Approaches to Optimise Neuroplasticity Induction in the Human Motor Cortex

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ABSTRACT

The human brain can change its connectivity with experience, and such neuroplasticity is critical for learning, memory, and recovery from brain injury. A number of non-invasive brain stimulation techniques can induce neuroplastic changes in the brain. In order to maximise the therapeutic potential of these techniques, we need to understand the factors influencing their effectiveness. This thesis investigates approaches to optimising neuroplasticity induction in the human motor cortex, focussing on a widely-used repetitive Transcranial Magnetic Stimulation (rTMS) paradigm, Theta Burst Stimulation (TBS).

Subject responses to neuroplasticity induction methods are characterised by high inter- and intra-individual variability. One factor which may contribute to this variability is the excitability state of the targeted cortex at the time stimuli are applied. In Chapter Two, I investigate whether power in several electroencephalography (EEG) frequency bands can be used as a state-marker to predict responses to experimental (TBS) and behavioural (visuomotor training) plasticity induction. The results suggest pre-stimulation EEG power is not useful for predicting responses to plasticity induction. However, an interesting finding is a large increase in alpha (8-12 Hz) power following visuomotor training, which positively correlates with changes in cortical excitability. Although speculative, this may be related to disengagement of the somatosensory system important for motor memory consolidation.

Inhibition in the cortex exerts a powerful modulatory influence over plasticity induction. Therefore, in Chapters Three and Four I examine approaches that might be useful for

optimising (reducing) the level of inhibition in the motor cortex during plasticity induction. Although Chapter Two provides no evidence that pre-stimulation EEG can predict responses to plasticity induction, the timing of stimuli relative to *ongoing* oscillatory activity may be more important. In Chapter Three, I question whether timing of stimuli to different phases of an intrinsic brain rhythm might allow plasticity-inducing stimuli to be applied during natural oscillations in inhibitory tone within the cortex. Alpha, a prominent rhythm in the resting human brain, has been proposed to reflect bouts of cortical inhibition. In this chapter I undertook the technical challenge to develop a method to trigger TMS on different phases of alpha. Whilst Short Interval Intracortical Inhibition (SICI) is unchanged by alpha phase, Motor Evoked Potential (MEP) amplitude is 30% greater on the downgoing phase compared to the upgoing phase. The results may suggest some other inhibitory network is down-regulated during this phase of the alpha rhythm, and provide an opportunity for enhancing neuroplasticity induction by applying stimuli during optimal temporal windows.

In Chapter 4, I investigate whether it is possible to selectively down-regulate activity in inhibitory cortical networks using a modified TBS technique. Many rTMS paradigms affect both inhibitory and excitatory pathways. As intracortical inhibitory pathways have a lower threshold for activation than excitatory pathways, I aimed to target cortical inhibitory circuitry by reducing the intensity of TBS. The results demonstrate it is indeed possible to reduce SICI without concurrent effects on the MEP.

In summary, this thesis provides approaches that may be useful for targeting or creating a more favourable cortical environment for neuroplasticity induction.

DECLARATION

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 to Suzanne McAllister 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.

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McAllister, S. M., Rothwell, J. C. and Ridding, M. C. (2011) Cortical oscillatory activity and the induction of plasticity in the human motor cortex. *Eur J Neurosci*, **33**(10), 1916-1924.

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GENERAL INTRODUCTION

One of the most intriguing features of the human nervous system is its ability to undergo structural and functional reorganisation in response to altered environmental input. For example, changes in the organisation of the motor cortex can be observed in professional musicians who have spent long hours practising the specific movement patterns required to play their musical instruments (Munte *et al.*, 2002). Changes in the nervous system may involve functional modifications, such as alterations in synaptic strength and the unmasking of 'silent' connections, or structural changes such as the growth of new neurons and synapses. The ability of the nervous system to undergo such changes is referred to as 'neuroplasticity' and likely underlies some of the most fascinating functions of the human brain, including the ability to learn and remember, as well as the capacity to undergo functional recovery following injury such as stroke (Traversa *et al.*, 1997; Thickbroom *et al.*, 2004). Neuroplastic changes in the nervous system are also seen in many neurological disorders, such as chronic pain conditions (Jaken *et al.*, 2010) and dystonias (Quartarone *et al.*, 2003). It has therefore been suggested that plasticity can also be maladaptive. Given the important role of neuroplasticity in health and disease, there is currently great interest in how we may be able to modulate the neuroplastic capabilities of the brain for therapeutic benefit.

Recent advances in neurophysiology have lead to the development of non-invasive brain stimulation techniques which can be used to experimentally induce plasticity in the cortex (Pascual-Leone *et al.*, 1994; Stefan *et al.*, 2000; Ridding *et al.*, 2001; Huang *et al.*, 2005). These techniques have been shown to improve some aspects of motor and cognitive performance when tested in patient populations (Khedr *et al.*, 2005; Talelli *et*

al., 2007). However, the usefulness of these plasticity-inducing paradigms is currently limited by the large degree of variability in subject responses and the small magnitude of the induced changes. In order to maximise the therapeutic potential of these techniques, we need to understand more about the mechanisms underlying the induced changes, and how current stimulation paradigms may be improved. In this thesis, I argue that an important determinant of responses to plasticity induction is the excitability state of the brain at the time plasticity inducing stimuli are applied, and investigate approaches to determining, targeting and creating a more favourable cortical environment for plasticity induction.

The thesis opens with a review of the literature, followed by three experimental chapters, which explore various approaches to optimising neuroplasticity induction in the human motor cortex. It closes with a discussion of the three studies in the context of optimising neuroplasticity induction for potential therapeutic use, examining the limitations of my research and speculating upon future possibilities in non-invasive brain stimulation.

CHAPTER ONE

A review of the literature

The following introductory chapter provides a brief overview of the anatomy and physiology of human motor control, followed by a discussion of non-invasive brain stimulation techniques that are available to investigate and induce neuroplastic change in the human motor cortex. It discusses the mechanisms that might underlie these changes, and argues that the excitability state of the brain is a key modulator of neuroplasticity induction; if we can target the cortex at a time when it is most receptive to stimuli, we might be able to maximise responses to plasticity-inducing interventions.

1.2. AN OVERVIEW OF HUMAN MOTOR CONTROL

The human motor system plays an indispensible role in our everyday lives. Effective motor control is essential for a wide array of daily activities, from tying our shoe laces to typing on a keyboard or signing our names. Without it, we would be unable to perform even the most basic and fundamental behaviours; we couldn't eat, breathe or communicate. Understandably, human motor control involves a wide network of cortical and subcortical regions, including the prefrontal cortex, somatosensory cortex and other parietal areas, the basal ganglia, the cerebellum and the thalamus. These brain regions are interconnected with the motor cortex, which, together with the spinal cord, plays the most direct role in human movement control. Whilst the motor cortex was once considered to be simply an output region of the cortex subserving movement, it is now also believed to contribute to many higher order cognitive functions, including speech perception (D'Ausilio *et al.*, 2009), movement recognition during action observation (Fadiga *et al.*, 2005; Press *et al.*, 2011), and a focus of this thesis: motor learning and memory (Hess & Donoghue, 1994; Donoghue, 1995; Muellbacher *et al.*, 2001).

The human motor cortex can be broadly classified into the primary motor cortex and secondary motor areas (the premotor cortex, supplementary motor area and cingulate cortex), although this classification is somewhat simplified and further subdivisions can be made based on histological analysis. The primary motor cortex is located in the posterior region of the frontal lobe of the brain, just anterior to the central sulcus (Brodmann's area 4). The motor cortex was first defined in canines and monkeys (Ferrier, 1874), and later in humans (Penfield & Boldrey, 1937; Rasmussen & Penfield,

1947), by electrically stimulating the cortical surface and observing which areas evoked movement. This was a rather crude definition as stimulation of various cortical areas could potentially evoke movement via current spread and cortico-cortical connections with motor regions, however the primary motor cortex had the lowest threshold for stimulation followed by the secondary motor areas. These pioneering neurophysiological studies revealed a key feature of the motor cortex: its somatotopic organisation. A somatotopic organisation of the motor cortex was first suggested by John Hughlings Jackson (Jackson, 1873), who observed how muscle activation spread in a specific pattern across the body during what are now known as 'Jacksonian' seizures. Jackson reasoned that the motor cortex must be somewhat compartmentalised for this pattern of activation to occur. Later, Canadian neurosurgeon Wilder Penfield provided evidence to support this hypothesis when he used electrical stimulation to investigate the functional topography of the human motor cortex in patients undergoing surgery for removal of epileptogenic tissue. A somatotopic 'map' emerged, known as the motor homunculus (Penfield & Boldrey, 1937; Rasmussen & Penfield, 1947). Stimulating medial areas of the motor cortex evoked movements in leg muscles. As the stimulating electrode was moved laterally, the arms, hands, fingers, face, jaw and masticatory muscles produced movements. A comparably large area of the cortex was devoted to control of the hand and fingers, and this was attributed to their role in fine motor movements, such as grasping. The jaw and mouth muscles also had a considerable cortical representation, likely important for the control of speech.

Although the somatotopic organisation of the motor cortex reveals a general separation of the lower and upper limbs and head, within these topographies, many

muscle representations converge and overlap and the same body part may be represented at multiple sites (Schieber, 2001). For example, the areas representing the thumb overlap extensively with regions controlling movement of the other digits and wrist (Schieber & Hibbard, 1993). The widespread distribution and overlap of motor representations is thought to provide a highly flexible network, important for producing the vast repertoire of novel and practised movements required by the human motor system (Schieber, 2001). It also gives the motor cortex a capacity to somewhat compensate for losses following brain injury (Schieber, 2001). Importantly, studies in both animal models and humans suggest that motor representations can change with skill acquisition (Pascual-Leone *et al.*, 1995; Classen *et al.*, 1998; Rioult-Pedotti *et al.*, 1998) or following damage to the peripheral (Sanes *et al.*, 1990; Cohen *et al.*, 1991) or central (Traversa *et al.*, 1997; Hallett, 2001) nervous system, providing evidence of the neuroplastic capabilities of the motor cortex.

The adaptable, flexible nature of the motor cortex is reflected in its cellular architecture. Neuronal density (measured as number of cell bodies per unit of cortical volume) is lower than any other cortical region, which results in there being a large volume available for dendritic branching and synaptic interactions (Porter & Lemon, 1993). The cytoarchitecture of the cerebral cortex is well described, with six distinct horizontal layers numbered from the more superficial layers (Laminae I-III) to the deeper layers (Laminae IV-VI). Aside from its laminar structure, the cerebral cortex also exhibits organisation in vertical modules called cortical columns (Mountcastle, 2005). These columns consist of neurons with shared input and output connections, and are thought to represent the basic computational units of cortical processing (Mountcastle, 2005),

although the specific function of cortical columns is still under debate (Horton & Adams, 2005).

Cortical columns contain the two main classes of neurons found in the cortex: the pyramidal cells (projection neurons) and the stellate cells (local interneurons). In the motor cortex, the pyramidal cells are located in Layers II-VI, with the majority of them found in Layers III and V (Porter & Lemon, 1993). Pyramidal cells have apical dendrites which can be very long, extending vertically all the way from Layer V into Layer I, and many basal dendrites extending radially from the cell body. The dendrites are covered in spines for receiving synaptic inputs (Gray, 1959). Dendritic spines are highly dynamic, morphologically diverse structures which are thought to play a role in modulating synaptic plasticity, and which may increase the surface area available for synaptic input (Yuste *et al.*, 2000). It has been estimated that a single pyramidal cell may have as many as 60 000 synaptic inputs (Cragg, 1975). The axons of pyramidal cells in the motor cortex project into other cortical and subcortical areas, including to the contralateral motor cortex via the corpus callosum, to parietal regions, and to the spinal cord via the pyramidal tract. Pyramidal cells primarily use glutamate as their neurotransmitter. A special type of large pyramidal cell exists in Layer V, known as the Betz cell (Porter & Lemon, 1993). The axons of these cells descend the corticospinal tract and directly synapse with alpha motor neurons. There are also non-Betz pyramidal cells which synapse directly with spinal motor neurons (Lemon, 2008). Examination of the primate phylogenetic tree reveals that these monosynaptic connections were likely critical for the evolution of independent finger movements; primates with a greater number of corticomotoneuronal projections have far more

dexterous hand function compared with primates that lack these projections (Lemon & Griffiths, 2005).

In contrast to pyramidal cells, interneurons project locally. Their neuronal targets are pyramidal cells and other interneurons. Cortical interneurons are thought to be important for the tight feed-forward and feed-back control of motor pathways, and maintaining a balance of inhibition and excitation within the cortex (Amaral, 2000). Interneurons also form an important substrate for plasticity (Kullmann & Lamsa, 2007). Although there are a wide variety of interneurons which can be classified based on their neurotransmitters and receptor subtypes, the interneurons in the cortex can be generally divided into two prominent classes: spiny and non-spiny stellate cells (Porter & Lemon, 1993). Spiny stellate cells are excitatory interneurons, using glutamate as their neurotransmitter. They are largely present in Layer IV of the cortex, although this layer is barely discernible in the motor cortex (Porter & Lemon, 1993). Non-spiny stellate cells are present in all layers of the motor cortex and use GABA as their neurotransmitter. It is estimated that these neurons make up 20-25% of the total neural content of the cortex (Amaral, 2000).

Output from the motor cortex reaches the spinal cord via the pyramidal tract. The majority of the one million fibres in the pyramidal tract are corticospinal fibres projecting from Layer V of the cortex to the spinal cord (Porter & Lemon, 1993). Other fibres (geniculate fibres) project to the motor neurons in the cranial nerve nuclei of the medulla oblongata, forming the corticobulbar tract, or to the pons and from there to the cerebellum, forming the corticopontine and pontocerebellar projections (Kuypers,

2011). Approximately 60% of corticospinal fibres originate from frontal motor areas, with the remaining 40% from the sensory and parietal cortices (Jane *et al.*, 1967). The majority of corticospinal fibres cross-over at the pyramidal decussation in the medulla and descend contralaterally in the lateral corticospinal tract to innervate distal muscles such as those controlling digit movement (Lemon, 2008; Kuypers, 2011). A small proportion of fibres descend ipsilaterally and continue in the medial corticospinal tract to innervate axial muscles important for postural control (Lemon, 2008). Other fibres descend ipsilaterally in the lateral corticospinal tract and do not cross-over until they reach the level of the spinal cord at which they terminate (Lemon, 2008). All corticospinal fibres terminate in the ventral horn of the spinal cord, where they synapse either directly with spinal motor neurons or indirectly via spinal interneurons. These interneuronal connections are involved in coordinating the activity of large groups of muscles, for example, those involved in walking (Amaral, 2000).

Whilst much of our knowledge of the motor cortex and corticospinal system comes from invasive electrophysiological recordings in non-human primates or from post-mortem studies in humans, in the late 1980s new techniques became available to non-invasively stimulate corticospinal pathways using electromagnetic induction. In the following section, I detail the development and use of non-invasive brain stimulation techniques for investigations of the human motor system.

1.3. NON-INVASIVE STIMULATION OF THE MOTOR CORTEX

The first reports of direct electrical stimulation of the human cortex appeared in the late nineteenth century, in patients with who had eroding tumours of the scalp and

skull or who had undergone skull-opening surgery following head trauma (reviewed by Zago *et al.*, 2008). These situations offered neurologists a unique opportunity to directly access the cortical surface. Not long after, renowned British neurosurgeon, Sir Victor Horsley, pioneered the use of electrical stimulation for the identification of epileptic seizure foci to be removed during surgery (Horsley, 1886). This approach became widely implemented as a treatment for epilepsy, and electrical stimulation of the cortex continued to be used in various neurosurgical procedures throughout the twentieth century. However, it wasn't until the early 1980s that non-invasive techniques to stimulate the cortex were first explored by Merton and Morton, who applied electrical stimulation through the intact scalp (Merton & Morton, 1980). Electrical pulses were delivered to two stimulating electrodes placed over the motor cortex, resulting in contralateral muscle twitches, most easily observed in the muscles of the hand. This technique became known as Transcranial Electrical Stimulation, or TES.

The main shortcoming of TES is that due to the resistance of the scalp and skull, only a small proportion of the applied current stimulates the underlying cortex, the remainder spreads through the scalp's surface between the two stimulating electrodes, causing local pain and muscle contraction. In 1985, Barker and colleagues overcame this problem when they described stimulation of the motor cortex using electromagnetic induction (Barker *et al.*, 1985). Transcranial Magnetic Stimulation (TMS) avoids excessive stimulation of scalp muscles and nociceptive afferents, minimising discomfort to the subject. Hence, the use of TMS has largely superseded that of TES. However, it should be noted that there are significant differences in the mechanism by which these

two techniques activate the motor cortex (discussed in *Section 1.2.1: Physiology of TMS and TES*).

TMS involves the use of a TMS coil connected to a magnetic stimulator unit. The coil is made of multiple strands of insulated copper wiring in a protective casing and acts as an inductor (Epstein, 2008). The magnetic stimulator unit contains a series of energy-storing capacitors which, when discharged, cause a large (~4kA), short-lasting (<1ms) current flow through the coil (Riehl, 2008). This rapidly changing electric current creates a magnetic field perpendicular to the current flow in the coil. When the coil is held over the scalp, the magnetic field passes painlessly through the scalp and induces eddy currents in the underlying neuronal tissue. If the stimulation intensity is high enough, some of the pyramidal cells of the motor cortex will be depolarised to threshold, causing a volley (or several volleys) of action potentials to descend the corticospinal tract (Di Lazzaro *et al.*, 2004). A population of spinal motor neurons will be depolarised and if threshold is reached, the motor units will discharge and a muscle response will be observed. This biphasic muscle response is known as a motor evoked potential, or MEP, and can be recorded using EMG electrodes placed over the skin's surface. The MEP represents the summation of motor unit action potentials produced by the pool of motor neurons activated by the descending volley/s and has a latency of approximately 20-25 ms for the hand muscles. This latency encompasses the time it takes the descending volley to traverse the corticospinal tract and activate the muscle via the spinal motor neurons, and can be used clinically as a measure of the conduction velocity of the corticospinal tract.

Given the somatotopy of the motor cortex, discussed earlier, different muscle groups can be activated depending on the position of the coil. Medial coil positions can activate the leg muscles, whilst the muscles of the hand and mouth/ jaw can be targeted by moving the coil more laterally. The muscles of the hand are most commonly studied, in part due to their relatively large, accessible somatotopic representation which allows easy targeting with a relatively low stimulation intensity compared to the deeper leg muscle representation, and without the discomfort associated with activating the temporalis and masseter muscles when stimulating the jaw/ tongue muscles. TMS coils come in many shapes, some designed specifically to activate these more difficult to access muscle representations (Epstein, 2008). However, the circular and figure-of-eight coils are the most commonly used. The figure-of-eight coil holds an advantage over the circular coil in that it can achieve more focal stimulation due to the concentration of current at the centre point where the copper wiring overlaps (Epstein, 2008). TMS stimuli can be delivered in numerous patterns, including single, paired and repetitive pulses. To gain an understanding of the potential uses of these stimulation techniques, it is first necessary to examine the physiological mechanisms underlying TMS.

1.3.3. Physiology of TMS and TES

It is widely accepted that TMS activates the pyramidal cells of the motor cortex transynaptically via cortical interneurons, whereas TES directly activates pyramidal cell axons (Di Lazzaro *et al.*, 2004). These conclusions were drawn from what was already known about electrical stimulation of the motor cortex in non-human primates, combined with neurophysiological recordings in humans following TES and TMS.

Patton and Amassian (1954) recorded descending volleys in the cat and monkey pyramidal tract following electrical stimulation applied directly to the cortical surface. They found that anodal electrical stimulation elicited a single descending volley at threshold, with a latency of 0.75 - 1.2 ms when recorded from the cervical cord. As the stimulation intensity was increased, additional descending volleys were recruited, with a periodicity of approximately 1.5 ms. These later descending volleys were more sensitive to anaesthesia, asphyxia and cortical damage than the first volley, and were not observed when the subcortical white matter was directly stimulated following removal of the cortical grey matter. It was thus concluded that the first volley resulted from *direct* excitation of pyramidal neurons, whereas the later volleys were likely due to *indirect* stimulation of pyramidal neurons via cortical interneurons. Hence, these volleys were respectively termed 'D' and 'I' waves. The I-waves were additionally named in order of their latency (I1, I2, I3 etc).

In humans, indirect evidence from surface EMG and single motor unit recordings (Day *et al.*, 1989) as well as direct evidence from recordings during spinal surgery (Boyd *et al.*, 1986; Berardelli *et al.*, 1990; Thompson *et al.*, 1991a; Burke *et al.*, 1993) suggest that the corticospinal tract responds to TES in a similar way to the observations in monkeys and cats following direct electrical stimulation. A short latency volley (D-wave) is seen at intensities near threshold, whereas at higher intensities longer latency volleys are recruited (I-waves). Because anaesthesia can severely depress I wave number and amplitude, studies of descending volleys following TMS in patients undergoing surgery were somewhat inconclusive (Di Lazzaro *et al.*, 2004). More recently, descending volleys have been recorded in awake, unanaesthetised patients who have epidural

electrodes implanted for the treatment of chronic pain (Kaneko *et al.*, 1996; Di Lazzaro *et al.*, 1998a). These recordings confirm that TMS preferentially recruits I waves at threshold. D-waves may also be observed at stimulation intensities much higher than threshold, or when using alternative coil shapes (round) or orientations (latero-medial induced current as opposed to posterior-anterior induced current) (Di Lazzaro *et al.*, 1998a; Di Lazzaro *et al.*, 2002b). Additionally, the nature of the D wave evoked by anodal TES and TMS may differ. Whilst the latency of the D wave is the same for anodal TES and focal TMS, the latency is longer for round coil TMS (Di Lazzaro *et al.*, 2002b). The amplitude of this D wave is altered by cortical excitability (increased during voluntary contraction) (Di Lazzaro *et al.*, 2002b). Together, these observations suggest the D wave evoked by round coil TMS is evoked closer to the pyramidal cell body, perhaps at the initial segment (Di Lazzaro *et al.*, 2002b).

The implication of the findings regarding the mechanisms of action of TES and TMS is that whilst at intensities close to threshold TES can be used to probe the excitability of the corticospinal tract from a subcortical level, TMS provides important additional information about intracortical excitability. A change in synaptic efficacy of the pathways involved in I-wave production, for example, should be observable in the MEP elicited by TMS. The D-wave recruited by TES is not sensitive to changes in cortical excitability, whereas the I-waves recruited by focal TMS are (Di Lazzaro *et al.*, 2004).

1.3.4. Single pulse TMS as a probe for cortical excitability

As mentioned previously, a single pulse of TMS of sufficient intensity delivered over the motor cortex will evoke a response (MEP) in the contralateral muscle of interest. There

are three commonly used measurements made using single pulse TMS. These are motor threshold, MEP amplitude and the silent period.

1.3.4.1. Motor threshold

Motor threshold is the minimum stimulator intensity at which an MEP is evoked in the target muscle. It can be measured either whilst the subject is at rest (resting motor threshold, RMT) or performing a weak voluntary contraction (active motor threshold, AMT). RMT is usually defined by finding the lowest stimulator intensity at which an MEP of 50 μ V is evoked in 50% of ten consecutive trials. AMT is measured similarly, except that the MEP must be at least 200 μ V in size and be distinguished from the ongoing EMG activity. Alternative methods of measuring motor thresholds, such as averaging a number of traces at each intensity, are sometimes used (Hanajima *et al.*, 2007). Pharmacological agents which block voltage-gated sodium channels increase motor thresholds (Ziemann *et al.*, 1996b; Chen *et al.*, 1997b). As such, motor threshold is considered a measure of intrinsic membrane properties/ axonal excitability of the corticospinal neurons activated by TMS. Additionally, motor threshold is influenced by the activity of neural inputs to pyramidal cells that affect their membrane excitability (for example, tonic inhibitory or excitatory drives), as well as the excitability of motor neurons in the spinal cord and neuromuscular junction and muscle factors (Ziemann *et al.*, 1996b; Chen *et al.*, 1997b). Motor thresholds are often used in TMS experiments as a reference point by which to set stimulation intensity by, in order to standardise stimulation intensities across subjects.

1.3.4.2. MEP amplitude

Because TMS activates pyramidal cells transynaptically, the amplitude of the MEP can be used as an indicator of cortical excitability. Measures of peak-to-peak MEP amplitude are often taken at baseline and following an intervention (a motor learning task for instance), in order to investigate changes in cortical excitability. An increase in MEP amplitude following an intervention may be due to changes in synaptic strength in the neural network recruited by TMS, or alternatively due to engagement of a wider neural network by TMS. Additionally, a change in MEP amplitude could be due to changes in the synaptic or intrinsic excitability of the pool of spinal motor neurons activated by the descending volley, as TMS tests the excitability of the entire corticospinal pathway. Thus, measures of subcortical or spinal excitability, such as brain stem stimulation, spinal H-reflexes and F-waves, are often used in addition to TMS in order to better interpret the changes in MEP amplitude. However, these techniques also come with their limitations. The H-reflex involves electrical stimulation of 1a afferents onto spinal motor neurons. It can thus be influenced by a host of factors that influence the activity of 1a afferents, rather than motor neuron excitability (for example, changes in pre-synaptic inhibition or homosynaptic post-activation depression of the 1a afferents) (Knikou, 2008). It is also difficult to evoke H-reflexes in the hand at rest and the H-reflex likely does not target the same population of spinal motor neurons as TMS. Similarly, F-waves represent activation of a small subset of spinal motor neurons and may not be representative of those motor neurons activated by TMS (in particular, they are biased towards large diameter, faster-conducting motor neurons). Also, F-waves involve antidromic activation of spinal motor neurons and may not represent the way these neurons would respond to synaptic events (Espiritu *et al.*,

2003). Perhaps the best option for measuring subcortical excitability changes is cervicomedullary electrical stimulation (Ugawa *et al.*, 1991). However, this type of stimulation involves a high voltage electric current passed between two electrodes over the mastoids, and is quite painful. It is, however, likely to activate similar pathways to TMS, and thus is thought to be the most appropriate control for subcortical excitability changes (Ugawa *et al.*, 1991; Taylor, 2006).

MEP amplitude increases in a sigmoidal fashion as stimulation intensity increases. At low stimulation intensities, with posterior-anterior stimulation, I1 waves are recruited, whereas at higher stimulation intensities later I-waves are also recruited (Di Lazzaro *et al.*, 1998a). In contrast, anterior-posterior stimulation preferentially recruits the later I3 wave (Di Lazzaro *et al.*, 1998a). It is important to remember that the MEP represents probing of both excitatory and inhibitory neural pathways. MEP amplitude can thus be affected by the numerous pharmacological agents which modulate these pathways, including GABAa receptor antagonists (Ziemann *et al.*, 1996a), dopamine agonists and antagonists (Ziemann *et al.*, 1997), noradrenalin agonists (Ilic *et al.*, 2003), acetylcholine antagonists (Di Lazzaro *et al.*, 2000b) and serotonin reuptake inhibitors (Ilic *et al.*, 2002a). There are various TMS techniques which have been developed to selectively probe inhibitory and excitatory pathways in the motor cortex. These are discussed below.

1.3.4.3. Silent Period

The silent period is a period of EMG silence following an MEP evoked during a tonic muscle contraction. The silent period can last up to 200-300 ms in hand muscles

depending on the intensity of stimulation. Spinal inhibitory mechanisms such as after-hyperpolarisation and recurrent inhibition are thought to contribute to the early (< 50 ms) part of the silent period, whilst the latter part is attributed to cortical inhibitory mechanisms (Inghilleri *et al.*, 1993; Ziemann *et al.*, 1993). $GABA_B$ –reuptake inhibitors lengthen the silent period duration and have thus implicated $GABA_B$ neurotransmission in the mechanisms underlying the cortical component of the silent period, (Werhahn *et al.*, 1999). The long duration of the silent period is consistent with the time course of $GABA_B$ receptor activation, however, other studies showing no effect of $GABA_B$ antagonists on the silent period have left the matter open to debate (Inghilleri *et al.*, 1996; Ziemann *et al.*, 1996a).

1.3.5. Paired pulse TMS techniques for investigating intracortical pathways

1.3.5.1. Short latency intracortical inhibition (SICI)

Short latency intracortical inhibition (SICI) is a paired-pulse TMS technique first developed by Kujirai and colleagues (Kujirai *et al.*, 1993). When a subthreshold 'conditioning' stimulus is delivered prior to a suprathreshold 'test' stimulus through the same coil, the MEP following the suprathreshold test stimulus is reduced in amplitude compared to if the suprathreshold test stimulus is delivered alone. The interval between the sub- and suprathreshold stimuli is usually 2 or 3 ms, though inhibition can be observed from 1-5 ms (Kujirai *et al.*, 1993). The subthreshold conditioning stimulus does not suppress the MEP elicited by TES, suggesting an intracortical origin of the inhibition (Kujirai *et al.*, 1993). SICI has also been shown to be reduced during weak voluntary contraction (Ridding *et al.*, 1995c) and increased following administration of various $GABA_A$ agonists (Ziemann *et al.*, 1996a; Palmieri *et al.*, 1999). It is therefore

highly likely that the MEP inhibition reflects the action of low threshold $GABA_A$-mediated intracortical inhibitory pathways activated by the subthreshold conditioning stimulus. Recordings of descending volleys in patients with epidural electrodes implanted for pain relief have shown that the amplitude of the I1 wave is unaffected by the conditioning stimulus, whereas the amplitude of later I-waves is reduced (Di Lazzaro *et al.*, 1998b).

The amount of SICI is highly dependent on the intensity of the conditioning and test stimuli as well as coil orientation (Hanajima *et al.*, 1998). A number of studies have shown that depending on the ISI and intensities employed, SICI can be contaminated by activation of intracortical excitatory pathways (discussed in *Section 1.2.3.3: Short Latency Intracortical Facilitation (SICF)* below) (Ziemann *et al.*, 1998b; Ilic *et al.*, 2002b).

1.3.5.2. Intracortical facilitation (ICF)

As the latency between the sub- and suprathreshold stimuli increases, inhibition switches to facilitation (Kujirai *et al.*, 1993). This facilitation is termed intracortical facilitation (ICF), and peaks at an ISI of approximately 10 ms. The source of this facilitation is not well understood, however NMDA receptor antagonists have been shown to reduce ICF (Ziemann *et al.*, 1998c). $GABA_A$ agonists also decrease ICF (Ziemann *et al.*, 1996a), suggesting ICF represents a mix of excitatory and inhibitory pathway activation, with a net facilitatory outcome. Interestingly, Di Lazzaro and colleagues (2006) found that I waves were unchanged by ICF. The authors suggest three possibilities: that the conditioning stimulus may have an effect on spinal excitability that is yet to be elucidated, that the composition of the descending volleys

is altered such that a greater proportion of activity is destined for the target muscle, or alternatively that the descending volley is not a good representation of *all* output destined for the target muscle, and that more dispersed activity may not be observable in the recordings (Di Lazzaro *et al.*, 2006).

