Intra- and interhemispheric cortical adaptations due to modulations of premotor and primary motor cortices

by

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Authour's declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Movement training modulates the excitability in several cortical and subcortical areas. Compared to training with a single arm, movement training with both arms yields a greater increase in motor related cortical regions. A short-term session of bimanual training (BMT) enhances cortical activity of motor preparation and execution areas in both hemispheres. The underlying neural mechanisms for this increased activation with BMT are unclear, but may involve interhemispheric connections between homologous primary motor cortex (M1) representations and input from motor preparatory areas (i.e. dorsal premotor cortex (PMd)). Also, it is unclear how selective up-regulation or down-regulation of specific motor-related areas may contribute to changes in M1 excitability when combined with BMT. The work in this thesis investigated modulation of M1 excitability in terms of in-phase versus anti-phase BMT (Study #1), potentially up-regulating the left dorsal premotor cortex (lPMd) via iTBS before BMT (Study #2), theoretically down-regulating contralateral (right) M1 homologous representation before BMT (Study #3), and finally the potential intracortical and interhemispheric cortical adaptations in M1 bilaterally due to the same interventions as Study #2 (Study #4). For Study #1, it was hypothesized that in-phase BMT would lead to an increased excitability in M1. For Studies #2-4, it was hypothesized that modulation of motor-related areas would cause an increase in the excitability of left M1, and this modulation would be greater when combined with BMT. Study #1 found that in-phase, and not anti-phase BMT, lead to increase M1 excitability. Study #2 found that iTBS to lPMd followed by BMT caused a unique increase in M1 excitability, in terms of increased spatial extent and global MEP amplitude. Study #3 found that the combination of cTBS to right M1 with

BMT caused greater excitability enhancements than either intervention alone. Finally, Study #4 found distinct modulations of cortical excitability within and across M1 bilaterally due to BMT, iTBS to lPMd and the combination of these interventions that involved long-interval inhibitory circuitry asymmetrically. Overall, this current work found that the modulation of remote cortical areas to M1 (i.e. lPMd and contralateral M1) in combination with movement training led to unique, and at times greater, excitability enhancements of M1 which could be advantageous in enhancing short-term plasticity in damaged M1.

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The work in this thesis could not have been completed without the love and support of my family. This thesis is dedicated to my father, brother and with a very special mention of my late mother.

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List of Abbreviations

AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMT	Active motor threshold
ADM	Adductor digiti minimi
APB	Abductor pollicis brevis
BMT	Bimanual movement training
BP	Bereitschaftspotential
CC	Corpus callosum
CSP	Cortical silent preriod
cTBS	Continuous theta burst stimulation
ECR	Extensor carpi radialis
EEG	Electroencephalography
EMG	Electromyography
EPSP	Excitatory post-synaptic potential
ERP	Event-related potential
FCR	Flexor carpi radialis
fMRI	Functional magnetic resonance imaging
GABA	Gamma-aminobutyric-acid
GMP	Generalized motor program
ICF	Intracortical facilitation
IHI	Interhemispheric inhibition
iTBS	Intermittent theta burst stimulation
IPSP	Inhibitory post-synaptic potential
LICI	Long-interval intracortical inhibition
LTP	Long-term potentiation
LTD	Long-term depression
M1	Primary motor cortex
MEP	Motor evoked potential
MRI	Magnetic resonance imaging
MRP	Movement-related potential
MT	Movement time
MVC	Maximum voluntary contraction
NMDA	N-methyl-D-aspartic acid
PM	Premotor
PMd	Dorsal premotor cortex
PPC	Posterior parietal cortex
Pre-SMA	Pre-supplementary motor area
rTMS	Repetitive transcranial magnetic stimulation
RMT	Resting motor threshold
RT	Reaction time
S1	Primary somatosensory cortex
SICI	Short-interval intracortical inhibition
sLORETA	Standardized low resolution brain electromagnetic tomography
SMA	Supplementary motor area
TBS	Theta burst stimulation
TMS	Transcranial magnetic stimulation

Chapter 1 -- Introduction

1.1 Overview of the thesis

Chapter 1 begins with the general objectives of the thesis. Relevant literature will then be reviewed concerning the anatomy and physiology of the motor preparatory and execution cortical loci, the modulation and relationship of these cortical nodes due to bimanual arm training, and modulatory effects of repetitive transcranial magnetic stimulation (rTMS) protocols. Chapter 1 concludes with the specific research objectives and hypotheses that motivated the work throughout this thesis. Chapters 2 through 5 entails the rationale, hypotheses, methods and results of the original research contributing to the thesis. Chapter 6 includes a general discussion of the findings of the thesis, the limitations, future directions and conclusion.

1.2 General objectives of the thesis

The general objective of this thesis is to investigate the modulation of the primary motor cortex (M1) excitability as influenced by connections with related intra- and interhemispheric motor preparatory and execution cortical regions. Further, cortical adaptations in M1 were investigated using bimanual visuomotor movement training (BMT), theta burst stimulation (TBS) protocols to remote but related cortical nodes and a combination of these interventions. Particularly, this thesis focused on M1 excitability changes due to different phases of upper-limb movement, potentially up-regulating the left dorsal premotor cortex (lPMd), theoretically down-regulating contralateral (right) M1 homologous representation, and the potential intracortical and interhemispheric cortical adaptations in M1 bilaterally due to these interventions.

There is extensive connectivity between premotor (PM) to motor areas within ipsilateral and contralateral hemispheres, as well as reciprocal connections between

homologous and non-homologous M1 representations (Asanuma & Okuda, 1962; Matsunami & Hamada, 1984; Gould et al., 1986; Meyer et al., 1995; Picard & Strick, 2001; Wahl et al., 2007; Nelson et al., 2009). Damage to the cortex (i.e. stroke related injuries) often leads to weakness in one side of the body (hemiparesis) due to damage to a motor cortical area (e.g. M1) (Staines et al., 2001). Damage to one hemisphere motor cortex leads to a misbalance in the interhemispheric interactions, where the unaffected cortex often increases in excitability and sends abnormally high inhibitory signals to the damaged hemisphere (Ferbert et al., 1992; Liepert et al., 2000; Murase et al., 2004). Therefore, understanding the underlying neural connections between these intra- and interhemispheric cortical regions and how they may be modulated may be beneficial in assisting with functional recovery. Certainly, studies have shown that PM areas can influence the excitability of downstream ipsilateral and contralateral M1 (Kalaska & Crammond, 1995; Geyer et al., 2000; Toni et al., 2001; Thoenissen et al., 2002). Several behavioural and neurophysiological studies have shown that M1 activity is facilitated when PM areas are engaged via sensorimotor tasks (Rushworth et al., 2003; O'Shea et al., 2007) and stimulation protocols (Bäumer et al., 2003; Rizzo et al., 2004; Chouinard & Paus, 2006; Huang et al., 2009; Koch & Rothwell, 2009; Ortu et al., 2009; Groppa et al., 2012), such as transcranial magnetic stimulation (TMS).

The M1 cortices are highly interconnected through dense reciprocal projections via the corpus callosum, particularly between homologous and non-homologous neighbouring muscle representations (Chen et al., 1997; Chen et al., 2003; Picard & Strick, 2001; Swinnen, 2002; Daskalakis et al., 2004; Wahl et al., 2007). Further, M1 in one hemisphere has influence over the opposite hemisphere M1 via facilitatory and

inhibitory reciprocal connections (Ferbert et al., 1992; Liepert et al., 2001; Stinear & Byblow, 2002; Smith & Staines, 2006, 2010, 2012; Duque et al., 2007; Avanzino et al., 2011; Sattler et al., 2012). Specifically, activity is enhanced in both M1 hemispheres when both limbs are active in a task compared to only one limb in healthy and stroke patient populations (Silvestrini et al., 1998; Staines et al., 2001; Smith & Staines, 2006, 2010, 2012), suggesting that similar M1 areas activated in both hemispheres may facilitate and/or release inhibition to one another. In fact, when the two upper limbs activate the same muscles simultaneously there is disinhibition of the contralateral M1 (Stinear & Byblow, 2002). Although the connectivity and cortical plasticity between PM and M1 areas have been explored in healthy and patient population, the neurophysiological mechanisms that underlie modulations between these areas still remain largely unclear.

Bimanual visuomotor movement training (BMT) is a useful way to understand the connections between these intra- and interhemispheric motor preparatory and execution areas. A short-term session of BMT has been shown to enhance the cortical activity of PM areas in both hemispheres (Smith & Staines, 2006, 2010, 2012) and M1 (Neva et al., 2012). These cortical modulations seem to occur particularly when the training includes time for motor preparation and requires the simultaneous activation of homologous muscle groups. The M1 excitability changes seem to be reflected by an increase in cortical territory occupied by the trained muscles (Neva et al., 2012). Since this task is bimanual and requires motor preparation, a potential contributor to the observed cortical modulations may include intra- and interhemispheric interactions between PM and M1. Another potential contributor to this modulation could be due to the interhemispheric

interactions between homologous muscle representations in M1 via the corpus callosum. It is currently unclear how these intra- and interhemispheric connections between motor preparatory and execution regions modulate M1 excitability due to BMT, rTMS protocols that selectively up-regulate or down-regulate these cortical nodes, and the combination of these interventions.

1.3 *Background of relevant literature*

1.3.1 *Motor execution and preparation cortical areas*

Primary motor cortex (M1)

Anatomy and functional connectivity

The M1 is located in the frontal lobe of the cortex, immediately anterior to the central sulcus, and is referred to as the pre-central sulcus (Brodmann area 4). The M1 has traditionally thought to be the final output from the cortex to generate simple voluntary movement via muscle contraction in the contralateral body. Studies in humans and monkeys using microstimulation from indwelling electrodes in the cortex revealed that M1 is organized in the form of a homunculus ("little man") (Foerster, 1931, 1936; Jackson, 1931; Penfield & Rasmussen, 1950). This orderly somatotopic organization of M1 along the gyrus generally has the more proximal muscles represented medially and the distal muscles more laterally, with the facial and vocalization muscles represented most laterally (Foerster, 1931, 1936; Penfield & Rasmussen, 1950; Sanes & Schieber, 2001; Schieber, 2001). These cortical motor maps were discovered in seizure patients experiencing a spread of depolarization along M1 (Jackson, 1931; Foerster, 1936). The areas representing the fingers, hand and face are disproportionally large compared to

other areas likely due to the greater skill and finer precision required during everyday tasks (Foerster, 1931, 1936; Penfield & Rasmussen, 1950; Woolsey, 1958; Sanes & Schieber, 2001; Schieber, 2001). Further study on the somatotopy of M1 has revealed that it is not so strictly organized with clear borders, but that it is more of a mosaic representation with blurred borders between muscle loci. Generally, there is an agreement among those who study M1 that the lower limbs and proximal structures are represented more medially, and that representation of the upper-limbs are represented more laterally (Hluštík et al., 2001; Sanes & Schieber, 2001; Schieber, 2001; Plow et al., 2010). However, there is less agreement on the specific representation of the arm and hand areas (Schieber & Hibbard, 1993; Hluštík et al., 2001; Indovina & Sanes, 2001; Plow et al., 2010). In fact, stimulation of one area in M1 will often activate more than one muscle, but rather groups of muscles (Hluštík et al., 2001; Schieber, 2001), likely due to converging (Woolsey, 1958; Woolsey et al., 1979; Schieber & Hibbard, 1993; Schieber, 2001), diverging (Buys et al., 1986; Lemon et al., 1986) and overlapping cortical output to the periphery, as well as horizontal cortico-cortical interconnections to neighbouring somatotopic regions (Huntley & Jones, 1991). Other studies have confirmed that corticospinal projections often diverge to several motor units in order to activate multiple muscle groups (Buys et al., 1986; Lemon et al., 1986; Schieber, 2001). Also, not only are the borders blurred between the somatotpic organization of M1, but activation of one particular muscle can occur in vastly different areas of M1 (Woolsey, 1958; Woolsey et al., 1979; Schieber & Hibbard, 1993; Schieber, 2001); this is particularly true of the cortical representations of smaller body parts, such as the forearm and hand (Woolsey, 1958; Kwan et al., 1978; Woolsey et al., 1979; Schieber & Hibbard,

1993; Schieber, 2001; Plow et al., 2010). The redundancy of limb representation within M1 may be advantageous to the coordination of diverse muscle synergies for vastly different and complex tasks. Of all the motor-related areas (motor, premotor and somatosensory), M1 has the greatest amount and densest projections to the brainstem and spinal cord. M1 is also the area requiring the lowest stimulus intensity to generate muscle contraction of the opposite limb (Murray & Coulter, 1981; Dum & Strick, 2002).

Several studies have revealed that M1 is involved in more than simple motor execution, demonstrating parameters of planning, spatial target location, hand position and velocity, joint configuration and patterns of muscle activation, different coordinated activity based on the desired goal of a particular movement (Hammond, 1956; Scott, 2003, 2008) and reorganization due to learning or injury (Pascual-Leone et al., 1995; Nudo & Milliken, 1996; Nudo et al., 1996; Kleim et al., 1998; Kleim et al., 2004; Sanes & Donoghue, 2000). The conclusion from the vast number of studies indicates that M1 is involved in basic production of motor execution and control, but is also involved in higher level processing of many different movement parameters (Scott, 2003, 2008) as well as a candidate cortical locus for storing learned motor memories (Pascual-Leone et al., 1995; Nudo & Milliken, 1996; Nudo et al., 1996; Classen et al., 1998; Kleim et al., 1998; Kleim et al., 2004; Sanes & Donoghue, 2000; Tyc et al., 2005; Neva et al., 2012).

The premotor cortices (SMA, PMd and PMv)

The PM areas are located in the frontal lobe, located immediately anterior to M1 and classified as Brodmann area 6. The PM areas contribute to the preparation and production of movement through projections to M1 and direct contributions to the corticospinal tracts (Murray & Coulter, 1981; Canedo, 1997; Picard & Strick, 2001; Dum

& Strick, 2002). Direct stimulation of PM areas produces movement like that of M1, except it requires greater stimulus intensity. Also, the movements produced from stimulation to PM produce more complex, coordinated multiple jointed movements compared to the relatively simple movements generated from M1 stimulation (Weinrich & Wise, 1982). The PM areas have pyramidal neurons directly projecting to the spinal cord that are more sparse and smaller in size to M1 pyramidal neurons (Murray & Coulter, 1981; He et al., 1993; Canedo, 1997; Dum & Strick, 2002). Generally, most of the projections from all PM areas are to M1. Each PM area receives unique input from cortical and subcortical structures, such as parietal areas 5 and 7 and prefrontal area 46. The inputs from the parietal areas contribute to combining visual and somatosensory information to form a movement plan (Murray & Coulter, 1981; Picard & Strick, 2001; Dum & Strick, 2002; Rushworth et al., 2003; Vesia & Crawford, 2012). Input from prefrontal area 46 has strong connections with the ventral PM, and is thought to be involved with keeping visual information about objects in working memory. There is dense connectivity between all PM areas themselves in order to integrate information for movement planning. Subcortical structures like the basal ganglia, cerebellum and thalamus all project to the PM with areas projecting back to the subcortical areas and the spinal cord (Murray & Coulter, 1981; Weinrich & Wise, 1982; Picard & Strick, 2001; Dum & Strick, 2002).

Recent research is advancing the understanding of PM cortices and at present there are six functionally defined areas with sub-classifications in each. These areas, from medial to lateral are the cingulate cortices (dorsal, rostral and ventral), SMA, PMd and the ventral PM cortex (PMv). Some of these areas are recognized contributors to

bimanual movement control and certainly these areas have been shown to contribute to the preparation and execution of motor control (Schluter et al., 1998, 2001; Johansen-Berg et al., 2002; Swinnen, 2002; Smith & Staines, 2006, 2010, 2012). For the purposes of this work, the SMA, PMd and PMv will be focused upon due to their involvement in bimanual coordination and contribution to the visuomotor transformation within the tasks of the current thesis.

The most medial of the PM areas is the SMA and it can be anatomically divided into two separate areas: the pre-SMA, which lies just anterior to the SMA proper. In humans, the division between these two areas is the VCA line which is at the level of the anterior commissure. The pre-SMA is associated in structure and function to operate more like a prefrontal cortical area, which deals with cognitive and motivational inputs for motor control. The evidence for this is that the pre-SMA only has connections with the SMA proper and is highly interconnected with prefrontal cortices, whereas the SMA proper connects directly with M1 and has direct corticospinal projections (Murray & Coulter, 1981; Picard & Strick, 2001; Dum & Strick, 2002). There is evidence that the pre-SMA is involved with tasks that require skilled visuomotor movement and also with the early learning phases of sequential motor tasks (Shima et al., 1996; Jäncke et al., 2000; Picard & Strick, 2001). In these studies the SMA proper seemed to be more involved in the motor execution aspects of the task and particularly when the task was learned (Hikosaka et al., 1996*a*; *b*; Picard & Strick, 2001). The SMA proper is also activated during preparation of movements during skilled motor learning (Picard & Strick, 2001). Therefore, the pre-SMA could be a relevant area recruited for acquisition

of the visuomotor associations involved with the BMT task in the current work and the SMA may be more involved in the execution of the bimanual coordination.

The lateral PM cortices include the dorsal and ventral portions (PMd-PMv). PMd, located lateral to SMA, in monkeys can be functionally and structurally divided in to rostral (PMd_r) and caudal (PMd_c) portions that are similar to the pre-SMA to SMA distinctions. PMd_r has a lot of similarities to pre-SMA and the same goes for the PMd_c with the SMA proper (Geyer et al., 2000; Dum & Strick, 2002). In general, PMd_r has many connections with prefrontal areas and the reticular formation and very sparse direct corticospinal projections like PMd_c (Dum & Strick, 2002). In human imaging studies, PMd_r shows greater activation learning visuomotor associations and PMd_c is active during hand and arm movement (Boussaoud, 2001). Therefore, similarly to the distinction between pre-SMA and SMA, the relevancy to the current work is similar with the division between more caudal and rostral portions of PMd. It may be that PMd_r is involved in the learned visuomotor transformation required in the BMT whereas the more PMd_c may contribute to the execution of the task (Geyer et al., 2000; Boussaoud, 2001; Picard & Strick, 2001).

The second and most lateral PM area, the PMv, which lies just below the arcuate sulcus, can be divided into two slightly different areas lying anterior and posterior to one another (Matelli et al., 1985; Picard & Strick, 2001). The posterior portion of PMv has very dense connections with M1 and direct corticospinal projections, similarly to PMd proper (He et al., 1993; Dum & Strick, 2002). In monkeys, this portion of PMv has connections with the posterior parietal cortex and has led to the idea that this area has to do with transforming visual data about objects into information that can be used by the

limbs to make reaching movements (Rizzolatti et al., 1998). This particular area has not been clearly demonstrated in humans. In monkeys, the anterior portion seems to be largely involved in visual information processing and it contains what are called 'cannonical' and 'mirror' neurons. Cannonical neurons respond to visual information with three-dimensional objects. Mirror neurons are activated during action observation (Rizzolatti et al., 1998; Geyer et al., 2000; Picard & Strick, 2001). Analogous areas have not been observed in humans through imaging studies. The PMv could assist in visual transformation during the bimanual training task, but likely the PMd as a whole contributes more significantly. Therefore, this work focuses its efforts on PMd in order to probe the contributions of motor preparation to the BMT task and its effects to downstream M1.

Interaction of premotor and motor cortices

It has been shown previously that motor preparation during motor tasks (Kalaska & Crammond, 1995; Sheliga et al., 1995; Deubel & Schneider, 1996; Nobre et al., 2000; Corbetta & Shulman, 2002; Thoenissen et al., 2002; Smith & Staines, 2006, 2010, 2012; Neva et al., 2012) and skill training may increase cortical excitability and improve behavioural performance (Deiber et al., 1996; Staines et al., 2001; Jennings & van der Molen, 2005; Smith & Staines, 2006, 2010, 2012). On the other hand, cortical activation is slightly decreased and task performance is generally worse when there is not the opportunity to prepare for upcoming movements (Deiber et al., 1996; Smith & Staines, 2010). Reaction times (RT) decrease when participants have prior knowledge of stimulus information, such as, spatial location or object features. Indeed, covertly and overtly preparing movements to a target stimulus decreases RTs, and behavioural and

neuroimaging studies suggest that both types of preparation are not separate mechanisms, but they are coded by similar neural networks (Sheliga et al., 1995; Deubel & Schneider, 1996; Nobre et al., 2000; Corbetta & Shulman, 2002). The PM cortices have well known roles in selection of appropriate actions for movement execution (Kalaska & Crammond, 1995; Thoenissen et al., 2002). Some neuroimaging and TMS research suggests that PMd in the left hemisphere has a dominant role in action selection for motor execution. Specifically, PMd seems to be particularly involved in movement selection with learned visuomotor associations, and not as specifically with reaching or grasping like PMv (Geyer et al., 2000; Toni et al., 2001). Furthermore, lPMd activity increases with action selection of one or both upper-limbs (Schluter et al., 2001). Additionally, when the right PMd is disrupted with inhibitory TMS, action selection is hindered in the contralateral hand alone. Conversely, disruption of lPMd leads to a disruption in action selection in both upper-limbs (Schluter et al., 1998; Johansen-Berg et al., 2002). Similarly, rTMS to lPMd causes faster preparation of complex sequences performed with the right hand (Stinear et al., 2009). These studies suggests that both hemispheres of PM cortices clearly have a role in movement preparation and action selection of the upper limbs. Also, enhanced activation of the PM cortices leads to an increased excitability of the downstream M1, and improved behavioural performance. Critically, the lPMd has a particularly relevant role in the selection of movement and learning of visuomotor behavioural associations with both upper-limbs, which may play an important role in the current work.

1.3.2 *Bimanual movement*

The act of coordinated motor behaviour entails precise synchronization of multiple muscles around several joints. Movement coordination is certainly complex with a single upper-limb, and becomes much more complex when both upper-limbs move simultaneously in order to interact with the surrounding environment. In order to perform many common upper-limb movements, there is a need to overcome the tendency for the nervous system to couple movements in terms of timing and spatial displacement. Research on rhythmic and discrete bimanual coordination has demonstrated that homologous activation of the upper-limbs is the naturally preferred mode of movement, which can be referred to as in-phase movements. There are many movements that necessitate an active de-coupling the natural predisposition towards in-phase movements of the upper-limbs. Anti-phase movements, involving antagonist movements of the upper-limbs, requires much more complex coordination. Since bimanual movement requires complex coordination of the upper-limbs, there have been a number of theories to explain how the CNS might mediate its control. There are three main theories/models proposed to understand bimanual coordination: (1) generalized motor program (GMP) theory, (2) the intermanual crosstalk model, and (3) the dynamic systems model (Cardoso de Oliveira, 2002; Swinnen, 2002).

The generalized motor program (GMP) theory suggests that movements involving both limbs could be stored in particular brain areas as a common motor plan (Schmidt et al., 1979). This common motor plan would organize an entire movement goal for the limbs, i.e. the 'shape' or destination of movement. When a movement is executed, since the goals of the movement have been specified by the GMP, all that is required is the particular parameters to be specified like the force, timing and spatial displacement. The

advantage of the GMP is that it is more efficient, in that the execution of a movement is initiated from a singular source, allowing more resources to be allocated to specifying movement parameters to complete the desired movement. This movement theory applies to both unimanual and bimanual movements alike, thus a common motor plan could account for two-handed wrist movements as well, such as in-phase bimanual movements (Swinnen, 2002).

As opposed to the GMP, the intermanual crosstalk model proposes that there are separate motor programs for each limb (Marteniuk & MacKenzie, 1980). Mutual influence and sharing of information between these separate motor programs are suggested to occur at two main levels, the spinal and cortical levels. Spinal level communication is thought to occur primarily due to the ventral corticospinal tract (VCST) that remains ipsilateral to the cortical projections, which at the spinal level projects bilaterally. This would cause communication with proximal and axial muscles on both sides of the body. Also, since most of the information for proximal and distal limb muscles projects contralaterally, there is the notion that the uncrossed tracts influence the crossed tracts and would cause one arm to move similarly to the opposite arm. The spinal level crosstalk is suggested to be non-flexible. However, crosstalk at the cortical level is presumed to be more flexible and occurs by connections between PM and M1 in each hemisphere mediated by transcallosal connections. These connections suggest a tendency towards homologous movements, with the ability to overcome this tendency due to the flexibility of these higher level cortical interactions (Cardoso de Oliveira, 2002; Swinnen, 2002).

Lastly, the dynamic systems model may incorporate ideas from the GMP and crosstalk models, however, it differs in that it does not assume a hierarchical organization. Generally, it proposes that there are oscillation patterns from two opposing stable patterns of movement: in-phase and anti-phase movements. There is a tendency towards in-phase movements when certain parameters change, such as increased movement frequency. This model does not assume that any one locus will serve to specify an abstract motor plan, or rigid stereotyped behaviour but rather bimanual coordination results from a distributed network that is highly adaptable to particular situations (Easton, 1972; Turvey et al., 1986; Cardoso de Oliveira, 2002; Swinnen, 2002).

1.3.3 *Cortical and subcortical regions involved in bimanual movement*

There is certainly evidence for one common location being the locus for bimanual coordination, but there is also a wealth of evidence supporting a distributed functional network. There are particular cortical and subcortical areas that have been identified as being associated with bimanual coordination, such as the supplementary motor area (SMA), M1, cingulate cortex, and to a lesser extent PMd, posterior parietal cortex, the basal ganglia and cerebellum.

The SMA is certainly a prime candidate for a locus of bimanual coordination due to dense interhemispheric connections between SMAs in both hemispheres (Rouiller et al., 1994). There is greater activation of particular SMA neurons during bimanual movement compared to unimanual movement (Lang et al., 1990; Jancke et al., 2000). In addition, greater activity in SMA occurs when movements are anti-phase rather than in-phase (Goerres et al., 1998; Toyokura et al., 1999). The SMA may be a cortical area that contributes to the de-coupling of the natural tendency toward mirrored movements of the

upper-limbs. There could be bimanual movement specific neurons that carry a common signal that codes an overall goal of movement with two limbs. This is supported by evidence that certain neurons in the SMA are only active during bimanual movement and are silent during unimanual movement (Farrar & Zipser, 1999). Moreover, several clinical and lesion studies to the SMA have demonstrated that these patients have specific difficulties with bimanual coordination while unimanual coordination remains intact (Brinkman, 1984). Therefore, the activity observed within the SMA may garner support the idea of the GMP for bimanual coordination.

M1 is an obvious candidate for the locus of bimanual coordination. The traditional view of M1 is that it is concerned with processing and execution of movement parameters for the contralateral limb such as muscle force, joint torque, and movement direction (Evarts, 1973, 1979; Kalaska et al., 1983; Kakei et al., 1999) as well as for more abstract parameters such as motor imagery (Georgopoulos et al., 1989), serial sequencing (Carpenter et al., 1999) and stimulus-response associations (Zhang et al., 1997; Scott, 2003, 2008). Much of the current research is casting light on a different view of M1, one that does not emphasize a rigid contralateral limb control, but a shared network between both M1 representations, particularly of homologous muscle representations (Chen et al., 1997; Chen et al., 2003; Kanouchi et al., 1997; Kobayashi et al., 2004). There is substantial ipsilateral limb representation in M1 (Wassermann et al., 1994) and neurons in one hemisphere M1 are active during unimanual and bimanual movement (Donchin et al., 1998; Kermadi et al., 1998) specifically when examining fMRI, local field potentials and even single neuron data (Toyokura et al., 1999; Donchin et al., 2001). From these lines of research we can see that there is not only contralateral dominant control of the

upper-limbs, but in fact there is substantial overlap in M1 representation for both upper limbs. It could be that the two M1s act as one cohesive unit in order to plan and execute a common bimanual movement plan (Cardoso de Oliveira, 2002; Swinnen, 2002).

Along with cortical structures there are subcortical structures that may be involved in the coordination of bimanual movement. These subcortical structures are namely the basal ganglia and cerebellum. The basal ganglia have been implicated in bimanual control due to studies on patients with Parkinson's and Huntington's disease (Serrien et al., 2000; van den Berg et al., 2000; Byblow et al., 2002). These patients have been shown in several studies to have difficulty executing bimanual coordination tasks. Both of these diseases result from localized cell death in a particular area (substantia nigra and striatum, respectively), but these damaged local areas likely affect basal ganglia-thalamic-cortical loops. Specifically, the SMA has been implicated as a cortical region having prominent connections with the basal ganglia. Therefore, the deficits due to these disease states may arise from indirectly affecting the cortical areas that have reciprocal connections with the basal ganglia.

Other than the specific cortical and subcortical structures involved there are particular pathways integral to bimanual movement coordination. As mentioned earlier, there are lower level spinal interactions due to ipsilateral corticospinal tracts. In addition, higher level interactions due to dense reciprocal connections between homologous M1 representations via the corpus callosum are a likely contributor to bimanual coordination. Certainly, split brain patients (those who have had a callostomy to sever interhemispheric connections) display deficits in spatial and temporal bimanual coordination and learning associations between the two upper limbs (Eliassen et al., 1999; Franz et al., 2000). The

specific sub-areas of the corpus callosum are associated with particular aspects of temporal and spatial coupling of the limbs. For instance, the posterior third of the corpus callosum seems to be involved in spatial coupling based on patients with damage to that area (Eliassen et al., 1999). There is much anatomical and physiological data that suggest crosstalk between homologous M1 representations and descending corticospinal tracts likely contribute to the coordination of complex bimanual motor control (Cardoso de Oliveira, 2002; Swinnen, 2002).

1.3.4 *Neurophysiology of plasticity in motor adaptation*

Prior to the last century of advances in understanding the central nervous system, the mainstream belief in medicine and research was that generally, the anatomy of the brain would never change. "Plasticity" is a term widely used in modern neuroscience that generally describes the ability of the brain to physically change its functional and structural characteristics throughout the lifespan. Over the past decades, the plasticity of the nervous system has been demonstrated in humans (Jacobs & Donoghue, 1991; Pascual-Leone et al., 1995; Karni et al., 1995, 1998; Nudo & Milliken, 1996; Nudo et al., 1996; Borsook et al., 1998; Kleim et al., 1998; Kleim et al., 2004; Bütefisch et al., 2000; Muellbacher et al., 2001; Korman et al., 2003; Nudo, 2006; Butler & Wolf, 2007), monkeys (Nudo & Milliken, 1996; Nudo et al., 1996) and rodents (Donoghue & Sanes, 1988; Sanes et al., 1988; Rioult-Pedotti et al., 2000) due to brain injury (Brion et al., 1989; Nudo & Milliken, 1996; Netz et al., 1997; Rossini et al., 1998; Nelles et al., 1999; Johansson, 2000; Sanes & Donoghue, 2000; Nudo, 2006; Butler & Wolf, 2007), amputation (Cohen et al., 1991; Ramachandran, 1993; Karl et al., 2001), injury to the

peripheral nervous system (Donoghue & Sanes, 1988; Sanes et al., 1988) and also experience-dependent skilled motor movement (Jacobs & Donoghue, 1991; Pascual-Leone et al., 1995; Nudo et al., 1996; Karni et al., 1998; Kleim et al., 1998; Kleim et al., 2004; Bütefisch et al., 2000; Muellbacher et al., 2001; Ungerleider et al., 2002; Korman et al., 2003; Tyc et al., 2005; Fox & Wong, 2005; Nudo, 2006; Butler & Wolf, 2007). It is generally agreed that neuronal plasticity, whether that be in the form of increased electrical activity or reorganization of cortical representation, must last for a certain time beyond the intervention in order to be properly defined as "plasticity" (Jacobs & Donoghue, 1991; Classen et al., 1998; Sanes & Donoghue, 2000; Stefan et al., 2004; Huang et al., 2005; Ziemann et al., 2006). However, it is not entirely agreed upon exactly how long this effect should last beyond the particular intervention. Early research demonstrated plastic effects on the nervous system that persisted for minutes, whereas more recent research has shown through artificial stimulation protocols that plastic changes in cortical excitability last for an hour in humans (Huang et al., 2005) and up to 4 hours in rodents (Bliss & Lomo, 1973). Similarly, for motor learning to properly be observed in any biological system, the effects of the training intervention (i.e. behavioural performance improvement) should be observable for a certain amount of time after initial learning (Pascual-Leone et al., 1995; Shadmehr & Brashers-Krug, 1997; Caithness et al., 2004; Krakauer et al., 2005; Yamamoto et al., 2006; Butler & Wolf, 2007).

