an essential property of the human nervous system and is critically important for an array of normal brain processes. The human motor system has a remarkable capacity for undergoing this neuroplastic change, enabling us to learn and continually refine the accuracy and efficacy of a large range of complex movements. Likewise, this motor cortical plasticity is important for the recovery of motor skills lost due to neurological injury. For instance, in the chronic stages following stroke, much of the recovery of motor function is likely to occur as a result of neuroplasticity. Accordingly, a central focus of neuroscientific research has been to develop therapeutic strategies which beneficially enhance this neuroplastic change. It is hoped that these strategies may someday be used either on their own or in conjunction with conventional rehabilitative therapies to drive neuroplasticity within the affected brain region and promote recovery of lost function. One such strategy exists in the form of non-invasive brain stimulation.

There is much promise of non-invasive brain stimulation techniques to be used as therapeutic agents in treating a range of neurological and psychiatric conditions. However, given the short lifetime and instability of their induced effects within the human motor system, as well as the high variability of individual responses, the implementation of these techniques in a clinical setting is, at present, far from established. Thus, the studies described in this thesis have aimed to optimise the

application of these non-invasive brain stimulation protocols such that they produce longer lasting and more robust neuroplastic effects within the human motor system.

Unlike the long-lasting synaptic plasticity induced experimentally in animal models using trains of electrical stimulation, the neuroplasticity induced within the human primary motor cortex using repetitive transcranial magnetic stimulation (rTMS) has a very short lifetime that rarely persists for more than 1 h. This discrepancy may be due to differences in the approaches used to apply stimulation trains: whereas rTMS is applied as a single train in humans, the stimulation protocols used in animals are often applied repeatedly in a spaced manner.

Following a review of the literature in Chapter 1, the first three experimental chapters of this thesis will examine the possible benefits of applying repeated trains of an rTMS paradigm (continuous theta burst stimulation; cTBS) to the human primary motor cortex. Specifically, I will examine the lifetime of the induced neuroplastic changes (Chapter 2), the motor networks at which these changes are likely to occur (Chapter 3), and also the stability of these changes in the presence of behaviourally-relevant physiological activity (Chapter 4). The final experimental chapter will shift focus to the stimulation parameters used for cTBS, with the aim of optimising a single cTBS application such that the induced neuroplastic changes in the human primary motor cortex are less variable between individuals (Chapter 5). This thesis will close with a discussion of the main findings, with a focus on the implications of this research for the therapeutic application of non-invasive brain stimulation.

1. A REVIEW OF THE LITERATURE

In this review, I will first provide a brief description of the anatomy and physiology of the structures of the central nervous system involved in the execution of skilled movements in humans. I will then discuss the techniques which may be used to modulate the excitability of these structures through the induction of neuroplastic change. Finally, I will review the therapeutic application of these techniques, as well as the possible approaches that could be used to enhance their potential as therapeutic agents.

1.1. THE NEURAL BASIS FOR HUMAN MOTOR CONTROL

Humans are capable of producing a large range of highly skilled and complex movements. Whilst the mechanical components of human anatomy such as the muscles and joints are important for implementing these skilled tasks, it is the responsibility of the central nervous system to exert control over movement. There are a range of structures within the human central nervous system that work synergistically to produce refined movement, and these will now be discussed in detail.

1.1.1. The cerebral cortex

The cerebral cortex in humans is composed primarily of neocortex, which itself is arranged into six distinct horizontal layers that differ in cellular composition and are numbered from I (corresponding to the outer-most layer) through to VI (corresponding to the inner-most layer). Cells within the neocortex are also arranged vertically into

columns, which make up the primary units of cortical processing (Mountcastle, 1997). Anatomically and functionally distinct cortical regions have been characterised based on the cytoarchitectural organisation of neurons within the cortical layers. In the early twentieth-century, German anatomist Korbinian Brodmann created cytoarchitectonic maps of these regions for a number of species, including humans (Brodmann, 1909). Of the cortical regions defined by Brodmann, area 4, which is located on the precentral gyrus anterior to the central sulcus, constitutes the main cortical region involved in the execution of movement, and is thus referred to as the primary motor cortex.

1.1.1.1. The primary motor cortex

The localisation of motor function to the cerebral cortex was apparent in the nineteenth-century prior to the study of cytoarchitectonic maps, with patients suffering lesions to different parts of the cortex exhibiting a range of different behavioural deficits. Perhaps most informative on the cortical localisation of motor function were the investigations made by the English neurologist John Hughlings Jackson on the spread of muscle spasms in epileptic patients during seizure. He suggested that the part of the body initially activated during the attack indicated the cortical focus on the precentral gyrus where epileptic discharges originate, and that the order in which different body parts convulsed represented the cortical spread of discharge to neighbouring foci (Jackson, 1873). From this it was proposed that a rough somatotopic organisation existed for the cortical representations of motor functions within the precentral gyrus.

The topography of muscle representations was later confirmed and refined by Canadian neurosurgeon Wilder Penfield, who systematically applied stimulation in the form of an electric current to the exposed cortical surface of conscious epileptic patients

undergoing brain surgery under local anaesthesia. He observed activation of leg and trunk muscles when the medial surface of the precentral gyrus was stimulated, and as he probed more laterally activations of the arm, hand and facial muscles were observed (Penfield and Boldrey, 1937; Penfield and Rasmussen, 1950). Additionally, the relative sizes of the cortical representations for different muscles differed disproportionate to the size of the muscle itself, with the muscles of the hands and fingers having larger representations compared to those for the leg and trunk muscles. This most likely reflects the greater requirement for fine motor control for muscles of the hand compared to those of the trunk.

It should be noted that whilst the organisation of different muscle representations within the primary motor cortex are, in general, arranged according to a rough topographical structure, these representations are not fixed. Indeed, there is considerable complexity, with wide-ranging and overlapping representations for different muscles allowing for a large degree of flexibility for primary motor cortical circuits (Gould et al., 1986; Donoghue et al., 1992; Nudo et al., 1992). This flexibility is thought to be intimately related to the cellular structure of the primary motor cortex. Compared to other cortical regions, the primary motor cortex is considerably thicker whilst containing roughly the same number of neurons (Sloper, 1973; Sloper et al., 1979; Rockel et al., 1980). This low density cellular packing affords more space for synaptic connections to form between neurons (Porter and Lemon, 1993). The large degree of interconnectivity between neurons of the primary motor cortex provides this region a great capacity for undergoing neuroplastic changes, and this may well underlie our ability to learn many different complex movements.

1.1.1.2. Cells of the primary motor cortex

As with the other regions of the neocortex, the two main cells of the primary motor cortex are the pyramidal cells and the stellate cells (Porter and Lemon, 1993). Pyramidal cells are the main output cells that connect the primary motor cortex with other cortical, sub-cortical and spinal structures, and are found predominately within cortical layers III and V. Compared to other cortical regions, pyramidal cells of the primary motor cortex come in a large variety of sizes (Jones and Wise, 1977; Meyer, 1987). The largest pyramidal cells, also known as Betz cells named after their discoverer, the Ukrainian anatomist Vladimir Betz (1874), populate cortical layer V and project axons vertically down the corticospinal tract to form excitatory connections with the alpha motor neurons and inhibitory interneurons that populate the spinal column. The many dendrites of pyramidal cells are covered with spines which can receive both excitatory and inhibitory synaptic inputs from cells of all cortical layers. It is estimated that a single pyramidal cell may receive as many as 60,000 synaptic inputs (Cragg, 1975).

The stellate cells comprise approximately 28% of cells within the primary motor cortex (Sloper et al., 1979), and unlike the pyramidal cells their axonal and dendritic projections do not extend beyond the cortical layers. As such, stellate cells serve as cortical interneurons, forming intrinsic connections with other neurons of a given cortical region. There are two types of stellate cells: those with dendritic spines and those without. The spiny stellate cells are found predominately within cortical layer IV and form excitatory synaptic connections, whereas the aspiny stellate cells populate all cortical layers and form inhibitory synaptic connections (Lund, 1973; Hendry and Jones, 1981).

1.1.1.3. Non-primary motor cortical and sub-cortical areas involved in movement

There are a number of non-primary motor cortical areas which have been identified as being important for motor control. For instance, the premotor cortex and supplementary motor area are situated anterior to the primary motor cortex on the lateral and medial portions of Brodmann's area 6, respectively, and contain projections which innervate neurons of the primary motor cortex (Ghosh et al., 1987). The premotor cortex is thought to be concerned with generating the appropriate motor plan in response to external stimuli and coordinating hand and eye movements during complex actions (Passingham, 1985; Pesaran et al., 2006), whereas the supplementary motor area is important for internally-generated motor plans and coordinating bimanual tasks (Brinkman, 1981; Passingham, 1987). Additional motor areas have been identified within the cingulate cortex, located on the medial surface of the cerebral hemispheres above the corpus callosum. These cingulate motor areas receive afferents from the limbic system and are involved in the emotional and motivational aspects of voluntary movement selection based on reward (Shima and Tanji, 1998).

In addition to motor regions of the neocortex, there are two main sub-cortical structures that each plays an important role in the control of movement: the basal ganglia and the cerebellum. Whilst these structures are not part of the cerebral cortex *per se*, they receive information through afferent inputs from motor and sensory cortical regions and send outputs back to the motor cortical regions *via* the thalamus, thus allowing modulation of motor function (Allen and Tsukahara, 1974; Alexander and Crutcher, 1990). In particular, modulation of motor cortex excitability *via* cerebello-thalamocortical projections has been implicated in motor learning (Torriero et al., 2011).

Although these non-primary motor areas are important for the higher-order control of complex voluntary movements in humans, the main focus for the experiments of this thesis is the function of the primary motor cortex and its output to the muscles *via* the descending pathways of the corticospinal tract.

1.1.2. The corticospinal tract

While motor plans are generated within the cerebral cortex, voluntary movement occurs in the periphery. The descending pathway through which motor commands are carried from the brain to the periphery *via* the spinal cord is the corticospinal tract. The corticospinal tract consists of axonal projections of the pyramidal cells located in layer V of the primary motor cortex and, to a lesser extent, the non-primary motor regions and sensory regions of the cerebral cortex (Coulter and Jones, 1977; Dum and Strick, 1991). It can be sub-divided into the lateral corticospinal tract and the ventral corticospinal tract. Fibres of the lateral corticospinal tract (which make up the majority of corticospinal tract fibres) decussate in the brainstem, crossing to the contralateral side and descend through the dorsolateral columns of the spinal cord. Conversely, fibres of the ventral corticospinal tract do not crossover to the contralateral side and descend through the ventromedial columns of the spinal cord, projecting (often bilaterally) to spinal motoneurons that innervate the axial muscles of the trunk (Rothwell, 1994).

One unique property of the corticospinal system that enables highly dexterous finger movements in higher order primates, most notably in humans, is the excitatory monosynaptic connections that can exist between the pyramidal cells of the cerebral

cortex and the motoneurons of the spinal cord that innervate the hand and forearm muscles. These monosynaptic cortico-motoneuronal connections allow for a fast and selective excitation of specific muscles and muscle groups involved in a given action, thus permitting fractionated movements of individual finger muscles (Buys et al., 1986).

1.1.3. Excitatory and inhibitory neurotransmission within the human motor cortex

An important role of the stellate cells in the primary motor cortex is to regulate the output of pyramidal cells by exerting both excitatory and inhibitory control over pyramidal cell firing. The main inhibitory and excitatory neurotransmitters that operate within the mammalian brain are gamma-aminobutyric acid (GABA) and glutamate, respectively.

There exist two main subclasses of GABA receptors: $GABA_A$ receptors and $GABA_B$ receptors (Enna, 2007). The ionotropic $GABA_A$ receptors are ligand-gated chloride ion channels that, when bound by GABA, allow an influx of chloride ions which hyperpolarise the post-synaptic cell, producing an inhibitory post-synaptic potential. Conversely, $GABA_B$ receptors are metabotropic receptors coupled to G proteins. When activated, the $GABA_B$ receptors initiate an intracellular cascade that increases the membrane conductance for potassium ions and decreases conductance for calcium ions, thus hyperpolarising the post-synaptic cell and producing an inhibitory post-synaptic potential. Whilst both GABA receptor subclasses result in hyperpolarisation of the post-synaptic cell membrane (thus making it more difficult for the post-synaptic cell to reach the firing threshold for generating an action potential), $GABA_A$ receptors act over a much shorter time course compared to $GABA_B$ receptors. Whilst $GABA_A$-mediated

inhibition occurs quite rapidly, $GABA_B$-mediated inhibition has a delayed onset, with a time course lasting hundreds of milliseconds (Solis and Nicoll, 1992; Pearce, 1993; Kerr and Ong, 1995). Additionally, $GABA_B$ receptors can act pre-synaptically to modulate the release of neurotransmitters into the synaptic cleft (Pierau and Zimmermann, 1973; Howe et al., 1987).

As with receptors for GABA, glutamate receptors may either be ionotropic or metabotropic. The ionotropic glutamate receptors are the main receptors involved in fast excitatory neurotransmission, and may be further divided into three families: N-methyl-D-aspartate (NMDA), a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors (Meldrum, 2000). Each of these receptors is a ligand-gated channel permeable to sodium and calcium ions, and when activated they produce depolarisation of the post-synaptic cell membrane resulting in an excitatory post-synaptic potential (thus bringing the post-synaptic cell closer to the firing threshold for generating an action potential). The NMDA glutamate receptors are particularly important for the induction of activity-dependent synaptic plasticity, and this will be discussed further in Section 1.3.1.

An altered balance in excitatory and inhibitory neurotransmission within the primary motor cortex has been associated with a number of pathological conditions. For instance, studies in dystonic patients have revealed that in addition to changes occurring at the levels of the spinal cord and brainstem, there is a reduction in cortical inhibition within the motor system that might explain the excessive, disordered movements associated with the disease (Ridding et al., 1995b; Chen et al., 1997a). Reduced motor cortical inhibition may also contribute to the disordered movements seen in patients

with Parkinson's disease (Ridding et al., 1995a) and cortical myoclonus (Brown et al., 1996), and may also be an important factor mediating cortico-motoneuronal hyperexcitability and excitotoxicity in patients with amyotrophic lateral sclerosis (Vucic et al., 2012).

Given the necessity of balanced cortical excitation and inhibition for normal brain functions, the study of the excitatory and inhibitory neuronal networks within the cerebral cortex has become an area of increasing importance. The emergence of non-invasive brain stimulation techniques has allowed researchers to test the excitability of these neuronal networks within the human primary motor cortex, and this may facilitate future research into the selective neuromodulation of these networks to correct imbalances in cortical excitation and inhibition (a concept explored further in Chapter 3). The following section will discuss the techniques that have been developed to non-invasively and painlessly stimulate the motor cortex in conscious human subjects.

1.2. NON-INVASIVE STIMULATION OF THE HUMAN BRAIN

1.2.1. Stimulation of the human motor cortex through the intact scalp and skull

Throughout the early-to-mid-twentieth century the direct excitation of exposed cortical tissue by electrical microstimulation contributed greatly to the understanding of the human motor cortex. However, towards the end of the twentieth century techniques that would allow transcranial stimulation of the motor areas through the intact scalp and skull were developed.

The first advancement in this area came in 1980 when Merton and Morton developed the method of transcranial electrical stimulation (TES). In their study, they employed a very brief, high voltage electric shock through electrodes attached to the scalp overlying the motor cortex. They found such stimulation to produce a twitch-like activation of the contralateral hand muscles when applied to the arm motor area and a similar activation of the foot muscles when applied to the leg motor area (Merton and Morton, 1980). The twitch-like movements produced by TES could be recorded as a biphasic electrical potential (i.e. motor evoked potential; MEP) through surface electrodes over the targeted hand or foot muscles, and their latencies were consistent with those observed following electrical stimulation of the exposed motor cortical surface (Milner-Brown et al., 1975). Although TES provided the first feasible method for non-invasively stimulating the motor cortex without the requirement of surgery to expose the cortical surface, given the high resistance of the scalp and skull to electrical stimulation, this method produced a high level of discomfort.

In 1985, Barker and colleagues introduced the method of transcranial magnetic stimulation (TMS). Whilst the electric current induced in cortical tissue by TES is produced by electrodes attached to the patients scalp, TMS utilises the process of electromagnetic induction to activate cortical circuits. This is achieved by passing a strong electric current though a magnetic coil, which in turn creates a very brief magnetic field with lines of flux perpendicular to the plane of the coil. The magnetic field is able to pass through the intact scalp and skull, inducing eddy currents in the underlying conductive cortical tissue. In much the same way as TES, when TMS was applied to the human motor cortex MEPs could be recorded from the hand muscles using surface electromyography (Barker et al., 1985). However, an important distinction

could be made between TES and TMS in the mode by which electric currents were delivered to the cortical tissue. Whilst the skull offers considerable resistance to electrical stimuli, the magnetic field of TMS is able to pass through the scalp and skull unimpeded. As a result, compared to the discomfort of TES, TMS is virtually pain-free.

1.2.2. Origin of descending activity elicited by TES and TMS

Using direct stimulation of the exposed motor cortical surface in monkeys, Patton and Amassian (1954) observed that activation of the pyramidal neurons with a single stimulus produced a series of discharges that propagated downward through the corticospinal tract. The first of these discharges occurring with the shortest latency arises as a result of direct activation of the pyramidal neurons, whilst the remaining discharges produced thereafter occur through indirect, trans-synaptic activation of the pyramidal neurons. Consequently, these electrical responses were termed direct (D) and indirect (I) waves, respectively. I-waves are generally numbered in order of their appearance, with I1 representing the earliest I-wave.

Recordings made from the cervical epidural space of conscious human subjects have shed light on the origin of descending corticospinal activity elicited by TES and TMS of the human primary motor cortex. At intensities around threshold, TES evoked D-wave activity whilst TMS (applied with a coil orientation such that the induced current flow in the brain was posterior-anterior) produced an I1-wave only (Di Lazzaro et al., 1998a). Only when the intensity of stimulation was increased were mixed D and I-waves evident for both TES and TMS. This suggests that whilst TES preferentially activates pyramidal cells of the corticospinal tract directly, TMS with a monophasic

posterior-anterior current preferentially activates the neuronal elements that project onto pyramidal cells. Evidence that increasing the excitability of the motor cortex by voluntary contraction increases the size and number of evoked I-waves suggests that TMS activates neuronal elements within the cortex (Di Lazzaro et al., 1998b). These neuronal elements are likely to consist of corticocortical afferent connections to the motor cortex, as well as local intracortical interneurons that synapse onto the pyramidal cells of the corticospinal tract (Di Lazzaro et al., 2008a).

It should be noted that the descending corticospinal activity evoked by focal TMS applied using a figure-of-eight shaped coil may differ depending on the orientation of the stimulating coil (and thus the direction of the induced current within the brain). Whereas TMS with an induced monophasic current that flows posterior-anterior preferentially evokes an I1-wave at threshold intensities, TMS with an anterior-posterior current direction preferentially evokes the later I3-wave in some subjects and D or I1 waves (with slightly longer peak latencies) in other subjects (Di Lazzaro et al., 2001b). Positioning of the coil such that the direction of induced current flow is lateral-medial recruits both a D-wave and an I1-wave at low stimulation intensities (Di Lazzaro et al., 1998a). These findings suggest that TMS with different current directions may activate different neuronal elements and/or different sites of the same neuronal elements within the primary motor cortex (Di Lazzaro et al., 2008a). This is important when considering the cortical site of descending activity elicited by TMS with a biphasic pulse waveform, which induces current in two opposing directions within the brain. In this instance, the descending corticospinal activity may be elicited by current flowing in either direction, depending on the intensity of stimulation and the relative threshold of each direction of current flow for recruiting D and I-wave activity (Di Lazzaro et al., 2001a).

1.2.3. Application of TMS to probe the excitability of the human motor system

The descending corticospinal activity evoked by TMS of the human primary motor cortex excites the alpha motoneurons of the spinal cord, which fire in synchrony to produce a twitch-like activation of the target muscle. This muscle activation may be recorded as a biphasic electrical potential (i.e. an MEP) using surface electromyography, and the peak-to-peak amplitude of this biphasic potential is influenced by the size and number of the descending waves that arrive at the spinal motoneurons. As the descending activity evoked by TMS is generated by trans-synaptic excitation of the corticospinal tract, the efficacy of synaptic connections within the motor cortex determines to a large extent the size of the induced MEP response. Thus, when changes in spinal excitability are controlled for, changes in the amplitude of MEPs evoked by TMS can be used as a sensitive marker for changes in the excitability of synaptic connections within the targeted primary motor cortical region. I will briefly describe in this section the various ways that TMS can be applied to test the excitability of different cortical networks, with a primary focus on those techniques used throughout the studies of this thesis.

1.2.3.1. Single-pulse TMS

There are several ways that single-pulse TMS can be applied to test cortical excitability. For instance, low intensity pulses can be used to assess the threshold for evoking MEPs, and this can be measured either during complete relaxation (i.e. resting motor threshold; RMT) or whilst the subject performs a mild voluntary contraction of the target muscle (i.e. active motor threshold; AMT). Measures of motor threshold are elevated by drugs

that block voltage-gated sodium ion channels, reflecting a dependence on the axonal membrane excitability of neuronal elements of the corticospinal tract (Mavroudakis et al., 1994; Ziemann et al., 1996b; Chen et al., 1997b; Boroojerdi et al., 2001). Furthermore, studies using diffusion-weighted magnetic resonance imaging show evidence that motor threshold may also reflect the microstructural properties of corticocortical and corticospinal fibres not limited to membrane excitability (Klöppel et al., 2008). Motor threshold may also be reduced by drugs that enhance fast excitatory neurotransmission through AMPA receptors, suggesting that the strength of excitatory synaptic connections within the cortical networks activated by TMS may also be an important factor mediating the threshold for evoking MEPs (Di Lazzaro et al., 2003).

Single-pulse TMS can also be used to produce a scalp map that reflects to some degree the distribution of underlying cortical representations of different muscles by systematically stimulating different cortical sites at a constant intensity above motor threshold (Wassermann et al., 1992). This method of motor mapping has been used to show expansions and reductions in cortical representations for different muscles and is sensitive to the neuroplastic changes in human motor cortical excitability that occur as a result of motor learning (Pascual-Leone et al., 1995), as well as those due to pathological changes within the central nervous system (Cohen et al., 1991; Topka et al., 1991; de Carvalho et al., 1999). Similar to motor mapping, changes in motor cortical excitability may also manifest as changes in the input/output characteristics of the motor cortex, assessed by applying TMS to a single cortical site at a range of different intensities (Ridding and Rothwell, 1997). The relationship between TMS intensity and the size of the MEP response is sigmoidal (Devanne et al., 1997), with the highest rate of change in MEP size occurring at intermediate intensities above RMT.

In line with the majority of current literature investigating neuroplasticity induction within the human motor system, for the experiments described throughout this thesis I have used TMS applied at single, intermediate intensities to detect changes in MEP size and show evidence for neuroplastic change within the human primary motor cortex. Studies using this method for assessing MEP change following some intervention have typically used a test TMS intensity that either: (1) evokes baseline MEPs of fixed amplitude (i.e. 1 mV, measured peak-to-peak) (Stefan et al., 2000; Stefan et al., 2002; Ziemann et al., 2004; Gentner et al., 2008), or (2) is relative to each subject's baseline measure of motor threshold (Maeda et al., 2000a; Wolters et al., 2003; Di Lazzaro et al., 2011). In practice, both approaches result in a test MEP of intermediate amplitude that lies within the linear portion of the sigmoidal input/output curve for most subjects. All experiments for this thesis have been conducted using the former approach.

1.2.3.2. Paired-pulse TMS

In a seminal study published in 1993, Kujirai and colleagues tested the impact of a subthreshold conditioning TMS pulse on the size of the MEP response to a second, suprathreshold TMS test pulse. They investigated a range of interstimulus intervals (ISIs) between 1 and 15 ms, and found that the conditioning stimulus inhibited the MEP response to the test pulse at ISIs between 1 and 6 ms and facilitated the MEP response at longer ISIs of 10 and 15 ms (Kujirai et al., 1993). These inhibitory and facilitatory effects of paired-pulse TMS have been referred to as short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF), respectively. SICI is thought to occur as a result of the conditioning pulse eliciting an inhibitory postsynaptic potential at pyramidal cells within the motor cortex, and most likely reflects the excitability of

$GABA_A$-mediated intracortical inhibitory circuits since administration of the $GABA_A$ receptor agonist lorazepam results in increased SICI (Ziemann et al., 1996a; Di Lazzaro et al., 2000). Conversely, the ICF observed with paired-pulse TMS at longer ISIs is likely due to the slightly delayed excitatory postsynaptic potential mediated by NMDA receptors, with reduced ICF observed following administration of the NMDA receptor antagonist dextromethorphan (Ziemann et al., 1998).

In addition to its utility in testing the excitability of $GABA_A$-mediated intracortical inhibitory circuits, paired-pulse TMS may also be used to test the excitability of inhibitory circuits dependent upon $GABA_B$ receptors. This is achieved by applying two suprathreshold pulses at long ISIs between 50 and 200 ms (Valls-Sole et al., 1992). The long ISIs required for this form of intracortical inhibition (termed long-interval intracortical inhibition; LICI) are consistent with the slow inhibitory postsynaptic potentials produced by activation of the metabotropic $GABA_B$ receptors, and this is supported by pharmacological evidence showing enhanced LICI following administration of the $GABA_B$ receptor agonist baclofen (McDonnell et al., 2006).

There is little doubt that the development of TMS as a method for assessing cortical excitability non-invasively in conscious human subjects has increased our understanding of human motor physiology. For several decades it has helped researchers to characterise the pathophysiological changes associated with a range of neurological disorders. However, for all the contributions made by TMS to date, perhaps its most significant clinical application has yet to be fulfilled. Its potential to not only detect pathological changes in the excitability of cortical circuits but also correct them makes TMS an appealing therapeutic strategy for a range of brain-related

disorders. Thus, the remainder of this review will focus on the potential of TMS to be used as a therapeutic tool for treating disease. I will first discuss the neuroplastic processes through which TMS is thought to change cortical excitability, followed by an evaluation of what will be required for its therapeutic potential to be realised.

1.3. NEUROPLASTICITY

Neuroplasticity is the process by which neuronal networks of the central nervous system reorganise the strength of their connections. Neuroplastic change can be triggered by a variety of different external and internal stimuli, and is largely an activity-dependent phenomenon. For instance, neuronal networks that are used frequently will tend to be favoured and will become stronger, whereas those used less frequently will be selected against and will become weaker. In this way, neuroplasticity is thought to represent the main process through which information is stored and retrieved within the central nervous system, and may well underlie learning and memory.

1.3.1. Neuroplastic change through long-term potentiation and depression of synaptic transmission

An early notion of neuroplasticity and its role in behaviour was conceptualised in the mid-twentieth century by the Canadian psychologist Donald Hebb. In his 1949 book entitled ‘The Organization of Behaviour’, Hebb presented his theory regarding the use-dependent changes in the strength of synaptic connections forming ‘traces’ in cortical circuits. This was immortalised in his Neurophysiological Postulate:

> *'When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.'* (Hebb, 1949).

Whilst Hebb's ideas were revolutionary for their time, the mechanisms through which such change might occur in a living organism remained largely unknown. However, experimental evidence was provided in 1966 when Terje Lømo presented his research to the Scandinavian Physiological Society (Lømo, 1966), which was later expanded upon and published in collaboration with Timothy Bliss in 1973 (Bliss and Lømo, 1973). The experiments were conducted in the hippocampus of the anaesthetised rabbit and showed that short trains of repetitive, high-frequency electrical stimulation applied to perforant path fibres produced long-lasting enhancements in synaptic transmission to the granule cells of the dentate gyrus. This long-lasting increase in synaptic efficacy, termed long-term potentiation (LTP), has since been shown at neuronal networks outside of the hippocampus and in a range of different species (Kirkwood et al., 1993; Hess and Donoghue, 1994; Urban et al., 1996). Similarly, a lasting suppression of synaptic transmission has been observed typically with low-frequency stimulation, and this has been referred to as long-term depression (LTD).

Whilst several cellular processes may underlie LTP and LTD induction, by far the most extensively studied and best understood are those involving the ionotropic NMDA receptor (Malenka and Bear, 2004). When the post-synaptically localised NMDA receptors are activated by pre-synaptically released glutamate, entry of calcium ions through the NMDA receptor cation channels is impeded by a voltage-dependent

magnesium ion blockade (Nowak et al., 1984). However, when the NMDA receptors bind glutamate concurrently with post-synaptic depolarisation, the magnesium block is released, allowing calcium ions to enter the post-synaptic cell (Cooke and Bliss, 2006). Large increases in intracellular calcium concentration activate various calcium-sensitive kinases, which increase the conductance and insertion of AMPA glutamate receptors into the post-synaptic cell membrane through protein phosphorylation (Derkach et al., 1999; Lee et al., 2003; Takahashi et al., 2003). This increased AMPA activity enhances the post-synaptic cells permeability and sensitivity to glutamate, making it more easily excitable by the pre-synaptic cell and resulting in an increased synaptic strength.

The nature of NMDA receptor-dependent LTP shares several similarities with the initial ideas that Hebb postulated in his 1949 work. Of these similarities, perhaps the most notable is the requirement of a high level of synchronicity between the repeated firing of two cells for synaptic strengthening to occur. In addition, this form of LTP exhibits several other properties that are closely related to Hebbian learning. For instance, LTP exhibits input specificity, with potentiation occurring only at synaptic connections activated by correlative pre and post-synaptic activity and not at neighbouring synapses (Andersen et al., 1980). LTP can also be associative, such that a weak tetanic stimulation protocol subthreshold for inducing LTP can produce lasting changes in synaptic efficacy if applied concurrently with a second induction protocol to another pathway (McNaughton et al., 1978; Levy and Steward, 1979). Finally, LTP can be made to be extremely durable, lasting for many days and even months (see Section 1.5).

Whilst LTP may occur at synapses as a result of coincident pre and post-synaptic activity, synaptic connections may undergo LTD and become weakened on occasions

where the pre-synaptic cell repeatedly fails to sufficiently fire a post-synaptic cell. Like LTP, LTD can be produced by NMDA receptor activation. However, unlike LTP, LTD is induced by mismatched pre and post-synaptic activity resulting in only minute calcium influx through the NMDA receptor cation channels (Nishiyama et al., 2000). The small rise in calcium concentration within the post-synaptic cell preferentially activates protein phosphatase cascades, which dephosphorylate AMPA receptors and promote their removal from the post-synaptic cell membrane (Lee et al., 1998; Carroll et al., 1999). This decreases the permeability and sensitivity of the post-synaptic cell to glutamate released from the pre-synaptic cell, thus decreasing synaptic strength.

1.3.2. The involvement of LTP in learning and memory: evidence from animal models

The involvement of activity-dependent synaptic plasticity in learning and memory has been well documented in studies investigating animal models. Morris et al. (1986) used the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP5) to block LTP induction in the rat hippocampus and found that rats infused with AP5 prior to being placed in a water maze exhibited impaired spatial learning when trying to locate a hidden platform (Morris et al., 1986). Other studies have shown similar results with other forms of hippocampal-dependent learning using NMDA receptor antagonists (see Danysz et al., 1995, for review), suggesting a possible role for NMDA receptor-dependent LTP in learning and memory in rodents. There also exist drugs which enhance both hippocampal LTP and learning (Staubli et al., 1994b; Staubli et al., 1994a; Larson et al., 1995; Shors et al., 1995; Rogan et al., 1997), thus further emphasising the link between activity-dependent synaptic plasticity and learning.

The use of genetically-altered mice has also demonstrated an association between hippocampal LTP and learning and memory. Tsien et al. (1996) tested the spatial memory of mutant mice possessing a *NMDA receptor 1* (*NR1*) gene knockout in the cornus ammonis 1 (CA1) subfield of the hippocampus only. NR1 is a required subunit for the formation of NMDA receptors, thus *NR1* gene knockout mice do not express the NMDA receptor. By restricting this gene knockout to the CA1 region of the hippocampus, not only were the investigators able to expand the lifetime of mutant mice into adulthood but it also ensured that any altered performance in the water maze task was due to the absence of NMDA receptors in the hippocampus and not in other regions of the brain. They found that, in addition to being unable to express LTP within the CA1 region, these mutant mice exhibited impaired spatial learning and memory (Tsien et al., 1996). These results indicate a strong involvement of NMDA receptor-dependent hippocampal LTP in learning and memory in mice.

In addition to the evidence for the involvement of LTP in learning and memory in the rat hippocampus, alterations in the strength of horizontal synaptic connections within the motor regions of the rat neocortex have also been demonstrated in response to motor learning (Rioult-Pedotti et al., 1998). These changes in synaptic efficacy are likely due to the induction of LTP, as evidenced by the reduced capacity to induce subsequent LTP in primary motor cortex slice preparations retrieved from rats that had previously undergone motor training (an effect specific to the forelimb representation of the hemisphere contralateral to the trained side) (Rioult-Pedotti et al., 1998; Rioult-Pedotti et al., 2000). This reduction of subsequent LTP induction following motor training is consistent with the saturation of LTP in response to motor skill learning. Indeed, the capacity for LTD induction was enhanced in slice preparations from trained rats

(Rioult-Pedotti et al., 2000), a result also consistent with LTP being the primary mechanism by which motor learning-induced changes in synaptic strength occur in the rat motor cortex.

1.3.3. Non-invasive brain stimulation as a means for neuroplasticity induction in the human cortex

As discussed in Section 1.2, brain stimulation techniques such as TMS offer a non-invasive and painless method for assessing cortical excitability in humans and may be used to detect neuroplastic changes in the excitability of motor neuronal networks. However, an important secondary application of TMS relates to its capacity to induce neuroplastic changes in human cortical excitability. This has been achieved by applying trains of repeated pulses of magnetic stimuli in a technique referred to as repetitive transcranial magnetic stimulation (rTMS). Whilst rTMS has been used to induce neuroplasticity within a number of different cortical regions (George et al., 1996; Boroojerdi et al., 2000; Nyffeler et al., 2006b), due to the relative ease of quantifying changes in motor cortical excitability by measuring changes in the size of MEPs evoked by single and paired-pulse TMS the majority of studies investigating rTMS-induced neuroplasticity have focused on the human primary motor cortex. Indeed, the changes in motor cortical excitability induced by rTMS protocols closely resemble those observed following motor learning and likely involve similar mechanisms (Stefan et al., 2002; Wolters et al., 2003; Ziemann et al., 2004; Stefan et al., 2006; Huang et al., 2007). The different ways in which rTMS may be applied to induce these neuroplastic changes will now be discussed.

1.3.3.1. Conventional rTMS

Early investigations studying the application of rTMS in humans employed simple protocols consisting of evenly spaced magnetic pulses. Generally, rTMS applied at low frequencies (i.e. ≤ 1 Hz) will reduce the size of the MEP response to single-pulse TMS, reflecting a decrease in motor cortical excitability (Chen et al., 1997c; Maeda et al., 2000a; Muellbacher et al., 2000; Gangitano et al., 2002). Conversely, rTMS applied at higher frequencies (i.e. ≥ 5 Hz) tends to produce facilitatory after-effects in the human primary motor cortex, increasing the size of the MEP response to single-pulse TMS (Berardelli et al., 1998; Maeda et al., 2000a; Gangitano et al., 2002). However, this relationship between frequency and response to rTMS may be complicated by a number of factors, for instance, the intensity at which rTMS trains are applied. Modugno et al. (2001) showed that short trains of high-frequency rTMS applied at low intensities suppressed MEPs, whilst the same frequency of stimuli applied at a higher intensity resulted in MEP facilitation. The duration of the stimulus train may also influence the response to rTMS, as well as the waveform (i.e. monophasic vs. biphasic) and number of pulses within a train (Maeda et al., 2000b; Taylor and Loo, 2007).

1.3.3.2. Paired associative stimulation

Another rTMS protocol which has been used to induce changes in motor cortical excitability is paired associative stimulation (PAS), which involves the application of trains of magnetic stimuli over the hand regions of the primary motor cortex paired with electrical stimulation of the median nerve at the wrist (Stefan et al., 2000). Like simple rTMS protocols, PAS-induced neuroplasticity may be excitatory or inhibitory depending on the time interval between electrical nerve stimulation and the TMS pulse. For instance, if stimulation of the median nerve occurs 25 ms before TMS is applied to

the primary motor cortex (i.e. PAS_{25ms}), the peripheral nerve stimulus will arrive at the primary motor cortex *via* the primary somatosensory cortex marginally before TMS, having a facilitatory effect on motor cortical excitability when repeated paired stimuli are applied (Stefan et al., 2000; Wolters et al., 2003). However, when the peripheral nerve is stimulated 10 ms before TMS (i.e. PAS_{10ms}), the synchronicity between the paired stimuli is lost, resulting in a suppression of motor cortical excitability (Wolters et al., 2003).

1.3.3.3. Theta burst stimulation

Animals engaging in exploration of a novel environment show bursts of high-frequency cellular firing within the hippocampus in phase with the 4 to 7 Hz theta frequency rhythm (O'Keefe and Nadel, 1978). Based on this pattern of neuronal activity during behaviours which require learning and memory, stimulation protocols used to induce LTP in animal models have been developed which involve the application of short bursts of high-frequency stimuli (i.e. 100 Hz) repeated at the theta frequency of 5 Hz (Larson et al., 1986). Using rTMS, Huang et al. (2005) adapted this so-called theta burst stimulation (TBS) pattern so it would be safe to use in humans (that is, bursts of three subthreshold TMS pulses at 50 Hz repeated at a frequency of 5 Hz). It was found that the direction of change in motor cortical excitability induced by TBS applied to the hand area of the human primary motor cortex was highly dependent on the temporal pattern of TBS delivery. When TBS was applied as a continuous train lasting 40 s (i.e. continuous theta burst stimulation; cTBS), a lasting suppression of MEP amplitudes was observed. However, when TBS with the same number of pulses was applied using intermittent 2 s trains at 10 s intervals for a total of 190 s (i.e. intermittent theta burst stimulation; iTBS), MEP amplitudes were facilitated (Huang et al., 2005). It was

suggested that the different effects of these two TBS protocols on motor cortical excitability may have been the result of TBS producing a mixture of facilitation and inhibition, with facilitation dominating during the initial stages of TBS application before saturating after a few seconds, allowing a slower and more prolonged build-up of inhibition to dominate.

1.3.3.4. Repetitive paired-pulse TMS

An additional rTMS protocol has utilised the repetitive application of paired suprathreshold TMS pulses at 1.5 ms ISIs, corresponding to the frequency at which I-waves are generated within the primary motor cortex following application of a single TMS pulse (Day et al., 1989). When applied every 5 s for 30 min, repetitive paired-pulse TMS at I-wave periodicity facilitated the MEP response to single-pulse TMS for a period of up to 10 min following stimulation (Thickbroom et al., 2006). Conversely, when paired stimuli were applied out of phase with I-wave periodicity using an ISI of 2 ms, this protocol induced MEP suppression (Cash et al., 2013).

Extending the repetitive paired-pulse TMS protocol developed by Thickbroom et al. (2006), Hamada et al. (2007) reasoned that increasing the number of pulses per train would enhance the neuroplastic modulation of motor cortical excitability. Therefore, in their study, trains of four suprathreshold TMS pulses were applied at 1.5 ms ISIs every 5 s for 30 min. This protocol (termed quadripulse stimulation) induced a facilitatory after-effect on MEP amplitudes that lasted at least 75 min in duration (Hamada et al., 2007). Similar to paired-pulse stimulation, the neuromodulatory effects of quadripulse stimulation of the human primary motor cortex may be either facilitatory or inhibitory depending on the intervals between TMS pulses, with short ISIs (1.5, 5 and 10 ms)

increasing MEP amplitudes and long ISIs (30, 50 and 100 ms) decreasing MEP amplitudes (Hamada et al., 2008).

1.3.3.5. Other neuroplasticity-inducing protocols

An alternative to rTMS for non-invasively inducing neuroplastic changes in human cortical excitability is transcranial direct current stimulation (tDCS), which involves applying a weak direct current (approximately 1 mA) to the brain through two pad electrodes (one anode and one cathode) attached to the surface of the scalp. Prolonged stimulation for around 10 min can produce a persistent change in motor cortical excitability that is either facilitatory or inhibitory, depending on the placement of the anodal and cathodal electrodes (Nitsche and Paulus, 2000, 2001). Placement of the anode to the scalp overlying the hand area of the primary motor cortex and the cathode to the contralateral orbit has been shown to increase MEP amplitudes measured from the hand muscles (i.e. anodal tDCS). Conversely, when this configuration is reversed by placing the cathode over the motor cortex and the anode over the contralateral orbit, there is a decrease in MEP amplitudes (i.e. cathodal tDCS). Although tDCS is a safe and effective method for inducing lasting neuroplastic changes within the human primary motor cortex, the experiments described in this thesis have focused primarily on optimising rTMS protocols to further their therapeutic value.