1.3.5.3. Short latency intracortical facilitation (SICF)

Short latency cortical facilitation (SICF) was developed as a method to investigate I-wave circuitry without using invasive epidural recordings (Tokimura *et al.*, 1996; Ziemann *et al.*, 1998b). Whilst these invasive studies have been seminal to our understanding of the physiological mechanisms underlying TMS, they can obviously only be used in the patient population. Again, SICF relies on pairing TMS stimuli. However, unlike SICI where a subthreshold stimulus precedes a suprathreshold stimulus, when SICF is measured a suprathreshold stimulus is delivered prior to a subthreshold stimulus. At latencies of approximately 1.3, 2.7 and 3.5 ms, peaks of MEP facilitation are seen (Tokimura *et al.*, 1996; Ziemann *et al.*, 1998b). These latencies closely align with I-wave latencies. The subthreshold stimulus is thought to activate neurons which were depolarised by the suprathreshold first stimulus but did not reach the threshold for action potential generation (Tokimura *et al.*, 1996; Ziemann *et al.*, 1998b). Thus, when the two stimuli are delivered together, MEP amplitude is greater than if the suprathreshold stimulus is delivered alone.

One can see that the ISIs used to record SICF potentially overlap with those of SICI. Whether inhibition or facilitation is observed depends critically on the intensities of stimuli delivered. SICI can be somewhat isolated by identifying the 'troughs' of SICF (i.e.

the latencies at which minimal facilitation is observed) and delivering stimuli at these ISIs, where the SICI response will not be contaminated with SICF (Peurala *et al.*, 2008). The relationship between SICI and SICF is, however, complicated. It might be expected that SICI would inhibit SICF pathways, however triple-pulse paradigms suggest that at low conditioning stimulus intensities, SICI can actually facilitate SICF (Wagle-Shukla *et al.*, 2009; Shirota *et al.*, 2010). Current models theorise that a second set of inhibitory interneurons mediate this effect, such that SICF is facilitated by SICI through disinhibition. A stronger conditioning stimulus may directly activate this second set of inhibitory neurons and thus reduce rather than increase SICF (Wagle-Shukla *et al.*, 2009; Shirota *et al.*, 2010).

1.4. NEUROPLASTICITY

The concept of neuroplasticity is not new, in fact ideas about the ability of the brain to undergo changes have been documented as early as the 19th century. The renowned Spanish neuroscientist, Santiago Ramon y Cajal, proposed the idea that the brain has an ability to adapt in his 1894 Croonian lecture to the Royal Society of London. However, he concluded later in his career that "in the adult centres the nerve paths are something fixed, ended and immutable" (Ramon y Cajal, 1928). This view of the static adult brain largely pervaded neuroscience until the second half of the 20th century, despite observations that functional recovery was possible following brain injury, and growing evidence of neuroplastic change in animal studies (e.g. Lashley, 1920). A key piece of the puzzle was missing: a mechanism for neuroplastic change.

In 1949, Canadian psychologist Donald Hebb provided a potential explanation of how activity at the cellular level might be involved in learning and memory in his book 'The Organisation of Behaviour; A Neuropsychological Theory':

> *Let us assume that the persistence or repetition of a reverberatory activity (or "trace") tends to induce lasting cellular changes that add to its stability. When an axon of cell A is near enough to excite a 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)*

The above statement is known as 'Hebb's postulate' and is commonly paraphrased as "neurons that fire together wire together." Whilst a cellular basis of learning was an attractive concept, it would be some two decades later before experimental evidence would support it. In the late 1960s, advances in molecular and cellular biology allowed Eric Kandel and his colleagues to investigate two simple forms of implicit learning - habituation and sensitisation - at the single neuron level in the marine invertebrate, *Aplysia californica* (Pinsker *et al.*, 1970; Pinsker *et al.*, 1973). These forms of learning were found to involve changes in synaptic transmission brought about by alterations in neurotransmitter release from the pre-synaptic cell (Castellucci *et al.*, 1970; Castellucci & Kandel, 1974; Castellucci & Kandel, 1976). Around the same time, Tim Bliss and Terje Lømo were exploring synaptic transmission in the more complex mammalian hippocampus, which was known from lesion studies to play an important role in

memory formation (Milner, 1959). In 1973, they discovered long term potentiation (LTP) in the rabbit hippocampus (Bliss & Lømo, 1973). Later, its counterpart, Long Term Depression (LTD), was reported (Stanton & Sejnowski, 1989; Dudek & Bear, 1992). LTP and LTD are candidate cellular mechanisms for more complex, associative learning, because they require simultaneous activation of pre- and post-synaptic neurons and thus lend support to Hebb's postulate (Bliss & Collingridge, 1993). Although the mechanisms underlying habituation/ sensitisation and the induction of LTP/ LTD are different, both involve functional changes in synaptic efficacy which can eventually lead to longer term changes in synaptic morphology. Together, these intriguing discoveries of potential cellular mechanisms underlying learning and memory heralded a new era in neuroscience, forming the foundations for the field of neuroplasticity, an area of research which has rapidly expanded over the past four decades.

1.4.1. MECHANISMS OF NEUROPLASTICITY

Neuroplasticity can involve rapid, short-term functional changes, as well as longer lasting structural changes which require time to develop. Rapid changes include the unmasking of previously silent synapses (Jacobs & Donoghue, 1991; Isaac *et al.*, 1995), changes in intrinsic neuronal excitability (non-synapse specific) (Desai *et al.*, 1999; Daoudal & Debanne, 2003), and activity-dependant changes in synaptic efficacy such as LTP and LTD (Bliss & Collingridge, 1993).

LTP and LTD are two of the most widely studied phenomena in neuronal plasticity. LTP was first discovered in the hippocampus of anaesthetised rabbits (Bliss & Lømo, 1973).

Bliss and Lømo (1973) applied repetitive electrical stimuli to the perforant path fibres, which terminate on the granule cells of the dentate gyrus in the hippocampus. Short conditioning trains of electrical stimuli delivered to the perforant path fibres (10-20 Hz for 10-15s or 100 Hz for 3-4s) resulted in an increase in the response of the granule cells. This increased response was measured as an increase in the population excitatory postsynaptic potential (EPSP). Population EPSP slope remained potentiated from half an hour up to ten hours post-stimulation, depending on the stimulation parameters employed. It was proposed that the increase in population EPSP was due to an enhancement in synaptic efficacy at individual synapses (Bliss & Lømo, 1973).

Much is now known about the mechanisms underlying these changes in synaptic efficacy. Ligand-gated calcium channels called N-methyl-D-aspartate (NMDA) receptors in the post-synaptic cell play an important role in the most commonly studied forms of LTP and LTD (Collingridge *et al.*, 1983; Harris *et al.*, 1984). These channels are activated by glutamate. However, even when glutamate is bound, calcium influx may be prevented by a magnesium ion blocking the channel of the NMDA receptor (Coan & Collingridge, 1985; Foster & Fagg, 1987). Magnesium is only expelled from the channel when the post-synaptic cell is sufficiently depolarised (Foster & Fagg, 1987). In this way, the NMDA receptor can act as a 'coincidence detector' for pre- and post-synaptic activity. Once the magnesium ion is removed, calcium can flow into the post-synaptic cell, where it activates various second messenger systems that lead to synaptic enhancement (Roberson *et al.*, 1996).

There are several properties that define NMDA-receptor dependant LTP. These are input specificity, cooperativity, associativity and persistence (Bliss & Collingridge, 1993). Input specificity refers to the fact that only the pathways stimulated undergo changes in synaptic efficacy, changes do not generalise to nearby synapses (Andersen *et al.*, 1977), although there are some exceptions to this rule. Cooperativity means that there is an intensity threshold for induction - LTP can be induced not only by a strong tetanic input but also by many weak inputs which together depolarise the postsynaptic cell enough to expel the magnesium ion (McNaughton *et al.*, 1978). Similarly, when a weak input is combined with a strong input, both pathways undergo LTP, explaining the associative nature of LTP (Levy & Steward, 1979; Barrionuevo & Brown, 1983; Levy & Steward, 1983). LTP is also characterised by persistence; changes in synaptic efficacy in the rat dentate gyrus have been shown to outlast the period of stimulation by hours, days, or even up to one year, depending on the initial parameters of stimulation and environmental exposure (Abraham *et al.*, 2002). LTP is thought to underlie long-term memory and could thus be envisioned to last a lifetime, depending on factors influencing its expression and stability (Adams & Dudek, 2005).

The amount of time LTP lasts helps define two phases of LTP - early and late. Early phase LTP generally lasts for only a few hours and is characterised by functional changes which enhance synaptic efficacy. For example, calcium influx into the post-synaptic cell activates calcium/calmodulin-dependant protein kinases which phosphorylate receptors in the cell membrane, causing a conformational change which increases their sensitivity to glutamate (Roberson *et al.*, 1996). Activation of calcium-mediated second messenger systems also cause the postsynaptic cell to release a

retrograde messenger (possibly nitric oxide (Schuman & Madison, 1991)), which enhances the release of glutamate from the pre-synaptic cell (Bliss & Collingridge, 1993). Late phase LTP often lasts for days or weeks, and involves structural changes which rely on gene transcription and protein synthesis (Adams & Dudek, 2005). For example, persistent calcium influx activates a protein kinase signalling cascade which mediates phosphorylation of the CREB protein in the nucleus (Impey *et al.*, 1996). CREB is a transcription factor which binds to DNA and up- or down-regulates transcription of downstream genes (Impey *et al.*, 1996). One of the genes modulated by CREB encodes brain derived neurotrophic factor (BDNF). BDNF plays many roles in the expression of LTP (Lu *et al.*, 2008) and has been shown to modulate axonal growth and dendritic morphology, providing a potential mechanism for how LTP may eventually lead to the growth of new synapses (Rex *et al.*, 2007).

Similarly to LTP, the induction of LTD also features an early phase involving functional changes and a late phase of morphological transformations (Collingridge *et al.*, 2010). Whether LTP or LTD is induced depends on the frequency and timing of conditioning stimuli. LTD can be induced with low frequency repetitive stimuli, or when stimuli are applied such that the post-synaptic action potential precedes depolarisation of the post-synaptic cell by pre-synaptic input within a short time window (e.g. when a strong stimulus to the post-synaptic cell precedes a weaker stimulus – changing this order induces LTP) (Caporale & Dan, 2008; Collingridge *et al.*, 2010). Like LTP, LTD relies on calcium influx and activation of NMDA receptors. This raises the question of what determines whether LTP or LTD is induced. The mechanisms are not well understood, however, one potential explanation may be that the kinetics of the rise in calcium

following NMDA receptor activation determine whether LTP or LTD is induced. Rapid, large increases in calcium induce LTP, whereas slower, sustained increases in calcium induce LTD (Yang *et al.*, 1999). Early phase LTD is characterised by reduced neurotransmitter release from the pre-synaptic cell (Stanton *et al.*, 2003) and internalisation of AMPA receptors (non-NMDA ion channels involved in neuronal depolarisation) in the post-synaptic cell (Snyder *et al.*, 2001; Brown *et al.*, 2005). Like late-phase LTP, late-phase LTD can be identified by changes in gene transcription and protein synthesis. In this case, persistent LTD leads to cellular anatomical changes including the shrinkage of dendritic spines and a reduction in synaptic bouton contacts (Zhou *et al.*, 2004; Becker *et al.*, 2008).

It should be noted that there are also forms of LTP/ LTD which are not dependant on NMDA receptor activity. For example, LTP in the mossy-fibre pathway of the hippocampus primarily involves activation of *pre-synaptic* second messenger systems which mediate increased neurotransmitter release (Johnston *et al.*, 1992; Nicoll & Malenka, 1995; Anwyl, 2006).

1.4.2. Metaplasticity and homeostatic plasticity

The changes in synaptic efficacy induced by LTP/ LTD present a problem; changes in synaptic strength would eventually saturate, creating a 'ceiling' or 'floor' effect whereby no further increases/ decreases could occur (Turrigiano & Nelson, 2000; Leslie *et al.*, 2001). Excitotoxicity could also occur, whereby pathologically high levels of neurotransmitter lead to cell damage and/ or death (McEachern & Shaw, 1999). Continued reduction in synaptic efficacy by LTD would result in neuronal quiescence

(Turrigiano & Nelson, 2000). In 1982, Bienenstock, Cooper and Munro theorised a mechanism by which neural pathways could be stabilised (Bienenstock *et al.*, 1982). This became known as the BCM rule. The BCM rule predicted that there is a sliding threshold for LTP/ LTD induction. High levels of synaptic activity reduce the threshold for LTD induction and increase the threshold for LTP induction and vice versa for low levels of synaptic activity (Bienenstock *et al.*, 1982). Studies in animal models and humans somewhat support the existence of such a mechanism (Huang *et al.*, 1992; Abraham *et al.*, 2001; Hamada *et al.*, 2008). For example, in the CA1 area of the hippocampus the threshold for LTP induction is not fixed and can be altered depending on the prior synaptic activity level (Huang *et al.*, 1992). The influence of past synaptic history on plasticity induction is referred to as 'metaplasticity': the plasticity of plasticity itself.

There is evidence that neurons possess a mechanism to detect levels of synaptic activity over time, and are able to scale synapses accordingly. This is known as homeostatic plasticity and is a slower, non-Hebbian form of plasticity (Turrigiano & Nelson, 2000). Homeostatic plasticity involves multiplicative or divisive scaling of all synaptic inputs to a neuron, such that the relative input of each synapse remains the same but the overall levels of activity are scaled up or down (Turrigiano & Nelson, 2000). How neurons actually achieve this scaling is not well understood, but it likely involves calcium-dependent second messengers which mediate trafficking of AMPA receptors to and from the cell membrane (Turrigiano, 2008).

1.4.3. LTP/LTD and learning and memory

Whilst LTP and LTD have been most extensively studied in the hippocampus, they have also been demonstrated in many other areas of the brain, including the cerebellum (Crepel & Jaillard, 1991), amygdala (Chapman *et al.*, 1990) and various neocortical areas including the visual and motor cortices (Sakamoto *et al.*, 1987; Hess & Donoghue, 1994). The induction of LTP/ LTD has been demonstrated at inhibitory (GABA-ergic) synapses (Komatsu & Iwakiri, 1993; McLean *et al.*, 1996; Castillo *et al.*, 2011) as well as excitatory (glutamatergic) synapses. The conservation of these mechanisms between different brain regions and across different classes of neurons supports their proposal as key mechanisms of learning and memory. Since their discovery, many studies have specifically investigated their role in these brain functions (Morris *et al.*, 1986; Tsien *et al.*, 1996; Tang *et al.*, 1999). Convincing evidence comes from studies in NMDA receptor knock-out rats. These rats cannot produce LTP and demonstrate impaired performance on the Morris water maze task, a task which assesses spatial learning (Tsien *et al.*, 1996). Conversely, over-expression of NMDA receptors improves spatial learning in rats (Tang *et al.*, 1999).

1.4.4. LTP/LTD as candidate mechanisms of neuroplasticity in the motor cortex

Whilst many studies have investigated the role of hippocampal LTP/ LTD in learning and memory, LTP and LTD are candidate mechanisms for neuroplasticity in many other brain regions, including the motor cortex. Neuroplasticity of the motor cortex is thought to be important for motor learning as well as recovery of motor function following injury. Indeed, changes in human motor cortex topography are well documented following deafferentation (Brasil-Neto *et al.*, 1993; Ziemann *et al.*, 1998a),

amputation (Hall *et al.*, 1990; Cohen *et al.*, 1991), limb immobilisation (Zanette *et al.*, 2004) and motor skill acquisition (Pascual-Leone *et al.*, 1995; Classen *et al.*, 1998). For example, transient ischemic nerve block of a forearm muscle increases corticospinal excitability in muscles immediately proximal to the deafferented region (Brasil-Neto *et al.*, 1993). The neural substrate for such changes is likely to be the extensive horizontal connections within the motor cortex. Reductions in GABAergic inhibition have been shown to lead to rapid map reorganisations in the rat motor cortex (Jacobs & Donoghue, 1991). Similarly, in the temporarily deafferented human motor cortex, changes in cortical maps are observed with a concomitant reduction in intracortical inhibition (SICI) (Ziemann *et al.*, 1998a). It is thought that these reductions in GABAergic inhibition are important for releasing tonic inhibition on present, but 'silent', synapses, thus allowing for very fast changes in cortical somatotopy (Jacobs & Donoghue, 1991; Ziemann *et al.*, 1998a). Reducing GABAergic inhibition is also known to facilitate LTP/ LTD induction in the rat motor cortex *in vitro* (Hess *et al.*, 1996) and LTP and LTD are likely to be an important mechanism of plasticity in the motor cortex. The capacity of the motor cortex to undergo LTP and LTD has been demonstrated numerous times in the rat motor cortex *in vitro* (Hess & Donoghue, 1994; Aroniadou & Keller, 1995; Hess *et al.*, 1996) and *in vivo* in the motor cortex of anaesthetised cats (Iriki *et al.*, 1989; Keller *et al.*, 1990) and awake rats (Werk & Chapman, 2003). Moreover, the induction of LTP in the motor cortex has been shown to be a contributing factor for motor learning. Rioult-Pedotti and colleagues (1998) demonstrated that motor skill learning in rats increased the strength of field potentials in the trained motor cortex and that *in vitro* electrical stimulation induced less LTP in the trained hemisphere compared with the untrained hemisphere – consistent with

saturation of LTP. This study provided convincing evidence that motor learning relies on LTP-like mechanisms. Whilst it is not possible to directly investigate LTP and LTD in the human motor cortex, non-invasive TMS measures can be used to infer such changes. Because TMS activates pyramidal cells transynaptically, changes in synaptic efficacy in the motor cortex should modulate MEP amplitude. The use of TMS can be combined with measures of spinal excitability and pharmacological agents such as NMDA-receptor antagonists in order to better elucidate the mechanisms underlying changes in MEP amplitude. For example, the changes in MEP amplitude induced by some experimental plasticity-inducing paradigms are abolished following administration of the NMDA-receptor antagonist dextromethorphan, providing evidence that these paradigms induce LTP/ LTD-like changes in the motor cortex (Stefan *et al.*, 2002; Huang *et al.*, 2007).

1.4.5. Motor cortex plasticity and brain injury

Plasticity of the motor cortex is important for optimising motor performance, but also critical for the recovery of motor function following injury such as stroke. It is well known that changes in the motor cortex occur following brain injury. In fact, the injured brain may have a unique capacity for neuroplasticity. Animal studies suggest focal brain injury can lead to widespread cortical changes, with a neural framework that is more able to undergo structural and functional change in response to afferent input than in the healthy adult brain (Bury & Jones, 2002; Ward & Frackowiak, 2006). Changes in motor cortical representations/ excitability following stroke correlate with improved motor function (Traversa *et al.*, 1997; Koski *et al.*, 2004; Thickbroom *et al.*, 2004) and it has been shown that experimental techniques which drive such

neuroplastic change can potentially improve motor performance following brain injury (Fraser *et al.*, 2002; Talelli *et al.*, 2007). It is thus of great interest to explore ways to exploit the neuroplastic capacity of the human motor cortex in order to improve current methods of neurorehabilitation in brain-injured patients.

1.5. INDUCING NEUROPLASTICITY IN HUMANS USING NON-INVASIVE METHODS OF BRAIN STIMULATION

There are now a wide array of experimental brain stimulation techniques available to experimentally induce plasticity in the human motor cortex. These techniques involve either peripheral stimulation (Hamdy *et al.*, 1998; Ridding *et al.*, 2000; Ridding & Uy, 2003), paired cortical and peripheral stimulation (Stefan *et al.*, 2000; Wolters *et al.*, 2003), or cortical stimulation alone (Pascual-Leone *et al.*, 1994; Nitsche & Paulus, 2000; Huang *et al.*, 2005; Fitzgerald *et al.*, 2006) . Whilst each of these techniques differ in their method of application, all induce changes in cortical excitability which outlast the period of stimulation. These changes are similar in nature to those observed following motor learning, and there is convincing evidence that similar neural pathways are involved in experimentally-induced and motor-learning induced plasticity (Ziemann *et al.*, 2004; Stefan *et al.*, 2006). For example, a period of prior motor training can block the subsequent induction of LTP-like plasticity but enhance the induction of LTD-like pathways in the motor cortex (Ziemann *et al.*, 2004; Stefan *et al.*, 2006), suggesting motor learning and plasticity induction techniques recruit similar pathways. It has therefore been proposed that these plasticity-inducing paradigms could be used to alter motor performance and thus be of neurorehabilitative value in the treatment of

patients with motor impairments (see Ridding & Rothwell, 2007, for review). An overview of various experimental stimulation techniques is followed by a discussion of their interaction with motor behaviour in healthy and neurologically-impaired individuals.

1.5.1. Peripheral nerve stimulation.

Afferent input is known to be a powerful driver of neuroplastic change. As discussed earlier, deafferentation (Garraghty *et al.*, 1994; Ziemann *et al.*, 1998a), limb immobilization (Liepert *et al.*, 1995) and amputation (Florence & Kaas, 1995) reduce sensory input and lead to significant cortical map reorganisation in areas adjacent to the deafferented/ amputated limb. Similarly, increasing sensory input can lead to changes in cortical excitability and topography. Hamdy and co-workers (1998) found that repetitive electrical pharyngeal stimulation at 10 Hz increased pharyngeal MEP amplitudes up to 30 minutes post-stimulation, whilst decreasing oesophageal MEP amplitudes (Hamdy *et al.*, 1998). Additionally the area of scalp over which TMS responses could be elicited was larger for the pharynx but smaller for the oesophagus following stimulation, suggesting an increase in the excitability of the cortical pharyngeal representation. Pharyngeal reflexes evoked by trigeminal or vagal nerve stimulation did not change following stimulation, nor did TES responses investigated in one subject. This suggested the changes in MEP amplitude were probably due to changes in the motor cortex. The interpretation of this data, however, was complicated by the fact that TMS investigation of the musculature involved in swallowing is somewhat difficult, as MEP responses are often very small and difficult to record (25 microvolts at baseline in this case (Hamdy *et al.*, 1998)).

Ridding and colleagues (2000) investigated whether repetitive electrical stimulation of hand muscle afferents could similarly be used to induce plasticity in the motor cortex. Following trains of 10 Hz electrical stimulation applied to the ulnar nerve there was an increase in MEP amplitude (Ridding *et al.*, 2000). Only those muscles which were innervated by the ulnar nerve (first dorsal interosseous and abductor digiti minimi) showed enhancement of MEP amplitude. Control experiments using F-waves and TES provided some evidence to suggest these changes were not due to alterations in spinal excitability. Further experiments demonstrated that the changes in excitability induced by peripheral nerve stimulation were accompanied by changes in the topography of the muscle representations (Ridding *et al.*, 2001). Following stimulation, MEPs could be evoked over a larger area of the scalp and there was a significant shift in the optimal location for evoking an MEP, suggesting changes in cortical somatotopy rather than just a change in cortical excitability.

Synchronous electrical stimuli to the motor points of two muscles is another peripheral stimulation technique which induces significant increases in MEP excitability (Ridding & Uy, 2003). One of the disadvantages of these techniques is the prolonged period of stimulation required to induce such changes (often one-two hours).

1.5.2. Paired associative stimulation (PAS)

The precise timing of neural inputs is paramount to Hebbian models of associative plasticity. If an input arrives at the postsynaptic cell at a time when the cell is depolarized, the synapse will be strengthened via LTP mechanisms (Levy & Steward,

1983). This has been demonstrated numerous times in both *in vivo* and *in vitro* studies of the motor cortex, by a number of pairing protocols (Baranyi & Feher, 1981; Iriki *et al.*, 1989; Hess & Donoghue, 1994; Hess *et al.*, 1996). Based on these protocols, Stefan and colleagues (2000) designed a paired TMS paradigm aimed at inducing LTP-like changes in the human motor cortex. The technique, known as paired associative stimulation (PAS), involved delivering an electrical stimulus to the median nerve 25 ms prior to a TMS stimulus applied over the contralateral motor cortex (Stefan *et al.*, 2000). Ninety pairs of stimuli were delivered at a rate of 0.05 Hz for a period of 30 minutes. The timing of the inputs was such that the two stimuli should coincide in the cortex. This paradigm increased MEP amplitude by 55%. ISIs of 25, 100, 525 and 5000 ms did not result in any changes in MEP amplitude, confirming the importance of the timing of the two stimuli. This paradigm was later further investigated using an ISI of 10 ms, such that the afferent input reached the motor cortex prior to the TMS stimulus (Wolters *et al.*, 2003). A reduction in MEP amplitude was observed, likened to an LTD-like change in cortical excitability (Wolters *et al.*, 2003). Animal studies have demonstrated that the timing of action potential firing and depolarization by afferent stimulation is essential in determining whether LTP or LTD is induced (Levy & Steward, 1983). The effects of PAS are highly likely to depend on LTP/LTD-like changes for several reasons. Apart from the fact it is based on the principles of associativity important for LTP/ LTD, PAS also demonstrates input specificity, because adjacent muscles not receiving afferent stimulation do not demonstrate changes in MEP amplitude (Stefan *et al.*, 2000). The changes in MEP amplitude outlast the period of stimulation, yet are also reversible. Most convincingly, the NMDA receptor antagonist dextromethorphan has been shown to block the effects of PAS on MEP amplitude (Stefan *et al.*, 2002).

1.5.3. Repetitive TMS (rTMS)

TMS can be applied in single pulses or in paired pulse paradigms as discussed earlier, in order to probe the excitability of the corticospinal pathways and various intracortical circuits. However, when replied repetitively in trains of pulses, TMS can alter the excitability of these pathways. This is known as repetitive TMS or rTMS. In the early 90s, the commercial availability of new magnetic stimulators which were able to deliver pulses at a much higher frequency lead to the development of rTMS. The wide array of rTMS paradigms can be divided into three classifications: low frequency, high frequency and patterned stimulation.

In general, low frequency rTMS (less than 5 Hz) results in a reduction in MEP amplitude, whereas high frequency rTMS (>5 Hz) tends to increase cortical excitability (see Fitzgerald *et al.*, 2006, for review). Low frequency rTMS almost always involves a single, long train of repetitive stimuli. High frequency rTMS, on the other hand, is characterised by high frequency repetitive stimuli applied in shorter trains, separated by an interval. The inter-train-interval varies widely between different paradigms, but is usually at least several seconds long in order to comply with current safety guidelines (see *Section 1.4.4: Safety of TMS and rTMS* below) (Rossi *et al.*, 2009). There is also a huge variation in stimulation parameters, including the number and frequency of TMS stimuli, and intensity of stimulation (ranging from sub- to suprathreshold stimulation intensities).

More recently, patterned stimulation techniques have been investigated. The most common of these is Theta Burst Stimulation (TBS), developed by Huang and colleagues

in 2005. TBS was designed based on stimulation parameters used induce LTP in animal models. Individual neurons in the rat hippocampus tend to produce short bursts of spikes in phase with theta oscillations, which are the dominant oscillatory activity during spatial learning/ exploratory behaviour (Larson *et al.*, 1986). Based on this observation, Larson and colleagues (1986) developed an electrical stimulation paradigm involving bursts of stimuli, with inter-burst intervals of 200 ms (i.e. 5 Hz) to mimic natural spiking activity. This pattern of stimulation induces LTP in hippocampal slices, evidenced by an increase in the slope of the EPSP (Larson *et al.*, 1986). Huang and colleagues (2005) used this stimulation paradigm as a model to design a new rTMS technique for use in human subjects.

Human TBS involves bursts of three TMS stimuli at 50 Hz repeated every 200 ms. There are three commonly used patterns of TBS, continuous (cTBS), intermediate (imTBS) and intermittent (iTBS). cTBS is TBS applied continuously for 40s (i.e. 600 pulses in total). imTBS is TBS applied for 5s, repeated every 15s, whilst iTBS is TBS applied for 2s, repeated every 10s. cTBS suppresses MEP amplitude, imTBS has no effect on MEP amplitude and iTBS increases MEP amplitude (Huang *et al.*, 2005). These effects last for up to twenty minutes following iTBS or one hour following cTBS (Huang *et al.*, 2005), longer than the after-effects seen with many other rTMS paradigms. Unlike PAS, these paradigms also have effects on SICI, with cTBS reducing SICI and iTBS increasing SICI (Huang *et al.*, 2005). TBS holds advantages over other rTMS paradigms in that it is rapidly applied; just 40 s of cTBS produces reductions in MEP amplitude similar to those seen with 15 minutes of 1 Hz low-frequency rTMS. It is also applied at very low-intensity (80% AMT), minimising discomfort for the participant and reducing the chance

of spinal effects as this intensity should evoke minimal descending activity. The ability of TBS to induce either increases or decreases in cortical excitability depending on the pattern of stimulation has also made it a popular choice for rTMS studies. TBS is considered by many to be the most effective rTMS paradigm, however individual responses to TBS vary widely (Goldsworthy *et al.*; Di Lazzaro *et al.*, 2008; Ridding & Ziemann, 2010).

The after-effects of both cTBS and iTBS are likely to be due to LTD and LTP-like mechanisms, given that administration of an NMDA-receptor antagonist abolishes their after-effects (Huang *et al.*, 2007; Teo *et al.*, 2007). It is thought that cTBS reduces the excitability of the excitatory synapses responsible for MEP production as well as suppressing the inhibitory pathways involved in SICI, whilst iTBS has the opposite effect (Huang *et al.*, 2005). Recordings of descending volleys evoked prior to and following TBS have further elucidated the mechanisms underlying these changes. Whilst there is a reduction in the amplitude of the I1 wave following cTBS, there is an increase in the amplitude of the I3 wave following iTBS. Thus it is proposed that cTBS has LTD-like effects on the synapses responsible for the I1 wave, whereas iTBS has LTP-like effects on the synapses responsible for the I3 wave (Di Lazzaro *et al.*, 2005; Di Lazzaro *et al.*, 2008).

1.5.4. Safety of TMS and rTMS

Single and paired-pulse TMS paradigms are generally considered to be very safe and well-tolerated in human subjects. Tissue heating from a single-pulse of TMS is less than 0.1 degree Celsius (Ruohonen & Ilmoniemi, 2002), and the total magnetic field

exposure time during a TMS or rTMS session is considered too short to carry a risk (Rossi *et al.*, 2009). The most commonly reported side-effect of TMS/rTMS is a mild, transient headache (Rossi *et al.*, 2009). The most serious adverse reaction to rTMS which has been reported is seizure induction. This is of particular concern when using high-frequency rTMS paradigms, due to their excitatory after-effects. However, these events are very rare; the most recent TMS safety review reports just 16 cases of seizure induction from several thousand TMS publications (although it should be noted such cases may be under-reported) (Rossi *et al.*, 2009). Often these events were associated with use of pro-convulsant medication, or a family history of epilepsy (Bernabeu *et al.*, 2004; Tharayil *et al.*, 2005). Seizures have been induced using rTMS when quite high frequency stimuli are used with a very short inter-train interval (less than 1s) (Pascual-Leone *et al*., 1993; Flitman *et al*., 1998). Thus, guidelines for safe parameters for rTMS have since been established (Wassermann, 1998; Rossi *et al.*, 2009). Additionally, participants are routinely screened for contraindications to TMS. Subjects with metal plates in the skull, who are taking certain neuromodulatory medications, or who have a family history of epilepsy are excluded (See *Appendix 1* for an example of the screening questionnaire used in my studies). The use of rTMS as a treatment for psychiatric disorders often involves the use of large numbers of stimuli, delivered daily or weekly over a number of months or even years. However, no serious adverse reactions have been reported following chronic rTMS exposure (Janicak *et al*., 2008; Di Lazzaro *et al*., 2009; Rossi *et al.*, 2009). Given the relative infancy of TMS techniques, it remains important to continue evaluating their safety, particularly through longitudinal studies.