Motor learning and adaptation

The definition of motor learning is controversial, but it generally denotes either (i) *learning a novel motor skill* and/or (ii) *adaption of a previously learned motor skill or association* to new and challenging environments (motor adaptation). *Learning a novel*

motor skill often involves the acquisition of new spatial and temporal muscle activation patterns to complete the task. This would be the case when an individual learns to play piano, where the timing and spatial location of the hands and digits must be coordinated in particular sequences to perform a musical piece. *Motor adaptation* can be functionally divided in two ways: (i) sensory-motor adaptation and (ii) conditional sensory-motor associations. Sensory-motor adaptation occurs when an accomplished pianist must learn a new musical score, where the individual would alter precise timing and spatial locations of hands and digits to move in proper sequence with the new piece. Conditional sensory-motor associations are similar, but involve more arbitrary visual cues associating with certain movement response, such as a red traffic light indicating to the driver to depress the brake pedal. Whether the motor task involves learning a brand new skill or adapting already learned skills and associations to novel environments, there is generally a progression of motor adjustments so the motor task is learned and performed with relative ease, and both scenarios involve similar cortical adaptations demonstrated in many studies. Following a certain amount of exposure, practice and repetition with the adapted motor task, it can be recalled and executed for long periods of time, and these behavioural adaptations may be associated with changes in cortical excitability (Friston et al., 1992; Grafton et al., 1992; Karni & Sagi, 1993; Jenkins et al., 1994; Kawashima et al., 1994; Pascual-Leone et al., 1995; Karni et al., 1995; Doyon et al., 1996; Nudo et al., 1996; Hikosaka et al., 1996*a*; Shadmehr & Brashers-Krug, 1997; Kleim et al., 2004; Sakai et al., 1998; Kleim et al., 1998; Sanes & Donoghue, 2000; Ungerleider et al., 2002; Korman et al., 2003; Caithness et al., 2004; Krakauer et al., 2005; Luft & Buitrago, 2005; Seitz & Roland, 2006; Yamamoto et al., 2006). Strictly speaking, since the current behavioural

tasks require short-term visuomotor movement training and motor performance adjustments, the research in this thesis will not refer to motor learning specifically. Therefore, the work in this thesis primarily involves and refers to modest changes in motor adaptation and performance.

Motor adaptation and cortical plasticity

The general progression of adapting a new motor skill tends to begin with pronounced initial errors in the behavioural task, followed by large improvements in performance over successive practice. This initial phase in motor adaptation is thought to be the 'fast' process of performance improvement which depends on a larger subset of cortical resources in order to rapidly improve performance in a trial-by-trial fashion (Karni & Sagi, 1993; Karni et al., 1998; Korman et al., 2003). More cortical and subcortical loci are involved in this initial adaptation process, such as prefrontal areas, PM areas, M1 as well as the cerebellum and basal ganglia (Friston et al., 1992; Grafton et al., 1992; Karni & Sagi, 1993; Jenkins et al., 1994; Kawashima et al., 1994; Karni et al., 1995, 1998; Doyon et al., 1996; Nudo & Milliken, 1996; Nudo et al., 1996; Hikosaka et al., 1996*a*; Kleim et al., 2004; Sakai et al., 1998; Kleim et al., 1998; Sanes & Donoghue, 2000; Ungerleider et al., 2002; Luft & Buitrago, 2005; Seitz & Roland, 2006). Once motor adaptation has progressed to the 'slow' phase, there is greater accuracy and fewer gains in performance (i.e. asymptomatic performance). In this 'slow' phase of motor adaptation, where the motor task is becoming more consolidated, cortical activity seems to shift to a more local subset of the aforementioned cortical areas, such as the PM and M1 cortices (Friston et al., 1992; Grafton et al., 1992; Karni & Sagi, 1993; Jenkins et al., 1994; Doyon et al., 1996; Hikosaka et al., 1996*a*; Karni et al., 1998; Sakai et al., 1998;

Sanes & Donoghue, 2000; Ungerleider et al., 2002; Kleim et al., 2004; Luft & Buitrago, 2005). The M1 cortices are certainly involved throughout all stages of motor adaptation; however, it seems that M1 is particularly involved in the longer term storage and consolidation of motor memories (Karni & Sagi, 1993; Jenkins et al., 1994; Hikosaka et al., 1996*a*; Karni et al., 1998; Sakai et al., 1998; Sanes & Donoghue, 2000; Ungerleider et al., 2002; Kleim et al., 2004; Luft & Buitrago, 2005). This has been demonstrated with several studies displaying greater activation in M1 when humans perform a practiced motor skill as compared to adaptation of a novel skill (Karni & Sagi, 1993; Jenkins et al., 1994; Karni et al., 1998), and also by an expanded M1 representation due to continued practice of a motor skill (Pascual-Leone et al., 1995; Nudo et al., 1996; Sanes & Donoghue, 2000; Ungerleider et al., 2002; Kleim et al., 2004).

Motor adaptation, neuronal and cellular mechanisms of plasticity

On a much smaller scale, there are several neurophysiological mechanisms underlying the progression in motor adaptation. A widely accepted cellular mechanism for the formation of motor memories is long-term potentiation (LTP) and the counterpart long-term depression (LTD) (Bliss & Lomo, 1973; Baranyi & Feher, 1978; Baranyi et al., 1991; Aroniadou & Keller, 1995; Hess & Donoghue, 1996; Martin et al., 2000; Rioult-Pedotti et al., 2000; Hess, 2004). These are thought to be due to changes in synaptic efficacy in the communication between the pre- and post-synaptic membranes of neurons. Induction of LTP results in strengthening connections between neurons whereas LTD results in decreasing the strength of synaptic connections (Sanes & Donoghue, 2000). LTP leads to a lasting increased response in magnitude of excitatory post-synaptic potentials (EPSPs) in the post-synaptic membrane, due to simultaneous firing of pre- and

post-synaptic neurons. Several studies have demonstrated this phenomenon in areas like the entorhinal cortex, hippocampus and M1 due to activity-dependent synaptic plasticity and cortical stimulation within animal models (Bliss & Lomo, 1973; Baranyi & Feher, 1978; Aroniadou & Keller, 1995; Andersen et al., 1996; Hess & Donoghue, 1996; Martin et al., 2000; Rioult-Pedotti et al., 2000; Hess, 2004). LTD, which decreases EPSPs lasting from hours to days (Moser et al., 1993; Martin et al., 2000; Rioult-Pedotti et al., 2000), is also thought to be a mechanism in the formation of motor memories. Generally, artificially inducing LTP requires high-frequency intracortical stimulation whereas inducing LTD requires low-frequency stimulation (Martin et al., 2000; Sanes & Donoghue, 2000). A model of a molecular basis for the initiation of LTP involves the increased concentration of calcium (Ca^{2+}) in the postsynaptic membrane. An increase in depolarization of the post-synaptic membrane is brought on by the opening of existing N-methyl-D-aspartic (NMDA) and non-NMDA channels. NMDA and non-NMDA are fast-acting ionotropic channels existing on the postsynaptic membranes and are receptive to glutamate, a neurotransmitter associated with excitatory responses (EPSPs). The opening of these postsynaptic channels results in the increased postsynaptic Ca^{2+} concentration, which is thought to send a retrograde messenger to the presynaptic dendritic spine. This leads to an increase in the production and release of neurotransmitters from the presynaptic dendritic spine, ultimately resulting in an increased depolarization response in the postsynaptic membrane (Gustafsson & Wigström, 1988; Kandel et al., 2012).

Motor adaptation and plasticity of intracortical circuitry

Another mechanism that could account for rapid plasticity in the brain due to motor adaptation is the unmasking of latent or existing connections in the sensorimotor

cortex. This type of adaptive neural mechanism has been shown in the somatosensory cortex due to amputation of a limb or digit (Cohen et al., 1991; Ramachandran, 1993; Karl et al., 2001) or peripheral nerve injury (Donoghue & Sanes, 1988; Sanes et al., 1988). Shortly after the digit was removed, the cortical area formally occupied by the amputated digit was now responding to sensory information from the surface area of the neighbouring digit (Cohen et al., 1991; Ramachandran, 1993; Karl et al., 2001). It is thought that these pre-existing connections are within the 2nd and 3rd layers of the cortex and run horizontally to connect neighbouring areas together for things like muscle synergy (Cohen et al., 1991; Jacobs & Donoghue, 1991; Ramachandran, 1993; Karl et al., 2001). Also, these pre-existing connections could be another means of communicating between adjacent regions of cortex after injury. It is suggested that the mechanism for strengthening pre-existing horizontal connections could be a release of the chief inhibitory receptor in the nervous system: gamma-aminobutyric acid-A (GABA-A). The release of inhibitory GABA-A receptor activity, in turn, induces excitatory horizontal signals between neighbouring regions of cortex, possibly leading to greater excitability and larger motor output maps (Jacobs & Donoghue, 1991; Hess & Donoghue, 1994). The involvement of horizontal cortical connections, both in terms of activating latent or silent pre-existing connections, is certainly a possible way that the cortex could rapidly reorganize itself in the short-term phases of motor adaptation (Jacobs & Donoghue, 1991; Hess & Donoghue, 1994; Isaac et al., 1995; Liao et al., 1995, 1999; Nudo et al., 1996; Gomperts et al., 1998; Kleim et al., 1998; Nusser et al., 1998; Petralia et al., 1999; Rioult-Pedotti et al., 2000). Silent synapses are neurons that do not have the α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA)-mediated glutamate receptors.

Therefore, presynaptic neurotransmitter release would have no effect on postsynaptic action potential (Isaac et al., 1995; Liao et al., 1995; Atwood & Wojtowicz, 1999). AMPA are non-NMDA ionotropic receptors that are responsible for fast transmission of excitatory signals which are receptive to the neurotransmitter glutamate and an analogue of glutamate 'AMPA', and are the most common receptors in the CNS. Activation, or 'awakening', of these existing but silent synapses occur due to the appearance of AMPA receptors within synaptic connections, which would then increase the rate and amount of signal transmission across neurons (Gomperts et al., 1998; Nusser et al., 1998; Atwood & Wojtowicz, 1999; Liao et al., 1999; Petralia et al., 1999). Latent horizontal connections may be active in the sense that they have these AMPA receptors, but may not participate in intracortical communication with adjacent areas until injury or activity-dependent changes occur to the nervous system (Jacobs & Donoghue, 1991; Hess & Donoghue, 1994, 1996; Huntley, 1997).

Additionally, it is commonly thought that increases in local cortical activity from motor adaptation could be due to the remodelling and formation of new synapses (synaptogenesis). Synaptogenesis has been demonstrated in several areas of the cortex due to motor adaptation, such as M1, somatosensory, visual areas and the cerebellum (Black et al., 1990; Kleim et al., 1998; Kleim et al., 2004; Butler & Wolf, 2007). Specifically, it is thought that synaptogenesis occurs in the M1 due to longer term motor adaptation with repeated exposure to specific muscle activation patterns (Kleim et al., 1998; Kleim et al., 2004).

Motor adaptation and M1 cortical reorganization

All of the mechanisms mentioned above at the molecular and cellular level are thought to contribute to a reorganization of the cortical territory occupied by the particular limbs involved in the skilled motor adaptation tasks. Several studies have shown that motor adaptation leads to modulation of the motor map which represents the specific muscles trained in the task in human (Pascual-Leone, Valls-Sole, et al., 1994; Pascual-Leone et al., 1995; Classen et al., 1998; Pearce et al., 2000; Muellbacher et al., 2001; Luft & Buitrago, 2005; Tyc et al., 2005; Butler & Wolf, 2007; Neva et al., 2012), monkey (Nudo et al., 1996; Plautz et al., 2000) and rodent (Kleim et al., 1998; Kleim et al., 2004) motor cortex. Reorganization of M1 territory has usually been demonstrated in the long-term aspects of motor skill consolidation due to repeated practice (Wilson et al., 1993; Pascual-Leone, Valls-Sole, et al., 1994; Pascual-Leone et al., 1995; Thickbroom et al., 1998; Kleim et al., 2004; Tyc et al., 2005), but some studies have shown this cortical reorganization during short term training as well (Pascual-Leone et al., 1995; Neva et al., 2012).

One such study demonstrated that skilled reaching caused an increase in the representation of distal muscles used in the task at the expense of more proximal muscles, with no overall increase in motor map size (Nudo et al., 1996). This demonstrates that skilled motor movement leads to an expansion of the particular trained muscles and this encroaches on the neighbouring untrained muscles. This also indicates that there is a finite area that M1 can expand. This is likely due to horizontal connections in layers 2, 3 and 4 of the cortex that remain unused or silent before either brain trauma or the onset of skilled movement training. Another study showed that reaching for food pellets which required skilled motor control lead to an areal increase of cortical representation whereas

retrieval of the food pellets with simple motor control demands (unskilled) did not cause this expansion in rat M1 (Kleim et al., 1998). Repetitive use or movement of the limbs in monkey in a similar task to retrieve food pellets showed no such increase or modulation in the M1 map, and it was concluded that no skill was adapted during this more simple movement task. This lends more evidence to suggest that skilled movement is required to produce such plastic changes in the cortex, such as M1 (Plautz et al., 2000). Another similar study showed that M1 map reorganization only took place due to long-term skilled motor training (Kleim et al., 2004). In this study the researchers suggest that initial stages of motor adaptation, where the rats improved performance at the task significantly from day 3 to 7 of training, is not the time period where expansion in M1 maps are observed. Increases in spatial representation of distal muscles involved were only seen after 10 days of training, where the motor task has been sufficiently practiced. The authors concede that changes in M1 excitability occur during early adaptation stages, and it is even possible that small changes in representational M1 maps may occur early as well. Pascual-Leone and colleagues (1993) used TMS to map the first dorsal interosseous (FDI) and adductor digiti minimi (ADM) muscles in both hands in proficient Braille readers and controls. The reading hand of the Braille readers FDI representation was significantly larger than the non-reading hand and both hands of the control group (Pascual-Leone et al., 1993). Conversely, studies have shown M1 map output can decrease in size quite rapidly when practice of a known skill ceases for a time (Pascual-Leone et al., 1993). Braille readers who followed a 6 hours daily training regime demonstrated enlarged FDI representation in M1. However, when these Braille readers spent 2 days without the usual practice regime a significant decrease in FDI

representation was observed. This occurs not only when a skill is not practiced for a period of time, but also when a limb is immobilized (Liepert et al., 1995). M1 output maps were acquired from the tibialis anterior (TA) of the immobilized and non-immobilized ankle. It was found that the immobilized TA representation was much smaller than the non-immobilized TA, and the amount of time the ankle was immobilized correlated to the decrease in map size. Interestingly, this affect could be reversed rapidly due to voluntary muscle contraction. Another study using TMS to measure the extents of the cortical representation of the wrist muscles before and after human participants learned a skilled finger tapping sequence on a piano, demonstrated that M1 maps can expand in size after a short-term session of training. This study showed that after 2 hours of training M1 representation was enhanced (Pascual-Leone et al., 1995). Interestingly, with additional training of 2 hour sessions over multiple days (total of 5) the cortical representation continued to expand.

Adaptation of fine motor skills as well as gross motor skills leads to reorganization of M1. This study used TMS to investigate the representation of corticomotor projection of the hand muscles in elite racquet players. Therefore, this was an investigation into the long-term modulations of M1 representation through continued high level skilled gross motor movement. The elite racquet players demonstrated higher MEP amplitude, and a shift in cortical representation compared to social racquet players. The interpretation of these results was that these modulations in cortical representation are associated with the initial adaptation and long-term retention of the motor skill (Pearce et al., 2000; Kleim et al., 2004; Tyc et al., 2005). Another similar study investigated the wrist and shoulder representation of elite volleyball players versus

runners (control group). The results revealed that the medial deltoid and ECR representations were larger in space and of greater MEP amplitude compared to runners. In addition, the comparison of dominant and non-dominant hemisphere within both groups showed that the elite volleyball players had larger map areas in the dominant compared to non-dominant hemispheres, whereas no such difference was observed in the runners. This further demonstrates that skilled motor training leads to reorganization of M1 and does not occur with mere aerobic exercise (Tyc et al., 2005). Other TMS studies have demonstrated a shift in the pattern of representation of particular trained muscles, due to short-term movement training (Classen et al., 1998). In this study, TMS was used to elicit right thumb flexion movements before and after 30 minutes of training thumb extension movements. After training, the same TMS pulses that elicited thumb flexion now produced thumb extension. This study showed that movement parameters that are likely coded in M1, such as movement direction and force, can be modulated due to short-term training (Classen et al., 1998). The current research clearly indicates that M1 reorganizes itself due to short-term skilled movement training, and this will not occur with simple repetitive movement, aerobic exercise or strength training (Pascual-Leone et al., 1995; Nudo et al., 1996; Kleim et al., 1998; Kleim et al., 2004; Pearce et al., 2000; Plautz et al., 2000; Tyc et al., 2005). Several of the above mentioned studies utilized a modern technique which can not only probe corticospinal and intra-cortical activity, but also transiently modulate this activity in a focal area, namely: TMS.

1.3.5 *Transcranial magnetic stimulation (TMS)*

Modern day TMS was introduced by Baker and colleagues (1985) as a way to map the cortical muscle representation in healthy and patient populations. TMS is a safe, painless and non-invasive way to probe the underlying activity within the cortex and even alter activity in a focal area transiently. TMS operates on the bases of electromagnetic induction wherein electric current is converted to magnetic fields (Rothwell, 1997; Terao & Ugawa, 2002). Specifically, electricity flows through the TMS figure of eight coil (or a single circular coil (round coil)) and this creates a focal transient magnetic field at the intersection of the two round components, which travels perpendicular to the induced electric current and then traverses the skull and generates an electric current within the underlying brain tissue in the opposite direction to the electrical flow within the coil. When the coil is held over M1, a single-pulse of TMS is thought to stimulate the descending corticospinal tracts indirectly, via interneurons likely in layers 2-4 of the cortex (Walsh & Cowey, 1999; Hallett, 2000; Butler & Wolf, 2007). This type of activity generated by a TMS pulse is said to produce an "indirect" wave (I-wave) in the horizontal interneurons that eventually reaches the descending pyramidal neurons in layer 5 (Di Lazzaro et al., 1998). Once these corticospinal neurons are activated, the descending volleys of electrical activity eventually reach the target muscles and produce a contraction. This induced activity that results in a motor evoked potential (MEP) can be recorded from surface electrodes overtop the target muscles (Rothwell, 1997; Walsh & Cowey, 1999; Hallett, 2000; Kammer et al., 2001; Butler & Wolf, 2007).

Single, paired-pulse and repetitive TMS

There are several different types of TMS that are employed to measure or alter the excitability of focal cortical areas in the brain. Single-pulse TMS is easy to administer

and is the most common form of TMS used. This type of TMS does not alter cortical activity, but is primarily used to generate corticospinal output that can be measured in the peripheral musculature through surface or indwelling electrodes. Although single-pulse TMS is primarily used over M1 to generate MEPs, it has been shown to elicit phosphenes when applied over the occipital cortex (Amassian et al., 1989). Since cortical excitability is variable among individuals, the TMS intensity used in experiments are most commonly adjusted to the excitability of the individual. M1 excitability is used to determine this since it is the only reliable and measurable physiological output from the cortex in the form of MEPs (Kammer et al., 2001). In order to adjust M1 excitability to each individual, the resting motor threshold (RMT) or active motor threshold (AMT) must be determined for each individual. The RMT is determined by finding the lowest stimulus intensity output required to elicit 5 out of 10 MEPs greater or equal to a peak-to-peak amplitude of 50 μV. The AMT is determined to be the lowest stimulus intensity that would elicit 5 out of 10 MEPs greater or equal to a peak-to-peak amplitude of 200 μV while maintaining a light contraction of the target muscle of approximately 10-20% of maximum voluntary contraction (MVC). Usually, a certain suprathreshold TMS intensity is used to evoke MEPs in the range of 100-150% of RMT (Rossini et al., 1994). Similarly, single-pulse TMS may probe long-latency inhibitory intracortical circuitry the cortical silent period (CSP). The CSP is evoked when participants hold a light voluntary contraction during the application of a single-pulse of TMS over the contralateral target muscle representation in M1, which produces a temporary suppression of EMG activity (Cantello et al., 1992; Kimiskidis et al., 2005). The CSP evoked in the upper-limb muscles results largely from cortical inhibitory mechanisms, although spinal mechanisms

are likely involved in the early portion (Fuhr et al., 1991; Inghilleri et al., 1993; Roick et al., 1993; Uncini et al., 1993; Chen et al., 1999). Assuming that spinal excitability remains the same, prolonged CSP indicates greater cortical inhibition and shorter CSP indicates less inhibition (Chen et al., 2008).

Another common type of TMS is called paired-pulse which can either excite or inhibit cortical motor output. The purpose of paired-pulse TMS is to understand the intra- and intercortical connections between two different hemispheres or the intracortical interactions within a local cortical area (Kujirai et al., 1993; Ziemann et al., 1996; Di Lazzaro et al., 1998; Di Lazzaro et al., 1999; Chen, 2004; Ni et al., 2011). Paired-pulse TMS is accomplished when a subthreshold conditioning stimulus (CS) is delivered before a suprathreshold test stimulus (TS), which would normally elicit an MEP. Depending on the interstimulus interval (ISI) between CS and TS, the resulting MEP will either be suppressed or enhanced. Suppressed MEPs occur with ISIs from 2-5 ms and enhanced MEPs result from ISIs of 6-11 ms. The former is referred to as short-intracortical inhibition (SICI) and the latter intracortical facilitation (ICF). Typically, the stimulus intensity of the CS is 60-90% of RMT and the TS is ~120 % of RMT. For SICI, it is thought that the CS, which is at a high enough intensity to evoke cortical activity, but not enough to produce corticopsinal output, primarily activates GABAergic interneurons that inhibit the successive TS that would, by itself, produce a greater MEP amplitude. Therefore, the resultant MEP amplitudes for SICI are smaller in comparison to single-pulse MEPs. With ICF, the resultant MEP amplitude is facilitated compared to a single-pulse. ICF is thought to involve interactions with glutamate receptors (Ziemann et al., 1998; Schwenkreis et al., 1999). It is suggested that subcortical or spinal interactions

may be involved as well (Di Lazzaro et al., 2006). Another type of paired-pulse TMS is called long-interval intracortical inhibition (LICI), where the ISIs between the suprathreshold CS and TS from 50-200 ms produce inhibition of corticospinal output (Wassermann et al., 1996; Nakamura et al., 1997; Chen et al., 1999, 2008). It is thought that LICI is mediated by GABA-B receptors (Roick et al., 1993; Werhahn et al., 1999; McDonnell et al., 2006), which are a metabotropic type of inhibitory receptor that has to do with long-latency synaptic modulation (Kandel et al., 2012).

Using the same general approach as above, dual-site paired-pulse TMS probes the intrahemispheric and interhemispheric connections between two cortical regions. Generally, this type of paired-pulse TMS entails a CS over one cortical area and a TS over M1, in order to observe the interactions between other cortical regions and M1 (Civardi et al., 2001; Koch et al., 2007; Bäumer et al., 2009). Several studies have investigated connections between PM (Civardi et al., 2001; Mochizuki et al., 2004; Koch et al., 2007; Bäumer et al., 2009; Groppa et al., 2012), sensory (Ziluk et al., 2010), parietal (Koch et al., 2007; Koch & Rothwell, 2009; Karabanov et al., 2012), frontal areas (Civardi et al., 2001) and M1, and also the interaction between M1 bilaterally (Ferbert et al., 1992; Netz et al., 1995; Stinear & Byblow, 2002; Bäumer et al., 2007; Nelson et al., 2009; Perez & Cohen, 2009; Ni et al., 2011; Sattler et al., 2012). Depending on the physical cortical distance between the CS and the TS (intra- or interhemispheric), the ISIs would be modified to investigate these intrinsic cortical connections and whether these are inhibitory or excitatory interactions (Civardi et al., 2001; Baumer et al., 2006; Koch et al., 2007; Nelson et al., 2009). For example, Civardi et al. (2001) showed that delivering a low intensity CS (~90% AMT) over PMd suppressed the MEP amplitude from a TS to

ipsilateral M1 at an ISI of 6 ms. However, increasing the CS intensity (110-120% AMT) led to a facilitation of M1 MEPs. Mochizuki et al (2004) showed that a CS over PMd (80% AMT) led to facilitation in MEPs from contralateral M1 when the ISI was 8 ms. Also, it has been shown that MEPs are suppressed when a CS over one M1 representation of a particular muscle is applied before a TS over the opposite M1 representation, at ISIs between 6-50 ms (Ferbert et al., 1992; Kujirai et al., 1993; Gerloff et al., 1998; Chen et al., 2003; Chen, 2004; Nelson et al., 2009). These results suggest that interhemispheric inhibition (IHI) dominates the interaction between homologous muscle representations across M1 hemispheres (Nelson et al., 2009). These paired-pulse TMS measures are certainly useful ways to measure intra- and interhemispheric cortical excitability due to training or rTMS protocols.

The third common type of TMS is rTMS, which is used to either enhance or suppress local cortical activity for a period of time beyond the application of the stimulation (Pascual-Leone et al., 1994; Chen et al., 1997; Walsh & Cowey, 1999; Hallett, 2000; Butler & Wolf, 2007). Corticospinal activity is suppressed after a session of >1 Hz suprathreshold rTMS over M1 with relatively little effect on intracortical inhibition (Chen et al., 1997; Fitzgerald et al., 2006; Heide et al., 2006). M1 corticospinal activity is generally enhanced when rTMS is applied at < 5 Hz with a reduction in intracortical inhibition (Pascual-Leone et al., 1994; Ziemann, 2004; Fitzgerald et al., 2006; Heide et al., 2006). Whether the effect of rTMS enhances or suppresses cortical excitability depends on the frequency, intensity and duration of the stimulation (Modugno et al., 2001; Ziemann, 2004; Houdayer & Degardin, 2008). Low frequency rTMS (> 1 Hz) applied at an intensity of 115% of RMT over M1 for ~15-30

min leads to a reduction in MEPs for ~15 min (Chen et al., 1997; Gilio et al., 2003). High frequency rTMS (< 5 Hz) applied to M1 at an intensity of 90% of RMT for 4 min leads to an increase in M1 excitability (Maeda et al., 2000). Also, rTMS applied to remote cortical locations, such as the opposite M1 and PMd, can influence cortical excitability between areas (e.g. PMd to M1) as well as the excitability of intracortical circuits within M1 (Wassermann et al., 1998; Chouinard et al., 2003; Rizzo et al., 2004; Suppa et al., 2008). These studies suggest that rTMS can modulate M1 activity in terms of its corticospinal output and intracortical networks by applying it directly to M1 and to other cortical loci as well. Although rTMS is used in many studies investigating its modulatory effects on cortical excitability, studies have indicated potential issues with its application in human participants (Huang et al., 2005).

Some of these issues with rTMS include the fact that the effects are minimal and are variable between individuals (Maeda et al., 2000), behaviour is scarcely effected in terms of simple motor parameters like speed or strength of movements (Muellbacher et al., 2000), stimulation to different cortical areas only produce modest changes in cognitive function (Evers et al., 2001) and results in neurological and psychological disorders have been difficult to interpret (Martin et al., 2002; Hausmann et al., 2004). In addition, there is a safety concern when applying the amount of stimulation to humans in rTMS studies which has limited the frequency of stimulation to be relatively low (usually > 10 Hz) for a short period of time (Wassermann et al., 1998; Hallett, 2000). Finally, rTMS is likely activating more than one focal neural system that may interact with each other, which makes it difficult to interpret the effects (Huang et al., 2005). For all of these reasons, a different type of repetitive stimulation with higher frequencies and

shorter duration used in animal models has been applied to rTMS in humans (Hess et al., 1996; Vickery et al., 1997; Huang et al., 2005).

This type of rTMS, which is becoming more commonly used in human neuroscience research, is theta burst stimulation (TBS). The benefit of this type of stimulation is that it can produce similar effects of traditional rTMS within a shorter time, but with potentially more reliable and focal after effects on the cortex (Huang et al., 2005). This type of stimulation utilizes high frequency bursts of 3 stimuli at 50 Hz in patterns separated by a time of 200 ms (5 Hz, hence 'theta burst'), for a total of 600 TMS pulses. A recent study demonstrated that different patterns of TBS produces unique plastic changes in M1 excitability (Huang et al., 2005). Continuous TBS (cTBS) suppresses M1 cortical excitability for up to 60 min post stimulation. Implied in the name, 'continuous' TBS applies the stimulation in the theta burst pattern constantly for the 600 pulses which lasts for 40 seconds. On the other hand, intermittent TBS (iTBS) employs the burst of 3 stimuli at 50 Hz for 2 seconds, followed by a pause of 8 seconds with no stimulation. This pattern is repeated until 600 pulses are completed after a total time of 190 seconds. iTBS applied to M1 leads to an increase in M1 excitability for approximately 60 min post stimulation. Not only is corticospinal activity either suppressed or enhanced due to TBS (cTBS or iTBS, respectively), but the intracortical excitability within M1 is modulated as well. Specifically, following cTBS to M1, SICI and ICF is suppressed for up to 20 min beyond the stimulation. Conversely, following iTBS to M1, SICI is enhanced and ICF is suppressed for a short time. This demonstrates that TBS applied to M1 modulates the output as well as the intracortical connectivity within M1 for a significant time beyond stimulation. However, recent work has revealed

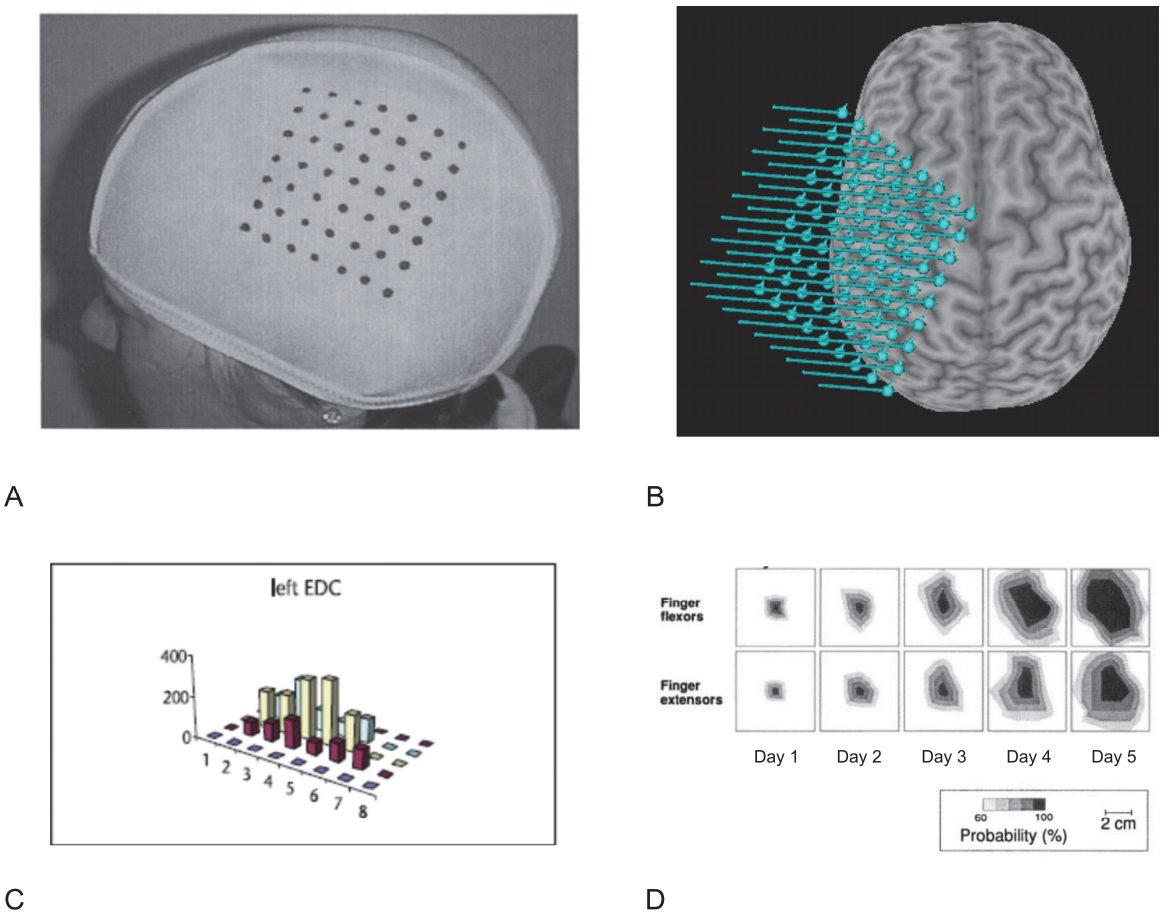
that the effects of TBS show substaintial variability across participants, which likely depends upon which interneuron populations are activated by the TMS pulse (Hamada et al., 2013).

Regardless, TBS has also been shown to modulate M1 excitability by applying stimulation to remote cortical loci, such as the PM cortex (Huang et al., 2009; Ortu et al., 2009; Stinear et al., 2009). These studies found that cTBS applied to PMd suppressed MEPs from the ipsilateral M1 to a greater extent than when applied to M1 itself. However, cTBS to PMd had no effect on local M1 SICI and ICF. Also, cTBS and iTBS applied over area 5 in the parietal cortex modulates M1 excitability, causing an increase in MEPs from M1 bilaterally for up to an hour, with no effect on SICI and ICF within M1 (Premji et al., 2011). iTBS caused a delayed increase in MEPs in contralateral M1 for up to an hour, with no effect on SICI or ICF (Premji et al., 2011). TBS has also been shown to modulate the excitability from M1 in one hemisphere to the other (Suppa et al., 2008; Meehan et al., 2011) and contralateral PMd (Stefan et al., 2008). Specifically, Suppa and colleagues (2008) found that cTBS to M1 caused an increase in MEPs and reduced SICI in the non-stimulated opposite M1, and iTBS caused a decrease in MEPs and increased SICI in the opposite M1. Stefan and colleagues (2008) found that cTBS to right PMd caused no change in contralateral M1 MEP amplitude. TBS has been used to effect motor behaviour and skilled movement in stoke patients when applied to contralesional M1 and S1 (Meehan et al., 2011) and in healthy individuals applied to ispilateral PMd (Mochizuki et al., 2005; Ortu et al., 2009; Stinear et al., 2009) and contralateral PMd (Stefan et al., 2008). Meehan and colleagues found that cTBS applied to contralesional M1 and S1 causes increases in movement speed and better motor control in terms of peak

velocity and acceleration of movement in the affected limb. Ortu and colleagues (2009) found that cTBS applied to M1 led to changes in motor preparation when making self-paced voluntary movements. Mochizuki and colleagues (2005) demonstrated delays in a cued reaction time task with both limbs due to TBS to PMd, with no detriment to accuracy. Interestingly, Stinear and colleagues (2009) found that iTBS to left PMd led to an increase in the preparation speed of complex sequence learning with no changes in MEP excitability in M1 bilaterally. However, cTBS to left PMd caused no change in performance of the sequence task, with an increased left M1 and decreased right M1 excitability. TBS has since been used in other focal regions of the cortex, such as the dorsal lateral prefrontal cortex (DLPFC), which modulated cortical activity during attention related tasks (Bolton & Staines, 2011). Specifically, this study found that the ability to ignore distractor stimuli not relevant to the particular task was impaired as well as the tactile event-related potentials (ERPs) after cTBS to the right DLPFC. Clearly, TBS is a useful method to transiently modulate cortical excitability in several nodes directly and remotely, which can also affect sensorimotor behaviour and motor adaptation.