1.3.4. The origin and mechanisms for rTMS-induced after-effects in humans

Whilst the amplitude of MEPs evoked by TMS reflects the excitability of synaptic connections within the human primary motor cortex, a significant contribution is also made by the excitability of neuronal elements intrinsic to the spinal cord. Measures of

spinal cord excitability such as H-reflexes and F-waves have been shown to be unaffected by intervention with various rTMS protocols (Stefan et al., 2000; Modugno et al., 2001; Wolters et al., 2003; Huang et al., 2005). Whilst there is some evidence that PAS induces changes in H-reflexes (Meunier et al., 2007; Lamy et al., 2010), studies have also shown the amplitude of MEPs generated from the direct activation of the corticospinal tract downstream of the cortex using electrical brainstem stimulation (Ugawa et al., 1991) to be unaffected by PAS (Stefan et al., 2000; Wolters et al., 2003), suggesting its effects on TMS-evoked MEPs are largely cortical.

Perhaps the best evidence for the cortical origin of rTMS-induced after-effects has come from directly recording descending corticospinal volleys from the cervical epidural space of conscious human subjects. For instance, Di Lazzaro et al. (2002b) showed an increase in the size and number of descending I-waves evoked during the course of a short train of suprathreshold 5 Hz rTMS. This I-wave facilitation outlasted the period of stimulation and was in close accord to the increase in MEP amplitudes shown in previous studies using the same rTMS paradigm (Di Lazzaro et al., 2002b). A similar facilitation of I-waves has been observed following the excitatory PAS_{25ms} protocol (Di Lazzaro et al., 2009), suggesting a cortical site of action for both simple rTMS protocols and PAS. Direct evidence also exists for the cortical origin of TBS-induced after-effects. Whilst cTBS had no apparent effect on the D-wave evoked by single-pulse TMS, there was significant suppression of the I1-wave (Di Lazzaro et al., 2005). In a separate study by the same group it was shown that iTBS facilitated later I-waves whilst the I1-wave remained unchanged (Di Lazzaro et al., 2008b). These results indicate a cortical origin for both cTBS and iTBS-induced changes in MEPs and suggest that these

two protocols exert their opposing effects on motor cortical excitability by way of different intracortical circuitry.

Although there is good evidence indicating a cortical site of action for rTMS, it remains difficult to conclusively verify the mechanisms responsible for these changes in cortical excitability. Several studies have shown the after-effects of various rTMS protocols to be influenced by prior motor learning (Ziemann et al., 2004; Stefan et al., 2006). Similarly, there is some evidence from studies on the human primary motor cortex to suggest that rTMS application may be able to interact with and modify voluntary movement (Huang et al., 2005) and motor learning (Muellbacher et al., 2002; Jung and Ziemann, 2009). As a result of the involvement of LTP and LTD in motor learning in animal models, it has been suggested that rTMS may alter the strength of synaptic connections within the cortex by way of similar processes. Indeed, several characteristics of rTMS-induced changes in motor cortical excitability are consistent with LTP and LTD-like mechanisms (see Hoogendam et al., 2010, for review). However, perhaps the best evidence for this has been presented in the human primary motor cortex using pharmacological intervention.

Consistent with *in vitro* studies in animal slice preparations demonstrating dopaminergic receptor activation to be a positive modulator of LTP and LTD (Huang et al., 2004), the application of the dopamine receptor agonist pergolide has been shown to enhance the suppressive effects of 1 Hz rTMS on human motor cortical excitability (Lang et al., 2008). Additionally, the administration of the NMDA receptor antagonist memantine blocked both iTBS and cTBS-induced changes in MEPs, suggesting that the after-effects produced by these rTMS protocols are, at least to some extent, NMDA

receptor-dependent (Huang et al., 2007). A similar dependency on NMDA receptor activity has also been shown for PAS-induced plasticity, with dextromethorphan blocking the changes in motor cortical excitability induced by both PAS_{25ms} and PAS_{10ms} (Stefan et al., 2002; Wolters et al., 2003). Given the critical importance of the NMDA receptor in mediating the induction of most forms of LTP and LTD, these results are consistent with the notion that rTMS paradigms exert their effects on human motor cortical excitability through LTP and LTD-like processes.

Despite the considerable pharmacological evidence linking the after-effects of various rTMS protocols with the LTP and LTD observed in animal models, there remain some features of rTMS-induced changes in human motor cortical excitability that are not entirely consistent with these mechanisms. In particular, compared to the long-lasting experimentally-induced changes in synaptic efficacy that can persist in awake animals for several weeks (Abraham, 2003), the changes in MEPs induced by even the strongest rTMS protocols do not persist for more than 1-1.5 h at most (Stefan et al., 2000; Wolters et al., 2003; Huang et al., 2005; Hamada et al., 2008). This may be due to the particular phase of LTP/LTD induced by current rTMS protocols: whilst the MEP changes following rTMS may involve similar mechanisms to those required during the *early-phase* of LTP/LTD induction, they lack the *late-phase* required for the long-term maintenance of these LTP/LTD-like effects. The different, mechanistically distinct phases of LTP and LTD will be discussed in more detail in Section 1.5.

1.4. THERAPEUTIC APPLICATION OF rTMS PROTOCOLS

1.4.1. Therapeutic application of rTMS in treating psychiatric disorders

As a result of their capacity to alter human cortical excitability, the possible therapeutic application of rTMS protocols in a variety of disorders characterised by abnormal cortical excitability has attracted much interest. The therapeutic value of rTMS has been explored for a number of different psychiatric conditions, including obsessive-compulsive disorder (Greenberg et al., 1997; Sachdev et al., 2001), schizophrenia (Hoffman et al., 2003; Chibbaro et al., 2005; Lee et al., 2005; Poulet et al., 2005; Bagati et al., 2009), post-traumatic stress disorder (Grisaru et al., 1998; Cohen et al., 2004) and addiction (Eichhammer et al., 2003; Camprodon et al., 2007; Amiaz et al., 2009; Mishra et al., 2010). Of those investigated, the psychiatric disorder in which the therapeutic application of rTMS has been studied most extensively is drug-resistant depression.

The use of rTMS as a treatment for depression is based on functional imaging studies showing reduced cerebral blood flow and metabolism in the left dorsolateral prefrontal cortex (Baxter et al., 1989; Martinot et al., 1990; Bench et al., 1993). Several clinical trials have observed promising anti-depressant effects of high-frequency rTMS applied to the left dorsolateral prefrontal cortex in patients with medication-resistant depression (George et al., 1995; Pascual-Leone et al., 1996; George et al., 2000; O'Reardon et al., 2007). This is perhaps best shown in a recent multicentre, sham-controlled study observing a strong anti-depressant effect of daily treatment with 10 Hz rTMS applied to the left prefrontal cortex of patients with medication-resistant depression (George et al., 2010). An alternative approach to treating depression with rTMS is based on the notion

that an inter-hemispheric imbalance exists between the left and right prefrontal regions, with excessive excitability within the right dorsolateral prefrontal cortex in patients with depression. Indeed, reducing the excitability of the right dorsolateral prefrontal cortex by low-frequency rTMS has been shown to be equally beneficial in relieving symptoms of depression (Fitzgerald et al., 2003; Isenberg et al., 2005; Fitzgerald et al., 2009).

Given its non-invasive nature and the minimal risk associated with its application when administered using parameters within the recommended limits (Rossi et al., 2009), rTMS has been heralded as a potential alternative to electroconvulsive therapy in treating major depression. However, whilst several studies have shown positive results of simple rTMS protocols in depression, any anti-depressant effects are variable and generally too small to be of clinical relevance (Holtzheimer et al., 2001; Burt et al., 2002; Martin et al., 2003; Mitchell and Loo, 2006).

1.4.2. Therapeutic application of rTMS in treating movement disorders

In addition to its implementation in clinical trials for various psychiatric conditions, the application of rTMS as a therapeutic intervention has also been examined for a number of movement disorders characterised by motor dysfunction. For instance, in Parkinson's disease patients, the application of a subthreshold train of 5 Hz rTMS to the primary motor cortex during performance of a movement task was able to significantly decrease choice reaction time and movement time, as well as improve performance in a grooved pegboard test (Pascual-Leone et al., 1994). When applied over consecutive days, suprathreshold 5 Hz rTMS of the lower limb and hand motor representations in un-medicated Parkinson's disease patients significantly improved motor function for at

least one month following the end of treatment (Khedr et al., 2003). Similar improvements in motor function have also been observed with rTMS applied at 25 Hz to the dorsolateral prefrontal cortex and motor cortex of both hemispheres in Parkinson's disease patients already receiving optimal therapy (Lomarev et al., 2006).

Another movement disorder for which rTMS intervention may be beneficial is dystonia. Based on evidence that dystonic patients exhibit reduced intracortical inhibition (Ridding et al., 1995b), the application of inhibitory rTMS paradigms have been proposed to reduce motor cortical excitability and restore inhibitory drive. Application of subthreshold 1 Hz rTMS to the primary motor cortex in patients with writer's cramp not only increased intracortical inhibition but also significantly (although transiently) improved handwriting (Siebner et al., 1999). Low-frequency rTMS over the premotor cortex has shown similar improvements in handwriting in dystonic patients (Murase et al., 2005).

Pilot studies have provided some evidence that inhibitory rTMS paradigms might have some potential for treatment of amyotrophic lateral sclerosis by reducing the excitatory drive within the corticospinal tract, thus ameliorating glutamate-mediated excitotoxicity. A preliminary study in two patients showed that 1 Hz rTMS applied to the primary motor cortex over several sessions slowed disease progression (Di Lazzaro et al., 2004). Similar results have also been shown in a double-blind, placebo-controlled trial, with regular cTBS treatment of both left and right primary motor cortices over a six month period significantly slowing the rate of deterioration in patients receiving active treatment (Di Lazzaro et al., 2006b).

1.4.3. The application of rTMS as an adjunctive treatment of motor dysfunction resulting from stroke

Commonly following stroke there is reduced motor function of the hand and arm muscles contralateral to the hemisphere with the infarct. Although this movement disability may persist for years following the initial insult, some spontaneous recovery may occur through neuroplastic reorganisation of the damaged hemisphere. This has resulted in the development of various rehabilitation therapies which aim to promote functional recovery through use-dependent neuroplasticity (Hallett, 2001; Schaechter, 2004). Given the capacity of rTMS to induce neuroplastic changes in the human cortex, its application in conjunction with conventional rehabilitation therapies may have the potential to enhance natural processes of neuroplasticity in the lesioned hemisphere, thus facilitating recovery and improving clinical outcome. Indeed, daily application of rTMS to the lesioned motor cortex in conjunction with normal physical therapies in early stroke patients improved recovery of motor function in comparison to sham rTMS (Khedr et al., 2005).

In addition to rTMS-induced facilitation of the lesioned primary motor cortex, inhibition of the intact hemisphere is thought to be another approach which may be beneficial in aiding functional recovery in stroke patients. There is evidence that motor cortical excitability of the intact hemisphere may be enhanced following stroke, resulting in an increased inhibitory drive through transcallosal fibres to the lesioned motor cortex and creating an inter-hemispheric imbalance that is suppressive of functional recovery (Murase et al., 2004). Reducing the excitability of the intact hemisphere using low-frequency rTMS has been effective at increasing motor function

of the paretic hand in several sham-controlled studies (Mansur et al., 2005; Takeuchi et al., 2005; Fregni et al., 2006), suggesting that this approach may too have the potential to promote recovery of motor performance in stroke patients.

1.4.4. Critical analysis of the therapeutic potential of rTMS protocols

In comparison to other methods of rTMS delivery, TBS protocols require only short application times and subthreshold stimulation intensities to produce neuroplastic changes in cortical excitability. These factors make TBS an attractive candidate as a possible therapeutic intervention to treat various neurological and psychiatric disorders. However, as has been the case with simple rTMS protocols, several studies have shown poor clinical efficacy of TBS. A recent sham-controlled study in chronic stroke patients tested the immediate and long-term benefits of adding TBS (either as iTBS to the lesioned motor cortex or cTBS to the intact motor cortex) to standardised physical therapy of the upper limb for 10 working days and found no significant difference between the real and sham TBS groups (Talelli et al., 2012).

Although the neuroplastic changes induced by TBS are considered stronger and longer lasting in comparison to the neuroplasticity induced by simple rTMS protocols, they are still too short-lived and too variable to provide any long-term therapeutic benefit. This may be due, at least in part, to the instability of experimentally-induced neuroplastic changes under normal physiological conditions. Behavioural engagement of the motor regions has been shown to disrupt rTMS-induced neuroplasticity within the human primary motor cortex, with the performance of a sustained sub-maximal voluntary contraction of the targeted hand muscle during rTMS application blocking its effects on

MEP amplitudes (Touge et al., 2001; Huang et al., 2008). There is also evidence that rTMS-induced neuroplasticity within the motor cortex may be influenced by voluntary contraction performed after rTMS application, with activation of the target muscle disrupting the rTMS-induced suppression of motor cortical excitability (Todd et al., 2006). Likewise, MEP suppression induced by cTBS was reversed when a voluntary contraction was applied immediately following cTBS application (Huang et al., 2008).

This instability of rTMS-induced neuroplasticity in the presence of normal, behaviourally-dependent physiological activity in the target cortical region is in accordance with studies performed in animal models. For example, Xu et al. (1998) demonstrated that LTP induced within the CA1 area of the adult rat hippocampus using high-frequency electrical stimulation was completely reversed following exploration of a novel environment. A similar reversal of synaptic plasticity was also observed in the developing *Xenopus* visual system, with spontaneous activation of the post-synaptic tectal neuron disrupting LTP and LTD induced by electrical stimulation at retinotectal synapses (Zhou et al., 2003). Synaptic plasticity induced by visual stimuli was similarly abolished in this study by random flashes of white light onto the retina, demonstrating a behavioural relevance for this reversal effect.

Functionally, this reversal of LTP and LTD (termed depotentiation and de-depression, respectively) is thought to protect against the stabilisation of synaptic modifications generated by random or incidental activity (Zhou and Poo, 2004). Considering the instability of neuroplastic changes induced in the human primary motor cortex using rTMS, its viability as a therapeutic agent must be considered, particularly if it is to be used in conjunction with conventional physical therapies to treat motor dysfunction in

patients with various movement disorders. In this instance, the movement task designed to benefit from the neuroplastic effects of rTMS may, in itself, disrupt these rTMS-induced changes. In light of this, greater stabilisation of rTMS-induced neuroplasticity in the presence of destabilising activity is likely to be necessary for these paradigms to be considered useful in a therapeutic sense.

1.5. THE CONSOLIDATION OF NEUROPLASTIC CHANGE

1.5.1. Increasing the persistence of LTP with repeated trains of stimulation in animal models

Shortly following the discovery of LTP in the anaesthetised rabbit hippocampus, Timothy Bliss and Tony Gardner-Medwin described a series of experiments investigating the induction of hippocampal LTP in chronically-prepared, unanaesthetised rabbits (Bliss and Gardner-Medwin, 1973). This preparation allowed them to examine plastic changes in synaptic function over a much longer time course than was possible in the anaesthetised animal. Among their observations, they found that whilst a single train of high-frequency stimulation could induce LTP which lasted up to 3 days, repeated trains of stimulation in one animal induced LTP which persisted for 16 weeks. Similar increases in the persistence of hippocampal LTP have been observed in the dentate gyrus of awake rats with repeated bouts of high-frequency stimulation (Barnes, 1979; Jeffery et al., 1990; Abraham et al., 1993; Abraham et al., 2002). Likewise, the repeated and spaced application of high-frequency stimulation trains is required to induce long-lasting LTP within the neocortex of awake, freely moving rats (Racine et al., 1995; Trepel and Racine, 1998).

The repeated application of high-frequency stimulation trains may also induce a more stable LTP that is less sensitive to reversal (i.e. depotentiation). In addition to depotentiation of induced LTP occurring as a result of behaviourally-relevant activity at the stimulated synaptic input, depotentiation may also occur following application of a weak, low-frequency train of electrical stimulation applied within a short time period following LTP induction (Barrionuevo et al., 1980; Staubli and Lynch, 1990; Fujii et al., 1991; Huang et al., 1999; Chen et al., 2001). However, Woo and Nguyen (2003) showed that LTP induced within the CA1 area of mouse hippocampal slice preparations using repeated trains of high-frequency stimulation was resistant to depotentiation by low-frequency stimulation. A similar stabilisation of LTP was observed in the developing *Xenopus* visual system, with repeated trains of electrical stimulation inducing LTP at retinotectal synapses that was resistant to depotentiation by spontaneous activation of the post-synaptic tectal neuron (Zhou et al., 2003). This stabilisation of LTP was only present when repeated trains were applied with a spaced pattern, with the massed delivery of the same number of stimuli inducing LTP which was completely abolished by spontaneous neuronal activity.

The enhanced duration and stability of synaptic modifications induced by repeated stimulation trains is likely the result of a consolidation of LTP through increases in *de novo* protein synthesis and gene transcription (Krug et al., 1984; Huang and Kandel, 1994; Nguyen et al., 1994; Woo and Nguyen, 2003). This consolidated, protein synthesis-dependent form of LTP is commonly referred to as late-phase LTP, and is mechanistically distinct from the short-lasting and easily disrupted early-phase that is independent of protein synthesis and gene transcription and occurs through post-

translational modifications of pre-existing proteins alone. The different phases of LTP will now be discussed in more detail.

1.5.2. Mechanistically distinct phases of synaptic modulation

An analysis of the decay rates of LTP induced *in vivo* in the animal dentate gyrus revealed at least three distinct phases of LTP with average decay time constants of approximately 2 h, 4 days and 20 days (Abraham and Otani, 1991). These phases (in accordance with the terminology employed by Racine et al. (1983)) were designated LTP1, LTP2, and LTP3 respectively, with LTP1 representing an early-phase of LTP dependent on the post-translational modifications of pre-existing proteins (but not protein synthesis) and LTP2 and LTP3 representing the protein synthesis-dependent late-phase of LTP. A further distinction has been made for LTP2 and LTP3 based on their respective dependence on gene transcription, with altered gene expression a feature of LTP3 but not LTP2 (Abraham et al., 1993).

The characteristic increase in *de novo* protein synthesis evident during LTP2 and LTP3 is thought to be the primary cause for the observed increase in LTP persistence. The local synthesis of new proteins within the dendritic spine may result in a range of morphological changes, including increases in the size and number of dendritic spines (see Yuste and Bonhoeffer, 2001, for review), as well as the synthesis of new calcium-sensitive kinases and AMPA receptor subunits (Nayak et al., 1998; Steward and Schuman, 2001; Ju et al., 2004). The increased persistence of LTP with altered gene expression during LTP3 is likely due to the synthesis of new gene transcripts and proteins within the cell body which specifically target the stimulated synapses through

the generation of a 'synaptic tag' (Frey and Morris, 1997), thus increasing the availability of the raw materials required to sustain these structural modifications long-term. The dependence on new protein synthesis and gene expression is also a feature for maintaining long-lasting depression of synaptic efficacy, with LTD also possessing a late-phase analogous to that described for LTP (Linden, 1996; Kauderer and Kandel, 2000).

1.5.3. The consolidation of neuroplastic change within the human cortex

Whilst considerable evidence exists demonstrating the consolidation of plastic changes with repeated and spaced stimulation trains in animal models, few studies have investigated this phenomenon in humans. The first experimental evidence for this consolidation effect in humans appeared several years ago in a study where repeated trains of rTMS were applied to the frontal eye field (Brodmann's area 8) of the human oculomotor system (Nyffeler et al., 2006a), which is a region of the frontal cortex anterior to the premotor region and is involved in voluntary saccadic eye movements (Pierrot-Deseilligny et al., 1995; Tehovnik et al., 2000). As a result of its critical involvement in saccadic movements, changes in the latency of horizontal saccades made towards a lateral visual target may be used as a quantifiable marker for neuroplasticity induction in the human frontal eye field.

The rTMS paradigm used by Nyffeler et al. (2006a) was a slightly modified cTBS protocol consisting of bursts of three subthreshold TMS pulses at 30 Hz repeated at a frequency of 6 Hz. In accordance with a previous study by the same group (Nyffeler et al., 2006b), the application of a single train of this modified cTBS paradigm suppressed

saccade triggering for around 30 min, as evidenced by an increase in saccade latencies. However, the application of two trains separated by 15 min extended these suppressive after-effects to well over 2 h (Nyffeler et al., 2006a). The magnitude by which saccade latencies were increased was also enhanced from 25% to 50% of baseline with the addition of a second train. To determine the impact of adding more trains in a single session, four cTBS trains were applied in a spaced manner to the frontal eye field of one subject. Whilst the magnitude of suppression was not enhanced with respect to that observed following two trains, the duration of the inhibitory after-effects was extended to approximately 10 h. Similar results have since been shown in the posterior parietal cortex of stroke patients with visual neglect, with four trains of cTBS applied to the contralesional hemisphere in a single session improving performance in a visual perception task for up to 32 h following intervention (Nyffeler et al., 2009). Additionally, eight trains of cTBS applied to the posterior parietal cortex over two consecutive days improved symptoms of spacial neglect for a period of 3 weeks (Cazzoli et al., 2012).

Assuming that the suppressive after-effects of cTBS applied to the frontal eye field and posterior parietal cortices are due to LTD-like processes at excitatory synapses, the prolongation of these after-effects with repeated trains of cTBS is consistent with the induction of a late-phase of LTD dependent on protein synthesis and perhaps altered gene expression. However, whilst at least several lines of indirect evidence for the involvement of LTP and LTD-like processes exist for rTMS-induced changes in human motor cortical excitability (for instance, the dependence on the NMDA receptor; see Section 1.3.1), the same cannot be said for other cortical regions. Similarly, it is difficult to gauge whether the observed consolidation of neuroplastic changes in these cortical

regions with repeated trains of cTBS are resistant to being disrupted by behaviourally-relevant physiological activity. Indeed, given the well-defined impact of ongoing cortical activity on rTMS-induced motor cortical plasticity (see Section 1.4.4), it is likely that the primary motor cortex (specifically the regions controlling the hand muscles) represents the best model in humans for studying the impact that repeated trains of rTMS has on the stability of neuroplastic modifications. Moreover, the enhancement of rTMS-induced neuroplastic changes in the human primary motor cortex is certainly relevant in a therapeutic sense considering the large number of movement disorders characterised by motor dysfunction.

1.5.4. Some considerations for the repeated application of rTMS to the human motor cortex

There are several matters that must be considered before the application of repeated trains of rTMS may be integrated into clinical studies for treating movement disorders. First, it has yet to be determined whether the repeated application of the same rTMS paradigm within a single session is capable of extending the lifetime of neuroplastic changes within the human primary motor cortex. On the contrary, there is some evidence to suggest the opposite.

Evidence from animal hippocampal slice preparations suggests that the history of synaptic activity may influence the induction of synaptic plasticity (Huang et al., 1992; Izumi et al., 1992; Wexler and Stanton, 1993; O'Dell and Kandel, 1994). At the systems level, this phenomenon – termed metaplasticity (Abraham and Bear, 1996) – is thought to maintain homeostasis within neuronal networks by preventing synaptic connections

from becoming saturated by runaway potentiation or depression. In 1982, Elie Bienenstock, Leon Cooper and Paul Munro developed a computational model to account for this phenomenon (Bienenstock et al., 1982). Simply put, their model (termed the Bienenstock-Cooper-Munro (BCM) theory) proposed that a history of elevated activity may increase the threshold for inducing subsequent LTP, whereas a history of low activity may decrease this threshold. This rule has since been shown to operate *in vivo* in various cortical networks in animal models (see Bear, 2003, for review), and has also been demonstrated in the human primary motor cortex using repeated bouts of various neuroplasticity-inducing techniques (Iyer et al., 2003; Siebner et al., 2004; Ziemann et al., 2004; Stefan et al., 2006; Müller et al., 2007; Hamada et al., 2008; Todd et al., 2009a).

So what does this mean for the consolidation of neuroplasticity within the human primary motor cortex using repeated applications of rTMS? If the LTP and LTD-like after-effects of rTMS are mediated by these homeostatic mechanisms within the human primary motor cortex, then presumably the cortical response to a second rTMS train would be highly biased by the first train such that motor cortical excitability is maintained near baseline levels. This would yield the opposite result to that expected for the consolidation of neuroplastic changes, which would require motor cortical excitability to be moved further from baseline levels with the addition of a second train. Whilst Nyffeler et al. (2006a) were able to show evidence for the consolidation of neuroplastic changes in the human frontal eye field using repeated cTBS protocols, it is unclear whether neuroplasticity induction within this cortical region is mediated by the same homeostatic mechanisms as the primary motor cortex.

Therefore, the first set of experiments of this thesis sought to determine whether the spaced application of repeated cTBS protocols prolonged the duration of induced neuroplastic changes within the human primary motor cortex (Chapter 2). The results show that whilst a single cTBS application did not significantly modify MEP amplitudes, the paired application of cTBS induced a strong MEP suppression that lasted for at least 2 h. To determine which synapses were likely affected by cTBS and contributed to the MEP change, the next set of experiments investigated the impact of repeated cTBS protocols on $GABA_A$ and $GABA_B$-mediated intracortical inhibition within the primary motor cortex (Chapter 3). Additionally, a third set of experiments were conducted to determine whether the MEP suppression induced by repeated cTBS protocols was stable in the presence of behaviourally-relevant physiological activity within the targeted motor regions (Chapter 4).

1.6. OPTIMISING TBS APPLICATION

1.6.1. Variability of the neuroplastic response to rTMS

The repeated and spaced application of stimulation trains may well provide a novel approach for improving the therapeutic potential of rTMS paradigms. However, this approach does not address the question of the efficacy of these paradigms for inducing neuroplastic effects when applied alone. In addition to the short lifetime and instability of induced responses, a fundamental problem associated with rTMS is the high response variability both within and between individuals. This variability is likely due to a range of factors, including the age, gender, physical activity level, and genetic profile of

subjects, as well as the time of day at which testing is performed (see Ridding and Ziemann, 2010, for review), and severely limits the therapeutic value of rTMS.

The short application times and subthreshold stimulation intensities utilised by TBS protocols make them a more attractive and efficient option for inducing neuroplasticity compared to other available rTMS protocols, which typically require higher intensities and application times lasting tens of minutes (Chen et al., 1997c; Muellbacher et al., 2000; Stefan et al., 2000; Wolters et al., 2003; Thickbroom et al., 2006; Hamada et al., 2007). However, as has been observed for other rTMS protocols, there exists considerable variability in the neuroplastic response to TBS. Although the initial report of TBS showed pronounced changes in MEP amplitudes that were relatively long-lasting (Huang et al., 2005), there have been several more recent studies that have described effects on MEPs that were significantly smaller in magnitude and shorter in duration (Gentner et al., 2008; Zafar et al., 2008; Swayne et al., 2009; Todd et al., 2009a; Di Lazzaro et al., 2011; McAllister et al., 2011). In a recent study, Hamada et al. (2013) found no overall effects of both iTBS and cTBS on motor cortical excitability in a group of 52 healthy individuals, with only a small percentage of individuals showing the expected response profile to both protocols. Similarly, Player et al. (2012) showed that whilst a small majority of subjects responded to iTBS with the expected facilitation of motor cortical excitability, greater than one third of subjects showed either no effect or an inhibitory response to stimulation.

It is possible that part of the variability in subject responses to TBS may be due to non-optimal stimulation parameters. Therefore, whilst the first three experimental chapters of this thesis describe studies which aimed to improve TBS application by using

repeated trains of stimulation, the final experimental chapter (Chapter 5) sought to improve the stimulation parameters used for a single TBS train.

1.7. SUMMARY

The capacity of rTMS to induce bidirectional and functionally-relevant neuroplastic changes in human cortical excitability has led to much research investigating its therapeutic application for a range of neurological and psychiatric disorders. In particular, its capacity to induce LTP and LTD-like neuroplastic changes in the human primary motor cortex have made rTMS an attractive therapeutic option for a number of movement disorders characterised by abnormal motor cortical excitability. Whilst there is enormous scope for its application in the clinical setting, the short duration, instability and high variability of subject responses to rTMS severely limits its therapeutic potential. The experiments described throughout this thesis have aimed to develop approaches for rTMS application that generate a longer lasting neuroplastic response within the human primary motor cortex that is less variable between subjects and is more stable under normal physiological conditions.

2. THE APPLICATION OF SPACED THETA BURST PROTOCOLS INDUCES LONG-LASTING NEUROPLASTIC CHANGES IN THE HUMAN MOTOR CORTEX

2.1. ABSTRACT

There is some limited evidence suggesting the spaced application of repetitive transcranial magnetic stimulation (rTMS) protocols may extend the duration of induced neuroplastic changes. However, this has yet to be demonstrated in the human primary motor cortex. We evaluated whether the paired application of an inhibitory rTMS paradigm (continuous theta burst stimulation; cTBS) at a 10 min interval prolonged the duration of induced motor cortical neuroplasticity. Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle before and following single and paired cTBS protocols applied using two intensities: 80% of active motor threshold (AMT_{80}) and 70% of resting motor threshold (RMT_{70}). Single cTBS protocols did not significantly influence MEP amplitudes. Whilst paired trains applied at AMT_{80} had no effect on MEPs, paired cTBS trains at RMT_{70} significantly reduced MEP amplitudes. MEPs remained suppressed for at least 2 h following the second train. Control experiments suggest that the contraction used to establish AMT prior to cTBS application may be responsible for blocking the effect of paired cTBS trains at the AMT_{80} intensity. The results suggest that the spaced application of cTBS protocols may be an effective approach for establishing long-lasting motor cortical neuroplasticity only

in the absence of prior voluntary motor activation. These findings may have important implications for the therapeutic application of rTMS.

2.2. INTRODUCTION

Over recent years there has been an explosion in the number of studies using non-invasive brain stimulation techniques to investigate the functioning of the human brain. Additionally, there is much interest in using these techniques to induce lasting neuroplastic changes that might be beneficial for the rehabilitation of patients with a wide range of neurological/psychiatric conditions including depression (Fitzgerald et al., 2003; George et al., 2010), stroke (Khedr et al., 2005; Fregni et al., 2006) and Parkinson's disease (Lomarev et al., 2006). Perhaps the most popular experimental paradigm to induce neuroplasticity within the human cortex involves the application of trains of transcranial magnetic stimuli (repetitive transcranial magnetic stimulation; rTMS), a technique which induces lasting plasticity via mechanisms similar to the long-term potentiation (LTP) and long-term depression (LTD) observed in animal models (Cooke and Bliss, 2006; Huang et al., 2007; Ridding and Rothwell, 2007).

Whilst there is good evidence that the plastic changes induced by rTMS are due to LTP/LTD-like mechanisms (Huang et al., 2007), the magnitude of such change is highly variable (Ridding and Ziemann, 2010) and has a duration commonly less than 1 h. This is considerably shorter in duration than the LTP/LTD induced in animal models, which may last anywhere from a few hours to several days (Malenka and Bear, 2004). Indeed, the comparatively short lifetime of rTMS-induced after-effects may limit the

therapeutic potential of rTMS, with any positive effects being too short to have any long-term therapeutic benefit.

One potential approach to prolong the duration of experimentally-induced human neuroplasticity involves the repeated application of rTMS protocols in a spaced manner. In animal models the repeated application of stimulation protocols can enhance the lifetime of activity-dependent synaptic plasticity (Bliss and Gardner-Medwin, 1973; Abraham et al., 1993; Abraham et al., 2002). Interestingly, a similar effect has been observed in human subjects with multiple applications of an inhibitory rTMS paradigm to the frontal eye field producing behavioural effects which lasted for several hours; significantly longer than the effects observed following a single train (Nyffeler et al., 2006a). Whilst evidence for the prolongation of neuroplastic effects has been observed in the human primary motor cortex using repeated applications of other non-invasive brain stimulation techniques (Monte-Silva et al., 2010), a similar extension in the duration of induced effects with the repeated application of rTMS protocols has yet to be demonstrated.

Therefore, the present study aimed to determine whether the paired application of rTMS protocols could prolong the duration of plasticity induced in the human primary motor cortex. To do this we employed the inhibitory rTMS paradigm continuous theta burst stimulation (cTBS); a well characterised technique for inducing neuroplasticity in the primary motor cortex (Huang et al., 2005). Given previous reports that prior voluntary motor activation can influence cTBS-induced motor cortical plasticity (Gentner et al., 2008), a secondary aim was to examine whether behavioural engagement of the motor cortical regions prior to applying cTBS influenced the outcome. This was achieved by

setting the cTBS intensity relative to either active motor threshold (AMT) or resting motor threshold (RMT).

2.3. MATERIALS AND METHODS

2.3.1. Subjects

All participants were screened for any contraindications to TMS (Rossi et al., 2009) and gave informed written consent prior to their involvement in this study. A total of twenty-two healthy subjects (eight males) between the ages of 19 and 47 years (mean age 24.2 ± 1.4 years) were investigated. Twelve subjects participated in Experiments 1-3 (five males; mean age 26.3 ± 2.3 years), with six of the twelve returning for Experiment 4 (three males; mean age 29.7 ± 4.0 years). Experiment 5 was performed on a total of nine subjects (four males; mean age 22.1 ± 3.7 years), two of whom had participated in Experiments 1-4, and Experiment 6 was performed on a total of seven subjects (four males; mean age 28.9 ± 3.5 years), four of whom had participated in Experiments 1-3. All experiments conformed to the Declaration of Helsinki and were approved by the University of Adelaide Human Research Ethics Committee.

2.3.2. Stimulation and recording

Subjects were seated in a comfortable chair for each experimental session and were directed to stay as relaxed as possible unless instructed otherwise. Changes in corticospinal excitability were assessed using single-pulse TMS to evoke motor evoked potentials (MEPs) from the right first dorsal interosseous (FDI) muscle. Surface

electromyographic recordings were made from the right FDI muscle using Ag-AgCl surface electrodes and a sampling rate of 5 kHz. Signals were amplified with a gain of 1000 and filtered (20-1000 Hz) (Cambridge Electrical Design 1401, Cambridge, UK) before being stored on a computer for offline analysis.

Single-pulse TMS with monophasic waveform was performed using a Magstim 200 magnetic stimulator (Magstim, Whitland, Dyfed, UK) connected to a figure-of-eight magnetic coil (external wing diameter, 90 mm) held tangentially to the skull over the left primary motor cortex with the handle pointing posteriorly and laterally at a 45° angle to the sagittal plane. A felt marker was used to mark the optimal scalp position for eliciting MEPs in the right FDI at rest. Single-pulse TMS was applied at an intensity sufficient to evoke baseline MEPs of approximately 1 mV amplitude (measured peak-to-peak). Two blocks of fifteen MEPs were recorded prior to the intervention in each experimental session and averaged to yield a baseline measure of corticospinal excitability. All pre and post-intervention MEPs were recorded at a rate of 0.2 Hz (using 10% variance) unless indicated otherwise. Any change in MEP amplitude following intervention was used as a marker for neuroplasticity in the primary motor cortex.

2.3.3. TBS paradigm

In all sessions, cTBS was applied in trains of repetitive biphasic magnetic pulses to the left primary motor cortex using a Magstim Super Rapid stimulator (Magstim, Whitland, Dyfed, UK) connected to an air-cooled figure-of-eight coil. Each cTBS train consisted of 600 pulses applied in bursts of three pulses at 50 Hz, with bursts repeated at a frequency of 5 Hz corresponding to a total train length of 40 s (Huang et al., 2005).

Where paired trains of cTBS were applied, an inter-train interval of 10 min was employed.

The stimulation intensity of cTBS was set relative to either AMT or RMT. AMT was defined as the minimum stimulus intensity necessary to evoke MEPs with peak-to-peak amplitudes greater than 200μV in at least 5 of 10 consecutive trials whilst the subject performed a sub-maximal isometric contraction of their right FDI muscle. The force of contraction during AMT measurement was maintained at 10% of maximum voluntary contraction using visual feedback displayed on an oscilloscope. RMT was defined as the minimum stimulus intensity necessary to evoke MEPs with peak-to-peak amplitudes greater than 50μV in at least 5 of 10 consecutive trials whilst the subject was at rest (i.e. right FDI muscle relaxed).

2.3.4. Sham stimulation

Sham stimulation was delivered using a sham rTMS coil (placebo coil PN 3285-00, Magstim, Whitland, Dyfed, UK) which produced the same number and pattern of auditory stimuli as real cTBS without inducing an electric current in the brain.

2.3.5. Experiments

A total of six experiments were included in this study (Figure 2-1). For Experiment 1 subjects attended two sessions, receiving a single train of cTBS at an intensity of 80% AMT (AMT_{80}) in one session and paired trains of cTBS (also at an intensity of AMT_{80}) in the other. For Experiment 2 subjects were again required to attend two sessions,

receiving a single train of cTBS in one session and paired cTBS trains in the other; however, in this experiment the intensity of cTBS trains was set at 70% of RMT (RMT_{70}) instead of AMT_{80} (Gentner et al., 2008). Additionally, for this experiment the single cTBS train was paired with a sham control (applied first). It should be noted that the sham stimulation condition was not used for Experiment 1. This was because we wanted the time interval between completion of AMT determination and the onset of cTBS application (the first application in the case of the paired cTBS intervention) to be the same for each of the relevant interventions.

Experiment 3 was a control study designed to investigate more closely the impact that the initial contraction used to establish AMT may have had on the response to paired cTBS trains. Subjects attended one session in which they received paired trains of cTBS applied at RMT_{70} (as in Experiment 2); however, prior to application of the first cTBS train subjects were instructed to sustain a sub-maximal isometric contraction of their right FDI muscle for 3 min (similar to the contraction performed during AMT measurement in Experiment 1). As with AMT measurement, subjects were provided visual feedback during the contraction to ensure force production was maintained at 10% maximum voluntary contraction.

Although we anticipated that the absolute intensities employed for RMT_{70} and AMT_{80} would be very similar we actually found that RMT_{70} stimulation resulted in a slightly higher intensity than that used for AMT_{80} (see Section 2.4). Therefore, in order to investigate whether this small difference in stimulation intensity might explain differences in the response to cTBS at RMT_{70} and AMT_{80} a second control experiment was conducted. For this experiment (i.e. Experiment 4) subjects attended one session in

which they again received paired cTBS trains; however, the intensity of stimulation was set at 65% of RMT (RMT_{65}).

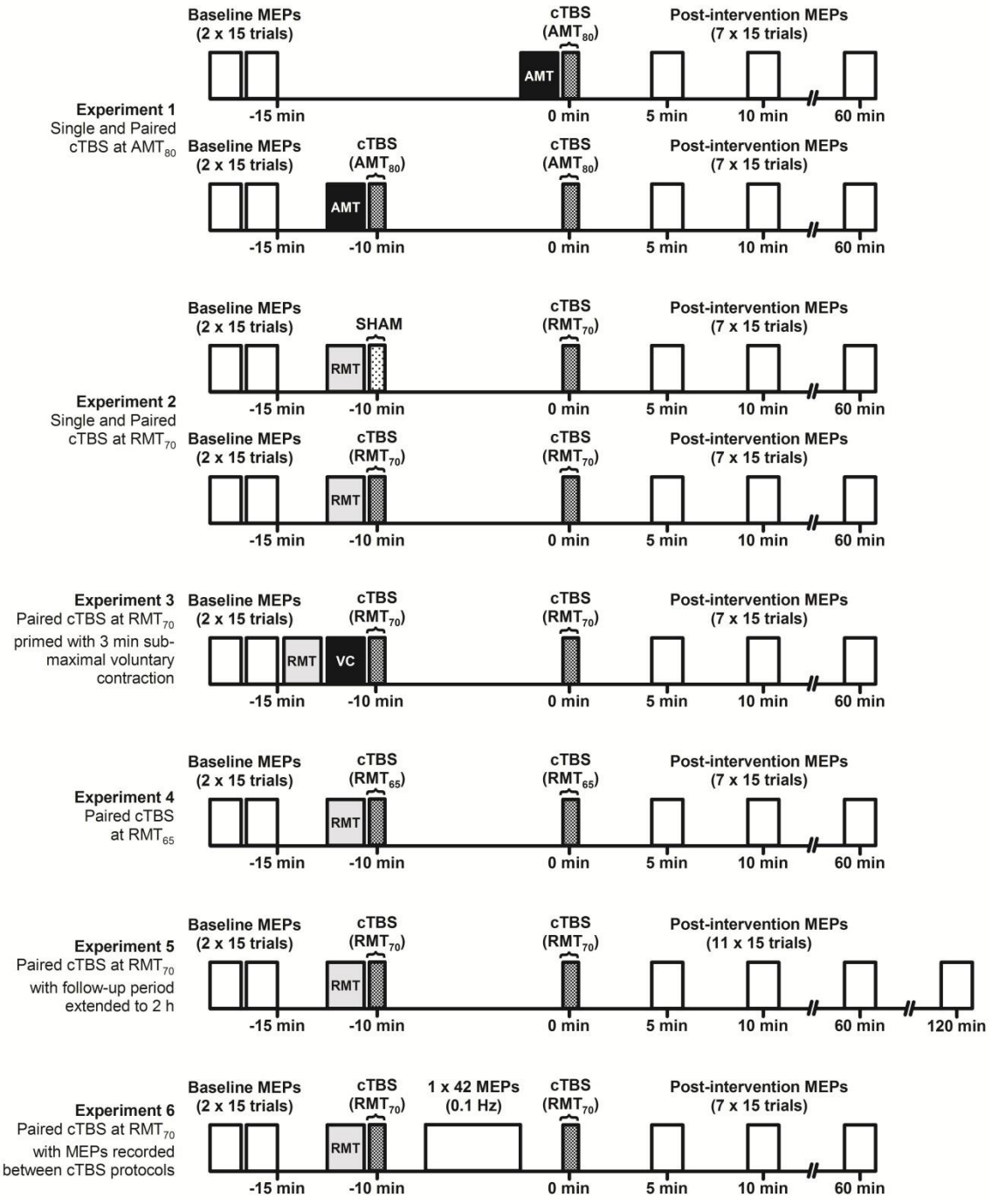

Figure 2-1: *Schematic overview of experimental design.*

For Experiments 1-4, blocks of fifteen MEPs were recorded at 5, 10, 20, 30, 40, 50 and 60 min following completion of the intervention period. Each session was separated by at least two days.