1.5.5. Functional effects of paradigms which alter cortical excitability

Many studies have looked at the functional effects of these plasticity-inducing paradigms. However the wide variation in participants (healthy vs neurologically impaired), parameters (frequency, intensity and duration of stimulation) and type of motor function investigated (motor learning vs motor performance, simple vs complex tasks) renders it difficult to compare results. Studies in healthy and neurologically impaired individuals should be considered separately. As discussed earlier, the injured brain may have a greater capacity for neuroplastic change. Studies in healthy participants using inhibitory 0.9-1 Hz rTMS paradigms have reported no effect on tapping speed or peak finger force and peak finger acceleration (Chen *et al.*, 1997a; Muellbacher *et al.*, 2000). Similarly, 5 Hz excitatory rTMS and iTBS have no effect on rapid index finger abductions (Agostino *et al.*, 2007; Agostino *et al.*, 2008). In contrast, Jancke (2004) found that 1 Hz inhibitory rTMS slowed tapping speed and in the first human TBS study, cTBS lengthened simple reaction time (Huang *et al.*, 2005). One of the problems with many of the tasks employed to examine the functional effects of rTMS paradigms is that task performance in healthy human subjects may be near optimal and, therefore, there is little room for improvement. Studies using more complex tasks may help address this issue. For instance, associative afferent stimulation can facilitate performance of a complex sensorimotor task, the grooved pegboard (McDonnell & Ridding, 2006a). Brain imaging studies suggest more complex tasks employ a wider cortical and subcortical network (Meister *et al.*, 2005) and therefore perhaps provide greater substrate for plastic change.

rTMS has been proposed as a treatment in a wide array of neurological disorders including Parkinson's disease (Khedr *et al.*, 2003), amyotrophic lateral sclerosis (Di Lazzaro *et al.*, 2010), tinnitus (Khedr *et al.*, 2009), schizophrenia (Blumberger *et al.*, 2010) and depression (see Daskalakis *et al.*, 2008 for review). One of the most widely investigated potential therapeutic uses of rTMS is in the treatment of patients with motor impairments following stroke. Whilst the effects of rTMS are likely to lack the specificity to target particular synapses, it is thought that rTMS may interact with and enhance the intrinsic ability of the cortex to undergo neuroplastic change following stroke and may be useful when combined with traditional physical therapy (Ridding & Rothwell, 2007). Indeed, studies in small groups of stroke patients have demonstrated improvement in a number of clinical measures of motor performance following application of various rTMS paradigms (Meehan *et al.*; Khedr *et al.*, 2005; Takeuchi *et al.*, 2005; Talelli *et al.*, 2007).

1.5.6. Variability in responses to plasticity inducing paradigms

One of the problems with these plasticity-inducing techniques is the large degree of variability both within and between subjects. Patient populations display a great deal of heterogeneity and the effects may depend upon the location and extent of brain damage. However, even healthy subject cohorts exhibit a wide variation in responses to plasticity-inducing paradigms. Subjects respond differently from each other and an individual's response may vary between repeated experimental sessions, even when using identical stimulation parameters. In some cases, a paradigm which resulted in facilitation of the MEP in one experimental session may result in MEP inhibition in another session. The reasons for this large inter- and intra-individual variability are not

well understood, although many factors are likely involved (Ridding & Ziemann, 2010). One factor which has been shown to be important in modulating neuroplasticity induction is the time of day. Sale and colleagues (2007) demonstrated that the neuroplastic response to PAS is greater when the stimulation is applied later in the afternoon compared to the morning. This effect was later found to be partly modulated by cortisol, which is under circadian influence, with high circulating levels in the morning that rapidly decline over time (Sale *et al.*, 2008). Oral administration of cortisol abolished the after-effects of PAS applied in the evening compared to a placebo (Sale *et al.*, 2008). Genetic factors are also likely to be very important determinants of neuroplastic capacity and response. For example, subjects with a particular BDNF polymorphism (*Val66Met* or *Met66Met* compared to *Val66Val*) show reduced responses to iTBS, cTBS and PAS (Cheeran *et al.*, 2008). However, carriers of these alleles have demonstrated increased responses to both inhibitory and excitatory tDCS compared to *Val66Val* carriers (Antal *et al.*, 2010), suggesting that the relationship between genotype and plasticity induction is not straightforward and likely depends upon the mechanisms by which plasticity is induced (i.e. different mechanisms underlie TBS/PAS and tDCs-induced plasticity). Other factors that may influence individual responses to plasticity-inducing paradigms include attention levels (Stefan *et al.*, 2004), physical activity (Cirillo *et al.*, 2009), the menstrual cycle (Inghilleri *et al.*, 2004), age (Rogasch *et al.*, 2009; Fathi *et al.*, 2010; Todd *et al.*, 2010) and pharmacological modulations (Stefan *et al.*, 2002; Inghilleri *et al.*, 2006; Huang *et al.*, 2007).

The following section argues that an additional important factor influencing the response to plasticity inducing paradigms is the current state of activity within the

cortex at the time stimuli are applied. The state-dependancy of neuroplasticity induction forms a central theme of this thesis.

1.6. STATE-DEPENDENCY OF NEUROPLASTICITY INDUCTION

Responses to single-pulse TMS are highly state-dependent. For example, MEP amplitude is much greater and MEP latency is reduced during contraction of the target muscle compared to relaxation. This effect is dependent on both cortical and spinal mechanisms (Hess *et al.*, 1986; Thompson *et al.*, 1991b; Ugawa *et al.*, 1995). Interestingly, just thinking about movement of the target muscle enhances MEP amplitude (Izumi *et al.*, 1995). Similarly, in studies of the visual cortex, visual mental imagery reduces the threshold for TMS-induced phosphenes (Sparing *et al.*, 2002).

The response to plasticity inducing paradigms also appears to be highly state-dependant. For example, contraction during cTBS or iTBS abolishes their effects on the MEP (Huang *et al.*, 2008). Another example of the state-dependence of plasticity paradigms is how various 'priming' techniques have been developed to maximise the responses to rTMS by exploiting metaplastic effects (see *Section 1.3.2: Metaplasticity and homeostatic plasticity*). These techniques generally involve applying a short period of rTMS prior to a traditional rTMS paradigm. For example, subthreshold 6Hz rTMS increases the reduction in MEP amplitude observed following 1 Hz inhibitory rTMS (Iyer *et al.*, 2003). Priming can also be applied over adjacent cortical areas. For example, rTMS over the supplementary motor area enhances responses to quadripulse rTMS applied over the motor cortex (Hamada *et al.*, 2009). tDCS can also be used as a

priming technique. In one study, anodal (facilitatory) tDCS enhanced suppression of MEP excitability by 1 Hz inhibitory rTMS whereas cathodal (inhibitory) tDCS reversed MEP suppression to MEP facilitation. When rTMS was preceded by sham tDCS, no change in cortical excitability was observed (Siebner *et al.*, 2004). Recent data from our lab has shown that the after-effects of cTBS on the MEP are prolonged when it is applied concurrently with slow-oscillatory tDCS (Doeltgen *et al.*, 2012, in press). tDCS is thought to modulate neuronal membrane excitability (Nitsche & Paulus, 2000). Thus, it appears the induction of plasticity depends critically upon the excitability state of the underlying neuronal population at the time stimuli are applied.

State-dependent effects have not only been observed in changes in MEP amplitude but also in the behavioural after-effects of rTMS. In one example, direction sensitive neurons in the visual cortex were targeted by having subjects view rightward or leftward motion during application of a modified inhibitory TBS protocol over the visual cortex (Silvanto *et al.*, 2007). In a subsequent visual discrimination task, subjects were required to detect the direction of movement of a small visual target. Detection of movement in the incongruent direction (i.e. the opposite direction to that which was viewed during TBS) was impaired. A control condition with TBS applied over the vertex showed no effect on direction detection. The authors suggest TBS preferentially suppressed the least active neural population (i.e. those encoding the incongruent direction) (Silvanto *et al.*, 2007), again supporting the idea that neural activity state can alter the after-effects of rTMS paradigms. Indeed, more invasive electrophysiological recordings have clearly demonstrated state-dependent effects of rTMS in the cat visual cortex (Pasley *et al.*, 2009). When short trains of rTMS were applied during a high

neural activity state, they were more likely to result in spontaneous neural discharge than application of the same rTMS trains during a low neural activity state (Pasley *et al.*, 2009). This raises a central question of this thesis – whether a marker of neural activity state could be used to predict responses to plasticity-inducing paradigms. In the next section, I discuss the use of oscillatory activity in the brain as an indicator of neural activity state, and its proposed role in neuroplasticity induction and cortical inhibitory processes.

1.6.1. Brain oscillations as a marker of neural state

In humans, an indication of the activity state of neural networks in the brain can be obtained using electroencephalography or EEG. EEG can be recorded intracranially, using electrodes placed directly over the brain surface, or non-invasively using electrodes placed over the surface of the scalp. The EEG represents the summation of post-synaptic potentials in the neuronal population underlying the electrodes, and thus gives some indication of the excitability state of the cortex. The activity observed in the EEG is usually evaluated according to pre-defined frequency bands, and is oscillatory in nature.

What is the function of oscillatory activity in the human brain? This is one of the most fascinating questions in neuroscience today and one that has intrigued researchers since electrical rhythms were first recorded from the brain's surface by Hans Berger in the 1920s (Berger, 1969 (English translation)). Rhythmic neural activity can be observed in most brain regions and at a range of different frequencies (Buzsaki & Draguhn, 2004). For many years, brain rhythms were considered to be epiphenomena,

simply by-products of brain activity. More recently, oscillations have been proposed to play an essential role in information processing in the brain, providing a key link between single neuron activity and behaviour by coordinating the activity of neuronal populations (Buzsaki & Draguhn, 2004). An important property of oscillatory activity is its ability to 'bind' cell assemblies through synchronisation (Buzsaki & Draguhn, 2004; Bazhenov *et al.*, 2008). Oscillations have the potential to synchronise activity in widely distributed but inter-connected neuronal populations and as such provide a potential mechanism for the transfer of information between distant, but functionally connected, brain regions (Buzsaki & Draguhn, 2004; Bazhenov *et al.*, 2008). Oscillatory activity has been shown to synchronise spatially distinct cortical columns (Gray *et al.*, 1989) and on a more global level, signal coherence is observed between EEG signals recorded from various brain regions (Anderson *et al.*, 2009). Such transient temporal coordination of cell assemblies is likely important for the encoding, storage and retrieval of information (Buzsaki & Draguhn, 2004). For example, synchronisation of convergent input would provide a potential mechanism to integrate information. In this way, brain oscillations may provide temporal 'windows' for synaptic integration (Buzsaki & Draguhn, 2004). It is perhaps not surprising that brain oscillations at particular frequencies have been implicated in the modulation of spike-timing-dependent plasticity (Jutras & Buffalo, 2010).

The precise mechanisms by which oscillatory activity is produced are not well understood, though it is thought that there are many distinct generators, at both cortical and subcortical levels (Buzsáki, 2006). Oscillatory activity appears to be an inherent property of cortical neuronal networks. For example, when the neocortex is

surgically isolated from the rest of the brain, spontaneous slow wave oscillations develop (Kellaway *et al.*, 1966). Many individual neurons have intrinsic oscillatory properties and demonstrate rhythmical fluctuations in membrane potentials when isolated from their neuronal network (Llinas, 1988). Others only oscillate when electrically coupled with other neurons (Lampl & Yarom, 1997). Oscillations can be recorded from single neurons as fluctuations in membrane potential using intracellular electrodes, or from the local neuronal population as the local field potential (LFP), using extracellular electrodes. Alternatively, the oscillatory activity of larger neural assemblies can be observed using EEG, as discussed earlier. The frequencies of oscillations recorded from the human brain vary from the slow delta rhythm (0-3 Hz) to the fast gamma oscillations (>30 Hz) (Buzsáki, 2006). Other commonly investigated frequency bands are theta (4-7 Hz), alpha (8-12 Hz) and beta (13-30 Hz). However, oscillations as fast as 900 Hz have been recorded (Hashimoto *et al.*, 1996). Oscillatory periods are limited by distance, such that fast oscillations involve small, local networks whereas slow oscillations are able to synchronise more widely spread neuronal populations (Buzsaki & Draguhn, 2004). Thus, oscillations at different frequencies not only provide temporal but also spatial coding. There is also evidence that different oscillatory frequencies interact; cross-frequency phase coupling has been observed and may be important for the integration of spectrally distributed functions (Palva *et al.*, 2005; van der Meij *et al.*, 2012).

Various EEG frequency bands have been implicated in the mechanisms underlying learning and memory. For example, the slow wave activity observed during sleep is thought to play a role in memory consolidation (Massimini *et al.*, 2009). Theta and

alpha rhythms, which are a focus of this thesis, are thought to play key roles in the modulation of neuroplasticity and cortical inhibitory processes.

1.6.1.1. Theta rhythm and neuroplasticity

The hippocampal theta rhythm is one of the most studied rhythms of the brain. This rhythm has been extensively investigated in animal models *in vitro* (Huerta & Lisman, 1995; Natsume & Kometani, 1997) and *in vivo* (Holscher *et al.*, 1997; Orr *et al.*, 2001) and more recently in human subjects (Cornwell *et al.*, 2008; Rutishauser *et al.*, 2010). There is a large and growing body of evidence to suggest that theta rhythm plays an important role in learning and memory. In the rodent, theta oscillations are dominant in the hippocampus during spatial learning and exploratory behaviour, and loss of the hippocampal theta rhythm by lesion of the medial septus has been shown to impair spatial memory in rats (Winson, 1978). Rats which retained theta rhythm, even in the presence of this lesion, retained their spatial memory abilities. LTP induction has been shown to be more effective during high levels of pharmacologically induced theta activity in the guinea pig hippocampus (Natsume & Kometani, 1997) and greater naturally-occurring theta power in the rabbit hippocampus predicts faster learning rates in a classical conditioning paradigm (Seager *et al.*, 2002). The importance of theta in memory formation is not just limited to the rodent hippocampus; increased theta power in the human hippocampus is observed during good as compared to poor performance of a virtual navigation task which relies on spatial learning (Cornwell *et al.*, 2008).

It is important to note that these studies investigating the relationship between theta power and learning and memory are mostly correlational. Perhaps more convincing evidence comes from investigations of the role of theta *phase* in neuroplasticity induction. Individual neurons in the hippocampus produce bursts of spikes which occur at particular points during the theta cycle (i.e. in phase with theta oscillations). Based on this observation, Larson and colleagues (Larson *et al.*, 1986) developed an electrical stimulation paradigm utilising bursts of stimuli at 5 Hz to mimic natural spiking activity. This paradigm induced LTP in hippocampal slices and has since been used to induce LTP in various brain regions *in vitro* and *in vivo*, including the visual and motor cortices (Hess & Donoghue, 1994; Givens & McMahon, 1995; Hess *et al.*, 1996; Wang & Daw, 2003). In fact, it is this stimulation paradigm upon which the human TBS rTMS paradigm for inducing plasticity in the motor cortex is modelled (Huang *et al.*, 2005). In the rat hippocampus, a single burst of high frequency stimuli applied on the positive phase of theta induces LTP, which can be depotentiated by stimuli applied on the negative phase of theta. This was first demonstrated during carbachol-induced theta oscillations in hippocampal slices (Huerta & Lisman, 1995), but the finding has since been replicated in the anaesthetised rat *in vivo* (Holscher *et al.*, 1997) and, notably, has been shown in the awake, freely behaving rat during naturally-occurring theta (Orr *et al.*, 2001), providing strong evidence that it is an important physiological mechanism. Even more intriguing is the fact that this finding has recently been extended to human subjects with intracranial electrodes implanted for the detection of seizure foci (Rutishauser *et al.*, 2010). Participants were asked to remember 100 images following visual presentation. Subsequently, they were shown 50 of the previous images inter-mixed with 50 new images and were required to report which ones they had seen

before. Participants were also asked to indicate their level of confidence in their memory of the seen image. During encoding, neurons in the amygdala and hippocampus produced spikes that were phase-locked to local field potential theta oscillations. The phase locking of neuronal spiking to the local theta oscillation was 50% higher during presentation of images that were later remembered compared to those that were later forgotten. Subjects were also asked to rate the confidence of their image memory. Phase-locking in the theta range was greater during those images remembered with high confidence than those remembered with lower confidence, which led the authors to suggest that the degree of phase-locking influences the strength of the memory (Rutishauser *et al.*, 2010). However, it is still difficult to determine cause and effect - an alternative explanation is that the theta phase locking is a consequence of memory formation, rather than an important underlying mechanism of memory formation.

Theta oscillations not only occur in the hippocampus, but also in various other brain regions, including the neocortex. In the cat visual cortex, pyramidal cells show spontaneous spiking which is tightly phase locked to local field theta (Allen *et al.*, 2007), similar to the theta phase-locking of spontaneous spikes in the hippocampus. Thus, though not coherent with hippocampal theta (Buzsaki, 2002), cortical theta shares some similar properties and may play a similar role. For example, similar to hippocampal theta, cortical theta has been implicated in memory formation. In one study, subjects studied lists of common nouns and then performed a series of simple arithmetic problems, whilst EEG activity was recorded intracranially (Sederberg *et al.*, 2003). Subjects were later asked to recall as many words as possible. Increased theta

power over the right temporal and frontal cortices was present during encoding of recalled words compared to unrecalled words (Sederberg *et al.*, 2003).

The relationship between cortical oscillations and responses to experimentally induced plasticity techniques such as rTMS is unknown, however the studies discussed above suggest theta activity would influence responses to neuroplasticity induction. Whilst to my knowledge, no studies have investigated the influence of cortical rhythms on responses to rTMS, rTMS *has* previously been shown to modulate cortical rhythms, providing evidence that the two may interact. For example, rTMS applied at a frequency to match at an individual's upper alpha frequency increases the power in that frequency band (Klimesch *et al.*, 2003). This increase in alpha appears to be functionally significant, leading to an increased event-related desynchronisation in alpha activity during a mental rotation task, which is associated with improved task performance (Klimesch *et al.*, 2003). Brief periods of low frequency (1-4 Hz) rTMS applied to the cat visual cortex transiently increases the power of high frequency activities (>40 Hz) and results in a longer lasting decrease in the power of low frequency activities (<40 Hz) (Allen *et al.*, 2007). Additionally, this rTMS paradigm affects the phase relationship between spontaneous spiking of individual pyramidal neurons and the local field potential. At baseline, spontaneous spikes are phase-locked to theta field oscillations. However, following rTMS there is a much greater desynchronisation (i.e. spontaneous spikes occurred more randomly in the theta cycle). The ability of rTMS to alter neuronal phase locking might suggest that it has the potential to influence spike-timing dependent plasticity. In Chapter Two of this thesis, I investigate the relationship between EEG measures at baseline and plasticity induction by cTBS and a behavioural

visuomotor learning task. Additionally, I examine whether cTBS and visuomotor learning have any effect on EEG parameters. Whilst the study initially arose from interest in the theta rhythm's potential role in modulating neuroplastic processes such as LTP and LTD, the study also investigates delta, alpha and beta rhythms. Alpha is a rhythm of particular interest because of its proposed relationship with inhibitory processes in the brain (Jensen & Mazaheri, 2010).

1.6.1.2. Alpha rhythm and inhibition

The alpha rhythm is one of the most easily observed oscillatory signals recorded from the human brain. Alpha rhythm was long considered to indicate an 'idling' state of the brain due to observations that alpha desynchronises during behavioural engagement of the cortex (Buzsaki, 2006). For example, alpha recorded over the sensorimotor cortex (also known as 'mu', 10-12 Hz) desynchronises during movement compared to rest, and alpha activity over the occipital cortex is greater during eyes closed conditions, desynchronising with incoming visual input when the eyes are opened. However, evidence is accumulating that alpha may in fact indicate an active inhibitory state rather than cortical idling. For instance, in the visual system, the appearance of phosphenes can be used to assess the responsiveness of the visual cortex to TMS. Phosphenes are more likely to be induced by TMS during low as compared to high alpha power (Romei *et al.*, 2008). In the motor system, a study by Sauseng and colleagues (2009) suggests that at barely suprathreshold TMS intensities, MEPs are more likely to be evoked during low rather than high alpha power. These studies suggest that high levels of alpha activity are associated with high levels of inhibition in the cortex. However, the use of the term 'inhibition' can be somewhat confusing;

although these studies might suggest cortical excitability is reduced during high alpha, it is unclear whether this represents increased inhibition *per se*, or rather a lack of facilitation. Some evidence for increased intracortical inhibition comes from a study by Hummel and colleagues (2002). Eighteen healthy volunteers were trained on a task which involved learning sequences of key presses with their fingers, with visual cues presented on a screen in front of them indicating which key to press. In a second experimental session on the following day, participants were required to perform either an 'activation' condition, where they needed to press the appropriate key in response to the visual cue, or an 'inhibition' condition, where the visual cue was presented but the participant was not allowed to move. During the 'inhibition' condition, a significant increase in 11-13 Hz activity over sensorimotor areas was observed, compared to a decrease in this oscillatory activity during the activation condition. Additionally, this increase in 11-13 Hz activity was not observed in a group of six patients with focal hand dystonia, a neurological disorder characterised by impaired intracortical inhibition (Hummel *et al.*, 2002).

Studies of alpha activity during directed attention also support a suppressive function of this rhythm (Cooper *et al.*, 2003). For example, reduced alpha activity is observed in the occipital cortex contralateral to an attended item, and increased alpha activity is observed ipsilateral to the attended item (Sauseng *et al.*, 2005). In these cases, the increased alpha activity is thought to reflect suppression of task-irrelevant visual stimuli in order to enhance processing of relevant visual stimuli (Sauseng *et al.*, 2005). Consistent with this, reaction time in a task involving detection of cued targets correlated with the lateralisation index of alpha (Thut *et al.*, 2006). Detection of

rightward targets was faster when left hemisphere alpha activity was less prevalent than right hemisphere alpha activity, and vice versa for leftward targets (Thut *et al.*, 2006). Task-specific suppression of alpha is not just observed between hemispheres but also between modalities. For example, attention to somatosensory nerve stimuli results in a reduction in alpha over the somatosensory cortex contralateral to the attended hand and increased alpha over the occipital lobe (Anderson & Ding, 2011). Similarly, Fu et al. (2001) demonstrated that compared to attention to visual cues, attention to auditory cues increased alpha over the occipital lobe. Again, this increased alpha was attributed to active disengagement of task-irrelevant regions. These findings have led to the proposal of a 'gating by inhibition' hypothesis (Jensen & Mazaheri, 2010), whereby information flow in the brain is 'gated' by inhibiting brain regions which are not required for performance of the task, ensuring that information is deployed only to task-relevant areas. In this way, pre-stimulus alpha activity may mould the functional architecture of the brain to enhance information processing in regions of the brain that are important for task performance (Jensen & Mazaheri, 2010). This hypothesis predicts that increased alpha in task-irrelevant regions should correlate with good task performance. Several studies support this idea. Haegens and colleagues (2011) applied sample stimuli of 6-10 Hz to the right median nerve for a period of 1 second. Probe stimuli were then applied at either the same frequency or +/- 1 Hz. Participants were required to report whether the probe stimulus was the same frequency as the previous sample stimulus by pressing a pedal with their foot. Increased alpha was observed over posterior regions, particularly the occipital lobe, and over the right sensorimotor cortex during the retention period of the task. Increased alpha activity in these regions correlated with good performance of the task.

Similarly, Meeuwissen et al. (2010) found that long-term memory of learned word sequences was predicted by greater alpha over the parietal-occipital region during the memory encoding phase.

Interestingly, alpha phase has been shown to predict the ability for visual detection. Mathewson and colleagues (2009) found that a visual target was more likely to be perceived if presented at the peak of the alpha oscillation compared to the trough. Current models of sensory disengagement by alpha suggest that the inhibitory effects of alpha arise from 'pulsed inhibition', with bouts of inhibition repeated every 100 ms or so (Jensen & Mazaheri, 2010). GABAergic interneuronal networks have been implicated in the physiological mechanism underlying the generation of alpha oscillations, and may be responsible for this pulsed inhibition (Jensen & Mazaheri, 2010). Notably, suppression of alpha activity by neurofeedback training is associated with a 150% reduction in $GABA_A$-mediated intracortical inhibition, measured using TMS (Ros *et al.*, 2010). Thus, although correlational only, there is some evidence that alpha activity is associated with inhibitory processes in the cortex, and that these inhibitory processes may be mediated by $GABA_A$. In Chapter Three of this thesis, I investigate this proposal further, by examining the relationship between ongoing alpha activity and SICI, a $GABA_A$-mediated form of intracortical inhibition which can be measured using TMS (see *Section 1.2.3.1. SICI*). I develop a technique to trigger TMS from the ongoing alpha rhythm in order to target different oscillatory phases. Given the known relationship between GABAergic inhibition and plasticity induction, if the alpha rhythm is related to modulations in inhibitory tone it may be highly relevant for the mechanisms underlying neuroplasticity; if we are able to target the cortex at times when GABA inhibition is

reduced, we may be able to enhance neuroplasticity induction. This idea is further explored in Chapter Four, where I modify an existing rTMS technique to selectively target cortical inhibitory pathways.

1.7. SUMMARY

It is possible to induce neuroplastic change in the human motor cortex and these changes may be of functional relevance. However, there is a large degree of variability in subject responses to plasticity induction. An important determinant of this variability is the state of the cortex at the time stimuli are applied. This thesis employs EEG and TMS techniques to explore the state-dependence of neuroplasticity induction; how we may be able to determine or target the optimal state for neuroplasticity induction, and how we might be able to create a more optimal cortical environment for neuroplasticity induction by reducing cortical inhibition.

CHAPTER TWO

Cortical oscillatory activity and the induction of plasticity in the human motor cortex

The first experimental chapter of my thesis considers the possibility of using characteristics of the EEG as a marker of brain state, in order to predict the response to plasticity inducing experimental paradigms. I investigate whether spectral power in the delta, theta, alpha and beta frequency bands at baseline predicts subject responses to neuroplasticity induction by non-invasive brain stimulation (continuous Theta Burst Stimulation (cTBS)) and behavioural motor learning (visuomotor training). Additionally, I explore whether cTBS and visuomotor learning have any lasting effect on the EEG power spectra.

2.1. ABSTRACT

BACKGROUND: Repetitive transcranial magnetic stimulation paradigms such as continuous Theta Burst Stimulation (cTBS) induce Long-Term Potentiation- and Long-Term Depression-like plasticity in the human motor cortex. However, responses to cTBS are highly variable and may depend on the activity of the cortex at the time of stimulation. We investigated whether power in different EEG (electroencephalogram) frequency bands predicted the response to subsequent cTBS, and conversely whether cTBS had after-effects on the EEG. cTBS may utilise similar mechanisms of plasticity as motor learning; thus we conducted a parallel set of experiments to test whether ongoing EEG could predict performance of a visuomotor training task, and whether training itself had effects on the EEG.

METHODS: Motor Evoked Potentials (MEPs) provided an index of cortical excitability pre and post-intervention. EEG was recorded over the motor cortex pre- and post-intervention and power spectra were computed.

RESULTS: cTBS reduced MEP amplitudes, however baseline power in the delta, theta, alpha or beta frequencies did not predict responses to cTBS or learning of the visuomotor training task. cTBS had no effect on delta, theta, alpha or beta power. In contrast, there was an increase in alpha power following visuomotor training which was positively correlated with changes in MEP amplitude post-training.

CONCLUSIONS: The results suggest the EEG is not a useful state-marker for predicting responses to plasticity-inducing paradigms. The correlation between alpha power and

changes in corticospinal excitability following visuomotor training requires further investigation, but may be related to disengagement of the somatosensory system important for motor memory consolidation.

2.2. INTRODUCTION

Neuroplasticity can be induced in the human motor cortex using various non-invasive neurophysiological techniques, including peripheral nerve stimulation (Ridding *et al.*, 2000; Charlton *et al.*, 2003), paired peripheral and cortical stimulation (Stefan *et al.*, 2000; Wolters *et al.*, 2003), and cortical stimulation alone in the form of repetitive transcranial magnetic stimulation (rTMS) (Di Lazzaro *et al.*, 2002a; Huang *et al.*, 2005; Fitzgerald *et al.*, 2006 for review). Neuroplasticity induced by these techniques manifests as a change in cortical excitability, measured as changes in Motor Evoked Potential (MEP) amplitude. These changes in cortical excitability are similar in nature to those seen following motor learning. Indeed, there is evidence that motor learning-induced, and experimentally-induced plasticity engage similar neural pathways (Ziemann *et al.*, 2004; Stefan *et al.*, 2006), likely involving changes in synaptic efficacy brought about by mechanisms similar to Long-Term Potentiation (LTP) and Long-Term Depression (LTD) (Stefan *et al.*, 2002; Huang *et al.*, 2007). Thus, there is great interest in whether plasticity-inducing interventions can modify motor behaviour and potentially be of therapeutic value for disorders characterised by motor impairment, such as stroke and Parkinson's disease (Ridding & Rothwell, 2007 for review). However, a major problem with these interventions is that their effectiveness is limited by the large variability in participant responses. The reasons for this variability are not well-understood, though multiple factors are likely involved (Ridding & Ziemann, 2010). One factor known to be important in modulating responses to rTMS is the initial physiological state/ history of activity of the cortex at the time stimuli are applied (Silvanto & Pascual-Leone, 2008). For instance, 6 Hz 'priming' rTMS applied prior to 1 Hz inhibitory rTMS enhances the depression of motor cortical excitability compared to

1 Hz inhibitory rTMS applied alone (Iyer *et al.*, 2003). State-dependent effects of rTMS have been demonstrated using invasive electrophysiological recordings in the cat visual cortex (Pasley *et al.*, 2009). Larger post-rTMS responses were predicted by higher pre-TMS local field power, a measure of the activity of local neural networks (Pasley *et al.*, 2009). In humans, ongoing activity of cortical neural networks can be monitored using electroencephalography (EEG) (Buzsaki & Draguhn, 2004). The EEG reflects the summing of postsynaptic potentials from the neuronal population underlying the recording electrodes and thus gives an indication of the activity state of the cortex (Buzsaki & Draguhn, 2004). We questioned whether EEG recorded at baseline can be used as a state-marker to predict responses to rTMS protocols. If the EEG can indeed be used as a state-marker, it may also be relevant for behaviourally-induced plasticity, such as motor learning. Finally, if the EEG yields a marker of brain activity relevant for motor learning, then learning should change the EEG. Thus the aims of the present study were three-fold; firstly, we wanted to investigate whether characteristics of the baseline EEG could be used to predict subject responses to cTBS. Secondly, we wished to examine whether the baseline EEG predicted learning of a visuomotor training task. Lastly, we explored whether either intervention changed the EEG.

2.3. METHODS

2.3.1. Participants

Participants were 23 healthy adults (13 females) with mean age 27.9 $\pm$ 8.3 years. All participants provided written, informed consent and the study was approved by the University of Adelaide Human Research Ethics Committee and performed in accordance

with the Declaration of Helsinki. All participants were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). All experiments took place in the afternoon in order to control for any time-of-day effects on plasticity induction (Sale *et al.*, 2007). In one experimental session participants received cTBS. In a second experiment participants performed a visuomotor training task. Only participants who were naïve to the visuomotor training task (n=13) took part in this session. The two experimental sessions were separated by at least one week. Data from two participants was excluded from the visuomotor analysis due to technical problems encountered during the experimental sessions.