The work in this thesis will primarily involve the use of single-pulse TMS in order to probe the excitability of M1 in terms of quantifying the distribution and amplitudes of MEP in the target muscles (Figure 1). This technique of mapping the M1 representation of particular muscles has been used since TMS was introduced to understand normal and pathological cortex (Levy et al., 1991; Wassermann et al., 1992; Wilson et al., 1993; Mortifee et al., 1994; Amassian et al., 1995; Thickbroom et al., 1998, 1999*a*; *b*, 2005; Pearce et al., 2000; Thielscher & Kammer, 2002; Uy et al., 2002).

Quantifying M1 maps in individuals over multiple time points has been shown to be very accurate and reproducible (Levy et al., 1991; Mortifee et al., 1994; Uy et al., 2002). Usually, it is more difficult to map proximal muscles (Levy et al., 1991) compared to distal muscles (Wassermann et al., 1992) due to a higher stimulation intensity required to produce MEPs. Mapping M1 has also been used before and after skilled motor training (Pascual-Leone et al., 1994; Pascual-Leone et al., 1995; Pearce et al., 2000; Tyc et al., 2005; Butler & Wolf, 2007; Neva et al., 2012). In addition to the intervention of BMT, this thesis will utilize TBS to a select portion of motor preparatory and execution cortical nodes to modulate the contributions of these nodes to the dominant M1 representation. In the final study of the thesis, paired-pulse TMS will be used to investigate the relationship and interconnections between the motor preparatory and execution areas due to short-term BMT and TBS.



Pascual-Leone et al. 1995; Bohning et al. 2001; Butler and Wolf 2007

Figure 1: Examples of TMS mapping procedures and display of data. A) Participant wearing swimming cap on which TMS mapping grid has been drawn ready for motor cortex mapping, with each position 1 cm apart from one another. B) Image of template MRI with an example rectangular grid as used in the neuronavigation software. C) Three-dimensional map of the extensor digitorum communis (EDC) muscle, which amplitude in microvolts on the Y-axis and the X- and Z- axis represent 1 cm spacing between each grid position. D) Examples of cortical motor output maps for the wrist flexor and extensor muscles on days 1-5 of practice of a finger sequencing task. Each map is based on 25 measured points, with each grid position 1 cm apart. The shade of grey represents the probability of an MEP (< 50μV) occurring.

1.4.1 Specific Research Objectives

1) To determine the effects of in-phase versus anti-phase bimanual movement training on the trained muscle representation in M1.

Hypothesis: In-phase and not anti-phase BMT will increase the M1 excitability of the wrist extensors with the greatest change from an emphasis on motor preparation.

2) To investigate the effects of potentially enhancing PM input on ipsilateral M1 and the potential combined effects when followed by BMT.

Hypothesis: Enhancing left PM input will enhance the excitable area of M1 representation of the wrist extensors. The combined effects of enhancing left PM input and short-term BMT will lead to a greater enhancement of M1 representation compared to either intervention alone.

3) To investigate the effects of theoretically suppressing the excitability of the contralateral homologous (right) M1 on (left) M1 representation and the potential combined effects when followed by BMT.

Hypothesis: Suppressing right M1 will increase excitability of the wrist extensors in left M1, and the addition of BMT will cause a greater increase in left M1 excitability.

4) To investigate the intracortical and interhemispheric excitability circuitry within and across M1 bilaterally due to short-term BMT, the enhancement of left PM input, and the combination of these interventions.

Hypothesis: BMT will enhance excitability within and between M1 bilaterally, 2) enhancement of left PM input will primarily enhance left M1 excitability, and 3) the combination of these interventions will cause a greater enhancement of bilateral M1 cortical excitability.

Chapter 2 - Study #1

Primary motor cortex excitability is modulated with bimanual training
Adapted from work seen in Neuroscience Letters *514 (2012) 147– 151*

Jason L. Neva, Wynn Legon, W. Richard Staines

2.1 Research objective
This study sought to address research objective 1:

1) To determine the effects of in-phase versus anti-phase bimanual movement training on the trained muscle representation in M1.

Abstract

Bimanual visuomotor movement has been shown to enhance cortical motor activity in both hemispheres, especially when movements require simultaneous activation of homologous muscle groups (in-phase movement). It is currently unclear if these adaptations are specific to motor preparatory areas or if they also involve changes in primary motor cortex (M1). The present study investigated the representation of wrist muscles within motor cortex before and following bimanual movement training that was in-phase, anti-phase with or without motor preparation. Motor evoked potentials (MEPs) for the extensor carpi radialis muscle (ECR) cortical territory were acquired and analyzed before and following bimanual movement. The cortical representation was quantified and compared in terms of spatial extent and MEP amplitude, in two different experiments involving distinct movement training types. In Experiment 1, participants performed bimanual wrist flexion/extension movements to targets which involved in-phase movements, either following a 2 s preparation period (In-phase preparation), or without the preparation period (In-phase *no* preparation). In Experiment 2, training involved antagonist muscle groups activated simultaneously (Anti-phase) with the addition of the 2 s preparation period. In-phase bimanual movement enhanced the spatial representation of

ECR in M1, and did not show a difference in MEP amplitude of the cortical area. It may be that simultaneous activation of homologous M1 representations in both hemispheres, in combination with activity from premotor areas, leads to a greater increase in plasticity in terms of increased M1 spatial extent of trained muscles.

Background

Movement training changes the excitability in several cortical and subcortical loci (Jacobs & Donoghue, 1991; Deiber et al., 1996; Classen et al., 1998; Kleim et al., 1998; Hallett et al., 1999; Staines et al., 2001; Cauraugh & Kim, 2002; Jennings & van der Molen, 2005; Butler & Wolf, 2007; Cauraugh et al., 2010). In stroke patients, movement training performed with the upper limb increases the excitability within primary motor cortex (M1) (Silvestrini et al., 1998; Staines et al., 2001). Compared to single arm training, movement training with both arms yields a greater increase in M1 cortical excitability (Silvestrini et al., 1998; Staines et al., 2001; Stinear & Byblow, 2002). Bimanual arm training also improves hand and arm function in stroke patients (Mudie & Matyas, 2000; Staines et al., 2001; Cauraugh & Kim, 2002; Luft et al., 2004; McCombe Waller & Whitall, 2008; Cauraugh et al., 2010).

Changes in cortical excitability that follow bimanual training may relate to the phase of movement and/or the opportunity to prepare for an upcoming movement. In-phase bimanual movement refers to the simultaneous activation of homologous muscle groups on each limb. Anti-phase bimanual movement refers to the simultaneous activation of antagonist muscles on each limb (Smith & Staines, 2006, 2010). In-phase movement training modulates preparatory activity as measured by electroencephalography (EEG) associated with premotor cortices and possibly M1. This

increase in preparation associated cortical activity was assessed when participants performed closely related unilateral movements not specifically trained. However, these increases in preparatory activity were not observed due to anti-phase movement training (Smith & Staines, 2006, 2010). The lack of effects involving anti-phase training may relate to the duration of training, the skill learning requirement or the sensitivity of the dependent measure. The opportunity to prepare for movement during training may also increase cortical excitability (Smith & Staines, 2006, 2010) and improve behavioural performance (Deiber et al., 1996; Sohn & Carlson, 2000; Jennings & van der Molen, 2005; Smith & Staines, 2006, 2010). Conversely, without the opportunity to prepare for movement, cortical activation is slightly decreased and task performance is worsened (Deiber et al., 1996; Smith & Staines, 2010).

Cortical excitability changes in M1 may also be measured using the amplitude and spatial distribution of motor evoked potentials (MEPs) following single pulse transcranial magnetic stimulation (TMS) over the cortical territory occupied by a particular muscle representation (Wise, 1985; Wassermann et al., 1992; Pascual-Leone et al., 1994; Pascual-Leone et al., 1995; Tyc et al., 2005; Butler & Wolf, 2007). The MEP amplitude is an index of cortical and spinal excitability for a particular target muscle while the cortical map of MEPs indicates the spatial extent of excitability for a given targeted muscle (Weinrich & Wise, 1982; Wise, 1985; Classen et al., 1998; Tyc et al., 2005; Butler & Wolf, 2007). Both measures are sensitive to changes induced by movement interventions (Weinrich & Wise, 1982; Wise, 1985; Classen et al., 1998; Tyc et al., 2005; Butler & Wolf, 2007).

Experiment 1 measures the MEP amplitude and spatial extent for the extensor carpi radialis (ECR) muscle within left-hemisphere M1 before and following in-phase bimanual training. Such training was performed with or without the opportunity to prepare for the upcoming movement. It was hypothesized that in-phase bimanual movement training would increase the excitability of M1 leading to an increase in the spatial extent of the cortical representation of ECR muscle with the greatest change exhibited by the addition of motor preparation. Experiment 2 performs identical measures during anti-phase bimanual movement training. Previous studies have shown that anti-phase training does not increase cortical excitability therefore we examined this question without *a priori* hypotheses.

Methods

Participants

Twenty-four healthy, self-reported right-handed participants (12 female, 12 male; average age= 27 years, range 20-41) took part in the study. Twenty individuals participated in Experiment 1 and ten individuals in Experiment 2. The experimental procedures were approved by the University of Waterloo Office of Research Ethics. All participants completed informed written consent and a transcranial magnetic stimulation (TMS) screening form.

Electromyographic (EMG) recording

Surface EMG was recorded from the right extensor carpi radials (ECR) muscle using a 9 mm diameter Ag-AgCl electrodes. Two active electrodes were placed over the muscle belly of the ECR with a ground electrode over the styloid process of the ulna. EMG recordings were amplified (2000X), band-pass filtered (DC-200 Hz), digitized with

a sample frequency of 1 kHz, and stored for later analysis, using customized LabVIEW software (National Instruments; Austin, Texas, USA).

TMS & Neuronavigation

Focal TMS was performed using a figure-8 (MCF-B65) 70 mm MagProx 100 stimulation coil (Medtronic, Minneapolis, MN, USA). BrainSight Neuronavigation (Rogue Research, Canada) was used to facilitate the location of the coil to the cortical target areas using a template MRI for all participants. The motor hot-spot for the ECR in M1 of the left hemisphere was acquired by placing the stimulation coil on the scalp at a 45˚ to the mid-sagittal line to induce a posterior to anterior current in the underlying neural tissue. The motor hot-spot was determined to be the location in left-hemisphere M1 to elicit an optimal MEP in the contralateral resting ECR. The resting motor threshold (RMT) was determined to be the lowest stimulus intensity that would elicit 5 out of 10 MEPs greater or equal to a peak-to-peak amplitude of 50 μV.

The spatial extent of ECR was determined before and immediately following a bimanual movement training paradigm. Specifically, Brainsight was used to create a rectangular grid, with positions separated by 1 cm, centered on the hot-spot for ECR as a reproducible template for stimulus delivery (Figure 2A). Ten stimulation samples were acquired from each grid position at a stimulus intensity of 120% of RMT with an interstimulus interval (ISI) of ~1-2 s. Background EMG activity of the target measure was quantified during the interstimulus interval at the hotspot. Neuronavigation was used to acquire MEPs initially at the hot-spot, then at the position 1 cm lateral, medial, posterior and anterior (order varied across subjects) followed by locations diagonal to the hot-spot. The identical pattern was continued until the MEPs did not meet the amplitude

criteria (≥ 30 μV peak-to-peak). These sites were considered "active" and were summed before and following bimanual training to determine changes in spatial extent. To assess changes in MEP amplitude ten stimulation samples from the nine sites closest to, and including, the hot-spot were averaged and compared before and after bimanual movement training.

Behavioural task - Experiment 1

Participants were seated in a well lit room facing a computer monitor, with the head and forearms supported. The ECR representation was mapped in the left hemisphere before and following bimanual movement training. Training consisted of 160 bimanual wrist flexion and extension movements to visually cued targets displayed on a computer monitor (Figure 2B). The left and right handles controlled movement of a circular cursor displayed on the monitor in the horizontal and vertical planes, respectively. The position of the handles were recorded by a potentiometer at the base of each handle and were sampled at a frequency of 1 kHz in a customized LabVIEW program. Participants were required to make wrist flexion/extension movements that moved a cursor to particular targets displayed in the upper and lower corners of the computer screen (Figure 2C). Targets were displayed as a box outlined in black (2.5 X 2.5 cm). Targets appeared at random distances from the center origin in the upper and lower corners. A 2 s time window was provided in order to move the cursor to the desired target. If the cursor did not reach the target within 2 s it was considered an incomplete trial.

Bimanual visuomotor movement training was performed by two different groups. Group 1 performed In-phase preparation, required simultaneous flexion or extension of the wrists with the addition of a 2 s preparation period where the cursor disappeared as

the target was visible. After 2 s, the cursor reappeared and a brisk bimanual wrist flexion or extension movement was made. Group 2 performed In-phase *no* preparation, identical to that above but without the 2 s preparation period.

Behavioural task - Experiment 2

The methods were identical to Experiment 1, except the training type was completed with an anti-phase movement. Ten individuals participated in Experiment 2, six of which also participated in Experiment 1. Anti-phase training involved simultaneous activation of antagonistic muscle groups with each arm with a 2 s preparation period before reaching to each target. Therefore, participants were using wrist flexion and extension movements to move the cursor to the opposite diagonal corners as the in-phase training groups (Figure 2C). Since previous studies have found no enhancement of preparatory motor activity due to anti-phase training, the current study did not require a further study of the effects of preparation during anti-phase training (Smith & Staines, 2006, 2010).

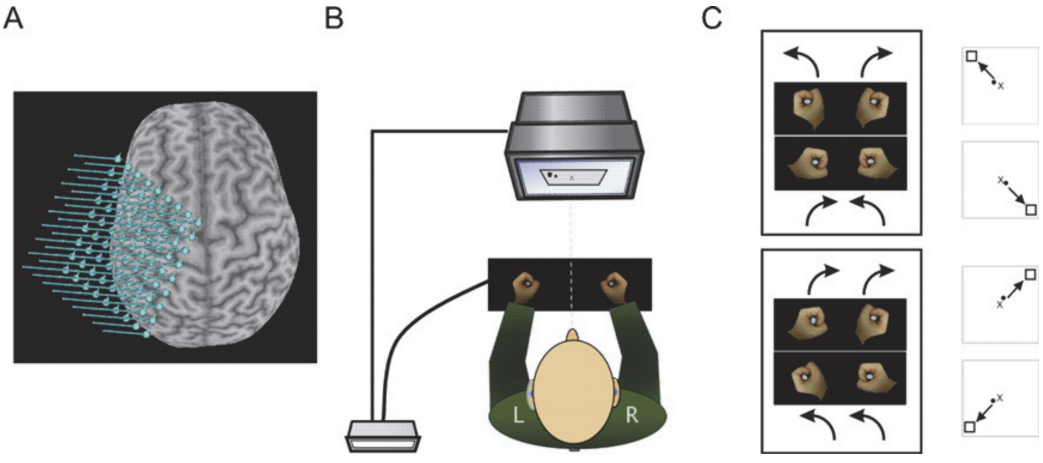


Figure 2: Experimental apparatus, neuronavigation and behavioural tasks. A) Image of template MRI with an example rectangular grid as used in the neuronavigation software. B) Above view of a participant performing the behavioural task, grasping the two handles and viewing both the target and cursor movement on the computer screen. C) *Top panel*: displays movements made during the In-phase movement task, with the corresponding cursor movement on the right. *Bottom panel*: displays movements made during the Anti-phase movement task, with the corresponding cursor movement on the right.

Statistical analysis

For Experiment 1, a 2-way ANOVA was performed on the spatial extent and MEP amplitude data with within-subject factor TIME (2 levels; before and after) and between subject-factor GROUP (2 levels; In-phase preparation, In-phase *no* preparation). Behavioural performance was quantified by taking the angle at peak velocity of the resultant cursor path, relative to a straight path to the visual target, for each movement trial. A 2-way ANOVA was performed on the first block (10 trials) and last block of trials of the behavioural performance data with within-subject factor BLOCK (2 levels; first block and last block of 10 trials) and between-subject factor GROUP (2 levels; In-phase preparation, In-phase *no* preparation). For Experiment 2, a two-tailed paired t-test was used to investigate differences between the before and after training measures for the cortical excitability and the same two-tailed paired t-test comparing the first and last block of trials for the behavioural performance data. Significance was set at $p \leq 0.05$.

Results

Experiment 1

The mean RMT for the left M1 was 48 ± 8.6% (range 34 - 63%) of maximum stimulator output. Figure 3A displays the spatial extent before and after movement for in-phase training groups. Shown are the average number of active sites (with standard error). There is a significant increase in spatial extent for the two groups for number of active sites indicating a modest territorial expansion in ECR cortical representation. Two-way ANOVA revealed a main effect of TIME ($F(1, 18)=12.99$, $p=0.002$), no effect of GROUP ($F(1, 18)=0.104$, $p=0.750$) and no interaction of TIME by GROUP ($F(1,$

18)=0.06, p=0.804). Data from individual participants for both training groups are shown in Figure 3B. For the measure of MEP amplitude, the ANOVA revealed no effect of TIME and no interaction (Mean Pre = 198 ± 38 μV (SE); Mean Post = 225 ± 45 μV (SE)).

Figure 5 displays the behavioural data of the in-phase training groups, with the angle at peak velocity (leftward panel) and movement time (rightward panel). A two-way ANOVA on angle at peak velocity revealed a main effect of BLOCK (F (1, 18)=6.445, p=0.021) with no interaction of BLOCK by GROUP (F (1, 18)=1.693, p=0.210) and no effect of GROUP (F (1, 18)=1.160, p=0.296). The main effect of BLOCK indicates that there was a decrease in deviation of cursor path from the initial to the final trials.

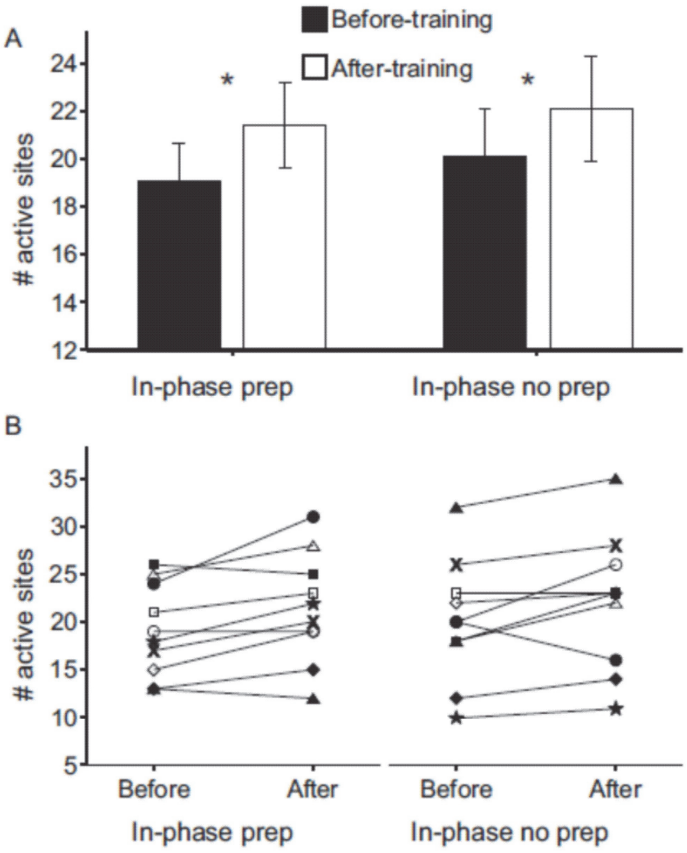


Figure 3: Spatial extent of ECR for in-phase training. Number of active sites for all participants and for in-phase movement training groups. A) Average number of active sites before (black) and after (white) movement training for each training group. Bars represent SEM. Asterisk indicates significance, $p < 0.05$. B) Number of active sites before and after training for each participant. Each participant is shown as a different symbol.

Experiment 2

Figure 4 displays the spatial extent (with standard errors) for the anti-phase training. Paired t-tests revealed that the spatial extent was unaltered following anti-phase training ($t(9) = -0.733$, $p=0.482$). This was also the case for MEP amplitude ($t(9) = 1.359$, $p=0.207$). A two-tailed paired t-test revealed no decrease in cursor path deviation from the beginning to the end of anti-phase training ($t(9)=1.080$, $p=0.308$) (Figure 5).

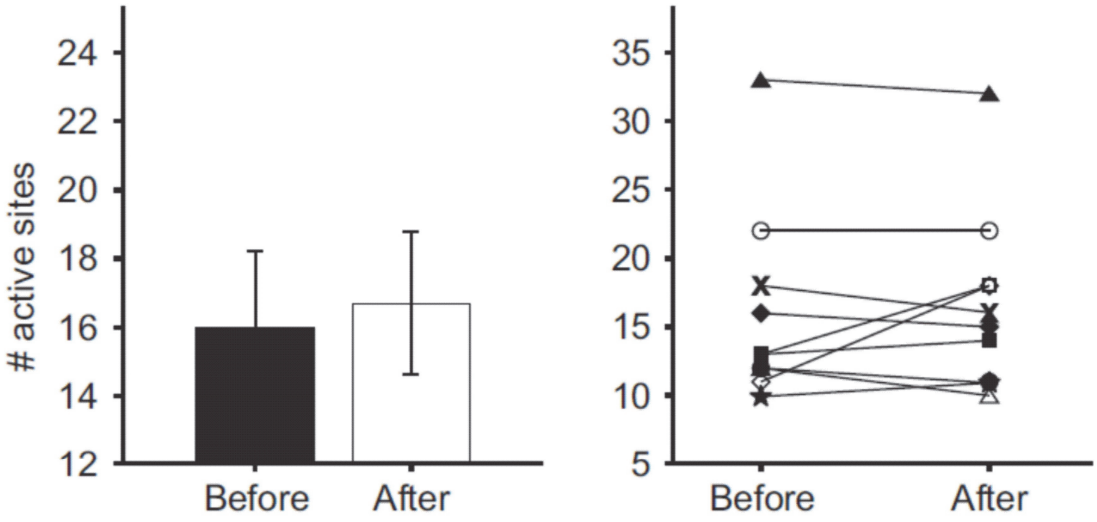


Figure 4: Spatial extent of ECR for Anti-phase training. Number of active sites for all participants and for Anti-phase movement training group. *Left* Average number of active sites before (black) and after (white) movement training for each training group. Bars represent SEM. *Right* Number of active sites before and after training for each participant. Each participant is shown as a different symbol.

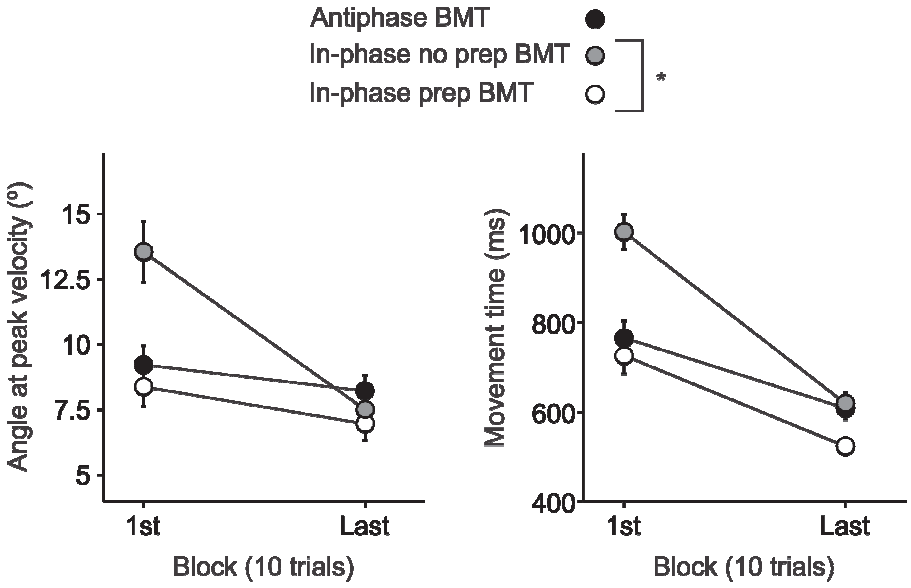


Figure 5: Behavioural data for anti-phase (black), in-phase without preparation (grey), and in-phase with preparation (white) bimanual training. *Left* Angle at peak velocity of the resultant cursor path. *Right* Movement time. All bars represent SEM. Asterisk indicates significance, $p < 0.05$.

Discussion

The present study demonstrated short-term bimanual visuomotor training enhancement of M1 excitability. Specifically the spatial representation of the ECR muscle was expanded only when bimanual wrist movements were performed in-phase. However, increased excitability was not represented in the amplitude of the MEP in the cortical region of the ECR muscle. Also, in-phase training with an emphasis on preparation did not further increase M1 excitability. It may be that simultaneous activation of homologous M1 representations of ECR across both hemispheres, in combination with activity from premotor areas, leads to an increase in excitability along the borders of M1 representation of the trained muscles.

Increases in cortical spatial extent are shown to occur with trained muscles at the expense of neighbouring muscle representations (Pascual-Leone et al., 1995; Nudo et al., 1996; Kleim, Barbay, et al., 1998). Modest increases in trained muscle representation are seen after two 2 hr sessions of finger sequence training in humans (Pascual-Leone et al.,

1995). However, more substantial increases in spatial extent of trained muscles appear to require more extensive training (Pascual-Leone et al., 1995; Nudo et al., 1996; Kleim et al., 1998; Kleim et al., 2004). The modest increase in spatial extent of ECR we observe may involve preliminary phases of unmasking existing neuronal connections (Jacobs & Donoghue, 1991) and early activity-dependent LTP-like mechanisms, possibly involving increased neurotransmitter release in cortical regions surrounding the ECR hot-spot (Rioult-Pedotti et al., 2000). The above studies indicate that motor skill training likely engages the aforementioned neural mechanisms (i.e. unmasking existing neuronal connections and increased synaptic transmission such as LTP) as an explanation for the expansion of cortical territory.

Changes in cortical excitability are exhibited by altered motor thresholds and MEP amplitude (Pascual-Leone et al., 1995; Muellbacher et al., 2001). Increases in MEP amplitude due to motor training likely reflect increased activity in neighbouring cortical area to the hot-spot (Hallett et al., 1999; Muellbacher et al., 2001), suggestive of an increase in the bordering regions of cortical representation found in the present study. Conversely, decreases in motor threshold represent a focal change in the hot-spot of a muscle representation (Hallett et al., 1999; Muellbacher et al., 2001). In addition, increases in MEP amplitude are correlated to increases in exerted force, particularly in short-term training (Muellbacher et al., 2001). In the present study, we did not observe a change in the MEP amplitude following any training type. One explanation is that the training used does not emphasize increased exertion of force, and engages different mechanisms for cortical plasticity that results in cortical expansion while the amplitude remains unchanged. Prolonged movement training decreases motor threshold, indicating

enhanced excitability at the hot-spot (Pascual-Leone et al., 1995). Our in-phase training could have been too brief to engage neuronal mechanisms that would increase MEP amplitude.

Cortical activity is enhanced in both hemispheres in damaged and healthy M1 when homologous muscles are activated together (Silvestrini et al., 1998; Staines et al., 2001). It is thought that transcollosal neural activity of homologous representations in M1 act to excite and/or release inhibition from the contralateral hemisphere (Stinear & Byblow, 2002), which may contribute to M1 plasticity. Intracortical inhibition is decreased in M1 when the two upper limbs co-activate the homologous muscles simultaneously, but inhibition remains when they are not activated simultaneously (Stinear & Byblow, 2002). Although the current study did not find a greater enhancement of M1 representation when in-phase training emphasized movement preparation, an alternative mechanism may involve contribution from premotor cortex. Premotor cortical areas, such as the dorsal premotor cortex (PMd), have extensive reciprocal neuronal projections with M1 (Weinrich & Wise, 1982). In-phase bimanual training increases fMRI activity in the lateral premotor cortical areas, including PMd (Seitz et al., 2004). Also, short-term in-phase bimanual training, particularly involving visual cues, has been associated with increases in lateral premotor cortical activity (Smith & Staines, 2006, 2010). In contrast, increases in premotor cortex are not necessarily accompanied by increases in M1 activity as measured using EEG (Smith & Staines, 2006, 2010). However, TMS may have an enhanced ability to detect subtle changes as we presently observe. Therefore, the liberation of inhibition due to homologous M1 ECR representations activated together, along with this activity engaging areas in PMd which

could further facilitate M1 ECR representation, may be the driving force for the observed changes in spatial extent.

Conclusion

Short-term bimanual visuomotor movement training leads to an increase in M1 excitability in terms of an expansion along the borders of cortical representation of trained muscles. It is possible that co-activation of M1 representation of trained muscles across both hemispheres, in combination with activity from premotor areas, leads to the greatest increase in plasticity along the borders of M1 representation of the trained muscle.

Chapter 3 - Study #2

Modulation of left primary motor cortex excitability after bimanual training and intermittent theta burst stimulation to left dorsal premotor cortex
Adapted from work seen in Behavioural Brain Research *2014, in press*

Jason L. Neva, Michael Vesia, Amaya M. Singh, W. Richard Staines

3.1 Research objective
This study sought to address research objective 2:

2) To investigate the effects of potentially enhancing PM input on ipsilateral M1 and the potential combined effects when followed by BMT.

Abstract

Bimanual visuomotor movement training (BMT) enhances the excitability of human preparatory premotor and primary motor (M1) cortices compared to unimanual movement. This occurs when BMT involves mirror symmetrical movements of both upper-limbs (in-phase) but not with non-symmetrical movements (anti-phase). The neural mechanisms mediating the effect of BMT is unclear, but may involve interhemispheric connections between homologous M1 representations as well as the dorsal premotor cortices (PMd). The purpose of this study is to assess how intermittent theta burst stimulation (iTBS) of the left PMd affects left M1 excitability, and the possible combined effects of iTBS to left PMd applied before a single session of BMT. Left M1 excitability was quantified using transcranial magnetic stimulation (TMS) in terms of both the amplitudes and spatial extent of motor evoked potentials (MEPs) for the extensor carpi radialis (ECR) before and multiple time points following 1) BMT, 2) iTBS to left PMd or 3) iTBS to left PMd and BMT. Although there was not a greater increase in either specific measure of M1 excitability due to the combination of the interventions, iTBS applied before BMT showed that both the spatial extent and global MEP amplitude

for the ECR became larger in parallel, whereas the spatial extent was enhanced with BMT alone and global MEP amplitude was enhanced with iTBS to left PMd alone. These results suggest that the modulation of rapid functional M1 excitability associated with BMT and iTBS of the left PMd could operate under related early markers of neuroplastic mechanisms, which may be expressed in concurrent and distinct patterns of M1 excitability. Critically, this work may guide rehabilitation training and stimulation techniques that modulate cortical excitability after brain injury.

Introduction

Visuomotor movement training modulates the excitability in several cortical areas, namely, motor (Jacobs & Donoghue, 1991; Pascual-Leone et al., 1995; Classen et al., 1998; Karni et al., 1998; Kleim et al., 1998; Nudo, 2006; Butler & Wolf, 2007), premotor (PM) (Deiber et al., 1996; Karni et al., 1998; Andres et al., 1999; Jennings & van der Molen, 2005; Smith & Staines, 2006, 2010, 2012), and parietal cortices as well as subcortical areas such as the basal ganglia and cerebellum (Clower et al., 1996; Doyon et al., 1997; Kleim et al., 1998; Seidler & Noll, 2008). In individual stroke patients, bimanual movement performed with the upper-limbs can increase the excitability within the damaged primary motor cortex (M1) (Silvestrini et al., 1998; Staines et al., 2001). Critically, bimanual visuomotor movement training (BMT) yields a greater increase in premotor (Smith & Staines, 2006, 2010, 2012) and M1 (Neva et al., 2012) cortical excitability than does unimanual movement training. Additionally, bimanual arm training has been shown to improve hand and arm function in stroke patients (Mudie & Matyas, 2000; Staines et al., 2001; Cauraugh & Kim, 2002; Luft et al., 2004; McCombe

Waller & Whitall, 2008; Cauraugh et al., 2010). Although BMT can modulate the excitability in motor preparation and execution areas as well as improve upper-limb function in patient populations, the underlying neural mechanisms remain unclear.