To more comprehensively characterise the duration of after-effects produced by paired cTBS trains applied at RMT_{70}, a fifth experiment (i.e. Experiment 5) was conducted. Subjects for this experiment attended one session in which they received paired trains of cTBS at RMT_{70}. MEPs were then recorded in blocks of fifteen trials at 5, 10, 20, 30, 40, 50, 60, 75, 90, 105 and 120 min following completion of the second cTBS train.

A final experiment (Experiment 6) was conducted to more fully explore the influence that a subject's response to the first cTBS in a pair might have on the response to a paired cTBS paradigm. For this, subjects again received paired cTBS trains at RMT_{70}, with MEPs recorded in blocks of fifteen trials at 5, 10, 20, 30, 40, 50 and 60 min following completion of the second cTBS train (as in Experiments 1-4); however, following the first train and prior to application of the second train, a block of forty-two MEPs were recorded at a frequency of 0.1 Hz to determine the response to the first cTBS train.

All experimental sessions were performed in the afternoon to prevent any time-of-day effects from influencing our results (Sale et al., 2007). Given the impact that physiological activity can have on TBS-induced neuroplasticity induction (Huang et al., 2008), surface electromyographic activity was monitored at all times in all subjects for each experiment to ensure the FDI muscle was completely at rest during periods where

a contraction was not required. Trials contaminated with background muscle activation prior to TMS application were excluded from analysis.

2.3.6. Data analyses

All statistical analyses were performed using PASW statistics version 17 (IBM SPSS, Armonk, New York, USA). MEP amplitudes were expressed as a percentage of baseline for comparisons between two or more interventions. Comparisons between single and paired cTBS for both AMT_{80} (Experiment 1) and RMT_{70} (Experiment 2) were performed using two-way repeated measures analysis of variance ($ANOVA_{RM}$) with INTERVENTION (two levels – single cTBS, paired cTBS) and TIME (seven levels – 5 min, 10 min, 20 min, 30 min, 40 min, 50 min, 60 min) as within-subject factors. A one-way $ANOVA_{RM}$ was then performed on raw data for each intervention separately (i.e. single cTBS at AMT_{80}, paired cTBS at AMT_{80}, single cTBS at RMT_{70} and paired cTBS at RMT_{70}) with TIME (eight levels – baseline plus the seven post-intervention time points) as a within-subject factor. If a significant main effect of time was observed, paired *t* tests were used to determine at which time points MEP amplitudes were significantly different to baseline. Multiple comparisons were corrected for using the false discovery rate procedure (FDRP) (Curran-Everett, 2000).

To investigate whether the response to paired cTBS trains might be influenced by the subject's response to a single train, correlations between the responses to single and paired cTBS trains were performed using a two-tailed Pearson correlation coefficient test for both the AMT_{80} (Experiment 1) and RMT_{70} (Experiment 2) intensities. All data were expressed as percentage of baseline, and all seven post-intervention time points for

the paired cTBS conditions were averaged for each subject prior to analysis. For the single cTBS data two average response variables were calculated: the average of the entire 60 min follow-up period (to give a measure of the overall response to the single cTBS train) and the average for the first 10 min following intervention (as differences in the size and direction of effects observed during this time may be more likely to impact on the response to a second train applied 10 min later).

For Experiment 3, the response to paired cTBS at RMT_{70} primed with an initial voluntary contraction was compared with that produced by paired trains of cTBS in Experiments 1 and 2 (i.e. paired cTBS at AMT_{80} and RMT_{70} respectively) using $ANOVA_{RM}$ with within-subject factors INTERVENTION (three levels) and TIME (seven levels). Similarly, $ANOVA_{RM}$ (with factors INTERVENTION and TIME) was used to compare the response to paired cTBS at RMT_{65} in Experiment 4 with that of the paired cTBS conditions in Experiments 1 and 2; however, analysis was performed only on the subjects from Experiments 1 and 2 who also participated in Experiment 4 (giving a total of six subjects for this comparison). One-way $ANOVA_{RM}$ was again performed on raw data for Experiments 3 and 4, and, contingent on a significant main effect of TIME, *post hoc* analyses were performed using paired *t* tests (corrected for multiple comparisons using FDRP).

For Experiment 5 testing the duration of after-effects induced by paired cTBS at RMT_{70}, a one-way $ANOVA_{RM}$ was performed on raw MEP data with TIME (twelve levels – baseline, 5 min, 10 min, 20 min, 30 min, 40 min, 50 min, 60 min, 75 min, 90 min, 105 min, 120 min) as the within-subject factor. Paired *t* tests were again used for *post hoc* analyses (corrected for multiple comparisons using FDRP).

Finally, data for Experiment 6 were analysed using two-tailed Pearson correlation coefficient tests to determine whether the response to the first train (average of 42 MEPs, expressed as a percentage of baseline MEP amplitude) influenced the outcome to intervention with paired cTBS applied at RMT_{70}. Three average response variables were calculated to characterise paired cTBS outcome: the average amplitude of MEPs (expressed as a percentage of baseline) recorded 5 min following the second cTBS train, the average amplitude of MEPs at the time of peak suppression, as well as the average amplitude of the MEPs across the entire 60 min follow-up period.

Data are presented as group means ± SEM unless otherwise indicated. Statistical significance was accepted at *P*-values < 0.05 for all analyses.

2.4. RESULTS

2.4.1. Experiment 1 – single cTBS at AMT_{80} vs. paired cTBS at AMT_{80}

Baseline MEP amplitudes for Experiment 1 were not significantly different between the single and paired cTBS interventions when applied at an intensity of AMT_{80} (0.96 ± 0.06 mV and 0.93 ± 0.07 mV respectively; $P > 0.05$). $ANOVA_{RM}$ also revealed no significant difference between the two interventions with respect to their effect on MEP amplitude ($F_{1,11} = 3.47$, $P = 0.089$). There appeared to be a mild suppression of MEP amplitudes following a single train of cTBS at AMT_{80} (Figure 2-2A); however, this change was not significant (TIME; $F_{7,77} = 1.56$, $P = 0.161$). There was no MEP

suppression following paired cTBS trains at AMT_{80} ($F_{3,29}$ = 1.34, P = 0.280) (Figure 2-2B).

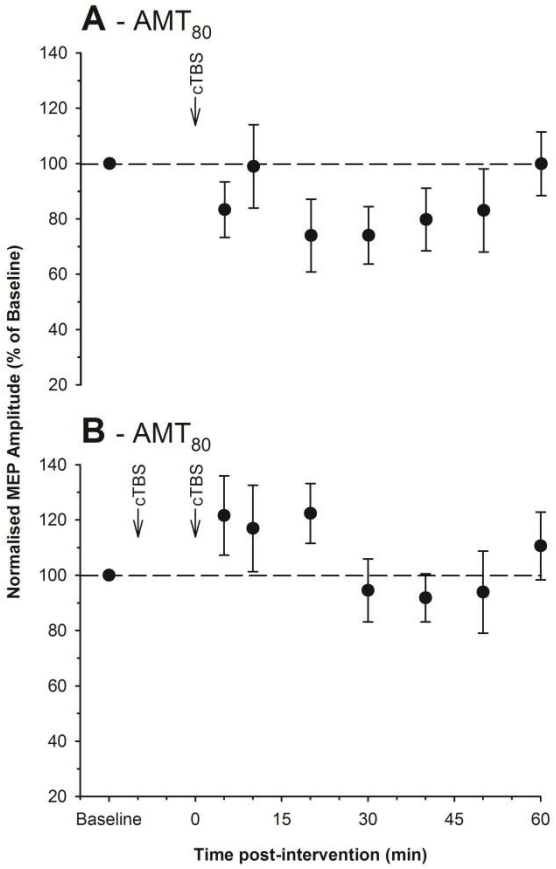

Figure 2-2: *The time course of change in MEP amplitudes (expressed as a percentage of baseline) following intervention with (A) a single train and (B) paired trains of cTBS applied at AMT_{80} (Experiment 1). Whilst a single train applied at AMT_{80} appeared to suppress MEP amplitudes, this did not reach statistical significance. When applied at AMT_{80}, paired cTBS trains did not alter MEP amplitudes.*

2.4.2. Experiment 2 – single cTBS at RMT_{70} vs. paired cTBS at RMT_{70}

Baseline MEP amplitudes for Experiment 2 were not different when the single and paired cTBS interventions at RMT_{70} were compared (1.05 ± 0.06 mV and 1.00 ± 0.07

mV respectively; $P > 0.05$). However, $ANOVA_{RM}$ revealed a highly significant main effect of intervention type on MEP amplitude (INTERVENTION; $F_{1,11} = 22.82$, $P = 0.0006$). No MEP suppression was observed following a single train of cTBS at RMT_{70} ($F_{3,29} = 0.646$, $P = 0.572$) (Figure 2-3A), however, paired trains of cTBS at RMT_{70} induced a strong suppression of MEP amplitudes (TIME; $F_{7,77} = 5.37$, $P = 0.00005$) (Figure 2-3B). *Post hoc* analyses revealed that MEP amplitude was suppressed compared with baseline when measured at 5, 10, 30, 40, 50 and 60 min after paired cTBS trains at RMT_{70} (for all, $P \leq 0.017$; corrected using FDRP), with peak suppression occurring 50 min following the second cTBS train (48% of baseline MEP amplitude).

2.4.3. Relationship between the responses to single and paired cTBS trains

There was no significant correlation between the responses to a single cTBS train (average of all post-intervention time points) and paired cTBS trains for either the AMT_{80} intensity paradigms ($r = 0.20$, $P = 0.532$) (Figure 2-4A) or the RMT_{70} intensity paradigms ($r = 0.40$, $P = 0.198$) (Figure 2-4B). Similarly, when time points were averaged only for the first 10 min following intervention for the single cTBS condition there was no significant correlation between single and paired cTBS responses regardless of whether AMT_{80} ($r = 0.26$, $P = 0.422$) (Figure 2-4C) or RMT_{70} paradigms ($r = 0.46$, $P = 0.134$) (Figure 2-4D) were used.

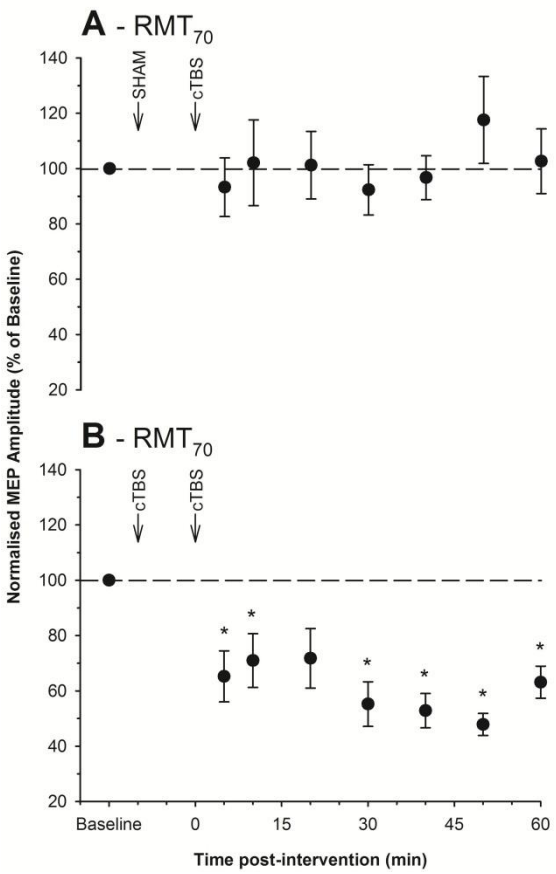

Figure 2-3: *The time course of change in MEP amplitudes (expressed as a percentage of baseline) following (A) a single train and (B) paired trains of cTBS applied at RMT_{70} (Experiment 2). Whilst a single train had no significant effect on MEP amplitudes, paired trains of cTBS applied at RMT_{70} induced suppression of MEPs which was still present 1 h following the second train. * denotes $P < 0.05$ when compared with baseline MEP amplitudes.*

2.4.4. Stimulation intensity of paired cTBS trains at AMT_{80} and RMT_{70}

Analysis of the intensity at which paired cTBS trains were applied in the first two experiments revealed that a slightly (but significantly) lower absolute stimulation intensity was used for paired cTBS at AMT_{80} than for RMT_{70} stimulation (34.4 ± 2.2%

of max. stimulator output and 38.5% ± 1.9% of max. stimulator output respectively; $P < 0.01$).

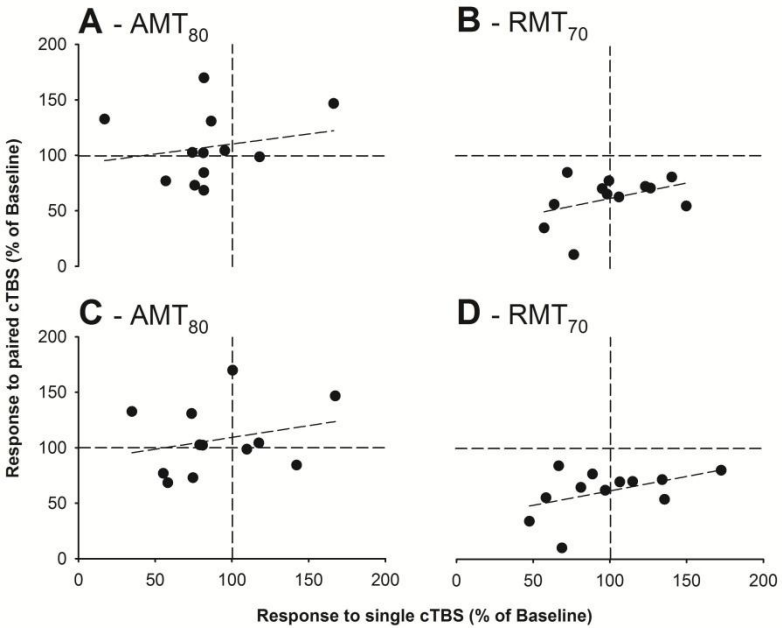

Figure 2-4: *Correlations between the MEP responses to single and paired cTBS trains. Data are the average of post-intervention MEP amplitudes for each subject (expressed as a percentage of baseline) across multiple time points, such that values below 100 indicate an overall inhibitory response to intervention and values above 100 indicate an overall facilitatory response. When all post-intervention time points were averaged for the single cTBS condition there was no correlation between the MEP responses to single and paired cTBS at either the (A) AMT_{80} ($r = 0.20$, $P = 0.532$) or (B) RMT_{70} ($r = 0.40$, $P = 0.198$) intensities. Likewise, when time points were averaged only for the first 10 min following intervention for the single cTBS condition, no correlation was observed for either (C) AMT_{80} ($r = 0.26$, $P = 0.422$) or (D) RMT_{70} ($r = 0.46$, $P = 0.134$).*

2.4.5. Experiment 3 – paired cTBS trains at RMT_{70} primed with an initial voluntary contraction

Baseline MEP amplitudes for the paired cTBS intervention applied at RMT_{70} and primed with an initial voluntary contraction were not significantly different to those for the paired cTBS interventions of Experiments 1 and 2 ($F_{2,22}$ = 1.37, P = 0.274). Comparison of the three conditions with respect to their effect on MEP amplitude revealed a main effect of INTERVENTION ($F_{2,22}$ = 7.63, P = 0.003). *Post hoc* tests showed that this was due to greater MEP suppression with paired cTBS at RMT_{70} (with no initial contraction) (Figure 2-3B) when compared with paired cTBS at AMT_{80} (Fig. 2-2B) (P = 0.002; corrected using FDRP) and paired cTBS at RMT_{70} primed with an initial contraction (Figure 2-5A) (P = 0.013; corrected using FDRP). There was no difference in MEP amplitude change when paired cTBS at AMT_{80} (Figure 2-2B) and paired cTBS at RMT_{70} primed with an initial contraction (Figure 2-5A) were compared (P = 0.341). In addition, there was no main effect of TIME for paired cTBS at RMT_{70} primed with an initial contraction ($F_{7,77}$ = 1.257, P = 0.283) (Figure 2-5A).

2.4.6. Experiment 4 – paired cTBS trains at RMT_{65}

In the subset of six subjects to participate in this experiment, there was no difference between baseline MEP amplitudes for the paired cTBS intervention applied at RMT_{65} and the paired cTBS interventions of Experiments 1 and 2 ($F_{2,10}$ = 0.135, P = 0.875). There was also no difference between the stimulation intensities used for paired cTBS at AMT_{80} and paired cTBS at RMT_{65} (34.2 ± 3.0% of max. stimulator output and 33.3 ± 3.0% of max. stimulator output respectively; $P > 0.05$). There was a main effect of the

different interventions on MEP amplitude (INTERVENTION; $F_{2,10}$ = 8.32, P = 0.007). This was due to a greater suppression of MEP amplitudes for paired cTBS at both RMT_{70} and RMT_{65} intensities compared with the paired cTBS intervention at AMT_{80} (P = 0.026 and P = 0.01 respectively; corrected using FDRP). There was no difference in the degree of MEP suppression induced following paired cTBS at RMT_{70} and paired cTBS at RMT_{65} (P = 0.601). One-way $ANOVA_{RM}$ revealed a main effect of TIME for paired cTBS at RMT_{65} ($F_{3,17}$ = 4.77, P = 0.011) (Figure 2-5B). This was due to suppression of MEPs measured at 10, 30, 40, 50 and 60 min after paired cTBS trains at RMT_{65} compared with those measured at baseline (for all, $P \leq 0.029$; corrected using FDRP), with peak suppression occurring 60 min following the second cTBS train (44% of baseline values).

2.4.7. Experiment 5 – duration of paired cTBS-induced after-effects when applied at RMT_{70}

Strong suppression of MEP amplitudes was again observed for paired cTBS at RMT_{70} with the post-intervention follow-up period extended to 2 h (TIME; $F_{5,41}$ = 4.472, P = 0.002) (Figure 2-6A). MEP amplitudes measured at all time points up to and including 2 h after the second train of stimulation were depressed when compared with those at baseline (for all, $P < 0.05$; corrected using FDRP), with peak suppression occurring around 75-90 min following the second cTBS train (38% of baseline values).

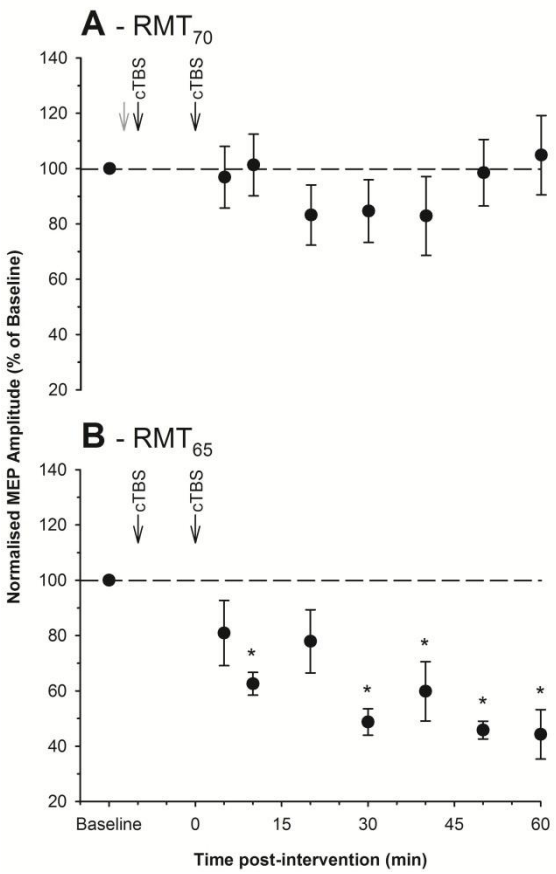

Figure 2-5: *The time course of change in MEP amplitudes (expressed as a percentage of baseline) following paired cTBS trains at (A) RMT_{70} primed with an initial voluntary contraction (grey arrow) (Experiment 3), and (B) RMT_{65} (Experiment 4). * denotes $P < 0.05$ when compared with baseline MEP amplitudes.*

2.4.8. Experiment 6 – relationship between the response to the first train and the outcome to paired cTBS applied at RMT_{70}

A significant positive linear correlation was observed between the response to the first cTBS train and all three post cTBS response variables (the response recorded at 5 min $r = 0.856$, $P = 0.014$; peak suppression $r = 0.913$, $P = 0.004$; and the average response of all time points $r = 0.891$, $P = 0.007$; Figure 2-6B).

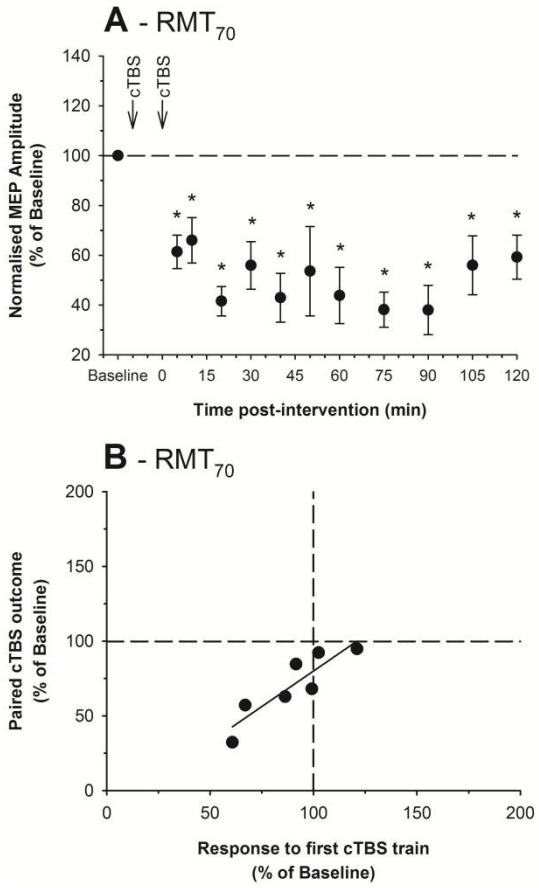

Figure 2-6: *Duration of paired cTBS-induced after-effects and the relationship between cTBS trains. (A) The time course of change in MEP amplitudes (expressed as a percentage of baseline) following paired cTBS at RMT_{70} with the post-intervention follow-up period extended to 2 h (Experiment 5). * denotes $P < 0.05$ when compared with baseline MEP amplitudes. (B) Correlation between the response to the first cTBS train and the outcome to intervention with paired cTBS trains at RMT_{70} (Experiment 6). Data are the average of MEP amplitudes for each subject (expressed as a percentage of baseline) such that values below 100 indicate an overall inhibitory response to intervention and values above 100 indicate an overall facilitatory response. Outcome to paired cTBS was characterised by averaging the response of all post-intervention time points. A significant positive linear correlation was observed between the response to the first cTBS train and paired cTBS outcome ($r = 0.891$, $P = 0.007$).*

2.5. DISCUSSION

The two main findings of this study are that spaced pairs of cTBS trains induce a significantly greater neuroplastic response than single cTBS protocols, and that a voluntary contraction prior to paired cTBS trains at RMT_{70} intensity abolishes the neuroplastic effect.

2.5.1. Single cTBS trains had no significant effect on corticospinal excitability

One unexpected finding from the present study was the absence of a significant response to a single cTBS protocol at either AMT_{80} or RMT_{70} intensities. This result contrasts with the strong suppression of corticospinal excitability observed by Huang et al. (2005) which, in their study, persisted for up to 1 h. Whilst the magnitude and duration of effects reported by Huang et al. were considerable, several more recent studies using the same TBS protocols have described changes in MEPs that were significantly smaller in magnitude and shorter in duration (Gentner et al., 2008; Zafar et al., 2008; Swayne et al., 2009; Todd et al., 2009a; Di Lazzaro et al., 2011; McAllister et al., 2011). Indeed, some have reported no significant difference between the changes induced by TBS protocols and those induced by simple 1 Hz (Zafar et al., 2008; Di Lazzaro et al., 2011) and 5 Hz rTMS (Zafar et al., 2008).

It should be noted that although the change in corticospinal excitability induced in the present study by a single cTBS protocol at the AMT_{80} intensity was insufficient to reach a statistically significant level, there was a strong trend towards a suppression of MEP amplitudes. Of the twelve subjects tested for this protocol, the majority responded to

intervention with an overall suppression of MEP amplitudes; however, for two subjects a facilitatory response to cTBS was observed (see Figure 2-4A). A similar variability between subject response profiles to a single cTBS protocol has been reported previously (Martin et al., 2006; McAllister et al., 2011). Taken together, these findings reflect the considerable inter-individual variability that is common for rTMS protocols and is likely due to a number of factors (Ridding and Ziemann, 2010), including genetic differences between subjects. For example, it has been demonstrated that a common single nucleotide polymorphism of the gene encoding brain derived neurotrophic factor (BDNF) can attenuate the response to a number of plasticity-inducing protocols (Kleim et al., 2006; Cheeran et al., 2008; Antal et al., 2010), including cTBS (Cheeran et al., 2008). Similarly, genetic variations in the gene encoding the NR2B subunit of the *N*-methyl-D-aspartate (NMDA) receptor have been linked to variations in the response profiles of subjects to the facilitatory intermittent TBS protocol (Mori et al., 2011). Whilst this has yet to be tested for cTBS, it is likely that at least some of the variability associated with cTBS protocols may also be mediated by differences in NMDA receptor genotype given the dependency of the cTBS-induced neuroplastic response on NMDA receptor function (Huang et al., 2007).

2.5.2. Paired cTBS trains at RMT_{70} induced a long-lasting suppression of corticospinal excitability

Whilst there was no significant change in MEP amplitudes following a single cTBS train applied at either AMT_{80} or RMT_{70}, paired cTBS trains applied at RMT_{70} significantly suppressed MEP amplitudes. Furthermore, this MEP suppression was present for all subjects; a response rate rare in conventional rTMS experiments (see

Figures 2-4B and D), and lasted at least 2 h. All currently published data suggests that MEP suppression induced by a single cTBS train does not exceed 1 h (Huang et al., 2005; Gentner et al., 2008; Gamboa et al., 2010). The fact that paired cTBS at RMT_{70} induced a strong MEP suppression exceeding 2 h in the present study suggests that the spaced application of multiple cTBS trains may be an effective approach for inducing long-lasting neuroplastic changes in corticospinal excitability when applied to the human primary motor cortex.

Although Experiment 5 was included in this study to determine the duration of MEP suppression induced by paired cTBS trains, the post-intervention follow-up period of 2 h was not long enough to capture a return of MEP amplitudes to baseline. Therefore, additional experiments will be required to determine how long the suppressive effects of spaced cTBS last.

2.5.3. Why are paired trains at RMT_{70} more effective than paired trains at AMT_{80}?

Several factors may have contributed to the differing response to paired cTBS trains when applied at RMT_{70} or AMT_{80}. Firstly, small differences in the absolute stimulation intensity used in the RMT_{70} and AMT_{80} protocols may have played a role. The rationale for using RMT_{70} was that the resultant absolute intensity would be well matched to that used for the AMT_{80} protocols (Gentner et al., 2008). However, the absolute intensity for the RMT_{70} protocol was slightly, but significantly, higher than that used for the AMT_{80} protocol. Therefore, we performed a control experiment (Experiment 4) where the response to paired cTBS at RMT_{65} was investigated. In this condition the absolute stimulation intensity employed was well matched to that of the AMT_{80} condition.

However, paired cTBS at RMT_{65} still suppressed MEPs to a similar degree to RMT_{70} stimulation. Therefore, it is unlikely that the small difference in absolute intensity employed in the AMT_{80} and RMT_{70} conditions was responsible for the different response profiles.

The second factor that may have been important for modifying the response to paired cTBS trains was the initial voluntary contraction used to establish AMT for the AMT_{80} protocols. In Experiment 3 we found that the MEP suppression seen following paired cTBS at RMT_{70} was abolished when a priming contraction (similar to that required during AMT assessment) was performed. Taken together, these findings suggest that the initial contraction performed during AMT assessment, and not the difference in stimulation intensity, was responsible for attenuating the response to paired cTBS trains.

2.5.4. Other factors which may have influenced the response to paired cTBS trains

To date, only one other study has examined the effect of repeated applications of cTBS to the human primary motor cortex. Gamboa et al. (2011) applied paired cTBS trains at an intensity of AMT_{80} with various inter-train intervals. Whilst a single cTBS train suppressed MEP amplitudes for up to 1 h, no suppression was observed when paired trains were applied at intervals of 2 and 5 min. Paired cTBS trains applied with a longer interval of 20 min reduced corticospinal excitability to a similar degree as a single cTBS train. It was proposed that some interaction, perhaps mediated by homeostatic mechanisms, was responsible for the lack of effects when two cTBS protocols were applied at short intervals, whilst at longer intervals the impact of the first train would have subsided such that no interaction with the second train occurred.

At first glance it would appear that our results are in contrast to the findings of Gamboa et al. (2011). However, several factors may preclude direct comparisons of the studies. Firstly, a single cTBS train at both AMT_{80} and RMT_{70} intensities failed to elicit significant MEP suppression in the present study, but Gamboa et al. observed a strong inhibitory effect with a single cTBS train. It is possible that the interaction between two cTBS trains differs depending on whether a single train modulated motor cortical excitability. We examined this possibility in two ways. Firstly, using the data from Experiments 1 and 2, we performed a correlation analysis between the magnitude of a subject's response to the single cTBS and paired cTBS conditions at both AMT_{80} and RMT_{70} intensities. Although these correlations were not significant there was a suggestion of a positive correlation (especially for the RMT_{70} condition; see Figures 2-4B and D). However, it should be noted that for this analysis subjects' responses to single and paired cTBS trains were investigated on different days, and it is possible that an individual's response to the single train protocol and the response to the first train in the paired protocol were different. Therefore, a second approach was employed to further investigate the influence of the response to the first cTBS train on the outcome to the paired trains. In Experiment 6 we took MEP measures between the cTBS trains to investigate the response to the first train. We found that the response to the first cTBS train was positively correlated with the outcome to paired cTBS (see Figure 2-6B), suggesting an interaction between the two cTBS trains that was non-homeostatic in nature. Thus, we consider it unlikely that the different response to paired cTBS trains in the present study and that reported by Gamboa et al. is due to a stronger inhibitory response to the first cTBS train in the pair in the Gamboa et al. study.

The rationale for employing a 10 min interval in the present study was based upon data from the rat hippocampus showing the repeated application of stimulation protocols at this interval to be highly effective in extending the duration of induced plasticity (Abraham et al., 2002). It is possible that the inter-train intervals used by Gamboa et al. (2011) were not optimal for prolonging the duration of cTBS-induced suppression of motor cortical excitability. Indeed, the degree to which repeated stimulation trains stabilise synaptic plasticity within the developing *Xenopus* visual system appears highly dependent on inter-train interval length, showing an inverted U-shaped relationship (Zhou et al., 2003). A similar relationship may influence the interaction of paired cTBS. Consequently, the intervals employed by Gamboa et al. may have been either too short or too long to interact in a manner which potentiates neuroplasticity.

Whilst the possibility exists that the use of sub-optimal intervals between cTBS protocols by Gamboa et al. may have prevented the progression of long-lasting suppression of motor cortical excitability, it should also be remembered that in their study AMT_{80} was used as the stimulation intensity of paired cTBS. Given our finding that an initial voluntary contraction may bias the response to paired cTBS trains, it is difficult to make direct comparisons between our results using paired cTBS at RMT_{70} and those of Gamboa and colleagues.

The induction of long-lasting neuroplastic change in regions other than the primary motor cortex has been reported using repeated applications of a slightly modified cTBS protocol (Nyffeler et al., 2006b). This modified cTBS paradigm, when applied to the human frontal eye field as a single train, induced delays in the triggering of saccadic eye movements that lasted for less than 1 h. However, when paired trains were applied at an

interval of 15 min the delay in saccade triggering lasted well over 2 h (Nyffeler et al., 2006a). Despite differences in the stimulation parameters, the method for assessing outcomes (i.e. behavioural as opposed to electrophysiological in the present study), and the cortical region investigated, the results of the present study using paired cTBS trains at RMT_{70} are largely consistent with the effects reported by Nyffeler et al. (2006a).

2.5.5. Mechanisms by which paired cTBS increases neuroplasticity induction

It is not clear from the present study how paired cTBS protocols induce a stronger and more lasting neuroplastic effect than single applications. It is possible that this increased strength of stimulation could simply be a product of applying a greater number of pulses. However, we consider this unlikely as a previous study showed that doubling the length of a cTBS train was not sufficient to increase suppression of MEP amplitudes; in fact, they found this to reverse the response from suppression to facilitation (Gamboa et al., 2010).

Whilst an initial voluntary contraction had an attenuative effect on the long-lasting MEP suppression induced by paired cTBS protocols in the present study, it is interesting to note that the less persistent changes induced by single cTBS protocols in a number of previous studies have been largely resistant to this same disruption (Huang et al., 2005; Gentner et al., 2008; Gamboa et al., 2011). This might suggest that different mechanisms may be responsible for mediating the response to repeated cTBS protocols. Data from animal studies suggest that LTP and LTD each consist of at least two mechanistically distinct phases: a transient early-phase dependent on post-translational modifications of pre-existing proteins lasting less than a few hours, as well as a more

persistent late-phase associated with new gene expression and increases in *de novo* protein synthesis which can last many hours or even days (Krug et al., 1984; Huang and Kandel, 1994; Nguyen et al., 1994; Linden, 1996). The repeated application of stimulation protocols in a spaced manner has proved an effective method for inducing this protein synthesis-dependent late-phase in animal models (Bliss and Gardner-Medwin, 1973; Abraham et al., 1993; Huang and Kandel, 1994; Abraham et al., 2002). Furthermore, there is evidence that certain types of priming stimulation may selectively impair late-phase LTP whilst having no impact on the protein synthesis-independent early-phase (Abraham et al., 2002; Woo and Nguyen, 2002; Young and Nguyen, 2005; Young et al., 2006). Although speculative at this stage, the long-lasting suppression of corticospinal excitability induced by paired cTBS protocols in the present study is consistent with the induction of late phase LTD-like effects at excitatory synapses within the human primary motor cortex. However, we cannot exclude the possibility that LTP-like effects at inhibitory synapses within the motor cortex or changes in intrinsic neuronal excitability may be contributing to the results of the present study.

In conclusion, we have demonstrated that the repeated application of cTBS trains in a spaced manner is a highly effective approach for inducing long-lasting neuroplastic changes in corticospinal excitability when applied to the human primary motor cortex. We also showed that this long-lasting suppression only occurred in the absence of prior engagement of the motor cortical regions. Whilst the mechanisms responsible for the disruptive effect of an initial voluntary contraction are unclear, these results do suggest that having subjects perform a voluntary contraction of the hand muscles prior to cTBS application may be undesirable when using spaced cTBS trains to induce lasting motor

cortical plasticity. The findings of this study may have significant implications for the therapeutic use of rTMS.

3. NEUROPLASTIC MODULATION OF INHIBITORY MOTOR CORTICAL NETWORKS BY SPACED THETA BURST STIMULATION

3.1. ABSTRACT

Continuous theta burst stimulation (cTBS) suppresses the excitability of motor networks responsible for generating motor evoked potentials (MEPs), and may also modulate the excitability of inhibitory motor networks. However, its effects on intracortical inhibition are modest in comparison to the effects on MEPs. The repeated, spaced, application of cTBS protocols results in more MEP suppression than seen with a single cTBS protocol, but whether this approach is also effective at modulating intracortical inhibition has not been tested. Therefore, this studied sought to determine whether the paired application of cTBS effectively modulates the excitability of intracortical inhibitory motor networks. Single and paired-pulse transcranial magnetic stimulation (TMS) were used to assess resting motor threshold (RMT), MEP amplitude, short-interval intracortical inhibition (SICI), and long-interval intracortical inhibition (LICI) before and during two time periods (0-10 and 30-40 min) following application of either a single or paired cTBS protocols. Both the single and paired cTBS conditions induced a significant reduction in both MEP amplitudes and the level of SICI. While paired cTBS produced a significantly greater MEP suppression than single cTBS, the effects on SICI were similar. Neither single nor paired cTBS had an effect on RMT or LICI. This suggests that although the repeated application of cTBS protocols may be effective for enhancing modulation of the MEP-generating excitatory motor networks, these

findings suggest that this approach offers little advantage when targeting intracortical inhibitory networks.

3.2. INTRODUCTION

Transcranial magnetic stimulation (TMS) applied to the human primary motor cortex in trains of repetitive stimuli (repetitive TMS; rTMS) can bidirectionally modify corticospinal excitability. These effects are largely thought to be the result of neuroplastic changes in the efficacy of excitatory glutamatergic synaptic connections between intracortical interneurons and the pyramidal neurons of the corticospinal tract, brought about by processes similar to those underlying long-term potentiation (LTP) and long-term depression (LTD) described in animal models (Di Lazzaro et al., 2005; Huang et al., 2007).

In a recent study, we were able to demonstrate an enhanced neuroplastic response to the theta burst pattern of rTMS (TBS) when repeated trains of stimulation were applied to the human primary motor cortex in a spaced manner (Goldsworthy et al., 2012). In this previous study we employed the continuous TBS (cTBS) protocol which suppresses motor evoked potentials (MEPs) for a period lasting less than 1 h (Huang et al., 2005). However, when two cTBS protocols were applied 10 min apart, there was a suppression of MEP amplitudes that lasted for at least 2 h following the second stimulation protocol (Goldsworthy et al., 2012).

Whilst the repeated application of cTBS protocols has been shown to be effective for inducing long-lasting suppression of the excitatory motor cortical circuits responsible

for generating MEPs, the effect that repeated cTBS protocols have on intracortical inhibitory networks has not been investigated. There is some evidence that cTBS may induce changes at gamma-aminobutyric acid (GABA)-mediated inhibitory synapses within the motor cortex. For example, in addition to demonstrating effects on MEP amplitudes, Huang et al. (2005) reported a reduction in $GABA_A$-mediated short-interval intracortical inhibition (SICI) following a single application of cTBS. Similar changes have been reported in other studies following application of TBS paradigms (Huang et al., 2008; Murakami et al., 2008; Suppa et al., 2008; McAllister et al., 2009). However, there have also been several studies that have been unable to demonstrate such effects (Di Lazzaro et al., 2011; Doeltgen and Ridding, 2011b, a). These contrasting results suggest that the effects of a single cTBS protocol on cortical inhibitory networks are variable and modest.

The capacity of plasticity-inducing paradigms to target the intracortical inhibitory networks could be beneficial for neurological conditions such as Parkinson's disease and focal hand dystonia, which are associated with abnormal excitability within inhibitory networks (Ridding et al., 1995a; Ridding et al., 1995b; Strafella et al., 2000; Bares et al., 2003; Butefisch et al., 2005). Therefore, in the present study we aimed to determine whether the repeated application of cTBS would be more effective for modulating the excitability of inhibitory synapses within the primary motor cortex than a single application. As well as investigating effects on $GABA_A$ inhibitory circuitry by examining SICI (Ziemann et al., 1996a; Di Lazzaro et al., 2000), we also investigated effects on $GABA_B$ circuitry by examining long-interval intracortical inhibition (LICI) (McDonnell et al., 2006).

3.3. MATERIALS AND METHODS

3.3.1. Subjects

A total of 16 healthy subjects (eight males) aged 18-34 (mean age, 23.3 ± 4.6 years) participated in this study. 14 subjects participated in Experiment 1 (seven males; mean age, 23.8 ± 4.7 years). Two of the 14 subjects from Experiment 1 were unable to participate in Experiment 2; therefore two additional subjects were recruited to bring the total to 14 subjects for this second experiment (seven males; mean age, 23.4 ± 5.0 years). All subjects were screened for any contraindications to TMS (Rossi et al., 2009) and gave their informed written consent prior to participation. This study was approved by the University of Adelaide Human Research Ethics Committee and performed in accordance with the 2008 Declaration of Helsinki.