2.3.2. Single-pulse TMS

Single-pulse TMS was applied over the left motor cortex hand area using a flat figure-of-eight coil (diameter 9cm) connected to a Magstim 200^2 stimulator (Magstim, Dyfed, UK). The coil was held tangentially to the skull, perpendicular to the estimated position of the central sulcus and over the optimal spot for evoking a response in the first dorsal interosseous (FDI) muscle. Silver-silver chloride electrodes were placed over the muscle belly and the metacarpophalangeal joint of the index finger for the cTBS session. For the visuomotor session the reference electrode was placed over the carpometacarpal joint to avoid interference with performance of the task. EMG signals were amplified (x1000) by a CED 1901 amplifier (CED, UK), filtered (20-1000 Hz) and digitised at 2 kHz by a CED 1401 analog-digital converter (CED, UK). Data was saved on a computer for offline analysis. Blocks of 15 MEPs were recorded from FDI at baseline and five and ten minutes following cTBS and visuomotor training. MEPs were also recorded between the first and second visuomotor training blocks. MEPs were evoked

using an intensity adjusted to give a mean peak-to-peak MEP amplitude of 0.5-1mV at baseline. This intensity was then used for all further MEP measurements during the experimental session. Stimuli were delivered every 7 seconds with 20% variance. 50 ms of EMG was recorded prior to the TMS trigger. Trials with EMG activity prior to the trigger were discarded offline.

2.3.3. Theta Burst Stimulation

Continuous Theta Burst Stimulation (cTBS) was applied using an air-cooled figure-of-eight coil connected to a Magstim Rapid^2 Stimulator (Magstim, Dyfed, UK). Three pulses at 50Hz were applied every 200 ms for a period of 40s (i.e. 600 pulses in total), as first described by Huang and colleagues (Huang *et al.*, 2005). The intensity of stimulation was set at 80% Active Motor Threshold (AMT). AMT was measured whilst participants were performing a light contraction of right FDI at approximately 10% of maximal voluntary contraction. AMT was defined as the minimum stimulator output necessary to evoke a response of at least 200 μV in 5 out of 10 consecutive trials, and ranged from 24% to 59% (mean 45 $\pm$ 9.5%). Subjects were instructed to keep FDI completely relaxed during and following the application of cTBS, as activation during or following cTBS can alter the after-effects (Huang *et al.*, 2008).

2.3.4. Visuomotor Training

The visuomotor training task was based on visuomotor tracking tasks described previously (Perez *et al.*, 2004; Dartnall *et al.*, 2009; Todd *et al.*, 2009). Subjects performed two training blocks, each of six minutes duration. Participants sat at a table with their right arm out-stretched in front of them and fixed in a moulded cast to

restrict movement. Their palm was facing downwards with their right index finger attached to a potentiometer to measure the angle of the metacarpophalangeal joint. Their range of movement was determined using a protractor, and the index finger was placed in a resting position at the centre of this movement range. The task involved tracking a blue target line moving across a display in front of the subject. Participants were required to track the target line by abducting and adducting their index finger. Their tracking line was displayed in red. At the beginning and end of each frame the blue target line returned to 0 degrees so that participants could prepare for the following frame. The target line moved a maximum of 10 degrees in either direction. The speed of movement depended on the slope of the line. The maximal speed was 40 degrees per second. Each frame lasted 10s and there were 36 frames in each training block. There were 36 target line patterns, the order of which was randomised for each training block. At the beginning of the task participants were given a demonstration of the target line moving across the screen and were told that the angle of their index finger joint was represented by the red line. They were informed that when given the instruction, they would need to track the blue target line as closely as possible by moving their index finger from side to side. They were not allowed to practise the task before recording. Participants were told when they were halfway through the training block and when they had five frames remaining. Signals were filtered (DC-100Hz) and sampled at 2 kHz by a CED 1401 analog-digital converter (CED, UK). Data was saved on a computer for later analysis offline.

2.3.5. Electroencephalography

EEG was recorded over the motor cortex using a bipolar montage with one Ag-AgCl electrode placed at C3 (active) and one at Fz (reference). A two-channel electrooculogram was recorded by placing an electrode on each temple with a reference electrode in the centre of the forehead. The electrooculogram was used to identify eye movement artefacts in the EEG for later removal offline. Impedance was kept at below 5 kOhms for all EEG recordings. EEG data was recorded following MEP measurements, immediately prior to the intervention (either cTBS or the visuomotor training task) and at 0 and 8 mins following the intervention. The participant was instructed to relax their face, jaw and hand muscles, to keep their eyes open and staring straight ahead, and to swallow before the experimenter counted down to the start of recording. Signals were amplified (x10000) by a CED 1901 amplifier, filtered (0.5-100 Hz) by a CED 1902 signal conditioner (CED, UK) and digitised (2.048 kHz) by a CED 1401 analog-digital converter (CED, UK).

2.3.6. Data Analysis

2.3.6.1. MEPs

All data were recorded using Signal version 4.6 (CED, UK). Mean peak-to-peak MEP amplitude was calculated for each block of 15 trials for each subject. The cTBS and visuomotor MEP data were then analysed with two separate one-way repeated-measures ANOVAs with the factor of TIME (Baseline, 5 and 10 mins for cTBS; baseline, between training blocks, 5 and 10 mins for the visuomotor training task). cTBS results in MEP inhibition in the majority of subjects. Three participants had facilitated MEPs following cTBS. It is unclear why some participants demonstrate facilitated MEPs

following cTBS, and it is possible that different mechanisms underlie these changes, compared to those subjects whose MEPs are suppressed following cTBS. Thus, these three subjects were excluded from all group data analysis. However, we thought it would be interesting to compare, qualitatively, the power spectra from those subjects who showed facilitation of MEPs following cTBS with those who demonstrated inhibition. We found no obvious similarities in the baseline EEG of those subjects who facilitated, nor in any changes in their power spectra following cTBS.

2.3.6.2. Electroencephalography

Eye movement artefacts were identified in the EEG data offline by visual inspection and removed. To control for spectral leakage a Cosine window was applied to all EEG data. Fast Fourier Transform was performed on 2s epochs, with a mean power spectrum calculated from the resulting 60 power spectra for each 120s EEG block. The resolution of the Fast Fourier Transform was 0.5 Hz. The power in each frequency band was calculated by summing the power in 0.5 Hz bins (delta:0.5-3.5 Hz; theta:4-7.5 Hz; alpha:8-13.5 Hz; beta:14-30 Hz). The power in each frequency band was then normalised to the total summed power from 0.5-40 Hz. Linear regression analysis was undertaken to examine the relationship between the normalised power in each frequency band at baseline and the change in MEP amplitude following cTBS and visuomotor training. Additionally, two separate two-way repeated-measures ANOVAs were performed on the raw power values for the cTBS and visuomotor data in order to determine whether the interventions had any effect on the power spectra. The within-subject factors analysed were TIME (Baseline, 0 and 8 mins) and FREQUENCY BAND (delta, theta, alpha, beta).

2.3.6.3. Visuomotor training

In order to assess learning of the visuomotor training task we used three measures: maximal cross correlation coefficient, lag time and absolute error (Perez *et al.*, 2004; Dartnall *et al.*, 2009; Todd *et al.*, 2009). The target angle and actual angle across each trial were cross correlated and the maximal cross correlation coefficient and lag time calculated. The absolute error was the mean, over all time points, of the absolute difference between the target angle and the actual angle (degrees). Mean maximal cross-correlation coefficient, mean lag time and mean absolute error were calculated for each training block for each participant. Each of these variables were then analysed by a student's paired *t*-test to compare training block one and two. An increase in maximal cross correlation coefficient and a decrease in lag time and absolute error are indicative of learning having occurred (Perez *et al.*, 2004; Dartnall *et al.*, 2009; Todd *et al.*, 2009).

The maximal cross-correlation coefficient, lag time and absolute error values from training block two were also normalised to training block one and linear regression analysis conducted to determine the relationship between baseline EEG power and learning of the visuomotor task. Pearson correlation analysis was conducted to examine the relationship between changes in MEP amplitude following training and learning of the task.

P-values < 0.05 were considered significant. All data are presented as the mean ± SEM unless stated otherwise. For the ANOVA analyses, Mauchly's test of sphericity was applied and in cases of significant non-sphericity a Greenhouse-Geisser correction was

used (ε value is stated in results). Post hoc-analysis was conducted where appropriate using the Bonferroni *t*-test method. Data analysis was conducted using PASW Statistics 17.0 (SPSS Inc., US) except for the visuomotor task cross-correlations which were performed using a purpose-written script in Matlab 7.6.0 (The Mathsworks Inc., US).

2.4. RESULTS:

2.4.1. cTBS

Figure. 2.1 summarises the MEP data. There was a significant effect of TIME on MEP amplitude (ε=0.65, $F_{1.3, 25}$=8.2, p=0.0052). Post-hoc analysis revealed that this effect was due to a reduction in MEP amplitude 5 mins following cTBS (Bonferroni corrected p<0.0001). MEP amplitudes were significantly reduced from 0.99 ± 0.078 mV at baseline to 0.67 ± 0.066 mV following cTBS. There was also a strong trend for MEPs amplitudes to be reduced compared to baseline at 10 mins post-cTBS (0.75 ± 0.078 mV, Bonferroni corrected p=0.084)

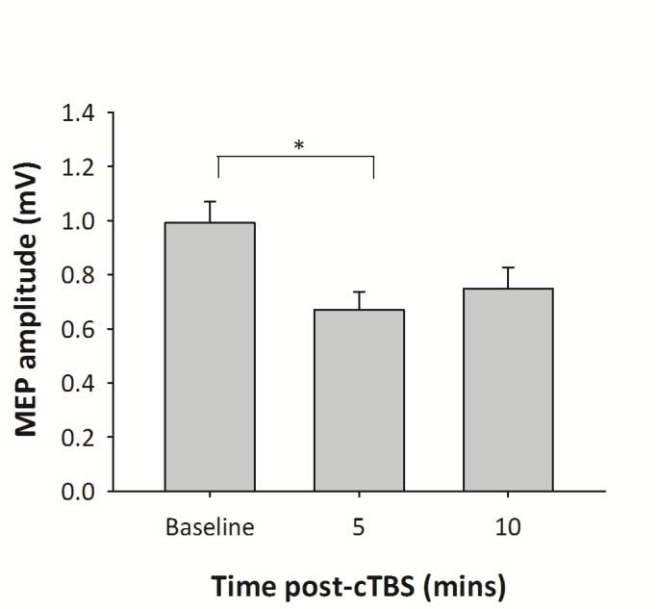

Figure 2.1. *Reduction in FDI MEP amplitude following cTBS. MEP amplitude was reduced 5 minutes following cTBS (n=20, p<0.0001).*

Figure. 2.2 presents the relationship between baseline spectral power (as a % of 0.5-40 Hz power) and the reduction in MEP amplitude 5 minutes following cTBS. Simple linear regression was performed to investigate the relationship between the reduction in MEP amplitude and baseline spectral power. The regression equations were not

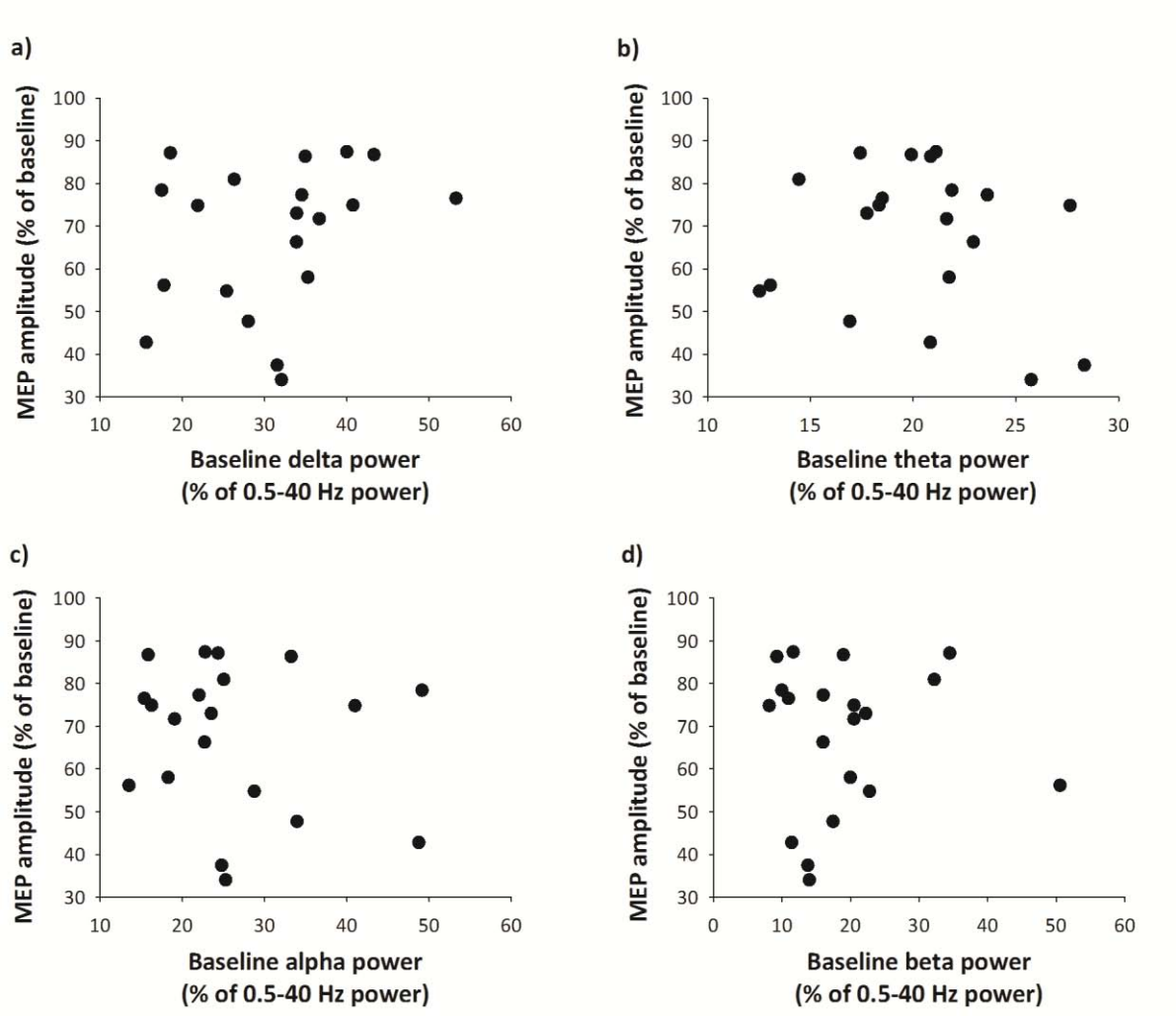

Figure 2.2. *The relationship between normalised baseline spectral power and the reduction in FDI MEP amplitude following cTBS. Normalised baseline power in the delta (a), theta (b), alpha (c) and beta (d) frequency bands did not predict changes in MEP amplitude 5 minutes following cTBS (n=20, p>0.05 for all)*

significant for any of the frequency bands analysed (delta: R^2=0.077, $F_{1,18}$=1.5, p=0.24; theta: R^2=0.034, $F_{1,18}$=0.63, p=0.44; alpha: R^2=0.025, $F_{1,18}$=0.47, p=0.50; beta: R^2=0.00012, $F_{1,18}$=0.002, p=0.96). There was also no relationship between baseline spectral power and reductions in MEP amplitude 10 minutes following cTBS (delta: R^2=0.16, $F_{1,18}$=3.5, p=0.077; theta: R^2=0.022, $F_{1,18}$=0.41, p=0.53; alpha: R^2=0.096, $F_{1,18}$=1.9, p=0.18; beta: R^2=0.0000014, $F_{1,18}$=0.000025, p=0.99). Thus, baseline spectral

power did not significantly predict the reduction in MEP amplitude for any of the frequency bands analysed.

An additional analysis was performed looking specifically at upper and lower alpha bands which were adjusted for each individual based on their peak alpha frequency. The lower alpha band was defined as individual peak alpha frequency minus 2Hz. cTBS. The regression equations were not significant (5 mins: lower alpha: R^2=0.004, $F_{1,18}$=0.076, p=0.79; upper alpha: R^2=0.062, $F_{1,18}$=1.2, p=0.29. 10 mins: lower alpha R^2=0.029, $F_{1,18}$=0.54, p=0.47; upper alpha: R^2=0.059, $F_{1,18}$=1.1, p=0.30; Figure 2.3).

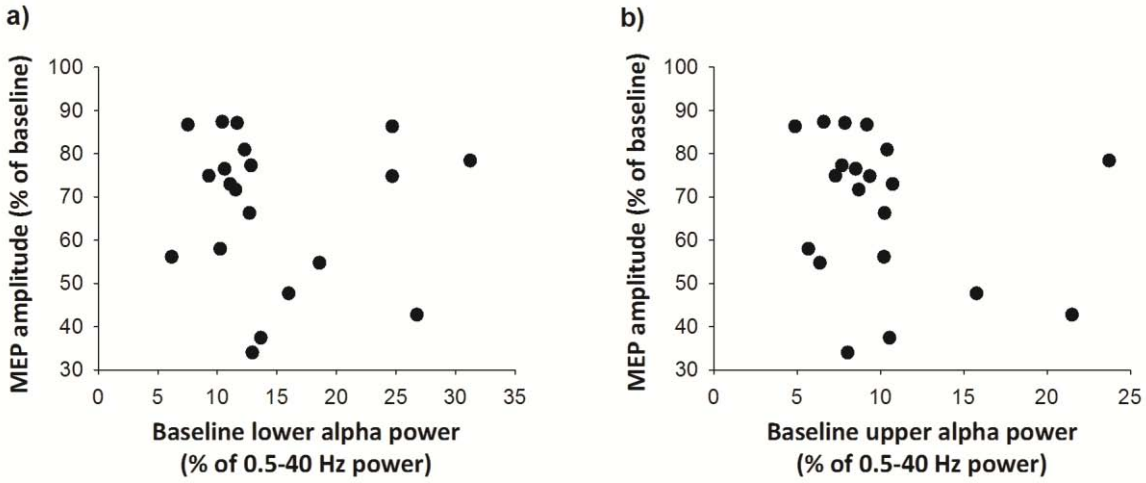

Figure 2.3. *The relationship between normalised baseline alpha power and the reduction in FDI MEP amplitude following cTBS. Normalised baseline power in the lower (a) and upper (b) alpha bands did not predict changes in MEP amplitude 5 minutes following cTBS (n=20, p>0.05 for both). Lower and upper alpha bands were calculated for each individual based on their dominant alpha frequency.*

The mean spectral power across time and frequency band is presented in Figure. 2.4. Two way repeated measures ANOVA showed no significant effect of FREQUENCY (ε=0.56, $F_{1.7,32}$=2.9, p=0.078) or TIME (ε=0.75, $F_{1.5,28}$=0.92, p=0.39) and no TIME x

FREQUENCY interactions (ε=0.42, $F_{2.5,47}$, p=0.63). Thus cTBS had no effect on the power spectra.

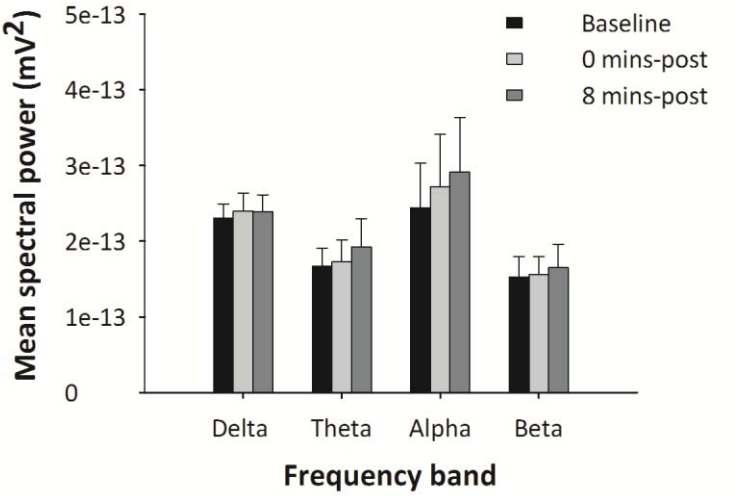

Figure 2.4. *Raw spectral power before and after cTBS. There were no changes in any of the power spectral frequency bands across time (n=20, p>0.05 for all comparisons).*

2.4.2. Visuomotor training

Paired t-tests showed there was a significant increase in maximal cross correlation coefficient (t(10)=-2.5, p=0.030) and a significant decrease in lag time (t(10)=4.0, p=0027) and absolute error (t(10)=3.3, p=0.0076) between training blocks 1 and 2, indicating improvement in performance of the visuomotor training task (Figure. 2.5).

Changes in MEP amplitude following the visuomotor training task were highly variable, with some participants demonstrating large decreases in MEP and others large increases. Thus, the one-way repeated-measures ANOVA showed no significant effect of TIME on the MEP amplitudes ($F_{3,30}$=0.85, p=0.48) (Figure. 2.6a).

Simple linear regression showed that changes in MEP amplitude 5 mins and 10 mins following visuomotor training were not predicted by baseline spectral power in any of the four frequency bands analysed (5 mins: delta: R^2=0.085, $F_{1,9}$=0.84, p=0.39; theta: R^2=0.11, $F_{1,9}$=1.1, p=0.31; alpha: R^2=0.038, $F_{1,9}$=0.36, p=0.56; beta: R^2=0.11, $F_{1,9}$=1.1, p=0.33. 10 mins: delta: R^2=0.007, $F_{1,9}$=0.067, p=0.80; theta: R^2=0.081, $F_{1,9}$=0.80, p=0.40; alpha: R^2=0.072, $F_{1,9}$=0.70, p=0.43; beta: R^2=0.034, $F_{1,9}$=0.32, p=0.59).

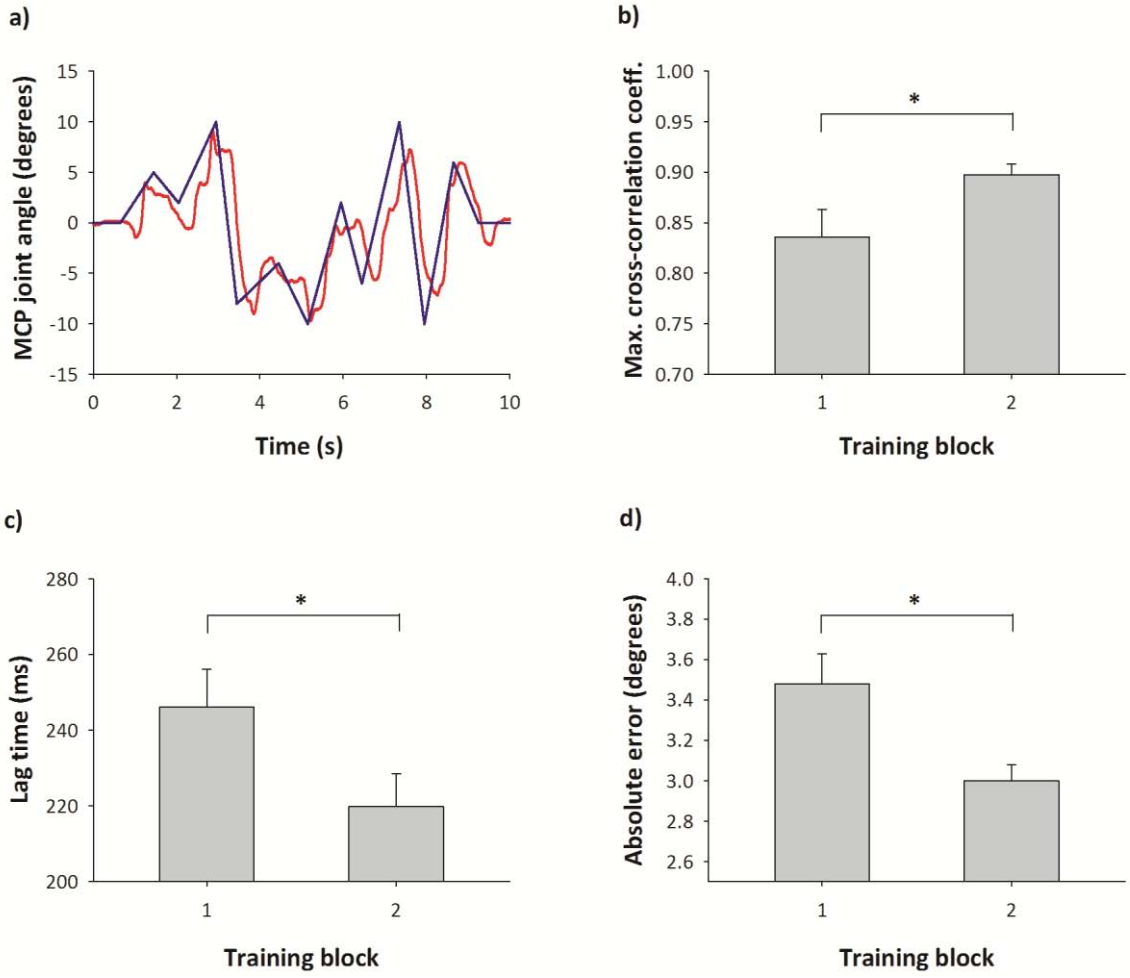

Figure 2.5. *Learning of the visuomotor training task. a) An example of one frame of the visuomotor training task. The blue line shows the target angle of the MCP joint, the red line is the participant's actual MCP joint angle. The target and actual line were cross-correlated. b) Mean maximal cross correlation coefficient was significantly increased from training block one to training block two (n=11, p=0.030). c) Mean lag time was significantly reduced from training block one to training block two (n=11, p=0.0027). d) Mean absolute error was significantly reduced from training block one to training block two (n=11, p=0.0076).*

There were also no correlations between baseline spectral power and changes in maximal cross correlation coefficient (delta: R^2=0.080, $F_{1,9}$=0.79, p=0.40; theta: R^2=0.097, $F_{1,9}$=0.96, p=0.35; alpha: R^2=0.034, $F_{1,9}$=0.32, p=0.59; beta: R^2=0.077, $F_{1,9}$=0.76, p=0.41), lag time (delta: R^2=0.20, $F_{1,9}$=2.2, p=0.17; theta: R^2=0.33, $F_{1,9}$=4.4, p=0.065; alpha: R^2=0.082, $F_{1,9}$=0.80, p=0.39; beta: R^2=0.042, $F_{1,9}$=0.39, p=0.55) and absolute error (delta: R^2=0.11, $F_{1,9}$=1.1, p=0.33; theta: R^2=0.18, $F_{1,9}$=2.0, p=0.19; alpha: R^2=0.0062, $F_{1,9}$=0.056, p=0.82; beta: R^2=0.012, $F_{1,9}$=0.11, p=0.74) , suggesting no relationship between baseline spectral power and learning of the visuomotor training task.

The two-way repeated-measures ANOVA looking at whether the power in the frequency bands was altered by visuomotor training showed a significant effect of FREQUENCY (ε=0.54, $F_{1.6,16}$=4.4, p=0.037), TIME ($F_{2,20}$=3.9, p=0.038) and a TIME x FREQUENCY interaction (ε=0.35, $F_{2.1,\ 21}$=5.7, p=0.010). Separate one-way repeated-measures ANOVAs were then performed on each of the frequency bands. There was a significant effect of time for the alpha frequency band ($F_{2,20}$=5.4, p=0.013). Post-hoc analysis showed that alpha power was significantly increased at 0 mins (Bonferonni corrected p=0.025) and 8 mins (Bonferonni corrected p=0.034) post-training (Figure. 2.6b). There was no correlation between increased alpha power and changes in max. cross correlation coefficient (p=0.53), lag time (p=0.24) or absolute error (p=0.41) . However there was a strong positive relationship between the increase in alpha power from baseline to 8 mins post-training and the change in MEP amplitude at 5 mins (r^2=0.64, p=0.0054) and 10 mins (r^2=0.46, p=0.031) following training (see Figure.2.6c and d). This correlation was not evident with the EEG recorded immediately following

training (p=0.47 and p=0.62 for MEPs at 5 and 10 mins respectively). Note that one participant's data was removed from the correlation analysis as their increase in alpha power was greater than three standard deviations from the mean increase in alpha power.

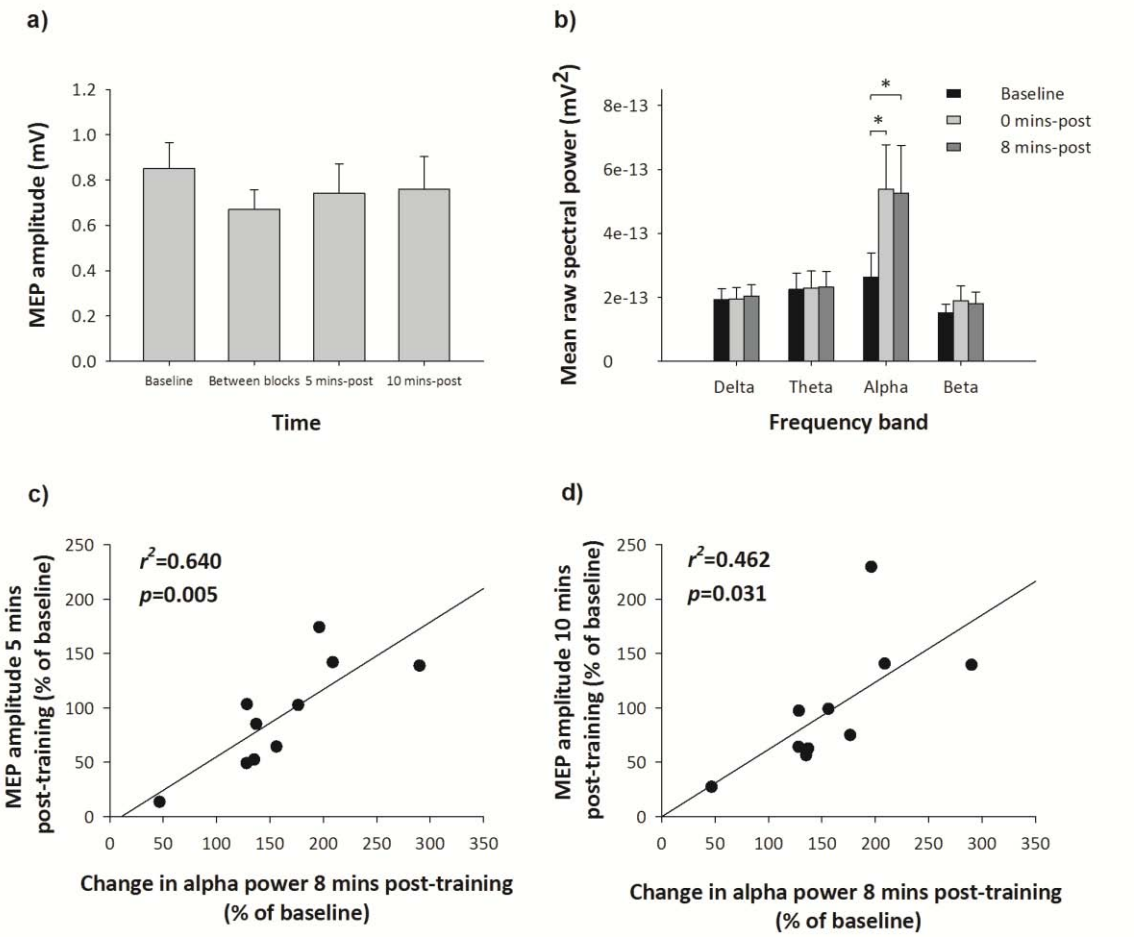

Figure 2.6. *Changes in MEP amplitude and the power spectrum following visuomotor training. a) Group means of MEP amplitude before and after cTBS. There were no significant differences in mean MEP amplitude across time (n=11, p>0.05). b) Mean raw spectral power in the delta, theta, alpha and beta frequency bands before and after visuomotor training. Raw alpha power was significantly increased at 0 and 8 mins following training (n=11, p=0.025 and p=0.034 respectively). There were no significant changes in any of the other power spectral frequency bands (p>0.05 for all). c) and d) The relationship between changes in normalised alpha power and changes in FDI MEP amplitude following visuomotor training. There was a significant positive linear relationship between the change in alpha power 8 mins post-training and changes in MEP amplitude at 5 (c) and 10 (d) mins post-training (n=10).*

Although there was no overall change in alpha power following cTBS, after observing the relationship between increased alpha power and changes in MEP amplitude following visuomotor training we decided to investigate whether there was a similar relationship between individual changes in alpha power following cTBS and the cTBS-induced reduction in MEP amplitude. There was no correlation between change in alpha power from baseline to immediately post-cTBS and the reduction in MEP amplitude 5 mins ($p=0.34$) and 10 mins ($p=0.80$) following cTBS. Similarly, there was no correlation between the change in alpha power from baseline to 8 mins post-cTBS and the reduction in MEP amplitude 5 mins ($p=0.55$) and 10 mins ($p=0.47$) following cTBS.