Modulation of cortical excitability after BMT likely relates to the phase of movement with some influence of emphasizing the motor preparatory aspect of the trained movements (Neva et al., 2012). Specifically, increases in motor preparatory and execution areas occur when BMT involves the simultaneous co-activation of homologous muscle groups (in-phase training), but not with co-activation of antagonist muscle groups (anti-phase training) (Smith & Staines, 2006, 2010, 2012; Neva et al., 2012). Electroencephalography (EEG) work suggests that in-phase BMT modulates preparatory activity in PM cortices and possibly M1. This increase in preparation-associated cortical activity was found during the performance of similar unilateral movements not specifically trained (Smith & Staines, 2006, 2010). Likewise, transcranial magnetic stimulation (TMS) work has shown that in-phase BMT, but not anti-phase, increases M1 excitability. Specifically, the excitable cortical territory of trained muscle representation increases along the borders without a concurrent increase in excitability of the central representation of that muscle (Neva et al., 2012). The lack of effect due to anti-phase training may relate to the reciprocal inhibition of active versus inactive agonist and antagonist muscle representations in the contralateral hemispheres (Stinear & Byblow, 2002). In addition, motor preparation associated with a goal-directed movement during training increases cortical excitability and, in turn, improves behavioural performance (Deiber et al., 1996; Sohn & Carlson, 2000; Jennings & van der Molen, 2005; Smith & Staines, 2006, 2010, 2012). Conversely, without this goal-directed motor preparation,

cortical activation is slightly decreased and task performance generally declines (Deiber et al., 1996).

Indeed, behavioural studies have shown that covertly and overtly preparing movements to a target stimulus decreases reaction times (RTs) and increases activity in PM cortices (Sheliga et al., 1995; Deubel & Schneider, 1996; Nobre et al., 2000; Corbetta & Shulman, 2002). The dorsal premotor cortex (PMd) has well-known roles in the selection of appropriate actions for movement execution (Kalaska & Crammond, 1995; Thoenissen et al., 2002; O'Shea et al., 2007; Groppa et al., 2012). Interestingly, neuroimaging and TMS research suggest that PMd in the left hemisphere has an important role in action selection for motor execution (Geyer et al., 2000; Toni et al., 2001). Specifically, PMd seems to be particularly involved in movement selection with learned visuomotor associations (Geyer et al., 2000; Toni et al., 2001). Also, left PMd activity increases with action selection of one or both upper-limbs (Schluter et al., 2001). Further, when the right PMd is disrupted with inhibitory TMS, action selection is hindered in the contralateral hand alone. Conversely, disruption of left PMd leads to a disruption in action selection of both upper-limbs (Schluter et al., 1998; Johansen-Berg et al., 2002). Similarly, repetitive TMS to left PMd causes faster preparation of complex sequences performed with the right hand (Stinear et al., 2009). This suggests that the left PMd has a particularly relevant role in movement selection with both upper-limbs and the learning of visuomotor behavioural associations.

Theta burst stimulation (TBS) is a type of repetitive TMS (rTMS) that has been shown to modulate the cortical excitability of M1 after a brief period of stimulation (Huang et al., 2005). Continuous theta burst stimulation (cTBS) decreases cortical

excitability of M1, and intermittent theta burst stimulation (iTBS) enhances the excitability of M1 as demonstrated by respective modulations in motor evoked potential (MEP) amplitude. Furthermore, cTBS to PMd decreases MEP amplitude of the ipsilateral M1 representation (Huang et al., 2009; Ortu et al., 2009). Subthreshold rTMS to PMd decreases ipsilateral M1 cortical excitability when delivered at 1 Hz, and increases excitability when delivered at 5 Hz (Gerschlager et al., 2001; Chouinard et al., 2003; Rizzo et al., 2004; O'Shea et al., 2007; Suppa et al., 2008). This suggests that M1 excitability may be differentially modulated by unique stimulation patterns to remote and related areas, like PMd. Specifically, there are strong excitatory anatomical connections between the PM and M1 cortices, particularly within the left hemisphere (Picard & Strick, 2001; Rushworth et al., 2003; Koch et al., 2007). Therefore, up-regulating the excitability of the left PMd may lead to a modulation in the excitability of the left (ipsilateral) M1. Furthermore, given that PMd has been shown to be specifically involved with action selection of learned associations with both upper-limbs, perhaps enhancing the excitability of ipsilateral PMd via iTBS will lead to a greater enhancement of M1 excitability when combined with BMT compared to BMT alone.

The current study investigates the effect of short-term in-phase BMT, iTBS to left PMd and the possible combined effects of iTBS to left PMd applied before BMT on left M1 cortical excitability. It was hypothesized that in-phase BMT would increase the corticospinal excitable area of left M1. Also, it was hypothesized that iTBS to left PMd would enhance the excitability of the M1. Finally, it was hypothesized that iTBS to left PMd would potentially enhance the excitable input from PMd to the motor cortices and, in turn, enhance M1 corticospinal excitability to a greater extent when followed by BMT.

Methods

Participants

Twenty healthy, self-reported right-handed participants (7 female; average age= 27 years, range 21-38) took part in the study. Participants were divided into three groups with different interventions: BMT alone (group 1), iTBS to left PMd alone (group 2) and iTBS to left PMd followed by BMT (group 3). Ten individuals participated in each of the three interventions in random order, with no participants performing the bimanual training twice. The experimental procedures were approved by the University of Waterloo Office of Research Ethics. All participants provided informed written consent and completed a TMS screening form (Keel et al., 2000).

Electromyographic (EMG) recording

Surface EMG was performed in the same way as Study #1.

TMS & Neuronavigation

Focal TMS was performed in a similar manner to Study #1 with some additions listed below. The active motor threshold (AMT) was defined as the lowest stimulus intensity that would elicit 5 out of 10 MEPs greater than or equal to a peak-to-peak amplitude of 200 μV while maintaining a light contraction of the ECR of 10% of maximum voluntary contraction. For iTBS, the theta burst pattern of stimulation (three stimuli delivered at 50 Hz, which were grouped and delivered at 5 Hz) was delivered in blocks of 2 seconds followed by a period of 8 seconds with no stimulation, for a total of 600 stimuli applied over 190 seconds (Huang et al., 2005; Stinear et al., 2009). We delivered iTBS to PMd in the left hemisphere (Huang et al., 2009; Stinear et al., 2009) at

80% of AMT. The location of PMd was determined to be 2.5 cm anterior to the ECR motor hotspot in left M1 (Picard & Strick, 2001; Huang et al., 2009; Stinear et al., 2009).

The modulation of M1 excitability in the left hemisphere was measured similarly to Study #1 with exceptions listed below. The spatial extent of ECR was measured before and multiple time points after 1) BMT alone (pre, 30 min post), 2) iTBS to left PMd alone (pre, 5, 30, 60 min post) or 3) iTBS to left PMd with BMT following (pre, 30, 60 min post) (Figure 7). Ten stimulation samples were acquired from each grid position at stimulus intensity of 110% of rMT with a random interstimulus interval (ISI) of ~2 seconds. Acquisition of MEPs from individual grid positions were sampled with a time interval of ~30-45 seconds between stimulation blocks.

The centre of gravity (CoG) was calculated by taking the average of the 10 MEPs from each grid position and dividing that by the average MEP amplitude for the entire spatial map.

Behavioural task

The behavioural task was performed in a similar way to Study #1 with a few additions listed below (see Figure 6C-D). Participants were required to make simultaneous in-phase wrist extension movements (Smith & Staines, 2006, 2010, 2012; Neva et al., 2012) that moved a cursor to targets displayed in the upper left from the lower right (starting point) quadrants of the computer screen (Figure 6D). Targets were displayed as a box outlined in black (2.5 X 2.5 cm), and appeared at one of three different locations in the upper left corner of the screen in pseudo-random order. BMT was performed by groups 1 and 3, with group 1 performing BMT alone and group 3 performing the same BMT as group 1 following iTBS to left PMd (Figure 7).

Statistical analysis

Analysis was performed in two ways. First, to specifically investigate the temporal factors of each intervention, analysis was performed within each group across all time points with the dependent measures of spatial extent, global, and central MEP amplitude. Therefore, for each group, a repeated measures ANOVA was performed with TIME as a factor (group 1: BMT alone – pre and 30 min post; group 2: iTBS to PMd alone – pre, 5, 30, 60 min post; group 3: iTBS to PMd + BMT – pre, 30, 60 min post). *Post hoc* analyses were performed with a Tukey correction method to investigate any other differences between time points. Second, as an exploratory measure that the combination of iTBS to left PMd and BMT would possibly yield a greater increase in M1 excitability than either intervention alone, a one-way ANOVA was performed on all three groups with the difference score between pre and post 30 min time points for the spatial extent, global, and central MEP amplitude data with between-subjects factor GROUP (BMT alone, iTBS to left PMd alone, iTBS to left PMd + BMT).

Behavioural performance for groups 1 and 3 were quantified in terms of the movement time for both hands and the resultant cursor movement to the targets displayed on the screen. Generally, both hands were active simultaneously and were similarly contributing to the resultant cursor movement across training trials in both groups. Specifically, the behavioural performance was quantified by taking the movement time and the angle at peak velocity of the resultant cursor path (wrist extension movements of both upper-limbs), relative to an ideal (straight) path to the visual target, for each movement trial (Neva et al., 2012). A two-way ANOVA was performed on the movement time and angle at peak velocity including within-subjects factor BLOCK (first

block and last block of 10 trials) and between-subjects factor GROUP (BMT alone and iTBS to left PMd + BMT). Where interactions were observed, separate paired t-tests were performed with factor BLOCK (first block and last block of 10 trials), in order to investigate the differences in performance between groups 1 and 3. Statistical significance was set at $p \leq 0.05$.

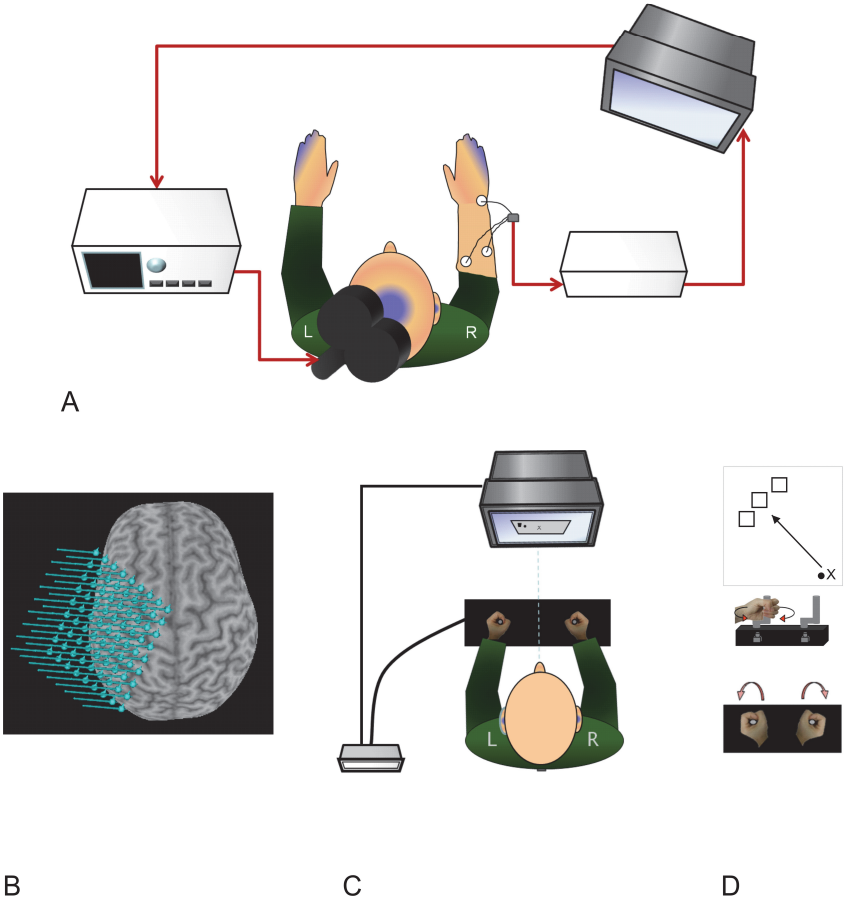


Figure 6: Experimental apparatus, neuronavigation and behavioural task. (A) Displays the experimental set up during TMS and collection of MEPs while participants were at rest (B) Image of template MRI with an example rectangular grid as used in the neuronavigation software. (C) Above view of a participant performing the behavioural task, grasping the two handles and viewing both the target and cursor movement on the computer screen. (D) Top panel: displays movements made during the bimanual movement training task. Participants began in the bottom right corner and made varying degrees of wrist extension movements to move the cursor to the remembered targets.

Results

The motor thresholds were consistent across groups (group 1 – mean rMT = 47 ± 6.8%; group 2 – mean rMT = 47 ± 7.2% and mean AMT = 39 ± 6.6%; group 3 – mean rMT = 49 ± 8.7% and mean AMT = 43 ± 8%). Similarly, the size of the stimulated spatial map area was similar across groups (average number of grid positions acquired in the pre: group 1 = 29 ± 6; group 2 = 29 ± 6; group 3 = 31 ± 8). Figure 7 displays the M1 cortical excitability maps of the right ECR muscle (leftward panel) of representative participants and the means of the resultant displacement (medial-lateral and anterior-posterior) of the CoG (rightward panel) for all groups at all time points. For group 1, the ECR cortical excitability map in the left hemisphere increased after a single session of BMT as shown previously (Figure 7A) (Neva et al., 2012). For group 2, the ECR cortical excitability map increased noticeably at 30 min post iTBS to left PMd and continued to 60 min post stimulation (Figure 7B). Lastly, for group 3, the cortical excitability map of ECR in the left hemisphere was increased immediately after iTBS to left PMd and BMT at both 30 min and 60 min post intervention (Figure 7C). Figure 7D shows that the average resultant displacement in CoG shifts slightly across groups and across time points, with no significant change.

To further analyze the data of all three groups, figure 8 displays the spatial map, global, and central MEP amplitude before and after the intervention of (1) in-phase BMT (white bars), (2) iTBS to left PMd (black bars), and (3) iTBS to left PMd followed by BMT (grey bars). Figure 8A shows the spatial extent of ECR in M1 in the left hemisphere as the group average number of active sites (with standard error), with figure 8B displaying individual data (unique symbols represent individual participants). There

was an increase in spatial extent of ECR in M1 for the two groups that performed the BMT (groups 1 and 3), as evidenced by the increased number of active sites. For group 1 (white), a one-way repeated measures ANOVA revealed an increase in active sites between before and after in-phase BMT alone ($F(1, 9)=16.943$, $p=0.003$). Additionally, for group 2 (black), a repeated measures ANOVA revealed no increase in active sites from iTBS to left PMd ($F(3, 26)= 2.21$, $p=0.111$). Finally, for group 3 (grey), a repeated measures ANOVA revealed an increase in active sites from iTBS to left PMd followed by BMT ($F(2, 18)= 9.57$, $p=0.002$). *Post hoc* analyses revealed differences between pre and 30 min and 60 min post ($p<0.05$), with no difference between 30 min and 60 min post ($p>0.05$). Additionally, there were no differences across groups between pre and the 30 min time point post, as a one-way ANOVA revealed no effect of GROUP ($F(2, 26)=0.841$, $p=0.443$).

Figure 8C shows the global MEP amplitude before and after the intervention of (1) in-phase BMT (white bars), (2) iTBS to left PMd (black bars), and (3) iTBS to left PMd followed by BMT (grey bars) (with standard error). There was an increase in global MEP amplitude for groups 2 and 3. A one-way repeated measures ANOVA performed on group 1 (white) revealed a slight increase in global MEP amplitude after BMT, which was near significance ($F(1, 9)=4.530$, $p=0.062$). Additionally, for group 2 (black), a repeated measures ANOVA revealed a significant increase in global MEP amplitude from iTBS to left PMd ($F(3, 26)= 4.01$, $p=0.018$). *Post hoc* analyses revealed differences between pre and 30 min post ($p<0.05$) and 60 min post ($p<0.05$), with no other differences. Finally, for group 3 (grey), a repeated measures ANOVA revealed an increase in global MEP amplitude due to iTBS to left PMd followed by BMT ($F(2,18)=$

4.51, p=0.026). *Post hoc* analyses revealed differences between pre and 30 min post ($p<0.05$) and 60 min post ($p<0.05$), with no difference between 30 min and 60 min post. In addition, a one-way ANOVA comparing the difference between pre and post 30 min revealed no difference across GROUP ($F(2, 26)=0.643$, $p=0.534$).

Figure 8D displays central MEP amplitude before and after the interventions of all three groups (with standard error). There was no excitability increase of the central area of ECR in left hemisphere M1 due to any intervention. A repeated measures ANOVA revealed no effect of BMT (group 1 – white) ($F(1, 9)=1.918$, $p=0.199$), iTBS to left PMd (group 2 – black) ($F(3, 26)=1.59$, $p=0.215$) and iTBS to left PMd followed by BMT (group 3 – grey) ($F(2, 18)=1.22$, $p=0.320$) on central MEP amplitude of ECR in the left hemisphere M1 across all time points. Additionally, a one-way ANOVA comparing the difference between pre and post 30 min revealed no difference across GROUP ($F(2, 26)=0.067$, $p=0.935$).

Figure 9 displays the behavioural data of groups 1 and 3, with the movement time (leftward panel) and angle at peak velocity (rightward panel). For the movement time, a two-way ANOVA revealed a main effect of BLOCK ($F(1, 18)=20.460$, $p<0.001$), no effect of GROUP ($F(1, 18)=0.598$, $p=0.451$) and no interaction of BLOCK x GROUP ($F(1, 18)=0.009$, $p=0.926$). This indicates that both groups 1 and 3 improved performance in movement time similarly. For the angle at peak velocity, a two-way ANOVA revealed a main effect of BLOCK ($F(1, 18)=8.513$, $p=0.009$) and an interaction of BLOCK x GROUP ($F(1, 18)=6.060$, $p=0.024$), but no effect of GROUP ($F(1, 18)=0.082$, $p=0.778$). The main effect of BLOCK indicates that there was a decrease in deviation of cursor path from the initial to the final trials (i.e., performance improvement). Further analysis on

factor BLOCK with separate paired t-tests revealed a significant improvement in performance for group 1 (t (9)=3.338, p=0.009), with no improvement for group 3 (t (9)=0.385, p=0.709), which may be due to the slightly lower initial angle at peak velocity of the resultant cursor path in group 3.

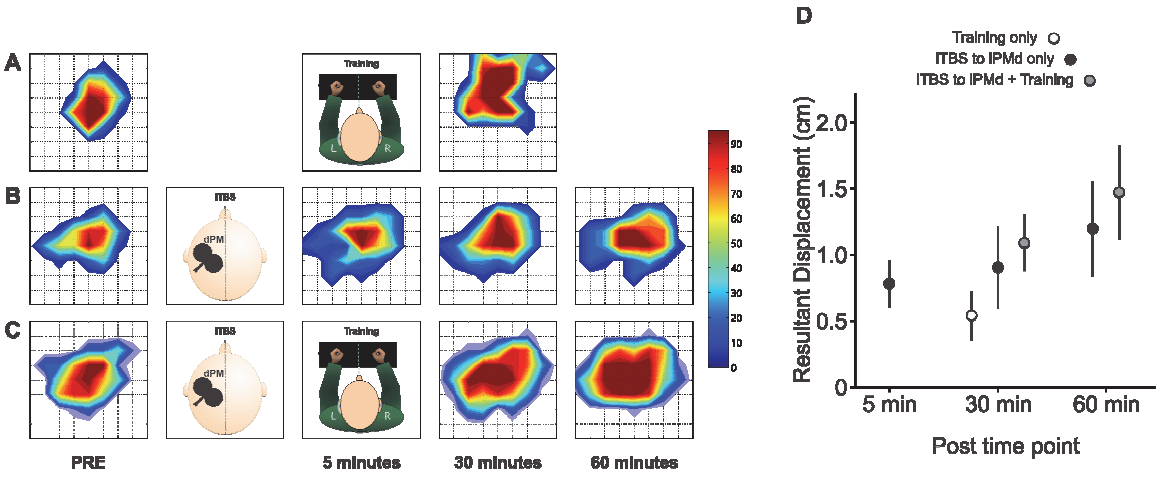


Figure 7: Representative examples of the M1 cortical excitability maps for the ECR muscle for groups 1 (A), 2 (B) and 3 (C) across all time points (post time points are relative to the pre). Red on the scale represents the greatest probability and blue represents the least probability to generate a MEP. An amplitude of ≥ 30 μV peak-to-peak in the ECR was considered an MEP. (D) Means of the resultant displacement (medial-lateral and anterior-posterior) of the center of gravity (CoG) for all participants (time points relative to the pre). All bars represent SEM. Asterisk indicates significance, $p < 0.05$.

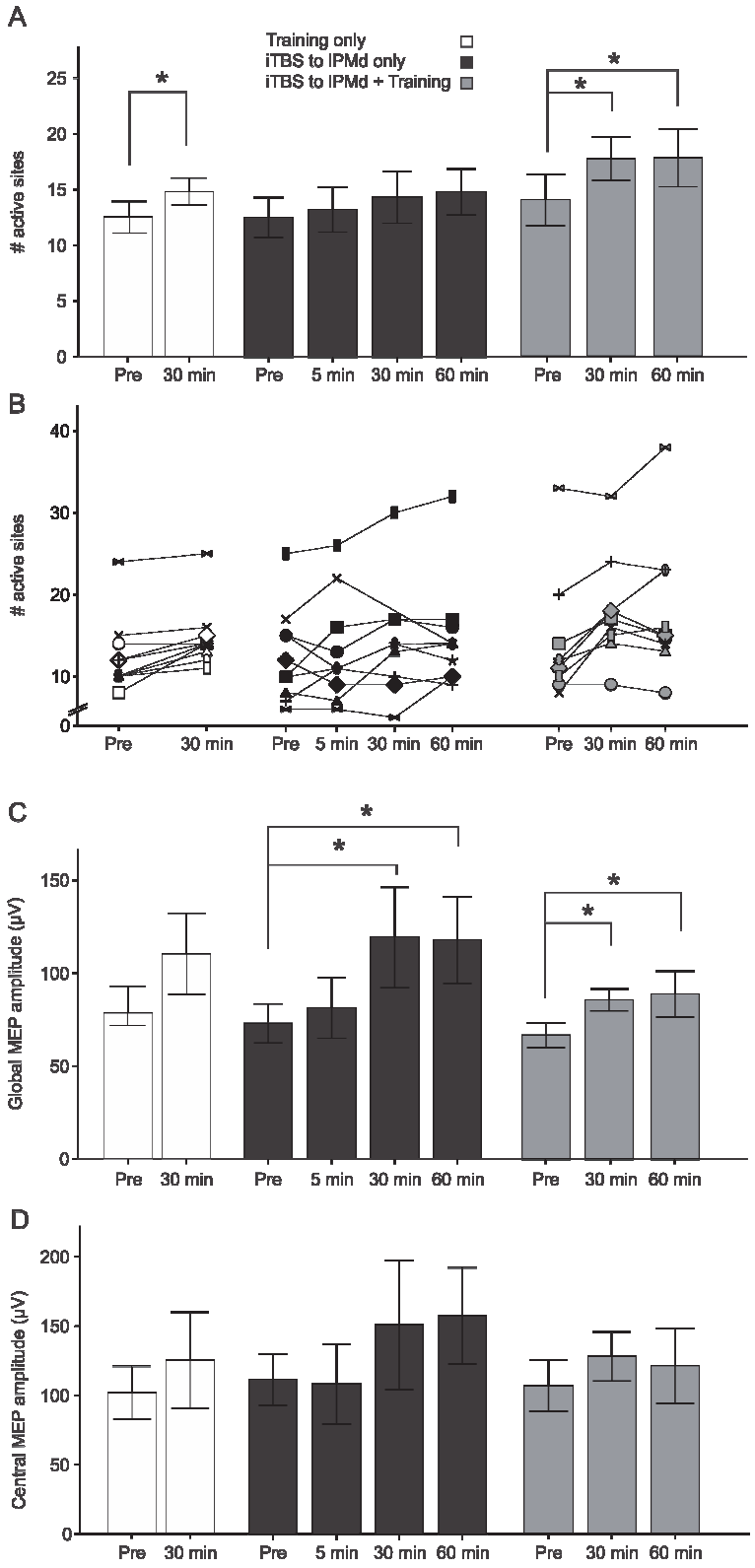


Figure 8: Means of all dependent measures for left M1 ECR excitability for all participants before and after bimanual training and/or iTBS to PMd. Group 1: BMT (white). Group 2: iTBS to PMd (black). Group 3: iTBS to PMd + BMT (grey). (A) Spatial maps displayed by number of active sites group mean (B)

individual participants (unique symbols represent each participant). (C) Global MEP amplitude mean. (D) Central MEP amplitude mean. All bars represent SEM. Asterisk indicates significance, $p < 0.05$.

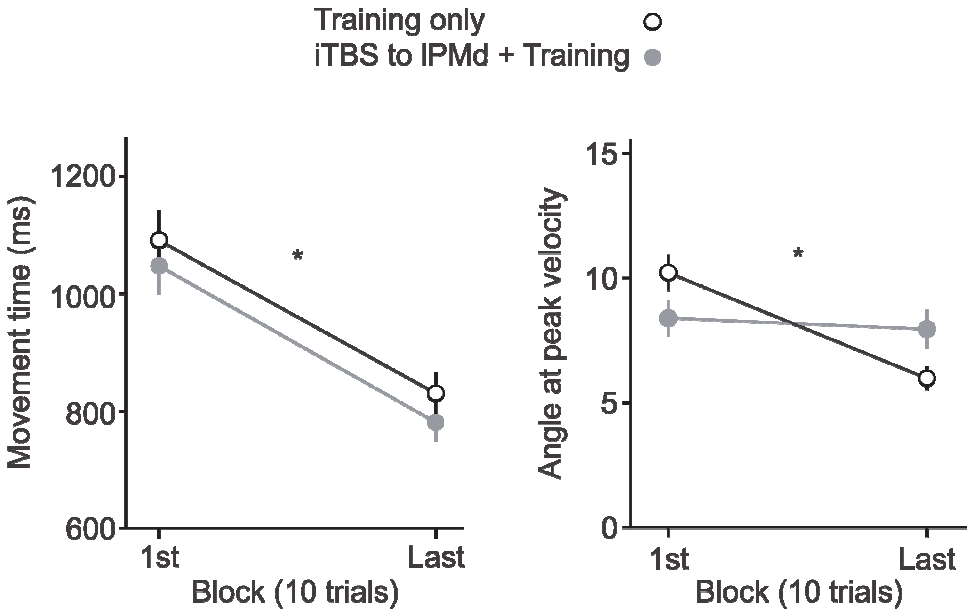


Figure 9. Behavioural data for groups 1 and 3. *Left* Movement time for Group 1 (white) and Group 3 (grey). *Right* Angle at peak velocity of the resultant cursor path for Group 1 (white) and Group 3 (grey). All bars represent SEM. Asterisk indicates significance, $p < 0.05$.

Discussion

The present study is the first to demonstrate the early markers of rapid functional human motor cortical plasticity associated with short-term BMT following iTBS to left PMd. Although there was not a greater increase in each measure due to the combination of iTBS to left PMd followed by BMT, the specific modulations in left M1 excitability resulting from each intervention indicate that they may operate under related neural mechanisms, which are possibly expressed in distinct patterns concurrently. Motor cortex mapping demonstrated that both the spatial extent and global MEP amplitude for the task-specific muscles became larger with iTBS to left PMd followed by short-term BMT, whereas the spatial extent was enhanced with BMT alone and the global MEP amplitude was enhanced with iTBS to left PMd alone. The effect of iTBS to left PMd alone indicates that motor preparatory areas modulate the excitability of the downstream

ipsilateral (left) M1, whereas the effect of BMT alone confirms that left M1 excitability is increased along the borders of the ECR representation (Neva et al., 2012).

The concurrent effects of iTBS to left PMd with bimanual training

This study is the first to demonstrate that applying iTBS to the left PMd before BMT increases excitability in the left M1 in terms of both the spatial extent and global MEP amplitude concurrently, along with the specific excitability changes due to BMT or iTBS to left PMd alone. One possibility is that the iTBS to left PMd enhanced the downstream ipsilateral connections to M1 (and possibly connections between left PMd and right M1) and facilitated BMT-induced excitability changes. As noted, the left PMd has a critical role in action selection for execution of learned associations for movements of either or both upper-limbs (Schluter et al., 1998; Rushworth et al., 2003). Inhibition of left PMd using TMS leads to a disruption during action selection when using both upper-limbs (Schluter et al., 1998; Johansen-Berg et al., 2002). Also, short-term BMT increases activity in the lateral PM cortex during a closely associated unimanual task (Smith & Staines, 2006, 2010, 2012). Further, iTBS to left PMd causes faster preparation of complex sequences performed with the right hand (Stinear et al., 2009). Given that the current study involved learning a skilled task that required movement of both upper-limbs simultaneously, the potential up-regulation of the left PMd could have induced a greater degree of excitatory input to M1 in both hemispheres during the training of the task. This up-regulation of the left PMd may have led to the slightly enhanced initial behavioural performance observed when followed by BMT, compared to BMT alone. Conversely, it is possible that the BMT slightly enhanced the effects of iTBS applied to PMd. Voluntary contraction of the right limb immediately after iTBS applied to left M1

enhances the facilitating effects of iTBS to MEP amplitude in the resting limb (Huang et al., 2008). It may be that increased cortical input to M1 due to iTBS to left PMd acts to facilitate excitability similarly with our BMT task to that observed due to voluntary contraction immediately after applying iTBS to M1 itself. Overall, it could be that simultaneous activation of homologous muscles, with the addition of an increased excitable input from left PMd, led to the slightly greater enhancement in left M1 ECR excitability observed in this study.

iTBS to left PMd on left M1 cortical excitability

This study found that iTBS to left PMd markedly increased the excitability of ipsilateral M1, in terms of global MEP amplitude. The lateral premotor cortex (i.e. PMd) has extensive reciprocal neuronal projections with the ipsilateral M1 (Picard & Strick, 2001; Rushworth et al., 2003). Perhaps iTBS increased the excitatory input from left PMd to ipsilateral M1 and, in turn, increased the resulting corticospinal excitability of the downstream M1. Other studies have shown that rTMS at 5 Hz to left PMd increases cortical excitability in the ipsilateral M1 (Gerschlager et al., 2001; Chouinard et al., 2003; Rizzo et al., 2004; O'Shea et al., 2007; Suppa et al., 2008). The increased excitability from left PMd to ipsilateral M1 may be due to neural projections to M1 in both hemispheres that may be particularly involved in motor preparatory sequences, particularly for visually cued tasks (Schluter et al., 1998; Modugno et al., 2001; Rushworth et al., 2003). Interestingly, similar to other studies using different TBS protocols to PMd (i.e. cTBS), the effects on corticospinal excitability in M1 are observed after a delay (Huang et al., 2009). Perhaps this results from left PMd reciprocal connections with M1 in both hemispheres, with interactions between both excitatory and

inhibitory projections, which may in turn also influence right M1 to left M1 projections (Asanuma & Okuda, 1962; Ferbert et al., 1992; Gerloff et al., 1998; Chen et al., 2003; Nelson et al., 2009), that could lead to a delay in the observable excitability changes in left M1. These studies along with the current results demonstrate that cortical excitability in M1 may be modulated by inputs from the upstream ipsilateral PMd.

Bimanual training effects on left M1 cortical excitability

Enhanced M1 cortical excitability represented by the enlargement of the spatial extent of the muscles involved in a skilled motor task has been shown to occur at the expense of neighbouring limb representations (Pascual-Leone et al., 1995; Nudo et al., 1996; Kleim et al., 1998; Kleim et al., 2004). Modest increases in M1 spatial extent of trained muscles have been observed in a 30 min session of BMT (Neva et al., 2012) and skilled finger sequence training for two 2 hr sessions (Pascual-Leone et al., 1995). However, more substantial increases in spatial extent of M1 require a greater amount of training (Pascual-Leone et al., 1995; Nudo et al., 1996). The increases in spatial extent of M1 observed in the current study resulting from BMT could be due to early stages of unmasking of pre-existing horizontal connections in M1 and the increased synaptic transmission of long-term potentiation (LTP) (Jacobs & Donoghue, 1991).

Cortical activity is enhanced in both hemispheres in damaged and undamaged M1 when homologous muscles are activated together in individual stroke patients (Silvestrini et al., 1998; Staines et al., 2001). Transcallosal neural activity of the homologous muscle representations in M1 could act to excite, but likely releases inhibition to the contralateral hemisphere (Ferbert et al., 1992; Gerloff et al., 1998; Stinear & Byblow, 2002; Chen et al., 2003), possibly leading to short-term M1 plasticity. Intracortical inhibition is

released when upper-limb movements are performed synchronously (in-phase) (Stinear & Byblow, 2002). It is possible that the mere co-activation of the homologous muscles involved in the current bimanual training task increases M1 excitability.

Conclusion

In sum, our findings suggest that iTBS to the left PMd followed by BMT caused a slightly different modulation of M1 excitability than either intervention alone, as shown by the concurrent increase in spatial extent and global MEP amplitude. iTBS to left PMd markedly increased the excitability of ipsilateral (left) M1, as reflected by an increase in the global MEP amplitude. Short-term BMT increased the spatial extent of M1 excitability, as revealed by the expansion along the borders of the trained muscles. These modulations in M1 cortical excitability resulting from BMT and iTBS suggest that they operate under related plasticity mechanisms that may be expressed in distinct ways concurrently. It is possible that the simultaneous activation of homologous M1 representations across both hemispheres, combined with neural input from PMd, promotes the observed concurrent increases in excitability of the trained muscle representations in M1. Critically, this work may guide rehabilitation training and stimulation techniques that modulate cortical plasticity after brain injury and other neurological diseases. It may be that the modulation of remote cortical areas to M1 (i.e. PMd) in combination with rehabilitation training could be advantageous in enhancing short-term plasticity in damaged motor cortex. However, further study is required to understand the potential implications of this research that could be applicable in clinical settings.