3.3.2. Stimulation and recording

Subjects were seated in a comfortable chair, with their right arm and hand in a relaxed position for each experiment. Two Ag-AgCl surface electrodes arranged in a belly-tendon montage were used to record surface electromyographic recordings from the right first dorsal interosseous (FDI) muscle. Signals were sampled at 5 kHz (Cambridge Electrical Design 1401, Cambridge, UK), amplified with a gain of 1000 and filtered (20-1000 Hz) (Cambridge Electrical Design 1902 amplifier, Cambridge, UK) before being stored on a computer for later offline analysis.

Single and paired-pulse TMS were applied with monophasic waveform using a figure-of-eight coil (external wing diameter, 90 mm) connected to two Magstim 200^2 magnetic stimulators coupled using a Magstim Bistim unit (Magstim, Whitland, UK). The coil was positioned over the left primary motor cortex, with the handle pointing posteriorly and laterally with an angle of approximately 45° to the sagittal plane. The optimal coil position for evoking MEPs in the right FDI muscle at rest was located, and a water-soluble felt marker was used to mark the site on the scalp.

Resting motor threshold (RMT) for the right FDI muscle was defined as the minimum stimulus intensity required to evoke an MEP with peak-to-peak amplitude > 50 μV in at least five out of 10 consecutive trials whilst the FDI was relaxed. Corticospinal excitability was assessed pre and post-intervention by recording MEPs in blocks of 15 trials at an interval of 7 s (using 10% variance), using a stimulus intensity that was sufficient to evoke MEPs with an amplitude approximately 1 mV at baseline.

3.3.3. Continuous theta burst stimulation

An air-cooled figure-of-eight coil connected to a Magstim Super Rapid stimulator (Magstim, Whitland, UK) was used to apply cTBS with biphasic pulse waveform to the optimal site for stimulating the right FDI. All cTBS protocols consisted of 600 pulses applied in bursts of three pulses at 50 Hz, repeated at 5 Hz for a total of 40 s (Huang et al., 2005). The intensity of stimulation was set to 70% of RMT, assessed prior to cTBS application using the rTMS coil (RMT_{bi}).

Sham cTBS was delivered using a sham rTMS coil (placebo coil PN 3285-00; Magstim, Whitland, UK) to the same scalp site as real cTBS.

3.3.4. Short-interval intracortical inhibition

Paired-pulse TMS was used to test SICI (Kujirai et al., 1993). Three conditioning stimulus (CS) intensities were investigated: (1) 60% of RMT, (2) 70% of RMT, and (3) 80% of RMT. The intensity of the test stimulus was that which produced MEPs with peak-to-peak amplitude of approximately 1 mV when applied in the absence of a CS, and the interval between conditioning and test stimuli (i.e. interstimulus interval; ISI) was set at 2 ms. Pre and post-intervention SICI were recorded in blocks of 40 trials (10 trials for each CS intensity, plus 10 trials for test alone, applied in a pseudo-randomised order). If necessary, test stimulus intensity was adjusted to match the size of the test MEP amplitude between the pre and post-intervention recording blocks.

3.3.5. Long-interval intracortical inhibition

LICI was also assessed using paired-pulse TMS (Valls-Sole et al., 1992). Two CS intensities were investigated: (1) 105% of RMT and (2) 115% of RMT. The test stimulus intensity was set at 120% of RMT, and 100 ms was used as the ISI. Pre and post-intervention LICI were recorded in blocks of 30 trials (10 trials for each CS intensity, plus 10 trials for test alone, applied in a pseudo-randomised order). If necessary, test stimulus intensity was adjusted to match the size of the test MEP amplitude between the pre and post-intervention recording blocks.

3.3.6. Experiments

The present study consisted of two experiments (Figure 3-1). For Experiment 1, subjects attended for a single session, receiving one sham cTBS protocol followed 10 min later by one real cTBS protocol (i.e. single cTBS). For Experiment 2, subjects again attended for one session; however, for this experiment, subjects received paired cTBS protocols separated by 10 min (i.e. paired cTBS). For each experiment, RMT (measured with monophasic TMS pulse waveform; RMT_{mono}), MEPs (15 trials), SICI (40 trials), and LICI (30 trials) measurements were made at baseline. These measurements were then repeated during the period 0-10 min (P1) and 30-40 min (P2) following each intervention. RMT_{mono} and MEP measurements preceded SICI and LICI measurements for all time periods. The order of the SICI and LICI measurements was randomised between subjects.

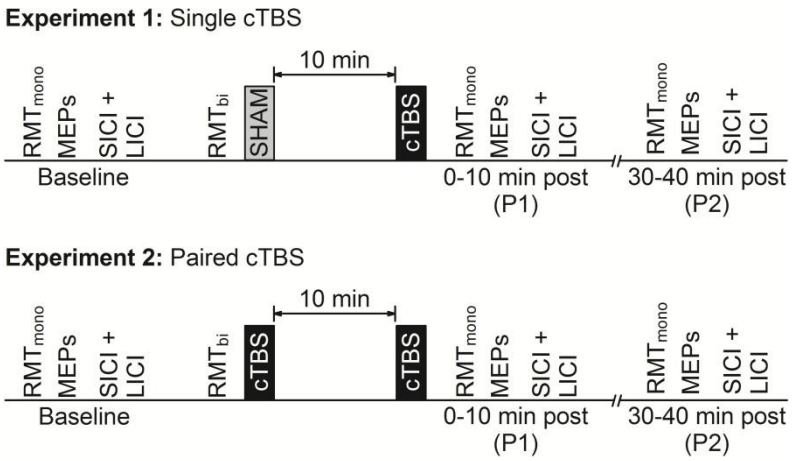

Figure 3-1: *Experimental timelines.*

3.3.7. Data analyses

All statistical analyses were performed with PASW statistics version 18 (IBM SPSS, Armonk, NY, USA). Repeated-measures analysis of variance ($ANOVA_{RM}$) and *post hoc* paired *t* tests (where appropriate) were performed on raw data for all analyses. One-way $ANOVA_{RM}$ with within-subject factor TIME (three levels – Baseline, P1, and P2) was performed for RMT and MEP measures and two-way $ANOVA_{RM}$ with within-subject factors TIME and CS INTENSITY (SICI: three levels – 60% RMT, 70% RMT, and 80% RMT; LICI: two levels – 105% RMT and 115% RMT) was performed for SICI and LICI measures for both the single cTBS (Experiment 1) and paired cTBS (Experiment 2) conditions. Also, two-tailed Pearson correlation coefficient tests were performed to determine whether a relationship existed between changes in MEP amplitude and changes in SICI from baseline measures following single and paired cTBS, as well as whether the basal excitability of SICI and LICI circuits (expressed as the average of all CS intensities) influenced the responses to single and paired cTBS. $ANOVA_{RM}$ for comparison between single and paired cTBS was performed in the subset of 12 subjects that participated in both experiments with CONDITION and TIME as within-subject factors for RMT and MEP measures and CONDITION, TIME, and CS INTENSITY for comparison of SICI and LICI measures.

Unless indicated otherwise, all data are presented as group means ± standard deviation. Statistical significance was accepted for *P*-values ≤ 0.05 for all analyses.

3.4. RESULTS

3.4.1. Experiment 1 – single cTBS protocol

3.4.1.1. Resting motor threshold and MEP amplitude

Whilst there was no change in RMT following the single cTBS protocol (TIME: $F_{2,26}$ = 0.58, P = 0.57) (Figure 3-2A), there was a significant main effect of TIME on MEP amplitudes ($F_{2,26}$ = 3.65, P = 0.04) due to significant suppression of MEPs at P1 when compared with baseline (P = 0.04) (Figure 3-2B). No significant correlation was observed between the degree of MEP suppression at P1 and baseline levels of SICI (r = -0.24, P = 0.41) and LICI (r = -0.05, P = 0.86).

3.4.1.2. Short-interval intracortical inhibition

There was no difference in the unconditioned test MEP amplitudes between time periods for the SICI assessments (TIME: $F_{2,26}$ = 0.35, P = 0.71). Two-way $ANOVA_{RM}$ revealed a significant main effect of CS INTENSITY ($F_{1,18}$ = 10.1, P = 0.003) for SICI, but only a non-significant trend of TIME ($F_{1,18}$ = 3.20, P = 0.08). Separate analysis of each of the CS intensities revealed a significant main effect of TIME for SICI with CS intensities of 60% ($F_{2,26}$ = 3.29, P = 0.05) and 80% of RMT ($F_{2,26}$ = 4.58, P = 0.02), but not 70% of RMT ($F_{1,19}$ = 0.43, P = 0.59). *Post hoc* analysis revealed that SICI recorded with a CS intensity of 60% RMT was suppressed at P1 (P = 0.04), and when recorded with a CS intensity of 80% RMT was suppressed at both P1 and P2 (P = 0.02 for both) (Figure 3-2C).

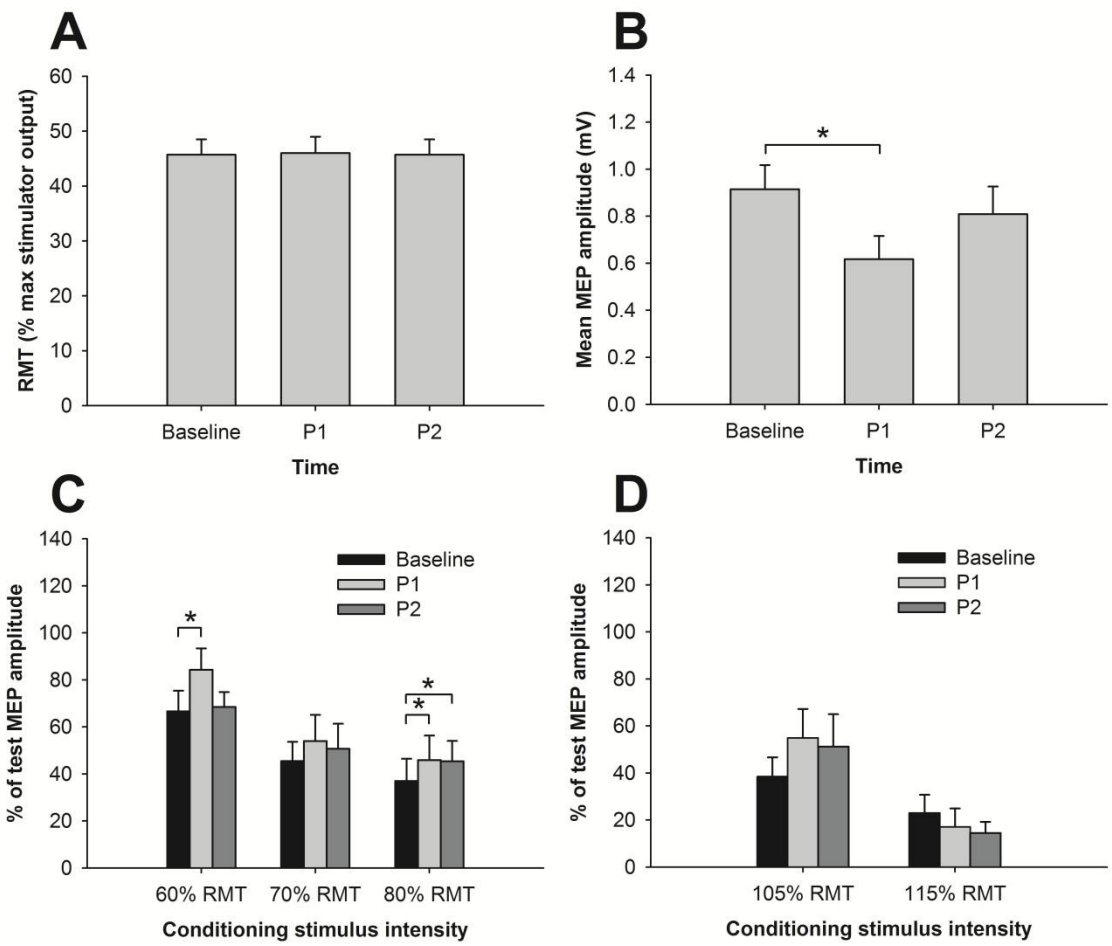

Figure 3-2: *The effect of a single cTBS protocol on (A) RMT, (B) MEP amplitude, (C) SICI, and (D) LICI. * denotes* $P \leq 0.05$*. Data are shown as group means ± SEM.*

No significant correlation was observed between the suppression of MEP amplitudes and the suppression of SICI at the P1 time period when SICI was recorded with CS intensities of 60% (r = 0.13, P = 0.67) and 80% of RMT (r = -0.51, P = 0.06).

3.4.1.3. Long-interval intracortical inhibition

As with SICI, there was no difference in the unconditioned test MEP amplitudes between time periods for the LICI assessments (TIME: $F_{2,26}$ = 0.70, P = 0.51). Also, the amplitude of conditioning MEPs were not different between time periods for both the

105% RMT (TIME: $F_{2,26}$ = 2.70, P = 0.09) and 115% RMT (TIME: $F_{2,26}$ = 1.73, P = 0.20) CS intensities. Two-way $ANOVA_{RM}$ revealed a significant main effect of CS INTENSITY ($F_{1,13}$ = 19.2, P = 0.001) and this was due to LICI being greater when conditioned at 115% RMT compared with 105% RMT. However, single cTBS had no effect on LICI during any time period (Figure 3-2D).

3.4.2. Experiment 2 – paired cTBS protocols

3.4.2.1. Resting motor threshold and MEP amplitude

As in Experiment 1, RMT did not change following paired cTBS protocols in Experiment 2 (TIME: $F_{2,26}$ = 1.53, P = 0.24) (Figure 3-3A). However, there was a strong suppression of MEP amplitudes (TIME; $F_{2,26}$ = 23.9, P < 0.001), with *post hoc* analysis revealing that MEP amplitudes were suppressed compared to baseline at both P1 and P2 (P < 0.001 for both) (Figure 3-3B). No significant correlation was observed between the degree of MEP suppression at both P1 and P2 and baseline levels of SICI (P1 time period, r = 0.15, P = 0.61; P2 time period, r = -0.10, P = 0.75) and LICI (P1 time period, r = -0.27, P = 0.35; P2 time period, r = -0.22, P = 0.45).

3.4.2.2. Short-interval intracortical inhibition

There was no difference in the unconditioned test MEP amplitudes when the three time periods for the SICI assessments were compared (TIME; $F_{2,26}$ = 0.32, P = 0.73). Two-way $ANOVA_{RM}$ of SICI data indicated main effects of TIME ($F_{1,18}$ = 5.58, P = 0.02) and CS INTENSITY ($F_{1,17}$ = 29.9, P < 0.001). Separate analyses of each of the CS intensities showed that this was due to suppressed SICI, when compared with baseline, at CS intensities of 60% ($F_{2,26}$ = 5.46, P = 0.01) and 70% of RMT ($F_{1,19}$ = 3.92, P =

0.05), but not at 80% of RMT ($F_{2,26}$ = 1.89, P = 0.17). *Post hoc* analyses further showed that SICI conditioned with both 60% and 70% RMT was suppressed compared to baseline at both P1 and P2 ($P \leq 0.02$ for all) (Figure 3-3C).

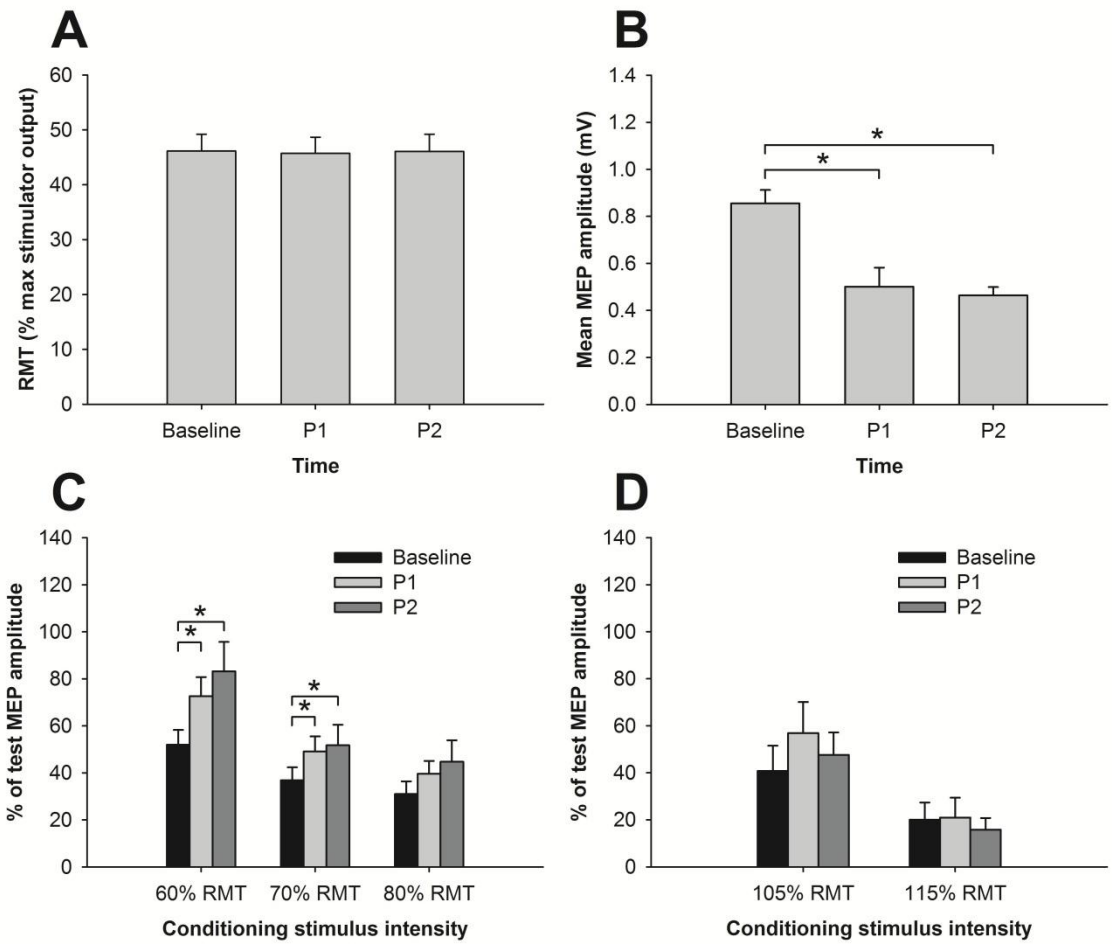

Figure 3-3: *The effect of paired cTBS protocols on (A) RMT, (B) MEP amplitude, (C) SICI, and (D) LICI. * denotes* $P \leq 0.05$. *Data are shown as group means ± SEM.*

No significant correlation was observed between the suppression of MEP amplitudes and the suppression of SICI at both the P1 and P2 time periods when SICI was recorded with CS intensities of 60% (P1 time period, r = -0.15, P = 0.62; P2 time period, r =

0.41, $P = 0.15$) and 70% of RMT (P1 time period, $r = -0.12$, $P = 0.68$; P2 time period, $r = 0.33$, $P = 0.25$).

3.4.2.3. Long-interval intracortical inhibition

There was no difference in the unconditioned test MEP amplitudes between time periods for the LICI assessments (TIME: $F_{2,26} = 2.32$, $P = 0.12$). The amplitudes of conditioning MEPs were also not different between time periods for both the 105% RMT (TIME: $F_{2,26} = 0.26$, $P = 0.77$) and 115% RMT (TIME: $F_{2,26} = 0.07$, $P = 0.94$) CS intensities. Two-way $ANOVA_{RM}$ revealed a significant main effect of CS INTENSITY ($F_{1,13} = 18.0$, $P = 0.001$), and, as with Experiment 1, this was due to LICI being greater at CS of 115% RMT than at 105% RMT. However, paired cTBS had no effect on LICI; baseline LICI was not different with that recorded during either of the two time periods following paired cTBS, regardless of the CS intensity (Figure 3-3D).

3.4.3. Comparison of single and paired cTBS conditions

Baseline RMT_{mono}, MEP, SICI and LICI measures for the single and paired cTBS conditions did not differ (Table 3-1), nor did cTBS intensities (38.3 ± 8.2% and 37.8 ± 8.2% of maximal stimulator output (MSO), respectively; $P = 0.51$). There was no difference between single and paired cTBS with respect to their effect on RMT. However, single and paired cTBS differed in their effects on MEP amplitudes (CONDITION: $F_{1,11} = 5.09$, $P = 0.04$; TIME: $F_{2,22} = 10.6$, $P = 0.001$), with a greater suppression of MEP amplitudes following paired cTBS.

Measure	***Single cTBS***	***Paired cTBS***	***P-value***
RMT_{mono} (% MSO)	45.7 ± 11.3	45.7 ± 12.3	1.00
MEP amp. (mV)	0.96 ± 0.40	0.85 ± 0.19	0.25
SICI: test MEP amp. (mV)	1.09 ± 0.82	0.92 ± 0.34	0.45
SICI: 60% RMT MEP amp. (% test)	61.8 ± 29.5	48.6 ± 23.2	0.10
SICI: 70% RMT MEP amp. (% test)	37.2 ± 22.1	34.7 ± 21.5	0.68
SICI: 80% RMT MEP amp. (% test)	31.3 ± 33.8	25.9 ± 16.2	0.57
LICI: test MEP amp. (mV)	0.99 ± 0.79	1.02 ± 0.67	0.90
LICI: 105% RMT MEP amp. (% test)	37.8 ± 31.6	37.2 ± 37.1	0.96
LICI: 115% RMT MEP amp. (% test)	19.4 ± 24.6	13.2 ± 21.3	0.24

Table 3-1: *Comparison of baseline measures for the single and paired cTBS conditions. Data are mean ± SD*

Three-way $ANOVA_{RM}$ revealed significant main effects of TIME ($F_{2,22}$ = 5.12, P = 0.02) and CS INTENSITY ($F_{2,22}$ = 25.3, P < 0.001) on SICI, but no effect of CONDITION. There was a mild CONDITION x TIME interaction, although this did not reach statistical significance ($F_{2,22}$ = 2.77, P = 0.08). There was no overall difference between single and paired cTBS in their effect on SICI when the three CS intensities were compared. As indicated previously, neither single nor paired cTBS altered LICI.

3.5. DISCUSSION

The present study confirms that application of a single cTBS protocol results in suppression of both MEP amplitudes and SICI. Also, our findings confirm that the paired application of cTBS protocols results in a significantly greater effect on MEPs than when a single cTBS protocol is applied. We have shown, for the first time, that the

paired application of cTBS reduces the excitability of the $GABA_A$-mediated SICI circuits but, in contrast, neither single or paired cTBS protocols modulate the $GABA_B$-mediated LICI.

3.5.1. Effects of single cTBS on MEP amplitude and SICI

In agreement with findings from previous studies (Di Lazzaro et al., 2005; Huang et al., 2005; Huang et al., 2007), the application of a single cTBS protocol in the present study induced a transient suppression of MEP amplitudes. The mechanisms underlying this MEP suppression have been well characterised. For instance, Di Lazzaro and colleagues (2005) have recorded the descending corticospinal volleys (evoked by single-pulse TMS) from implanted cervical spinal electrodes in a small group of chronic pain patients before and following single cTBS. They found that cTBS reduces the magnitude of the I1-wave, suggesting that the MEP suppression is due to reduction in the excitability of excitatory synapses within the primary motor cortex (Di Lazzaro et al., 2005). Also, pharmacological intervention with memantine, a NMDA receptor antagonist, has been shown to abolish the MEP suppression induced by cTBS (Huang et al., 2007). Together, these results provide strong evidence that the suppression of MEP amplitudes induced by cTBS is likely due to a LTD-like suppression of the excitability of glutamatergic synapses within the primary motor cortical regions.

As well as effects on the excitatory circuitry responsible for generating MEPs, there is also some evidence that cTBS can influence inhibitory circuitry. Whilst several studies have reported a reduction in the excitability of inhibitory $GABA_A$-mediated SICI circuits following cTBS application (Huang et al., 2005; Huang et al., 2008; Murakami

et al., 2008; Suppa et al., 2008; McAllister et al., 2009), others have failed to replicate such findings (Di Lazzaro et al., 2011; Doeltgen and Ridding, 2011b, a). The reasons for these discrepant findings are not clear, but it may be that the effects on these inhibitory networks are modest and variable, and suboptimal testing parameters may limit the capacity to identify such changes. For example, it may be that the CS intensities employed in previous studies were not optimal for detecting a change in SICI. Most studies have only investigated a single CS intensity, and given the high variability of SICI (Orth et al., 2003) and the subtle effects of cTBS on SICI circuits, it is possible that any change in SICI might have been missed. Indeed, in the present study we investigated SICI at a range of CS intensities using the 2 ms ISI (thus was unlikely to have been contaminated with intracortical facilitatory effects (Peurala et al., 2008)), and were able to show a significant cTBS-induced suppression of SICI but only at CS intensities of 60% and 80% of RMT, with no significant effect at 70% of RMT. These findings highlight the importance of testing SICI with a range of CS intensities.

3.5.2. Effects of paired cTBS on MEP amplitude and SICI

Whilst in the present study the effect of a single cTBS protocol on MEP amplitudes was relatively modest and short-lasting, the MEP suppression induced by the paired cTBS protocols was significantly greater. This is consistent with our recent finding that repeated cTBS protocols applied to the human primary motor cortex induce a significantly greater suppression of MEP amplitudes than that evoked with a single protocol (Goldsworthy et al., 2012).

As the repeated application of cTBS protocols has been shown to be an effective approach for enhancing suppression of the excitatory circuits responsible for generating MEPs, the present study investigated whether the same was true for the inhibitory motor cortical circuits. We show for the first time that application of paired cTBS protocols reduces the excitability of $GABA_A$-mediated SICI circuits. Also, we showed that this suppression was strongest when lower CS intensities (i.e. 60% and 70% of RMT) were used to test SICI. Whilst there was a general trend towards the paired cTBS protocols producing a greater suppression of SICI (particularly at the lower CS intensities) than a single cTBS at the later P2 time period, this was not statistically significant. This suggests that even repeated cTBS protocols, which result in robust and lasting effects on MEPs, have relatively modest effects on SICI circuitry.

It is worth noting that different CS intensities were optimal for detecting a change in SICI following each condition, with the higher 80% of RMT being most effective for the single cTBS condition and the lower 60% of RMT being most effective for the paired cTBS condition. The reasons for this are unclear and may just be a reflection of the variability in SICI measures and the modest effect of these cTBS paradigms on SICI. However, once again this emphasises the importance of using a range of CS intensities to test SICI.

3.5.3. Effects of single and paired cTBS protocols on LICI

Even though we employed multiple CS intensities, as well as an ISI optimal for evoking LICI and unlikely to be influenced by changes at the spinal level (Nakamura et al., 1997; Chen et al., 1999; Di Lazzaro et al., 2002a), there was no significant change in the

$GABA_B$-mediated LICI following either single or paired cTBS conditions. Few studies have investigated the excitability of LICI circuits following TBS. Suppa et al. (2008) found no change in LICI measured in the contralateral hemisphere following both cTBS and iTBS. However, LICI was not assessed for the stimulated hemisphere. Another method which has been used to assess $GABA_B$-mediated intracortical inhibition is to measure the duration of the cortical silent period following single-pulse TMS applied during a tonic voluntary contraction (Paulus et al., 2008). A recent study showed no effect of cTBS on cortical silent period recorded from the hand muscles both contralateral and ipsilateral to the stimulated hemisphere (Di Lazzaro et al., 2011). Therefore, the findings in the present study provide additional evidence that this form of stimulation does not modulate the excitability of $GABA_B$-mediated intracortical inhibitory circuits.

The reasons why cTBS has relatively modest effects on the excitability of inhibitory motor networks are unclear, although it may be related to the temporal characteristics of pulses within cTBS protocols being more optimal for evoking LTD-like neuroplasticity at excitatory synapses than at inhibitory ones (Caporale and Dan, 2008).

3.5.4. Inferences regarding the mechanisms of paired cTBS-induced MEP suppression

Although the primary aim for the present study was not to investigate the mechanisms by which repeated cTBS protocols induce MEP suppression, the findings do allow some inferences to be made. First, we show that application of paired cTBS protocols has no effect on RMT. Findings from pharmacological studies provide evidence that RMT is influenced by the intrinsic membrane excitability of corticospinal axons (Ziemann et al.,

1996b; Ziemann et al., 1998; Di Lazzaro et al., 2003). Therefore, this result suggests that changes in the intrinsic excitability of corticospinal circuitry generating the MEP are unlikely to play a major role in the MEP suppression. Also, as the excitability of GABA-mediated intracortical inhibitory networks is either suppressed (in the case of the $GABA_A$-mediated SICI) or unchanged (in the case of the $GABA_B$-mediated LICI) following the paired application of cTBS protocols, it is likely that increased inhibitory drive was not responsible for the enhanced reduction of MEP amplitude. This was also supported by the correlation analyses, which show no significant relationship between the degree of MEP suppression and the degree to which the excitability of the SICI circuits were modulated. Therefore, we suggest that the MEP suppression induced by the repeated application of spaced cTBS protocols is likely the result of an enhanced induction and/or maintenance of LTD-like effects at excitatory motor networks, and not the result of LTP-like facilitation in inhibitory motor networks. It is possible that this enhancement of LTD-like plasticity may be due, at least in part, to a gating mechanism (Ziemann and Siebner, 2008; Siebner, 2010), with the first cTBS protocol inducing a reduction in intracortical inhibition thereby enhancing the neuroplastic response to the second cTBS protocol. However, using correlation analyses, we were unable to show a relationship between the basal excitability of the inhibitory networks and the degree of MEP suppression following either the single or paired cTBS conditions. Whilst this does not necessarily exclude the possibility that gating mechanisms could be involved, it does suggest that other factors may be important.

In conclusion, we have confirmed that the repeated application of cTBS protocols in a spaced manner is an effective approach for inducing robust changes in the excitability of networks responsible for generating MEPs. However, while paired cTBS applications

result in significantly greater effects on MEPs than a single cTBS application, the effects on SICI generating networks were not significantly different. We also show that both single and paired cTBS protocols have no significant effect on $GABA_B$-mediated LICI. In sum, these findings suggest that cTBS protocols may not be an optimal approach for modulation of intracortical inhibitory networks. These findings may be important when considering possible therapeutic applications of spaced cTBS protocols, and suggest that this approach may be better suited to targeting conditions where the modulation of excitability in facilitatory (rather than inhibitory) networks would be beneficial.

4. DE-DEPRESSION & CONSOLIDATION OF NEUROPLASTIC CHANGES IN THE HUMAN MOTOR CORTEX

4.1. ABSTRACT

There is evidence that continuous theta burst stimulation (cTBS) induces long-term depression (LTD)-like changes in human primary motor cortex excitability. These LTD-like changes are subject to reversal (i.e. de-depression) following behavioural engagement of the motor cortical regions, and this severely limits the therapeutic potential of cTBS under normal physiological conditions. Experiments in animal models suggest that the repeated, spaced, application of stimulation trains may consolidate synaptic plasticity, making it resistant to reversal by normal physiological activity. Whilst the repeated application of cTBS protocols has been shown to prolong LTD-like motor cortical neuroplasticity in humans, whether these longer lasting effects are also resistant to reversal has yet to be tested. In this study we investigated whether the neuroplastic effects of paired cTBS protocols (applied at 10 min intervals) were stable following behavioural engagement of the motor cortex by a sustained, sub-maximal voluntary contraction of the hand muscles. In the absence of cTBS, the voluntary contraction had no effect on motor evoked potentials (MEPs) recorded from the right first dorsal interosseous muscle in response to single-pulse transcranial magnetic stimulation of the left primary motor cortical hand area. Whilst the LTD-like MEP suppression induced by a single cTBS was abolished by subsequent voluntary contraction, paired cTBS induced MEP suppression that was resistant to reversal. This

MEP suppression was also resistant to reversal when an experimental de-depression protocol was used instead of a voluntary contraction. These findings suggest that repeated cTBS applications may consolidate LTD-like neuroplastic changes within the human primary motor cortex. This may have significant implications for the clinical application of non-invasive brain stimulation protocols.

4.2. INTRODUCTION

Neuronal networks within the human central nervous system undergo neuroplastic modulation throughout life in response to a variety of experiences. One technique which has been used to induce and study neuroplastic change in the human cortex in recent years is repetitive transcranial magnetic stimulation (rTMS). When applied to the human primary motor cortex, rTMS can induce lasting changes in motor cortical excitability that resemble the long-term potentiation (LTP) and long-term depression (LTD) observed in animal models (Huang et al., 2007).

A feature of LTP and LTD induced in animal models is their susceptibility to reversal, either by subsequent physiological activity at the stimulated synaptic input (Xu et al., 1998; Zhou et al., 2003) or by delivery of a weak stimulation protocol following plasticity induction (Fujii et al., 1991; Huang et al., 1999; Chen et al., 2001). This reversal of LTP and LTD (termed depotentiation and de-depression, respectively) most likely acts as a safeguard preventing consolidation of random activity (Zhou and Poo, 2004). Similar depotentiation and de-depression phenomena have also been observed in humans, with behavioural engagement of the stimulated motor regions reversing rTMS-induced changes in motor cortical excitability (Huang et al., 2008). As with the

depotentiation and de-depression observed in animals, the reversal of rTMS-induced neuroplasticity may also be triggered by weak rTMS protocols which, when applied alone, do not change motor cortical excitability (Huang et al., 2010).

An important property of the LTP and LTD described in animal experiments is that they may become consolidated by applying repeated trains of electrical stimulation in a spaced manner (Bliss and Gardner-Medwin, 1973; Trepel and Racine, 1998). This consolidation is critical for the persistence of synaptic modifications under normal physiological conditions (Zhou et al., 2003), and is likely due to increases in *de novo* protein synthesis and gene transcription (Krug et al., 1984; Huang and Kandel, 1994; Nguyen et al., 1994; Woo and Nguyen, 2003). Similar to animal experiments showing long-lasting synaptic modifications with repeated induction protocols, the spaced application of rTMS has been shown to prolong the duration of induced neuroplasticity in the human cortex (Nyffeler et al., 2006a; Nyffeler et al., 2009; Goldsworthy et al., 2012). However, whether these longer lasting neuroplastic changes are also resistant to reversal has yet to be tested.

The capacity of rTMS to induce stable neuroplasticity under normal physiological conditions is critically important for its implementation as a therapeutic tool for treating disease. Therefore, the present study aimed to determine whether the repeated application of rTMS protocols could induce stable neuroplasticity resistant to reversal. We employed the continuous theta burst rTMS pattern (cTBS) which, when applied as a single train, induces LTD-like suppression of motor cortical excitability lasting less than 1 h (Di Lazzaro et al., 2005; Huang et al., 2005; Huang et al., 2007). Two de-depression methods were used to test the stability of induced neuroplasticity: (1) behavioural

engagement of the primary motor cortex during a sustained, sub-maximal voluntary contraction, and (2) stimulation of the primary motor cortex with a novel de-depression TBS protocol (Huang et al., 2010).

4.3. MATERIALS AND METHODS

4.3.1. Subjects

A total of 30 healthy subjects (thirteen females) aged 19-49 [24.4 ± 5.7 years (mean age ± SD)] gave informed written consent to participate in this study. Ten participants were included for each of Experiments 1 (seven females; 23.4 ± 3.6 years), 2 (five females; 23.7 ± 3.1 years) and 3 (six females; 24.7 ± 4.0 years), with four subjects participating in all three experiments (four females; 23.8 ± 1.3 years). Experiment 4 was performed on a total of eight participants (two females; 23.0 ± 3.5 years), and ten participants were included for Experiment 5 (three females; 26.4 ± 8.6 years), five of whom had participated in Experiment 4. All participants were screened for any contraindications to TMS prior to their involvement in the study (Rossi et al., 2009). All experiments were performed in accordance with the 2008 Declaration of Helsinki and were approved by the University of Adelaide Human Research Ethics Committee and the ethics committee of the medical faculty of the Goethe-University of Frankfurt am Main.

4.3.2. Stimulation and recording

Subjects were seated in a comfortable chair for each experimental session, and were directed to keep their right hand and arm as relaxed as possible unless instructed

otherwise. Surface electromyography was used to record motor evoked potentials (MEPs) from the right first dorsal interosseous (FDI) muscle using two Ag-AgCl electrodes arranged in a belly-tendon montage. Signals were sampled at a rate of 5 kHz (Cambridge Electrical Design 1401, Cambridge, UK), amplified with a gain of 1000 and band-pass filtered between 20 Hz and 1000 Hz (Cambridge Electrical Design 1902 amplifier, Cambridge, UK) or 2000 Hz (Counterpoint Mk2 electromyograph, Dantec, Denmark). Samples were stored on a laboratory computer for later offline analysis.

Single-pulse TMS was applied with monophasic current waveform using a figure-of-eight magnetic coil (external wing diameter, 90 mm) connected to a Magstim 200 magnetic stimulator (Magstim, Whitland, UK). The TMS coil was held tangential to the skull over the left primary motor cortex with the handle pointing posterolaterally at a 45° angle to the sagittal plane. The optimal coil position for eliciting MEPs in the right FDI was identified and marked on the subject's scalp using a felt marker. The intensity of stimulation was adjusted to evoke MEPs with peak-to-peak amplitude of approximately 1 mV at baseline.

4.3.3. TBS for plasticity induction

All TBS paradigms were applied with biphasic current waveform using either Magstim Super Rapid (Magstim, Whitland, Dyfed, UK) or MagPro X100 (MagVenture, Farum, Denmark) magnetic stimulators. The same type of stimulator was used for each session of an experiment for each subject. The standard pattern of TBS was employed, and consisted of bursts of three pulses at 50 Hz repeated using an inter-burst frequency of 5 Hz (Huang et al., 2005). All cTBS protocols were applied for 40 s and consisted of 600

stimuli. The intensity of stimulation was set to 70% of subjects' resting motor threshold, which was assessed for each experimental session prior to cTBS application using the rTMS coil and was defined as the minimum stimulus intensity required to evoke an MEP from the relaxed right FDI muscle with peak-to-peak amplitude greater than 50 μV in at least five out of ten consecutive trials. Sham cTBS was delivered to the same scalp site as real cTBS using a sham rTMS coil (either PN 3285-00, Magstim; or MCF-P-B65, MagVenture).

4.3.4. TBS for plasticity reversal

A shortened form of the intermittent TBS (iTBS) protocol was applied in Experiment 5 to test the reversal of motor cortical neuroplasticity by externally-generated network activity. The stimulation parameters were the same as those described by Huang et al. (2010), and consisted of the standard TBS pattern applied in 2 s trains repeated at 10 s intervals for a total of 150 pulses (i.e. iTBS150). The intensity of stimulation was the same as that used for cTBS.

4.3.5. Experiments

The present study consisted of five experiments (Figure 4-1). For Experiment 1, subjects received paired sham cTBS protocols separated by 10 min (i.e. no cTBS). For Experiment 2, subjects received one sham cTBS protocol followed 10 min later by one real cTBS protocol (i.e. single cTBS). For Experiment 3, subjects received paired real cTBS protocols separated by 10 min (i.e. paired cTBS). Each of Experiments 1-3 consisted of two sessions. In one session, subjects were instructed to keep their hand

completely relaxed for the entire post-intervention recording period (i.e. FDI relaxed). In the other session, subjects were instructed to produce a sub-maximal isometric contraction of their right hand by pinching their right thumb and index finger at 15 min following the first stimulation protocol (i.e. FDI contract). Subjects were required to sustain this voluntary contraction for 2 min, and visual feedback was provided to ensure constant force production (set to 0.45 kg force for each subject). Three maximal voluntary contractions (MVCs) were recorded at the end of each FDI contract session to determine the intensity of each subject's contraction relative to their maximal effort. MVC's were performed at the end of the session to prevent the voluntary muscle activation from influencing the response to cTBS (Gentner et al., 2008; Goldsworthy et al., 2012). Based on unpublished pilot data, we anticipated that the force of contraction would roughly equate to 10% of MVC for most subjects. The duration and intensity of contraction were chosen to produce the greatest likelihood of de-depression whilst minimising fatigue (Sogaard et al., 2006).

Experiment 4 was a control study investigating the impact that the time-interval separating cTBS and the sub-maximal voluntary contraction may have had on the de-depression response. Similar to Experiment 2, subjects for Experiment 4 received a single cTBS protocol; however, the order of sham/real cTBS delivery was reversed such that the real cTBS protocol was applied 10 min prior to the sham cTBS protocol [i.e. single cTBS (control)]. As with Experiments 1-3, subjects for Experiment 4 were required to attend for two sessions: one FDI relaxed and the other FDI contract. The voluntary contraction was performed at 15 min following cTBS, and was maintained for the same duration and at the same intensity as the first three experiments.