Individual changes in MEP amplitude 5 mins following visuomotor training did not correlate with changes in lag time ($p=0.91$) or absolute error ($p=0.35$). However there was a significant negative correlation between changes in MEP amplitude and maximal cross-correlation coefficient ($r^2=0.44$, $p=0.026$). People who had reduced MEPs 5 mins following visuomotor training had the largest increases in maximal cross-correlation coefficient (see Figure. 2.7). Individual changes in MEP amplitude 10 mins following visuomotor training did not correlate with changes in maximal cross correlation coefficient ($p=0.095$), lag time ($p=0.83$) or absolute error ($p=0.66$).

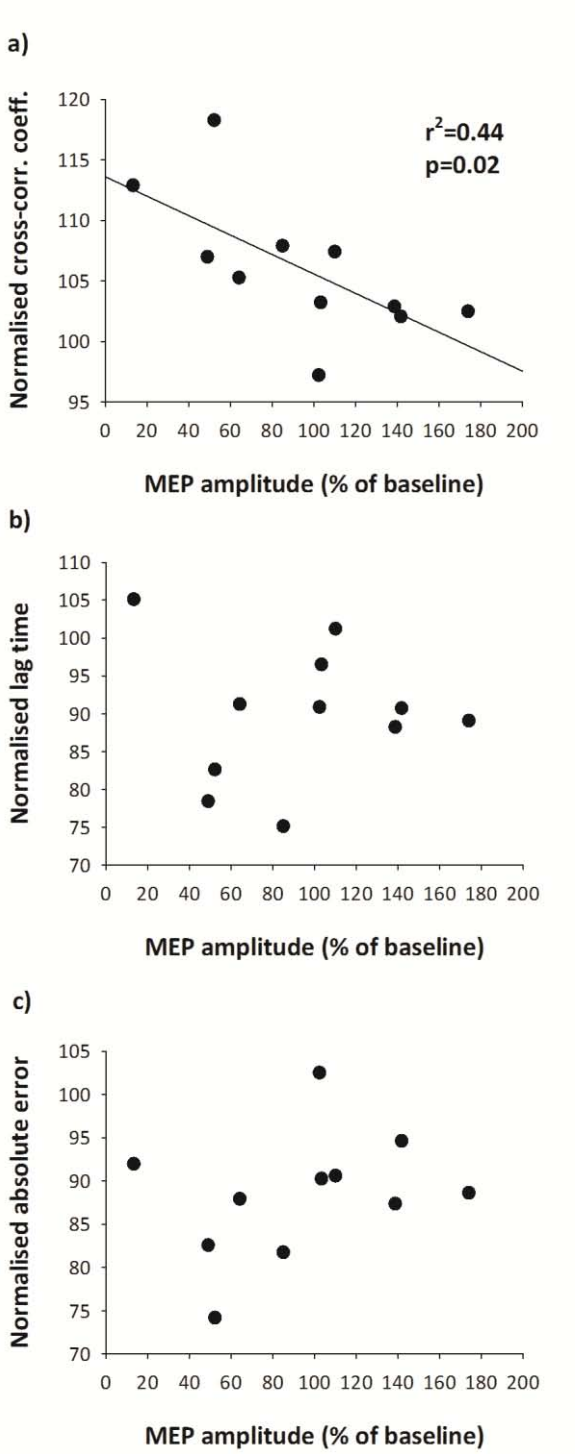

Figure 2.7. *The relationship between changes in FDI MEP amplitude and learning of the visuomotor training task. There was a significant negative linear relationship between normalised MEP amplitude at 5 mins post-training and normalised maximal cross correlation coefficient (n=11, p=0.026). However there were no significant correlations between normalised MEP amplitude and normalised lag time or normalised absolute error (n=11, p>0.05 for all).*

2.5. DISCUSSION

The present study found no evidence to suggest the EEG can be used as a state-marker to predict the response of the motor cortex to cTBS or the ability of subjects to learn a new task. Additionally, cTBS had no effect on the EEG (0.5-40 Hz) power spectra. In contrast, there was a large and significant increase in alpha power following visuomotor training, which lasted at least eight minutes post-training. This increase in alpha power was positively correlated with changes in MEP amplitude at five and ten minutes post-training, such that participants with the largest increase in alpha power also had the largest increase in MEP amplitude.

Rhythmic activity can be recorded from many different brain regions, in a range of frequency bands. Although the role of neuronal oscillations in brain function is not well understood, they are thought to be important for binding cell assemblies, allowing the transfer of information between distributed but synchronised neuronal populations (Buzsaki & Draguhn, 2004). This transient temporal coordination of neuronal groups may be important for the encoding, storage and retrieval of information (Buzsaki, 2002; Sederberg *et al.*, 2003; Buzsaki & Draguhn, 2004). In particular, oscillatory activities may play a role in integrating information by synchronising convergent inputs (Buzsaki & Draguhn, 2004). This implies a potential role for oscillations in spike-timing dependent plasticity. Indeed, a recent study demonstrated that human memory formation is predicted by how tightly neuronal spiking is phase-locked with the local theta oscillations in the hippocampus (Rutishauser *et al.*, 2010). In fact there is a large body of evidence, mostly from animal studies, suggesting theta oscillations modulate plasticity induction in the hippocampus. Individual neurons in the rodent hippocampus

produce short bursts of spikes in phase with theta oscillations, which are the dominant oscillatory activity during spatial learning/ exploratory behaviour (Larson *et al.*, 1986). During theta oscillations induced by carbachol in hippocampal slices, a single burst of high frequency stimuli applied on the positive phase of the theta peak induces LTP, which can be reversed or 'depotentiated' by stimuli applied on the negative phase of theta (Huerta & Lisman, 1995). The induction of LTP in the guinea pig hippocampus has been shown to be greater during periods of high theta activity (Natsume & Kometani, 1997). More recently, human hippocampal theta has been investigated using magnetoencephalography (MEG) and source analysis techniques (Cornwell *et al.*, 2008). High levels of hippocampal theta were associated with better performance of a virtual Morris water maze task (Cornwell *et al.*, 2008), implicating human hippocampal theta in spatial memory and extending the large body of work in the rodent hippocampus to humans.

A number of studies have also linked cortical oscillatory activity in many frequency bands with human memory formation. For instance, alpha power over posterior and central regions has been shown to increase with the number of items held in working memory (Jensen *et al.*, 2002) and increased theta activity was recorded over the right temporal and frontal cortices during encoding of recalled but not unrecalled words when subjects were asked to study and remember a list of nouns (Sederberg *et al.*, 2003). Although in these studies the EEG was not being treated as a state-marker as such but rather as a part of the learning process itself, the lack of relationship between cortical oscillatory power and the induction of plasticity in the present study may be somewhat surprising. One possible reason why no relationship was observed between

baseline EEG power and changes in MEP amplitude following cTBS could be that the EEG represents the summation of post-synaptic potentials from a large population of neurons underlying the electrodes, not just those projecting to the target muscle from which MEPs were recorded. A study by Mitchell and colleagues (2007) found no relationship between prestimulus EEG power and variability in MEP responses to single-pulse TMS applied during contraction. They did, however, show that MEP responses co-varied with oscillatory activity recorded from EMG during the tonic contraction. The authors suggest that EMG could potentially be used as a 'surrogate' measure of cortical oscillatory activity that more specifically reflects cortical output to the muscle of interest. Unfortunately, this approach would prove problematic with cTBS studies, as a sustained contraction before or during cTBS may alter its after-effects (Gentner *et al.*, 2008; Huang *et al.*, 2008). In contrast to Mitchell and colleagues (2007)(Mitchell *et al.*, 2007), Sauseng and colleagues (2009) applied TMS while subjects were at rest. They found that at intensities near resting motor threshold, an MEP was more likely to be evoked during periods of low rather than high alpha power. The present study was also performed at rest. Since we saw no relationship between baseline alpha power and post-cTBS changes in MEP we conclude that relationships between prestimulus EEG and single-pulse TMS effects may be of little relevance to long term changes in MEP size caused by cTBS-induced plasticity.

cTBS had no effect on the EEG power spectrum in the present study. Studies in the visual and auditory systems suggest that cortical rhythms can entrain to rhythmic visual or auditory stimuli, enhancing processing and perception (Lakatos *et al.*, 2008). Whether a similar entrainment occurs during rTMS is unclear, although a study by

Klimesch and colleagues (2003) may support this idea, showing that rTMS applied at individual upper alpha frequency over the mesial frontal and right parietal cortices enhanced the power in that frequency band. However, cTBS is applied at theta frequency, and the present study did not find an enhancement of theta power following cTBS. In fact, cTBS had no effect on any of the frequency bands analysed. This is consistent with several other studies which have found no effect of subthreshold rTMS at various frequencies on EEG power (Strens *et al.*, 2002; Oliviero *et al.*, 2003). In contrast, Fuggetta et al. (2008) found changes in event-related power in the alpha and beta frequency bands following trains of subthreshold and suprathreshold rTMS. However these effects were found within a very short time frame (a few seconds) following rTMS. Brignani et al. (2008) found synchronisation in the alpha band following 1 Hz rTMS, however this was only significant during the later third of a 10 minute paradigm and the rTMS was applied at a much higher intensity than in the present study (110% RMT). Another study showed increased EEG synchronisation in all frequency bands in the stimulated hemisphere compared to the unstimulated hemisphere following a modified theta burst stimulation paradigm (30 Hz bursts repeated at 6 Hz) (Schindler *et al.*, 2008). However, stimulation was applied over the right frontal eye field rather than the motor cortex and a higher intensity of stimulation was used (80% RMT) (Schindler *et al.*, 2008). Our results may suggest that short, low-intensity rTMS paradigms do not synchronise a large enough population of neurons to detect changes in the EEG power spectra or that the synchronisation is very short-lasting and thus undetected with the current methodology. Results may depend critically on the stimulation parameters employed, including intensity, duration and location of stimulation. Although cTBS has been shown to alter cortico-muscular

coherence in the beta frequency range (Saglam *et al.*, 2008), to our knowledge no studies of cTBS applied to the motor cortex have investigated its effects on the power spectrum *per se*. The results of the present study together with those of Saglam and colleagues (2008) might suggest the effects of cTBS on cortical oscillatory activity may be better observed in altered cortico-muscular coupling. It is also possible cTBS has effects on cortico-cortical coupling, though this remains to be investigated with multi-channel EEG. The effects of rTMS on oscillatory activity have been studied more invasively in the cat visual cortex; brief periods of low frequency (1-4 Hz) rTMS transiently increased the power of high frequency activities (>40 Hz) and resulted in a longer-lasting decrease in the power of low-frequency activities (<40 Hz) (Allen *et al.*, 2007). Additionally, this rTMS paradigm reduced phase-locking of spontaneous spiking of individual pyramidal neurons with the local theta field potential (Allen *et al.*, 2007). Whilst cTBS had no effect on the EEG power spectra, its effects on neuronal phase-locking are unknown, and difficult to explore in human subjects.

There was also no relationship between EEG power at baseline and the learning of a visuomotor task. Again, there are a number of factors that may mask any relationship between motor cortical EEG activities and the degree of task performance improvement. For example, given the more complex nature of the visuomotor task it is possible that changes in the motor cortex are just a small component of much more significant and widespread changes in a wide cortical and subcortical network .

Nevertheless, the fact that visuomotor training lead to a large, sustained increase in alpha power over the motor cortex of all eleven volunteers suggests that there must be

some role for the motor cortex in performance of this task. Relatively few studies have investigated changes in EEG power following motor training. Increased theta power over the frontal midline and increased beta-band coherence between the frontal midline and right temporal region has previously been reported following a sequential finger tapping task (Zhu *et al.*, 2010). However EEG was not recorded over the motor cortices (electrode positions C3 and C4) in this study. Our findings are perhaps best compared to those of Smith et al. (1999), who observed increased alpha power over the sensorimotor regions following performance of a virtual reality computer game with a visuomotor tracking component. Alpha power increased both during the training session as well as across several training sessions. In this study, the authors conclude that the increased alpha power is likely to reflect disengagement of the sensorimotor areas which are no longer needed as the task is learned.

Increased alpha power is sometimes regarded as indicating an 'idling' or 'deactivated' cortical state (Smith *et al.*, 1999), but more recently it has been interpreted as indicative of active inhibition. Thus increased sensorimotor alpha might reflect active disengagement of somatosensory processing in order to reduce interfering external input during performance of internal tasks (Jensen *et al.*, 2002; Hanslmayr *et al.*, 2005). In the present experiments we propose that the increase in alpha power – lasting at least eight minutes post-training, is consistent with a consolidation-like effect on motor cortical excitability. Increased alpha would then reflect reduced sensorimotor coupling at the cortex that facilitates motor consolidation by reducing potential interference from peripheral inputs. Unfortunately, as only a single channel of EEG was recorded we

are unable to further differentiate the location of the increased alpha (i.e. motor or sensory cortex?).

This hypothesis might also account for the fact that increased alpha did not correlate with increased performance in the task. If the increased alpha power is related to a consolidation-like effect, it is more likely that a relationship may be seen during a second, later performance of the task rather than after the first stage of practice. Further investigation with multi-channel EEG and repeated training bouts is required.

Finally, we found that the increased alpha power accompanying visuomotor learning was associated with larger MEP amplitudes five and ten minutes following the task. Whilst MEP amplitude was unchanged overall, there was a large variability in MEP changes amongst subjects. Subjects who exhibited little increase in alpha power post-training had decreased MEPs following training, whereas subjects with large increases in alpha power had increased MEPs following training. Although we can only speculate on the cause of this relationship, it may be that the more actively this early consolidation proceeds in the motor cortex, the more excitable is the cortical output. Interestingly, this appears to contrast with the previously mentioned findings of Sauseng and colleagues (2009), who demonstrate that an MEP is more likely to be evoked during low rather than high alpha activity. However, the important difference is that in the present task, the increased alpha represents active disengagement of sensorimotor coupling that allows consolidation to occur, whereas in the Sauseng example, it is more likely to represent a period of 'idling' and hence relative inexcitability. It should also be noted that the lack of overall effect of visuomotor

training on MEP amplitude in the present task is consistent with studies that show simple ballistic motor tasks increase cortical excitability whereas more complex (visuomotor) tasks such as grooved pegboard training transiently suppress MEP amplitude (McDonnell & Ridding, 2006b).

In conclusion, the present study provides no support for the use of the EEG as a state-marker to predict the responses to plasticity induction by either cTBS or behavioural motor learning. However, that is not to say a relationship between baseline cortical oscillatory activity and plasticity induction does not exist, but may be undetectable due to current methodological constraints. Whilst the potential benefit of rTMS techniques lies in their non-invasive nature, we are also limited to indirect methods of investigating the mechanisms underlying their effects in human subjects. Understanding the reasons contributing to the variability in responses to rTMS and other plasticity-inducing interventions will be fundamental for optimising these techniques for potential therapeutic use. An interesting finding was the increase in alpha power following visuomotor training which correlated with changes in motor cortical excitability. This requires further investigation but may be related to consolidation of the motor memory.

CHAPTER 3

Modulations in human motor cortical excitability associated with alpha oscillatory state: a study using EEG-triggered Transcranial Magnetic Stimulation

Whilst Chapter Two found no relationship between pre-stimulation EEG and the induction of plasticity by cTBS or visuomotor learning, it may be that the timing of stimuli relative to the ongoing EEG state is important. In particular, the alpha rhythm has been proposed to represent bouts of inhibition, repeated every 100 ms or so. If this is the case, the alpha rhythm may provide temporal windows for neuroplasticity induction, as cortical inhibition is a powerful modulator of neuroplasticity. In this chapter I investigate how the MEP and a measure of cortical $GABA_A$-ergic activity (SICI) are modulated on different phases of the alpha rhythm (up-going state or down-going state), as a first step towards the development of EEG-triggered rTMS.

3.1. ABSTRACT

BACKGROUND: Alpha rhythm (8-12 Hz), recorded in the human electroencephalogram (EEG), may reflect bouts of cortical inhibition. Triggering stimuli on particular phases of the alpha rhythm may provide an approach to optimise neuroplasticity induction by targeting the cortex at times when inhibitory tone is reduced. However, firstly, the relationship between alpha phase and cortical excitability requires investigation. We aimed to develop a technique to trigger Transcranial Magnetic Stimuli (TMS) on the up- or down-going phase of ongoing alpha rhythm, and to use this technique to investigate phasic modulation of cortical excitability.

METHODS: Short interval Intracortical Inhibition (SICI) and Motor Evoked Potential (MEP) amplitude were measured on the up- and down-going phase of the alpha rhythm, recorded over the motor cortex in 13 subjects. Additionally, we investigated the relationship between alpha power/ amplitude preceding the TMS trigger, and SICI and MEP amplitude.

RESULTS: MEP amplitude was 30% greater on the down-going phase compared to up-going phase ($p<0.05$). Motor thresholds and SICI were unchanged by alpha phase. Alpha power and alpha amplitude had no influence on MEP or SICI amplitude.

CONCLUSIONS: The pathways responsible for MEP production are more excitable on the down-going phase of the alpha rhythm. Targeting stimuli to the down-going phase of alpha may provide a useful approach for enhancing plasticity induction, although this remains to be tested.

3.2. INTRODUCTION

A variety of non-invasive brain stimulation techniques are available to induce neuroplastic changes in the human motor cortex, most commonly measured as changes in cortical excitability. Perhaps the most popular of these techniques is repetitive transcranial magnetic stimulation (rTMS). The changes induced by rTMS and other non-invasive brain stimulation paradigms are thought to rely upon Long Term Potentiation and Long Term Depression-like mechanisms (Stefan et al., 2002; Huang et al., 2007). Whilst these techniques are being investigated as potential therapeutic tools in a wide array of neurological disorders, from depression and schizophrenia to recovery of motor function following stroke, they are currently limited by a large degree of variability in responses, both within and between subjects (Ridding & Ziemann, 2010). It is therefore important to understand the determinants of this variability, and how we may be able to manipulate these factors in order to optimise non-invasive brain stimulation paradigms.

One factor known to affect plasticity induction is modulations in inhibitory tone. For example, *in vitro*, pharmacological modulations that reduce Gamma-Amino Butyric Acid $(GABA)_A$ receptor activity facilitate the induction of LTP in hippocampal (Wigstrom & Gustafsson, 1983; Bramham & Sarvey, 1996; Evans & Viola-McCabe, 1996) and motor cortex slices (Hess et al., 1996). *In vivo,* $GABA_A$ antagonism enhances LTP induction in the rat somatosensory cortex (Komaki et al., 2007), and promotes cortical remodelling in a mouse stroke model (Clarkson et al., 2010). There is some evidence that modulations in inhibition are also important for the induction of plasticity in humans. For example, the rapid changes in cortical somatotopy following limb deafferentation

are accompanied by a reduction in $GABA_A$ inhibition (Ziemann *et al.*, 1998a). Given the role of GABA inhibition in modulating plasticity induction, one approach to enhancing neuroplasticity induction may be to target the cortex at times when GABA inhibition is reduced.

Alpha rhythm (8-12 Hz) is one of the most prominent rhythms recorded from the human electroencephalogram (EEG), and has been associated with cortical inhibitory processes, although the exact nature of this 'inhibition' is unknown (Klimesch *et al.*, 2007; Jensen & Mazaheri, 2010; Mathewson *et al.*, 2011). It has been proposed that alpha represent bouts of inhibition ('pulsed inhibition'), repeated every 100 ms or so (Jensen & Mazaheri, 2010; Mathewson *et al.*, 2011). Indeed, a recent study in the macaque found that pyramidal cell firing is enhanced on the down state of alpha, and attributed this finding to a phasic reduction in inhibitory tone (Haegens *et al.*, 2011b). In the human visual cortex, the detection of TMS induced phosphenes is modulated by alpha phase (Dugué *et al.*, 2011). However, a study in the human motor cortex found that alpha phase had no effect on MEP excitability (Mäki & Ilmoniemi, 2010). This study used the dominant alpha frequency in the prestimulus EEG to predict alpha phase at the time TMS was delivered. This may not be ideal, as cortical rhythms are highly dynamic. In the present study, we develop a technique for triggering TMS stimuli on different phases of the *ongoing* alpha rhythm in the human EEG, and investigate how a measure of $GABA_A$-ergic inhibition, short latency intracortical inhibition (SICI), is modulated during the up and down state of the alpha rhythm. Additionally, we explore how Motor Evoked Potential (MEP) amplitude changes during the different alpha states. We demonstrate that MEP amplitude is greater when

stimuli are applied on the down-going phase compared with the up-going phase, whilst SICI is unchanged during the alpha cycle. The results are discussed in the context of their potential implications for neuroplasticity induction in the human motor cortex.

3.3. METHOD

3.4.5. Participants

Fifteen right-handed participants took part in the study, however the data from two of these subjects was excluded from analysis due to excessive background EMG activity. The mean age of the thirteen remaining participants was 27.8 ± 6.7 years, with seven females and six males. All participants provided written, informed consent and completed a TMS safety screen prior to their inclusion in the study. The study was conducted in accordance with the Declaration of Helsinki and was approved by the University of Adelaide Human Research Ethics Committee.

3.4.6. TMS

3.4.6.1. Equipment

Participants were seated in a recliner with their forearms resting in a comfortable, supported position on a pillow on their lap. EMG activity was recorded from the right first dorsal interosseous (FDI) muscle using two Ag-AgCl electrodes (Ambu blue sensor, M-00-S/50) in a belly-tendon montage. EMG was amplified (x1000) and filtered (20-1000 Hz) using a CED 1902 amplifier (CED, UK) and sampled at 2000 Hz by a CED 1401 A-D converter (CED, UK). Signal V4.0 software (CED, UK) was used to acquire the EMG data. In addition to this, EEG and EMG data was acquired using ASA-Lab (ANT,

Netherlands) (See *Section 3.3.3. EEG-triggered TMS* below for details). Participants were seated in front of a screen displaying this data, and were instructed to stay as relaxed as possible, keeping the EMG signal a straight line. Single and paired pulse TMS was applied using a Magstim Bistim^2 module connected to a 9 mm diameter Magstim coil (Magstim, UK). The coil was held tangentially to the motor cortex, with an angle of approximately 45 degrees to the midline, over the optimal spot for evoking a response in the relaxed FDI. The position of the coil was marked on the EEG cap, in order to keep placement consistent within an experimental session. TMS was triggered on the up or downgoing alpha state using a custom-made Spike II V6.16 (CED, UK) script (detailed below in *Section 3.3.3. EEG-triggered TMS*).

3.4.6.2. Setting TMS intensities

At the beginning of the experiment, three TMS intensities were measured. Half of the participants had these measurements taken with TMS triggered on the upgoing state of alpha, whilst the other half had these measurements taken with TMS triggered on the downgoing state. Subjects were randomly allocated to these groups. The three intensities measured were:

1. The intensity of TMS to evoke an MEP of between 0.5 and 1.5 mV, where a stable MEP could be evoked with a range of modulation in order to avoid 'floor' and 'ceiling' effects. This intensity was used as the test pulse intensity for SICI measures.
2. Resting Motor Threshold (RMT). RMT was determined as the intensity at which five out of ten MEPs in the relaxed FDI muscle were at least 50 μV.

3. Active Motor Threshold (AMT). AMT was determined as the intensity at which five out of ten MEPs were at least 200 μV in amplitude whilst the subject was performing a mild tonic contraction, approximately 10% of maximal voluntary contraction. 80% AMT was used as the conditioning stimulus intensity.

At the end of the experimental session, these three intensities were measured on the opposite alpha state, with the intention of repeating the MEP and SICI measurements using these new intensities if they differed by more than 2% of stimulator output to the intensities measured on the opposite state at the beginning of the experiment. However, this criterion was not met for any of the subjects.

3.4.6.3. Measuring SICI

Following the setting of TMS intensities, four blocks of SICI were recorded, with 28 trials within a block. Two of the blocks were triggered on the upgoing state of alpha and two on the downgoing state, with the order of the up and downgoing blocks randomised. The test pulse was delivered alone in half (14) of the trials within a block. The order of conditioned and test trials was pseudo-randomised for each recording block using a purpose-written Matlab (MathWorks, US) script. The interstimulus interval used for all SICI measurements was 2 ms.

It is difficult to determine the phase of alpha at which the TMS was triggered post-recording, as the large TMS artefact distorts the filtered signal. Therefore, prior to each SICI block, a block of data with the TMS stimulator switched off was recorded. This was later analysed to determine trigger phase and hit rate, in order to evaluate the accuracy of the Spike script used to trigger on the different alpha states.

3.4.7. EEG-triggered TMS

EEG was recorded using a 64 cap electrode system (Waveguard, Germany) connected to a high density amplifier (ANT, Netherlands). The data was acquired using ASA V4.7.3 software (ANT, Netherlands), and C3 referenced to Fz was displayed on the screen in front of the subject, along with the EMG signal. Display filters were set at 20-1000 Hz for EMG and 0.5-1000 Hz for EEG. Impedance was kept below 5 kOhms.

We used a custom-made Spike script (Spike II V6.16, CED, UK) with a sequencer for sending triggers to perform the signal processing required to deliver TMS triggers on the up and downgoing states of alpha. In order to do this, we first needed to extract the EEG signal from the ASA-lab system pre-amplification. C3 was referenced to Fz and amplified (x 10000) and filtered (0.5-100 Hz) by a CED 1902 amplifier (CED, UK). The signal was sampled at 2000 Hz using a CED 1401 micro A-D converter (CED, UK). The sent triggers were also recorded as an input by the CED 1401, so that we could estimate the delays between deciding to send the trigger and actually receiving the trigger (See Figures 3.3 and 3.4).

It was essential to extract alpha band EEG from the acquired signal, which contains several frequency bands of EEG and some noise. However, extraction techniques, such as filtering, cause delays, and the extracted signal is therefore only able to represent the alpha band signal at a certain time before (depending on the filter group delay and the system computation time). However, it is feasible to predict or capture a period of upgoing or downgoing alpha band EEG signal based on the filtered alpha band signal. It is predicted that the alpha band EEG signal will be in the upgoing state during a period

of 3/4 ~ 5/4 cycle *T* (*T=1/f)* from a time point when the alpha band EEG signal crosses zero with positive slope. As alpha band EEG signal frequency *f* is between 8 to 12 Hz, *T* is in a range of 83.3 to 125.0 (ms). The period of time that the signal will be in the upgoing state obviously varies for the different frequencies 8-12 Hz. However, as shown in Figure 3.1, there is a period of time during which the alpha band signal will be in the upgoing state for any frequency from 8-12 Hz. This period is 93.8- 104.2 ms from the point in time when the alpha band EEG signal crosses zero with positive slope. The same applies for capturing the downgoing state, 93.8-104.2 ms from the point in time when the alpha band EEG signal crosses zero with a negative slope.

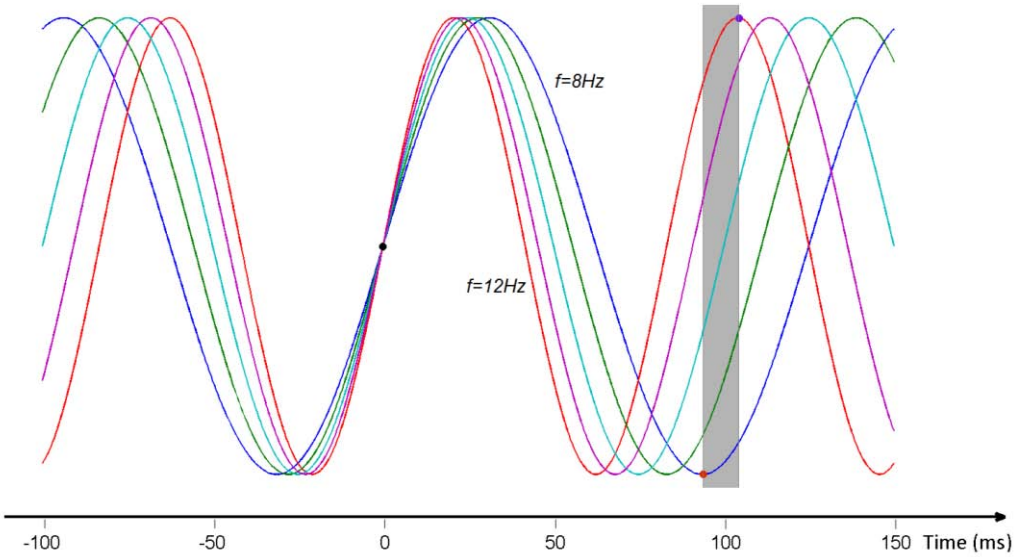

Figure 3.1. *Overlapping upgoing states for different frequencies within the alpha band. Each line represents a frequency between 8 Hz (blue line) and 12 Hz (red line). Sending stimuli between 94-104 ms following the time the positive slope passes through 0 ensures the alpha state is upgoing regardless of the frequency (8-12 Hz) of the next alpha cycle (see grey bar). Thus, even with a dynamic rhythm, a good hit rate can be achieved.*

In order to obtain a constant group delay for the signal at different frequencies, a 311th order Finite Impulse Response (FIR) alpha band pass filter was used, with a Kaiser

window for spectral leakage suppression. Its group delay was 155 samples (75.5 ms). In this study, there was a trade-off between filter performance and group delay; using a higher order filter would give better filter performance, but the filtered signal might be detected later than the overlapped alpha upgoing/ downgoing period, as discussed above. Fortunately, the power of theta and beta, the two EEG bands next to alpha, are normally much weaker than alpha band under our experimental conditions, which makes the filter technical requirements much lower.

It is important to estimate the delays in the system and process. It is supposed there was a negligible delay from the EEG cap to the CED 1902 amplifier, since we used the unamplified analog signal extracted from the high density ASA-lab amplifier; there was no extra processing done by the system. According to the CED1902 manual, there is a ~0.35 ms delay in the CED 1902 amplifier. After filtering, the trigger was sent using the CED sequencer. Our extensive testing has shown that the time interval between deciding to send the trigger and actually receiving the trigger varies between 5.2 to 19.8 ms (with mean 10.7 ms and standard deviation 4.3 ms), which is caused by computation in running the Spike script and sequencer. Considering that the overlapped upgoing/downgoing state is approximately 94 to 104 ms from the time the alpha band EEG signal crosses zero with positive/negative slope, and there is a 20 ms computational delay, the filter delay needed to be shorter than 84 ms.