Chapter 4 - Study #3

Selective modulation of left primary motor cortex excitability after continuous theta burst stimulation to right primary motor and bimanual training
Prepared for submission.

Jason L. Neva, Amaya M. Singh, Michael Vesia, W. Richard Staines

4.1 Research objective
This study sought to address research objective 3:

3) To investigate the effects of theoretically suppressing the excitability of the contralateral homologous (right) M1 on (left) M1 representation and the potential combined effects when followed by BMT.

Abstract

Activity in motor related cortical areas are enhanced after a single session of bimanual visuomotor training (BMT), and occur specifically when training requires simultaneous activation of homologous muscles (in-phase) and is characterized by an increase in the excitable cortical territory occupied by the trained muscles within the primary motor cortex (M1). This modulation may include connectivity with premotor regions and interhemispheric interactions between homologous muscle representations in M1. Continuous theta burst stimulation (cTBS) to M1 suppresses motor evoked potentials (MEPs) from the stimulated M1. Few studies suggest that cTBS to right hemisphere M1 (rM1) increases corticospinal activity in lM1. Also, motor function of the affected limb improves in stroke patients after applying cTBS to the contralesional M1 or S1 in combination with movement training. The purpose of this study is to investigate the effects of cTBS to rM1 on wrist extensor representation in lM1, and its potential effects when followed by BMT. This experiment tests the hypothesis that cTBS to rM1 will increase excitability of the extensor carpi radialis (ECR) in lM1, and the addition of BMT will cause a greater increase. lM1 excitability was quantified using transcranial magnetic

stimulation (TMS) in terms of both the amplitudes and spatial extent of motor evoked potentials (MEPs) for the extensor carpi radials (ECR) muscle representation before and multiple time points following 1) BMT, 2) cTBS to rM1 or 3) cTBS to rM1 and BMT. The combination of cTBS to rM1 and BMT demonstrated an increased shift in the center of gravity (CoG) compared to either intervention alone. Spatial extent of lM1 excitability was prolonged to 60 minutes when cTBS to rM1 was combined with BMT compared to cTBS to rM1 alone. Both spatial extent and map volume were enhanced with BMT alone and cTBS to rM1 alone at 30 min post stimulation, without an increase when cTBS to rM1 was combined with BMT. These results suggest that modulation of one M1 may alleviate ongoing interhemispheric inhibition (or increase facilitation) to the opposite M1 in healthy individuals via transcallosal or subcortical connections. Critically, this work may guide rehabilitation training and stimulation techniques that modulate cortical plasticity after brain injury.

Introduction

Visuomotor movement training modulates the excitability in several cortical areas, namely, motor (Jacobs & Donoghue, 1991; Pascual-Leone et al., 1995; Classen et al., 1998; Karni et al., 1998; Kleim et al., 1998; Nudo, 2006; Butler & Wolf, 2007), premotor (PM) (Deiber et al., 1996; Karni et al., 1998; Andres et al., 1999; Jennings & van der Molen, 2005; Smith & Staines, 2006, 2010, 2012), and parietal cortices as well as subcortical areas such as the basal ganglia and cerebellum (Clower et al., 1996; Doyon et al., 1997; Kleim et al., 1998; Seidler & Noll, 2008). Critically, bimanual visuomotor movement training (BMT) yields a greater increase in premotor (Smith & Staines, 2006, 2010, 2012) and M1 (Neva et al., 2012) cortical excitability compared to unimanual

movement training. Further, in select stroke patients, bimanual movement performed with the upper-limbs can increase the excitability within the damaged and undamaged primary motor cortex (M1) (Silvestrini et al., 1998; Staines et al., 2001). Additionally, bimanual arm training has been shown to improve hand and arm function in stroke patients (Mudie & Matyas, 2000; Staines et al., 2001; Cauraugh & Kim, 2002; Luft et al., 2004; McCombe Waller & Whitall, 2008; Cauraugh et al., 2010). Although BMT can modulate the excitability in motor preparation and execution areas as well as improve upper-limb function in patient populations, the underlying neural mechanisms remain unclear.

Modulation of cortical excitability after BMT likely relates to the phase of movement with some influence of emphasizing the motor preparatory aspect of the trained movements (Neva et al., 2012). Specifically, increases in motor preparatory and execution areas occur when BMT involves the simultaneous co-activation of homologous muscle groups (in-phase training), but not with co-activation of antagonist muscle groups (anti-phase training) (Smith & Staines, 2006, 2010, 2012; Neva et al., 2012). Electroencephalography (EEG) work suggests that in-phase BMT modulates preparatory activity in PM cortices and possibly M1. More specifically to M1, transcranial magnetic stimulation (TMS) work has shown that in-phase BMT, but not anti-phase, increases M1 corticospinal excitability. Explicitly, the excitable cortical territory of trained muscle representation increases along the borders without a concurrent increase in excitability of the central representation of that muscle (Neva et al., 2012). The lack of effect due to anti-phase training may relate to the reciprocal inhibition of active versus inactive agonist

and antagonist muscle representations in the contralateral hemispheres (Stinear & Byblow, 2002).

Many animal and human studies indicate that there are extensive reciprocal interhemispheric connections between homologous muscle representations in M1 (Asanuma & Okuda, 1962; Matsunami & Hamada, 1984; Gould et al., 1986; Meyer et al., 1995; Picard & Strick, 2001; Nelson et al., 2009). There are both inhibitory and excitatory connections between the homologous M1 representations, yet inhibition between the hemispheres seems to dominate (Asanuma & Okuda, 1962; Ferbert et al., 1992; Gerloff et al., 1998; Chen, 2004; Nelson et al., 2009). Further, local cortical inhibition in M1 is released between homologous M1 representations the upper-limbs are moved synchronously (in-phase), but inhibition remains with asynchronous (anti-phase) movements (Stinear & Byblow, 2002, 2004). These studies suggest that interhemispheric connections between M1 representations may be a potential neural mechanism, with presumed GABAergic local M1 disinhibition, which underlies the corticospinal modulations observed due to BMT tasks.

Transcranial magnetic stimulation (TMS) has become a useful way to measure and modulate the intracortical and subsequent corticospinal excitability in local areas of the brain. Repetitive TMS (rTMS) can induce lasting modulations of cortical excitability. A specific type of rTMS, known as theta burst stimulation (TBS) (Huang et al., 2005) modulates local cortical excitability with a short period of rapid stimulation. Specifically, when continuous theta burst stimulation (cTBS) is applied to M1, the amplitude of MEPs from the stimulated M1 are suppressed for up to 60 min post stimulation (Huang et al., 2005; Suppa et al., 2008; Ortu et al., 2009), with this effect showing variability across

participants depending upon which interneuron populations are activated by the TMS pulse (Hamada et al., 2013). Additionally, few studies suggest that cTBS applied to the right hemisphere M1 (rM1) increases corticospinal activity in the left M1 (lM1) (Suppa et al., 2008; Meehan et al., 2011). Also, motor function of the affected limb improves in stroke patients after applying cTBS to the contralesional M1 or S1 in combination with movement training (Meehan et al., 2011). Therefore, there is evidence that modulation of the one hemisphere homologous M1 representation can remotely influence excitability of the opposite M1. Although M1 excitability may be modulated by altering the excitability of the opposite M1, the underlying neural mechanisms remain unclear. Furthermore, it is unclear whether the remote modulation of M1 will be additive with the cortical excitability changes observed due to BMT (Smith & Staines, 2006, 2010, 2012; Neva et al., 2012). Therefore, suppression of the rM1 excitability may lead to an increase in the excitability of the lM1 representation of the wrist extensor muscles. Further, since it has been shown that BMT increases cortical excitability of the lM1, perhaps enhancing the excitability of the lM1 (by means of suppressing rM1), may cause an additional enhancement of corticospinal excitability of lM1 when cTBS to the rM1 is followed by BMT.

The current study investigates the effect of cTBS to rM1 on the opposite hemisphere M1 (lM1) in terms of the spatial representation and MEP amplitude of the extensor carpi radialis (ECR) muscle over time. Additionally, this study explores the possible combined effects of cTBS to rM1 applied before BMT on lM1 corticospinal excitability. This study also includes data from a previous study in order to compare each intervention to BMT alone (Neva et al., 2014). Group 1 measures ECR corticospinal

excitability before and three time points following cTBS to rM1. It was hypothesized that cTBS to rM1 would enhance the excitability of the lM1 ECR representation. Group 2 measures ECR corticospinal excitability before and following cTBS to rM1 followed by BMT. It was hypothesized that cTBS to rM1 would enhance the excitability in the lM1, which would potentially cause a greater enhancement of ECR corticospinal excitability when followed by BMT.

Methods

Participants

Twenty-seven healthy, self-reported right-handed participants (12 female; average age= 26±4 years) took part in the study. Participants were divided into three groups with different interventions: BMT (group 1), cTBS to rM1 alone (group 2) and cTBS to rM1 followed by BMT (group 3). Ten individuals participated in group 1, while twelve individuals participated in group 2 and 3 in random order, with five individuals participating in both group 2 and 3. The experimental procedures were approved by the University of Waterloo Office of Research Ethics. All participants provided informed written consent and completed a TMS screening form (Keel et al., 2000).

Electromyographic (EMG) recording

Surface EMG was recorded from the right and left extensor carpi radials (ECR) in the same way as Study #2 (Figure 6).

TMS & Neuronavigation

Focal TMS was performed in the same way as Study #2, with the exception of acquiring the AMT from rM1 (Figures 6 & 7). For cTBS, the theta burst pattern of stimulation (three stimuli delivered at 50 Hz, which were grouped and delivered every 5

Hz) was delivered in continuous blocks for a total of 600 stimuli applied over 40 seconds (Huang et al., 2005). We delivered cTBS to rM1 (Suppa et al., 2008; Meehan et al., 2011) at 80% of AMT.

The modulation of M1 excitability in the left hemisphere was measured in the same way as Study #2 with a few additions listed below. The excitability of lM1 ECR was measured before and multiple time points after i) cTBS to rM1 alone and ii) cTBS to rM1 followed by BMT. Additionally, changes in map volume were assessed similarly to global MEP amplitude, with the exception of summing all of the "active sites" rather than averaging (Wolf et al., 2004; Kleim et al., 2007). To assess changes in the MEP amplitude of the hotspot were averaged and compared before and after cTBS to rM1 and cTBS to rM1 followed by BMT.

Behavioural task

Refer to Study #2 and Figure 6.

Statistical analysis

Analysis was performed in two ways. First, to specifically investigate the temporal factors of each intervention, analysis was performed within each group across all time points with the dependent measures of spatial extent, global, central, hotspot MEP amplitude and map volume. Therefore, for each group, a repeated measures ANOVA was performed with TIME as a factor (group 1: BMT – pre, 30 min post; group 2: cTBS to rM1 alone – pre, 5, 30, 60 min post; group 3: cTBS to rM1 + BMT – pre, 30, 60 min post). In addition, for groups 2 and 3, *pre-planned* contrasts were performed between pre and 30 and 60 min post intervention to test the hypothesis that lM1 excitability would be greatest at 30 minutes post intervention and then would return to

baseline levels at 60 min post intervention. *Post hoc* analyses were performed with the Tukey correction method to investigate any other differences between time points. Second, as an exploratory measure that the combination of cTBS to rM1 and BMT would possibly yield an additional increases in M1 excitability than cTBS to rM1 alone, a one-way ANOVA was performed on all groups with the difference score between pre and post 30 min time points for the spatial extent, global, central MEP, hotspot amplitude and map volume data with between-subjects factor GROUP (BMT only, cTBS to rM1 only, cTBS to rM1 + BMT). Similarly, to explore whether there were any additional increases in M1 excitability between cTBS to rM1 alone (group 2) and cTBS to rM1 followed by BMT (group 3), a one-way ANOVA was performed on all groups with the difference score between pre and post 60 min time points for the spatial extent, global, central, hotspot MEP amplitude and map volume data with between-subjects factor GROUP (cTBS to rM1 only, cTBS to rM1 + BMT).

Behavioural performance for group 1 (Neva et al., 2014) and group 3 were quantified in the same way as in Study #2.

Results

Participants, motor thresholds and map distributions

For group 2, one data point (post 30 min) for one participant was not able to be collected due to technical difficulties, and thus has been excluded from analysis. The motor thresholds were consistent across groups (group 1 – mean rMT = 47 ± 6.8%; group 2 – mean left M1 rMT = 46 ± 8%, right M1 rMT = 47 ± 4%, and mean right M1 AMT = 44 ± 6%; group 3 – mean left M1 rMT = 45 ± 7%, right M1 rMT = 55 ± 6%, and mean right M1 AMT = 44 ± 6%). Similarly, the size of the stimulated spatial map area was

similar across groups (average number of grid positions acquired in the pre: group 1 = 29 ± 6; group 2 = 30 ± 9; group 3 = 26 ± 6).

Contour maps and center of gravity (CoG)

Figure 10 shows the representative cortical output maps of the right ECR muscle (leftward panel) and the means of the resultant displacement (medial-lateral and anterior-posterior) of the center of gravity (CoG) (rightward panel) for all groups at all time points. For group 1, the ECR cortical excitability map in the left hemisphere increased after a single session of BMT as shown previously (Figure 10A) (Neva et al., 2012). For group 2, the center of the ECR cortical representation increased slightly immediately after cTBS to rM1, and there was further increase at 30 min post (Figure 10B). For group 3, the size of the cortical representation of ECR in the left hemisphere was increased after cTBS to rM1 and BMT at both 30 min and 60 min post intervention (Figure 10C). Figure 2D shows that the average resultant displacement in CoG shifts across groups and across time points, with a one-way ANOVA revealing an effect of GROUP ($F(2, 31)=4.767$, $p=0.016$) at 30 min post. *Post hoc* analyses revealed a greater shift of CoG in group 3 (cTBS to rM1 + BMT) compared to group 1 (BMT only) ($p<0.05$). A one-way ANOVA showed no difference between group 2 and 3 for 60 min post ($F(1, 22)=0.001$, $p=0.978$).

Spatial extent

To further analyze the data of all groups, figure 11 displays the spatial map, global, and central MEP amplitude before and after the intervention of (1) in-phase BMT only (white bars), (2) cTBS to rM1 (black bars), and (3) cTBS to rM1 followed by BMT (grey bars). Figure 11A shows the spatial extent of left M1 as the average number of active sites (with standard error). There was an increase in spatial extent for groups 1 and

3, which performed the BMT after cTBS to rM1, as evidenced by the increased number of active sites. For group 1 (white), a one-way repeated measures ANOVA revealed an increase in active sites between before and after in-phase BMT alone ($F(1, 9)=16.943$, $p=0.003$). Additionally, for group 2 (black), a repeated measures ANOVA revealed no increase in active sites from cTBS to rM1 ($F(3, 33)= 2.22$, $p=0.105$). However, *pre-planned contrast* analyses revealed a significant increase between pre and 30 min ($F(1, 32)=5.81$, $p=0.022$). Since there were no other statistical differences, this indicates no increase immediately after cTBS to rM1 and a return to baseline levels 60 min post stimulation. Finally, for group 3 (grey), a repeated measures ANOVA revealed an increase in active sites from cTBS to rM1 combined with bimanual training ($F(2, 22)= 10.06$, $p=0.0008$). *Pre-planned contrast* revealed a significant increase in active sites between pre and 30 min ($F(1, 32)=9.26$, $p=0.006$). *Post hoc* analyses revealed an unexpected increase in active sites between pre and 60 min post ($p<0.05$), with no difference between 30 min and 60 min post ($p>0.05$). Additionally, there were no differences across all groups between pre and the 30 min time point post, as a one-way ANOVA revealed no effect of GROUP ($F(2, 31)=0.072$, $p=0.931$). Interestingly, there was a significant increase in active sites for group 3 (cTBS to rM1 + BMT) when comparing the difference between pre and the 60 min post time point between group 2 and 3, as a one-way ANOVA revealed an effect of GROUP ($F(1, 22)=6.832$, $p=0.016$).

Global MEP amplitude

Figure 11B *left* shows the global MEP amplitude before and after the intervention of (1) in-phase BMT (white bars), (2) cTBS to rM1 (black bars), and (3) cTBS to rM1 combined with BMT (grey bars) (with standard error). There was an increase in global

MEP amplitude for groups 1 and 2. A one-way repeated measures ANOVA performed on group 1 (white) revealed a slight increase in global MEP amplitude after BMT, which was near significance ($F(1, 9)=4.530$, $p=0.062$). Additionally, for group 2 (black), a repeated measures ANOVA revealed no increase in global MEP amplitude from cTBS to rM1 ($F(3, 33)= 2.13$, $p=0.115$). *Pre-planned contrast* revealed a near significant increase in global MEP amplitude from pre to post 30 min time point ($F(1,33)=4.11$, $p=0.06$). Finally, for group 3 (grey), a repeated measures ANOVA revealed no increase in global MEP amplitude due to cTBS to rM1 combined with BMT ($F(2,22)= 1.9$, $p=0.173$). Similarly, *pre-planned contrast* revealed no increase in global MEP amplitude. In addition, a one-way ANOVA comparing the difference between pre and post 30 min revealed no difference across GROUP ($F(2, 31)=0.078$, $p=0.926$). Similarly, there was no increase in global MEP amplitude when comparing the difference between pre and the 60 min post time point between group 2 and 3, as a one-way ANOVA revealed no effect of GROUP ($F(1, 22)=0.010$, $p=0.921$).

Central MEP amplitude

Figure 11C *left* displays central MEP amplitude before and after the interventions of all three groups (with standard error). There was no increase in central representation of M1 excitability of ECR in the left hemisphere. A repeated measures ANOVA revealed no effect of BMT (group 1 – white) ($F(1, 9)=1.918$, $p=0.199$), cTBS to rM1 (group 2 – black) ($F(3, 33)=1.43$, $p=0.250$) and cTBS to rM1 combined with BMT (group 3 – grey) ($F(2, 22)=0.74$, $p=0.490$) on central MEP amplitude of M1 ECR representation in the left hemisphere across all time points. Similarly, *pre-planned contrasts* revealed no increases in central MEP amplitude. Additionally, a one-way ANOVA comparing the difference

between pre and post 30 min revealed no difference across GROUP (F (2, 31)=0.211, p=0.811). Similarly, there was no increase in central MEP amplitude when comparing the difference between pre and the 60 min post time point between group 2 and 3, as a one-way ANOVA revealed no effect of GROUP (F (1, 22)=0.094, p=0.763).

Hotspot MEP amplitude

Figure 11D *right* displays hotspot MEP amplitude before and after the interventions of all three groups (with standard error). There was a slight decrease in M1 excitability at the hotspot for the ECR representation in the left hemisphere for groups 2 and 3. A repeated measures ANOVA revealed no effect of BMT (group 1 – white) (F (1, 9)=1.918, p=0.199), cTBS to rM1 (group 2 – black) (F (3, 33)=2.12, p=0.117) and cTBS to rM1 combined with BMT (group 3 – grey) (F (2, 22)=1.9, p=0.173) on hotspot MEP amplitude of M1 ECR representation in the left hemisphere across all time points. Similarly, *pre-planned contrasts* revealed no increases in hotspot MEP amplitude. Additionally, a one-way ANOVA comparing the difference between pre and post 30 min revealed no difference across GROUP (F (2, 31)=2.594, p=0.091). Similarly, there was no increase in hotspot MEP amplitude when comparing the difference between pre and the 60 min post time point between group 2 and 3, as a one-way ANOVA revealed no effect of GROUP (F (1, 22)=0.118, p=0.735).

Map Volume

Figure 11E *right* shows the map volume before and after the intervention of (1) in-phase BMT (white bars), (2) cTBS to rM1 (black bars), and (3) cTBS to rM1 combined with BMT (grey bars) (with standard error). There was an increase in map volume for groups 1 and 2. A one-way repeated measures ANOVA performed on group

1 (white) revealed an increase in map volume after BMT ($F(1, 9)=6.310$, $p=0.033$). Additionally, for group 2 (black), a repeated measures ANOVA revealed a slight increase in global MEP amplitude from cTBS to rM1 ($F(3, 33)= 2.58$, $p=0.070$). *Pre-planned contrast* revealed a significant increase in map volume from pre to post 30 min time point ($F(1, 33)=5.75$, $p=0.022$). Finally, for group 3 (grey), a repeated measures ANOVA revealed no increase in map volume due to cTBS to rM1 combined with BMT ($F(2, 22)= 2.77$, $p=0.084$). Similarly, *pre-planned contrast* revealed no increase in map volume. In addition, a one-way ANOVA comparing the difference between pre and post 30 min revealed no difference across GROUP ($F(2, 31)=0.178$, $p=0.838$). Similarly, there was no increase in map volume when comparing the difference between pre and the 60 min post time point between group 2 and 3, as a one-way ANOVA revealed no effect of GROUP ($F(1, 22)=0.008$, $p=0.930$).

Behavioural performance

Figure 12 displays the behavioural data of groups 1 (Neva et al. 2014) and 3, with the movement time (leftward panel) and angle at peak velocity (rightward panel). For movement time, a two-way ANOVA revealed a main effect of BLOCK ($F(1, 18)=20.460$, $p<0.001$), no effect of GROUP ($F(1, 18)=0.598$, $p=0.451$) and no interaction of BLOCK x GROUP ($F(1, 18)=0.009$, $p=0.926$). This indicates that both groups 1 and 3 had similar decreases in movement time. For the angle at peak velocity, a two-way ANOVA revealed a main effect of BLOCK ($F(1, 20)=19.252$, $p<0.0001$), an interaction of BLOCK x GROUP ($F(1, 20)=6.227$, $p=0.021$), and a main effect of GROUP ($F(1, 20)=7.439$, $p=0.013$). The main effect of BLOCK indicates that there was a decrease in deviation of cursor path from the initial to the final trials (i.e., performance

improvement). Further analysis on factor BLOCK with separate paired t-tests revealed a significant improvement in performance for group 3 (t (11)=2.969, p=0.013), and an improvement for group 1 (t (9)=3.338, p=0.009). Further, independent samples t-tests with factor GROUP revealed a significantly lower initial angle at peak velocity in the first block in group 3 compared to group 1 (t (20)=-2.930, p=0.008) with no difference between groups at the final block of trails (t (20)=-0.182, p=0.858). Therefore, the differing results between these groups seem to be due to the slightly lower initial angle at peak velocity of the resultant cursor path in group 3.

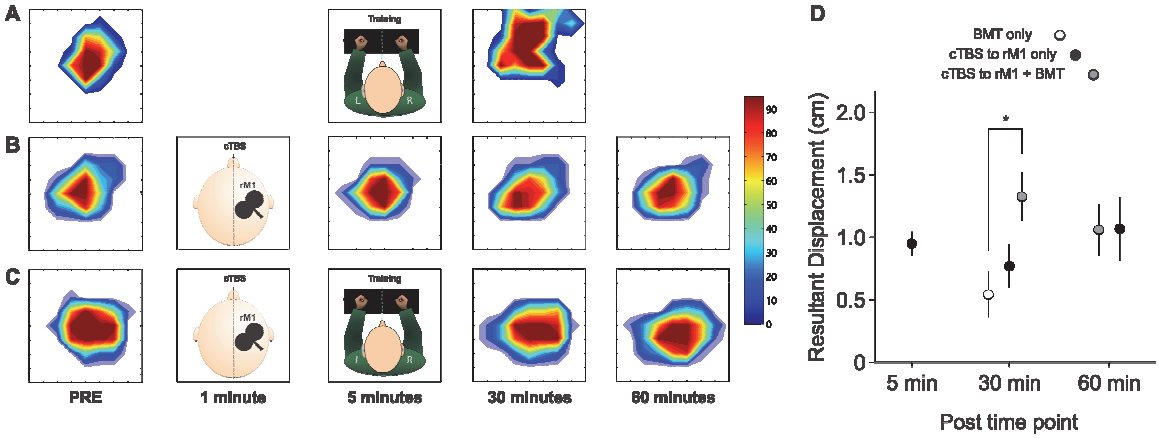


Figure 10. Representative examples of the M1 cortical excitability maps for the ECR muscle for groups 1 (A), 2 (B) and 3 (C) across all time points (post time points are relative to the pre). Red on the scale represents the greatest probability and blue represents the least probability to generate a MEP. An amplitude of ≥ 30 μV peak-to-peak in the ECR was considered an MEP. (D) Means of the resultant displacement (medial-lateral and anterior-posterior) of the center of gravity (CoG) for all participants (time points relative to the pre). All bars represent SEM. Asterisk indicates significance, $p < 0.05$.

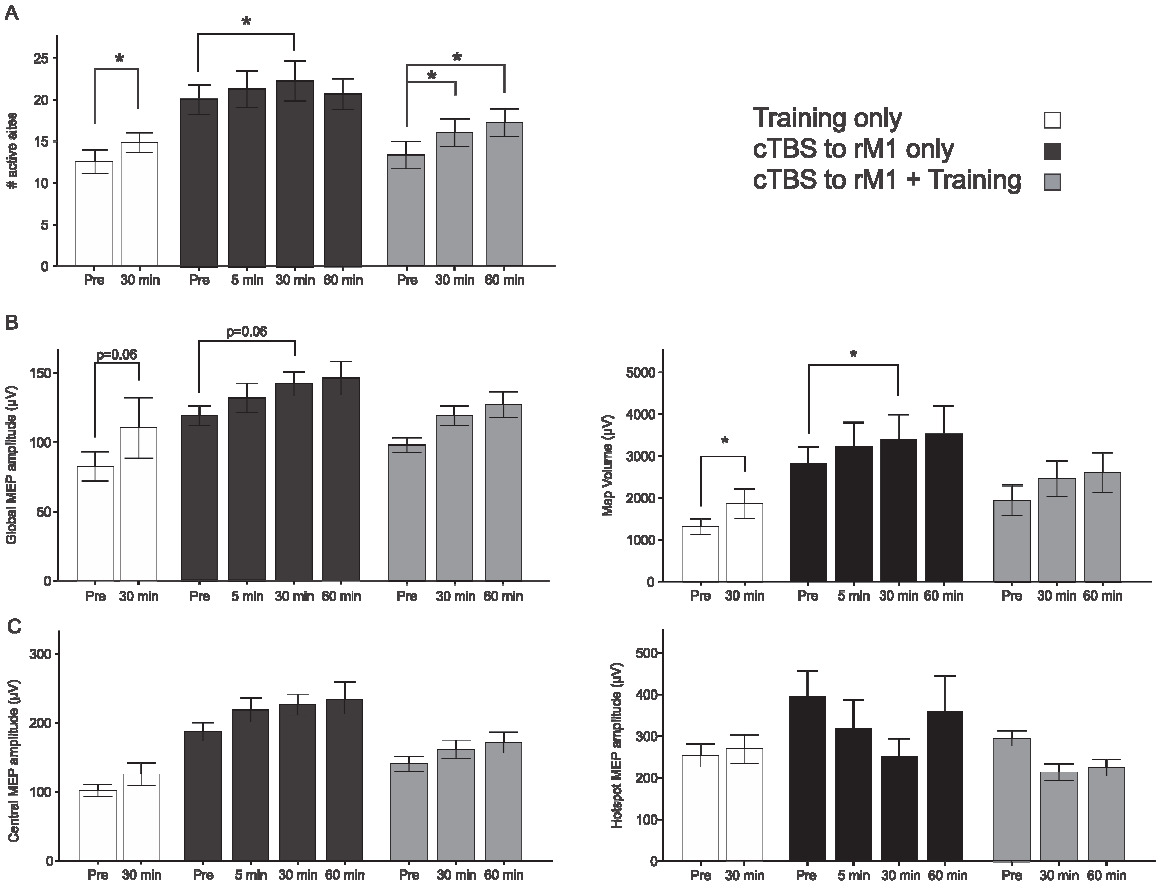


Figure 11. Means of all dependent measures for left M1 ECR excitability for all participants before and after bimanual training and/or cTBS to rM1. Group 1: BMT (white). Group 2: cTBS to rM1 (black). Group 3: cTBS to rM1 + BMT (grey). (A) Spatial maps displayed by number of active sites. (B) *Left* Global MEP amplitude, *Right* Map volume. (C) *Left* Central MEP amplitude, *Right* Hotspot MEP amplitude. All bars represent SEM. Asterisk indicates significance, $p < 0.05$.

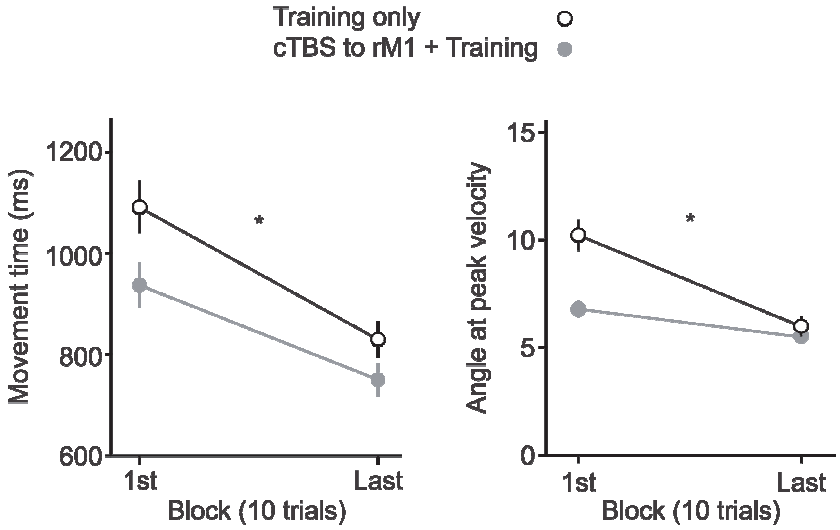


Figure 12. Behavioural data for groups 1 and 3. *Left* Movement time for Group 1 (white) and Group 3 (grey). *Right* Angle at peak velocity of the resultant cursor path for Group 1 (white) and Group 3 (grey). All bars represent SEM. Asterisk indicates significance, $p < 0.05$.

Discussion

The current study is the first to demonstrate the early indicators of motor cortex plasticity associated with short-term BMT following cTBS to rM1. The specific modulations in left M1 excitability resulting from each intervention indicate that they may operate under related neural mechanisms, possibly additively facilitating changes in cortical excitability. The combination of cTBS to rM1 and BMT demonstrated an increased shift in the center of gravity (CoG) compared to either intervention alone. Additionally, the increased spatial extent of M1 excitability was prolonged to 60 minutes when cTBS to rM1 was combined with BMT compared to cTBS to rM1 alone. Motor cortex mapping showed that both spatial extent and map volume were enhanced with BMT alone (Neva et al., 2014) and cTBS to rM1 alone at 30 min post stimulation, without an increase when cTBS to rM1 was combined with BMT. The effect of cTBS to rM1 alone indicates that homologous right M1 may remotely modulate the excitability of the left hemisphere M1, and the effect of BMT alone confirms that left M1 excitability is enhanced along the borders of ECR representation (Neva et al., 2012, Neva et al., 2014).

The combined effects of cTBS to rM1 with BMT

This study is the first to demonstrate that applying cTBS to rM1 before BMT increases excitability in the left M1 in terms of a shift in the centre of gravity and a prolonged increase in spatial extent. One possibility is that cTBS to rM1 released inhibition (or increased facilitation) from the right to left hemisphere M1 and facilitated the BMT-induced excitability enhancements. The homologous M1 to M1 reciprocal

connections have been demonstrated in many animal and human studies, with inhibitory connections dominating between the hemispheres (Asanuma & Okuda, 1962; Matsunami & Hamada, 1984; Gould et al., 1986; Asanuma & Keller, 1991; Meyer et al., 1995; Picard & Strick, 2001; Nelson et al., 2009). Local inhibition between the homologous M1 representations of the upper-limbs is released when movements are made synchronously (in-phase) (Stinear & Byblow, 2002, 2004). Additionally, cTBS applied to the right hemisphere M1 (rM1) increases corticospinal activity in the left M1 (lM1) (Suppa et al., 2008; Meehan et al., 2011). Also, motor function of the affected limb improves in stroke patients after applying cTBS to the contralesional M1 or S1 in combination with movement training (Meehan et al., 2011). These studies suggest that interhemispheric connections between M1 representations may be a potential neural mechanism, with presumed GABAergic local M1 disinhibition, which underlies the corticospinal modulations observed due to BMT tasks. It is possible that cTBS applied to the opposite M1 before performing BMT caused a release of inhibition (or increased facilitation) from the right to the left hemisphere, which leads to increased and prolonged effects when combined with BMT. This release of inhibition (or increased facilitation) from right to left M1 may have led to the enhanced initial behavioural performance observed when followed by BMT, compared to BMT alone. Overall, it could be that simultaneous activation of homologous muscles, with the addition of an increased excitable input from rM1, led to the slightly greater enhancement in left M1 ECR excitability observed in this study.

cTBS to right M1 on left M1 cortical excitability

This study found that cTBS to rM1 increased the excitability of contralateral M1, in terms of spatial extent and map volume. The homologous M1 to M1 reciprocal connections have been demonstrated in many animal and human studies, with inhibitory connections dominating between the hemispheres (Asanuma & Okuda, 1962; Matsunami & Hamada, 1984; Gould et al., 1986; Asanuma & Keller, 1991; Meyer et al., 1995; Picard & Strick, 2001; Nelson et al., 2009). It is thought that these interhemispheric M1 connections are primarily mediated by projections through the body of the corpus callosum (CC), as paired pulse TMS studies have shown decreased or absent interhemispheric interactions in patients without a an intact CC (Ferbert et al., 1992; Chen et al., 2002; Daskalakis et al., 2004; Avanzino et al., 2007). It has been shown that these interhemispheric connections between M1s can also be influenced by rTMS over either hemisphere M1. Several studies have shown differing results using rTMS, with some research showing an increased excitability after applying rTMS at 1 Hz to the contralateral M1 and some showing a decreased excitability (Wassermann et al., 1998; Gilio et al., 2003; Gorsler et al., 2003; Plewnia et al., 2003; Schambra et al., 2003; Pal et al., 2005; Heide et al., 2006). Possible reasons for these conflicting results may be the different intensities used during rTMS, the orientation of the TMS coil (i.e. anterior-posterior, posterior-anterior), and stimulation of either the dominant or non-dominant hand representation. However, a recent study suggests that hand dominance does not play a role in the effects produced by rTMS protocols such as theta burst stimulation (TBS) (Suppa et al., 2008).