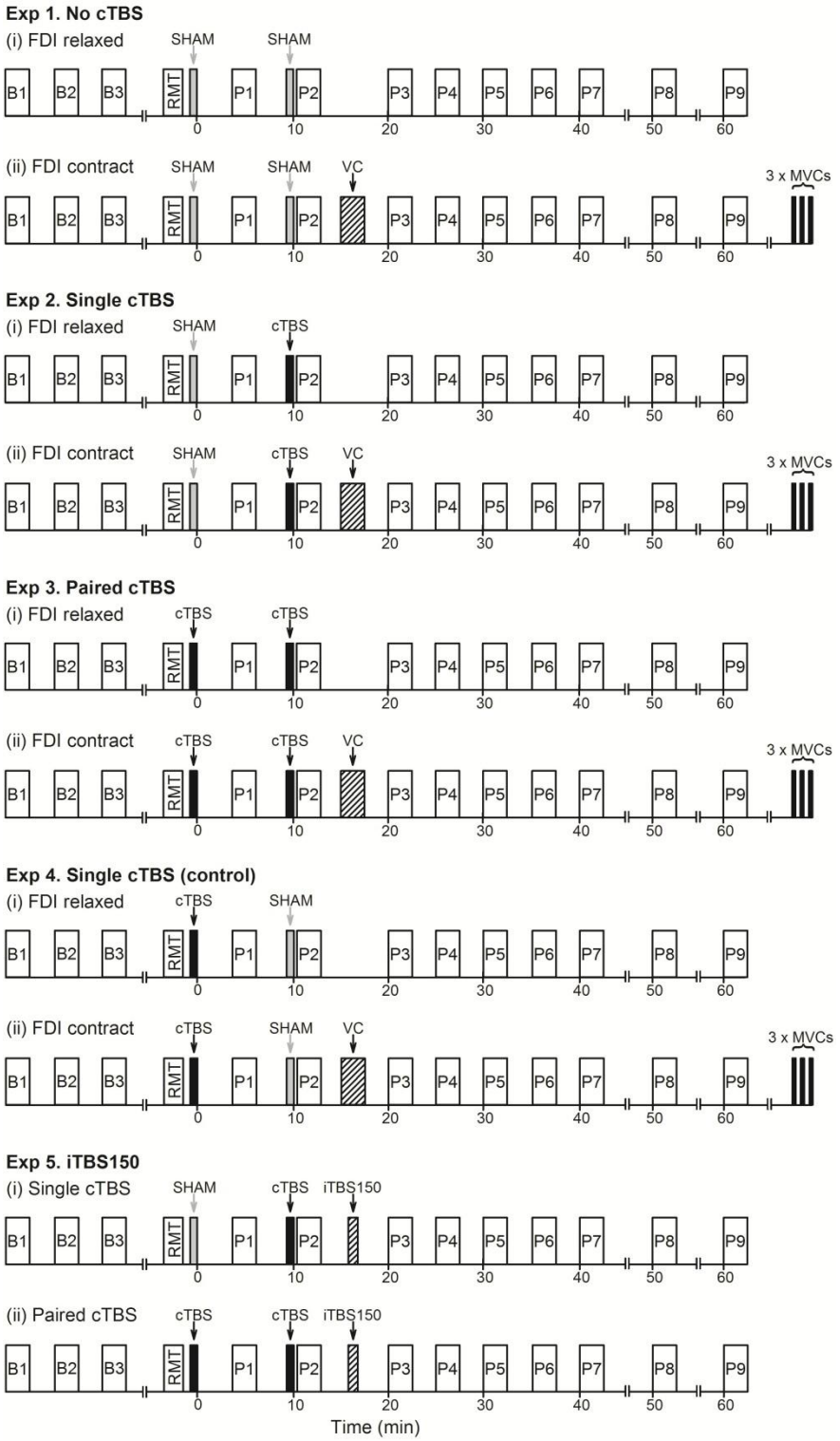

Figure 4-1: *Schematic overview of experimental design. All experiments were conducted with the FDI at rest. "FDI contract" as a condition only refers to the 2 min sub-maximal voluntary contraction (VC) performed after the stimulation protocols and not during MEP measurement.*

Experiment 5 was included to determine whether neuroplasticity induced in the primary motor cortex by paired cTBS was resistant to reversal by an iTBS150 paradigm designed to de-depress cTBS-induced MEP suppression (Huang et al., 2010). For this experiment subjects were required to attend two sessions. For one session subjects received single cTBS (i.e. one sham cTBS protocol followed 10 min later by one real cTBS protocol), whilst for the other session they received paired cTBS (i.e. two real cTBS protocols separated by 10 min). In both sessions a single train of iTBS150 was applied 15 min following the first stimulation protocol.

MEPs were recorded in blocks of 15 trials for all experiments. Three blocks were recorded at baseline (B1, B2, and B3), and a total of nine post-intervention blocks were recorded following the first stimulation protocol. One post-intervention block was recorded between stimulation protocols (i.e. P1), and another was recorded immediately following the second stimulation protocol and prior to the voluntary contraction (i.e. P2). Seven blocks were recorded after the voluntary contraction, with recordings at 20 min (P3), 25 min (P4), 30 min (P5), 35 min (P6), 40 min (P7), 50 min (P8), and 60 min (P9) following the first stimulation protocol.

At least two days separated each experimental session. The order in which subjects attended each of the sessions for each experiment was randomised and all experimental sessions were performed in the afternoon to control for time-of-day effects on neuroplasticity induction (Sale et al., 2007). Background surface electromyography was monitored at all points during and between recording blocks to ensure complete relaxation of subjects' right FDI muscle during periods where a voluntary contraction

was not required. Trials that contained background muscle activation during the 100 ms prior to TMS application were excluded from analysis.

4.3.6. Data analyses

All statistical analyses were performed with IBM SPSS Statistics 20 (IBM SPSS, Armonk, NY, USA). Mean peak-to-peak MEP amplitudes were calculated for each recording block for each subject. Baseline data were analysed using two-way repeated measures analysis of variance ($ANOVA_{RM}$) with CONDITION (Experiments 1-4: two levels – FDI relaxed and FDI contract; Experiment 5: two levels – single cTBS and paired cTBS) and BLOCK (three levels – B1, B2, and B3) as within-subject factors to assess the stability of baseline MEP amplitudes between conditions and between baseline recording blocks for each experiment. The three blocks of baseline MEPs were averaged for each subject and post-intervention MEP amplitudes were expressed as a percentage of the average baseline for comparisons between experimental conditions. Additionally, one-way $ANOVA_{RM}$ were performed on raw MEP data for each experimental condition separately with BLOCK (10 levels: the average baseline, as well as P1, P2, P3, P4, P5, P6, P7, P8, and P9) as the within-subject factor.

For Experiments 1-4, the impact that the voluntary contraction had on MEP amplitudes was assessed using two-way $ANOVA_{RM}$ with CONDITION (two levels – FDI relaxed and FDI contract) and BLOCK (seven levels – P3, P4, P5, P6, P7, P8, and P9) as within-subject factors. *Post hoc* comparisons were performed on pooled MEP data using paired *t* tests. Data were pooled into two time periods: an 'early response' period representing the average response over the period 20-35 min following the first

stimulation protocol (calculated as the average of P3, P4, P5, and P6) and a 'late response' period representing the average response over the period 40-60 min following the first stimulation protocol (calculated as the average of P7, P8, and P9). The definitions of the early and late response time periods were based on the duration of MEP suppression observed in Experiment 2 for the single cTBS, FDI relaxed condition (see Section 4.4).

Pearson correlation coefficient tests were performed on data from Experiment 2 to further characterise the impact that the voluntary contraction may have had on single cTBS-induced MEP suppression. We looked to determine whether the de-depression of MEP suppression by a voluntary contraction for each subject was influenced by two factors: (1) the initial level of MEP suppression prior to the voluntary contraction (i.e. the mean MEP amplitude at P2, expressed as a percentage of the average baseline), and (2) the relative intensity of the voluntary contraction (expressed for each subject as a percentage of their MVC). A measure of de-depression was calculated for each subject by subtracting the pooled MEP amplitudes recorded during the early response period for the FDI relaxed condition from that recorded during the same period for the FDI contract condition.

For Experiment 5, separate one-way $ANOVA_{RM}$ were performed on raw MEP data for the single and paired cTBS conditions with BLOCK (10 levels: the average baseline, as well as P1, P2, P3, P4, P5, P6, P7, P8, and P9) as the within-subject factor. The impact of iTBS150 on single and paired cTBS-induced changes in MEP amplitudes was assessed by comparing these data (expressed as a percentage of the average baseline) to that for the FDI relaxed conditions of Experiment 2 (for single cTBS) and Experiment 3

(for paired cTBS) using two-way mixed-design $ANOVA_{RM}$ with CONDITION [two levels – FDI relaxed (i.e. no iTBS150) and iTBS150] as the between-subject factor and BLOCK (seven levels – P3, P4, P5, P6, P7, P8, and P9) as the within-subject factor. *Post hoc* comparisons were performed on MEP data pooled into early and late response time periods using independent-samples *t* tests.

Where necessary, the degrees of freedom for $ANOVA_{RM}$ were adjusted using the Huynh-Feldt correction for non-sphericity. All statistical analyses were two-tailed, and unless indicated otherwise, all data represent group means ± standard deviation. Statistical significance was accepted for P values ≤ 0.05.

4.4. RESULTS

4.4.1. Experiment 1 – no cTBS

Average baseline MEP amplitudes for the FDI relaxed and FDI contract conditions for Experiment 1 were 0.94 ± 0.29 mV and 0.93 ± 0.18 mV respectively. There was no difference in baseline MEP amplitudes between conditions ($F_{1,9} = 0.08$, $P = 0.79$), nor was there a difference between baseline recording blocks ($F_{2,18} = 1.27$, $P = 0.31$). Likewise, there was no change in MEP amplitudes from baseline values during the post-intervention recording period for both the FDI relaxed ($F_{6,52} = 0.37$, $P = 0.89$) and FDI contract ($F_{5,46} = 0.40$, $P = 0.85$) conditions (Figure 4-2A).

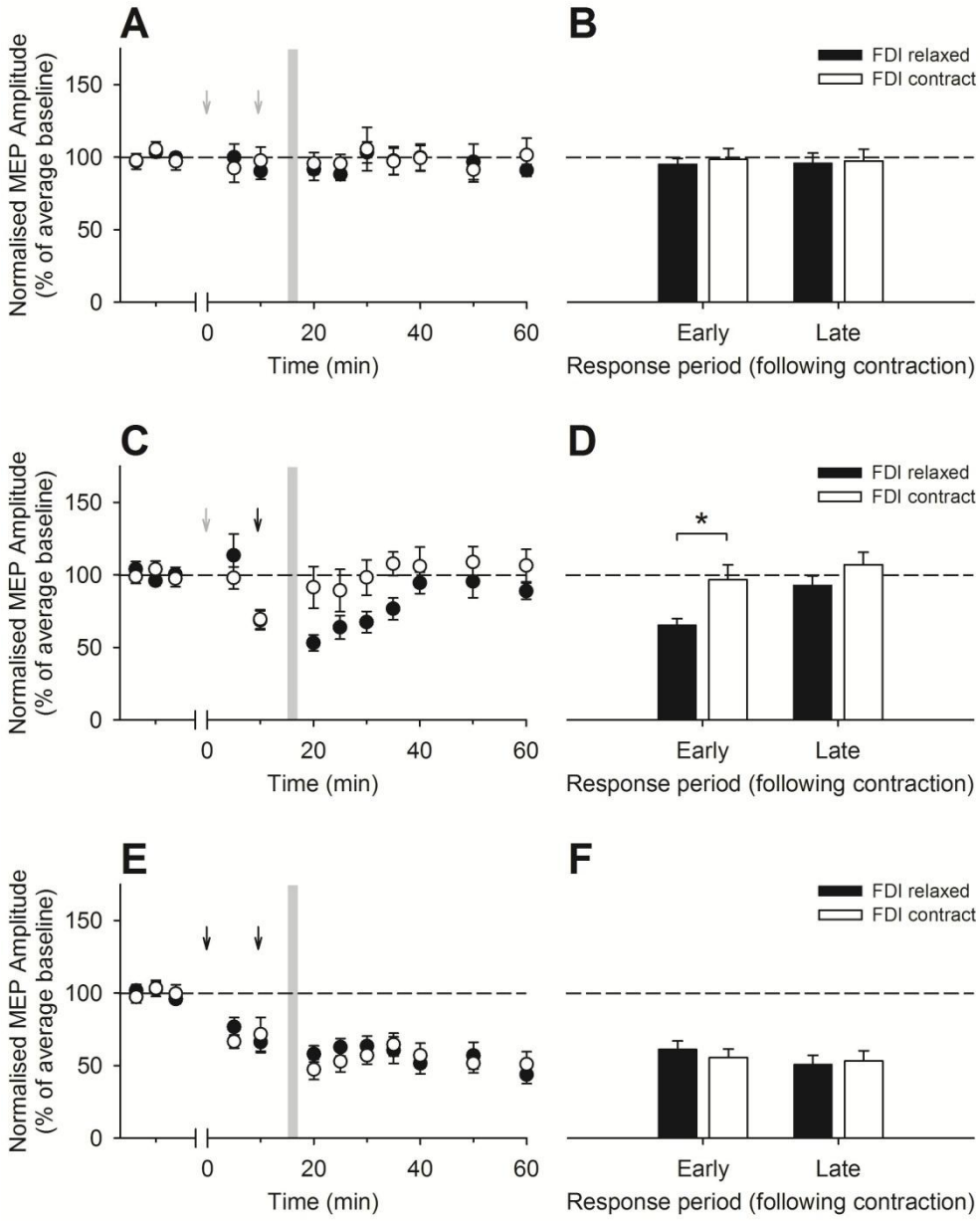

Figure 4-2: *The influence of a sub-maximal voluntary contraction (grey column) on MEP responses (expressed as a percentage of the average baseline) to no cTBS (Experiment 1; A and B), single cTBS (Experiment 2; C and D), and paired cTBS (Experiment 3; E and F). Grey arrows indicate delivery of sham cTBS, whilst the black arrows indicate delivery of real cTBS. (A) There was no change in MEP amplitudes from baseline levels following paired sham protocols for both the FDI relaxed (filled circles) and FDI contract (open circles) conditions. (B) Likewise, post-contraction MEPs pooled into early and late response periods did not differ between the conditions. (C), MEP amplitudes were suppressed following single cTBS for the FDI relaxed*

*condition; however, no MEP suppression was observed following the contraction for the FDI contract condition. (D), A significant de-depression of MEP suppression was observed for MEPs pooled into the early response period following the contraction. (E) There was pronounced suppression of MEP amplitudes from baseline levels following paired cTBS protocols for both the FDI relaxed and FDI contract conditions. (F) Post-contraction MEPs pooled into early and late response periods did not differ between the conditions. *P ≤ 0.05 when FDI relaxed and FDI contract conditions are compared. Data are shown as group means ± SEM.*

The average intensity of the voluntary contraction used for the FDI contract condition of Experiment 1 was 10.1 ± 3.6% of MVC. Analysis of the post-intervention MEP recording blocks that followed the contraction showed no difference between the two experimental conditions (CONDITION: $F_{1,9} = 0.08$, $P = 0.78$; CONDITION x BLOCK: $F_{5,45} = 0.29$, $P = 0.92$). There were also no differences between the FDI relaxed and FDI contract conditions when MEP data were pooled into early (FDI relaxed: 95.1 ± 12.9% of average baseline, FDI contract: 98.4 ± 24.1% of average baseline; paired $t_9 = -0.42$, $P = 0.69$) and late (FDI relaxed: 95.8 ± 22.3% of average baseline, FDI contract: 97.3 ± 25.7% of average baseline; paired $t_9 = -0.13$, $P = 0.90$) response periods following the contraction (Figure 4-2B).

4.4.2. Experiment 2 – single cTBS

Average baseline MEP amplitudes for the FDI relaxed and FDI contract conditions for Experiment 2 were 0.91 ± 0.20 mV and 0.90 ± 0.14 mV respectively. There was no difference in baseline MEP amplitudes between conditions ($F_{1,9} = 0.03$, $P = 0.88$), nor was there a difference between baseline recording blocks ($F_{2,18} = 0.09$, $P = 0.92$). There

was a significant suppression of post-intervention MEP amplitudes for the FDI relaxed condition ($F_{7,65}$ = 5.72, P < 0.001), with suppression of MEPs at P2, P3, P4, P5, and P6 compared to baseline ($P \leq 0.02$ for all). There was no change in MEP amplitudes from baseline values during the post-intervention recording period for the FDI contract condition ($F_{3,31}$ = 1.51, P = 0.23) (Figure 4-2C).

The average intensity of the voluntary contraction used for the FDI contract condition of Experiment 2 was 9.5 ± 3.4% of MVC. Analysis of the post-intervention MEP recording blocks that followed the contraction revealed significant differences between conditions ($F_{1,9}$ = 5.22, P = 0.05) and also between recording blocks ($F_{6,54}$ = 3.96, P = 0.002), although there was no interaction between the two factors ($F_{6,54}$ = 0.79, P = 0.58). *Post hoc* comparisons of pooled MEP data revealed a significant difference between conditions for the early response period following the contraction (paired t_9 = -2.40, P = 0.04), with suppression observed for the FDI relaxed condition (65.2 ± 14.3% of average baseline) but not FDI contract (96.6 ± 32.7% of average baseline). There was no difference between the FDI relaxed and FDI contract conditions for the late response period (FDI relaxed: 92.8 ± 19.9% of average baseline, FDI contract: 107.0 ± 27.1% of average baseline; paired t_9 = -1.32, P = 0.22) (Figure 4-2D).

4.4.3. Experiment 3 – paired cTBS

Average baseline MEP amplitudes for the FDI relaxed and FDI contract conditions for Experiment 3 were 0.88 ± 0.19 mV and 0.92 ± 0.15 mV respectively. There was no difference in baseline MEP amplitudes between conditions ($F_{1,9}$ = 0.73, P = 0.42), nor was there a difference between baseline recording blocks ($F_{2,18}$ = 0.49, P = 0.62). There

was a significant suppression of post-intervention MEP amplitudes for both the FDI relaxed ($F_{9,81}$ = 9.00, P < 0.001) and the FDI contract ($F_{5,45}$ = 7.24, P < 0.001) conditions, with suppression of MEPs recorded at all post-intervention time points compared to baseline for both conditions ($P \leq 0.04$ for all) (Figure 4-2E).

The average intensity of the voluntary contraction used for the FDI contract condition of Experiment 3 was 10.5 ± 2.5% of MVC. Analysis of the post-intervention MEP recording blocks that followed the contraction revealed significant differences between recording blocks ($F_{6,54}$ = 3.21, P = 0.009) but not between conditions ($F_{1,9}$ = 0.22, P = 0.65), and there was no interaction between CONDITION and BLOCK ($F_{6,54}$ = 1.28, P = 0.29). No differences were observed between the FDI relaxed and FDI contract conditions when MEP data were pooled into early (FDI relaxed: 61.2 ± 18.4% of average baseline, FDI contract: 55.4 ± 19.2% of average baseline; paired t_9 = 1.12, P = 0.29) and late (FDI relaxed: 50.7 ± 20.2% of average baseline, FDI contract: 53.1 ± 22.6% of average baseline; paired t_9 = -0.45, P = 0.66) response periods following the contraction (Figure 4-2F).

4.4.4. Factors affecting MEP de-depression following contraction

Correlation analyses on single cTBS data for Experiment 2 indicated a trend of a positive linear relationship between normalised MEP amplitudes recorded during the FDI contract session at P2 (i.e. just prior to the contraction) and the level of MEP de-depression observed during the early response period following the contraction, although this did not reach significance (r = 0.53, P = 0.12) (Figure 4-3A). There was a significant positive linear relationship between the intensity of contraction (expressed as

a percentage of MVC) and the level of MEP de-depression (r = 0.90, P = 0.001, excluding one outlier whose contraction intensity was more than two standard deviations above the group mean) (Figure 4-3B).

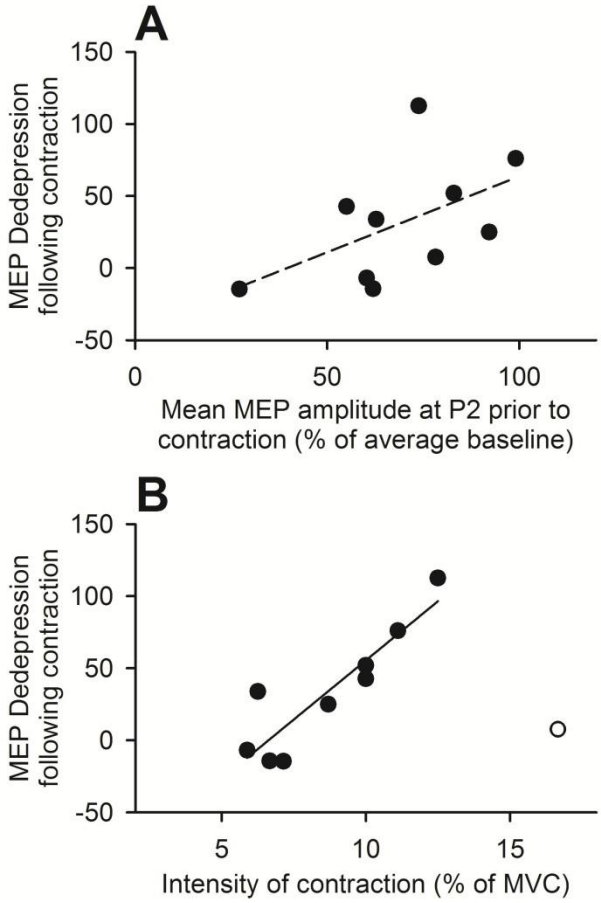

Figure 4-3: *Factors affecting the de-depression of single cTBS-induced MEP suppression by a voluntary contraction (Experiment 2). Each data point represents results from an individual subject. MEP de-depression was calculated by subtracting the pooled MEP data (expressed as a percentage of the average baseline) recorded during the early response period following the contraction for the FDI relaxed condition from that recorded during the same period for the FDI contract condition (i.e. values > 0 indicate MEP de-depression) (A) There tended to be less MEP de-depression in subjects that responded to single cTBS with greater MEP suppression (shown as a smaller mean MEP amplitude recorded prior to the contraction at P2, expressed as a percentage of the average baseline). However, this relationship did not reach statistical*

significance (r = 0.53, P = 0.12). (B) Subjects that performed a higher intensity contraction (expressed as a percentage of their MVC) showed a greater MEP de-depression [r = 0.90, P = 0.001, excluding an outlier (open circle)].

4.4.5. Experiment 4 – single cTBS (control)

Average baseline MEP amplitudes for the FDI relaxed and FDI contract conditions for Experiment 4 were 0.92 ± 0.21 mV and 0.86 ± 0.22 mV respectively. There was no difference in baseline MEP amplitudes between conditions ($F_{1,7}$ = 0.90, P = 0.38), nor was there a difference between baseline recording blocks ($F_{2,14}$ = 0.28, P = 0.76). There was a significant suppression of post-intervention MEP amplitudes for the FDI relaxed condition ($F_{7,52}$ = 3.12, P = 0.007), with suppression of MEPs recorded at P1, P2, P3, P4, and P5 compared to baseline ($P \leq 0.02$ for all). A significant main effect of BLOCK was also observed for the FDI contract condition ($F_{9,63}$ = 3.79, P = 0.001), and this was due to suppression of MEPs compared to baseline at P1 (P = 0.006). There was a trend towards MEP facilitation (compared to baseline levels) at recording blocks P4, P5, and P8, although this did not reach statistical significance ($P \geq 0.08$ for all) (Figure 4-4A).

The average intensity of the voluntary contraction used for the FDI contract condition of Experiment 4 was 9.3 ± 2.1% of MVC. Analysis of the post-intervention MEP recording blocks that followed the contraction revealed significant differences between conditions ($F_{1,7}$ = 13.4, P = 0.008) and recording blocks ($F_{6,42}$ = 2.76, P = 0.02), as well as a significant interaction between the two factors ($F_{6,42}$ = 3.17, P = 0.01). *Post hoc* comparisons of pooled MEP data revealed a significant difference between each of the conditions for the early response period following the contraction (paired t_7 = -4.67, P =

0.002), with suppression observed for the FDI relaxed condition (61.7 ± 28.3% of average baseline) but not FDI contract (113.2 ± 32.5% of average baseline). There appeared to be a slight difference in MEP amplitudes between the FDI relaxed and FDI contract conditions for the late response period, although this did not reach statistical significance (FDI relaxed: 85.3 ± 33.2% of average baseline, FDI contract: 123.3 ± 48.5% of average baseline; paired t_7 = -2.25, P = 0.06) (Figure 4-4B).

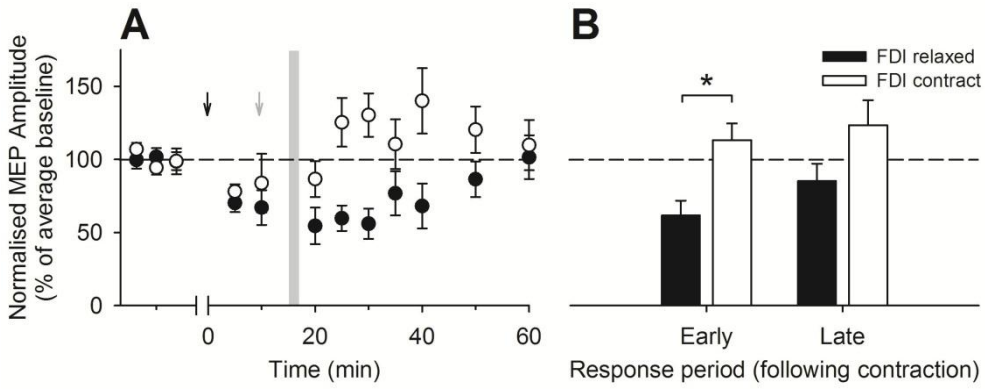

Figure 4-4: *The effect of the time-interval separating single cTBS and the voluntary contraction on MEP de-depression (Experiment 4). The grey arrow indicates delivery of sham cTBS, whilst the black arrow indicates delivery of real cTBS. (A) As with Experiment 2, MEP amplitudes were suppressed following single cTBS for the FDI relaxed condition (filled circles). Despite using a 15 min (instead of 5 min) interval between cTBS and the contraction, there was still MEP reversal back to baseline following the contraction for the FDI contract condition (open circles). (B) Analysis of pooled MEP data showed a significant de-depression of MEP suppression for MEP data pooled into the early response period following the contraction. *P ≤ 0.05 when FDI relaxed and FDI contract conditions are compared. Data are shown as group means ± SEM.*

4.4.6. Experiment 5 – de-depression by iTBS150

Average baseline MEP amplitudes for the single and paired cTBS conditions for Experiment 5 were 0.88 ± 0.27 mV and 0.86 ± 0.30 mV respectively. There was no difference in baseline MEP amplitudes between conditions ($F_{1,9}$ = 0.07, P = 0.80), nor was there a difference between baseline recording blocks ($F_{2,18}$ = 1.79, P = 0.20).

MEP amplitudes did not differ between recording blocks for the single cTBS-iTBS150 condition ($F_{9,81}$ = 1.79, P = 0.08). Analysis of the post-intervention MEP recording blocks that followed iTBS150 revealed a significant difference compared to the FDI relaxed condition of Experiment 2 (i.e. single cTBS without subsequent voluntary contraction or iTBS150) ($F_{1,18}$ = 7.31, P = 0.02), with a trend for an interaction between CONDITION and BLOCK ($F_{6,108}$ = 2.11, P = 0.06). This was due to less MEP suppression compared to the FDI relaxed condition of Experiment 2 during the early response period following iTBS150 application (independent t_{13} = -3.59, P = 0.003) (Figure 4-5A and B).

There was a significant suppression of post-intervention MEP amplitudes for paired cTBS-iTBS150 ($F_{9,81}$ = 4.56, P < 0.001), with suppression of MEPs recorded at all post-intervention time points compared to baseline ($P \leq 0.02$ for all). Analysis of the post-intervention MEP recording blocks that followed iTBS150 revealed no difference compared to the FDI relaxed condition of Experiment 3 ($F_{1,18}$ = 0.001, P = 0.98), nor was there an interaction between CONDITION and BLOCK ($F_{6,108}$ = 0.65, P = 0.69). Likewise, no differences were observed when MEP data were pooled into early and late

response periods following iTBS150 application (independent t_{18} = 0.18 and -0.35 respectively, $P > 0.05$ for both) (Figure 4-5C and D).

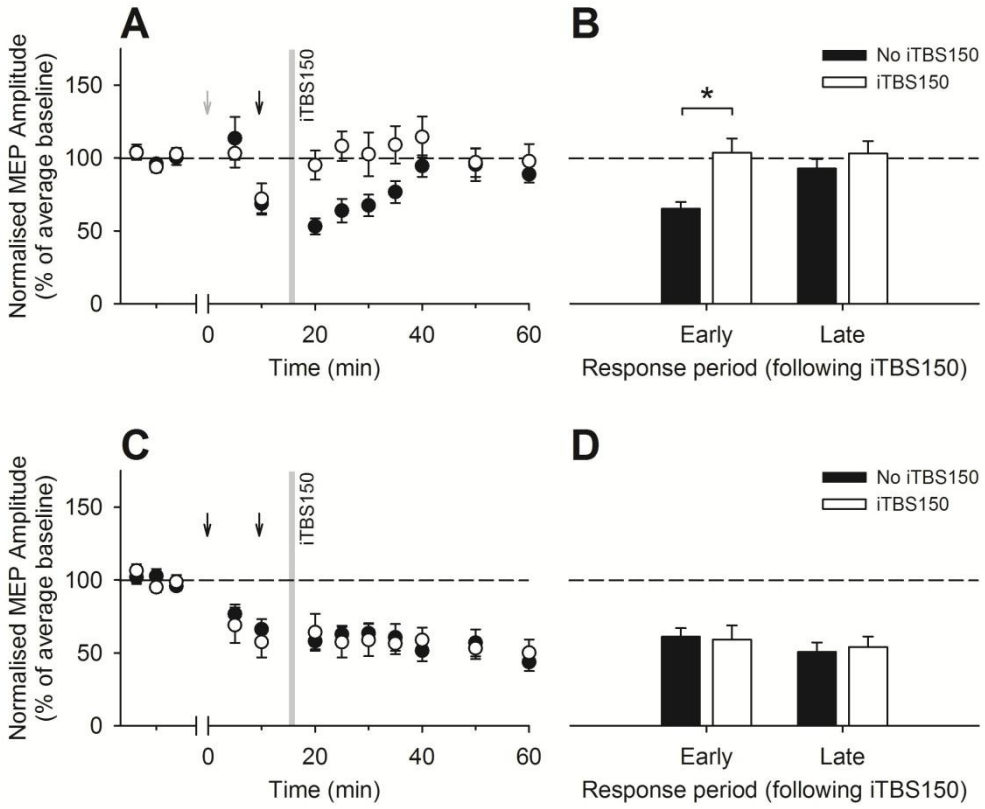

Figure 4-5: *The influence of iTBS150 (grey column) on MEP responses (expressed as a percentage of the average baseline) to single cTBS (A and B) and paired cTBS (C and D) (Experiment 5). The grey arrow indicates delivery of sham cTBS, whilst the black arrows indicate delivery of real cTBS. (A) Compared to the MEP suppression observed for the FDI relaxed condition of Experiment 2 (i.e. no iTBS; filled circles), there was no change in MEP amplitudes from baseline levels following single cTBS for the iTBS150 condition (open circles). (B) Analysis of pooled MEP data showed that this was due to reduced MEP suppression during the early response period following iTBS150 application. (C) As with the FDI relaxed condition of Experiment 3 (i.e. no iTBS; filled circles), there was a pronounced suppression of MEP amplitudes from baseline levels following paired cTBS for the iTBS150 condition (open circles). (D) Post-iTBS150 MEPs pooled into early and late response periods did not differ between the conditions.*

**P ≤ 0.05 when the iTBS150 condition is compared to the FDI relaxed condition of Experiment 2 (i.e. no iTBS). Data are shown as group means ± SEM.*

4.5. DISCUSSION

The present study confirms that behavioural engagement of the primary motor cortex by voluntary contraction abolishes LTD-like MEP suppression induced by a single cTBS protocol. Also, we have shown for the first time that the spaced application of repeated cTBS protocols induces MEP suppression that is resistant to disruption by voluntary contraction. We show that this MEP suppression is also resistant to reversal when an experimental de-depression protocol is used instead of a voluntary contraction.

The MEP suppression induced by single cTBS in this study was comparable to that observed previously (Huang et al., 2005), and is likely due to LTD-like changes at excitatory synaptic connections within the primary motor cortex (Di Lazzaro et al., 2005; Huang et al., 2007). A voluntary contraction applied following cTBS abolished this MEP suppression, and the extent to which MEPs were reversed was greater in subjects that contracted at higher intensities relative to their maximal effort. The reversal of LTD-like effects by behavioural engagement of the motor cortical regions is consistent with findings from Huang et al. (2008), which showed that a sub-maximal voluntary contraction applied immediately following cTBS reversed MEP suppression. Likewise, Thirugnanasambandam et al. (2011) found that a mild voluntary contraction reversed both MEP facilitation and suppression induced by tDCS (transcranial direct current stimulation), a non-invasive brain stimulation protocol which, like TBS, can be used to produce LTP and LTD-like plasticity within the human primary motor cortex

(Nitsche et al., 2003). Similar reversals of LTP and LTD (referred to as depotentiation and de-depression, respectively) have been shown in animal models when normal physiological activity within the stimulated network follows an induction protocol (Xu et al., 1998; Zhou et al., 2003). Therefore, the reversal of cTBS-induced MEP suppression by behavioural engagement of the hand motor regions in the present study may reflect a de-depression-like event within the human primary motor cortex.

An alternate explanation for the reversal of MEP suppression following the voluntary contraction in Experiment 2 may have been due to a facilitatory effect of the voluntary contraction itself. Whilst the voluntary contraction applied in the absence of cTBS in Experiment 1 had no lasting effects on MEP amplitude, it is possible that the reduced excitability of the primary motor cortex following cTBS application in Experiment 2 may have initiated homeostatic regulatory mechanisms, thus lowering the threshold for induction of LTP-like effects in accordance with the Bienenstock-Cooper-Munro (BCM) theory (Bienenstock et al., 1982). Such homeostatic regulation of neuroplastic change has been shown to occur within the human primary motor cortex (Siebner et al., 2004; Ziemann et al., 2004; Stefan et al., 2006; Müller et al., 2007), and may have promoted facilitation of MEPs following voluntary contraction. To investigate this further, correlation analyses were performed to determine whether the level of MEP suppression following cTBS and prior to the contraction in Experiment 2 was related to the extent to which MEPs were reversed following the contraction. Although the relationship did not reach statistical significance, there was a tendency for a smaller reversal of MEPs in subjects who responded to cTBS with greater MEP suppression. Thus, we consider it unlikely that the reversal of MEPs following a contraction in

Experiment 2 was due to homeostatic processes initiated by a cTBS-induced suppression of motor cortical excitability.

Whilst MEP suppression induced by a single cTBS protocol was reversed by a voluntary contraction, the MEP suppression induced by paired cTBS remained stable. One possible factor which may have contributed to this finding was the difference in the time-interval separating cTBS application and the voluntary contraction for the single and paired cTBS conditions. For single cTBS, subjects performed the contraction at 5 min following stimulation. However, for the paired cTBS condition the time-interval between the first cTBS protocol and the voluntary contraction was 15 min. Experiments in both hippocampal slice preparations and freely-moving animals have shown a time-dependency of reversal effects, with less reversal observed when disruptive stimuli were applied after a certain time period (usually tens of minutes) following plasticity induction (Fujii et al., 1991; Xu et al., 1998; Huang et al., 1999; Chen et al., 2001). Therefore, subjects for Experiment 4 received single cTBS with the order of the sham and real cTBS protocols reversed such that the interval between the real cTBS and the contraction was 15 min. Despite the interval being the same as that used for the paired cTBS condition of Experiment 3, the voluntary contraction was still able to reverse single cTBS-induced MEP suppression. Therefore, the stability of MEP suppression observed in Experiment 3 was likely due to the repeated application of cTBS and not the timing of the contraction.

The increased stability of paired cTBS-induced MEP suppression in the present study extends our previous finding that paired cTBS prolongs the duration of MEP suppression when applied to the human primary motor cortex (Goldsworthy et al.,

2012). Similarly, repeated cTBS has been found to produce robust after-effects when applied in a single session to the human frontal eye field (Nyffeler et al., 2006a) and parietal (Nyffeler et al., 2009; Cazzoli et al., 2012) cortical regions. In contrast to these results, several studies have shown homeostatic interactions between paired cTBS protocols when applied to the human primary motor cortex (Gamboa et al., 2011; Murakami et al., 2012; Mastroeni et al., 2013). However, these studies have required subjects to sustain a voluntary contraction of the targeted hand muscle prior to paired cTBS application to set the stimulation intensity, and this may have influenced the way in which the two cTBS protocols interacted to produce changes in MEP amplitudes (Goldsworthy et al., 2012). Additionally, we employed a slightly different interval between cTBS protocols compared to these previous studies. Studies in both animals (Zhou et al., 2003) and humans (Gamboa et al., 2011) have shown that the length of time separating successive stimulation protocols is important in determining the neuroplastic response to repeated stimulation. Therefore, this may have accounted for some of the differences in the results of the present study.

The prolonged duration of cTBS-induced effects with repeated applications bears resemblance to data in animal studies showing long-lasting synaptic plasticity following repeated stimulation protocols (Bliss and Gardner-Medwin, 1973; Abraham et al., 1993; Huang and Kandel, 1994; Trepel and Racine, 1998; Abraham et al., 2002). Furthermore, Zhou et al. (2003) showed that the repeated application of simulation protocols to the developing *Xenopus* visual system in a spaced manner produced lasting LTP at retinotectal synapses that was resistant to depotentiation by spontaneous activation of the post-synaptic tectal neuron. A similar resistance to depotentiation has been achieved in the rodent hippocampus using repeated stimulation protocols (Woo and Nguyen,

2003), and was likely due to consolidation of LTP through increases in *de novo* protein synthesis and gene transcription (Krug et al., 1984; Huang and Kandel, 1994; Nguyen et al., 1994; Woo and Nguyen, 2003). Thus, a similar consolidation of LTD-like effects following repeated cTBS applications may underlie the resistance to de-depression by voluntary contraction observed in the present study.

A feature of consolidated synaptic plasticity in animal models is that it is stable not only in the presence of behaviourally-relevant physiological activity but also when the network is stimulated artificially shortly after plasticity induction (Woo and Nguyen, 2003). The stimulation protocols used to reverse plasticity are typically weaker than those used for plasticity induction and do not produce lasting effects when applied on their own (Zhou and Poo, 2004). A recent study has shown that a shortened iTBS protocol (i.e. iTBS150), which, on its own, produced no lasting effects on motor cortical excitability, may be used as an artificial de-depression protocol for reversing cTBS-induced MEP suppression (Huang et al., 2010). A novel finding of the present study was that whilst single cTBS-induced MEP suppression was reversed by iTBS150, the MEP suppression induced by paired cTBS remained stable. We suggest that this provides an additional line of evidence linking the motor cortical excitability changes induced by TBS protocols with the LTP and LTD observed in animal models.

The instability of rTMS-induced neuroplasticity in the face of normal physiological activity impacts greatly on the therapeutic potential of rTMS protocols. The results of the present study have shown that the repeated application of cTBS may be an effective approach for consolidating MEP suppression, making it resistant to reversal by behavioural engagement of the motor regions. We also show that this MEP suppression

is stable in the presence of a stimulation protocol designed to reverse cTBS-induced neuroplasticity. These findings may have significant implications for the clinical application of rTMS.

5. A COMPARISON OF TWO DIFFERENT CONTINUOUS THETA BURST STIMULATION PARADIGMS APPLIED TO THE HUMAN PRIMARY MOTOR CORTEX

5.1. ABSTRACT

The application of repetitive transcranial magnetic stimulation (rTMS) in bursts at theta frequencies (TBS) may produce lasting neuroplastic changes in the human cortex. However, there exists high variability in subjects' responses, possibly due to non-optimal stimulation characteristics. Here we compare the efficacy of two variations of continuous TBS (cTBS) for producing neuroplastic change in the human primary motor cortex. The two cTBS paradigms were: (1) standard cTBS ($cTBS_{std}$) (three stimuli at 50 Hz, repeated at 5 Hz), and (2) modified cTBS ($cTBS_{mod}$) (three stimuli at 30 Hz, repeated at 6 Hz with intensity). Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle before, as well as at 0, 5, 10, 20 and 30 min following each paradigm. Both $cTBS_{std}$ ($P = 0.05$) and $cTBS_{mod}$ ($P < 0.0001$) induced a suppression of MEP amplitudes. However, MEP suppression following $cTBS_{mod}$ was greater ($ANOVA_{RM}$; $P = 0.02$). Experiments using magnetic brainstem stimulation provided evidence that $cTBS_{mod}$ induced MEP suppression through cortical mechanisms. These results suggest that the neuroplastic response of the human primary motor cortex to cTBS is highly dependent on the stimulation parameters employed. These findings may have significant implications for the clinical application of cTBS paradigms.