In order to send the TMS trigger on a strong and stable alpha rhythm rather than a transient alpha signal, an amplitude threshold was used to select the alpha rhythm cycles on which to send TMS. Also, the designed filter delay is shorter than the

minimum requirement, which provided a potential margin for sending TMS when the filtered signal reached a certain threshold after crossing zero. We performed an offline simulation using a large quantity of previously recorded EEG data in order to determine the threshold that should be used. The use of empirically set thresholds as a first step toward sending TMS at a given alpha state is perhaps not optimal, because it is based on a limited set of data observations, and is not modified for the individual subject. However, considering the dynamic nature of the EEG, variable computational delay, and limitations of FIR filter order, it has worked well in most experiments we have done (See Results *Section 3.4.1. Hit rates and trigger phase*). Additionally, we are currently testing some new adaptive methods for choosing the threshold that will allow the processing to adjust threshold automatically to send TMS during strong alpha signal oscillations.

3.4.8. Data Analysis and Statistics

3.4.8.1. Hit rate and trigger phase

The data with triggers sent by Spike but the TMS stimulator switched off was analysed to determine trigger phase and hit rate. The data was zero-phase IR filtered (alpha band) and the phase each trigger was sent on was calculated using Matlab (MathsWorks, US). Mean hit phase was calculated for each subject and for the group for the upgoing and downgoing states. Additionally, the hit rate was calculated for each recorded block (1-(number of hits on the incorrect phase/number of hits on the correct phase)). The mean hit rate was calculated for the upgoing and downgoing states for each subject and expressed as a percentage. The mean hit rate on the upgoing and downgoing states was compared using a two-tailed student's paired t-test.

3.4.8.2. Alpha power and amplitude

It is possible that any effect of alpha phase is dependant on the level of alpha activity present. Therefore, alpha power and amplitude were extracted from the recorded signal in order to analyse the relationship between alpha power/amplitude, alpha phase and MEP and SICI amplitude. The data in 500 ms (1000 samples) prior to the first TMS trigger were used for power spectral analysis. After removing the DC component, the data was zero padded to 2000 samples for the purpose of interpolation on the spectrum, and Fourier Transformed. Then the power in alpha band was extracted and normalised as showed in the following equation:

$$P = \frac{2\sum_{f=8}^{12} S_f^2}{N^2}$$

where *P* is the total power in alpha band, f is the frequency, S_f is power sepectrum density at frequency *f* and *N* is the length of data (Heinzel *et al.*, 2002).

Mean alpha power for TMS trials and non-TMS trials was calculated for each subject and compared using a two-tailed student's paired t-test, in order to determine the validity of estimating the accuracy of the Spike script using non-TMS trials (if alpha is not different between the TMS and non-TMS trials we can assume the accuracy of the script should be similar for the trials with TMS).

We also looked at the amplitude of three alpha cycles prior to the TMS pulse, in order to examine the EEG immediately prior to the TMS trigger. To calculate alpha band EEG signal amplitude, firstly the EEG data was alpha band pass filtered. Peaks and troughs of signal in the 500 ms (1000 samples) prior to each TMS pulse were then captured, and

the 'unreasonable' peaks and troughs were removed, for example, peaks with negative values, troughs with positive values, and peaks and troughs too close to zero (See Figure 3.2). Where the interval between two peaks was too short for an alpha cycle, a single peak value was interpolated. Finally, the peak and rectified trough values were averaged and used to represent the mean alpha amplitude prior to TMS. Only the three peaks/troughs prior to the TMS pulse were averaged.

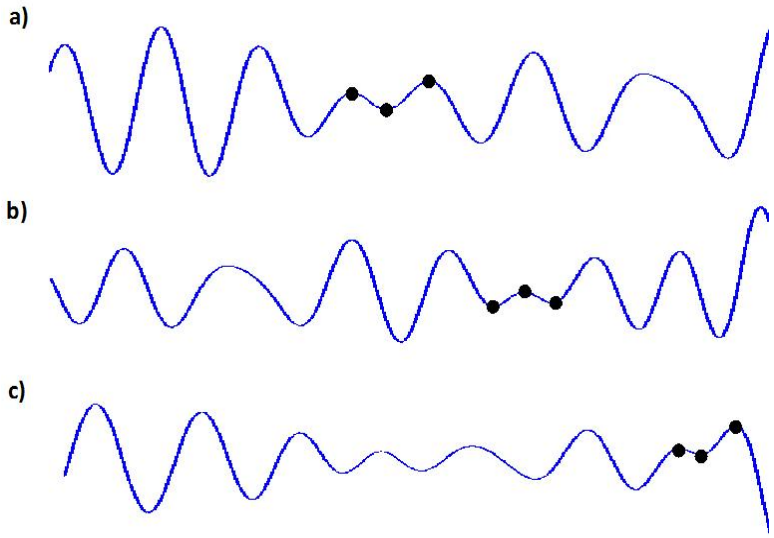

Figure 3.2 *Examples of 'unreasonable' alpha peaks and troughs that were excluded from the analysis of alpha amplitude. a) Peaks and troughs too close to 0°. b) Peak with a negative value, toughs too close to 0°. c) Peaks too close together, trough with a positive value.*

3.4.8.3. MEPs

Peak-to-peak MEP amplitude was calculated using a purpose written Matlab script. For each MEP block, a median split was performed to separate MEPs into trials with high and low alpha power. The mean MEP amplitude for the downgoing phase and upgoing phase was calculated for the high and low alpha power trials for each subject. There

were two upgoing blocks and two downgoing blocks for each subject, thus the mean MEP amplitude was calculated for the upgoing and downgoing states by averaging the two blocks for each state. The MEP data was analysed using a two-way repeated measures ANOVA with factors ALPHA POWER (low and high) and ALPHA PHASE (downgoing and upgoing).

Similarly, a median split was used to separate MEPs into trials with high and low alpha amplitude, and the data was analysed using a two-way repeated measures ANOVA with factors ALPHA AMPLITUDE (low and high) and ALPHA PHASE (downgoing and upgoing).

3.4.8.4. SICI

For SICI, conditioned MEP trials were divided into low and high alpha power trials using a median split. Mean conditioned MEP amplitude was calculated for each recording block for high and low alpha power, and expressed as a percentage of mean test MEP amplitude. Mean SICI was calculated for the upgoing and downgoing states for the high and low alpha power trials. The SICI data was analysed using a two-way repeated measures ANOVA with factors ALPHA POWER (low and high) and ALPHA PHASE (downgoing and upgoing).

Again, the effect of alpha amplitude was analysed, by splitting the conditioned MEP trials into high and low alpha amplitude trials, and analysing the data using a two-way repeated measures ANOVA with factors ALPHA AMPLITUDE (low and high) and ALPHA PHASE (downgoing and upgoing).

3.5. RESULTS

3.5.1. Hit rate and trigger phase

Mean trigger phase and hit rate were calculated using the trials with no TMS. In order to determine the validity of estimating the accuracy of the Spike script using non-TMS trials, mean alpha power was compared between trials with ($0.0070 \pm 0.019\ \mu V^2$) and without ($0.0063 \pm 0.017\ \mu V^2$) TMS (i.e. if alpha power is not different between the TMS and non-TMS trials we assume the accuracy of the script should be similar for the trials with TMS). A paired t-test confirmed there was no difference ($t(12) = -1.33$, $p=0.21$). For the upgoing state, the mean phase at which the trigger was sent by Spike was -1.9 ± 3.0 degrees and the mean phase at which the trigger was received was 19.4 ± 3.4 degrees. For the downgoing state, the mean phase the trigger was sent was 175.7 ± 3.8 degrees and the mean phase the trigger was received was 197.9 ± 3.4 degrees. Phase distribution diagrams are presented in Figure 3.3. An example of the filtering and triggering process is presented in Figure 3.4. The mean hit rate of the sent trigger was 92.3 ± 1.1 % for the upgoing state and 92.6 ± 1.2 % for the downgoing state. The mean hit rate of the received trigger was 82.4 ± 1.7% for the upgoing state and 82.7 ± 2.2 % for the downgoing state. There was no significant difference between the hit rates on the upgoing and downgoing states for either the sent trigger ($t(12) = -0.18$, $p=0.86$) or the received trigger ($t(12) = -0.19$, $p=0.85$).

3.5.2. Motor Thresholds

RMT was not significantly different when measured on the upgoing state in comparison to the downgoing state (upgoing: 54.1 ± 4.3 %; downgoing: 54.3 ± 4.3 %; $t(12) = -0.76$,

p=0.46). AMT was also unaffected by alpha state (upgoing: 39.9 ± 3.1 %; downgoing; 40.1 ± 3.2 %; t(12)= −1.00, p=0.34).

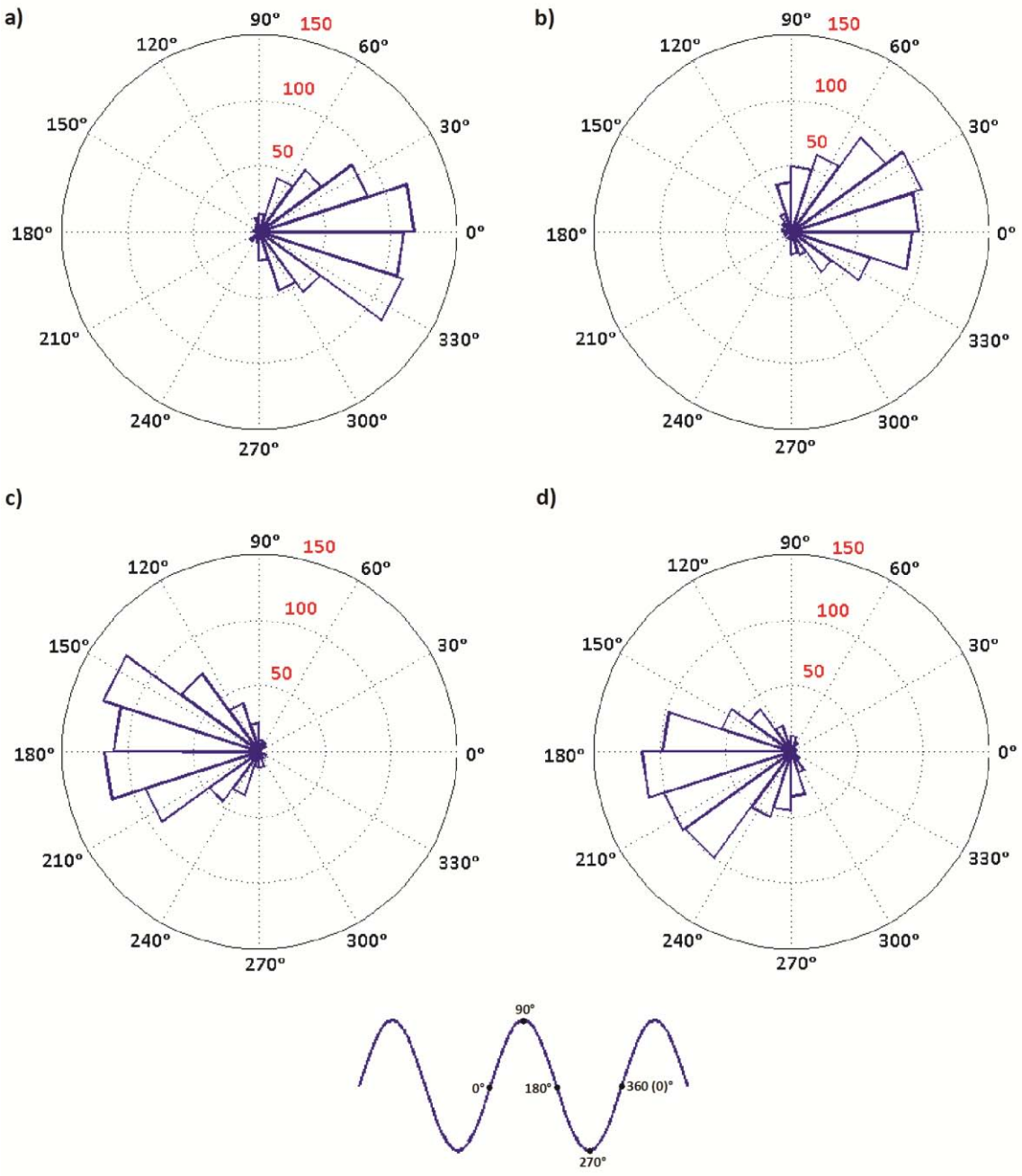

Figure 3.3 *Phase distribution diagrams for the pooled individual non-TMS trials from 13 subjects. Diagrams represent the phase at which the trigger was a) sent on the upgoing state; b) received on the upgoing state; c) sent on the downgoing state; d) received on the downgoing state. Red numbers indicate the number of individual trials at a particular phase.*

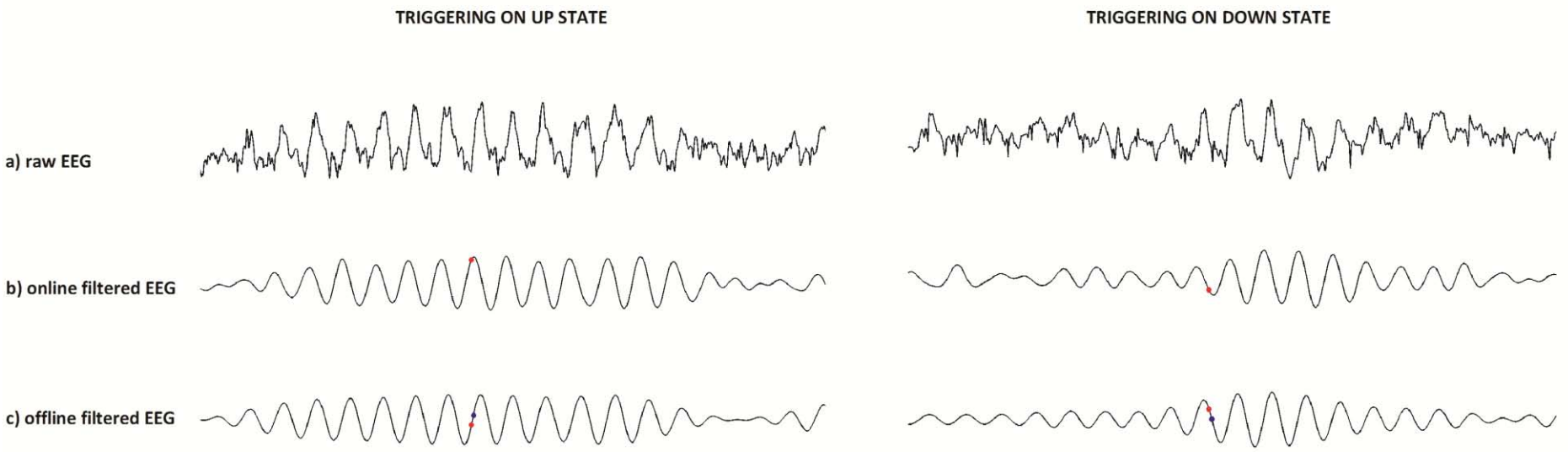

Figure 3.4. *Example of the filtering and triggering process. The raw EEG (a) is filtered online (b) and the 'up'/down state is identified as the signal passes through 0 with a positive/negative slope. When the signal passes through a predefined threshold, a trigger is sent (red circle). Note the delay induced by online filtering when comparing (b) with (a). The raw EEG is later zero-phase filtered offline (c). The red circle again indicates the time the trigger was sent. Note that the trigger was actually sent on the upgoing state of the alpha cycle that follows the one detected in the online filtered EEG. The blue circle indicates the time the trigger was received as an iput by the CED 1401 . The true TMS trigger time lies somewhere between these two points.*

3.5.3. MEPs

MEP amplitude was modulated by alpha phase. When MEP trials were binned by low and high alpha power (Figure 3.5a) the two way ANOVA revealed an effect of ALPHA PHASE ($F_{1,12}$=9.88, p=0.0085) but no effect of ALPHA POWER ($F_{1,12}$=1.51, p=0.24) and no ALPHA POWER x ALPHA PHASE interaction ($F_{1,12}$=1.11, p=0.31). Similarly, when MEP trials were binned according to low or high alpha amplitude (Figure 3.5b), there was a significant effect of ALPHA PHASE ($F_{1,12}$=11.65, p=0.0050) but no effect of ALPHA AMPLITUDE ($F_{1,12}$=3.68, p=0.079) or ALPHA AMPLITUDE x ALPHA PHASE interaction ($F_{1,12}$=0.44, p=0.52). MEP amplitude was approximately 30% larger when TMS was triggered on the downgoing state compared to the upgoing state (pooling high and low alpha trials: upgoing: 0.91 $\pm$ 0.12 mV; downgoing: 1.20 $\pm$ 0.17 mV). This difference was quite consistent across subjects, with 11 out of the 13 subjects exhibiting enhanced MEP amplitude on the downgoing phase (Figure 3.5c).

3.5.4. SICI

When SICI trials were binned according to low and high alpha power (Figure 3.6a), there was no effect of ALPHA POWER ($F_{1,12}$=0.046, p=0.84) or ALPHA PHASE ($F_{1,12}$=0.000040, p=0.98). There was, however, a significant ALPHA POWER x ALPHA PHASE interaction ($F_{1,12}$=8.34, p=0.014). Similarly, when SICI trials were binned according to low and high alpha amplitude (Figure 3.6b), there was no effect of ALPHA AMPLITUDE ($F_{1,12}$=0.0040, p=0.95) or ALPHA PHASE ($F_{1,12}$=0.054, p=0.82), but there was a significant ALPHA AMPLITUDE x ALPHA PHASE interaction ($F_{1,12}$=5.50, p=0.037).

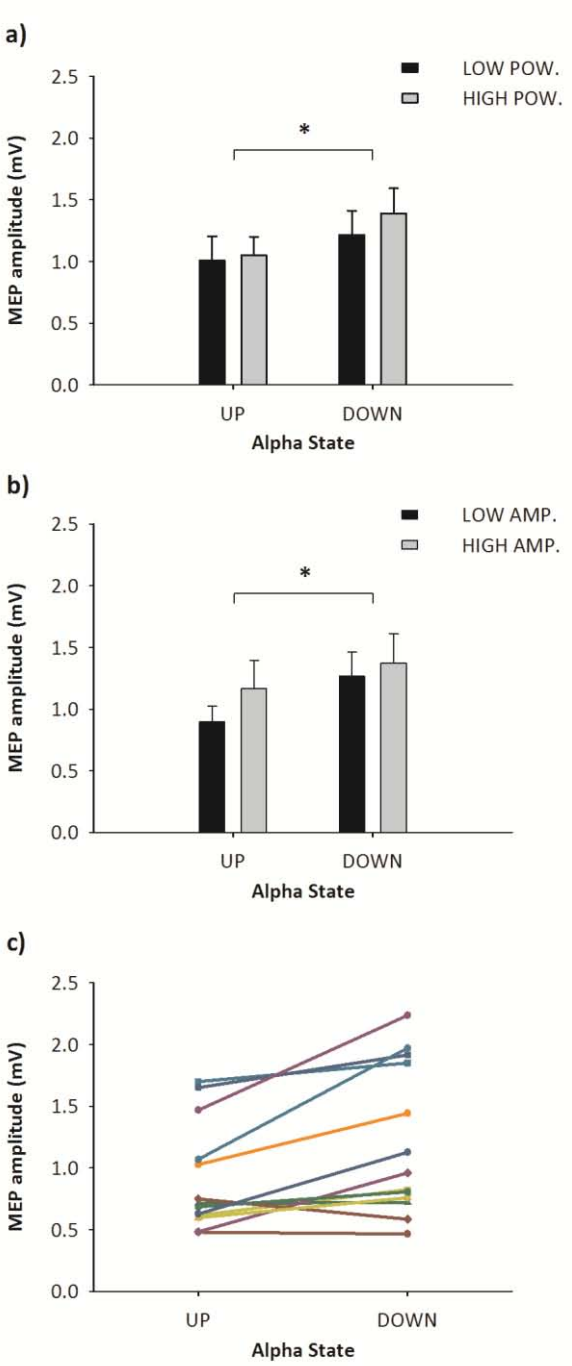

Figure 3.5. *MEP data. a) Group mean MEP amplitudes with TMS triggered on the up and downgoing states, separated into low and high alpha power trials. There was a significant effect of alpha phase (n=13, p=0.008). b) Group mean MEP amplitudes with TMS triggered on the up and downgoing states of alpha, separated into low and high alpha amplitude trials. There was a significant effect of alpha phase (n=13, p=0.005). c) Individual MEP data during up and downgoing states of alpha (low and high alpha trials pooled).*

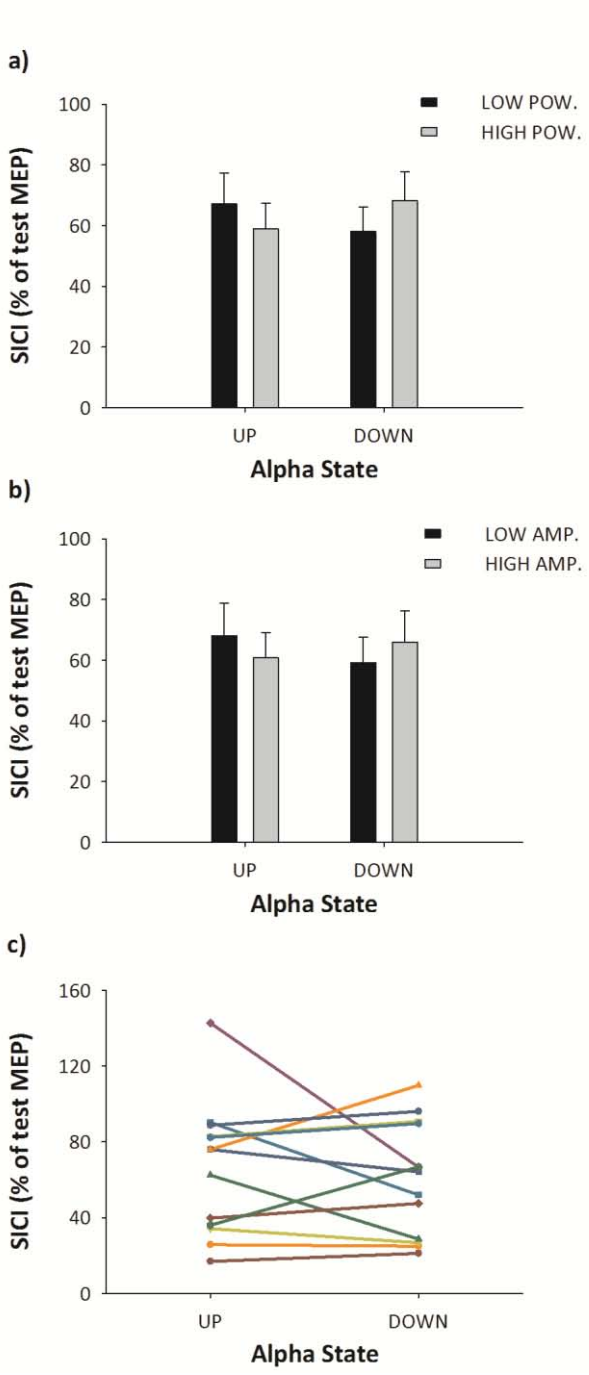

Figure 3.6. *SICI data. a) Group mean SICI with TMS triggered on the up and downgoing states, separated into low and high alpha power trials. There was a significant interaction between phase and power (n=13, p=0.014) but no main effect of either. b) Group mean SICI with TMS triggered on the up and downgoing states of alpha, separated into low and high alpha amplitude trials. There was a significant interaction between phase and power (n=13, p=0.037) but no main effect of either. c) Individual SICI data during up and downgoing states of alpha (high and low alpha trials pooled).*

3.6. DISCUSSION

This study presents the novel finding that motor cortical excitability is modulated on different phases of the alpha rhythm. Whilst MEP amplitude was increased when TMS was triggered on the downgoing alpha state compared to the upgoing state, SICI was unchanged by alpha state. Resting and active motor thresholds were also unchanged by alpha state. The level of alpha activity, as measured by alpha power and alpha amplitude, had no influence on either MEP or SICI amplitudes.

The function of the alpha rhythm, first described by Hans Berger in the 1920s (Berger, 1969 (English translation)), is not well understood. The rhythm was long thought to represent cortical 'idling', due to its enhancement during periods of behavioural disengagement. The most common examples of this are alpha recorded over the visual cortex, which synchronises when the eyes are closed and desynchronises when the eyes are opened and alpha recorded over the motor cortex (mu), which synchronises during muscle relaxation and desynchronises during muscle contraction. However, the idea that alpha is merely a signature of a 'default' inactive cortical state has recently been questioned. In fact, alpha has been proposed to play a functional role in signal processing, through active inhibition of cortical processing (Klimesch et al., 2007). This inhibition serves to 'gate' sensory input, inhibiting task-irrelevant information (Klimesch *et al.*, 2007; Jensen & Mazaheri, 2010; Mathewson *et al.*, 2011). Support for this proposal comes from studies of directed attention. Generally, decreased alpha activity is seen in the occipital cortex contralateral to an attended item, and increased alpha is observed ipsilateral to the attended item (Sauseng et al., 2005). The enhancement of alpha activity ipsilateral to the attended item is thought to indicate suppression of task-

irrelevant brain regions, important for enhancing task performance. Indeed, in a task involving detection of cued targets, reaction time correlated with the lateralisation index of alpha, such that detection of rightward targets was faster when the left hemisphere alpha activity was reduced compared to right hemisphere alpha activity and vice versa for leftward targets (Thut et al., 2006). This task-specific suppression of alpha is also observed between modalities. For example, attention to somatosensory nerve stimuli results in a reduction in alpha over the somatosensory cortex contralateral to the attended hand and increased alpha over the occipital lobe (Anderson & Ding, 2011). Whilst these and several other studies suggest alpha may play a role in blocking interfering sensory information during sensory perception tasks, evidence is emerging that alpha may have a more generalised inhibitory effect. Indeed, TMS studies suggest that alpha may indicate modulations in cortical excitability. For example, a study by Sauseng and colleagues (2009) reported that a suprathreshold MEP was more likely to be evoked during periods of low alpha power than high alpha power, and similarly, in the visual cortex, TMS-induced phosphenes are more likely to be perceived during low alpha power (Romei *et al.*, 2008). Together, these findings suggest the cortex is less excitable during periods of high alpha, and that modulation of cortical excitability may provide a mechanism by which high alpha inhibits cortical processing in task-irrelevant brain regions. It is also possible that alpha is an emergent property of modulations in cortical excitability.

Given the oscillatory nature of alpha, it might be expected that cortical excitability will exhibit phasic modulation. This has recently been demonstrated in the human visual cortex by Dugué and colleagues (2011), who found that the probability of perceiving

TMS-induced phosphenes differed by 15% between stimuli delivered at the peak and trough of alpha. Current theoretical models suggest alpha represents 'pulsed inhibition', that is, bouts of inhibition repeated every 100 ms or so (Jensen & Mazaheri, 2010; Mathewson *et al.*, 2011). Evidence to support the pulsed inhibition hypothesis comes from a recent study that investigated neuronal firing during different phases of alpha oscillations in the monkey somatosensory, motor and premotor cortex (Haegens *et al.*, 2011b). Neuronal spike rate was enhanced during the trough compared to the peak of the local field potential alpha oscillation. The authors interpreted this finding as rhythmical inhibition of neuronal firing on the peaks of the alpha rhythm, although the mechanism underlying this 'inhibition' is unclear. The present study provides indirect evidence of such a mechanism in the human motor cortex. We demonstrate that MEP amplitude is enhanced on the downgoing alpha state compared to the upgoing alpha state. TMS is thought to activate pyramidal output cells of the motor cortex transynaptically via excitatory cortical interneurons, producing the MEP (Di Lazzaro et al., 2004). The MEP represents a mixture of both excitatory and inhibitory influences in the cortex. If the excitatory neuronal pathways responsible for producing the MEP (I-wave pathways) are disinhibited during the trough of the alpha oscillation, MEP amplitude should be increased, as observed.

However, it is important to note that from the present data, the mechanism underlying enhanced MEP amplitude on the downgoing phase of alpha is unclear, and the above explanation is likely over-simplified. SICI was not modulated on the different phases of alpha and thus the present study provides no evidence for a role of SICI in the modulation of MEP amplitude (besides which, SICI and MEP effects can occur

independently of each other (Ziemann *et al.*, 1995; Liepert *et al.*, 2001)). It is possible that a different inhibitory pathway is involved, for example, the slower receptor kinetics of $GABA_B$ pathways may be more suited to alpha frequency oscillations. $GABA_B$ pathways can also be investigated using TMS, as Long Interval Intracortical Inhibition (LICI) or the Cortical Silent Period (CSP) (Werhahn *et al.*, 1999). However these measurements present methodological difficulties; firstly, LICI is measured using much longer interstimulus intervals (~100ms), which would encompass two alpha cycles, and given the dynamic nature of the rhythm it is unlikely the second stimulus would consistently trigger at the same phase as the first. Due to the stimulus artefact from the first trigger, it would not be possible to reliably determine where the second trigger was sent. Secondly, CSP is measured during contraction, during which alpha desynchronises.

Another possibility is that the inhibitory drive associated with alpha oscillations comes from a subcortical source. For example, although the mechanisms underlying alpha rhythm generation are not well understood, it is generally accepted that alpha rhythm generated in thalamic pathways modulates cortical alpha rhythm via thalamocortical relay loops (Bollimunta *et al.*, 2011). It is also possible that the 'inhibition' associated with alpha operates via a more indirect mechanism, such as suppression of facilitatory input. It is important to emphasise that my data only demonstrates phasic modulation of the pathways involved in MEP production. MEP amplitude is influenced by both excitatory and inhibitory input, and the alpha phase-dependent modulation of MEP amplitude observed in the present study may result from a complex interaction of

inhibitory and excitatory influences, determined by intrinsic properties of the oscillating neural network, rather than a simple 'reduction in inhibition'.

It is also necessary to clarify that in the present study, the up and downgoing phase of alpha were targeted, rather than the peak or trough. As Figure 3.4 suggests, stimuli delivered on the upgoing phase were triggered, on average, just after the positive slope of the EEG signal passed through 0°. Stimuli on the downgoing phase were triggered just after the negative slope of the EEG signal passed through 0°. In this way, the stimuli were triggered close to 0°, but biased towards either the peak or trough. Due to the predictive nature of the Spike script, it is difficult to target the peak or trough without over- or under-shoot, however it would be interesting to investigate whether the difference in MEP amplitude is more pronounced when stimuli are delivered at the peak or trough, or how it is modulated through different phase angles.

Unlike MEP amplitude, RMT and AMT were not different between the up and downgoing alpha state. Motor threshold is modulated by drugs that affect Calcium and Sodium channel permeability, and is thus thought to reflect the membrane potential of a subpopulation of corticospinal cells, and factors that influence this (for example, tonic inhibitory or excitatory inputs), as well as spinal, neuromuscular junction and muscle factors (Ziemann *et al.*, 1996b). The alpha rhythm reflects the synchronous activity of a large assembly of cortical neurons. Whether the activity of subpopulations of neurons (such as those responsible for threshold measures) are in synchrony with the overall rhythm is unclear. It is feasible that MEP amplitude is more closely associated with the alpha rhythm as it is measured at greater TMS intensities and reflects activation of a

larger population of intracortical and corticospinal neurons than TMS delivered at threshold intensities.