Results from other rTMS protocols (i.e. TBS) has shown conflicting results on the non-stimulated contralateral M1. Few studies have shown that cTBS applied to the right

hemisphere M1 (rM1) increases cortical excitability in the left M1 (lM1) (Stefan et al., 2008; Suppa, Bologna, et al., 2008; Meehan et al., 2011), whereas one such study showed a decrease excitability in the contralateral M1 (Ishikawa et al., 2007). The differing results of the mentioned studies may be due to the slightly different intensities used to apply the CTBS (70% versus 80% of AMT). Specifically, it seems when cTBS is applied to the rM1 with an anterior-posterior coil orientation, MEPs are increased in the lM1 as well as a reduction in local inhibition (i.e. short-interval intracortical inhibition (SICI)), while it decreased MEPs and increase SICI in the stimulated rM1 (Suppa et al., 2008). It is possible that the application of cTBS to the right hemisphere M1 in the current study lead to a decreased rM1 excitability, decreasing SICI and an increasing excitability in the left M1, which resulted in the enhanced spatial extent and map volume of the entire ECR representation in left M1.

BMT

The effects of in-phase BMT enhancing M1 cortical excitability has been shown and discussed in previous studies (Neva et al., 2012; Neva et al., 2014).

Conclusion

In summary, the findings of the current study suggest that cTBS to the right M1 followed by BMT demonstrated an increased shift in the center of gravity (CoG) compared to either intervention alone and an increased spatial extent of M1 excitability up to 60 minutes post intervention. cTBS to rM1 alone and BMT alone increased spatial extent and map volume, increasing the cortical excitability along the borders of the trained muscle representation. These modulations in M1 cortical excitability resulting from BMT and cTBS suggest that they operate under related plasticity mechanisms that

may be expressed distinctly. It is possible that the simultaneous activation of homologous M1 representations across both hemispheres, combined with enhanced neural input from right M1, promotes the observed combined increases in excitability of the trained muscle representation in left M1. Critically, this work may guide rehabilitation training and stimulation techniques that modulate cortical plasticity after brain injury and other neurological diseases. It may be that the modulation of related cortical areas to M1 (i.e. contralateral M1) in combination with rehabilitation training could be advantageous in enhancing short-term plasticity in damaged motor cortex. However, further study is required to understand the potential implications of this research that could be applicable in clinical settings.

Chapter 5 - Study #4

Cortical adaptations within and between the primary motor cortices after bimanual training and theta burst stimulation to the left dorsal premotor cortex
Prepared for submission.

Jason L. Neva, Michael Vesia, Amaya M. Singh, W. Richard Staines

5.1 Research objective
This study sought to address research objective 4:

4) To investigate the intracortical and interhemispheric excitability circuitry within and across M1 bilaterally due to short-term BMT, the enhancement of left PM input, and the combination of these interventions.

Abstract

Activity in motor related cortical areas are enhanced after a single session of bimanual visuomotor training (BMT) involved in motor preparation and execution. These changes in cortical excitability occur specifically when training requires simultaneous activation of homologous muscles (in-phase) and is characterized by an increase in the excitable cortical territory occupied by the trained muscles within the primary motor cortex (M1). These modulations may include interhemispheric interactions between homologous muscle representations in M1 and connectivity with premotor regions, like the dorsal premotor cortex (PMd). Specifically, the effects of short-term in-phase BMT was enhanced when training was preceded by intermittent theta burst stimulation (iTBS) to the left hemisphere PMd (lPMd). This study investigates the possible intracortical and interhemispheric modulations of the extensor carpi radials (ECR) in M1 bilaterally due to: 1) BMT, 2) iTBS to lPMd, and 3) the combination of these interventions. This study tests three related hypotheses in three separate experiments: 1) BMT will enhance excitability within and between M1 bilaterally, 2) iTBS to lPMd will primarily enhance

lM1 excitability, and 3) the combination of these interventions will cause a greater enhancement of bilateral M1 cortical excitability. This study quantified MEPs, short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), long-interval intracortical inhibition (LICI), cortical silent period (CSP), and interhemispheric inhibition (IHI) for the ECR in M1 bilaterally. BMT alone resulted in facilitated MEPs in both hemispheres, an increase in CSP in the right M1 and a decrease in IHI from the left to right M1. iTBS to the lPMd increased the CSP in the left M1, and when iTBS to lPMd preceded BMT there was increased MEPs and decreased LICI in M1. These results demonstrate the possible neural mechanisms that may underlie the early indications of rapid functional plasticity associated with BMT and iTBS to lPMd, which may be related to a decreases of in long-latency inhibitory mechanisms within and between M1s. Critically, this work may guide rehabilitation training and stimulation techniques that modulate cortical plasticity after brain injury.

Introduction

Visuomotor movement training modulates the excitability in several cortical areas, namely, motor (Jacobs & Donoghue, 1991; Pascual-Leone et al., 1995; Classen et al., 1998; Karni et al., 1998; Kleim et al., 1998; Nudo, 2006; Butler & Wolf, 2007), premotor (PM) (Deiber et al., 1996; Karni et al., 1998; Andres et al., 1999; Jennings & van der Molen, 2005; Smith & Staines, 2006, 2010, 2012), and parietal cortices as well as subcortical areas such as the basal ganglia and cerebellum (Clower et al., 1996; Doyon et al., 1997; Kleim et al., 1998; Seidler & Noll, 2008). Critically, bimanual visuomotor movement training (BMT) yields a greater increase in premotor (Smith & Staines, 2006, 2010, 2012) and M1 (Neva et al., 2012) cortical excitability compared to unimanual

movement training. Further, in select stroke patients, bimanual movement performed with the upper-limbs can increase the excitability within the damaged and undamaged primary motor cortex (M1) (Silvestrini et al., 1998; Staines et al., 2001). Additionally, bimanual arm training has been shown to improve hand and arm function in stroke patients (Mudie & Matyas, 2000; Staines et al., 2001; Cauraugh & Kim, 2002; Luft et al., 2004; McCombe Waller & Whitall, 2008; Cauraugh et al., 2010). Although BMT can modulate the excitability in motor preparation and execution areas as well as improve upper-limb function in patient populations, the underlying neural mechanisms remain unclear.

Modulation of cortical excitability after BMT likely relates to the phase of movement and emphasizing the motor preparatory aspect of the trained movements (Neva et al., 2012). Specifically, increases in the excitability of motor preparatory and execution areas occur when BMT involves the simultaneous co-activation of homologous muscle groups (in-phase training), but not when co-activation of antagonist muscle groups (anti-phase training) (Smith & Staines, 2006, 2010, 2012; Neva et al., 2012). Electroencephalography (EEG) work suggests that in-phase BMT modulates preparatory activity in PM cortices and possibly M1. More specifically to M1, transcranial magnetic stimulation (TMS) work has shown that in-phase BMT, but not anti-phase, increases M1 excitability. Specifically, the excitable cortical territory of trained muscle representation increases along the borders without a concurrent increase in excitability of the central representation of that muscle (Neva et al., 2012). The lack of effect due to anti-phase training may relate to the reciprocal inhibition of active versus inactive agonist and antagonist muscle representations in the contralateral hemispheres (Stinear & Byblow,

2002). In addition, motor preparation associated with a goal-directed movement during training increases cortical excitability and, in turn, improves behavioural performance (Deiber et al., 1996; Sohn & Carlson, 2000; Jennings & van der Molen, 2005; Smith & Staines, 2006, 2010, 2012). Conversely, without this goal-directed motor preparation, cortical activation is slightly decreased and task performance generally declines (Deiber et al., 1996).

Covertly and overtly preparing movements to a target stimulus decreases reaction times (RTs) and increases activity in PM cortices (Sheliga et al., 1995; Deubel & Schneider, 1996; Nobre et al., 2000; Corbetta & Shulman, 2002). The dorsal premotor cortex (PMd) has well-known roles in the selection of appropriate actions for movement execution (Kalaska & Crammond, 1995; Thoenissen et al., 2002; O'Shea et al., 2007; Groppa et al., 2012). Interestingly, neuroimaging and TMS research suggest that PMd in the left hemisphere has an important role in action selection for motor execution (Geyer et al., 2000; Toni et al., 2001). Specifically, PMd seems to be particularly involved in movement selection with learned visuomotor associations (Geyer et al., 2000; Toni et al., 2001). Also, left PMd activity increases with action selection of one or both upper-limbs (Schluter et al., 2001). Further, when the right PMd is disrupted with inhibitory TMS, action selection is hindered in the contralateral hand alone. Conversely, disruption of left PMd leads to a disruption in action selection of both upper-limbs (Schluter et al., 1998; Johansen-Berg et al., 2002). Similarly, repetitive TMS to left PMd causes faster preparation of complex sequences performed with the right hand (Stinear et al., 2009). Additionally, iTBS applied to lPMd before performing a short-term session of BMT leads to enhanced M1 excitability in the left hemisphere compared to either BMT or iTBS to

lPMd alone, in terms of both increases in spatial extent and global MEP amplitude (Neva et al., 2014). This suggests that the lPMd has a particularly relevant role in movement selection as well as the visuomotor movement training with both upper-limbs simultaneously.

There are extensive reciprocal interhemispheric connections between homologous muscle representations in M1 (Asanuma & Okuda, 1962; Matsunami & Hamada, 1984; Gould et al., 1986; Meyer et al., 1995; Picard & Strick, 2001; Nelson et al., 2009). Although there are inhibitory and excitatory connections between the homologous M1 representations, inhibition seems to dominate (Asanuma & Okuda, 1962; Ferbert et al., 1992; Gerloff et al., 1998; Chen, 2004; Nelson et al., 2009). Local cortical inhibition in M1 is decreased between homologous M1 representations the upper-limbs are moved synchronously (in-phase), but inhibition remains with asynchronous (anti-phase) movements (Stinear & Byblow, 2002, 2004). These studies suggest that interhemispheric connections between M1 representations may be a potential neural mechanism mediating cortical excitability changes due to synchronous upper-limb movements, with presumed GABAergic local M1 disinhibition. However, it is unclear if these interhemispheric connections are modulated by altering cortical excitability of remote but related cortical areas (i.e. lPMd), and in combination with short-term BMT.

Transcranial magnetic stimulation (TMS) is a useful way to measure and modulate the intracortical and subsequent corticospinal excitability in local areas of the brain. Repetitive TMS (rTMS) can induce lasting modulations of cortical excitability. A specific type of rTMS, known as theta burst stimulation (TBS) (Huang et al., 2005) modulates local cortical excitability with a short period of rapid stimulation. Specifically,

when continuous theta burst stimulation (cTBS) is applied to M1, the amplitude of MEPs from the stimulated M1 are suppressed for up to 60 min post stimulation (Huang et al., 2005; Suppa et al., 2008; Ortu et al., 2009), with this effect showing variability across participants depending upon which interneuron populations are activated by the TMS pulse (Hamada et al., 2013). Further, cTBS to PMd decreases MEP amplitude of the ipsilateral M1 representation (Huang et al., 2009; Ortu et al., 2009). Subthreshold rTMS to PMd decreases ipsilateral M1 excitability when delivered at 1 Hz, and increases excitability when delivered at 5 Hz (Gerschlager et al., 2001; Chouinard et al., 2003; Rizzo et al., 2004; O'Shea et al., 2007; Suppa et al., 2008). This suggests that M1 excitability may be differentially modulated by unique stimulation patterns to remote and related areas, like PMd. Specifically, there are strong excitatory anatomical connections between the PM and M1 cortices, particularly within the left hemisphere (Picard & Strick, 2001; Rushworth et al., 2003; Koch et al., 2007). Left hemisphere M1 excitability is enhanced by applying iTBS to lPMd, in terms of the overall MEP amplitudes of the entire ECR representation for up to 60 min post stimulation (Neva et al., 2014). Although there were specific modulations of M1 excitability due to BMT, iTBS to lPMd and the combination of these interventions, the underlying neural mechanisms are unclear.

Despite the known anatomical connectivity between PMd and M1, and the known roles of PMd in motor preparation, action selection and visuomotor associations, little is known about the functional significance of PMd to M1 in both hemispheres. Further, it is not understood how BMT alone, iTBS to lPMd alone or the combination of these interventions influences the excitatory and inhibitory networks within and across M1

bilaterally. This study investigates modulations of MEPs, CSP as well as intracortical and interhemispheric circuitry (SICI, ICF, IHI, LICI) within and across M1 extensor carpi radials (ECR) representation bilaterally due to 1) BMT, 2) iTBS to lPMd and 3) iTBS to lPMd before BMT. This study tests three related hypotheses in three separate experiments: 1) BMT will enhance excitability within and between M1 bilaterally, 2) iTBS to lPMd will primarily enhance lM1 excitability, and 3) the combination of these interventions will cause a greater enhancement of bilateral M1 cortical excitability.

Methods

Participants

Twenty-seven, self-reported right-handed participants (14 female; average age= 26 years, ± 3.3) took part in the study. Participants were divided into 3 experiments with different interventions: BMT alone (experiment 1), iTBS to lPMd (experiment 2), and iTBS to lPMd followed by BMT (experiment 3). Fourteen individuals participated in experiments 2 and 3 in random order, and these experiments were separated by at least one week. The experimental procedures were approved by the University of Waterloo Office of Research Ethics. All participants provided informed written consent and completed a TMS screening form (Keel et al., 2000).

Electromyographic (EMG) recording

Surface EMG was recorded from the right and left extensor carpi radials (ECR) muscle using 9 mm diameter Ag-AgCl electrodes. Two active electrodes were placed over the muscle belly of the right and left ECR with a ground electrode over the right styloid process of the ulna. EMG recordings were amplified (1000X), band-pass filtered (2-2500 Hz) (Intronix Technologies Corporation Model 2024F, Canada), digitized at a

sample frequency of 5 kHz by an analog-to-digital interface (Micro1401, Cambridge Electronics Design, Cambridge, UK), and stored for later analysis.

TMS & Neuronavigation

Single and paired-pulse magnetic stimulation were delivered using two custom built 50 mm inner diameter figure-of-eight branding coils connected to two Magstim 200^2 stimulators (Magstim, Whitland, UK). TBS was applied in a similar manner as Study #2. The motor hotspot for the ECR in M1s bilaterally were acquired similarly to the previous studies (#1-#3) (see Figure 13).

Behavioural task

Refer to Studies #2 and #3 (see Figure 13).

Experiment 1: BMT

Thirteen participants (7 female; average age= 28 years, ± 3) performed a short-term session of in-phase BMT (Neva et al., 2012, 2014). In thirteen individuals MEPs, short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), long-interval intracortical inhibition (LICI), and interhemispheric inhibition (IHI) was recorded from the ECR bilaterally before and immediately after BMT, as depicted in Figure 13D. Cortical silent period (CSP) was collected in twelve of the thirteen participants. For MEPs, 15 single TMS pulses were applied over the left and right M1. TMS intensity was set at 120% of rMT for both the left and right M1 ECR representation. For SICI and ICF, both the conditioning and test stimuli were applied over M1 with the same coil connected to a Magstim 200^2 stimulator operating via a Bistim module. The paired-pulse paradigms, SICI and ICF, were performed as previously (Kujirai et al., 1993), where a subthreshold conditioning stimulus (CS) is followed by a suprathreshold rest stimulus

(TS) to the M1 hotspot for ECR. The interstimulus interval (ISI) for SICI and ICF was 3 and 10 ms respectively, to produce intracortical inhibition and facilitation (Kujirai et al., 1993; Di Lazzaro et al., 2006). To measure SICI and ICF, a block of TMS pulses consisted of TS alone, ISI of 3 ms (SICI) and ISI of 10 ms (ICF). Each ISI and TS alone trials were randomly presented 15 times during the pre and post collections. The CS was set at 80% of rMT for SICI and ICF, which was determined before BMT and kept consistent throughout the experiment. The TS intensity was adjusted to evoke MEPs in the contralateral ECR of 0.3-0.5 mV before and after BMT (Perez & Cohen, 2008). Fifteen trials with an inter-trial interval of 6 seconds were collected for SICI and ICF in the left and right M1 ECR. LICI was elicited by suprathreshold CS and TS with an ISI of 100 ms (Inghilleri et al., 1993; Nakamura et al., 1997; Chen et al., 1999; Chen, 2004) over M1 ECR representation in both hemispheres. The CS and TS intensities were adjusted to evoke MEPs in the contralateral ECR of 0.3-0.5 mV, just as in the TS of the SICI and ICF conditions (Perez & Cohen, 2008), along with the same number of trials and inter-trial interval. IHI was tested in both cortical directions (left M1 → right M1 and vice versa), with the CS and TS adjusted to evoke MEPs in the contralateral ECR of 0.3-0.5 mV, just as in the LICI condition. The ISIs for IHI were 10 and 40 ms, to produce short and long IHI (SIHI and LIHI) (Ferbert et al., 1992; Chen et al., 2003; Chen, 2004; Perez & Cohen, 2008; Nelson et al., 2009). Similarly to SICI and ICF, a block of TMS pulses consisted of TS alone, ISI of 10 ms (SIHI) and ISI of 40 ms (LIHI). Each ISI and TS alone trials were randomly presented 15 times during the pre and post collections. Finally, CSP (Terao & Ugawa, 2002) was tested with participants maintaining a light contraction of the contralateral ECR of 20% MVC and fifteen single pulses of TMS was

applied to the left and right M1 at an intensity of 130% rMT. The duration of the CSP was acquired from the TMS stimulus onset to the re-onset of muscle activity within the ECR muscle.

Experiment 2: iTBS to lPMd

Fourteen participants (7 female; average age= 24 years, ± 4) received iTBS over PMd in the left hemisphere at 80% of AMT using the 600 pulse protocol (Huang et al., 2005; Ishikawa et al., 2007; Stefan et al., 2008). The location of PMd was determined to be 2.5 cm anterior to the ECR motor hotspot in left M1 (Picard & Strick, 2001; Huang et al., 2009; Stinear et al., 2009). In ten participants MEPs and CSP were recorded, and SICI/ICF, LICI, CSP and IHI were recorded from all fourteen participants using the same methodology as in Experiment 1, with the addition of collection immediately after iTBS was applied to lPMd of MEPs, SICI/ICF and IHI in M1s bilaterally. This was then followed by recording all of the dependent measures as in Experiment 1.

Experiment 3: iTBS to lPMd followed by BMT

The same fourteen individuals (7 female; average age= 24 years, ± 4) received iTBS over PMd in the left hemisphere at 80% of AMT using the 600 pulse protocol (Huang et al., 2005; Ishikawa et al., 2007; Stefan et al., 2008), which was followed by in-phase BMT that was performed in Experiment 1. The location of PMd was determined to be 2.5 cm anterior to the ECR motor hotspot in left M1 (Picard & Strick, 2001; Huang et al., 2009; Stinear et al., 2009). In ten participants MEPs and CSP were recorded, and SICI/ICF, LICI, CSP and IHI were recorded from all fourteen participants using the same methodology as in Experiment 1.

Statistical analysis

Analysis was performed in two ways. First, to specifically investigate the temporal factors of each intervention, analysis was performed within each group across all time points. Therefore, Experiments 1 and 3 used one-way repeated measures analyses of variance (ANOVA) with within-subject factor TIME (2 levels: pre, post) for each dependent measure (MEPs, SICI, ICF, LICI, CSP, IHI) for the left and right ECR. Experiment 2 used one-way repeated measures ANOVA using within-subject factor TIME (3 levels; pre, post 1 min and post 30 min) for each dependent measure as in Experiments 1 and 3. Additionally, *post hoc* analyses were performed with the Tukey correction method to investigate any other differences between time points. Second, as an exploratory measure that the combination of iTBS to lPMd and BMT would possibly yield an additional modulations of M1 intracortical and interhemispheric excitability than iTBS to lPMd or BMT alone, a one-way ANOVA was performed on all groups with the difference score between pre and post 30 min time points for all of the dependent measures (MEPs, SICI, ICF, LICI, CSP, IHI) with between-subjects factor EXPERIMENT (Exp. 1: BMT alone, Exp. 2: iTBS to lPMd only, Exp. 3: iTBS to lPMd + BMT). Where appropriate, *post hoc* analyses were performed with the Tukey correction method to investigate potential differences between experimental interventions. Significance was set at $p \leq 0.05$.

Behavioural performance for Experiments 1 and 3 were quantified in the same way in Studies #2 and #3.

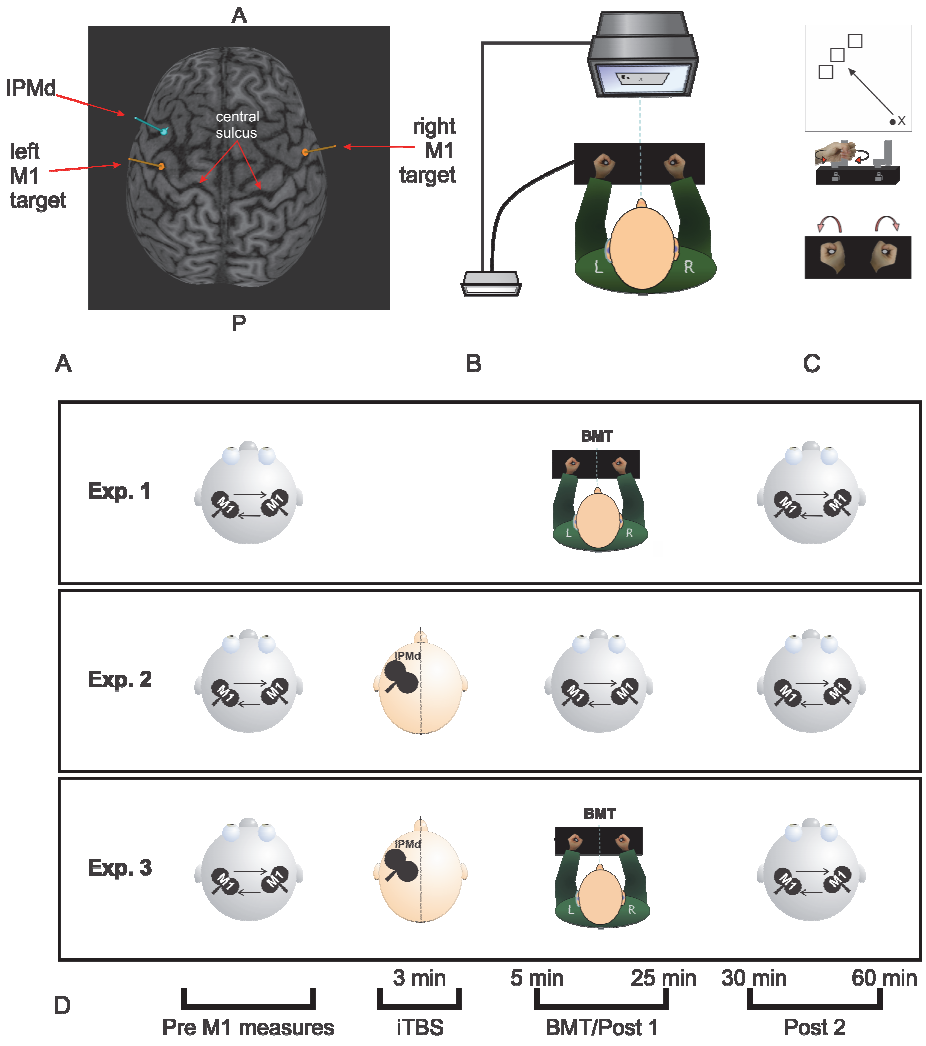


Figure 13. Neuronavigation, experimental set up, and behavioural task. (A) TMS target locations. Template MRI from one session demonstrating the targets used for iTBS in lPMd and M1 bilaterally. A (anterior), P (posterior). Orange lines indicate location of TMS coil placement over M1 ECR representation, and the blue line indicates TMS coil placement over left PMd. (B) Above view of a participant performing the behavioural task, grasping the two handles and viewing both the target and cursor movement on the computer screen. (C) Displays movements made during the bimanual movement training task. Participants began in the bottom right corner and made varying degrees of wrist extension movements to move the cursor to the remembered visual targets. (D) Experimental Time Course. Graphic representation depicting the order of data collection and each experimental interventions. Exp. (experiment), M1 (primary motor cortex), lPMd (left dorsal premotor cortex), iTBS (intermittent theta burst stimulation), BMT (bimanual training), Post 1 (bilateral collection of MEPs, SICI, ICF and IHI in experiment 2 only, immediately after iTBS to lPMd), Post 2 (bilateral collection of MEPs, SICI, ICF, IHI, LICI and CSP).

Results

Experiment 1: BMT

MEPs. The motor thresholds were consistent among participants (mean left M1 rMT = 40 ± 4%, right M1 rMT = 41 ± 6%). Figure 14A displays the MEPs for the left (top) and right (bottom) M1 ECR representations. A one-way repeated measures ANOVA revealed an increase in amplitude in the left M1 ($F(1, 11)=5.858$, $p=0.034$) and a near significant increase in the right M1 ($F(1, 11)=4.358$, $p=0.061$).

SICI/ICF. Two participants (one for each hemisphere) were removed for SICI due to not displaying inhibition in the pre measure. Figure 14B displays the SICI and ICF data for the left (top) and right (bottom) M1 ECR representations. For SICI, a one-way repeated measures ANOVA revealed no changes in the left M1 ($F(1, 10)=0.390$, $p=0.546$) or right M1 ($F(1, 10)=0.582$, $p=0.463$). For ICF, a one-way repeated measures ANOVA revealed no change for left M1 ($F(1, 11)<0.0001$, $p=0.995$) and near significant decrease in right M1 ($F(1, 11)=4.703$, $p=0.053$).

IHI. Three participants were removed in the left M1 (SIHI and LIHI) and two participants were removed in the right M1 (LIHI) due to not displaying inhibition in the pre measure. Figure 14C shows the IHI data for the left (top) and right (bottom) M1 representations for both the SIHI and LIHI. For SIHI, a one-way repeated measures ANOVA revealed no change from the right to the left M1 ($F(1, 8)=0.043$, $p=0.840$) or left to right M1 ($F(1, 11)=0.006$, $p=0.942$). For LIHI, a one-way repeated measures ANOVA revealed no change in the right to left M1 ($F(1, 8)=0.253$, $p=0.629$), with a significant decrease from the left to right M1 ($F(1, 9)=6.602$, $p=0.030$).

LICI. Conditioning stimulus MEPs were similar in the pre and post measures for the left (t (12)=1.095, p=0.295) and right M1 (t (12)=0.460, p=0.653). Figure 14D shows the LICI data for the left (top) and right (bottom) M1 representations. A one-way repeated measures ANOVA revealed a slight decrease in the left M1 (F (1, 11)=3.422, p=0.091) and no change in the right M1 (F (1, 11)=0.617, p=0.449).

CSP. Figure 14E displays the CSP data for the left (top) and right (bottom) M1. A one-way repeated measures ANOVA revealed no change in the left M1 (F (1, 10)=2.530, p=0.143), with a significant increase in the right M1 (F (1, 10)=8.327, p=0.016).

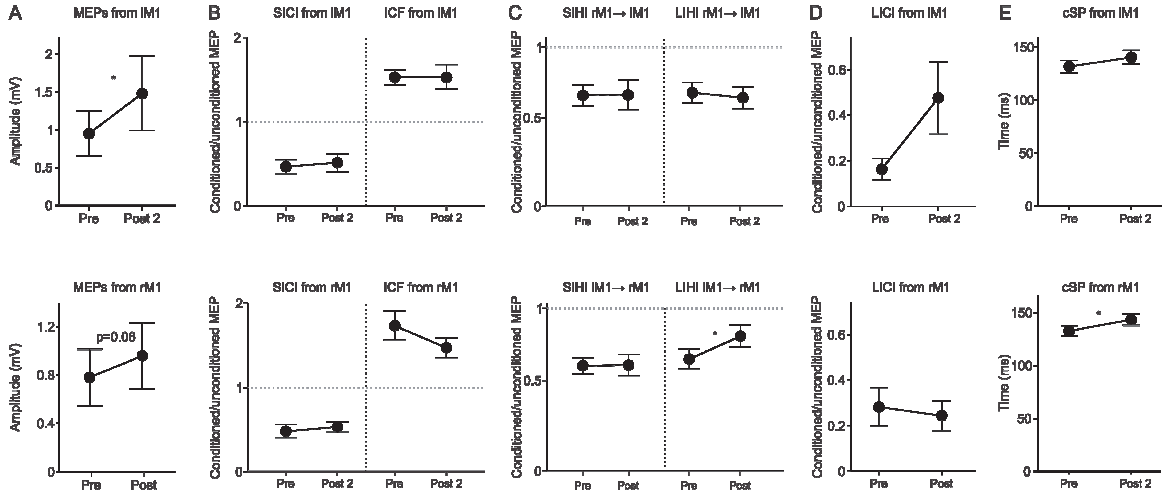


Figure 14. BMT alone. Group-averaged data acquired from the left (top) and right (bottom) M1 ECR representation A. MEPs. B. SICI/ICF. C. IHI. D. LICI. E. CSP. Pre, Post 1 (immediately following iTBS), Post 2 (30-60 min post). All bars represent SEM. * $p \leq 0.05$.

Experiment 2: iTBS to lPMd

MEPs. The motor thresholds were consistent among participants (mean left M1 rMT = 42 ± 7%, right M1 rMT = 42 ± 7%, and mean left M1 AMT = 39 ± 8%). Figure 15A displays the MEPs for the left (top) and right (bottom) M1 ECR representations. A one-way repeated measures ANOVA revealed no changes across all time points for MEP

amplitude in the left M1 (F (2, 18)=0.852, p=0.443) the right M1 (F (2, 18)=2.414, p=0.118).

SICI/ICF. Three participants were removed in the left M1 for not displaying the expected inhibition and one was removed for not displaying facilitation in the pre measures. Similarly, two participants were removed in the right M1 due to not displaying inhibition and two were removed due to not displaying facilitation in the pre measure. Figure 15B displays the SICI and ICF data for the left (top) and right (bottom) M1 ECR representations. For SICI, a one-way repeated measures ANOVA revealed no changes in the left M1 (F (2, 20)=0.682, p=0.517) or right M1 (F (2, 20)=2.373, p=0.117). For ICF, a one-way repeated measures ANOVA revealed no change for left M1 (F (2, 24)=1.075, p=0.357) or right M1 (F (2, 22)=0.477, p=0.627).

IHI. Two participants were removed in the left M1 (SIHI and LIHI), two participants were removed for SIHI and three were removed for LIHI in the right M1 due to not displaying inhibition in the pre measure. Figure 15C shows the IHI data for the left (top) and right (bottom) M1 representations for both the SIHI and LIHI. For SIHI, a one-way repeated measures ANOVA revealed no change from the right to the left M1 (F (2, 22)=2.252, p=0.129) or left to right M1 (F (2, 22)=1.087, p=0.355). For LIHI, a one-way repeated measures ANOVA revealed no change in right to left M1 (F (2, 22)=2.009, p=0.158), and no significant change from the left to right M1 (F (2, 20)=1.296, p=0.296).

LICI. Three participants were removed in left M1 for not displaying inhibition in the pre measure. Conditioning stimulus MEPs were similar in the pre and post measures for the left (t (13)=1.650, p=0.123) and right M1 (t (13)=0.016, p=0.988). Figure 15D shows the LICI data for the left (top) and right (bottom) M1 representations. A one-way

repeated measures ANOVA revealed no change in the left M1 (F (1, 10)=0.118, p=0.739) or right M1 (F (1, 13)=0.559, p=0.468).

CSP. Figure 15E displays the CSP data for the left (top) and right (bottom) M1. A one-way repeated measures ANOVA revealed an increase in the left M1 (F (1, 9)=7.045, p=0.026), or the right M1 (F (1, 9)=0.530, p=0.485).