5.2. INTRODUCTION

In recent years, the therapeutic promise of non-invasive, neuroplasticity-inducing brain stimulation techniques, in treating a range of neurological disorders, has led to a major research focus on identifying the most effective stimulation protocols. Of the different neuroplasticity-inducing paradigms investigated, perhaps the most widely studied involves the application of trains of repetitive magnetic stimuli, so-called repetitive transcranial magnetic stimulation (rTMS). One rTMS paradigm in particular has shown much promise, and involves applying short bursts of high frequency magnetic stimuli at the 4-7 Hz theta frequency band (theta burst stimulation; TBS) (Huang et al., 2005).

When applied to the hand representations of the human primary motor cortex, TBS has been shown to produce changes in the amplitude of motor evoked potentials (MEPs) measured from the hand muscles which, depending on the pattern of its application, may be facilitatory (intermittent TBS; iTBS)) or suppressive (continuous TBS; cTBS) (Huang et al., 2005). There is evidence that the MEP amplitude changes following iTBS and cTBS are cortical in origin and N-methyl-D-aspartate (NMDA) receptor dependent (Di Lazzaro et al., 2005; Huang et al., 2007; Di Lazzaro et al., 2008b), and thus are thought to occur, respectively, via increases and decreases in the strength of synaptic connections within the primary motor cortex by way of mechanisms similar to the long-term potentiation (LTP) and depression (LTD) observed in animal models.

In addition to their shorter stimulation times and sub-threshold stimulation intensities, the capacity of TBS paradigms to evoke lasting LTP and LTD-like changes in human cortical excitability has seen them become an appealing option for inducing

functionally-beneficial effects in a variety of neurological and psychiatric disorders (Di Lazzaro et al., 2006a; Talelli et al., 2007; Cardenas-Morales et al., 2010). However, as with similar non-invasive brain stimulation techniques, there still exists considerable variability in the response to conventional TBS paradigms, both within and between subjects (see Ridding and Ziemann, 2010, for review). It is possible that this variability may be, at least in part, the result of non-optimal stimulation parameters.

In the present study we contrasted the response of subjects to standard cTBS ($cTBS_{std}$) (Huang et al., 2005) and a slightly modified variant (modified cTBS; $cTBS_{mod}$). Although similar, these paradigms are characterised by small differences in frequency and intensity. The $cTBS_{mod}$ paradigm has been reported to produce lasting after-effects on behaviour when applied to the frontal eye field region of the human oculomotor cortex (Nyffeler et al., 2006b), and has also proved effective for inducing behavioural changes on visual exploration when applied to the posterior parietal cortex of both healthy subjects (Nyffeler et al., 2008; Cazzoli et al., 2009) and stroke patients suffering from visual neglect (Nyffeler et al., 2009). The strong, behaviourally-relevant, impact of the $cTBS_{mod}$ paradigm raises the possibility that it may be an effective protocol for inducing neuroplastic change. Therefore, here we compare its effects with those of the $cTBS_{std}$ paradigm on neurophysiological measures of human primary motor cortical plasticity.

5.3. MATERIALS AND METHODS

5.3.1. Subjects

A total of 16 healthy subjects (seven males) aged 18-47 (mean age, 24.8 ± 7.7 years) participated in this study, all of whom were blinded to the purpose of the study. 12 subjects participated in Experiment 1 (six males; mean age, 23.7 ± 8.1 years), and two subjects participated in Experiment 2 (one male; mean age, 37.5 ± 13.4 years), one of whom had also participated in Experiment 1. Experiment 3 was conducted on five subjects (three males; mean age, 27.0 ± 9.9), two of whom had also participated in Experiment 1. All subjects were screened for any contraindications to TMS (Rossi et al., 2009) and gave their informed written consent prior to participation. This study was approved by the University of Adelaide Human Research Ethics Committee and performed in accordance with the 2008 Declaration of Helsinki.

5.3.2. Stimulation and recording

Subjects were seated in a comfortable chair for all procedures. Single-pulse TMS was used to evoke MEPs from the right first dorsal interosseous (FDI) muscle. Electromyographic recordings were made from the right FDI using two Ag-AgCl surface electrodes arranged in a belly-tendon montage. Signals were sampled at a rate of 5 kHz, amplified (x 1000) and filtered (20-1000 Hz) (Cambridge Electrical Design 1401, Cambridge, UK) before being stored on a computer for offline analysis.

Single-pulse TMS with monophasic waveform was applied using a Magstim 200 magnetic stimulator (Magstim, Whitland, Dyfed, UK) connected to a figure-of-eight magnetic coil (external wing diameter, 90 mm). The coil was held tangentially to the skull over the left primary motor cortex, with the handle pointing 45° posterolaterally. The optimal scalp site for evoking MEPs in the right FDI was identified and marked using a water-soluble felt marker, and the intensity of stimulation was adjusted to evoke baseline MEPs of approximately 1 mV amplitude (measured peak-to-peak).

5.3.3. Magnetic brainstem stimulation (BST)

BST was applied using a Magstim 200 magnetic stimulator (Magstim, Whitland, Dyfed, UK) connected to a double-cone coil (wing diameter, 110 mm). The coil was held over the inion, with the current in the coil directed downward (Ugawa et al., 1994). The site of stimulation was marked using a water-soluble felt marker, and the intensity of stimulation was adjusted to evoke an MEP of approximately 0.5-1 mV amplitude (measured peak-to-peak) at baseline whilst the subjects performed a mild isometric contraction of their right FDI muscle. The force of contraction was maintained at a constant level (approximately 5% of their maximal voluntary contraction) by providing subjects visual feedback displayed on an oscilloscope.

5.3.4. TBS paradigms

cTBS was applied with biphasic waveform using a Magstim Super Rapid stimulator (Magstim, Whitland, Dyfed, UK). Two cTBS paradigms with varying pulse configurations were employed in this study (Figure 5-1). $cTBS_{std}$ consisted of a total of

600 stimuli applied in bursts of 3 stimuli at 20 ms intervals (50 Hz), with bursts repeated at 200 ms intervals (5 Hz) (Huang et al., 2005). In the $cTBS_{mod}$ paradigm, bursts consisted of 3 stimuli applied at intervals of 33.3 ms (30 Hz), with bursts repeated at 167 ms intervals (6 Hz) (Nyffeler et al., 2006b). As with the $cTBS_{std}$ paradigm, $cTBS_{mod}$ consisted of a total of 600 stimuli.

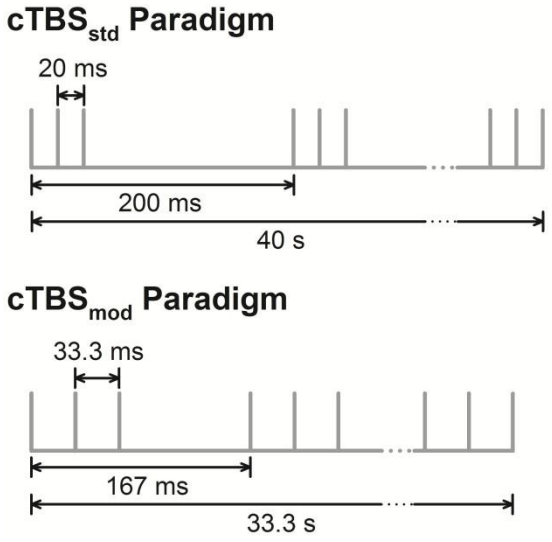

Figure 5-1: *The pulse configurations used for each of the cTBS paradigms.*

The intensity of cTBS application was set relative to active motor threshold (AMT) or resting motor threshold (RMT) (see Section 5.3.5), measured using a biphasic pulse waveform with the rTMS coil. AMT was defined as the minimum stimulus intensity sufficient to evoke MEPs with amplitudes greater than 200μV in at least five of 10 consecutive trials whilst the subject sustained a sub-maximal isometric contraction (20% of their maximum voluntary effort) of their right FDI muscle. Visual feedback of the contraction intensity was displayed to subjects on an oscilloscope. RMT was defined

as the minimum stimulus intensity sufficient to evoke MEPs in the right FDI at rest with peak-to-peak amplitudes of at least 50μV in five of 10 consecutive trials.

5.3.5. Experimental design

The present study consisted of three experiments, all of which were performed in the afternoon to minimise possible time-of-day effects influencing the results (Sale et al., 2007). For the first experiment (i.e. Experiment 1), subjects attended two sessions no less than four days apart, receiving the $cTBS_{std}$ paradigm applied at 80% of AMT (Huang et al., 2005) in one session and the $cTBS_{mod}$ paradigm applied at 80% of RMT (Nyffeler et al., 2006b) in the other. The order in which they received each paradigm was randomised between subjects. In both sessions, MEPs were recorded in blocks of fifteen trials before (baseline measure), as well as at 0, 5, 10, 20, and 30 min following cTBS application.

Experiment 2 investigated the level at which $cTBS_{mod}$ induced effects on corticospinal pathway excitability. For this experiment, two subjects attended for a single session where they received the $cTBS_{mod}$ paradigm applied at 80% of RMT. In addition to recording MEPs evoked by TMS of the left primary motor cortex hand representations, BST-evoked MEPs were also recorded before and at two time points (5 min and 15 min) following $cTBS_{mod}$ application. TMS-evoked MEPs were recorded whilst the subjects maintained a relaxed right FDI muscle (resting condition) and also whilst subjects performed a mild isometric contraction (approximately 5% of their maximal voluntary contraction) of their right FDI muscle (active condition) matched to that used

during BST. TMS-evoked MEPs were recorded in blocks of fifteen trials, and BST-evoked MEPs were recorded in blocks of ten trials.

Experiment 3 was performed to contrast the effects of $cTBS_{std}$ and $cTBS_{mod}$ when the stimulus intensities were matched. As with Experiment 1, subjects for Experiment 3 were tested twice with no less than four days between sessions, receiving the $cTBS_{std}$ paradigm in one session and the $cTBS_{mod}$ paradigm in the other. However, the stimulation intensity for both paradigms was set at 80% of AMT. In both sessions, MEPs were recorded in blocks of fifteen trials before, as well as at 0, 5, 10, 20, and 30 min following cTBS application.

5.3.6. Data analyses

All statistical analyses were performed using PASW statistics version 17 (IBM SPSS, Armonk, NY, USA). MEP amplitudes were expressed as a percentage of the baseline MEP amplitude, and comparison between the two cTBS paradigms in Experiments 1 and 3 were performed using two-way repeated measures analysis of variance ($ANOVA_{RM}$) with PARADIGM (two levels; $cTBS_{std}$ and $cTBS_{mod}$) and TIME (five levels; 0, 5, 10, 20, and 30 min) as within-subject factors. Separate one-way $ANOVA_{RM}$ were then performed on raw data for both the $cTBS_{std}$ and $cTBS_{mod}$ paradigms, with TIME (six levels; baseline, 0, 5, 10, 20, and 30 min) as the within-subject factor. Contingent on a significant main effect of TIME, *post hoc* comparisons were performed using paired *t* tests to determine at which post-intervention time points MEP amplitudes were significantly different to baseline. Multiple comparisons were corrected for using the false discovery rate procedure (FDRP) (Curran-Everett, 2000).

To investigate whether the intensity of stimulation had an impact on the subjects' responses to the two cTBS paradigms in Experiment 1, Pearson's correlation coefficient analyses were performed between the ratio of the average responses to $cTBS_{std}$ and $cTBS_{mod}$ for each subject and the difference in stimulation intensities used for the two paradigms. Differences in stimulation intensity were calculated in two ways: (1) the ratio of the stimulation intensity used for the $cTBS_{std}$ paradigm (i.e. 80% of AMT) to that used for the $cTBS_{mod}$ paradigm (i.e. 80% of RMT) for each subject, and (2) the absolute difference in the stimulation intensity used for the two cTBS paradigms. Additionally, correlations were performed between each subject's average response and the absolute intensity of stimulation for both the $cTBS_{std}$ and $cTBS_{mod}$ paradigms. Average response variables were determined by calculating mean MEP amplitude (expressed as a percentage of baseline) across all post-intervention time points.

Data are presented as group means ± standard deviation unless otherwise indicated. For all analyses, tests were two-tailed and statistical significance was accepted at $P \leq 0.05$.

5.4. RESULTS

5.4.1. Experiment 1 – comparison of $cTBS_{std}$ and $cTBS_{mod}$ paradigms

There was no difference in the baseline MEP amplitudes prior to the application of the $cTBS_{std}$ and $cTBS_{mod}$ paradigms (1.03 ± 0.37 mV and 0.99 ± 0.24 mV, respectively; $P > 0.05$). However, when comparing post-intervention MEP amplitudes, $ANOVA_{RM}$ revealed a significant main effect of PARADIGM ($F_{1,11} = 5.55$, $P = 0.04$), as well as a

significant PARADIGM x TIME interaction ($F_{4,44}$ = 3.39, P = 0.02). This was due to greater suppression of MEP amplitudes following the $cTBS_{mod}$ paradigm. MEP amplitudes were suppressed after both $cTBS_{mod}$ (TIME: $F_{5,55}$ = 8.39, P < 0.0001) and $cTBS_{std}$ (TIME: $F_{5,55}$ = 2.45, P = 0.05), but *post hoc* testing revealed that MEP amplitude was suppressed for longer following $cTBS_{mod}$. MEPs were suppressed compared to baseline at 0 and 5 min following the $cTBS_{std}$ paradigm ($P \leq 0.01$ for both, corrected with FDRP) (Figures 5-2 and 5-3A), whereas following the $cTBS_{mod}$ paradigm, MEP amplitudes recorded at all post-intervention time points (0, 5, 10, 20, and 30 min) were suppressed compared to baseline ($P \leq 0.02$ for all, corrected with FDRP) (Figures 5-2 and 5-3B). No significant correlation was observed between subjects' average responses to the $cTBS_{std}$ and $cTBS_{mod}$ paradigms (r = 0.40, P = 0.20).

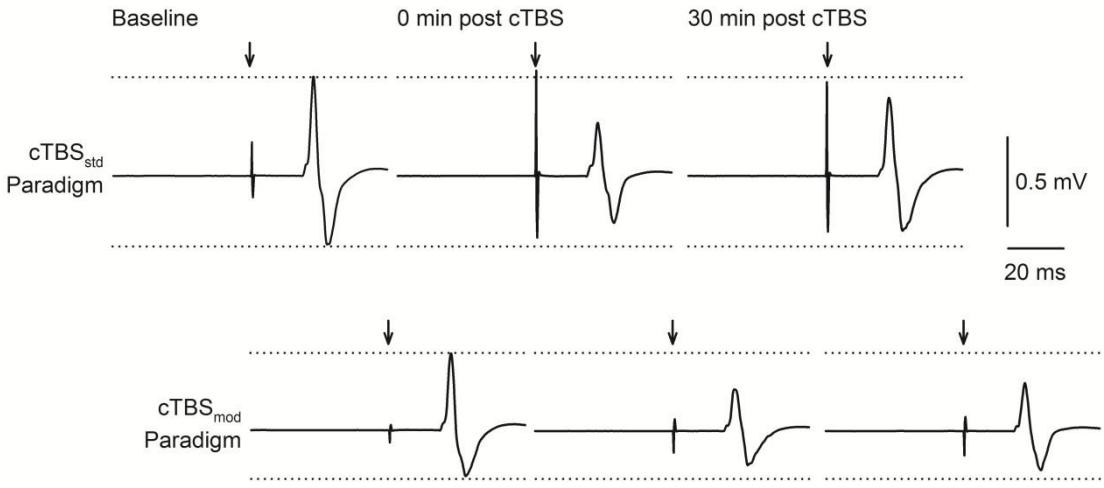

Figure 5-2: *Raw electromyographic data traces from one representative subject showing MEPs recorded at baseline and at 0 and 30 min after the $cTBS_{std}$ paradigm and $cTBS_{mod}$ paradigm. Both cTBS paradigms had an immediate suppressive effect on MEP amplitudes; however, this suppression persisted only for the $cTBS_{mod}$ paradigm. Traces are the average of 15 trials, and arrows indicate delivery of single-pulse TMS.*

Although an overall suppression of MEP amplitudes was observed following the $cTBS_{std}$ paradigm (Figure 5-3A), there was a large variability in the subjects' response profiles (Figure 5-4A). Of the 12 subjects tested, 8 showed an overall suppressive response when mean MEP amplitude was calculated across all post-intervention time points. Of the remaining subjects, two showed no overall change in MEP amplitudes and two showed an overall facilitatory response (Figure 5-4A). In contrast, the subjects' response profiles were far more consistent for the $cTBS_{mod}$ paradigm, with all 12 subjects exhibiting an overall suppression of MEP amplitudes (Figure 5-4B).

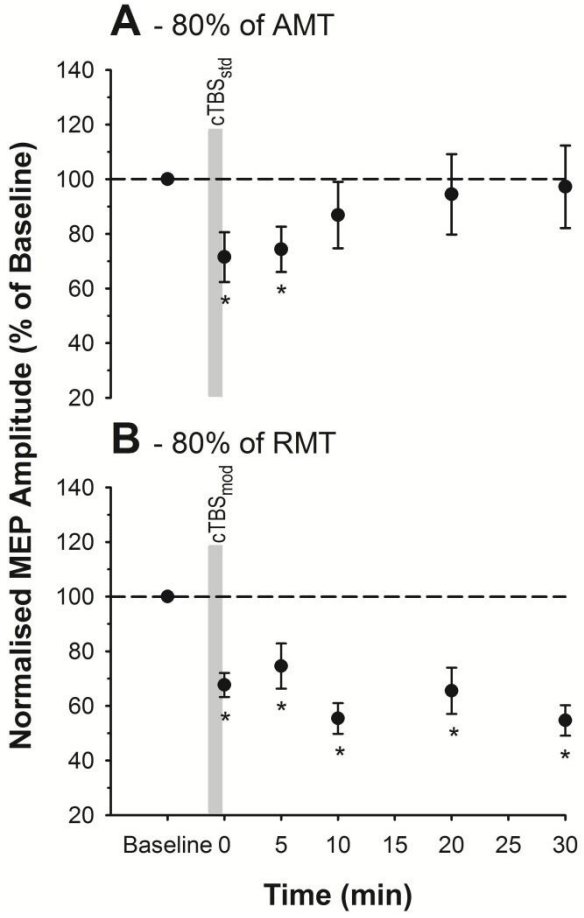

Figure 5-3: *The time course of change in MEP amplitudes (expressed as a percentage of baseline) following (A) the $cTBS_{std}$ paradigm and (B) the $cTBS_{mod}$ paradigm (Experiment 1). MEP amplitudes were suppressed at 0 and 5 min following $cTBS_{std}$,*

*whereas $cTBS_{mod}$ induced a suppression of MEPs at all time points. * denotes $P < 0.05$ when compared to baseline MEP amplitudes. Data points are group means ± SEM.*

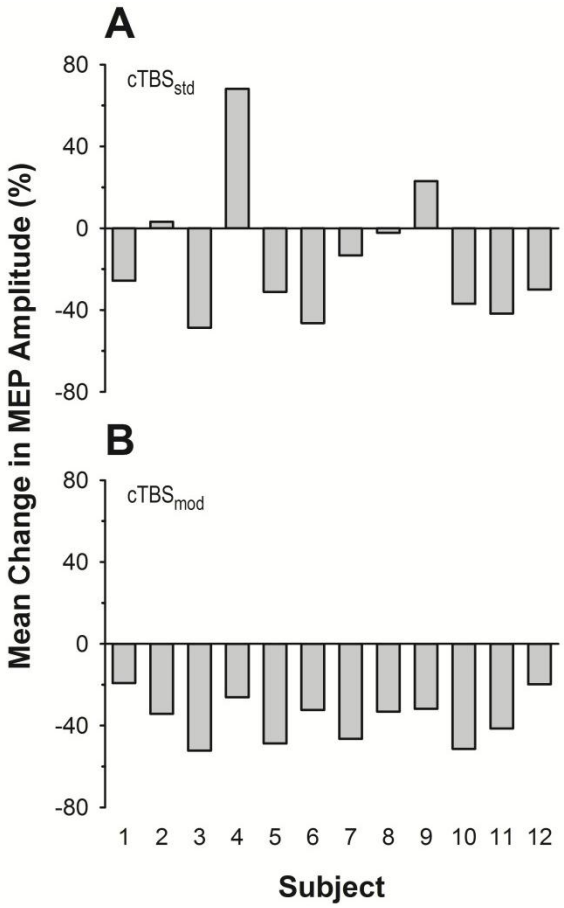

Figure 5-4: *Comparison of the inter-individual variability in the response profiles to (A) the $cTBS_{std}$ paradigm and (B) the $cTBS_{mod}$ paradigm for 12 subjects. Each individual subject's response bar is the mean of all post-intervention time points, expressed as the mean percentage change in MEP amplitude from baseline for that subject.*

Comparison of the stimulation intensities used for each of the cTBS paradigms revealed that a significantly higher absolute intensity of stimulation was used for the $cTBS_{mod}$ paradigm when compared with the $cTBS_{std}$ paradigm (45.6 ± 8.7% of maximum stimulator output and 30.4 ± 6.3% of maximum stimulator output, respectively; $P <$

0.0001). However, there was no correlation between the ratio of the average responses to $cTBS_{std}$ and $cTBS_{mod}$ for each subject and the difference in stimulation intensities used for the two paradigms, regardless of whether the ratio of the stimulation intensities (r = -0.23, P = 0.47) (Figure 5-5) or the absolute difference in intensity (r = 0.14, P = 0.66) was used in the analysis. Likewise, there was no relationship between each subject's average response and the absolute intensity of stimulation for both the $cTBS_{std}$ paradigm (r = -0.16, P = 0.62) and the $cTBS_{mod}$ paradigm (r = 0.07, P = 0.83).

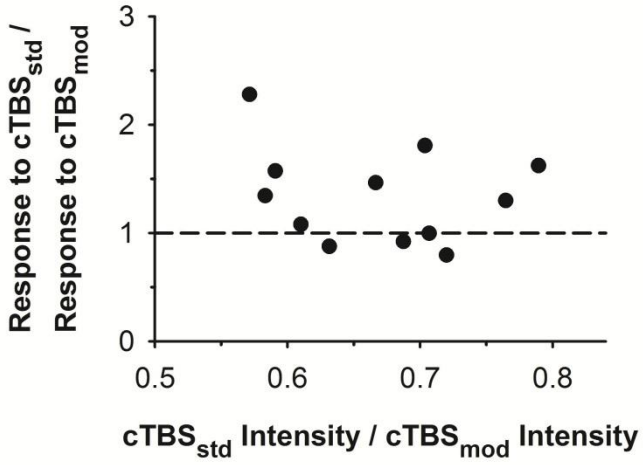

Figure 5-5: *Correlation between the ratio of the stimulation intensity used for the $cTBS_{std}$ paradigm to that used for the $cTBS_{mod}$ paradigm and the ratio of the average response to $cTBS_{std}$ and $cTBS_{mod}$ for each subject. Average response variables were determined by calculating mean MEP amplitude (expressed as a percentage of baseline) across all post-intervention time points. Values greater than one designate subjects that responded with greater MEP suppression to the $cTBS_{mod}$ paradigm, whereas values less than one designate subjects that responded with greater MEP suppression to the $cTBS_{std}$ paradigm. No significant correlation was observed (r = -0.23, P = 0.47).*

5.4.2. Experiment 2 – site of action for the $cTBS_{mod}$ paradigm

Figure 5-6 shows the effect of the $cTBS_{mod}$ paradigm on both TMS-evoked (resting and active) and BST-evoked MEPs for the two subjects studied in this experiment. There was a pronounced suppression in the amplitude of MEPs evoked by TMS of the left primary motor cortex in both the resting (Figure 5-6A) and active (Figure 5-6B) conditions following the $cTBS_{mod}$ paradigm in both subjects. However, MEPs evoked by BST were not suppressed in either subject following $cTBS_{mod}$ application (Figure 5-6C).

5.4.3. Experiment 3 – control for the intensity of stimulation

There was no difference in the baseline MEP amplitudes prior to the application of the $cTBS_{std}$ and $cTBS_{mod}$ paradigms in Experiment 3 (0.90 ± 0.29 mV and 0.81 ± 0.14 mV, respectively; $P > 0.05$), nor was there a difference in the absolute stimulation intensities used for each paradigm (31.8 ± 5.4% of maximum stimulator output and 33.0 ± 6.5% of maximum stimulator output, respectively; $P > 0.05$). As with Experiment 1, $ANOVA_{RM}$ revealed a significant main effect of PARADIGM ($F_{1,4} = 25.5$, $P < 0.01$) with there being more MEP suppression following $cTBS_{mod}$ than following $cTBS_{std}$. Both $cTBS_{mod}$ (TIME: $F_{5,20} = 6.92$, $P < 0.001$) (Figure 5-7A) and $cTBS_{std}$ (TIME: $F_{5,20} = 2.90$, $P = 0.04$) (Figure 5-7B) resulted in significant suppression of MEP amplitudes. *Post hoc* analyses revealed that MEPs were suppressed compared to baseline at 5 min following the $cTBS_{std}$ paradigm ($P < 0.01$, corrected with FDRP), whereas MEPs were suppressed compared to baseline at all post-intervention time points following the $cTBS_{mod}$ paradigm ($P < 0.05$ for all, corrected with FDRP).

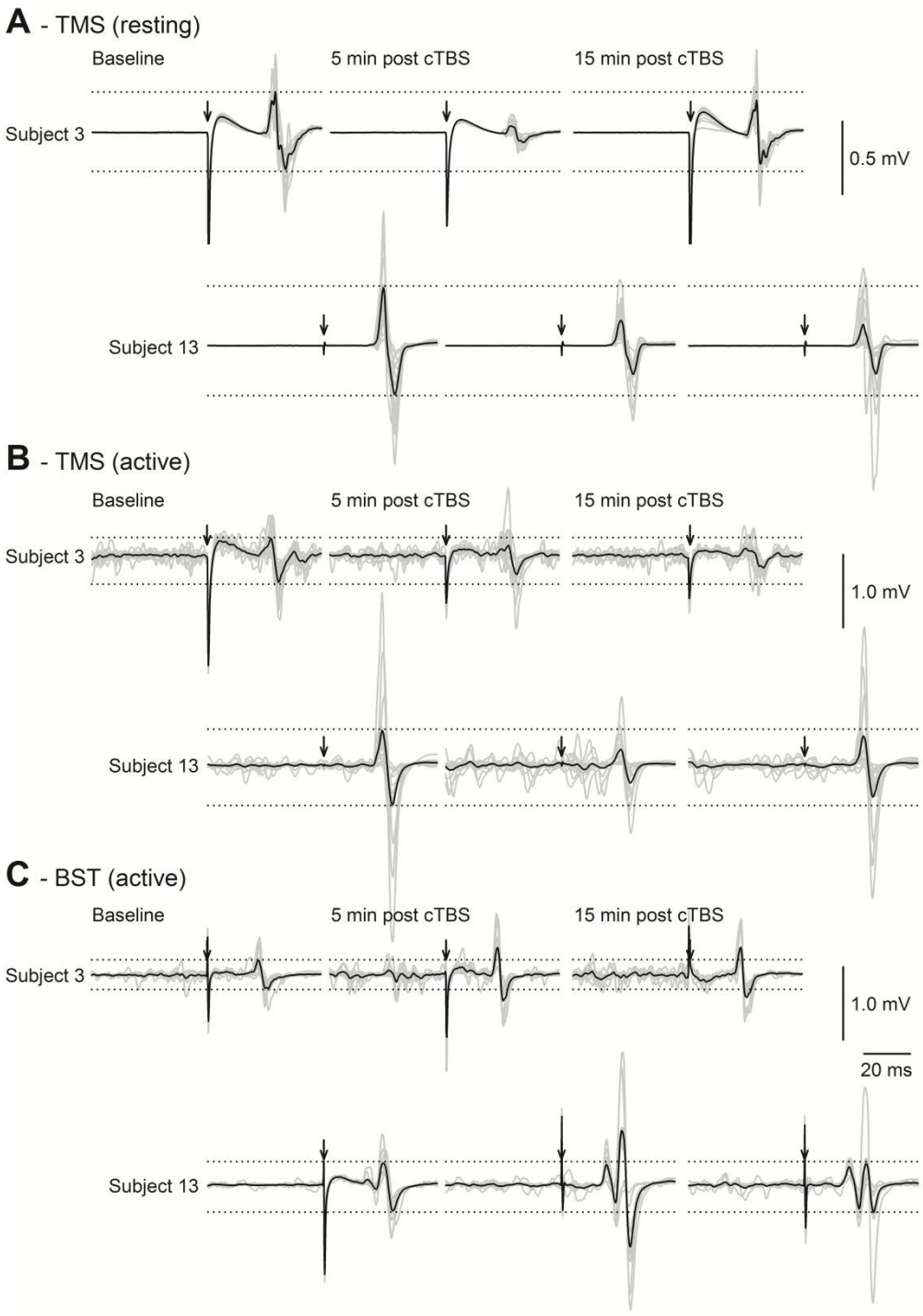

Figure 5-6: *Raw electromyographic data traces from two subjects showing MEPs evoked by TMS of the left motor cortex in the (A) resting and (B) active conditions, and by (C) BST in the active condition, recorded at baseline and at 5 and 15 min after* $cTBS_{mod}$*. Individual trials are shown in grey, and the average of all trials (15 for TMS-evoked MEPs and 10 for BST-evoked MEPs) are shown in black. Arrows indicate delivery of single-pulse TMS (A and B) and BST (C).*

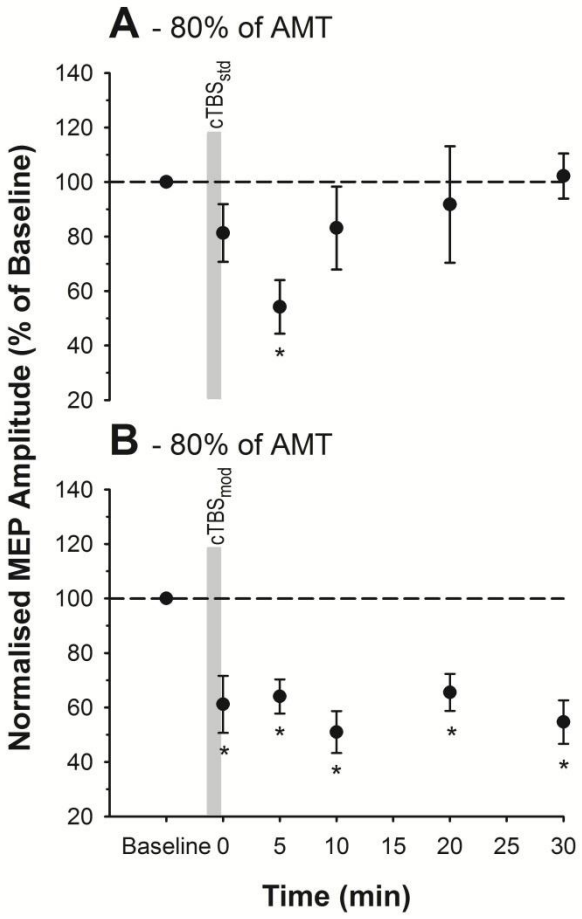

Figure 5-7: *The time course of change in MEP amplitude (expressed as a percentage of baseline) following (A) the $cTBS_{std}$ paradigm and (B) the $cTBS_{mod}$ paradigm applied at 80% of AMT (Experiment 3). MEP amplitudes were suppressed at 5 min following $cTBS_{std}$, whereas $cTBS_{mod}$ (applied at the same intensity) induced a suppression of MEPs at all time points. * denotes $P < 0.05$ when compared to baseline MEP amplitudes. Data points are group means ± SEM.*

5.5. DISCUSSION

The results of the present study show that small changes in the stimulation parameters used for applying cTBS can significantly modify the efficacy of neuroplasticity

induction in the human primary motor cortex. Whilst $cTBS_{std}$ induced a short-lasting suppression of MEP amplitudes that was variable between subjects, $cTBS_{mod}$ reliably induced MEP suppression which persisted for the entire post-intervention recording period and was highly consistent between subjects. The results also provide evidence that the MEP suppression seen following $cTBS_{mod}$ is likely to be due to effects within the motor cortex.

The two cTBS paradigms compared in this study differ in three ways: the method for setting stimulation intensity, the frequency at which bursts are applied (i.e. the inter-burst frequency), and the frequency of pulses within each burst (i.e. the intra-burst frequency). The rationale for comparing these paradigms was that they are the only variations of cTBS to have been tested in humans so far. It should be noted that the aim of this study was not to explore different combinations of the stimulation parameters used for the two paradigms, but rather, to compare their effectiveness for inducing a neuroplastic response. Whilst a variety of cortical regions have been studied using these two TBS paradigms (Di Lazzaro et al., 2005; Huang et al., 2005; Martin et al., 2006; Nyffeler et al., 2006b; Gentner et al., 2008; Cazzoli et al., 2009; Nyffeler et al., 2009), there has been no study comparing the effectiveness of these paradigms on the same cortical region in the same group of subjects.

5.5.1. Comparison of the $cTBS_{std}$ and $cTBS_{mod}$-induced after-effects with those observed in other studies

The suppression of corticospinal excitability induced by the $cTBS_{std}$ paradigm in the present study was short-lasting, with MEP amplitudes returning to baseline by 10 min

following the intervention. This short duration of MEP amplitude suppression contrasts with the results observed in the Huang et al. (2005) study, which reported a suppression of corticospinal excitability lasting up to 1 h in duration. However, more recent studies have reported responses to $cTBS_{std}$ that are of lesser magnitude and a more variable nature. In the present study the majority of subjects (eight of 12) responded with an overall suppression of MEP amplitudes. However, for two subjects there was, on average, no change in MEP amplitude across the 30 min post-intervention recording period, and two of the 12 subjects showed a facilitatory response to the $cTBS_{std}$ paradigm (see Figure 5-4A). Such inter-individual variability in the magnitude and direction of responses to $cTBS_{std}$ is consistent with these more recent reports (Martin et al., 2006; McAllister et al., 2011; Goldsworthy et al., 2012), and is likely due to a number of factors (see Ridding and Ziemann, 2010).

Unlike the $cTBS_{std}$ paradigm, there has been no previous study examining the effect of $cTBS_{mod}$ on corticospinal excitability. The $cTBS_{mod}$ paradigm has been investigated most extensively in humans to target the frontal eye field cortical region in order to study its behavioural effects on horizontal saccadic eye movements. Nyffeler et al. (2006b) demonstrated that this cTBS paradigm applied to the human frontal eye field significantly delayed saccade triggering for up to 30 min following its application. Although the present study investigated a different cortical region and employed electrophysiological measures instead of behavioural measures to assess outcomes, the duration and magnitude of after-effects observed following $cTBS_{mod}$ are consistent with those reported by Nyffeler et al. (2006b).

5.5.2. Cortical site of $cTBS_{mod}$-induced MEP suppression

Whilst there is evidence to suggest that the MEP suppression induced by the $cTBS_{std}$ paradigm is due to cortical mechanisms (Di Lazzaro et al., 2005), the locus for the effects induced by $cTBS_{mod}$ has not previously been investigated. Therefore, in Experiment 2 we investigated whether the site of $cTBS_{mod}$-induced MEP suppression was cortical or spinal. We used magnetic BST to activate the corticospinal pathways at the level of the pyramidal decussation (Ugawa et al., 1994). TMS-evoked MEPs were suppressed following $cTBS_{mod}$ both at rest (similar to Experiment 1) (Figure 5-6A) and during a small tonic contraction (Figure 5-6B). However, there was no suppression of BST-evoked MEPs (Figure 5-6C) during a matched low-level contraction. Indeed, there appeared to be a mild facilitation of BST-evoked responses. The reasons for this are unclear, although a similar discrepancy between changes in cortical and spinal excitability (assessed with H-reflexes) has been observed in a previous study investigating the effects of a 5 Hz rTMS paradigm (Berardelli et al., 1998). Therefore, although these studies were only conducted in a small number of subjects, they provide evidence that the MEP suppression induced by $cTBS_{mod}$ was cortical, rather than spinal, in origin.

5.5.3. Using RMT instead of AMT to set the stimulation intensity

The absolute intensity used for the $cTBS_{mod}$ paradigm for the first experiment of this study was significantly greater than that used for the $cTBS_{std}$ paradigm. This higher intensity of stimulation could explain why $cTBS_{mod}$ yielded a greater suppression of corticospinal excitability compared to the $cTBS_{std}$ paradigm. To more closely examine

whether the differences in stimulation intensities might have accounted for the differences observed between the subjects' response profiles for each of the cTBS paradigms, a correlation analysis was performed between the difference in stimulation intensity between the paradigms (expressed as a ratio as well as the absolute difference) and the ratio of subjects' responses to $cTBS_{std}$ and $cTBS_{mod}$ (Figure 5-5). No significant correlation was observed, indicating that the relative effectiveness of the $cTBS_{mod}$ and $cTBS_{std}$ paradigms was not related to the magnitude of the difference in stimulation intensities used for the two cTBS paradigms. Likewise, for each of the cTBS paradigms, subject responses were not correlated with the absolute intensity of stimulation. These results provide some evidence that the difference in response to the two cTBS paradigms was not simply due to different stimulus intensities.

Apart from a difference in the absolute stimulation intensity, there is an additional methodological difference between the two paradigms related to the setting of stimulus intensity that might be important in influencing the magnitude of the response. In the $cTBS_{mod}$ paradigm, stimulation intensity was set relative to RMT, while in the $cTBS_{std}$ paradigm, stimulation intensity was set relative to AMT. Therefore, subjects performed a voluntary contraction prior to the $cTBS_{std}$ paradigm, but not before the $cTBS_{mod}$ paradigm. Several studies have shown that behavioural engagement of the cortical region to be targeted by rTMS paradigms can influence the subsequent response (Gentner et al., 2008; Huang et al., 2008; Todd et al., 2009b; Goldsworthy et al., 2012). Therefore, we cannot exclude the possibility that the difference in the activation history of the motor cortex prior to application of the two different paradigms in Experiment 1 was, at least in part, responsible for the different response patterns.

To further investigate whether the different methods for setting stimulation intensity for $cTBS_{mod}$ could have accounted for its greater efficacy for inducing MEP suppression, a control experiment (i.e. Experiment 3) was performed in which both $cTBS_{std}$ and $cTBS_{mod}$ were applied using 80% of AMT to set stimulation intensity. Despite both paradigms being applied at similar intensities and with the same history of prior motor activation, application of $cTBS_{mod}$ still resulted in a greater MEP suppression than that seen following $cTBS_{std}$. Therefore, in summary, we consider it unlikely that the difference in the responses to the two cTBS paradigms in Experiment 1 was due to the differences in stimulus intensities or the history of contraction prior to cTBS application.

Although there has been little study of the influence of stimulus intensity on the response to $cTBS_{std}$ it is interesting to note that the $cTBS_{mod}$ paradigm was similarly effective across a moderate range of stimulus intensities (80% AMT - 80% RMT).

5.5.4. Variations in inter and intra-burst frequencies

We suggest that the most likely cause for the difference in response to the two paradigms lies with variations in inter and/or intra-burst frequencies. Although these changes appear relatively small (6 Hz vs. 5 Hz inter-burst frequency and 30 Hz vs. 50 Hz intra-burst frequency), there is already good evidence that alterations in the temporal pattern of stimuli can have significant effects on response profiles. For example, introducing breaks in stimulation of the basic TBS stimulation pattern can reverse the direction of excitability change induced (e.g. iTBS) (Huang et al., 2005). Likewise, small changes in the interval between pulses within trains of quadripulse stimulation, an

rTMS protocol which, like TBS, has been shown to produce lasting neuroplastic changes in corticospinal excitability (Hamada et al., 2007), may have a significant impact on the direction and duration of the induced neuroplastic response (Hamada et al., 2008). There is evidence that various frequencies of the rhythmic activity within the theta band are associated with specific forms of learning and memory. For example, in the animal hippocampus, movement-related exploratory behaviour is associated with burst activity at the upper end of the theta frequency range, whereas non-movement or immobility-related behaviours are associated with burst activity at the lower end (Vanderwolf, 1969; Bland, 1986). Although highly speculative, given the relevance to voluntary motor behaviour, this might explain why the slightly higher 6 Hz inter-burst frequency employed in the $cTBS_{mod}$ paradigm might be more effective for inducing motor cortical neuroplasticity than the 5 Hz frequency used in the $cTBS_{std}$ paradigm. However, based on the current data, we cannot say whether alterations in inter or intra-burst frequency is a more important influence on the response to $cTBS_{mod}$. Therefore, further studies are required to determine which combination of stimulation parameters achieves the most optimal response to cTBS.