As stated earlier, there was no difference in SICI amplitude when paired stimuli were delivered on the up and downgoing state of alpha. Whilst we did not control for the difference in MEP amplitude between up and down-going state when measuring SICI, it has previously been demonstrated that the amount of SICI is unchanged with low (<1mV) and high (>2mV) test amplitudes (Ridding *et al.*, 1995c). The enhancement of MEP amplitude in the present study was comparatively small (~30 % increase). Whilst to our knowledge, no studies have investigated the relationship between alpha phase and SICI, alpha *power* has previously been associated with SICI. For example, suppression of alpha activity by neurofeedback training is associated with a 150% reduction in SICI (Ros et al., 2010). In another study, subjects learned a sequence of cued key presses and then had to perform either an activation condition, where they were required to press the appropriate key upon presentation of the cue, or an inhibition condition, where the cue was presented but they were not allowed to move (Hummel et al., 2002). A significant increase in 11-13 Hz activity was seen over the motor cortex during the inhibition condition compared to a decrease in the activation condition. This was not observed in a group of six patients with focal hand dystonia, who typically display impaired SICI. However, the present study provides little evidence for a modulation in SICI associated with alpha power/ amplitude, although there was a significant interaction between alpha power/ amplitude and phase, such that SICI was greater when triggered on the upgoing state during high alpha activity, but greater when triggered on the downgoing state during low alpha activity. The differences

between high and low power in each state were, however, very small (~10%) and non-significant and the interaction is somewhat difficult to explain. It is possible that the result is a consequence of the fixed threshold for triggering, which may mean that during low alpha activity, the trigger is sent closer to the peak/trough than during high alpha activity. The effect of triggering on different phase angles remains to be investigated.

The present study also found no evidence for a relationship between alpha power/ amplitude and MEP amplitude. However, alpha power has previously been associated with changes in cortical excitability. For example, Sauseng and colleagues (2009) found that at intensities close to threshold, MEPs are more likely to be evoked during low than high alpha power and Romei and colleagues (2010) found TMS-induced phosphenes were more likely to be perceived during low than high alpha power. In contrast, Mäki and colleagues (2010) found no relationship between alpha power or alpha amplitude and MEP amplitude measured at RMT. It might have been expected that the effects of alpha phase would be greater during strong alpha activity, as presumably there would be a larger neuronal population oscillating in synchrony. However it is important to acknowledge the limitations of the experimental method for examining a potential relationship between alpha power/amplitude and MEP amplitude and SICI. A notable problem is that we do not know an individual's normal range of alpha power/amplitude, as EEG was only recorded in one experimental session. Thus, when we group MEPs/SICI according to 'low' and 'high' intra-session alpha power, it may not be a true representation of 'high' and 'low' alpha power for that subject. Even so, the results of Sauseng and colleagues (2009) and Romei and colleagues (2010)

suggest that within-session effects are possible. It is possible that the results may be related to the triggering threshold, which would have prevented triggers from being sent on very low alpha power trials. Additionally, because the triggering threshold was fixed, during low alpha power trials, triggers would have been sent closer to the peak/trough compared to higher alpha trials. Due to the limitations of analysing data with TMS artefacts, the relationship between the phase angle at which the trigger was sent and MEP amplitude cannot be further explored at this stage.

Importantly, the present study developed a new technique for targetting TMS stimuli to different phases of endogenous brain rhythms. The development of EEG-triggered TMS techniques may open up new doors for non-invasive brain stimulation techniques, allowing the application of stimuli to be temporally guided by ongoing brain rhythms in order to target windows of enhanced excitability, when neurons may be more receptive to stimuli. This may be particularly useful for repetitive TMS paradigms. Some studies have applied stimuli at individual peak alpha frequency (Klimesch et al., 2003), however, to our knowledge, no studies have investigated applying stimuli in phase with alpha oscillations. Studies in animal models have demonstrated LTP can be induced by stimuli applied on the peak of theta rhythm, which can be depotentiated by stimuli delivered on the troughs (Huerta & Lisman, 1995; Holscher *et al.*, 1997; Orr *et al.*, 2001). It would be fascinating to replicate these findings in the human motor cortex, by modifying the theta burst stimulation paradigm to apply stimuli on either the up or downgoing state of ongoing EEG theta rhythm. However, the theta rhythm is more dynamic than alpha (Gasser et al., 1985) and there may also be interference in the filtered signal from the neighbouring delta band, which is typically large. On the

other hand, the theta rhythm is slower than alpha rhythm, which means a better performing, higher order filter with a longer delay time could potentially be employed. To our knowledge, only one other study has investigated triggering TMS on different phases of an ongoing oscillation. Bergmann and colleagues (2012) used an external hardware filter to trigger TMS on the slow oscillatory rhythm during sleep, and found a modulation in MEP excitability during the up and down states. Their results, together with the findings of the present study, suggest EEG-triggered TMS is a feasible method for targeting stimuli to periods of low or high cortical excitability.

In summary, we have developed a novel technique for triggering TMS on different phases of the ongoing alpha rhythm. We demonstrate that motor cortical excitability is modulated on different alpha states, such that MEP amplitude is enhanced on the downgoing state compared to the upgoing state. SICI is unchanged during the alpha cycle, although other inhibitory pathways remain to be investigated. Although yet to be trialled, EEG-triggered TMS may provide a new frontier for optimising plasticity induction by non-invasive brain stimulation, allowing stimuli to be targeted to optimal temporal windows, identified in the ongoing brain rhythms.

CHAPTER FOUR

Selective modulation of intracortical inhibition by low-intensity Theta Burst Stimulation

Results from the previous chapter suggest that using alpha rhythm to identify periods of reduced inhibition might not be achievable. Therefore, in Chapter Four I seek to examine an alternative approach that might be useful for applying rTMS during periods of reduced inhibition. Because intracortical inhibitory pathways have a lower threshold for activation than excitatory pathways, I investigate whether it is possible to selectively target intracortical inhibitory pathways by reducing the intensity of conventional TBS. This may be useful in two ways. Firstly, as discussed in the previous chapter, reduction in GABAergic inhibition may be important for the induction of plasticity, so if we were able to selectively downregulate inhibition, we may be able to use this technique as a priming stimulation to enhance neuroplasticity induction. Secondly, selective modulation of intracortical inhibition itself may have important therapeutic potential, as SICI pathways appear to be impaired in some neurological disorders, such as focal hand dystonia, and therefore a technique to enhance SICI may be useful. For this reason, in addition to cTBS, we also investigated iTBS – which at conventional intensities, increases MEP amplitude and SICI.

4.1. ABSTRACT

BACKGROUND: Theta Burst Stimulation (TBS) is a repetitive transcranial magnetic stimulation paradigm which has effects on both excitatory and inhibitory intracortical pathways when applied at an intensity of 80% of active motor threshold. As intracortical inhibitory pathways have a lower threshold for activation than excitatory pathways, we sought to determine whether it was possible to selectively target cortical inhibitory circuitry by reducing the intensity of TBS to 70% of active motor threshold.

METHODS: Motor Evoked Potentials (MEPs), short latency intracortical facilitation (SICF), intracortical facilitation (ICF) and short interval intracortical inhibition (SICI) were measured at baseline, 5-20 and 20-35 minutes following continuous (cTBS) and intermittent (iTBS) low-intensity TBS in nine healthy subjects.

RESULTS: Low-intensity cTBS significantly reduced SICI 5-20 minutes following stimulation, whilst having no effect on MEPs, SICF or ICF. Low-intensity iTBS had no effect on SICI, MEPs, SICF or ICF.

CONCLUSIONS: It is possible to selectively target intracortical inhibitory networks for modulation by low-intensity TBS, however, responses may critically depend upon the particular paradigm chosen. These findings have important implications for the treatment of neurological disorders where abnormal levels of intracortical inhibition are present, such as focal hand dystonia. Additionally, given that the reduction of GABAergic inhibition is important for induction of plasticity, low-intensity cTBS may be useful as a priming stimulation to enhance plasticity induction by other techniques.

4.2. INTRODUCTION

Repetitive transcranial magnetic stimulation (rTMS) can be used to modify the excitability of the human motor cortex, evidenced by a change in the amplitude of motor evoked potentials (MEPs). The changes in MEP amplitude induced by rTMS are thought to be due to alterations in the synaptic efficacy of excitatory glutamatergic cortical interneurons which project onto corticospinal neurons, brought about by long term potentiation (LTP) and long term depression (LTD)-like mechanisms (Huang *et al.*, 2007).

There is currently great interest in whether the changes in MEP amplitude induced by rTMS are of any functional relevance both in healthy and brain damaged subjects. Increases in MEP amplitude are observed following motor training and these changes correlate positively with improved task performance (Muellbacher *et al.*, 2001). These training-induced changes in MEP amplitude are similar in nature to those seen following rTMS, raising the possibility of whether rTMS modulation of these intracortical excitatory pathways could potentially improve motor performance. However, a number of neurological conditions are characterised by impairments in intracortical inhibitory pathways. For instance, short interval intracortical inhibition (SICI) is reduced in Parkinson's disease patients (Ridding *et al.*, 1995a) and in individuals with focal hand dystonia (Ridding *et al.*, 1995b; Butefisch *et al.*, 2005). Thus an alternative therapeutic approach might be to target intracortical inhibitory pathways for modulation by rTMS, rather than the excitatory pathways responsible for the generation of the MEP. Additionally, targeting inhibitory pathways may be useful for modulating the induction of plasticity (Ziemann *et al.*, 1998a).

In general, low frequency (<5 Hz) rTMS paradigms reduce MEP amplitude and higher frequency (>5 Hz) rTMS paradigms increase MEP amplitude. Although animal studies have demonstrated the induction of LTP and LTD at inhibitory synapses following electrical stimulation (Nugent & Kauer, 2008), many rTMS paradigms have no effect on measures of intracortical inhibitory function such as short latency intracortical inhibition (SICI) (Fitzgerald *et al.*, 2002; Daskalakis *et al.*, 2006). However, one exception is the recently developed Theta Burst Stimulation (TBS), a paradigm which involves bursts of three high frequency stimuli (50 Hz) repeated every 200 ms (Huang *et al.*, 2005). The effects of TBS on MEP amplitude can be blocked by administration of an NMDA-receptor antagonist, providing strong evidence that the paradigm induces LTP/ LTD-like changes in the excitatory pathways involved in MEP generation (Huang *et al.*, 2007). Two main patterns of stimulation are employed; continuous TBS (cTBS) and intermittent TBS (iTBS). cTBS reduces MEP amplitude and SICI, whereas iTBS increases MEP amplitude and SICI (Huang *et al.*, 2007). Thus cTBS and iTBS paradigms have effects on both intracortical excitatory and inhibitory pathways. Given that intracortical inhibitory pathways have a lower threshold for activation than the excitatory pathways (Kujirai *et al.*, 1993), we sought to determine whether it was possible to selectively target the intracortical inhibitory circuitry by reducing the intensity of TBS from the conventional 80% of active motor threshold to 70% of active motor threshold.

We hypothesised that low-intensity cTBS and iTBS would reduce and increase SICI respectively, whilst having no effect on MEPs or short latency intracortical facilitation (SICF), an indirect measure of the excitability of the excitatory I-wave circuitry responsible for MEP generation (Ziemann *et al.*, 1998b). The results demonstrate that it is indeed

possible to selectively target cortical inhibitory pathways for modulation with TBS by reducing the stimulation intensity. However the response to low-intensity TBS may critically depend upon the specific TBS paradigm employed.

4.3. METHODS

4.3.1. Participants

Nine healthy individuals with no relevant medical history or contraindications for TMS were recruited for the study (4 males, 5 females). The mean age of subjects was 28.3 +/- 11.1 years and all were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). Written informed consent was obtained from all participants before commencing the study, and the experiments were approved by the local Human Research Ethics Committee and conducted in accordance with the Declaration of Helsinki. Subjects were seated in a comfortable armchair for the duration of the experiment.

4.3.2. Design

Subjects received cTBS and iTBS in two different experimental sessions separated by approximately one week, in a randomised, cross-over design study. All experimental sessions were conducted in the afternoon, as there is evidence that there may be time of day differences in subjects responsiveness to plasticity-inducing paradigms (Sale *et al.*, 2007).

4.3.3. TMS measurements

Single and paired pulse TMS were applied using a flat figure-of-eight coil connected to two Magstim 200^2 stimulators via a Magstim Bistim^2 module (Magstim, Dyfed, UK). The coil was held perpendicular to the estimated position of the central sulcus with the handle pointing backward so that the current induced in the brain flowed posterior-anterior. The optimal site for evoking a response in the right first dorsal interosseous (FDI) muscle was determined and marked on the scalp using a marker in order to ensure consistent coil placement. Electromyographic (EMG) signals were recorded from FDI using two Ag-AgCl electrodes placed approximately 2cm apart. The EMG signal was amplified (gain 1000), filtered (bandpass 20-1000 Hz; CED 1902, CED, Cambridge, UK) and digitised at 2 kHz (CED 1401, CED, Cambridge, UK) for off line analysis. The inter-trial interval for all TMS measurements was 5 seconds with ±10% variation.

4.3.4. Thresholds

Resting motor threshold (RMT) and active motor threshold (AMT) were measured. RMT was defined as the minimum stimulator intensity required to evoke MEPs of at least 50 μ V in five out of ten consecutive trials. AMT was defined as the minimum stimulator intensity required to evoke MEPs of at least 200 μ V in five out of ten consecutive trials whilst the subject maintained a weak voluntary contraction of FDI (20% of maximum voluntary contraction). RMT and AMT were measured at the beginning of each experiment and again 5-20 minutes following TBS.

4.3.4.1. Motor Evoked Potentials:

To investigate changes in corticospinal excitability fifteen MEPs were recorded at baseline and 5-20 and 20-35 minutes following TBS. Stimulation intensity was set at a level to evoke MEPs of approximately 1 mV at baseline, and this intensity was maintained for MEP measurements throughout the experiment. MEP amplitudes were measured peak-to-peak.

4.3.4.2. Short interval intracortical inhibition and intracortical facilitation

SICI and ICF were recorded as described previously (Kujirai *et al.*, 1993). A subthreshold conditioning stimulus was initially delivered at an intensity of 5% of stimulator output below AMT, followed by a suprathreshold test stimulus at an intensity to evoke MEPs of approximately 1 mV. The conditioning stimulus intensity was then adjusted (if necessary) to produce SICI trials where the mean conditioned MEP amplitude was approximately 50% of the mean MEP amplitude when the test stimulus was given alone. The interstimulus interval was 3 ms for SICI and 10 ms for ICF. There were twelve trials each for SICI, ICF and the test stimulus given alone, thus in each block there were 36 trials. The order of the three conditions was pseudorandomised throughout the trials. If necessary, the intensity of the test stimulus was adjusted post-TBS in order to match the test MEP amplitudes pre- and post-TBS.

4.3.4.3. Short latency intracortical facilitation:

SICF was measured using the protocol first described by Ziemann et al. (Ziemann *et al.*, 1998b). A supratheshold first stimulus (S1) was delivered at an intensity to evoke an MEP of approximately 1 mV. This was followed by a subthreshold second stimulus (S2)

delivered at 90% RMT. The interstimulus intervals investigated were 1.3, 2.7 and 4.4 ms. These intervals were chosen as they lie within the peaks of facilitation seen with this technique (Ziemann *et al.*, 1998b; Ridding & Taylor, 2001). There were twelve trials for each of the four stimulation conditions: the three ISIs and the suprathreshold test condition which consisted of S1 given alone. Thus there were 48 trials. The order in which the conditions were presented during each trial was pseudorandomised. If necessary, the intensity of the test stimulus was adjusted post-TBS in order to match the test MEP amplitudes pre- and post-TBS.

The order in which SICF and SICI/ ICF trials were conducted was also randomised within and between subjects. These measurements were taken at baseline and 5-20 and 20-35 minutes post-TBS, following the MEP recordings (see Figure 4.1 for timeline).

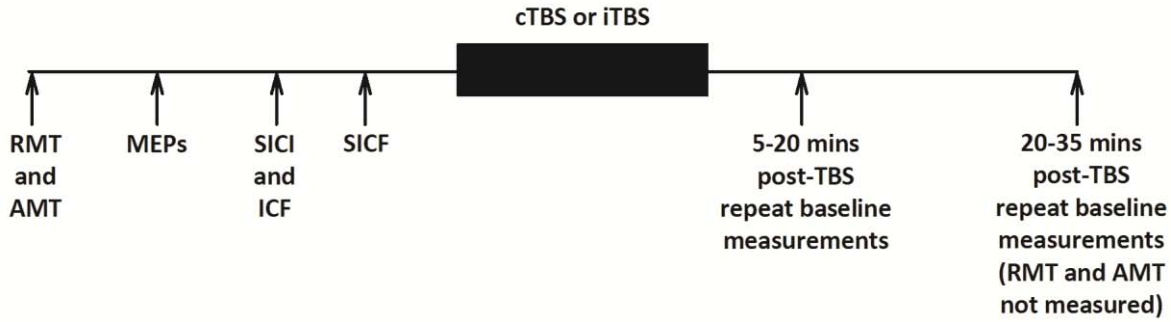

Figure 4.1. *Timeline of TMS measurements during experimental sessions. The order of measurement of SICI/ICF and SICF was randomised.*

4.3.5. Theta Burst Stimulation

TBS was applied using the method originally described by Huang et al. (Huang *et al.*, 2005). However the intensity of stimulation was reduced from the conventional 80% AMT to 70% AMT. This was done because pilot studies indicated that at this intensity TBS had no significant effect on MEP amplitudes. TBS was applied using an air-cooled figure-of-eight

coil connected to a Magstim Rapid^2 stimulator (Magstim, Dyfed, UK). The coil was held over the FDI motor hotspot. Continuous TBS (cTBS) consisted of bursts of three stimuli at 50 Hz presented every 200 ms for 40 s. Intermittent TBS (iTBS) consisted of the same burst pattern except that instead of being applied continuously for 40s, it was applied in trains of length 2 s, delivered every 10 s for 190 s. Thus, 600 stimuli were applied for both cTBS and iTBS.

4.3.6. Data Analysis

SICI/ICF and SICF values are the mean conditioned MEP amplitude expressed as a percentage of the mean test (unconditioned) MEP amplitude. Motor threshold data was analysed by two-way repeated-measures ANOVA with factors of INTERVENTION (cTBS or iTBS) and TIME (baseline and 5-20 mins post-TBS). MEP amplitudes, SICI, ICF values and test MEP amplitudes (from paired pulse studies) were all analysed with separate two-way repeated-measures ANOVAs with factors INTERVENTION (cTBS or iTBS) and TIME (baseline, 5-20 and 20-35 mins post-TBS). SICF was analysed by a three-way repeated-measures ANOVA with factors INTERVENTION (cTBS or iTBS), ISI (1.3, 2.7 and 4.4 ms) and TIME (baseline, 5-20 and 20-35 mins post-TBS). This was followed by separate two-way repeated-measures ANOVAs for cTBS and iTBS with factors ISI (1.3, 2.7 and 4.4 ms) and TIME (baseline, 5-20 and 20-35 mins post-TBS). Post hoc analyses were conducted where appropriate using the Bonferroni t-test method. All statistical analyses were performed using Sigma Stat, version 3.11 software (Systat Software Inc.), except for the three-way repeated-measures ANOVA, which was performed using SPSS, version 15.0 (SPSS Inc.). All data are given as mean ± SD. p-values <0.05 were considered significant.

4.4. RESULTS

4.4.1. Thresholds and stimulus intensities

RMT was unchanged by cTBS (pre: 44.8 ± 8.1 %; post: 45.2 ± 8.0 %) or iTBS (pre: 47.0 ± 6.4 %; post: 47.1 ± 7.4 %). Likewise, AMT was not affected by cTBS (pre: 34.0 ± 6.8 %; post: 33.9 ± 6.8 %) or iTBS (pre: 36.0 ± 6.5 % ; post: 35.7 ± 6.2 %). The baseline AMT values were not different between the cTBS and iTBS conditions, and thus stimulation was applied at a similar intensity for both interventions (cTBS: 28.7 ± 5.7 %; iTBS 30.8 ± 4.2 %).

4.4.2. Corticospinal excitability

Figure 4.2 summarises the group MEP data. There was no significant effect of TIME or INTERVENTION and no TIME x INTERVENTION interactions. Thus MEP amplitude was unchanged by cTBS and iTBS when applied at 70% AMT.

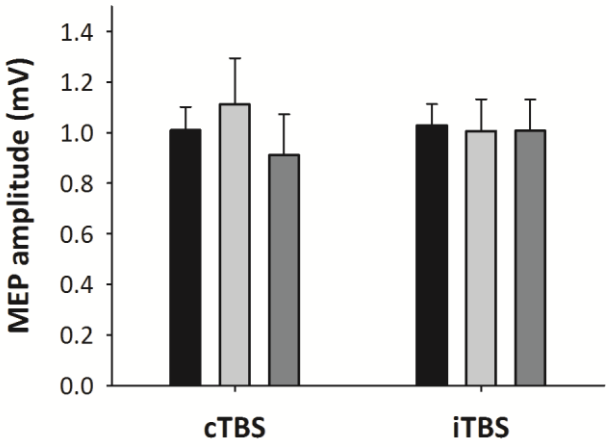

Figure 4.2. *Mean MEP amplitudes in FDI before and after low-intensity cTBS and iTBS. Black bars are pre-TBS, light grey bars are 5-20 mins post-TBS and dark grey bars are 20-35 mins post-TBS. The graph shows group data from nine subjects. MEP amplitudes were not significantly different over time for either cTBS or iTBS.*

4.4.3. Intracortical excitability

Figure 4.3 summarises the group SICF data. Figure 4.3a shows the MEP amplitude when the suprathreshold S1 was given alone. There were no effects of TIME or INTERVENTION and no TIME x INTERVENTION interaction, confirming that test MEP sizes were not different over time or between the cTBS and iTBS interventions. The three-way repeated measures ANOVA results showed a significant INTERVENTION by ISI interaction ($F_{2,16}$=4.3, p=0.03). Therefore two-way repeated-measures ANOVAs were performed separately for each intervention. There was no effect of ISI or TIME and no ISI x TIME interactions for either of the interventions (Figures 4.3b and 4.3c).

The test MEP sizes for SICI/ ICF trials are presented in Figure 4.4a. There was no effect of INTERVENTION or TIME and no TIME x INTERVENTION interactions confirming that test MEP sizes were consistent across time and between the interventions. Figure 4.4b presents the results for ICF. There was no significant effect of TIME or INTERVENTION and no TIME x INTERVENTION interaction. Therefore cTBS and iTBS had no significant effect on ICF. Figure 4.4c demonstrates the effect of low-intensity TBS on SICI. There was a significant effect of INTERVENTION ($F_{1,16}$=7.9, p=0.02) and a significant TIME x INTERVENTION interaction ($F_{2,16}$=4.8, p=0.02). Therefore, two one-way ANOVAs were performed on the cTBS and iTBS SICI data. This revealed a significant time effect following cTBS ($F_{2,16}$=4.8, p=0.02) but no significant effect following iTBS. Post-hoc analysis showed that for the cTBS data this significant effect was due to a reduction in SICI 5-20 minutes following cTBS (Bonferroni t-test: p=0.02). In contrast to the results seen following cTBS, SICI was unchanged by iTBS. Figure 4.5 depicts individual SICI responses to cTBS and iTBS.

Of the nine subjects, eight showed a reduction in SICI following cTBS. However responses to iTBS were far more variable.

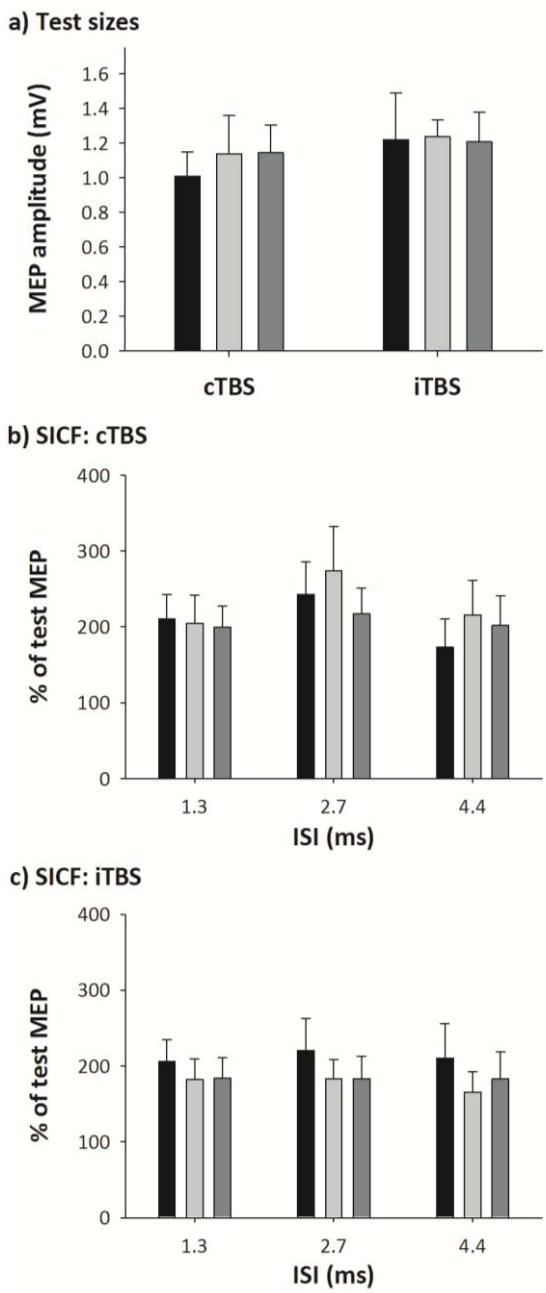

Figure 4.3. *SICF data. All graphs show group data from nine subjects, recorded from FDI. Black bars are pre-TBS, light grey bars are 5-20 mins post-TBS and dark grey bars are 20-35 mins post-TBS. a) Mean test MEP amplitudes. Test MEP amplitude is the response to stimulus 1 presented alone. There were no differences in the test MEP amplitudes across time for cTBS or iTBS. b-c) Mean SICF at three different ISIs before and after low-intensity cTBS (b) and low-intensity iTBS (c). On the y-axes the MEP response to the two SICF stimuli given together is expressed as a percentage of the MEP response to stimulus 1 given alone. There were no differences in SICF across time for any of the ISIs tested for either cTBS or iTBS.*

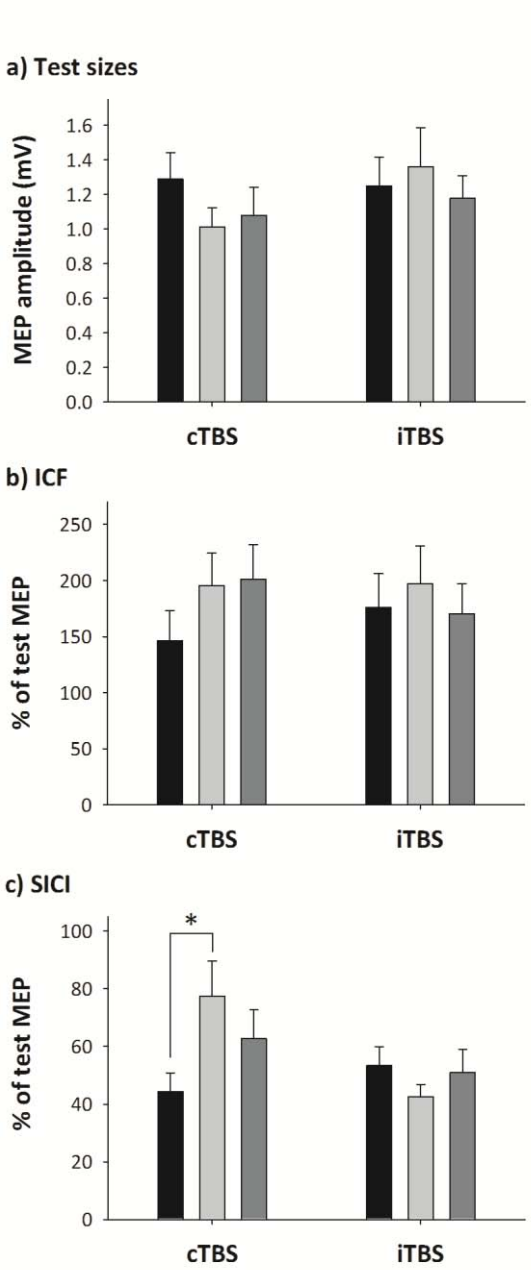

Figure 4.4. *SICI and ICF data. All graphs show group data from nine subjects, recorded from FDI. Black bars are pre-TBS, light grey bars are 5-20 mins post-TBS and dark grey bars are 20-35 mins post-TBS. a) Mean test (unconditioned) MEP amplitudes for SICI/ ICF. There were no differences in the test MEP amplitudes across time for cTBS or iTBS . b) Mean ICF before and after low-intensity cTBS and iTBS. ICF was measured at an ISI of 10 ms and is expressed as the mean conditioned MEP amplitude as a percentage of the mean test MEP amplitude. There were no changes in ICF across time for either cTBS or iTBS. c) Mean SICI before and after low-intensity cTBS and iTBS. SICI was measured at an ISI of 3 ms and is expressed as the mean conditioned MEP amplitude as a percentage of the mean test MEP amplitude. SICI was significantly reduced 5-20 mins following cTBS (p=0.02). However, SICI was unchanged following iTBS.*

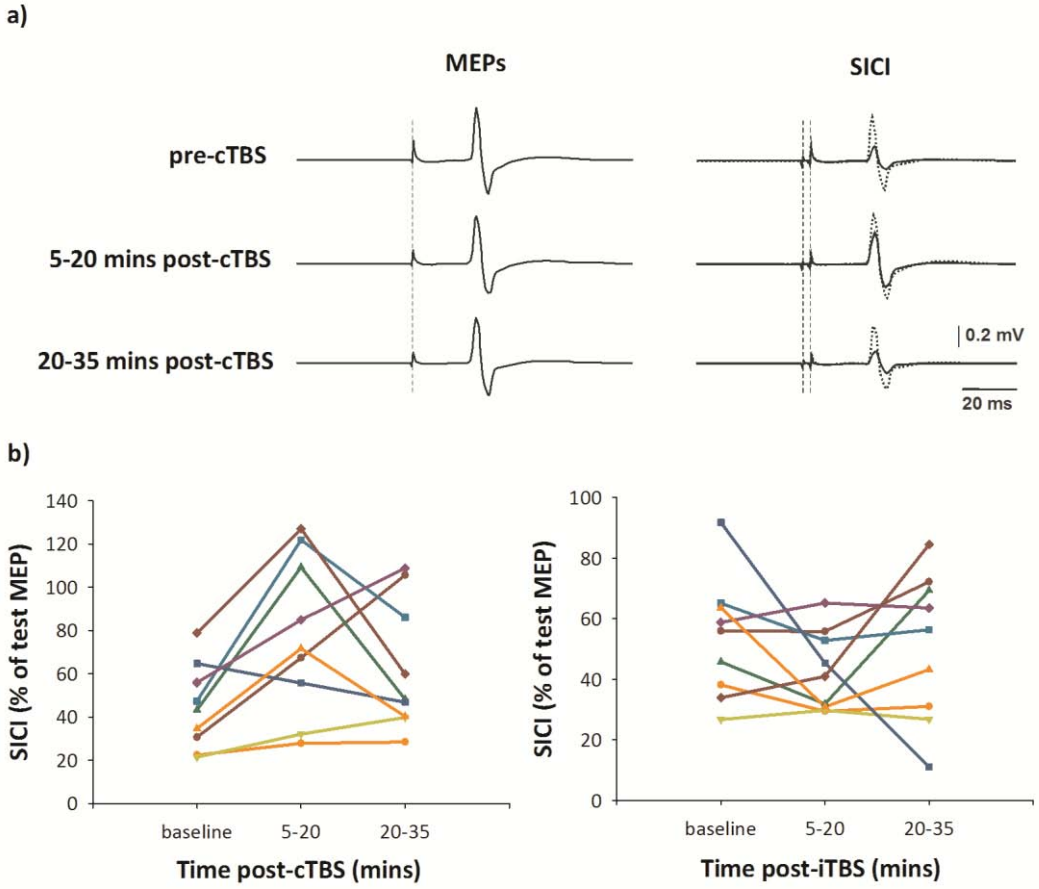

Figure 4.5. *Individual subject data for SICI. All data was recorded from FDI. a) Raw data from a representative subject following low-intensity cTBS. MEP amplitudes are shown on the left, with the vertical dashed line indicating delivery of the TMS pulse. MEP amplitude was unchanged by low-intensity cTBS. SICI is shown on the right. The dotted line is the test (unconditioned) MEP response. The solid line is the conditioned MEP response. The two vertical dashed lines indicate delivery of the subthreshold conditioning stimulus and the suprathreshold test stimulus 3 ms later. SICI was reduced 5-20 mins following low-intensity cTBS, but had returned to baseline levels by 20-35 mins post-cTBS in this subject. b) Individual SICI data from all nine subjects. Each line on the graphs represents one subject. Numbers on x-axis indicate time following TBS. SICI was measured at an ISI of 3 ms and is expressed as the mean conditioned MEP amplitude as a percentage of the mean test MEP amplitude. Note the reduction in SICI 5-20 mins following low-intensity cTBS in eight of the nine subjects. In contrast, SICI responses following low-intensity iTBS were highly variable between subjects.*

4.5. DISCUSSION

The major finding of the present study is that cTBS at an intensity below the threshold for inducing changes in excitatory pathways can modulate excitability in intracortical inhibitory networks; with SICI being reduced from approximately 44% to 78% five minutes following cTBS. Resting and active motor thresholds, MEP amplitude, SICF and ICF were unchanged by cTBS. In contrast, iTBS at this low intensity did not have any significant effect on either intracortical inhibitory or excitatory pathways.