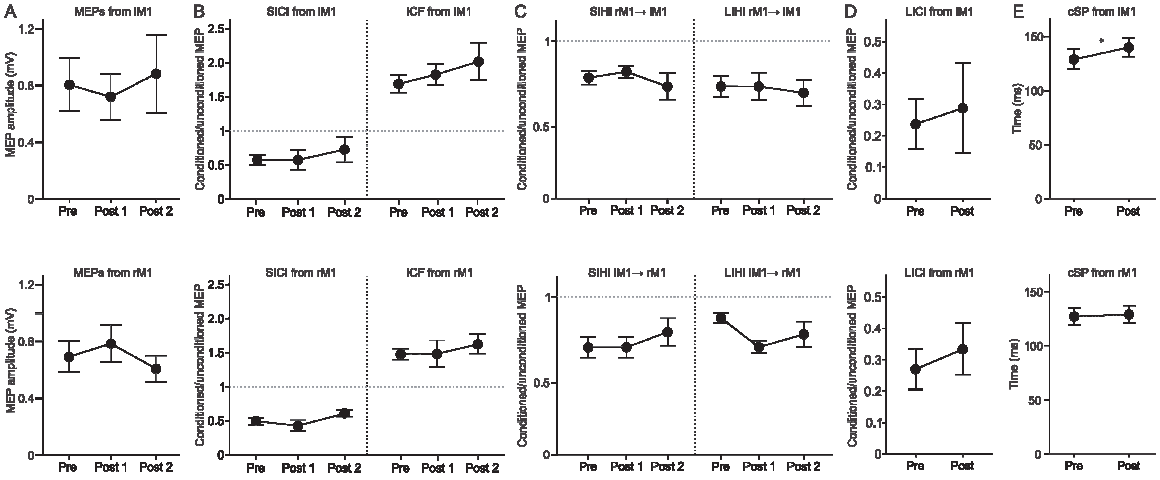


Figure 15. iTBS to lPMd alone. Group-averaged data acquired from the left (top) and right (bottom) M1 ECR representation A. MEPs. B. SICI/ICF. C. IHI. D. LICI. E. CSP. Pre, Post 1 (immediately following iTBS), Post 2 (30-60 min post). All bars represent SEM. * $p \leq 0.05$.

Experiment 3: iTBS to lPMd + BMT

MEPs. The motor thresholds were consistent among participants (mean left M1 rMT = 42 ± 7%, right M1 rMT = 43 ± 7%, and mean left M1 AMT = 39 ± 8%). Figure 16A displays the MEPs for the left (top) and right (bottom) M1 ECR representations. A one-way repeated measures ANOVA revealed an increase in amplitude in the left M1 (F (1, 9)=2.145, p=0.177) and a near significant increase in the right M1 (F (1, 9)=4.434, p=0.065).

SICI/ICF. Two individuals were removed for left M1 and right M1 for not displaying inhibition (SICI) in the pre measure. One individual was removed for right M1

for not displaying facilitation (ICF) in the pre measure. Figure 16B displays the SICI and ICF data for the left (top) and right (bottom) M1 ECR representations. For SICI, a one-way repeated measures ANOVA revealed no changes in the left M1 ($F(1, 11)=0.816$, $p=0.386$) or right M1 ($F(1, 11)=1.557$, $p=0.238$). For ICF, a one-way repeated measures ANOVA revealed no change for left M1 ($F(1, 13)=0.140$, $p=0.714$) or right M1 ($F(1, 12)=0.576$, $p=0.463$).

IHI. Four individuals were removed for the left M1 for both SIHI and LIHI due to not displaying inhibition in the pre measures. Two individuals were removed for the right M1 for LIHI and one individual was removed for SIHI due to not displaying inhibition in the pre measure. Figure 16C shows the IHI data for the left (top) and right (bottom) M1 representations for both the SIHI and LIHI. For SIHI, a one-way repeated measures ANOVA revealed no change from the right to the left M1 ($F(1, 9)=1.945$, $p=0.197$) or left to right M1 ($F(1, 12)=2.816$, $p=0.119$). For LIHI, a one-way repeated measures ANOVA revealed no change in right to left M1 ($F(1, 9)=0.462$, $p=0.514$), and no significant change from the left to right M1 ($F(1, 11)=1.226$, $p=0.292$).

LICI. One individual was removed for the right M1 due to not displaying the expected inhibition in the pre measure. Conditioning stimulus MEPs were similar in the pre and post measures for the left ($t(13)=1.149$, $p=0.271$) and right M1 ($t(13)=1.124$, $p=0.282$). Figure 16D shows the LICI data for the left (top) and right (bottom) M1 representations. A one-way repeated measures ANOVA revealed no change in the left M1 ($F(1, 13)=1.377$, $p=0.262$), with a decrease in the right M1 ($F(1, 12)=6.880$, $p=0.022$).

CSP. Figure 16E displays the CSP data for the left (top) and right (bottom) M1. A one-way repeated measures ANOVA revealed no change in the left M1 ($F(1, 9)=0.193$, $p=0.670$), or the right M1 ($F(1, 9)=0.014$, $p=0.909$).

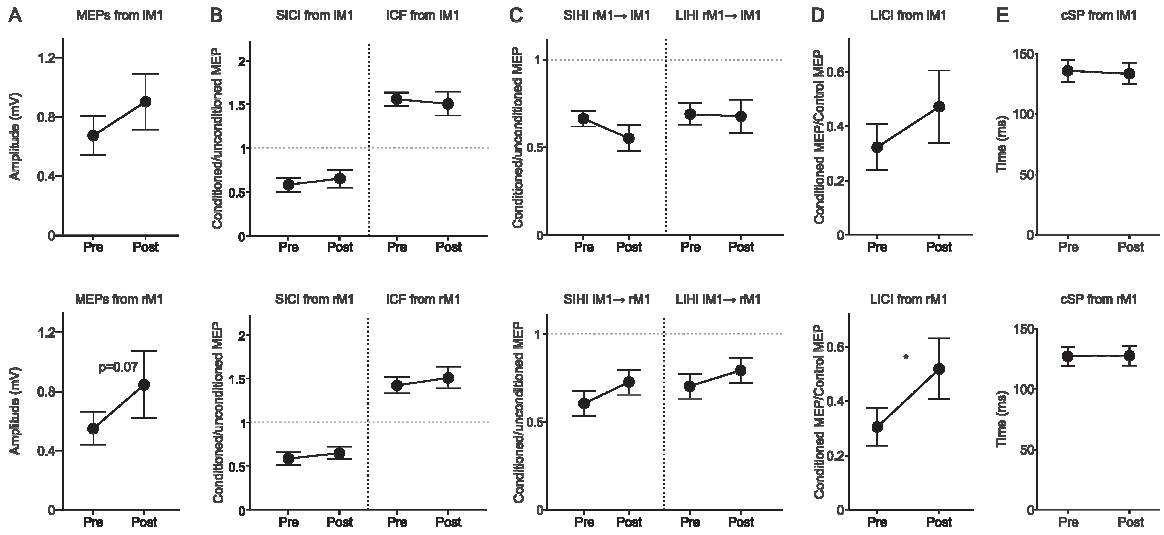


Figure 16. iTBS to lPMd followed by BMT. Group-averaged data acquired from the left (top) and right (bottom) M1 ECR representation A. MEPs. B. SICI/ICF. C. IHI. D. LICI. E. CSP. Pre, Post 2 (30-60 min post). All bars represent SEM. * $p \leq 0.05$.

Comparison across Experiments

As a secondary exploratory measure that the combination of iTBS to lPMd and BMT would possibly yield additional modulations of M1 intracortical and interhemispheric excitability than iTBS to lPMd or BMT alone, a one-way ANOVA was performed on all groups with the difference score between pre and post 30 min time points with between-subjects factor GROUP (Exp. 1: BMT alone, Exp. 2: iTBS to lPMd only, Exp. 3: iTBS to lPMd + BMT).

MEPs. Figure 17A shows that there were no differences between pre and 30 min time point post, as a one-way ANOVA revealed no effect of EXPERIMENT in left M1 ($F(2, 29)=1.708$, $p=0.199$), but there was a significant difference in right M1 ($F(2, 29)=3.386$, $p=0.048$). *Post Hoc* analyses revealed a difference between Experiment 2 and

3 (p=0.045), showing that iTBS to lPMd alone caused a decrease but iTBS to lPMd followed by BMT caused an increase in MEP amplitude.

SICI/ICF. Figure 17B and C shows SICI and ICF data. For SICI, there were no differences between pre and 30 min time point post, as a one-way ANOVA revealed no effect of EXPERIMENT in left M1 (F (2, 31)=0.231, p=0.795), or in right M1 (F (2, 32)=0.226, p=0.799). For ICF, there were no differences between pre and 30 min time point post, as a one-way ANOVA revealed no effect of EXPERIMENT in the left M1 (F (2, 36)=0.226, p=0.799), and a near significant difference in the right M1 (F (2, 34)=3.009, p=0.063).

IHI. Figure 17D shows IHI data. For SIHI, there were no differences between pre and 30 min time point post, as a one-way ANOVA revealed no effect of EXPERIMENT in left M1 (F (2, 28)=0.547, p=0.585), or in right M1 (F (2, 34)=0.723, p=0.492). For LIHI, there were no differences between pre and 30 min time point post, as a one-way ANOVA revealed no effect of EXPERIMENT in the M1 (F (2, 28)=0.558, p=0.579), and a near significant difference in right M1 (F (2, 30)=2.896, p=0.071).

LICI. Figure 17E shows LICI data. There were no differences between each intervention, as a one-way ANOVA revealed no effect of EXPERIMENT in left M1 (F (2, 34)=0.761, p=0.475) and a trend towards a difference among experiments in right M1 (F (2, 36)=2.722, p=0.079).

CSP. Figure 17F shows CSP data. There were no differences in CSP as shown by a one-way ANOVA with factor EXPERIMENT in left M1 (F (2, 28)=1.923, p=0.165) or right M1 (F (2, 28)=2.607, p=0.092).

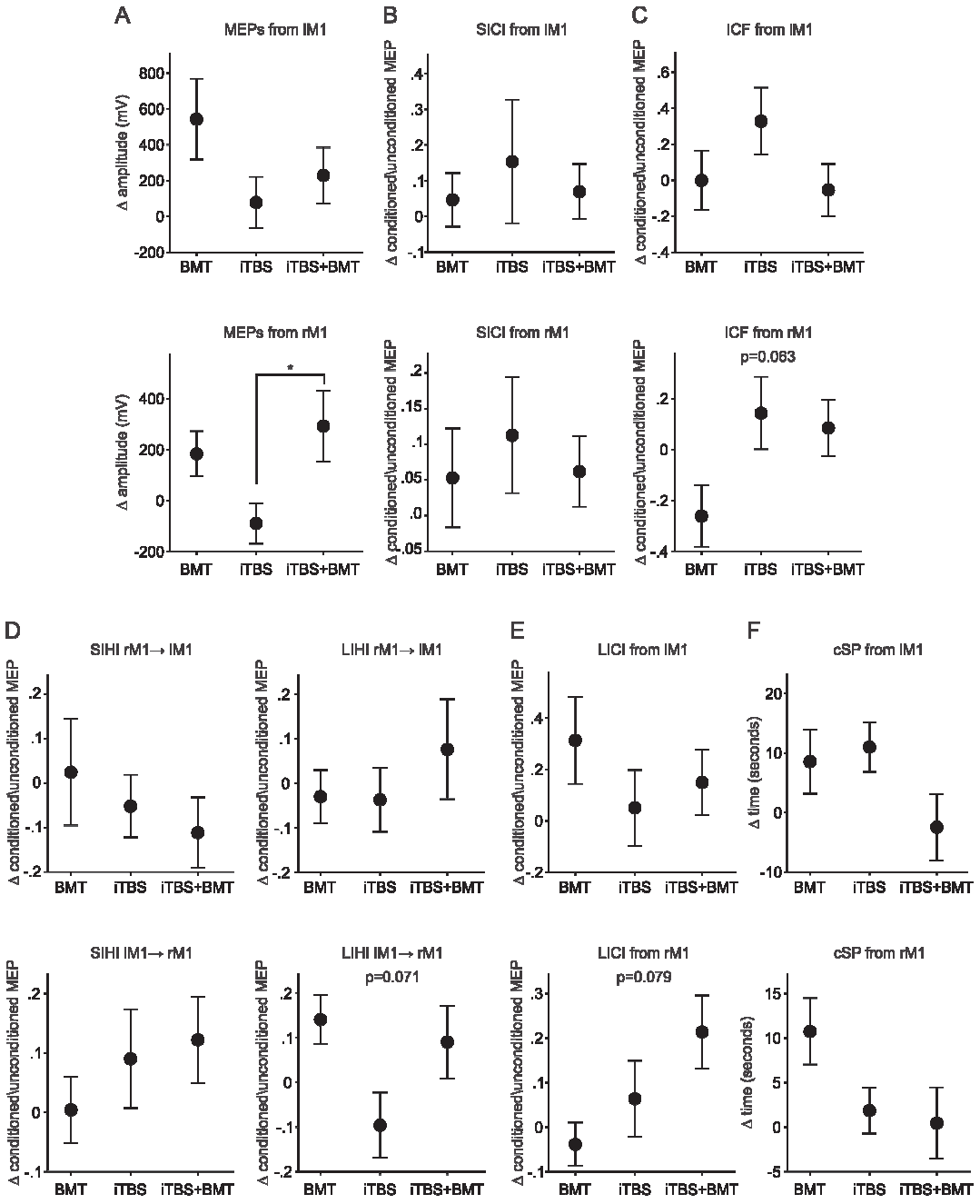


Figure 17. Comparison across experiments. Group-averaged difference score for all experimental conditions from the left (top) and right (bottom) M1 ECR for (A) MEPs, (B) SICI, (C) ICF, (D) IHI, € LICI and (F) CSP. All bars represent SEM. * $p \leq 0.05$.

Behavioural performance

Figure 18 displays the behavioural data of experiments 1 and 3, with the movement time (leftward panel) and angle at peak velocity (rightward panel). For the

movement time, a two-way ANOVA revealed a main effect of BLOCK ($F(1, 24)=27.071$, $p<0.0001$), no effect of EXPERIMENT ($F(1, 24)=0.007$, $p=0.935$) and no interaction of BLOCK x EXPERIMENT ($F(1, 24)=0.081$, $p=0.779$). For the angle at peak velocity, a two-way ANOVA revealed a main effect of BLOCK ($F(1, 24)=9.527$, $p=0.005$), no effect of EXPERIMENT ($F(1, 24)=0.0003$, $p=0.987$), and no interaction of BLOCK X EXPERIMENT ($F(1, 24)=1.762$, $p=0.201$). The main effect of BLOCK indicates that there was a decrease movement time and in deviation of cursor path from the initial to the final trials (i.e., performance improvement) similarly between experiments 1 and 3. *Post Hoc* analyses revealed no other differences between experiments with factor TIME.

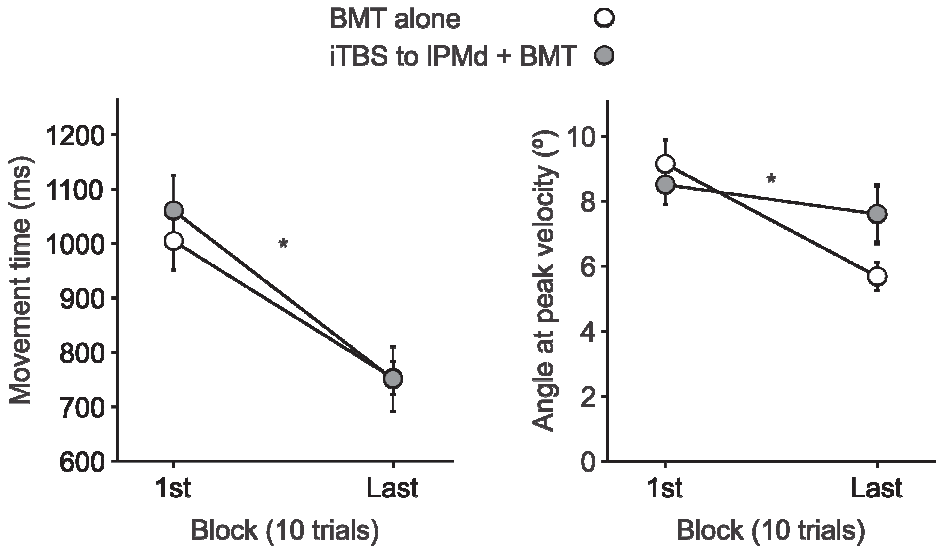


Figure 18. Behavioural data for experiments 1 and 3. *Left* Movement time for Group 1 (white) and Group 3 (grey). *Right* Angle at peak velocity of the resultant cursor path for Group 1 (white) and Group 3 (grey). All bars represent SEM. * $p \leq 0.05$.

Discussion

The experiments in the current work are the first to investigate the changes in cortical excitability in intracortical and interhemispheric circuitry within M1 associated

with short-term BMT following iTBS to lPMd. There were distinct modulations of intracortical and interhemispheric excitability due to the interventions within each of the experiments, which were primarily associated with changes in long-latency inhibitory mechanisms. Short-term BMT alone was associated with increases in bilateral M1 excitability, with a decrease in long interhemispheric inhibition from the left to right M1, and an increase in long-latency local inhibition in right M1. Surprisingly, iTBS to lPMd alone was only associated with an increase in long-latency local inhibition in the left M1. When short-term BMT is preceded by iTBS to lPMd there is a slight increase in excitability along with a decrease in long-latency local inhibition in right M1. Collectively, these data suggest that BMT asymmetrically modulates the excitability within and between homologous M1 representations, lPMd primarily modulates inhibition in the ipsilateral M1, and the potential up-regulation of lPMd before BMT leads to modulation in the contralateral (right) M1 inhibitory mechanisms.

Short-term BMT effects of bilateral M1 neural mechanisms

Enhancements in cortical excitability have been shown in several studies as an increase in the cortical area represented by the muscles involved in the specifically trained task (Pascual-Leone et al., 1995; Nudo et al., 1996; Kleim et al., 1998, 2004). Increases in M1 excitability of trained muscles have been observed after a 30 min session of BMT (Neva et al., 2012, 2014) and skilled digit sequence training for two 2 hour sessions (Pascual-Leone et al., 1995). Previous research has shown that a short-term session of BMT leads to an increase in the left M1 in terms of the cortical area occupied by the trained muscles, without a significant increase in the central MEP amplitude (Neva

et al., 2012, 2014). The current study found an increase in MEP amplitude in the left and a near significant increase in the right M1 ECR hotspots. The lack of consistency between the current and former studies may be due to slight variations in the experimental procedures. For example, the short-term BMT task in the former study involved skilled wrist extension and flexion, whereas the current study focused on skilled wrist extension movements. Additionally, the former study used a biphasic single pulse TMS to acquire MEPs from the left M1, which may recruit a slightly different population of neurons than the monophasic single pulse TMS employed in the current study (Kammer et al., 2001). Regardless of the difference in results with the former studies, it is not entirely surprising that there is an increase MEP amplitude at the trained ECR hotspot bilaterally. Many studies have found increased MEP amplitude due to movement training (Liepert et al., 1999; Pearce et al., 2000; Muellbacher et al., 2001; Perez et al., 2004; Jensen et al., 2005). MEP amplitude increases due to skilled thumb (Liepert et al., 1999), hand (Muellbacher et al., 2001), arm (Jensen et al., 2005) and ankle (Perez et al., 2004) movement training. In one study, participants performed repetitive (1 Hz) thumb movements while completely relaxing the other muscles of the hand (skilled motor component). Following training this task MEP amplitudes from the thumb muscle (trained muscle) was specifically enhanced compared to neighbouring hand muscles (Liepert et al., 1999). Another study where participants practiced ballistic pinch contractions caused increase in force and acceleration of the pincer grip and were also correlated with increased MEP amplitude in the trained muscle (Muellbacher et al., 2001). Further, another study comparing spatial distribution and amplitudes of MEPs in the upper-limb representation between highly skilled racket players and non-skilled

players showed that skilled racket players have not only the expected increased spatial distribution of MEPs compared to non-skilled players, but skilled players also have higher MEP amplitudes (Pearce et al., 2000). Changes in cortical excitability are also exhibited by altered motor thresholds (Pascual-Leone et al., 1995). A decrease in motor threshold due to prolonged training would indicate a focal change in the hotspot of a muscle representation, however a change in MEP amplitude likely reflects overall excitability of the muscle representation (cortical and spinal excitability). Since MEP amplitude reflects overall corticospinal tract activity at the moment of single pulse TMS stimulation, it is an index of the sum of cortical and spinal motor output excitability. The current study indicates that the sum of cortical and spinal motor output excitability of the trained muscle (ECR) representation was enhanced in both M1 hemispheres due to a short-term session of BMT. The short-term BMT in the current study could have been too brief to engage neural mechanisms that would induce a change in motor threshold.

Cortical activity is enhanced in both hemispheres in damaged and healthy M1 when homologous muscles are simultaneously activated (Silvestrini et al., 1998; Staines et al., 2001). It is thought that transcollosal activity between homologous M1 representations act to excite and/or release inhibition from the contralateral hemisphere (Stinear & Byblow, 2002), which could facilitate M1 plasticity observed in the current and previous (Neva et al., 2012; Neva et al., 2014) studies. Specifically, intracortical inhibition is decreased in M1 when both upper-limbs are moving in a mirror-symmetrical pattern, where both agonist and antagonist muscles are extending and contracting simultaneously. However, inhibition remains when the upper-limbs are moving asymmetrically (Stinear & Byblow, 2002; Byblow et al., 2012). Similarly, left M1

excitability of the wrist extensor muscles are enhanced when bimanual movement training is made with the two upper-limbs co-activated the homologous muscles simultaneously, with no increase when they are not activated simultaneously (Neva et al., 2012; Neva et al., 2014). The current study found a decrease in IHI specifically from the left to right M1 homologous representations. Since previous research found that when both upper-limbs are moving symmetrically, intracortical inhibition was decreased in both hemispheres (Stinear & Byblow, 2002), and that unimanual movement decreases IHI in both directions (Nelson et al., 2009), it is surprising that the current study found reduced IHI only in one direction (left to right M1). However, this specific finding may be explained by the specific movement requirements of the BMT.

During BMT, participants were required to make simultaneous wrist extension movements of both upper-limbs to three different targets (35º, 45º, 55º relative to start position). The 45º target requires simultaneous co-contraction of the ECR muscles and to the same magnitude. However, the 35º and 55º target locations require a slightly different magnitude of co-contraction of both limbs. The asymmetrical reduction in IHI could be due to the requirement of this different magnitude of co-contraction of the wrist muscles. Moreover, all participants in these studies were right-hand dominant. Even though previous studies did not find a difference in IHI with hand dominance (Nelson et al., 2009), it is possible that the differing amounts of co-contraction required for 66.7% of the training (35º, 55º target locations) along with individuals with right-hand dominance may have led to the reduction of inhibition from the dominant to non-dominant hemisphere. Furthermore, there is a plethora of evidence suggesting that asymmetrical bimanual movement is less stable than strictly mirror symmetrical movements (Kelso et al., 1979;

Cardoso de Oliveira, 2002; Swinnen, 2002; Carson, 2005). Therefore, asymmetrical bimanual movements likely require more complex patterns of neural activity, which is a potential explanation for the release of inhibition from the dominant to non-dominant hemisphere due to BMT. Interestingly, this reduction in IHI was only in the long interval IHI (LIHI, 40 ms ISI) and not with the shorter interval IHI. The underlying mechanism mediating SIHI is largely unknown (Meyer et al., 1995), while LIHI likely involves GABA-B-mediated inhibition since it has a relatively longer time course and is increased with baclofen, a GABA-B receptor agonist (Irlbacher et al., 2007). Therefore, we specifically found that short-term BMT caused an asymmetrical long-latency reduction in IHI from the dominant (left) to non-dominant (right) hemisphere, which could have been necessary to provide increase excitable input to the non-dominant hand for the skilled requirement of the BMT task.

The excitability of cortical inhibitory networks is integral to motor control and the motor cortex (Chen et al., 1999; Ljubisavljevic, 2006). Specifically, the 'cortical silent period' (CSP) duration induced from a single pulse of TMS while holding a light voluntary contraction contralateral to the hemisphere of stimulation is thought to indicate the state of spinal and cortical inhibitory networks (Inghilleri et al., 1993; Chen et al., 1999; Terao & Ugawa, 2002; Ljubisavljevic, 2006). Several studies suggest that the initial portion of the CSP is thought to be due to spinal mechanisms, and the latter portion due to long-interval cortical inhibition, that is associated with GABA-B-like mechanisms (Terao & Ugawa, 2002). The current study found an increase in the CSP duration in the right hemisphere due to short-term BMT. Therefore, similarly to the IHI data, BMT could possibly increase GABA-B inhibitory activity asymmetrically in the non-dominant (right)

hemisphere. This increase in inhibition may be associated with increased motor control required of the non-dominant hand, due to the asymmetrical co-contraction when moving the cursor to the peripheral targets during our BMT task. One such study in support of this idea, had participants actively move one upper-limb in response to the other which was passively moved. When movements were entirely mirror-symmetrical, there was a decrease in intracortical inhibition, however this inhibition remained when movements of the upper-limbs were asymmetrical (Stinear & Byblow, 2002). Other research supports the idea that the dominant hemisphere has the ability to disinhibit the non-dominant hemisphere during mirror-symmetrical upper-limb movements (Stinear & Byblow, 2004). Although the current study required both symmetrical and non-symmetrical movements of the upper-limbs, the asymmetrical nature of the movements could have been so slight that the movements were essentially symmetrical, leading to the asymmetrical increase in right M1 inhibition found in the current study and previous studies (Stinear & Byblow, 2004).

iTBS to lPMd effects of on M1 neural mechanisms

This study found that iTBS to lPMd increased the excitability of inhibitory newtworks of ipsilateral M1, in terms of CSP. The PMd has extensive reciprocal neuronal projections with the ipsilateral M1 (Picard & Strick, 2001; Rushworth et al., 2003). Perhaps iTBS increased the excitatory input from left PMd to ipsilateral M1 and, in turn, increased the resulting long latency inhibitory networks of the downstream M1. Other studies have shown that rTMS at 5 Hz to left PMd increases cortical excitability in the ipsilateral M1 (Gerschlager et al., 2001; Chouinard et al., 2003; Rizzo et al., 2004; O'Shea et al., 2007; Suppa et al., 2008). Further, cTBS to lPMd reduces MEP amplitudes

from ipsilateral M1 for a longer period of time than cTBS directly over M1. However, cTBS over lPMd did not cause any changes in inhibitory or excitatory intracortical networks (Huang et al., 2009; Ortu et al., 2009). Another study demonstrated increased MEP amplitudes due to cTBS to lPMd and no effects due to iTBS to lPMd (Stinear et al., 2009). All of these studies did not report any effects on long-latency inhibitory mechanisms like LICI or CSP. Huang and colleagues found that iTBS to M1 increases MEP amplitudes and also increases inhibitory networks like SICI (Huang et al., 2005). It is possible that the increase in CSP in ipsilateral (left) M1 selectively enhances inhibitory intracortical networks similarly as if iTBS was applied directly to M1. Perhaps this results from left PMd reciprocal connections with M1 in both hemispheres, with interactions between both excitatory and inhibitory projections, which may in turn also influence right M1 to left M1 projections (Asanuma & Okuda, 1962; Ferbert et al., 1992; Gerloff et al., 1998; Chen et al., 2003; Nelson et al., 2009), that could lead to an increased inhibition in left M1. These studies along with the current results demonstrate that cortical excitability in M1 may be modulated by input from the upstream ipsilateral PMd.

The effects of iTBS to left PMd with bimanual training

This study is the first to investigate the effects of applying iTBS to the left PMd before BMT to the circuitry within and between M1, along with the specific excitability changes due to BMT or iTBS to left PMd alone. One possibility is that the iTBS to left PMd enhanced the downstream ipsilateral connections to M1 (and possibly connections between left PMd and right M1) and combined with BMT-induced excitability changes to produce a unique set of modulations of excitability in M1. Specifically, there was a slight increase in MEP amplitude in the right hemisphere M1 due to iTBS to lPMd followed by

short-term BMT. Strictly speaking, there was not an additive effect due to the specific interventions of BMT and iTBS to lPMd, but rather there were unique modulations of excitability due to each intervention. Similarly to the effects due to BMT alone, the asymmetrical modulations in excitability in the right M1 from iTBS to lPMd and BMT could be due to the differing amounts of co-contraction required for 66.7% of the training (35º, 55º target locations) along with individuals with right-hand dominance may have led to the reduction of inhibition in the non-dominant hemisphere.

The current study also found a decrease in right M1 LICI due to iTBS to lPMd followed by short-term BMT. LICI is elicited by two suprathreshold TMS pulses with an ISI of 50-200 ms, and it likely relates to long-latency inhibitory activity like the CSP (Chen, 2004). There is some evidence that both the latter half of the CSP and LICI are associated with GABA-B-like activity (Werhahn et al., 1999). The concurrent effects of increased MEP amplitude and decreased LICI in the right hemisphere are consistent with the idea that presynaptic GABA-B receptor inhibition is associated with decreased MEP amplitude (Sanger et al., 2001; Chen, 2004). Additionally, a recent study where participants train upper-limb movements by having one upper-limb passively moved and either having to actively match that movement with the contralateral upper-limb (mirror symmetrical movement) or to move the contralateral limb in an alternating manner (asymmetrical movement) (Byblow et al., 2012). This study found that LICI decreased in the passively moved limb when movements were symmetrical and there was a slight increase in LICI when movements were asymmetrical. These findings are consistent with the current findings of a decrease in LICI when performing BMT alone in the left M1 and when iTBS to lPMd was applied before BMT in the right M1. Previous research has

shown that BMT performed in this study has shown increases in lateral premotor cortex (i.e. PMd) activity (Seitz et al., 2004) in both hemispheres (Smith & Staines, 2006, 2010, 2012). It may be the case that pre-conditioning the lPMd before BMT increased the neural input from the premotor cortex to both the left and right M1, which in turn, caused an associated release of inhibition and increased excitability due to BMT. The resulting specific increase in the right hemisphere M1 in the current study may be related to the increased skill requirement of the non-dominant (left) upper-limb of the varying degrees of co-contraction of the upper-limbs, as discussed previously.

Previously, it was found that iTBS over lPMd preceding in-phase BMT led to a concurrent increase in the spatial distribution and also the amplitude of MEPs in left M1 (Neva et al., 2014). Interestingly, an increase in spatial distribution of left M1 was only seen due to BMT alone, and an increase in amplitude of MEPs occurred due to iTBS to lPMd alone. Primarily, these enhancements in M1 excitability were found on the boarders of M1 representation (Neva et al., 2012, 2014). This study attempted to establish the potential intracortical and interhemispheric excitability changes that may have contributed to the interventions of iTBS and short-term BMT, but found that the effects were primarily in the right hemisphere, which was not tested in the previous studies. One possible explanation for why effects were not seen in the left hemisphere is due do the fact that all TMS measurements were taken only at the hotspot for the ECR representation. The previous studies mentioned took MEPs from the entire spatial distribution of ECR representation. Perhaps there were effects not captured in the current studies due to taking measures only at the centre of the M1 representation. Further study will have to be done to investigate the intracortical and interhemispheric adaptations that

may occur due to TBS and BMT interventions across the entire trained muscle representation(s).

Conclusion

In sum, our findings suggest that iTBS to the left PMd followed by BMT caused distinct modulations of M1 excitability than either intervention alone, which are mainly associated with changes in long-latency inhibitory neural mechanisms. Short-term BMT increased MEP amplitudes bilaterally, with an asymmetrical reduction of left to right M1 interhemispheric inhibition and an increased right M1 inhibition. iTBS to lPMd increased ipsilateral (left) M1 cortical inhibition. Finally, iTBS to lPMd followed by BMT caused an asymmetrical increase MEP amplitude and reduction of inhibition in the right M1. These modulations in M1 intracortical and interhemispheric excitability resulting from BMT and iTBS suggest that they operate under related plasticity mechanisms that may be expressed in distinct ways concurrently. It is possible that BMT, iTBS to lPMd and the combination of these interventions engage distinct neural circuitry associated with GABA-B-like activity. Critically, this work may guide rehabilitation training and stimulation techniques that modulate cortical plasticity after brain injury and other neurological diseases. It may be that the modulation of remote cortical areas to M1 (i.e. PMd) in combination with rehabilitation training could be advantageous in distinctly enhancing short-term plasticity in damaged motor cortex. However, further study is required to understand the potential implications of this research that could be applicable in clinical settings.

Chapter 6

6.1 General Discussion

The work in this thesis incorporated an investigation of the excitability modulation of the primary motor cortex (M1) as influenced by connections with related intra- and interhemispheric motor preparatory and execution cortical regions. Cortical adaptations in M1 were investigated using bimanual visuomotor movement training (BMT), theta burst stimulation (TBS) protocols to remote but related cortical nodes and a combination of these interventions. Particularly, this thesis investigated modulation of M1 excitability in terms of in-phase versus anti-phase BMT (Study #1), potentially up-regulating the left dorsal premotor cortex (lPMd) via iTBS before BMT (Study #2), theoretically down-regulating contralateral (right) M1 homologous representation before BMT (Study #3), and finally the potential intracortical and interhemispheric cortical adaptations in M1 bilaterally due to the same interventions as Study #2 (Study #4).

Based on the findings of the four studies covered in this work, this thesis proposes four related models to account for the observed modulations in M1 excitability: 1) released inhibition (or increased excitation) in lateral premotor cortices (i.e. PMd), 2) released inhibition (or increased excitation) with interhemispheric projections between homologous M1 representations, 3) modulations in long-interval inhibitory mechanisms within and between M1 representations, and 4) excitability increases along the borders of trained muscle representation in M1, which potentially relate to changes in long-term potentiation/depression (LTP/D) (Woody et al., 1991; Hess & Donoghue, 1996, 1999; Hess et al., 1996; Martin et al., 2000), and unmasking of pre-existing horizontal connections (Jacobs & Donoghue, 1991; Malinow et al., 2000).