5.5.5. Mechanisms responsible for the MEP suppression

Whereas there is good evidence that the suppression of MEP amplitudes induced by the $cTBS_{std}$ paradigm is the result of LTD-like changes in the efficacy of excitatory synapses within the primary motor cortex (Huang et al., 2005; Huang et al., 2007), the mechanisms by which $cTBS_{mod}$ suppresses cortical excitability have not been previously investigated. In the present study we sought to establish a relationship between the responses to the two cTBS protocols to provide evidence of a common mechanism.

However, although we could not demonstrate such a relationship, we suggest that this may largely be due to the variability that can exist in subjects' responses between experimental sessions and the large inter-individual variability (this was particularly evident for the $cTBS_{std}$ paradigm). We provide evidence that the effects following $cTBS_{mod}$ are due to cortical mechanisms and propose that, like $cTBS_{std}$, it is most likely that the changes seen following $cTBS_{mod}$ are due to LTD-like changes at excitatory synaptic connections within the primary motor cortex. However, we cannot rule out the possibility that other forms of synaptic plasticity (for instance, LTP-like facilitation of inhibitory circuits) may have been involved. It would be possible, and useful, to investigate these possibilities in future studies further by performing short-interval intracortical inhibition and facilitation measures.

Interestingly, although only a small number of subjects were tested, we show here that $cTBS_{mod}$-induced MEP suppression was still present in the actively contracting muscle. Several previous studies investigating neuroplastic changes in the human primary motor cortex using various experimental paradigms have been unable to demonstrate changes in MEP amplitude when the targeted muscle is in an active state (Touge et al., 2001; Todd et al., 2009b). The reason for lack of MEP modulation during a voluntary contraction is not clear but it is possible that behavioural engagement of the targeted cortex disrupts transient induced changes in synaptic efficacy. Therefore, the finding that $cTBS_{mod}$ suppresses MEPs evoked in both the resting and active conditions might be evidence that the changes induced by $cTBS_{mod}$ are more robust than those seen with $cTBS_{std}$.

5.5.6. The spaced application of $cTBS_{mod}$

In a previous study, we were able to show that applying repeated trains of $cTBS_{std}$ to the human primary motor cortex in a spaced manner significantly prolonged the duration of induced MEP suppression (Goldsworthy et al., 2012). Additionally, there was evidence that a strong response to the first $cTBS_{std}$ train predicted a better outcome to paired trains. Based on this, it is likely that the strong MEP suppression induced by $cTBS_{mod}$ may be further enhanced by applying repeated trains in a spaced manner, producing a neuroplastic response that is stronger and longer lasting than the robust after-effects induced by spaced $cTBS_{std}$ trains. Considering the importance of inducing lasting neuroplastic effects for the therapeutic application of cTBS, further studies will be required to determine whether spaced applications of $cTBS_{mod}$ prolong the duration of induced neuroplastic changes in the human primary motor cortex.

In conclusion, the present study has shown that the response to cTBS of the human primary motor cortex is highly dependent on the stimulation parameters employed. The $cTBS_{mod}$ paradigm induces a significantly greater neuroplastic response within the human motor regions than the $cTBS_{std}$ paradigm. The results of this study may have implications for the development and clinical application of cTBS paradigms.

6. GENERAL DISCUSSION

Over recent years, there has been much interest in the development of non-invasive brain stimulation techniques such as rTMS that are capable of inducing lasting neuroplastic changes in human cortical excitability. By being able to painlessly induce increases, as well as decreases, in the excitability of different cortical regions within the conscious human brain, these techniques have been identified as potential therapeutic options for a range of different neurological and psychiatric disorders. Although the potential of rTMS is clear, any positive effects induced by these protocols are typically short-lasting under normal physiological conditions and are highly variable between subjects. Thus, the experiments described within this thesis have investigated novel approaches for rTMS application that generate a longer lasting neuroplastic response in the human primary motor cortex that is less variable between subjects and is more stable under normal physiological conditions.

6.1. THE REPEATED APPLICATION OF cTBS

One approach that has been highly effective at prolonging the duration of experimentally-induced synaptic plasticity in studies investigating animal models is the repeated application of stimulation trains in a spaced manner (Bliss and Gardner-Medwin, 1973; Barnes, 1979; Jeffery et al., 1990; Abraham et al., 1993; Racine et al., 1995; Trepel and Racine, 1998; Abraham et al., 2002). Therefore, the experiments described in Chapter 2 investigated whether the repeated and spaced application of rTMS was similarly capable of extending the lifetime of induced neuroplastic effects in the human primary motor cortex. The rTMS paradigm employed for this study was

cTBS, which has previously been shown to produce LTD-like changes in human motor cortical excitability (Huang et al., 2007). The findings of this study showed that, in the absence of an initial voluntary contraction, the paired application of cTBS (applied at an interval of 10 min) induced a significantly greater neuroplastic response compared to a single cTBS application, with a suppression of MEP amplitudes lasting for at least 2 h following stimulation.

A feature of the LTP and LTD induced experimentally in animal models is that they can be extremely durable, with changes in synaptic efficacy that may last several weeks (Abraham, 2003). Whilst there is considerable pharmacological evidence linking rTMS-induced after-effects with the LTP and LTD observed in animals (Stefan et al., 2002; Wolters et al., 2003; Huang et al., 2007; Lang et al., 2008), the response to rTMS is typically very short-lasting. Although cTBS is considered one of the stronger rTMS paradigms, its effects on MEPs when applied as a single train do not persist beyond 1 h (Huang et al., 2005; Gentner et al., 2008; Gamboa et al., 2010). The longer lasting response to paired cTBS trains in this study is more compatible with the duration of LTD observed in animal models. However, further investigations into the underlying mechanisms for this effect are required.

The duration of after-effects induced by paired cTBS applied to the human primary motor cortex in this study is largely consistent to that described previously in the frontal eye field cortical region of the human oculomotor system. Whilst a single train of a modified cTBS variant applied to the frontal eye field induced delays in the triggering of saccadic eye movements that lasted less than 1 h, paired cTBS trains (applied at an interval of 15 min) induced delays in saccade triggering that lasted over 2 h (Nyffeler et

al., 2006a). Furthermore, when four cTBS trains were applied in a spaced manner, the induced delays in saccade triggering lasted up to 10 h in duration. A similar prolongation of behavioural effects has been observed following the application of repeated cTBS protocols to the posterior parietal cortex of the intact hemisphere in stroke patients with spatial neglect, with four trains of cTBS applied in a single session improving patients' performance in a visual perception task for up to 32 h following intervention (Nyffeler et al., 2009). These findings were extended in a recent study, which showed improvements in spatial neglect symptoms which lasted for at least 3 weeks following eight trains of cTBS applied over two consecutive days (Cazzoli et al., 2012).

Given the long-lasting after-effects observed following four (Nyffeler et al., 2006a; Nyffeler et al., 2009) and eight (Cazzoli et al., 2012) trains of cTBS applied to non-motor regions, it is possible that the application of a greater number of cTBS trains than that investigated in Chapter 2 may have generated an even greater neuroplastic response in the human primary motor cortex with longer lasting effects. This is supported by the finding of a non-homeostatic interaction between paired cTBS trains applied to the human primary motor cortex, with a strong response to the first cTBS train resulting in a greater MEP suppression upon application of a second train. This suggests that a summation of LTD-like effects may be possible with repeated, spaced, cTBS applications. Therefore, future studies should address whether a greater number of cTBS trains applied in a spaced manner to the human primary motor cortex are capable of inducing MEP suppression lasting days or even weeks. The induction of such highly persistent neuroplastic changes is critical for the therapeutic application of rTMS protocols.

The 10 min interval used to separate the paired cTBS trains in Chapter 2 was based on similar experiments performed in the adult rat hippocampus (Abraham et al., 2002), and although this proved effective at prolonging MEP suppression in this study, it is important to note that other time intervals were not tested. There is evidence from investigations in animal models that the efficacy of spaced stimulation trains for inducing stable synaptic plasticity is highly dependent on inter-train interval length, showing an inverted U-shaped relationship (Zhou et al., 2003). Whether a similar relationship exists for the repeated application of cTBS trains in the human motor cortex is unclear. Therefore, in addition to optimising the number of trains, the optimal spacing between cTBS trains should also be addressed in future studies.

An important finding of the experiments described in Chapter 2 was that a voluntary contraction of the hand muscles prior to the paired application of cTBS abolished the long-lasting MEP suppression. The mechanisms responsible for the negative effect of a prior contraction are unclear, although performance of a short, sub-maximal voluntary contraction (similar to that used in this study) has previously been shown to interact with and modulate the neuroplastic response to a short, 300 pulse train of cTBS (Gentner et al., 2008). It is possible that the synaptic activity associated with behavioural engagement of the primary motor cortex during voluntary contraction initiated some form of metaplastic priming effect, influencing subsequent neuroplasticity induction by repeated cTBS protocols. Experiments in mouse hippocampal slices found that prior synaptic activity (evoked using a low-frequency electrical stimulation train) blocked the subsequent induction of a protein synthesis-dependent late-phase of LTP by repeated trains of high-frequency electrical stimulation

(Woo and Nguyen, 2002). Interestingly, this priming stimulation had no effect on the short-lasting and protein synthesis-independent early-phase of LTP induced by a single high-frequency stimulation train. A similar selective impairment of late-phase LTP by priming stimulation has been observed in other studies investigating mouse hippocampal slices (Young and Nguyen, 2005; Young et al., 2006), and this may have been due to increased phosphatase activity preventing the synaptic capture of gene products required for the long-term maintenance of synaptic changes (Young et al., 2006).

Whilst it is possible that the voluntary contraction employed in Chapter 2 may have acted in a similar manner to the priming stimulation protocols investigated in these hippocampal slice preparations, further studies will be required to support this claim. However, these findings do highlight the impact that a prior voluntary contraction can have on subsequent neuroplasticity induction by non-invasive brain stimulation. Therefore, it is recommended that neuroplasticity-inducing paradigms (and in particular, repeated cTBS) should be applied without a prior voluntary contraction to ensure that any induced changes in cortical excitability are not biased by prior activation of the motor cortical regions.

6.2. MOTOR NETWORKS TARGETED BY REPEATED cTBS

Although the long-lasting MEP suppression that followed the repeated application of cTBS in Chapter 2 was consistent with the induction of LTD-like effects at excitatory synapses within the human primary motor cortex, it is unclear to what extent the inhibitory synaptic connections of the human primary motor cortex were affected by

repeated cTBS applications. To investigate this point further, the experiments described in Chapter 3 assessed the impact of both single and paired cTBS on the excitability of the inhibitory motor networks. As well as using single-pulse TMS to probe the excitability of the excitatory motor networks responsible for generating MEPs, paired-pulse TMS was used to assess $GABA_A$ and $GABA_B$-mediated intracortical inhibition (i.e. SICI and LICI, respectively). Consistent with the findings of Chapter 2, the paired application of cTBS in this study resulted in a significantly greater suppression of MEP amplitudes compared to that observed following a single cTBS application. Whilst paired cTBS also induced a significant suppression of the $GABA_A$-mediated SICI circuits, this did not differ to that observed following a single cTBS. Neither single nor paired cTBS modulated the $GABA_B$-mediated LICI circuits.

There is evidence that the excitability of the intracortical inhibitory motor networks is reduced in patients with various neurological conditions, including focal hand dystonia (Ridding et al., 1995b; Chen et al., 1997a), Parkinson's disease (Ridding et al., 1995a) and cortical myoclonus (Brown et al., 1996), and this reduced inhibition may contribute to the disordered movements observed in these patients. As a result, there has been much interest in identifying neuroplasticity-inducing paradigms that may be used to restore normal levels of cortical inhibition by selectively targeting and modulating the excitability of inhibitory motor networks. The possible neuroplastic effect of cTBS on SICI has been investigated in a number of previous studies with conflicting results. Although some studies have observed reduced SICI following cTBS application (Huang et al., 2008; Murakami et al., 2008; Suppa et al., 2008; McAllister et al., 2009), a number of other studies have shown no impact of cTBS on the excitability of SICI circuits (Di Lazzaro et al., 2011; Doeltgen and Ridding, 2011b, a). The results of

Chapter 3 showed that whilst a single cTBS protocol reduced SICI, this effect was quite modest. Furthermore, in contrast to the strong suppression of MEPs induced by the paired application of cTBS, the reduction in SICI induced by paired cTBS protocols was no greater than that observed following a single cTBS. Together with the absence of an effect on the $GABA_B$-mediated LICI circuits following either single or paired cTBS, these findings suggest that cTBS might not be an optimal approach for modulating the excitability of the inhibitory synaptic connections within the human primary motor cortex.

The reasons why cTBS has relatively modest effects on the excitability of inhibitory motor networks are unclear. Part of this may be related to the temporal characteristics of pulses within cTBS protocols being more optimal for evoking LTD-like neuroplasticity at excitatory synapses than at inhibitory ones (Caporale and Dan, 2008). However, another possible reason for the modest effects of cTBS on intracortical inhibition could be that the intensity of cTBS used in this study was not optimal for selectively targeting the inhibitory motor networks, which have a lower threshold for activation compared to the excitatory networks (Kujirai et al., 1993). Indeed, McAllister et al. (2009) showed that cTBS applied with reduced stimulation intensity (i.e. 70% of AMT instead of the conventional 80% of AMT) suppressed the excitability of SICI circuits whilst having no effect on MEP amplitudes, suggesting that lower intensities of cTBS may be required to selectively modulate the excitability of inhibitory synaptic connections. It may therefore be of interest to repeat the experiments of Chapter 3 using cTBS at lower stimulation intensities to determine whether the repeated and spaced application of low-intensity cTBS can induce long-lasting suppression of inhibitory motor networks.

A secondary aim of the experiments described in Chapter 3 was to provide additional information relating to the neurophysiological mechanisms by which paired cTBS reduces MEP amplitudes. In particular, because the excitability of the inhibitory motor networks was either suppressed (in the case of the $GABA_A$-mediated SICI) or unchanged (in the case of the $GABA_B$-mediated LICI) following paired cTBS protocols, it is highly unlikely that increased activity of the inhibitory motor networks was responsible for the reduction in MEP amplitudes. This is an important finding, and suggests that the long-lasting MEP suppression induced by the repeated application of cTBS in Chapter 2 was likely the result of enhanced LTD-like effects at excitatory synapses within the human primary motor cortex rather than LTP-like effects at inhibitory synaptic connections.

6.3. CONSOLIDATION BY REPEATED cTBS

Whilst Chapters 2 and 3 have shown that the repeated application of cTBS to the human primary motor cortex induced a longer lasting suppression of MEP amplitudes than a single cTBS application, whether these longer lasting effects were also more stable under normal physiological conditions was not addressed. Therefore, the experiments described in Chapter 4 investigated whether the repeated application of cTBS protocols could consolidate cTBS-induced neuroplastic changes in human motor cortical excitability, making them resistant to reversal by normal physiological activity within the stimulated motor cortical regions. The findings of this study showed that whilst behavioural engagement of the primary motor cortex by a sustained, sub-maximal voluntary contraction of the hand muscles abolished the LTD-like MEP suppression induced by a single cTBS protocol, the long-lasting MEP suppression induced by paired

cTBS protocols was resistant to reversal by subsequent voluntary contraction. Similar results were shown using a shortened, 150 pulse train of iTBS (i.e. iTBS150) instead of a voluntary contraction to externally generate activity within the motor regions.

Experiments in animal models have shown that experimentally-induced LTP and LTD are susceptible to reversal (i.e. depotentiation and de-depression, respectively) by either subsequent physiological activity at the stimulated synaptic input (Xu et al., 1998; Zhou et al., 2003) or synaptic activity generated externally by delivery of a weak stimulation protocol shortly after plasticity induction (Fujii et al., 1991; Huang et al., 1999; Chen et al., 2001). There is also evidence that the repeated application of stimulation protocols in a spaced manner can consolidate synaptic plasticity in animal models, inducing more stable changes in synaptic efficacy that are less sensitive to these reversal effects (Woo and Nguyen, 2003; Zhou et al., 2003), and this is likely due to the induction of a late-phase of synaptic plasticity characterised by increases in *de novo* protein synthesis and gene transcription (Krug et al., 1984; Huang and Kandel, 1994; Nguyen et al., 1994; Woo and Nguyen, 2003).

Reversal effects similar to those described in animals have been shown in the human primary motor cortex following neuroplasticity induction with both rTMS (Todd et al., 2006; Huang et al., 2008) and tDCS (Thirugnanasambandam et al., 2011), and are typically evoked by behavioural engagement of the stimulated motor cortical regions by a short, sub-maximal voluntary contraction. Likewise, Huang et al. (2010) showed that the increases and decreases in motor cortical excitability induced by iTBS and cTBS, respectively, could be reversed by externally-generated network activity evoked using 150 pulse trains of TBS which, when applied alone, do not change motor cortical

excitability. The reversal of single cTBS-induced MEP suppression by both voluntary contraction and iTBS150 in this study is consistent with these previous findings. However, the experiments of Chapter 4 have shown for the first time that, similar to the consolidated synaptic plasticity observed in animal models, the repeated application of spaced cTBS protocols induced a suppression of MEPs that was resistant to reversal by both normal physiological activity within the stimulated motor cortical regions and externally-generated activity within the motor cortex by iTBS150. These findings are consistent with the notion that a late-phase of synaptic plasticity similar to that described in animal models may have been responsible for the long-lasting suppression of MEPs induced by repeated cTBS of the human primary motor cortex.

As well as providing an additional line of evidence linking the after-effects of TBS protocols with synaptic plasticity in animals, the results of Chapter 4 also have significant implications for therapeutic application of rTMS protocols. The capacity of rTMS to induce a lasting and durable neuroplastic change under normal physiological conditions is critically important for its implementation as a therapeutic tool for treating disease. In particular, there is a great deal of interest in the therapeutic application of rTMS as an adjunctive treatment in chronic stroke patients with upper-limb motor dysfunction. Typical rehabilitation following stroke requires ongoing physical activity to promote use-dependent neuroplastic reorganisation of the damaged motor cortical regions and facilitate functional recovery (Hallett, 2001; Schaechter, 2004). It is anticipated that rTMS, applied in conjunction with these standard physical therapies, will improve clinical outcome in stroke patients by enhancing these natural neuroplastic processes. However, the instability of rTMS-induced neuroplastic effects under normal physiological conditions severely limits its application in this setting. The finding that

the repeated application of cTBS protocols not only prolongs the duration of induced neuroplastic effects in the human primary motor cortex, but also stabilises these effects in the presence of subsequent voluntary motor activity, suggests that this may be an effective approach for applying rTMS in conjunction with physical therapy in stroke patients.

Although these findings show evidence that a consolidation of LTD-like effects may occur through repeated and spaced applications of cTBS to the human primary motor cortex, whether repeated applications of a facilitatory rTMS protocol (for instance, iTBS) is able to induce LTP-like effects that are comparable in duration and stability under normal physiological conditions was not tested. The capacity to produce stable facilitation of human motor cortical excitability is important for a range of neurological disorders, including stroke. It has been proposed that an LTD-like reduction in the excitability of the intact hemisphere in stroke patients may be functionally beneficial in correcting inter-hemispheric imbalances between the motor cortical areas (Murase et al., 2004). Whilst this may be the case, there is also evidence that the intact motor cortex may contribute to some of the recovery of motor function following stroke (Lotze et al., 2006), and as a result, a cTBS-induced reduction of the excitability of this region may have detrimental effects in some patients (Ackerley et al., 2010). Therefore, further studies are required to determine whether the long-lasting and stable neuroplastic changes in human motor cortical excitability induced by repeated stimulation protocols are achievable using facilitatory rTMS protocols.

Another limitation in the interpretation of these results is that, although the repeated application of cTBS was shown to produce long-lasting effects on MEP amplitudes (an

electrophysiological measure of corticospinal function), the functional relevance of these effects is unclear. Likewise, it is not certain whether the effects observed in the human primary motor cortex will be the same in other cortical regions, for example, the dorsolateral prefrontal cortex, which has been the target of rTMS in several clinical trials for the treatment of medication-resistant depression (see Section 1.4.1). Therefore, additional studies should address the impact of repeated cTBS protocols on behavioural measures of motor function, and should also investigate the effects of applying repeated, spaced cTBS trains to non-motor cortical regions.

6.4. OPTIMISING THE PARAMETERS FOR SINGLE cTBS

One notable observation from the experiments described in Chapters 2, 3, and 4 is the variable neuroplastic response to a single cTBS protocol. This was particularly evident in Chapter 2. In contrast to the initial study by Huang et al. (2005) which showed a relatively strong suppression of human motor cortical excitability following cTBS, the application of a single cTBS protocol (at either the 70% of RMT or 80% of AMT stimulation intensities) in Chapter 2 failed to produce a significant effect on MEP amplitudes. This was due primarily to between-subject variability: although the majority of subjects responded to cTBS with MEP suppression, a portion of subjects showed an unexpected facilitatory response to stimulation. Whilst this may have been the result of a number of factors (Ridding and Ziemann, 2010), it is unclear whether the use of non-optimal stimulation parameters for cTBS application contributed to this variability. Therefore, the experiments described in Chapter 5 compared the efficacy of the standard cTBS paradigm (i.e. $cTBS_{std}$) (Huang et al., 2005) to that of a slightly modified variant (i.e. $cTBS_{mod}$) (Nyffeler et al., 2006b) for inducing suppression of human motor cortical

excitability. Whilst $cTBS_{std}$ induced MEP suppression that was short-lived and highly variable, $cTBS_{mod}$ induced a lasting MEP suppression that was consistent between subjects. These results suggest that the lesser-used $cTBS_{mod}$ paradigm may be more effective for inducing neuroplastic change in the human primary motor cortex.

Experiments controlling for the method of setting the stimulation intensity suggest that the difference in the neuroplastic response between the two cTBS paradigms was likely a result of slight variations in the inter and/or intra-burst frequencies. However, based on the findings from Chapter 5, it is unclear which of these factors was more important in determining the response to cTBS. Likewise, it remains to be seen whether similar improvements in the efficacy of the facilitatory iTBS are possible using these (or other) stimulation parameters. Therefore, further experiments will be required to determine which combinations of stimulation parameters produce the strongest and most consistent responses to both cTBS and iTBS.

Prior to this study, there has been little work investigating the optimal stimulation parameters for cTBS (and iTBS) application. Based on the vast number of different combinations that are possible, a systematic study of all combinations of stimulation parameters seems impractical. Nonetheless, it remains critically important that we arrive at a set of parameters that induce strong neuroplastic effects in *all* subjects with minimal variability. Indeed, this has implications for the repeated application of stimulation trains in a spaced manner. As was shown in Chapter 2, a strong response to the first cTBS train predicted a better outcome to paired cTBS. Therefore, the capacity of repeated cTBS protocols to reliably induce long-lasting neuroplastic change in the motor cortex rests largely on the efficacy of a single cTBS application. This warrants

further investigation into the TBS parameters that produce the optimal neuroplastic response in the human primary motor cortex.

6.5. IMPLICATIONS FOR THE THERAPEUTIC USE OF rTMS

The development of rTMS as a treatment for various pathological conditions has become an important area of neuroscientific research. However, despite its introduction to the clinical research setting occurring more than a decade ago, it is still unclear which methods of rTMS application are optimal. A common approach has been to perform repeated daily sessions of rTMS over the course of several days or weeks. Although this has yielded some promising results in patients suffering various neurological (Khedr et al., 2003; Khedr et al 2005; Fregni et al., 2006) and psychiatric (George et al., 2000; Lee et al., 2005; Amiaz et al., 2009; Bagati et al., 2009; Fitzgerald et al., 2009; George et al., 2010) disorders, the rationale is not entirely clear for rTMS paradigms such as cTBS. Whereas the low intensity requirements and short stimulation times make cTBS an attractive therapeutic option, the short duration and instability of the induced neuroplastic response to a single application will likely mean that any positive effects following stimulation will subside long before the next train is delivered. Likewise, because of the high variability of subject responses, some patients will inevitably respond in the opposite manner to stimulation, producing effects that are detrimental to functional recovery.

The results of Chapters 2, 3, and 4 of this thesis show that the repeated and spaced application of cTBS trains *within* a single session may prove to be an effective approach for enhancing the duration and stability of induced neuroplastic effects within the

human primary motor cortex. Furthermore, I show in Chapter 5 that slight variations to the stimulation parameters of a single cTBS train can drastically reduce between-subject variability. It is possible that repeated trains of this modified variant will induce an even longer lasting neuroplasticity persisting for several hours, possibly even days or weeks with the addition of a greater number of trains, and will result in a more consistant response profile that will benefit a greater proportion of patients. Several key points remain to be investigated, including the optimal number and spacing of stimulation trains, the optimal pulse frequencies, and whether these promising results extend to facilitatory, as well as suppressive, rTMS protocols and to the non-motor cortical regions. However, it is hoped that these early investigations will lead to the development of a new framework for the application of rTMS in the clinical setting.

6.6. CONCLUDING REMARKS

The therapeutic potential of rTMS is limited by the short duration, instability and high variability of induced neuroplastic effects. This thesis has demonstrated for the first time that the repeated application of cTBS protocols in a spaced manner induces long-lasting neuroplastic changes in the human primary motor cortex that are stable during behaviourally-relevant physiological activity. Additionally, it was shown that slight variations in the stimulation parameters used for the application of cTBS can have a significant impact on its efficacy for inducing a neuroplastic response in the motor cortical regions. The findings of these studies may have significant implications for the therapeutic application of rTMS protocols.

7. APPENDICES

7.1. APPENDIX I: TRANSCRANIAL MAGNETIC STIMULATION (TMS) ADULT SAFETY SCREEN

Name:
Date:
Age:

Please answer the following:

Do you have epilepsy or have you ever had a convulsion or a seizure?	❍Yes	❍No
Have you ever had a fainting spell or syncope? If *yes*, please describe in which occasions in the space provided below.	❍Yes	❍No
Have you ever had severe (i.e., followed by loss of consciousness) head trauma?	❍Yes	❍No
Do you have any hearing problems or ringing in your ears?	❍Yes	❍No
Are you pregnant or is there a chance you might be?	❍Yes	❍No
Do you have cochlear implants?	❍Yes	❍No
Do you have an implanted neurostimulator? (e.g., DBS, epidural/subdural, VNS)	❍Yes	❍No
Do you have a cardiac pacemaker or intracardiac lines or metal in your body	❍Yes	❍No
Do you have a medication infusion device?	❍Yes	❍No
Are you taking any medications? *(Please list)*	❍Yes	❍No
Have you had a surgical procedure to your spinal cord?	❍Yes	❍No
Do you have spinal or ventricular derivations?	❍Yes	❍No
Did you ever undergo TMS in the past?	❍Yes	❍No
Did you ever undergo MRI in the past?	❍Yes	❍No

Subject signature:	
Experimenter name:	**Signature:**

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

7.2. APPENDIX II: PUBLICATIONS ARISING FROM THIS THESIS

Goldsworthy MR, Pitcher JB, Ridding MC (2012) The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. Eur J Neurosci 35:125-134.

Goldsworthy MR, Pitcher JB, Ridding MC (2012) A comparison of two different continuous theta burst stimulation paradigms applied to the human primary motor cortex. Clin Neurophysiol 123:2256-2263.

Goldsworthy MR, Pitcher JB, Ridding MC (2013) Neuroplastic modulation of inhibitory motor cortical networks by spaced theta burst stimulation protocols. Brain Stimul 6:340-345.

7.3. APPENDIX III: CHAPTER 2 STATEMENT OF AUTHORSHIP

Goldsworthy MR, Pitcher JB, Ridding MC (2012) The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. Eur J Neurosci 35:125-134.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Mitchell Goldsworthy		
Contribution to the Paper	Experimental design, the collection and analysis of data, interpretation of data, and writing the manuscript.		
Signature		Date	28/05/13

Name of Co-Author	Julia Pitcher		
Contribution to the Paper	Supervision of the project, interpretation of data, and editing the manuscript		
Signature		Date	24/5/13

Name of Co-Author	Michael Ridding		
Contribution to the Paper	Supervision of the project, experimental design, interpretation of data, and editing the manuscript		
Signature		Date	24/05/13

7.4. APPENDIX IV: CHAPTER 3 STATEMENT OF AUTHORSHIP

Goldsworthy MR, Pitcher JB, Ridding MC (2013) Neuroplastic modulation of inhibitory motor cortical networks by spaced theta burst stimulation protocols. Brain Stimul 6:340-345.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Mitchell Goldsworthy		
Contribution to the Paper	Experimental design, the collection and analysis of data, interpretation of data, and writing the manuscript.		
Signature		Date	28/05/13

Name of Co-Author	Julia Pitcher		
Contribution to the Paper	Supervision of the project, interpretation of data, and editing the manuscript		
Signature		Date	24/5/13

Name of Co-Author	Michael Ridding		
Contribution to the Paper	Supervision of the project, experimental design, interpretation of data, and editing the manuscript		
Signature		Date	24/05/13

7.5. APPENDIX V: CHAPTER 4 STATEMENT OF AUTHORSHIP

Goldsworthy MR, Müller-Dahlhaus F, Ridding MC, Ziemann U. De-depression and consolidation of neuroplastic changes in the human motor cortex. *Manuscript in preparation.*

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Mitchell Goldsworthy		
Contribution to the Paper	Experimental design, the collection and analysis of data, interpretation of data, and writing the manuscript.		
Signature		Date	

Name of Co-Author	Florian Müller-Dahlhaus		
Contribution to the Paper	Experimental design, interpretation of data, and editing the manuscript		
Signature		Date	26/05/2013

Name of Co-Author	Michael Ridding		
Contribution to the Paper	Supervision of the project, experimental design, interpretation of data, and editing the manuscript		
Signature		Date	25/05/13

Name of Co-Author	Ulf Ziemann		
Contribution to the Paper	Supervision of the project, experimental design, interpretation of data, and editing the manuscript		
Signature		Date	24/05/2013

7.6. APPENDIX VI: CHAPTER 5 STATEMENT OF AUTHORSHIP

Goldsworthy MR, Pitcher JB, Ridding MC (2012) A comparison of two different continuous theta burst stimulation paradigms applied to the human primary motor cortex. Clin Neurophysiol 123:2256-2263.

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Name of Principal Author (Candidate)	Mitchell Goldsworthy		
Contribution to the Paper	Experimental design, the collection and analysis of data, interpretation of data, and writing the manuscript.		
Signature		Date	28/05/13

Name of Co-Author	Julia Pitcher		
Contribution to the Paper	Supervision of the project, interpretation of data, and editing the manuscript		
Signature		Date	24/5/13

Name of Co-Author	Michael Ridding		
Contribution to the Paper	Supervision of the project, experimental design, interpretation of data, and editing the manuscript		
Signature		Date	24/05/13

8. REFERENCES

Abraham WC (2003) How long will long-term potentiation last? Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 358:735-744.

Abraham WC, Otani S (1991) Macromolecules and the maintenance of long-term potentiation. In: Kindling and synaptic plasticity (Morrel F, ed), pp 92-109. Boston: Birkhäuser.

Abraham WC, Bear MF (1996) Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci 19:126-130.

Abraham WC, Logan B, Greenwood JM, Dragunow M (2002) Induction and experience-dependent consolidation of stable long-term potentiation lasting months in the hippocampus. J Neurosci 22:9626-9634.

Abraham WC, Mason SE, Demmer J, Williams JM, Richardson CL, Tate WP, Lawlor PA, Dragunow M (1993) Correlations between immediate early gene induction and the persistence of long-term potentiation. Neuroscience 56:717-727.

Ackerley SJ, Stinear CM, Barber PA, Byblow WD (2010) Combining theta burst stimulation with training after subcortical stroke. Stroke 41:1568-1572.

Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13:266-271.

Allen GI, Tsukahara N (1974) Cerebrocerebellar communication systems. Physiological reviews 54:957-1006.

Amiaz R, Levy D, Vainiger D, Grunhaus L, Zangen A (2009) Repeated high-frequency transcranial magnetic stimulation over the dorsolateral prefrontal cortex reduces cigarette craving and consumption. Addiction 104:653-660.

Andersen P, Sundberg SH, Sveen O, Swann JW, Wigstrom H (1980) Possible mechanisms for long-lasting potentiation of synaptic transmission in hippocampal slices from guinea-pigs. J Physiol 302:463-482.

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 Stimul 3:230-237.

Bagati D, Nizamie SH, Prakash R (2009) Effect of augmentatory repetitive transcranial magnetic stimulation on auditory hallucinations in schizophrenia: randomized controlled study. Aust N Z J Psychiatry 43:386-392.

Bares M, Kanovsky P, Klajblova H, Rektor I (2003) Intracortical inhibition and facilitation are impaired in patients with early Parkinson's disease: a paired TMS study. Eur J Neurol 10:385-389.

Barker AT, Jalinous R, Freeston IL (1985) Non-invasive magnetic stimulation of human motor cortex. Lancet 1:1106-1107.

Barnes CA (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J Comp Physiol Psychol 93:74-104.

Barrionuevo G, Schottler F, Lynch G (1980) The effects of repetitive low frequency stimulation on control and "potentiated" synaptic responses in the hippocampus. Life sciences 27:2385-2391.

Baxter LR, Jr., Schwartz JM, Phelps ME, Mazziotta JC, Guze BH, Selin CE, Gerner RH, Sumida RM (1989) Reduction of prefrontal cortex glucose metabolism common to three types of depression. Arch Gen Psychiatry 46:243-250.

Bear MF (2003) Bidirectional synaptic plasticity: from theory to reality. Philos Trans R Soc Lond B Biol Sci 358:649-655.

Bench CJ, Friston KJ, Brown RG, Frackowiak RS, Dolan RJ (1993) Regional cerebral blood flow in depression measured by positron emission tomography: the relationship with clinical dimensions. Psychol Med 23:579-590.

Berardelli A, Inghilleri M, Rothwell JC, Romeo S, Curra A, Gilio F, Modugno N, Manfredi M (1998) Facilitation of muscle evoked responses after repetitive cortical stimulation in man. Exp Brain Res 122:79-84.

Betz W (1874) Anatomischer Nachweis zweier Gehirncentra. Centralblatt für die medizinischen Wissenschaften 12:578-580, 595-599.

Bienenstock EL, Cooper LN, Munro PW (1982) Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. J Neurosci 2:32-48.

Bland BH (1986) The physiology and pharmacology of hippocampal formation theta rhythms. Prog Neurobiol 26:1-54.

Bliss TV, Lømo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232:331-356.

Bliss TV, Gardner-Medwin AR (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol 232:357-374.

Boroojerdi B, Prager A, Muellbacher W, Cohen LG (2000) Reduction of human visual cortex excitability using 1-Hz transcranial magnetic stimulation. Neurology 54:1529-1531.

Boroojerdi B, Battaglia F, Muellbacher W, Cohen LG (2001) Mechanisms influencing stimulus-response properties of the human corticospinal system. Clin Neurophysiol 112:931-937.

Brinkman C (1981) Lesions in supplementary motor area interfere with a monkey's performance of a bimanual coordination task. Neurosci Lett 27:267-270.

Brodmann K (1909) Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Leipzig: Barth.

Brown P, Ridding MC, Werhahn KJ, Rothwell JC, Marsden CD (1996) Abnormalities of the balance between inhibition and excitation in the motor cortex of patients with cortical myoclonus. Brain 119 (Pt 1):309-317.

Burt T, Lisanby SH, Sackeim HA (2002) Neuropsychiatric applications of transcranial magnetic stimulation: a meta analysis. Int J Neuropsychopharmacol 5:73-103.

Butefisch CM, Boroojerdi B, Chen R, Battaglia F, Hallett M (2005) Task-dependent intracortical inhibition is impaired in focal hand dystonia. Mov Disord 20:545-551.

Buys EJ, Lemon RN, Mantel GW, Muir RB (1986) Selective facilitation of different hand muscles by single corticospinal neurones in the conscious monkey. J Physiol 381:529-549.

Camprodon JA, Martinez-Raga J, Alonso-Alonso M, Shih MC, Pascual-Leone A (2007) One session of high frequency repetitive transcranial magnetic stimulation (rTMS) to the right prefrontal cortex transiently reduces cocaine craving. Drug Alcohol Depend 86:91-94.

Caporale N, Dan Y (2008) Spike timing-dependent plasticity: a Hebbian learning rule. Annu Rev Neurosci 31:25-46.

Cardenas-Morales L, Nowak DA, Kammer T, Wolf RC, Schonfeldt-Lecuona C (2010) Mechanisms and applications of theta-burst rTMS on the human motor cortex. Brain Topogr 22:294-306.

Carroll RC, Lissin DV, von Zastrow M, Nicoll RA, Malenka RC (1999) Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. Nat Neurosci 2:454-460.

Cash RF, Mastaglia FL, Thickbroom GW (2013) Evidence for high-fidelity timing-dependent synaptic plasticity of human motor cortex. J Neurophysiol 109:106-112.

Cazzoli D, Wurtz P, Muri RM, Hess CW, Nyffeler T (2009) Interhemispheric balance of overt attention: a theta burst stimulation study. Eur J Neurosci 29:1271-1276.

Cazzoli D, Muri RM, Schumacher R, von Arx S, Chaves S, Gutbrod K, Bohlhalter S, Bauer D, Vanbellingen T, Bertschi M, Kipfer S, Rosenthal CR, Kennard C, Bassetti CL, Nyffeler T (2012) Theta burst stimulation reduces disability during the activities of daily living in spatial neglect. Brain 135:3426-3439.

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. J Physiol 586:5717-5725.

Chen R, Lozano AM, Ashby P (1999) Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. Exp Brain Res 128:539-542.

Chen R, Wassermann EM, Canos M, Hallett M (1997a) Impaired inhibition in writer's cramp during voluntary muscle activation. Neurology 49:1054-1059.

Chen R, Samii A, Canos M, Wassermann EM, Hallett M (1997b) Effects of phenytoin on cortical excitability in humans. Neurology 49:881-883.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, Cohen LG (1997c) Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 48:1398-1403.

Chen YL, Huang CC, Hsu KS (2001) Time-dependent reversal of long-term potentiation by low-frequency stimulation at the hippocampal mossy fiber-CA3 synapses. J Neurosci 21:3705-3714.

Chibbaro G, Daniele M, Alagona G, Di Pasquale C, Cannavo M, Rapisarda V, Bella R, Pennisi G (2005) Repetitive transcranial magnetic stimulation in schizophrenic patients reporting auditory hallucinations. Neurosci Lett 383:54-57.

Cohen H, Kaplan Z, Kotler M, Kouperman I, Moisa R, Grisaru N (2004) Repetitive transcranial magnetic stimulation of the right dorsolateral prefrontal cortex in posttraumatic stress disorder: a double-blind, placebo-controlled study. Am J Psychiatry 161:515-524.

Cohen LG, Bandinelli S, Findley TW, Hallett M (1991) Motor reorganization after upper limb amputation in man. A study with focal magnetic stimulation. Brain 114 (Pt 1B):615-627.

Cooke SF, Bliss TV (2006) Plasticity in the human central nervous system. Brain 129:1659-1673.

Coulter JD, Jones EG (1977) Differential distribution of corticospinal projections from individual cytoarchitectonic fields in the monkey. Brain Res 129:335-340.

Cragg BG (1975) The density of synapses and neurons in normal, mentally defective ageing human brains. Brain 98:81-90.

Curran-Everett D (2000) Multiple comparisons: philosophies and illustrations. Am J Physiol Regul Integr Comp Physiol 279:R1-8.

Danysz W, Zajaczkowski W, Parsons CG (1995) Modulation of learning processes by ionotropic glutamate receptor ligands. Behav Pharmacol 6:455-474.

Day BL, Dressler D, Maertens de Noordhout A, Marsden CD, Nakashima K, Rothwell JC, Thompson PD (1989) Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. J Physiol 412:449-473.

de Carvalho M, Miranda PC, Luis ML, Ducla-Soares E (1999) Cortical muscle representation in amyotrophic lateral sclerosis patients: changes with disease evolution. Muscle & nerve 22:1684-1692.

Derkach V, Barria A, Soderling TR (1999) Ca2+/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. Proc Natl Acad Sci U S A 96:3269-3274.

Devanne H, Lavoie BA, Capaday C (1997) Input-output properties and gain changes in the human corticospinal pathway. Exp Brain Res 114:329-338.

Di Lazzaro V, Ziemann U, Lemon RN (2008a) State of the art: Physiology of transcranial motor cortex stimulation. Brain Stimul 1:345-362.

Di Lazzaro V, Oliviero A, Meglio M, Cioni B, Tamburrini G, Tonali P, Rothwell JC (2000) Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. Clin Neurophysiol 111:794-799.

Di Lazzaro V, Oliviero A, Saturno E, Pilato F, Dileone M, Sabatelli M, Tonali PA (2004) Motor cortex stimulation for amyotrophic lateral sclerosis. Time for a therapeutic trial? Clin Neurophysiol 115:1479-1485.

Di Lazzaro V, Dileone M, Pilato F, Contarino MF, Musumeci G, Bentivoglio AR, Tonali PA, Rothwell JC (2006a) Repetitive transcranial magnetic stimulation of the motor cortex for hemichorea. J Neurol Neurosurg Psychiatry 77:1095-1097.

Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, Mazzone P, Tonali P, Rothwell JC (1998a) Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. Electroencephalogr Clin Neurophysiol 109:397-401.

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC (1998b) Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol 508 (Pt 2):625-633.

Di Lazzaro V, Oliviero A, Mazzone P, Insola A, Pilato F, Saturno E, Accurso A, Tonali P, Rothwell JC (2001a) Comparison of descending volleys evoked by monophasic and biphasic magnetic stimulation of the motor cortex in conscious humans. Exp Brain Res 141:121-127.

Di Lazzaro V, Oliviero A, Saturno E, Pilato F, Insola A, Mazzone P, Profice P, Tonali P, Rothwell JC (2001b) The effect on corticospinal volleys of reversing the direction of current induced in the motor cortex by transcranial magnetic stimulation. Exp Brain Res 138:268-273.

Di Lazzaro V, Dileone M, Pilato F, Profice P, Ranieri F, Musumeci G, Angelucci F, Sabatelli M, Tonali PA (2006b) Repetitive transcranial magnetic stimulation for ALS. A preliminary controlled study. Neurosci Lett 408:135-140.

Di Lazzaro V, Oliviero A, Mazzone P, Pilato F, Saturno E, Insola A, Visocchi M, Colosimo C, Tonali PA, Rothwell JC (2002a) Direct demonstration of long latency cortico-cortical inhibition in normal subjects and in a patient with vascular parkinsonism. Clin Neurophysiol 113:1673-1679.

Di Lazzaro V, Oliviero A, Berardelli A, Mazzone P, Insola A, Pilato F, Saturno E, Dileone M, Tonali PA, Rothwell JC (2002b) Direct demonstration of the effects of repetitive transcranial magnetic stimulation on the excitability of the human motor cortex. Exp Brain Res 144:549-553.

Di Lazzaro V, Oliviero A, Profice P, Pennisi MA, Pilato F, Zito G, Dileone M, Nicoletti R, Pasqualetti P, Tonali PA (2003) Ketamine increases human motor cortex excitability to transcranial magnetic stimulation. J Physiol 547:485-496.

Di Lazzaro V, Dileone M, Pilato F, Profice P, Oliviero A, Mazzone P, Insola A, Capone F, Ranieri F, Tonali PA (2009) Associative motor cortex plasticity: direct evidence in humans. Cereb Cortex 19:2326-2330.

Di Lazzaro V, Pilato F, Saturno E, Oliviero A, Dileone M, Mazzone P, Insola A, Tonali PA, Ranieri F, Huang YZ, Rothwell JC (2005) Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. J Physiol 565:945-950.

Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, Insola A, Ranieri F, Meglio M, Tonali PA, Rothwell JC (2008b) The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol 586:3871-3879.

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. J Neurophysiol 105:2150-2156.

Doeltgen SH, Ridding MC (2011a) Low-intensity, short-interval theta burst stimulation modulates excitatory but not inhibitory motor networks. Clin Neurophysiol 122:1411-1416.

Doeltgen SH, Ridding MC (2011b) Modulation of cortical motor networks following primed theta burst transcranial magnetic stimulation. Exp Brain Res 215:199-206.

Donoghue JP, Leibovic S, Sanes JN (1992) Organization of the forelimb area in squirrel monkey motor cortex: representation of digit, wrist, and elbow muscles. Exp Brain Res 89:1-19.

Dum RP, Strick PL (1991) The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci 11:667-689.

Eichhammer P, Johann M, Kharraz A, Binder H, Pittrow D, Wodarz N, Hajak G (2003) High-frequency repetitive transcranial magnetic stimulation decreases cigarette smoking. J Clin Psychiatry 64:951-953.

Enna SJ (2007) The GABA receptors. In: The GABA receptors (Enna SJ, Möhler H, eds), pp 1-21. Totowa: Humana Press.

Fitzgerald PB, Hoy K, Daskalakis ZJ, Kulkarni J (2009) A randomized trial of the anti-depressant effects of low- and high-frequency transcranial magnetic stimulation in treatment-resistant depression. Depression and anxiety 26:229-234.

Fitzgerald PB, Brown TL, Marston NA, Daskalakis ZJ, De Castella A, Kulkarni J (2003) Transcranial magnetic stimulation in the treatment of depression: a double-blind, placebo-controlled trial. Arch Gen Psychiatry 60:1002-1008.

Fregni F, Boggio PS, Valle AC, Rocha RR, Duarte J, Ferreira MJ, Wagner T, Fecteau S, Rigonatti SP, Riberto M, Freedman SD, Pascual-Leone A (2006) A sham-controlled trial of a 5-day course of repetitive transcranial magnetic stimulation of the unaffected hemisphere in stroke patients. Stroke 37:2115-2122.

Frey U, Morris RG (1997) Synaptic tagging and long-term potentiation. Nature 385:533-536.

Fujii S, Saito K, Miyakawa H, Ito K, Kato H (1991) Reversal of long-term potentiation (depotentiation) induced by tetanus stimulation of the input to CA1 neurons of guinea pig hippocampal slices. Brain Res 555:112-122.

Gamboa OL, Antal A, Moliadze V, Paulus W (2010) Simply longer is not better: reversal of theta burst after-effect with prolonged stimulation. Exp Brain Res 204:181-187.

Gamboa OL, Antal A, Laczo B, Moliadze V, Nitsche MA, Paulus W (2011) Impact of repetitive theta burst stimulation on motor cortex excitability. Brain Stimulation 4:145-151.

Gangitano M, Valero-Cabre A, Tormos JM, Mottaghy FM, Romero JR, Pascual-Leone A (2002) Modulation of input-output curves by low and high frequency repetitive transcranial magnetic stimulation of the motor cortex. Clin Neurophysiol 113:1249-1257.

Gentner R, Wankerl K, Reinsberger C, Zeller D, Classen J (2008) Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: evidence of rapid polarity-reversing metaplasticity. Cereb Cortex 18:2046-2053.

George MS, Wassermann EM, Williams WA, Callahan A, Ketter TA, Basser P, Hallett M, Post RM (1995) Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. Neuroreport 6:1853-1856.

George MS, Wassermann EM, Williams WA, Steppel J, Pascual-Leone A, Basser P, Hallett M, Post RM (1996) Changes in mood and hormone levels after rapid-rate transcranial magnetic stimulation (rTMS) of the prefrontal cortex. J Neuropsychiatry Clin Neurosci 8:172-180.

George MS, Nahas Z, Molloy M, Speer AM, Oliver NC, Li XB, Arana GW, Risch SC, Ballenger JC (2000) A controlled trial of daily left prefrontal cortex TMS for treating depression. Biol Psychiatry 48:962-970.

George MS, Lisanby SH, Avery D, McDonald WM, Durkalski V, Pavlicova M, Anderson B, Nahas Z, Bulow P, Zarkowski P, Holtzheimer PE, 3rd, Schwartz T, Sackeim HA (2010) Daily left prefrontal transcranial magnetic stimulation therapy for major depressive disorder: a sham-controlled randomized trial. Arch Gen Psychiatry 67:507-516.

Ghosh S, Brinkman C, Porter R (1987) A quantitative study of the distribution of neurons projecting to the precentral motor cortex in the monkey (M. fascicularis). The Journal of comparative neurology 259:424-444.

Goldsworthy MR, Pitcher JB, Ridding MC (2012) The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. Eur J Neurosci 35:125-134.

Gould HJ, 3rd, Cusick CG, Pons TP, Kaas JH (1986) The relationship of corpus callosum connections to electrical stimulation maps of motor, supplementary motor, and the frontal eye fields in owl monkeys. The Journal of comparative neurology 247:297-325.

Greenberg BD, George MS, Martin JD, Benjamin J, Schlaepfer TE, Altemus M, Wassermann EM, Post RM, Murphy DL (1997) Effect of prefrontal repetitive transcranial magnetic stimulation in obsessive-compulsive disorder: a preliminary study. Am J Psychiatry 154:867-869.

Grisaru N, Amir M, Cohen H, Kaplan Z (1998) Effect of transcranial magnetic stimulation in posttraumatic stress disorder: a preliminary study. Biol Psychiatry 44:52-55.

Hallett M (2001) Plasticity of the human motor cortex and recovery from stroke. Brain Res Brain Res Rev 36:169-174.

Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC (2013) The Role of Interneuron Networks in Driving Human Motor Cortical Plasticity. Cereb Cortex 23:1593-1605.

Hamada M, Terao Y, Hanajima R, Shirota Y, Nakatani-Enomoto S, Furubayashi T, Matsumoto H, Ugawa Y (2008) Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. J Physiol 586:3927-3947.

Hamada M, Hanajima R, Terao Y, Arai N, Furubayashi T, Inomata-Terada S, Yugeta A, Matsumoto H, Shirota Y, Ugawa Y (2007) Quadro-pulse stimulation is more effective than paired-pulse stimulation for plasticity induction of the human motor cortex. Clin Neurophysiol 118:2672-2682.

Hebb DO (1949) The organization of behaviour: a neuropsychological theory. New York: Wiley.

Hendry SH, Jones EG (1981) Sizes and distributions of intrinsic neurons incorporating tritiated GABA in monkey sensory-motor cortex. J Neurosci 1:390-408.

Hess G, Donoghue JP (1994) Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. J Neurophysiol 71:2543-2547.

Hoffman RE, Hawkins KA, Gueorguieva R, Boutros NN, Rachid F, Carroll K, Krystal JH (2003) Transcranial magnetic stimulation of left temporoparietal cortex and medication-resistant auditory hallucinations. Arch Gen Psychiatry 60:49-56.

Holtzheimer PE, 3rd, Russo J, Avery DH (2001) A meta-analysis of repetitive transcranial magnetic stimulation in the treatment of depression. Psychopharmacol Bull 35:149-169.

Hoogendam JM, Ramakers GM, Di Lazzaro V (2010) Physiology of repetitive transcranial magnetic stimulation of the human brain. Brain Stimul 3:95-118.

Howe JR, Sutor B, Zieglgansberger W (1987) Baclofen reduces post-synaptic potentials of rat cortical neurones by an action other than its hyperpolarizing action. J Physiol 384:539-569.

Huang CC, Liang YC, Hsu KS (1999) A role for extracellular adenosine in time-dependent reversal of long-term potentiation by low-frequency stimulation at hippocampal CA1 synapses. J Neurosci 19:9728-9738.

Huang YY, Kandel ER (1994) Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. Learn Mem 1:74-82.

Huang YY, Colino A, Selig DK, Malenka RC (1992) The influence of prior synaptic activity on the induction of long-term potentiation. Science 255:730-733.

Huang YY, Simpson E, Kellendonk C, Kandel ER (2004) Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. Proc Natl Acad Sci U S A 101:3236-3241.

Huang YZ, Chen RS, Rothwell JC, Wen HY (2007) The after-effect of human theta burst stimulation is NMDA receptor dependent. Clin Neurophysiol 118:1028-1032.

Huang YZ, Rothwell JC, Edwards MJ, Chen RS (2008) Effect of physiological activity on an NMDA-dependent form of cortical plasticity in human. Cereb Cortex 18:563-570.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC (2005) Theta burst stimulation of the human motor cortex. Neuron 45:201-206.

Huang YZ, Rothwell JC, Lu CS, Chuang WL, Lin WY, Chen RS (2010) Reversal of plasticity-like effects in the human motor cortex. J Physiol 588:3683-3693.

Isenberg K, Downs D, Pierce K, Svarakic D, Garcia K, Jarvis M, North C, Kormos TC (2005) Low frequency rTMS stimulation of the right frontal cortex is as

effective as high frequency rTMS stimulation of the left frontal cortex for antidepressant-free, treatment-resistant depressed patients. Annals of clinical psychiatry : official journal of the American Academy of Clinical Psychiatrists 17:153-159.

Iyer MB, Schleper N, Wassermann EM (2003) Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. J Neurosci 23:10867-10872.

Izumi Y, Clifford DB, Zorumski CF (1992) Inhibition of long-term potentiation by NMDA-mediated nitric oxide release. Science 257:1273-1276.

Jackson JH (1873) Observations on the localisation of movements in the cerebral hemispheres, as revealed by cases of convulsion, chorea, and" aphasia". West Riding Asylum Medical Reports 3:175-195.

Jeffery KJ, Abraham WC, Dragunow M, Mason SE (1990) Induction of Fos-like immunoreactivity and the maintenance of long-term potentiation in the dentate gyrus of unanesthetized rats. Brain Res Mol Brain Res 8:267-274.

Jones EG, Wise SP (1977) Size, laminar and columnar distribution of efferent cells in the sensory-motor cortex of monkeys. The Journal of comparative neurology 175:391-438.

Ju W, Morishita W, Tsui J, Gaietta G, Deerinck TJ, Adams SR, Garner CC, Tsien RY, Ellisman MH, Malenka RC (2004) Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. Nat Neurosci 7:244-253.

Jung P, Ziemann U (2009) Homeostatic and nonhomeostatic modulation of learning in human motor cortex. J Neurosci 29:5597-5604.

Kauderer BS, Kandel ER (2000) Capture of a protein synthesis-dependent component of long-term depression. Proc Natl Acad Sci U S A 97:13342-13347.

Kerr DI, Ong J (1995) GABAB receptors. Pharmacology & therapeutics 67:187-246.

Khedr EM, Farweez HM, Islam H (2003) Therapeutic effect of repetitive transcranial magnetic stimulation on motor function in Parkinson's disease patients. Eur J Neurol 10:567-572.

Khedr EM, Ahmed MA, Fathy N, Rothwell JC (2005) Therapeutic trial of repetitive transcranial magnetic stimulation after acute ischemic stroke. Neurology 65:466-468.

Kirkwood A, Dudek SM, Gold JT, Aizenman CD, Bear MF (1993) Common forms of synaptic plasticity in the hippocampus and neocortex in vitro. Science 260:1518-1521.

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. Nat Neurosci 9:735-737.

Klöppel S, Baumer T, Kroeger J, Koch MA, Buchel C, Munchau A, Siebner HR (2008) The cortical motor threshold reflects microstructural properties of cerebral white matter. NeuroImage 40:1782-1791.

Krug M, Lossner B, Ott T (1984) Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. Brain Res Bull 13:39-42.

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. J Physiol 471:501-519.

Lamy JC, Russmann H, Shamim EA, Meunier S, Hallett M (2010) Paired associative stimulation induces change in presynaptic inhibition of Ia terminals in wrist flexors in humans. J Neurophysiol 104:755-764.

Lang N, Speck S, Harms J, Rothkegel H, Paulus W, Sommer M (2008) Dopaminergic potentiation of rTMS-induced motor cortex inhibition. Biol Psychiatry 63:231-233.

Larson J, Wong D, Lynch G (1986) Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. Brain Res 368:347-350.

Larson J, Lieu T, Petchpradub V, LeDuc B, Ngo H, Rogers GA, Lynch G (1995) Facilitation of olfactory learning by a modulator of AMPA receptors. J Neurosci 15:8023-8030.

Lee H-K, Kameyama K, Huganir RL, Bear MF (1998) NMDA Induces Long-Term Synaptic Depression and Dephosphorylation of the GluR1 Subunit of AMPA Receptors in Hippocampus. Neuron 21:1151-1162.

Lee HK, Takamiya K, Han JS, Man H, Kim CH, Rumbaugh G, Yu S, Ding L, He C, Petralia RS, Wenthold RJ, Gallagher M, Huganir RL (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell 112:631-643.

Lee SH, Kim W, Chung YC, Jung KH, Bahk WM, Jun TY, Kim KS, George MS, Chae JH (2005) A double blind study showing that two weeks of daily repetitive TMS over the left or right temporoparietal cortex reduces symptoms in patients with schizophrenia who are having treatment-refractory auditory hallucinations. Neurosci Lett 376:177-181.

Levy WB, Steward O (1979) Synapses as associative memory elements in the hippocampal formation. Brain Res 175:233-245.

Linden DJ (1996) A Protein Synthesis-Dependent Late Phase of Cerebellar Long-Term Depression. Neuron 17:483-490.

Lomarev MP, Kanchana S, Bara-Jimenez W, Iyer M, Wassermann EM, Hallett M (2006) Placebo-controlled study of rTMS for the treatment of Parkinson's disease. Mov Disord 21:325-331.

Lømo T (1966) Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. Acta physiol scand 68:128.

Lotze M, Markert J, Sauseng P, Hoppe J, Plewnia C, Gerloff C (2006) The role of multiple contralesional motor areas for complex hand movements after internal capsular lesion. J Neurosci 26:6096-6102.

Lund JS (1973) Organization of neurons in the visual cortex, area 17, of the monkey (Macaca mulatta). The Journal of comparative neurology 147:455-496.

Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A (2000a) Modulation of corticospinal excitability by repetitive transcranial magnetic stimulation. Clin Neurophysiol 111:800-805.

Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A (2000b) Interindividual variability of the modulatory effects of repetitive transcranial magnetic stimulation on cortical excitability. Exp Brain Res 133:425-430.

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron 44:5-21.

Mansur CG, Fregni F, Boggio PS, Riberto M, Gallucci-Neto J, Santos CM, Wagner T, Rigonatti SP, Marcolin MA, Pascual-Leone A (2005) A sham stimulation-controlled trial of rTMS of the unaffected hemisphere in stroke patients. Neurology 64:1802-1804.

Martin JL, Barbanoj MJ, Schlaepfer TE, Thompson E, Perez V, Kulisevsky J (2003) Repetitive transcranial magnetic stimulation for the treatment of depression. Systematic review and meta-analysis. Br J Psychiatry 182:480-491.

Martin PG, Gandevia SC, Taylor JL (2006) Theta burst stimulation does not reliably depress all regions of the human motor cortex. Clin Neurophysiol 117:2684-2690.

Martinot JL, Hardy P, Feline A, Huret JD, Mazoyer B, Attar-Levy D, Pappata S, Syrota A (1990) Left prefrontal glucose hypometabolism in the depressed state: a confirmation. Am J Psychiatry 147:1313-1317.

Mastroeni C, Bergmann TO, Rizzo V, Ritter C, Klein C, Pohlmann I, Brueggemann N, Quartarone A, Siebner HR (2013) Brain-derived neurotrophic factor--a major player in stimulation-induced homeostatic metaplasticity of human motor cortex? PloS one 8:e57957.

Mavroudakis N, Caroyer JM, Brunko E, Zegers de Beyl D (1994) Effects of diphenylhydantoin on motor potentials evoked with magnetic stimulation. Electroencephalogr Clin Neurophysiol 93:428-433.

McAllister SM, Rothwell JC, Ridding MC (2009) Selective modulation of intracortical inhibition by low-intensity Theta Burst Stimulation. Clin Neurophysiol 120:820-826.

McAllister SM, Rothwell JC, Ridding MC (2011) Cortical oscillatory activity and the induction of plasticity in the human motor cortex. Eur J Neurosci 33:1916-1924.

McDonnell MN, Orekhov Y, Ziemann U (2006) The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. Exp Brain Res 173:86-93.

McNaughton BL, Douglas RM, Goddard GV (1978) Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. Brain Res 157:277-293.

Meldrum BS (2000) Glutamate as a neurotransmitter in the brain: review of physiology and pathology. The Journal of nutrition 130:1007S-1015S.

Merton PA, Morton HB (1980) Stimulation of the cerebral cortex in the intact human subject. Nature 285:227.

Meunier S, Russmann H, Simonetta-Moreau M, Hallett M (2007) Changes in spinal excitability after PAS. J Neurophysiol 97:3131-3135.

Meyer G (1987) Forms and spatial arrangement of neurons in the primary motor cortex of man. The Journal of comparative neurology 262:402-428.

Milner-Brown SH, Girvin JP, Brown WF (1975) The effects of motor cortical stimulation on the excitability of spinal motoneurons in man. The Canadian journal of neurological sciences Le journal canadien des sciences neurologiques 2:245-253.

Mishra BR, Nizamie SH, Das B, Praharaj SK (2010) Efficacy of repetitive transcranial magnetic stimulation in alcohol dependence: a sham-controlled study. Addiction 105:49-55.

Mitchell PB, Loo CK (2006) Transcranial magnetic stimulation for depression. Aust N Z J Psychiatry 40:406-413.

Modugno N, Nakamura Y, MacKinnon CD, Filipovic SR, Bestmann S, Berardelli A, Rothwell JC (2001) Motor cortex excitability following short trains of repetitive magnetic stimuli. Exp Brain Res 140:453-459.

Monte-Silva K, Kuo MF, Liebetanz D, Paulus W, Nitsche MA (2010) Shaping the optimal repetition interval for cathodal transcranial direct current stimulation (tDCS). J Neurophysiol 103:1735-1740.

Mori F, Ribolsi M, Kusayanagi H, Siracusano A, Mantovani V, Marasco E, Bernardi G, Centonze D (2011) Genetic variants of the NMDA receptor influence cortical excitability and plasticity in humans. J Neurophysiol 106:1637-1643.

Morris RG, Anderson E, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319:774-776.

Mountcastle VB (1997) The columnar organization of the neocortex. Brain 120 (Pt 4):701-722.

Muellbacher W, Ziemann U, Boroojerdi B, Hallett M (2000) Effects of low-frequency transcranial magnetic stimulation on motor excitability and basic motor behavior. Clin Neurophysiol 111:1002-1007.

Muellbacher W, Ziemann U, Wissel J, Dang N, Kofler M, Facchini S, Boroojerdi B, Poewe W, Hallett M (2002) Early consolidation in human primary motor cortex. Nature 415:640-644.

Müller JF, Orekhov Y, Liu Y, Ziemann U (2007) Homeostatic plasticity in human motor cortex demonstrated by two consecutive sessions of paired associative stimulation. Eur J Neurosci 25:3461-3468.

Murakami T, Muller-Dahlhaus F, Lu MK, Ziemann U (2012) Homeostatic metaplasticity of corticospinal excitatory and intracortical inhibitory neural circuits in human motor cortex. J Physiol 590:5765-5781.

Murakami T, Sakuma K, Nomura T, Nakashima K, Hashimoto I (2008) High-frequency oscillations change in parallel with short-interval intracortical inhibition after theta burst magnetic stimulation. Clin Neurophysiol 119:301-308.

Murase N, Duque J, Mazzocchio R, Cohen LG (2004) Influence of interhemispheric interactions on motor function in chronic stroke. Ann Neurol 55:400-409.

Murase N, Rothwell JC, Kaji R, Urushihara R, Nakamura K, Murayama N, Igasaki T, Sakata-Igasaki M, Mima T, Ikeda A, Shibasaki H (2005) Subthreshold low-frequency repetitive transcranial magnetic stimulation over the premotor cortex modulates writer's cramp. Brain 128:104-115.

Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H (1997) Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. J Physiol 498 (Pt 3):817-823.

Nayak A, Zastrow DJ, Lickteig R, Zahniser NR, Browning MD (1998) Maintenance of late-phase LTP is accompanied by PKA-dependent increase in AMPA receptor synthesis. Nature 394:680-683.

Nguyen PV, Abel T, Kandel ER (1994) Requirement of a critical period of transcription for induction of a late phase of LTP. Science 265:1104-1107.

Nishiyama M, Hong K, Mikoshiba K, Poo MM, Kato K (2000) Calcium stores regulate the polarity and input specificity of synaptic modification. Nature 408:584-588.

Nitsche MA, Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 527 Pt 3:633-639.

Nitsche MA, Paulus W (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 57:1899-1901.

Nitsche MA, Fricke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N, Henning S, Tergau F, Paulus W (2003) Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. J Physiol 553:293-301.

Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462-465.

Nudo RJ, Jenkins WM, Merzenich MM, Prejean T, Grenda R (1992) Neurophysiological correlates of hand preference in primary motor cortex of adult squirrel monkeys. J Neurosci 12:2918-2947.

Nyffeler T, Cazzoli D, Hess CW, Muri RM (2009) One session of repeated parietal theta burst stimulation trains induces long-lasting improvement of visual neglect. Stroke 40:2791-2796.

Nyffeler T, Wurtz P, Luscher HR, Hess CW, Senn W, Pflugshaupt T, von Wartburg R, Luthi M, Muri RM (2006a) Extending lifetime of plastic changes in the human brain. Eur J Neurosci 24:2961-2966.

Nyffeler T, Wurtz P, Luscher HR, Hess CW, Senn W, Pflugshaupt T, von Wartburg R, Luthi M, Muri RM (2006b) Repetitive TMS over the human oculomotor cortex: comparison of 1-Hz and theta burst stimulation. Neurosci Lett 409:57-60.

Nyffeler T, Cazzoli D, Wurtz P, Luthi M, von Wartburg R, Chaves S, Deruaz A, Hess CW, Muri RM (2008) Neglect-like visual exploration behaviour after theta burst transcranial magnetic stimulation of the right posterior parietal cortex. Eur J Neurosci 27:1809-1813.

O'Dell TJ, Kandel ER (1994) Low-frequency stimulation erases LTP through an NMDA receptor-mediated activation of protein phosphatases. Learn Mem 1:129-139.

O'Keefe J, Nadel L (1978) The hippocampus as a cognitive map. Oxford: Oxford University Press.

O'Reardon JP, Solvason HB, Janicak PG, Sampson S, Isenberg KE, Nahas Z, McDonald WM, Avery D, Fitzgerald PB, Loo C, Demitrack MA, George MS, Sackeim HA (2007) Efficacy and safety of transcranial magnetic stimulation in the acute treatment of major depression: a multisite randomized controlled trial. Biol Psychiatry 62:1208-1216.

Orth M, Snijders AH, Rothwell JC (2003) The variability of intracortical inhibition and facilitation. Clin Neurophysiol 114:2362-2369.

Pascual-Leone A, Rubio B, Pallardo F, Catala MD (1996) Rapid-rate transcranial magnetic stimulation of left dorsolateral prefrontal cortex in drug-resistant depression. Lancet 348:233-237.

Pascual-Leone A, Valls-Sole J, Brasil-Neto JP, Cammarota A, Grafman J, Hallett M (1994) Akinesia in Parkinson's disease. II. Effects of subthreshold repetitive transcranial motor cortex stimulation. Neurology 44:892-898.

Pascual-Leone A, Nguyet D, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M (1995) Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. J Neurophysiol 74:1037-1045.

Passingham R (1987) Two cortical systems for directing movement. Motor areas of the cerebral cortex. Ciba Found Symp 132:151-164.

Passingham RE (1985) Premotor cortex: sensory cues and movement. Behavioural brain research 18:175-185.

Patton HD, Amassian VE (1954) Single and multiple-unit analysis of cortical stage of pyramidal tract activation. J Neurophysiol 17:345-363.

Paulus W, Classen J, Cohen LG, Large CH, Di Lazzaro V, Nitsche M, Pascual-Leone A, Rosenow F, Rothwell JC, Ziemann U (2008) State of the art: Pharmacologic effects on cortical excitability measures tested by transcranial magnetic stimulation. Brain Stimul 1:151-163.

Pearce RA (1993) Physiological evidence for two distinct GABAA responses in rat hippocampus. Neuron 10:189-200.

Penfield W, Boldrey E (1937) Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain 60:389-443.

Penfield W, Rasmussen T (1950) The cerebral cortex of man. New York: Macmillan.

Pesaran B, Nelson MJ, Andersen RA (2006) Dorsal premotor neurons encode the relative position of the hand, eye, and goal during reach planning. Neuron 51:125-134.

Peurala SH, Muller-Dahlhaus JF, Arai N, Ziemann U (2008) Interference of short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF). Clin Neurophysiol 119:2291-2297.

Pierau FK, Zimmermann P (1973) Action of a GABA-derivative on postsynaptic potentials and membrane properties of cats' spinal motoneurones. Brain Res 54:376-380.

Pierrot-Deseilligny C, Rivaud S, Gaymard B, Muri R, Vermersch AI (1995) Cortical control of saccades. Ann Neurol 37:557-567.

Player MJ, Taylor JL, Alonzo A, Loo CK (2012) Paired associative stimulation increases motor cortex excitability more effectively than theta-burst stimulation. Clin Neurophysiol 123:2220-2226.

Porter R, Lemon R (1993) Corticospinal function and voluntary movement. Oxford: Clarendon Press.

Poulet E, Brunelin J, Bediou B, Bation R, Forgeard L, Dalery J, d'Amato T, Saoud M (2005) Slow transcranial magnetic stimulation can rapidly reduce resistant auditory hallucinations in schizophrenia. Biol Psychiatry 57:188-191.

Racine RJ, Milgram NW, Hafner S (1983) Long-term potentiation phenomena in the rat limbic forebrain. Brain Res 260:217-231.

Racine RJ, Chapman CA, Trepel C, Teskey GC, Milgram NW (1995) Post-activation potentiation in the neocortex. IV. Multiple sessions required for induction of long-term potentiation in the chronic preparation. Brain Res 702:87-93.

Ridding MC, Rothwell JC (1997) Stimulus/response curves as a method of measuring motor cortical excitability in man. Electroencephalogr Clin Neurophysiol 105:340-344.

Ridding MC, Rothwell JC (2007) Is there a future for therapeutic use of transcranial magnetic stimulation? Nat Rev Neurosci 8:559-567.

Ridding MC, Ziemann U (2010) Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. J Physiol 588:2291-2304.

Ridding MC, Inzelberg R, Rothwell JC (1995a) Changes in excitability of motor cortical circuitry in patients with Parkinson's disease. Ann Neurol 37:181-188.

Ridding MC, Sheean G, Rothwell JC, Inzelberg R, Kujirai T (1995b) Changes in the balance between motor cortical excitation and inhibition in focal, task specific dystonia. J Neurol Neurosurg Psychiatry 59:493-498.

Rioult-Pedotti MS, Friedman D, Donoghue JP (2000) Learning-induced LTP in neocortex. Science 290:533-536.

Rioult-Pedotti MS, Friedman D, Hess G, Donoghue JP (1998) Strengthening of horizontal cortical connections following skill learning. Nat Neurosci 1:230-234.

Rockel AJ, Hiorns RW, Powell TP (1980) The basic uniformity in structure of the neocortex. Brain 103:221-244.

Rogan MT, Staubli UV, LeDoux JE (1997) AMPA receptor facilitation accelerates fear learning without altering the level of conditioned fear acquired. J Neurosci 17:5928-5935.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A (2009) Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 120:2008-2039.

Rothwell JC (1994) Control of human voluntary movement. London: Chapman and Hall.

Sachdev PS, McBride R, Loo CK, Mitchell PB, Malhi GS, Croker VM (2001) Right versus left prefrontal transcranial magnetic stimulation for obsessive-compulsive disorder: a preliminary investigation. J Clin Psychiatry 62:981-984.

Sale MV, Ridding MC, Nordstrom MA (2007) Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp Brain Res 181:615-626.

Schaechter JD (2004) Motor rehabilitation and brain plasticity after hemiparetic stroke. Prog Neurobiol 73:61-72.

Shima K, Tanji J (1998) Role for cingulate motor area cells in voluntary movement selection based on reward. Science 282:1335-1338.

Shors TJ, Servatius RJ, Thompson RF, Rogers G, Lynch G (1995) Enhanced glutamatergic neurotransmission facilitates classical conditioning in the freely moving rat. Neurosci Lett 186:153-156.

Siebner HR (2010) A primer on priming the human motor cortex. Clin Neurophysiol 121:461-463.

Siebner HR, Tormos JM, Ceballos-Baumann AO, Auer C, Catala MD, Conrad B, Pascual-Leone A (1999) Low-frequency repetitive transcranial magnetic stimulation of the motor cortex in writer's cramp. Neurology 52:529-537.

Siebner HR, Lang N, Rizzo V, Nitsche MA, Paulus W, Lemon RN, Rothwell JC (2004) Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for homeostatic plasticity in the human motor cortex. J Neurosci 24:3379-3385.

Sloper JJ (1973) An electron microscopic study of the neurons of the primate motor and somatic sensory cortices. Journal of neurocytology 2:351-359.

Sloper JJ, Hiorns RW, Powell TP (1979) A qualitative and quantitative electron microscopic study of the neurons in the primate motor and somatic sensory cortices. Philos Trans R Soc Lond B Biol Sci 285:141-171.

Sogaard K, Gandevia SC, Todd G, Petersen NT, Taylor JL (2006) The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. J Physiol 573:511-523.

Solis JM, Nicoll RA (1992) Pharmacological characterization of GABAB-mediated responses in the CA1 region of the rat hippocampal slice. J Neurosci 12:3466-3472.

Staubli U, Lynch G (1990) Stable depression of potentiated synaptic responses in the hippocampus with 1-5 Hz stimulation. Brain Res 513:113-118.

Staubli U, Rogers G, Lynch G (1994a) Facilitation of glutamate receptors enhances memory. Proc Natl Acad Sci U S A 91:777-781.

Staubli U, Perez Y, Xu FB, Rogers G, Ingvar M, Stone-Elander S, Lynch G (1994b) Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation in vivo. Proc Natl Acad Sci U S A 91:11158-11162.

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J (2000) Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 123 Pt 3:572-584.

Stefan K, Kunesch E, Benecke R, Cohen LG, Classen J (2002) Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J Physiol 543:699-708.

Stefan K, Wycislo M, Gentner R, Schramm A, Naumann M, Reiners K, Classen J (2006) Temporary occlusion of associative motor cortical plasticity by prior dynamic motor training. Cereb Cortex 16:376-385.

Steward O, Schuman EM (2001) Protein synthesis at synaptic sites on dendrites. Annu Rev Neurosci 24:299-325.

Strafella AP, Valzania F, Nassetti SA, Tropeani A, Bisulli A, Santangelo M, Tassinari CA (2000) Effects of chronic levodopa and pergolide treatment on cortical excitability in patients with Parkinson's disease: a transcranial magnetic stimulation study. Clin Neurophysiol 111:1198-1202.

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. J Physiol 586:4489-4500.

Swayne OB, Teo JT, Greenwood RJ, Rothwell JC (2009) The facilitatory effects of intermittent theta burst stimulation on corticospinal excitability are enhanced by nicotine. Clin Neurophysiol 120:1610-1615.

Takahashi T, Svoboda K, Malinow R (2003) Experience strengthening transmission by driving AMPA receptors into synapses. Science 299:1585-1588.

Takeuchi N, Chuma T, Matsuo Y, Watanabe I, Ikoma K (2005) Repetitive transcranial magnetic stimulation of contralesional primary motor cortex improves hand function after stroke. Stroke 36:2681-2686.

Talelli P, Greenwood RJ, Rothwell JC (2007) Exploring Theta Burst Stimulation as an intervention to improve motor recovery in chronic stroke. Clin Neurophysiol 118:333-342.

Talelli P, Wallace A, Dileone M, Hoad D, Cheeran B, Oliver R, VandenBos M, Hammerbeck U, Barratt K, Gillini C, Musumeci G, Boudrias MH, Cloud GC, Ball J, Marsden JF, Ward NS, Di Lazzaro V, Greenwood RG, Rothwell JC (2012) Theta burst stimulation in the rehabilitation of the upper limb: a semirandomized, placebo-controlled trial in chronic stroke patients. Neurorehabilitation and neural repair 26:976-987.

Taylor JL, Loo CK (2007) Stimulus waveform influences the efficacy of repetitive transcranial magnetic stimulation. J Affect Disord 97:271-276.

Tehovnik EJ, Sommer MA, Chou IH, Slocum WM, Schiller PH (2000) Eye fields in the frontal lobes of primates. Brain Res Brain Res Rev 32:413-448.

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. Clin Neurophysiol 117:61-66.

Thirugnanasambandam N, Sparing R, Dafotakis M, Meister IG, Paulus W, Nitsche MA, Fink GR (2011) Isometric contraction interferes with transcranial direct current stimulation (tDCS) induced plasticity: evidence of state-dependent

neuromodulation in human motor cortex. Restorative neurology and neuroscience 29:311-320.

Todd G, Flavel SC, Ridding MC (2006) Low-intensity repetitive transcranial magnetic stimulation decreases motor cortical excitability in humans. J Appl Physiol 101:500-505.

Todd G, Flavel SC, Ridding MC (2009a) Priming theta-burst repetitive transcranial magnetic stimulation with low- and high-frequency stimulation. Exp Brain Res 195:307-315.

Todd G, Rogasch NC, Flavel SC, Ridding MC (2009b) Voluntary movement and repetitive transcranial magnetic stimulation over human motor cortex. J Appl Physiol 106:1593-1603.

Topka H, Cohen LG, Cole RA, Hallett M (1991) Reorganization of corticospinal pathways following spinal cord injury. Neurology 41:1276-1283.

Torriero S, Oliveri M, Koch G, Lo Gerfo E, Salerno S, Ferlazzo F, Caltagirone C, Petrosini L (2011) Changes in cerebello-motor connectivity during procedural learning by actual execution and observation. Journal of cognitive neuroscience 23:338-348.

Touge T, Gerschlager W, Brown P, Rothwell JC (2001) Are the after-effects of low-frequency rTMS on motor cortex excitability due to changes in the efficacy of cortical synapses? Clinical Neurophysiology 112:2138-2145.

Trepel C, Racine RJ (1998) Long-term potentiation in the neocortex of the adult, freely moving rat. Cereb Cortex 8:719-729.

Tsien JZ, Huerta PT, Tonegawa S (1996) The Essential Role of Hippocampal CA1 NMDA Receptor-Dependent Synaptic Plasticity in Spatial Memory. Cell 87:1327-1338.

Ugawa Y, Rothwell JC, Day BL, Thompson PD, Marsden CD (1991) Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. Ann Neurol 29:418-427.

Ugawa Y, Uesaka Y, Terao Y, Hanajima R, Kanazawa I (1994) Magnetic stimulation of corticospinal pathways at the foramen magnum level in humans. Ann Neurol 36:618-624.

Urban NN, Henze DA, Lewis DA, Barrionuevo G (1996) Properties of LTP induction in the CA3 region of the primate hippocampus. Learn Mem 3:86-95.

Valls-Sole J, Pascual-Leone A, Wassermann EM, Hallett M (1992) Human motor evoked responses to paired transcranial magnetic stimuli. Electroencephalogr Clin Neurophysiol 85:355-364.

Vanderwolf CH (1969) Hippocampal electrical activity and voluntary movement in the rat. Electroencephalogr Clin Neurophysiol 26:407-418.

Vucic S, Ziemann U, Eisen A, Hallett M, Kiernan MC (2012) Transcranial magnetic stimulation and amyotrophic lateral sclerosis: pathophysiological insights. J Neurol Neurosurg Psychiatry.

Wassermann EM, McShane LM, Hallett M, Cohen LG (1992) Noninvasive mapping of muscle representations in human motor cortex. Electroencephalogr Clin Neurophysiol 85:1-8.

Wexler EM, Stanton PK (1993) Priming of homosynaptic long-term depression in hippocampus by previous synaptic activity. Neuroreport 4:591-594.

Wolters A, Sandbrink F, Schlottmann A, Kunesch E, Stefan K, Cohen LG, Benecke R, Classen J (2003) A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. J Neurophysiol 89:2339-2345.

Woo NH, Nguyen PV (2002) "Silent" metaplasticity of the late phase of long-term potentiation requires protein phosphatases. Learn Mem 9:202-213.

Woo NH, Nguyen PV (2003) Protein synthesis is required for synaptic immunity to depotentiation. J Neurosci 23:1125-1132.

Xu L, Anwyl R, Rowan MJ (1998) Spatial exploration induces a persistent reversal of long-term potentiation in rat hippocampus. Nature 394:891-894.

Young JZ, Nguyen PV (2005) Homosynaptic and heterosynaptic inhibition of synaptic tagging and capture of long-term potentiation by previous synaptic activity. J Neurosci 25:7221-7231.

Young JZ, Isiegas C, Abel T, Nguyen PV (2006) Metaplasticity of the late-phase of long-term potentiation: a critical role for protein kinase A in synaptic tagging. Eur J Neurosci 23:1784-1794.

Yuste R, Bonhoeffer T (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity. Annu Rev Neurosci 24:1071-1089.

Zafar N, Paulus W, Sommer M (2008) Comparative assessment of best conventional with best theta burst repetitive transcranial magnetic stimulation protocols on human motor cortex excitability. Clin Neurophysiol 119:1393-1399.

Zhou Q, Poo MM (2004) Reversal and consolidation of activity-induced synaptic modifications. Trends Neurosci 27:378-383.

Zhou Q, Tao HW, Poo MM (2003) Reversal and stabilization of synaptic modifications in a developing visual system. Science 300:1953-1957.

Ziemann U, Siebner HR (2008) Modifying motor learning through gating and homeostatic metaplasticity. Brain Stimul 1:60-66.

Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W (1996a) The effect of lorazepam on the motor cortical excitability in man. Exp Brain Res 109:127-135.

Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W (1996b) Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. Ann Neurol 40:367-378.

Ziemann U, Chen R, Cohen LG, Hallett M (1998) Dextromethorphan decreases the excitability of the human motor cortex. Neurology 51:1320-1324.

Ziemann U, Ilic 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. J Neurosci 24:1666-1672.