At the conventional stimulation intensity of 80% of active motor threshold, cTBS reduces both MEP amplitude and SICI whereas iTBS increases MEP amplitude and SICI (Huang *et al.*, 2005). The changes in MEP amplitude can outlast the stimulation period by 20-60 minutes, depending on the TBS paradigm used, the changes in SICI, though less well-studied, generally last 10-20 minutes (Huang *et al.*, 2005; Murakami *et al.*, 2008). It is currently thought that the changes in MEP amplitude induced by TBS are due to LTP/ LTD-like changes in the excitatory I-wave pathways responsible for the generation of the MEP (Di Lazzaro *et al.*, 2004; Di Lazzaro *et al.*, 2008). A single TMS pulse evokes several descending volleys, the I-waves, with a periodicity of approximately 1.5 ms (see (Di Lazzaro *et al.*, 2004) for review). Although the mechanisms underlying I-wave generation are unclear, it is believed they result from the excitation of glutamatergic cortical interneuronal pathways, with separate pathways generating the early (I1) and later (I2, I3) I-waves. Direct recordings of I-waves after conventional TBS have been made in small numbers of patients with epidural electrodes implanted for the treatment of chronic pain (Di Lazzaro *et al.*, 2005; Di Lazzaro *et al.*, 2008). These recordings suggest that the reduction in MEP amplitude following cTBS is primarily due to a reduction in the

amplitude of the I1 wave (Di Lazzaro *et al.*, 2005), whereas the facilitation of the MEP following iTBS is associated with an increased amplitude of the I3 wave (Di Lazzaro *et al.*, 2008). The changes in MEP amplitude can be blocked by administration of the NMDA-receptor antagonist, memantine, providing strong evidence for an LTP/ LTD-like mechanism underlying TBS (Huang *et al.*, 2007). Taken together, the results suggest the reduction in MEP amplitude following cTBS is due to LTD-like changes in the cortical circuits responsible for generating the I1 wave, whereas the facilitation of MEP amplitude following iTBS is due to LTP-like changes in the cortical circuits which generate the I3 wave. The changes in SICI observed following TBS may rely on similar LTP/LTD-like mechanisms to those responsible for changes in MEPs although the clearly different time-course of the two effects makes this possibility open to considerable debate. Future pharmacological studies may help to address this issue.

It is known that cortical inhibitory pathways have a lower threshold for activation than excitatory pathways (Kujirai *et al.*, 1993) and previous studies have shown that at rest SICI pathways are recruited with S1 intensities as low as 60% AMT, whereas SICF circuits are only activated when S1 is equal to or greater than 80% AMT (Ilic *et al.*, 2002b; Ortu *et al.*, 2008). Pilot studies suggested that TBS at 70% of active motor threshold had no effect on MEP amplitudes and, therefore, in this study we applied TBS at this lower intensity with the aim of selectively targeting intracortical inhibitory circuits. The present results confirmed these observations. The lack of change in motor thresholds following low-intensity cTBS and iTBS is consistent with previous TBS studies (Huang *et al.*, 2005) and indicates there were no changes in the intrinsic excitability of corticospinal neurons. We also measured SICF using the paradigm first described by Ziemann et al. (1998). SICF is

considered an indirect measure of the excitability of the I-wave circuitry involved in the generation of the MEP. The intervals between the first suprathreshold and the second subthreshold SICF stimuli at which facilitation is seen (approximately 1.3, 2.7 and 4.4 ms) closely parallel the periodicity of I-waves. It is thought that the peaks of facilitation seen at these interstimulus intervals are due to summation of depolarising currents arriving at corticospinal neurons (Hanajima *et al.*, 2002). There was no significant change in SICF measures at any of the interstimulus intervals following low-intensity cTBS and iTBS. This finding suggests neither paradigm had any effect on the excitatory I-wave circuitry and is consistent with the unchanged MEP amplitudes.

Additionally, no changes in ICF were observed following low-intensity cTBS or iTBS. At the conventional intensity of 80% AMT cTBS reduces and iTBS increases ICF (Huang *et al.*, 2005). Little is known of the mechanisms underlying ICF, however it is thought to test excitatory neuronal pathways and may be NMDA-receptor mediated (Ziemann, 2004). ICF pathways are recruited at higher intensities than SICI (Ziemann *et al.*, 1996c) and this may explain why no change in ICF was observed in the present study following low-intensity cTBS and iTBS. Together, the results for MEP amplitudes, SICF and ICF suggest that low-intensity cTBS and iTBS had no LTD/ LTP-like effects on intracortical excitatory circuits.

However, low-intensity cTBS did produce a reduction in intracortical inhibition, investigated using the SICI paradigm. This technique, developed by Kujirai et al. (1993), involves delivering a subthreshold conditioning stimulus followed by a suprathreshold test stimulus. At interstimulus intervals of 1-6 ms, the amplitude of the MEP is reduced

compared to if the test stimulus was given alone. It is thought that the subthreshold conditioning stimulus activates $GABA_A$-ergic inhibitory interneuronal pathways which terminate on corticospinal neurons, suppressing the response to the test stimulus. Administration of a $GABA_A$ agonist enhances SICI (Di Lazzaro *et al.*, 2000a), providing support for this hypothesis. Thus our results suggest low-intensity cTBS produced a decrease in the excitability of intracortical GABAaergic inhibitory circuits responsible for SICI. We can only speculate on the mechanisms underlying this reduction in inhibition. However, given the proposed mechanism by which conventional TBS has its effect (see above) the results may be compatible with the induction of LTD in low threshold SICI circuits.

In contrast to cTBS, low-intensity iTBS had no significant effect on SICI. This is puzzling given that conventional iTBS facilitates the MEP and increases SICI. It was expected that low-intensity iTBS would enhance inhibition whilst having no effect on MEP amplitude or SICF. However, the present results and those from other studies suggest that iTBS may be a less robust paradigm than cTBS for inducing changes in intracortical inhibitory circuits. In the original report of TBS by Huang et al. (2005), cTBS reduced SICI by approximately 25%, whereas iTBS only increased SICI by approximately 10% (see Figure 3 of Huang et al. (2005)). These results are closely paralleled in another study investigating inhibition following TBS (Figure 5 of Murakami et al. (2008)). In both studies, the reduction in SICI following cTBS is greater and longer lasting than the increase in SICI following iTBS. The reasons for this difference in effectiveness are unclear. The intensity of the conditioning pulse was adjusted to produce a baseline where the conditioned MEP was approximately 50% of the test MEP so it is unlikely the lack of change in SICI following iTBS is due to

'floor' or 'ceiling' effects. One possibility to explain the findings is that the temporal pattern of the iTBS trains is not optimal for inducing LTP-like changes in inhibitory interneurons. Alternatively, it may be that the inhibitory synapses involved in measures of SICI are more prone to undergo LTD than LTP-like change; it is interesting to note that almost all studies which have reported changes in SICI following rTMS have reported a reduction rather than an increase in SICI (Fitzgerald *et al.*, 2006). A study in the rat hippocampus has demonstrated that a stimulation paradigm which induces LTP at excitatory synapses also produces changes in the synaptic efficacy of inhibitory interneurons, however the direction of change depends on the interneuronal location (Mendoza *et al.*, 2006). The stimulation paradigm induces LTD at dendritic inhibitory synapses, whereas it induces LTP at somatic inhibitory synapses (Mendoza *et al.*, 2006). Therefore it may be that the specific inhibitory interneuron population targeted by TBS is more likely to undergo LTD rather than LTP-like changes due to intrinsic neuronal properties. This may also explain why some studies have reported reduced SICI following 5 Hz stimulation; a paradigm which is usually considered to be a 'facilitatory' paradigm, or causing LTP-like changes rather than LTD-like changes (Di Lazzaro *et al.*, 2002a; Quartarone *et al.*, 2005). In a study by Quartarone and colleagues (2005) increases in MEP amplitude were found following 5 Hz rTMS, accompanied by a *reduction* in SICI. Additionally, animal studies have provided evidence that the same induction paradigm can have opposite effects on excitatory and inhibitory pathways. For instance, in the xenopus retinotectal system, electrical stimulation of the optic nerve at theta frequency produces LTP of glutamatergic inputs but LTD of GABAa-ergic inputs to the same tectal neuron (Lien *et al.*, 2006).

Another possibility that might explain the apparent lack of effect of iTBS on SICI is that the after-effects of iTBS are highly intensity-dependant. An intensity of 70% of active motor threshold may not be optimal for modulation of SICI using iTBS. Alternatively, it may be that the after-effects of low-intensity iTBS are very short-lived and thus were not observed during the first recording period 5-20 minutes post-TBS. The present study employed cTBS consisting of 600 stimuli (cTBS600) rather than the 300 stimuli (cTBS300) used by Huang et al. (2005) when investigating the effects of cTBS on SICI. The effects of cTBS600 on MEPs last longer (up to one hour) than those seen with either cTBS300 or iTBS (up to 20 mins; Huang et al. (2005)). Additionally, a study employing cTBS600 suggests that the after-effects on SICI outlast those of iTBS (Murakami et al. (2008), see Figure 5). Therefore, in the present study it is possible that very short lasting effects on SICI induced by iTBS were missed, whereas those induced by cTBS, which may have a longer-lasting time course, were observed in the 5-20 min post stimulation period.

In vitro, addition of a $GABA_A$ antagonist reduces GABAaergic inhibition and greatly facilitates the induction of LTP (Hess *et al.,* 1996). It remains to be investigated whether low-intensity cTBS could similarly enhance the induction of neuroplasticity in the motor cortex if used as a priming stimulation technique. Additionally, intracortical inhibition is reduced in various neurological disorders, such as focal hand dystonia (Ridding *et al.*, 1995b; Butefisch *et al.*, 2005), and likely contributes to impaired movement. We have demonstrated that it is possible to target these intracortical inhibitory networks by reducing the intensity of TBS. It will now be important to investigate whether it is possible to optimise the technique to bidirectionally modulate the levels of inhibition.

CHAPTER FIVE

General discussion

This thesis aimed to investigate approaches to optimising plasticity induction in the human motor cortex. This concluding chapter summarises the main findings of the thesis, and discusses them in the context of enhancing the therapeutic potential of plasticity-inducing paradigms. I discuss the limitations of my work, as well as ideas for future research.

This thesis aimed to develop potential methodologies for optimising plasticity protocols in the human motor cortex. These methodologies were built upon the central concept that brain excitability state is important in determining responses to TMS and plasticity induction. The studies presented questioned how we may determine, target or create the optimal brain state for neuroplasticity induction.

A large body of evidence in animal models suggests that neural oscillatory activity, particularly the hippocampal theta rhythm, has an important role in the modulation of plasticity (Huerta & Lisman, 1995; Natsume & Kometani, 1997; Orr *et al.*, 2001). Additionally, invasive studies in the cat visual cortex have demonstrated that neural activity state is an important determinant of responses to rTMS (Pasley *et al.*, 2009). In Chapter Two I investigated whether EEG spectral power could be used as a marker of brain excitability state in order to predict responses to plasticity induction in the human motor cortex. Two methods of plasticity induction were employed - non-invasive brain stimulation (cTBS) and behavioural motor learning (a visuomotor training task). However, none of the spectral frequencies investigated (delta, theta, alpha and beta power) predicted responses to experimentally induced or behaviourally induced neuroplasticity. These findings suggest that spectral power in the prestimulation EEG is not useful as a state marker for determining responses to plasticity induction. Two possible explanations underlie this finding - either prestimulation cortical oscillatory activity is of little relevance to subsequent plasticity induction, or the surface EEG holds limited value for investigating this relationship.

Surface EEG has several limitations. Firstly, it lacks spatial resolution. There is a low signal-noise ratio due to attenuation of the signal by the cerebrospinal fluid, skull and scalp and although strong rhythms such as alpha are often observable in the raw EEG traces, weaker rhythms, such as theta, are only observable where there are strong amplitude oscillations over a large cortical area. In the absence of multichannel recordings, it is also difficult to localise the source of observed changes. Most studies that have demonstrated correlations between human theta activity and learning and memory have been done in patients with implanted EEG electrodes for the detection of seizure foci. Intracranial EEG has a much higher signal-noise ratio and allows the investigation of subcortical (for example, hippocampal) theta using advanced source localisation techniques (Raghavachari *et al.*, 2001; Caplan *et al.*, 2003; Sederberg *et al.*, 2003; Anderson *et al.*, 2009; Rutishauser *et al.*, 2010). Nevertheless, a weak theta spectral peak is observable in the scalp-recorded EEG and many studies have demonstrated alterations in fronto-midline recorded theta power related to cognitive performance (Klimesch, 1999; Staudigl *et al.*, 2010).

Aside from the limitations of surface EEG, the results of Chapter Two may be related to the fact that rhythmic activity in the brain is highly dynamic (Buzsaki & Draguhn, 2004), and the prestimulation activity may not necessarily reflect activity during the stimulation period. It may be that the *ongoing* oscillatory activity is more important for determining responses to TMS. This relationship is difficult to investigate with high frequency TBS, due to the interruption of the EEG signal with TMS artefacts. The period between stimuli bursts could be examined, but with very limited spectral frequency resolution due to the short length of time (200 ms). It may, however, be

possible to explore the relationship between ongoing spectral power and plasticity induction with slower paradigms (e.g. <1 Hz rTMS). It is also important to note that whilst there is a significant amount of research to suggest there is a relationship between oscillatory activity and plasticity induction, the majority of this work has been performed in the rodent hippocampus, and it may not be appropriate to extrapolate these findings to the human motor cortex. To begin with, cortical theta and hippocampal theta are generated by different neuronal machinery. The generation of cortical theta is not well understood, however it is thought to have many local generators and may be modulated by thalamo-cortical relay loops (Raghavachari *et al.*, 2001). Although the hippocampal theta rhythm has long been thought to rely largely upon the medial septum diagonal band of Broca (Buzsaki, 2002), the hippocampus has recently been shown to be capable of generating theta independent of extrinsic inputs (Goutagny *et al.*, 2009). However, although the isolated hippocampus has sufficient neuronal machinery to produce the theta rhythm, this likely does not reflect how the rhythm is generated/ modulated in the in-tact brain. Theta rhythm is one of the most dominant rhythms of the hippocampus, and can be easily observed during active movement and exploratory behaviour (Buzsaki, 2002). Cortical theta, as mentioned earlier, is often difficult to even observe in the raw EEG and is relatively weak compared to neighbouring alpha and delta frequency bands. The frequency range defined as hippocampal theta also varies widely (Kramis *et al.*, 1975; Bragin *et al.*, 1995; Orr *et al.*, 2001) and can be much larger than cortical theta (3-12 Hz cf. 4-7 Hz). Although it is not clear whether cortical theta performs a similar function to hippocampal theta, it is known that cortical neurons, like hippocampal neurons, exhibit phase locking to the local theta field potential (Jacobs *et al.*, 2007) and thus it is feasible

that these oscillations could be important in modulating spike timing dependent plasticity. However, given the above-mentioned differences between hippocampal and cortical theta, caution should be taken in suggesting these rhythms perform analogous functions.

Aside from the main aim of investigating the influence of prestimulus EEG on plasticity induction, Chapter Two also examined the influence of cTBS and visuomotor training on the EEG itself, by comparing the post-intervention power spectra with that recorded at baseline. EEG power spectra were unchanged by cTBS, however, visuomotor training produced a large, sustained increase in alpha power, lasting at least 8 mins post-training. Although there is some limited evidence that some rTMS paradigms can entrain endogenous cortical rhythms (Klimesch *et al.*, 2003), the influence of cTBS on cortical oscillatory activity is unknown. Chapter Two provides no evidence for a lasting modulation of cortical theta rhythm by cTBS, or an effect on any other oscillatory frequencies. Because cTBS is applied at low intensity and for a short period of time (40s), it may not be sufficient to synchronise a large enough neuronal population to see changes in the EEG power spectra. Many studies have failed to demonstrate after-effects of subthreshold rTMS on EEG power spectra (Strens *et al.*, 2002; Oliviero *et al.*, 2003). Brignani et al. (2008) found synchronisation in the alpha band following 1 Hz rTMS, however this was only significant towards the end of a 10 minute paradigm and the rTMS was applied at a much greater intensity than in my study (110% RMT) . Another study found enhancement of event-related power in the alpha and beta frequency bands following trains of both suprathreshold AND subthreshold TMS. However these effects were found within a very short time frame (a few seconds)

following rTMS (Fuggetta *et al.*, 2008). Such short-lasting effects may have been missed in our study, due to the delay between stimulation and EEG recording. Interestingly however, a recent study found enhancement of event related power in the theta and beta band for up to half an hour following just 20s of cTBS compared to sham (Noh *et al.*, 2012). However, this study involved performance of a choice reaction time task both before and after the intervention, and thus an effect from an interaction between the two cannot be ruled out. In summary, intervention effects on the EEG may depend critically on the stimulation parameters, including intensity, duration and location of stimulation, and history of cortical activity.

In contrast to cTBS, there was a large increase in alpha power following visuomotor training, and this increase was sustained up to 8 mins post- training. This is in line with a study by Smith and colleagues (1999), who found that alpha power over sensorimotor areas increased both within and across sessions when subjects were repeatedly trained on a computer game with a visuomotor tracking component. In my study, the change in alpha power was positively correlated with changes in MEP amplitude, such that participants with largest increases in alpha also demonstrated large increases in MEP amplitudes. Although speculative, the timing of the alpha increase is in-line with a consolidation-like effect on MEP excitability. There is a large body of evidence to suggest the alpha rhythm is involved in disengagement of task-irrelevant brain regions in sensory perception tasks (Smith *et al.*, 1999; Sauseng *et al.*, 2005; Thut *et al.*, 2006; Haegens *et al.*, 2011a). It is possible that a reduction in interfering sensory input is also important for consolidation of motor memory following learning. Thus, the increased alpha may reflect disengagement of the sensory cortex. In my study, EEG was only

recorded over C3, so it is not possible to localise the increased alpha. Even with high density, multi-channel EEG, it would be difficult to differentiate between sensory and motor alpha. However, the proposal of enhanced post-training alpha as a mechanism involved in motor memory consolidation itself requires further investigation, as the design of my study did not involve repeated training bouts.

Whilst Chapter Two provided no evidence that the baseline (pre-stimulation) EEG influences plasticity induction, as stated earlier, the *ongoing* EEG activity (at the time of TMS application) may be more relevant. The alpha rhythm is thought to represent oscillations in inhibitory tone in the cortex (Jensen & Mazaheri, 2010; Mathewson *et al.*, 2011) and inhibitory tone is known to be an important modulator or plasticity induction (Ziemann *et al.*, 1998a). For example, *in vitro*, the induction of LTP in hippocampal and motor cortical slices is favoured in conditions that reduce $GABA_A$ receptor activity (Wigstrom & Gustafsson, 1983; Bramham & Sarvey, 1996; Evans & Viola-McCabe, 1996; Hess *et al.*, 1996). Evidence suggests modulations in inhibition are important for the induction of plasticity in humans also. For example, the rapid changes in cortical somatotopy following limb deafferentation are accompanied by a reduction in $GABA_A$ inhibition (Ziemann *et al.*, 1998a). If we can target the cortex at times when inhibition is reduced, we may be able to target the cortex at times when it is more receptive to plasticity-inducing stimuli. In order to investigate this, in Chapter Three I developed a novel method to trigger TMS on different phases of the alpha rhythm in the ongoing EEG.

Several approaches to triggering TMS from the ongoing EEG rhythm were considered. These included building an external analog filter, online wavelet analysis, and online filtering. The first option, building an external analog filter, was considered problematic. In order to have a constant and predictable phase delay an FIR filter would be required which requires a higher order than an IIR filter and is difficult to build, requiring many components. Instead, I began with an online wavelet analysis approach to decompose the EEG signal into its different frequency components and to use these frequency components to predict the next oscillation in series. However, this approach was laced with complications. Firstly, a third party Matlab interface for CED was employed. The interface sampled segments of data, rather than recording continuously, and the delays in communication between this interface and the CED A-D converter were unclear. This interface essentially negated any benefits of wavelet temporal resolution, as the analysis was limited by the length of data sampled, as with Fourier transform. The frequency resolution of the analysis was poor due to the length of the sampled segments, however in order to improve the frequency resolution I would have needed to sample longer data lengths, and this would then limit the temporal resolution. Secondly, I used subject's peak alpha frequency to determine the wavelet frequencies (10 and 11 Hz were used for most subjects). However, not all subjects had an obvious alpha peak, and the rhythm is highly dynamic. Good hit rates were only achieved on subjects with a strong and stable alpha rhythm. I therefore looked for an alternative approach, where the computational and system delays could be more easily and accurately determined and I would not be limited by non-continuous data sampling. This approach involved digital filtering of the EEG signal online using CED software. An FIR filter was designed such that the group delay,

combined with computational/system delay allowed the TMS to be triggered on the next alpha cycle at a time when the alpha state would be upgoing (or downgoing), regardless of the frequency 8-12 Hz. This approach worked well, with hit rates of >80%. However, it still has its limitations. One problem is that the accuracy of the Script can only be determined from data without TMS triggers, as the TMS artefact is large and obscures the filtered signal. Secondly, it is difficult to target the peaks or troughs of the rhythm without overshoot or undershoot given it's dynamic nature. Another limitation of the script is that a pre-defined threshold was used to determine when the trigger was sent. We have since been working on development of an adaptive threshold, which can be modified for the individual subject, in order to send triggers only during high levels of alpha activity.

Nevertheless, Chapter Three produced some interesting results. I measured SICI and MEP amplitude and found that MEP amplitude was approximately 30% greater on the downgoing phase of alpha compared to the upgoing phase. SICI was unchanged between the up and down-going phases. These results build on evidence in the visual cortex that alpha phase is associated with modulations in cortical excitability, evidenced as a change in perception of TMS-induced phosphenes between the peak and trough of the alpha oscillation (Dugué *et al.*, 2011). However, in my study, stimuli were delivered just after the signal passed through 0 degrees, rather than at the peak or trough. Further experiments should investigate the effect of phase angle. The results of my study extend upon the findings of recent studies of alpha phase effects by developing a technique that can be used to *target* particular phases of alpha, and demonstrating an effect in the motor cortex. Given the modulation in cortical

excitability associated with alpha phase, this may provide a way to target the cortex at a time when it is more receptive to stimuli, and thus provide an opportunity for enhancing plasticity induction.

This technique could be further adapted to investigate other rhythms, though each rhythm comes with its own set of limitations. Stimuli applied on the upgoing phase of hippocampal theta rhythm induces LTP that can be depotentiated by stimuli on the downgoing phase (Huerta & Lisman, 1995; Orr *et al.*, 2001), and the idea of EEG-triggered TBS is an interesting one. The effects of TBS are highly variable between (and within) subjects, and it is possible that by timing stimuli relative to the individual's endogenous theta rhythm, this variability could be reduced. However, there are several limitations that need to be overcome before this is a possibility. Firstly, the method we designed relies on artefact-free data to predict the next oscillatory cycle. Although the artefacts from subthreshold rTMS are smaller and shorter lasting than those induced by higher intensity single-pulse TMS, they may interfere with the filtering analysis, and it may be necessary to reduce the frequency of TBS in order to overcome this problem (e.g. apply TBS to every second theta cycle). A second issue is whether TBS induces a phase reset of theta (or other) rhythms. There is some evidence that single pulse TMS induces a synchronisation at beta frequency (Paus *et al.*, 2001). If TBS does produces a phase-reset it may be difficult to predict the next oscillation. Again, this may not be such a problem if TBS bursts are not sent on every theta cycle. Another notable problem is that there is no overlapping up/down state that incorporates all frequencies within the traditionally defined theta band (4-7.5 Hz), and thus a narrower frequency band would need to be chosen (e.g. 5-7 Hz). The theta rhythm is also very

weak in the surface EEG, and the filtered signal would be highly influenced by the strong neighbouring delta rhythm. Thus a better performing, higher order filter would be required, with a longer delay time. This might be possible given the slower frequency of the theta rhythm allowing for a longer computational time. Another option may be to experimentally synchronise brain rhythms, by applying oscillating tDCS (Marshall *et al.,* 2006) or tACS (Antal *et al.,* 2008). In this way, stimuli could be more easily delivered on the appropriate phase. The relationship between experimentally-induced rhythms and endogenous rhythms is unclear, although there is some evidence that applied rhythms can entrain endogenous oscillators (Zaehle *et al.,* 2010; Thut *et al.,* 2011 for review). Other studies (Bergmann *et al.*, 2009; Doeltgen *et al.*, 2012, in press) provide little evidence for an entrainment of intrinsic brain rhythms by these techniques.

As Chapter Three did not provide any evidence of a modulation in SICI on different phases of alpha, in Chapter Four, I investigated an alternative way of modulating the excitability of inhibitory circuits as an approach to optimise plasticity induction. Conventional cTBS reduces MEP amplitude and SICI, whereas iTBS increases MEP amplitude and SICI (Huang *et al.*, 2005). Because the threshold for stimulation of cortical inhibitory pathways is lower than that of excitatory pathways (Kujirai *et al.*, 1993), I investigated whether lowering the intensity of cTBS could selectively target and decrease the activity of cortical inhibitory pathways. The results suggested it is possible to selectively decrease the activity of cortical inhibitory networks (in particular, SICI) without concurrent effects on the MEP or SICF. However, it seems more difficult to selectively enhance the activity of cortical inhibitory networks, as low intensity iTBS had

no effect on SICI. Although the mechanism of reduced inhibition following low-intensity cTBS was not investigated, it has previously been shown that the after-effects of conventional TBS on MEP amplitude are NMDA-receptor dependant, and thus rely on LTP and LTD-like mechanisms (Huang *et al.*, 2007; Teo *et al.*, 2007). The results of Chapter Four would be consistent with an LTD-like effect on the pathways responsible for the production of SICI. In this study, 70% AMT was chosen as the stimulation intensity based on a pilot study that suggested this intensity was sufficient for an effect on SICI without concurrent effects on the MEP. However, whether this intensity is optimal is unknown. A recent study from our laboratory suggests low-intensity cTBS 300 (cTBS 600 was used here) (Doeltgen & Ridding, 2011) does not modulate SICI, and highlights the importance of the stimulation paradigm chosen.

Low intensity cTBS may be useful as a priming technique to enhance plasticity induction by reducing $GABA_A$ergic inhibition, however this remains to be tested.

Concluding remarks

Responses to non-invasive brain stimulation techniques are highly variable in healthy subjects and even more-so in heterogeneous patient populations. Brain excitability state is an important determinant of this variability and methods for tailoring neuroplasticity induction techniques to the individual may be useful. With further development, EEG-triggered TMS may provide new frontiers in non-invasive brain stimulation by allowing plasticity-inducing stimuli to be targeted to optimal temporal windows based on ongoing neural activity. Additionally, selectively down-regulating

the activity of cortical inhibitory pathways may provide an opportune cortical environment for plasticity induction.

6. APPENDICES

6.1. APPENDIX 1. TRANSCRANIAL MAGNETIC STIMULATION 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):*

_____
_____
_____
_____
_____
_____
_____

6.2. APPENDIX 2. PUBLICATIONS ARISING FROM THIS THESIS

McAllister, S. M., Rothwell, J. C. and Ridding, M. C. (2009) Selective modulation of intracortical inhibition by low-intensity Theta Burst Stimulation. *Clin Neurophysiol*, **120**(4), 820-826.

McAllister, S. M., Rothwell, J. C. and Ridding, M. C. (2011) Cortical oscillatory activity and the induction of plasticity in the human motor cortex. *Eur J Neurosci*, **33**(10), 1916-1924.

6.3. APPENDIX 3. STATEMENT OF AUTHORSHIP FOR CHAPTER TWO

McAllister, S. M., Rothwell, J. C. and Ridding, M. C. (2011) Cortical oscillatory activity and the induction of plasticity in the human motor cortex. *Eur J Neurosci*, **33**(10), 1916-1924.

Suzanne M. McAllister

Statement of contribution:

Designed and conducted experiments, recruited participants, analysed and interpreted the data, wrote the manuscript.

Certification that the statement of contribution is accurate:

Signed: *Date:*

Professor John C. Rothwell

Statement of contribution:

Aided interpretation of the data and made suggestions for additional analyses, provided critical evaluation of the manuscript.

Certification that the statement of contribution is accurate:

Signed *Date:.....* *Date: 15 June 2012*

Associate Professor Michael C. Ridding

Statement of contribution:

Supervised development of the study, aided interpretation of the data and made suggestions for additional analyses, provided critical evaluation of the manuscript.

Certification that the statement of contribution is accurate:

Signed: *Date:*

6.4. APPENDIX 4. STATEMENT OF AUTHORSHIP FOR CHAPTER FOUR

McAllister, S. M., Rothwell, J. C. and Ridding, M. C. (2009) Selective modulation of intracortical inhibition by low-intensity Theta Burst Stimulation. *Clin Neurophysiol*, **120**(4), 820-826.

Suzanne M. McAllister

Statement of contribution:

Designed and conducted experiments, recruited participants, analysed and interpreted the data, wrote the manuscript.

Certification that the statement of contribution is accurate:

Signed: *Date:*

Professor John C. Rothwell

Statement of contribution:

Aided interpretation of the data and made suggestions for additional analyses, provided critical evaluation of the manuscript.

Certification that the statement of contribution is accurate:

Signed: *Date: 15 June 2012*

Associate Professor Michael C. Ridding

Statement of contribution:

Supervised development of the study, aided interpretation of the data and made suggestions for additional analyses, provided critical evaluation of the manuscript.

Certification that the statement of contribution is accurate:

Signed: *Date:*

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