(1) ***Released inhibition (or increased excitation) in lateral premotor cortices (PMd)***

The work in the current thesis provides evidence that the lateral PM cortices (the left dorsal (lPMd) portion in particular) likely contributes to the BMT-induced cortical adaptations observed. It is possible that the current BMT training task itself is responsible for the potential release in inhibition (or increased excitation) in the lateral PM that results in increased M1 excitability. Since the lateral PM responds to externally cued tasks (i.e. visual and auditory) (Jäncke et al., 2000; Sugiura et al., 2001; Koch et al., 2006), it is likely that PMd activity is enhanced during performance of the current visually cued BMT task. In addition, it is possible that this BMT task involving co-contraction of homologous muscle representations in M1 led to a release of inhibition of the homologous muscle representations in the premotor cortices, resulting in increased excitability in M1. The potential mechanisms driving this effect could involve interhemispheric communication between both hemispheres of PMd (Boussaoud et al., 2005) and interacting with the homologous M1 representations via the corpus callosum. Since there was disinhibition between the homologous M1 representations due to short-term BMT found in Study #4 (experiment 1), it is possible that the same homologous representations within the PM cortices followed the same pattern of decreased inhibition. In fact, previous research has confirmed that lateral PM (i.e. left and right PMd) excitability increases due to short-term in-phase BMT (Smith & Staines, 2006, 2010, 2012), during index finger-thumb opposition (Seitz et al., 2004), in the selection of appropriate actions for movement execution (Kalaska & Crammond, 1995; Thoenissen et al., 2002; O'Shea et al., 2007; Groppa et al., 2012), and with learned visuomotor associations of both upper-limbs (Geyer et al., 2000; Toni et al., 2001). Specifically,

when visually cued in-phase movements are trained, the Bereitschaftspotential (BP) (a self-paced, rather than externally cued movement related potential (MRP)), associated with motor preparatory activity within the SMA (Shibasaki et al., 1980; Shibasaki & Hallett, 2006), is not enhanced. Furthermore, source localization (sLORETA) identified PMd to be the cortical region generating the excitability changes in the early component of the MRP due to BMT (Smith & Staines, 2012). Although the contribution of lPMd to M1 excitability cannot be directly confirmed in these studies, there is evidence in previous and confirmed and extended in the current work that lPMd likely contributes to modulations in M1 excitability.

Study #2 found that iTBS to left PMd markedly increased the excitability of ipsilateral M1, demonstrating the vital influence of PM areas on the excitability of downstream M1. It is possible that the extensive reciprocal projections from the PM to M1 cortices (Weinrich & Wise, 1982; Wise, 1985) were enhanced by the application of iTBS to lPMd, leading to the modulations in ipsilateral M1 excitability observed. Other studies have shown that rTMS at 5 Hz to left PMd increases cortical excitability in the ipsilateral M1 (Gerschlager et al., 2001; Chouinard et al., 2003; Rizzo et al., 2004; O'Shea et al., 2007; Suppa et al., 2008). The increased excitability from left PMd to ipsilateral M1 may be due to neural projections to M1 in both hemispheres that may be involved in motor preparatory sequences, particularly for visually cued tasks (Schluter et al., 1998; Modugno et al., 2001; Rushworth et al., 2003). Other studies using TBS protocols to lPMd have shown modulation of the excitability of downstream M1 ipsilaterally (Huang et al., 2009) and contralaterally (Stinear et al., 2009).

Therefore, when neural input was potentially enhanced from lPMd before BMT due to iTBS, this may have led to increased input from PM during the cued BMT to ipsilateral and contralateral M1. This enhanced input from lPMd may have been partially responsible for the enhanced M1 excitability observed when iTBS to lPMd was followed by BMT. It is likely that both hemispheres of the PMd were involved during the cortical adaptations during this task, since they are highly connected interhemispherically (Boussaoud et al., 2005). The left PMd has a critical role in action selection for execution of learned associations for movements of either or both upper-limbs (Schluter et al., 1998; Rushworth et al., 2003). Inhibition of left PMd using TMS leads to a disruption during action selection when using both upper-limbs (Schluter et al., 1998; Johansen-Berg et al., 2002). Also, short-term BMT increases activity in the lateral PM cortex during a closely associated unimanual task (Smith & Staines, 2006, 2010, 2012). Further, iTBS to left PMd causes faster preparation of complex sequences performed with the right hand (Stinear et al., 2009). Given that the current study involved learning a skilled task that required movement of both upper-limbs simultaneously, the potential up-regulation of the left PMd could have induced a greater degree of excitatory input to M1 in both hemispheres during the training of the task. On the other hand, it is possible that the BMT slightly enhanced the effects of iTBS applied to PMd. Voluntary contraction of the right limb immediately after iTBS applied to left M1 enhances the facilitating effects of iTBS to MEP amplitude in the resting limb (Huang et al., 2008). Therefore, these studies along with the Study #1, #2 and #4 suggest that lPMd has a particularly relevant role in movement selection with both upper-limbs and the adaptation to visuomotor

movement associations, like the short-term cued in-phase BMT used in the studies of the current thesis.

It is likely that PM cortices, along with other important cortical nodes (e.g. the homologous M1 representations), potentially contribute to the modulations of cortical excitability due to in-phase BMT.

2) *Released inhibition (or increased excitation) with interhemispheric projections between homologous M1 representations*

The work in this thesis suggests a modulation of inhibition due to co-activation of homologous muscle representations due to BMT, via transcallosal projections. Specifically, Study #1 found that M1 excitability increased due to BMT with simultaneous homologous muscle activity (Neva et al., 2012), and these results were confirmed with Study #2 using similar BMT. Furthermore, modulating the excitability of the contralateral homologous M1 representation prior to BMT led to greater enhancements of M1 excitability as shown in Study #3. Finally, Study #4 specifically revealed a decrease in IHI between homologous muscle representations due to in-phase BMT. Several other studies support these findings by suggesting that transcallosal projections via homologous M1 representations may serve as a mediator of short-term plasticity due to BMT. For example, excitability in motor related areas are enhanced in both hemispheres in damaged and healthy cortices when homologous muscles are activated together (Silvestrini et al., 1998; Staines et al., 2001). It is thought that neural connections between homologous M1 representations via the corpus callosum act to excite and/or release inhibition to the contralateral hemisphere (Stinear & Byblow, 2002), which could lead to M1 plasticity. Specifically, local intracortical inhibition in M1 is released when homologous M1 representations of the upper-limbs are activated

synchronously (in-phase), but inhibition remains with asynchronous (anti-phase) activation (Stinear & Byblow, 2002, 2004). These studies suggest that interhemispheric connections between M1 representations may be a potential neural mechanism, with presumed GABAergic local M1 disinhibition, which underlies the corticospinal modulations observed due to BMT tasks. It is not surprising, therefore, that Study #1 specifically found increased M1 excitability due to in-phase (mirror-symmetrical/synchronous movements) and not anti-phase (asymmetrical/non-synchronous movements) BMT. Further, it follows that Study #4 found a release of inhibition across homologous M1 hemispheres due to in-phase BMT.

Modulation of the contralateral homologous M1 representation prior to in-phase BMT in Study #3 caused a greater change in M1 excitability compared to BMT alone or modulation of contralateral M1 alone. Other work supports these findings and suggest that this enhanced excitability found in Study #3 may be due to additional reduction of interhemispheric inhibition by combining in-phase BMT and suppressive rTMS to the opposite homologous M1 (Stinear & Byblow, 2002; Suppa et al., 2008; Meehan et al., 2011; Byblow et al., 2012). When suppressive rTMS (cTBS) is applied to rM1 corticospinal excitability in lM1 increases in healthy individuals (Suppa et al., 2008; Meehan et al., 2011) and with those who have suffered stroke (Meehan et al., 2011). Also, motor function of the affected limb improves in stroke patients after applying cTBS to the contralesional M1 (or S1) in combination with movement training (Meehan et al., 2011). The current study found no enhancements in behavioural performance when cTBS was applied prior to in-phase BMT, so this effect may only occur with more extensive training or with those displaying movement impairment due to stroke.

Although there is evidence from Study #3, #4 and the previous studies mentioned that increases to M1 excitability may be mediated through transcallosal pathways, it cannot be definitively stated that this was the driving neural mechanism in Study #3. Future study on the specific interhemispheric pathways and their potential modulation due to rTMS and movement training is required to further understand these effects.

More evidence for transcallosal pathways potentially mediating the cortical excitability effects observed was found in studies with split-brain patients (partial or full severing of the corpus callosum). These patients are unable to perform discrete anti-phase bimanual movements, and cannot perform continuous in-phase or anti-phase bimanual movements (Kennerley et al., 2002). This demonstrated the importance of the corpus callosum in the spatial coupling and coordination of complex bimanual movement tasks, such as bimanual circle drawing (representing anti-phase like movements of the digits). The BMT task in Studies #2, #3 and #4 required a complex coordination of both upper-limbs, involving simultaneous co-contraction of varying degrees in order to move a cursor to multiple visual targets (Neva et al., 2014). Therefore, it is likely that interhemispheric projections via the corpus callosum were necessary to perform the skilled motor movement required during the current studies and potentially mediate the cortical adaptations observed.

The lack of effect due to anti-phase training (in Study #1) may relate to the reciprocal inhibition of active versus inactive agonist and antagonist muscle representations in the contralateral hemispheres (Stinear & Byblow, 2002; Byblow et al., 2012). Other studies have also demonstrated that anti-phase BMT does not lead to increases in motor preparatory activity through EEG (Smith & Staines, 2006, 2010, 2012). The findings of

previous research and the current thesis does not discount the potential use of anti-phase BMT as a useful training strategy to enhance cortical excitability. The medial premotor areas (i.e. the supplementary motor area (SMA)) have been implicated as a cortical locus that mediates bimanual motor control (Swinnen et al., 1997; Johnson et al., 1998; Almeida et al., 2002, 2003; Cardoso de Oliveira, 2002; Serrien et al., 2002; Swinnen, 2002). Particularly, rTMS applied to disrupt SMA activity leads to an interruption of the spatial and temporal coordination of anti-phase bimanual movements as opposed to in-phase movements (Serrien et al., 2002). Additionally, Parkinson's patients with indicated disruption of the SMA has shown similar disorders of anti-phase bimanual movement production with external cueing (Swinnen et al., 1997; Johnson et al., 1998; Almeida et al., 2002, 2003). Of course, the lateral PM is likely to be involved along with the SMA in some aspect anti-phase bimanual movement training. Conversely, it is certainly possible that SMA is partially involved in the cortical adaptations observed in the in-phase BMT utilized in the current thesis. It is possible that the particular BMT emphasized and the methods used to measure the M1 excitability modulations were not sensitive to measure the involvement of the SMA. Future studies involving specific anti-phase BMT, fMRI and modulation of SMA through rTMS could be useful in understanding the contribution of medial PM areas to training-induced modulations of M1 cortical excitability.

3) ***Modulations in long-interval inhibitory mechanisms within and between M1 representations***

The effects observed in Study #4, which was investigating the intracortical and interhemispheric adaptations due to BMT and/or iTBS to lPMd, were surprisingly primarily modulations in long-interval inhibitory connections, and many of these were

observed asymmetrically in one hemisphere. BMT (experiment 1) led to a reduction in long-interval interhemispheric inhibition (LIHI) to rM1 and an increase in long-interval inhibition due to prolonged cortical silent period (CSP) in rM1. iTBS to lPMd (experiment 2) caused an increase in long-interval inhibition by a prolonged CSP in lM1. Finally, iTBS to lPMd followed by BMT (experiment 3) caused a reduction of long-interval intracortical inhibition (LICI) in rM1. There has been little research on the specific effects of long-interval inhibitory mechanisms due to skilled movement training and few on the effects due to iTBS protocols. These will be discussed below.

The effect of long-latency interhemispheric inhibition (LIHI)

Study #4 (experiment 1) found that short-term in-phase BMT led to an asymmetrical reduction of LIHI from the left to the right M1. It is generally accepted that IHI exists to suppress unwanted simultaneous movement of both upper-limbs, therefore, it follows that IHI would be reduced when simultaneous movements are made with both limbs, particularly with homologous muscle activation. Since previous research found that when both upper-limbs are moving symmetrically, intracortical inhibition was decreased in both hemispheres (Stinear & Byblow, 2002), and that unimanual movement decreases IHI in both directions (Nelson et al., 2009), it is initially surprising that the current study found reduced IHI only in one direction (left to right M1). However, this specific finding may be explained by the particular movement parameters of the in-phase BMT.

During BMT in the current thesis (Studies #2-#4), participants were required to make simultaneous wrist extension movements of both upper-limbs to three different targets (35º, 45º, 55º relative to start position) (see Figure 6D). The 45º target required

simultaneous co-contraction of the ECR muscles to the same magnitude. However, the 35º and 55º target locations required a slightly different magnitude of co-contraction of both limbs. The asymmetrical reduction in IHI could be due to the requirement of different magnitudes of wrist muscle co-contraction. Moreover, all participants in these studies were right-hand dominant. It is thought that there is a reduction in IHI to produce contralateral unimanual movements (Duque et al., 2007). Furthermore, some studies have not found a hemispheric difference in IHI with hand dominance during voluntary contraction and unimanual tasks (Nelson et al., 2009). Conversely, there is evidence for lateralization of M1, showing that there is increased IHI from the dominant to non-dominant hemisphere at rest (Netz et al., 1995; Bäumer et al., 2007), which is how IHI was tested bilaterally in the current studies. The suggestion by this work was that this increased inhibition due to handedness may reflect dominant usage and experience with skilled manipulation of the dominant hand. Additionally, IHI in the wrist extensors and flexors is unchanged during light contraction and rest conditions from the non-dominant (right) to dominant (left) M1 compared to the reverse scenario (Sattler et al., 2012), providing evidence for asymmetrical increased IHI from the dominant to non-dominant M1. It is possible that the differing amounts of co-contraction required for two-thirds of the training (35º and 55º target locations), along with individuals with right-hand dominance may have led to the asymmetrical reduction of inhibition from the dominant to non-dominant hemisphere. Therefore, a certain amount of IHI may have remained in order to suppress full co-contraction of the upper-limbs. This remainder of inhibition could have been more likely to suppress activity in the more experienced (dominant, right) upper-limb, leading to stable IHI from the right to left M1. Since there is evidence

that right-hand dominant people would initially demonstrate enhanced inhibition from the left to right hemisphere at rest (Netz et al., 1995; Bäumer et al., 2007; Sattler et al., 2012), it follows that this inhibition may be 'released' in order for the non-dominant hemisphere to be more efficiently engaged during the complex bimanual movement task of the current thesis. Furthermore, there is a plethora of evidence suggesting that asymmetrical bimanual movement is less stable than strictly mirror symmetrical movements (Kelso et al., 1979; Cardoso de Oliveira, 2002; Swinnen, 2002; Carson, 2005). Therefore, asymmetrical bimanual movements likely require more complex patterns of neural activity, which is another potential explanation for the release of inhibition from the dominant to non-dominant hemisphere due to our specific BMT task.

Interestingly, this reduction in interhemispheric inhibition was only in LIHI and not with SIHI. The underlying mechanism mediating SIHI is largely unknown (Meyer et al., 1995), while LIHI likely relates to the ipsilateral silent period (Chen et al., 2003) and involves GABA-B-mediated inhibition since it has a relatively longer time course and is increased with baclofen, a GABA-B receptor agonist (Irlbacher et al., 2007). Although both SIHI and LIHI are believed to be mediated by excitatory transcollosal connections (Lee et al., 2007), the connections to contralateral inhibitory interneurons may differ (Chen, 2004). Previous studies found that a unimanual movement task compared to rest led to a reduction in both SIHI and LIHI bi-directionally from M1 to M1 (Nelson et al., 2009). The results of Study #4 (experiment 1) indicate that SIHI and LIHI are likely mediated by different inhibitory mechanisms, which has been confirmed by previous work (Meyer et al., 1995; Irlbacher et al., 2007), since if the SIHI and LIHI followed

similar inhibitory pathways, both would likely be modulated in a similar way due to the training tasks in the current thesis.

The effects on cortical silent period (CSP)

The CSP is affected by damage to M1 itself and other cortical nodes while sparing M1 (von Giesen et al., 1994; Classen et al., 1997). Specifically, von Giesen and colleagues (1994) showed that patients who have suffered stroke to the sensorimotor cortex resulted in a shortening of CSP, whereas damage to the ipsilateral premotor cortex to M1 resulted in prolonged CSP in the contralateral muscles. These results suggest that the shortening of CSP indicates that this inhibition is primarily generated within M1, and that the damage to remote areas (i.e. premotor cortex) results in a decreased inhibition of cortical interneurons. The results of Study #4 (experiment 2), which demonstrated a prolonged CSP due to iTBS to lPMd, may be an indication of disinhibition within M1 or interneurons from the PM cortex to M1.

Additionally, the CSP can be modified by high frequency rTMS of the stimulated M1 (Daskalakis et al., 2006; Khedr et al., 2007) and by particular pharmacological agents that indicate the CSP is at least partially mediated by GABA-B receptors (Ziemann et al., 1996; Ziemann et al., 1996; Mohammadi et al., 2006). Specifically, Daskalakis and colleagues (2006) found that CSP was prolonged after 600 stimuli delivered at 6 Hz applied to M1. This indicates that cortical inhibition was increased due to high frequency rTMS, similar to the effects of Study #4 (experiment 2) where iTBS was applied to ipsilateral PMd. It is possible that increasing the excitability of M1 directly (Daskalakis et al., 2006) and to remote but closely related motor areas (i.e. left PMd) may have enhanced the excitability of the GABA-B-mediated cortical inhibitory circuitry. PMd has

extensive reciprocal neuronal projections with the ipsilateral M1 (Picard & Strick, 2001; Rushworth et al., 2003), and rTMS at 5 Hz to lPMd increases cortical excitability in the ipsilateral M1 (Gerschlager et al., 2001; Chouinard et al., 2003; Rizzo et al., 2004; O'Shea et al., 2007; Suppa et al., 2008). Perhaps iTBS to lPMd increased the excitatory input from lPMd to ipsilateral M1 and, in turn, increased the excitability of long-interval inhibitory circuitry specifically mediating CSP in downstream M1. The increased excitability from lPMd to ipsilateral M1 may be due to neural projections to M1 in both hemispheres (Schluter et al., 1998; Modugno et al., 2001; Rushworth et al., 2003). Interestingly, cTBS over lPMd suppresses corticospinal excitability in left M1, with no changes in SICI or ICF (Huang et al., 2009). Additionally, cTBS applied over lPMd suppressed corticospinal activity in the contralateral (right) M1 (Stinear et al., 2009). Perhaps the findings from Study #4 (experiment 2) result from lPMd reciprocal connections with M1 in both hemispheres, with interactions between both excitatory and inhibitory projections, which may in turn also influence right M1 to left M1 projections (Asanuma & Okuda, 1962; Ferbert et al., 1992; Gerloff et al., 1998; Chen et al., 2003; Nelson et al., 2009), that could lead to the enhancement in long-interval inhibition in left M1. Interestingly, lower frequency rTMS (< 1 Hz) has produced inconsistent effects on CSP, with some studies showing no change (Fitzgerald et al., 2002), others showing shortening (Fierro et al., 2001) and still others showing lengthening (Romeo et al., 2000). The evidence from most studies on rTMS to CSP seem to indicate that the effects depend largely on the frequency and intensity of stimulation (Daskalakis et al., 2006; Khedr et al., 2007; Chen et al., 2008). Overall, there is evidence from all of these studies along

with the Study #4 (experiment 2) that CSP can be modulated by applying rTMS to motor-related cortical nodes.

As for the prolonged CSP in rM1 following short-term in-phase BMT (Study #4, experiment 1), it is possible that an increase in inhibitory mechanisms could be concurrent with increased requirement of precise motor control. Studies have demonstrated that CSP is reduced in patients with ALS (Mills, 2003), PD (Cantello et al., 1991; Siebner et al., 2000), in some patients with HD (Lefaucheur et al., 2006) and dystonia involving the upper-limb (Filipović et al., 1997). This suggests that a decrease in CSP is detrimental to motor control and coordination. Many of these studies attempted to administer pharmacological aids or rTMS protocols to rebalance inhibition by prolonging CSP. Therefore, the increase in CSP-related inhibition may be indicative of the heightened motor control requirement during the bimanual task in the current studies. The increased motor control parameters of the BMT task is likely due to the varying degrees of simultaneous co-contractions of the wrist musculature in order to successfully move the visual cursor to the cued targets. Additionally, this increased motor control requirement could have been more pressing for the non-dominant (left) upper-limb, which is why increased inhibition was observed in rM1 instead of lM1. Furthermore, it may be that the reduction of IHI from the left to the right M1 may have resulted in an increased excitability in rM1, as shown by increased MEP amplitudes. This increased excitatory input to rM1 could have resulted in increased excitability in the long-interval inhibition mediating the CSP. Conversely, an increased CSP in rM1 may be an indication of a reduction in inhibition of cortical interneurons within or to rM1, as evidenced by similar effects to those with damage to remote cortical areas (i.e. PM) (von

Giesen et al., 1994). Altogether, the results of the current thesis and previous studies provide potential explanation for the increased asymmetric long-interval inhibitory circuitry related to the CSP in M1. Other than the CSP-related inhibitory modulations, related but distinct LICI was uniquely modulated due to a combination of iTBS to lPMd and in-phase BMT.

The effects of long-interval intracortical inhibition (LICI)

To the best of our knowledge, Study #4 is the first to investigate the combined effects of iTBS to lPMd with in-phase BMT on LICI. Furthermore, it is one of the few studies that have investigated the effects of movement training on LICI (Meunier et al., 2012), and the first to study the effects of BMT on LICI. The we found a reduction in LICI due to iTBS to lPMd followed by BMT (experiment 3) and a slight reduction in lM1 LICI due to BMT alone (experiment 1). LICI has been shown to decrease with increased test stimulus intensity, which indicates that low threshold corticospinal neurons are more sensitive to LICI than high threshold corticospinal neurons (Sanger et al., 2001). It is possible that lower threshold corticospinal neurons in the area surrounding the hotspot were heightened in excitability due to the interventions, and were therefore activated during the testing of LICI. This idea follows the previous findings of Study #2 (group 3) that combined iTBS to lPMd and BMT in the same way as Study #4 (experiment 3), which demonstrated an increased M1 excitability in terms of both the amplitude and spatial distribution of MEPs along the borders of the trained muscle representation, without a concurrent increase in the hotspot MEP amplitude (Neva et al., 2014). It could be that the modulation in LICI reflects a reduction in intracortical inhibition in the area surrounding the central representation of the specifically trained muscles, similar to the

findings of previous work (Neva et al., 2014). Similarly, the effects due to BMT alone may reflect changes in intracortical inhibition surrounding the hotspot of the wrist muscle representation (Neva et al., 2012). Additionally, a recent study has shown that LICI is reduced in the presence of LIHI (Udupa et al., 2010). This study specifically demonstrated that these two long-interval inhibitory circuits influence each other by reducing the amount of inhibition each population produces by itself when both of these protocols are delivered together, suggesting that they share common properties. It is suggested that both of these networks (LIHI, LICI) are mediated by pre-synaptic and post-synaptic GABA-B receptors (Huang, 2006). It is possible that BMT induced a slight decrease in the dominant (left) M1, which could have concomitantly reduced the LIHI from the left to rM1 through related GABA-B receptor mediated mechanisms. The specific underlying processes cannot be confirmed in this work, therefore future study potentially utilizing LICI in the presence of LIHI (Udupa et al., 2010) bilaterally in M1 after in-phase BMT would further elucidate the intracortical modulations observed.

Few studies have demonstrated modulations in LICI circuitry due to movement training, (Meunier et al., 2012) rTMS protocols of motor-related cortical nodes (Suppa, Ortu, et al., 2008), and other plasticity inducing protocols like paired-associative stimulation (PAS) (Russmann et al., 2009; Meunier et al., 2012). Similar to the results of Study #4 (experiment 3), recent work has shown a prolonged reduction in LICI due to movement training involving precisely timed pinch grip or PAS (Meunier et al., 2012). This study further supports a reduction in LICI due to movement training, likely involving GABA-B related transmission. Previous studies found reductions in SICI due to motor training (Nordstrom & Butler, 2002; Liepert et al., 2004; Perez et al., 2004;

Rosenkranz & Rothwell, 2006; Rosenkranz et al., 2007) and the aforementioned work found a decrease in LICI due to motor training tasks (Meunier et al., 2012), therefore this was suggestive of a post-synaptic GABA-B related mechanism. Particularly since baclofen (an agonist of post-synaptic GABA-B receptors), decreased PAS-induced plasticity in human M1 (McDonnell et al., 2007). Also, this is supported by the fact that LICI circuitry is believed to actively inhibit SICI connections pre-synaptically (Sanger et al., 2001; Chen, 2004; Ni et al., 2011). This particular effect was not supported in the current study due to no observed modulation in SICI. Therefore, it could be that the reduction of LICI observed (Study #4, experiment 3) could be equally due to pre- and/or post-synaptic GABA-B-related inhibitory circuitry. One study investigated the effects of cTBS over either hemisphere M1 and found no change in LICI (Suppa et al., 2008). To our knowledge, Study #4 (experiment 3) is the first to measure LICI modulations due to the combination of rTMS and movement training, particularly using both upper-limbs. Therefore, the potentially increased cortical input from the lPMd in combination with in-phase BMT-induced plasticity may be required to produce a selective modulation of GABA-B-related LICI circuitry in rM1. Again, the specific asymmetrical reduction in inhibition may be due to the requirement of more efficient engagement of the non-dominant upper-limb to co-activate in varying degrees with the contralateral upper-limb (see above).

The lack of effect of short-interval intracortical circuitry

Study #4 did not support the hypothesis that BMT and iTBS to lPMd would lead to modulations in both SICI and ICF, and a combination of these interventions would lead to a greater modulations. Previous research has demonstrated reductions in SICI due

to motor training (Nordstrom & Butler, 2002; Liepert et al., 2004; Perez et al., 2004; Rosenkranz & Rothwell, 2006; Rosenkranz et al., 2007) and TBS protocols over M1 (Huang et al., 2005; Suppa et al., 2008). Many of these studies and others found no concurrent change in ICF. Specifically, no changes in ICF have been found due to unimanual sequential visuomotor training (Winkler et al., 2012), or due to a visuomotor tracking task involving unimanual wrist flexion and extension (Smyth et al., 2010). Also, 5 Hz suprathreshold rTMS applied to PMd resulted in no change of SICI (Suppa et al., 2008). In addition, iTBS to lPMd has demonstrated no effect of SICI or ICF on ipsilateral or contralateral M1 (Stinear et al., 2009), or when iTBS to lPMd is followed by unimanual movement training (Stinear et al., 2009). The findings of the current Study #4 were somewhat surprising, but there are factors that may account for the lack of observed changes in SICI and ICF due to each intervention. The disparity between the previous studies and the findings of the current thesis (Study #4, experiment 1) may be reconciled due to vast difference between the motor tasks. The previous studies that demonstrated reductions in SICI were with expert musicians performing unimanual tasks (Nordstrom & Butler, 2002), phasic unimanual finger adductions (Liepert et al., 2004), gait training recorded from the tibialis anterior (Perez et al., 2004), rapid thumb abduction, muscle vibration and PAS (Rosenkranz & Rothwell, 2006; Rosenkranz et al., 2007). All of these tasks were vastly different from the current in-phase BMT in terms of the unimanual upper-limb(s) (or lower limbs) use, the extents of the training that was required to produce the changes in SICI, and the fact that the intrinsic hand muscles were used in many of the tasks instead of the wrist musculature. Similarly, all TMS measures were

recorded from intrinsic hand muscles or a lower leg muscle, which could likely explain the differences in results.

It has been shown that SICI is the lowest threshold neural circuitry activated by TMS in the upper-limb area (Davey et al., 1994; Ziemann et al., 1996; Awiszus et al., 1999), so it could be that the modulations in SICI were too slight due to the interventions to be detected at the hotspot. Although SICI is a useful measure for assessing minor changes in cortical excitability (Modugno et al., 2003; Bagnato et al., 2005), other intracortical and interhemispheric networks such as LICI and LIHI may interact with SICI and ICF circuitry (Udupa et al., 2010). LICI circuitry actively inhibits SICI circuitry (Chen, 2004; Udupa et al., 2010), and LIHI circuitry can modulate SICI and ICF connections. Therefore, it is possible that the training and stimulation protocols (in combination and separately) in the current studies may interact to result in undetectable changes in the short-interval intracortical inhibitory and facilitatory circuitry bilaterally. Since Study #4 demonstrated changes in LICI due to BMT alone and iTBS to lPMd followed by BMT, it is possible that the long-interval circuitry affected short-interval circuitry by inhibiting its potential modulation.

However, other studies performing symmetrical bimanual movement tasks have demonstrated a reduction in SICI (Stinear & Byblow, 2002). The difference between the results of the current thesis (Study #4, experiment 1) and the previous study may be due to the constraints of the tasks themselves. The previous study had one upper-limb passively moved and the task was to match the movement with the opposite limb to perform mirror symmetrical movements. The BMT task in the current study had

participants actively move both upper-limbs to a visually cued target, and then extend both wrists simultaneously to move the visual cursor to the target (Neva et al., 2012).

Since the studies of the current thesis (Studies #1-#3) found increased excitability in the cortical area surrounding the hotspot, it follows that modulations would not be observed in SICI or ICF directly over the hotspot. Furthermore, it is possible that the conditioning-test pulse paradigm of SICI and ICF could not have been at a high enough intensity to recruit neurons in the area surrounding the ECR hotspot (Chen et al., 2008). Therefore, future study of the effects of BMT and TBS protocols to PMd on SICI and ICF in the cortical territory adjacent to the hotspot would be necessary.

4) Excitability increases along the borders of trained muscle representation in M1, which potentially relate to changes in long-term potentiation/depression (LTP/D), and unmasking of pre-existing horizontal connections.

The findings from Study #4 are indicative of modulations in GABA-related inhibition, specifically the long-interval GABA-B-related inhibition. However, the methodology of Study #4 did not allow for the testing the cortical area outside of hotspot where the previous studies observed many of the enhancements in cortical excitability. The cortical reorganization and increases in M1 excitability observed in the current studies (Study #1, #2, #3) were found in the areas surrounding the hotspot (Neva et al., 2012; Neva et al., 2014). These specific excitability increases could potentially relate to the early stages of changes in long-term potentiation/depression (LTP/D) (Woody et al., 1991; Hess & Donoghue, 1996, 1999; Hess et al., 1996; Martin et al., 2000), and unmasking of pre-existing horizontal connections in M1 (Jacobs & Donoghue, 1991; Malinow et al., 2000).

Several other studies have demonstrated that cortical reorganization and the enlargement of spatial cortical excitability of trained muscle representation occurs due to many different types of movement training (Pascual-Leone et al., 1995; Nudo et al., 1996; Classen et al., 1998; Kleim et al., 1998; Liepert et al., 1999; Kleim et al., 2004; Tyc et al., 2005; Adkins et al., 2006). Increases in M1 spatial extent of trained muscles have been observed in a 30 min session of in-phase BMT (Neva et al., 2012). These modest increases in short-term BMT can be slightly enhanced by applying rTMS (i.e. iTBS or cTBS) to remote and related cortical nodes such as PMd and the contralateral homologous M1 representation before movement training (Neva et al., 2014; Study #3). Further, movement training involving skilled finger sequence training for two 2 hr sessions (Pascual-Leone et al., 1995) leads to an expansion of the excitable area in M1. Other studies have shown that gross motor movement training also leads to an expansion of the trained proximal muscle representation in M1 in skilled volleyball players (Tyc et al., 2005). The studies of the current thesis (Studies #1, #2, #3) support these previous findings and also extend them by demonstrating that BMT and TBS protocols also lead to expansion of the excitable area of trained muscles in M1.

The interventions of the current studies (Study #2 and #3) demonstrated slight changes in cortical reorganization in terms of the centre of gravity (CoG) due to short-term BMT, iTBS to lPMd, iTBS with BMT, with the greatest change in CoG due to cTBS to rM1 followed by BMT. A shift in CoG is an amplitude-weighted center of the map and hotspot indicating the scalp position of maximum response in the target muscle (Ljubisavljevic, 2006). Although a shift in CoG and the overall spatial distribution of cortical map are related, they could both be sensitive to slightly distinct modulations in

overall M1 excitability. Cortical reorganization reflected in a shift in CoG has been demonstrated in previous studies (Classen et al., 1998; Liepert et al., 1999). Liepert and colleagues (1999) found that synchronised hand and foot movements led to a shift of the two muscle representations closer towards each other. Interestingly, no shifts in the M1 maps were seen when asynchronous hand and foot movements were trained. Additionally, Study #3 showed that the shift in CoG of the trained muscle representations remained up to 60 min post movement training. Further support for a shift in CoG due to movement training was found by another study which trained participants to perform thumb movements in a different direction than were consistently produced by single-pulse TMS over the abductor pollicis brevis (APB) hotspot (Classen et al., 1998). After training thumb movements in the opposite direction for approximately 30 min, the same single-pulse TMS over the APB representation evoked thumb movements in the trained direction. This modulation in M1 excitability likely reflected a shift in the CoG of the trained muscle representation. The shift in CoG mentioned is similar to the CoG shift due to cTBS to rM1 followed by short-term BMT that lasted up to 60 min beyond the interventions. Interestingly, not only did the CoG shift due to cTBS to rM1 followed by BMT, but the spatial map increased in size as well. Further, cTBS to rM1 alone caused a slight shift in CoG without a concurrent increase in spatial extent of cortical excitability. This could indicate that CoG and total M1 excitability may share related plasticity mechanisms that may be expressed in slightly different patterns across the entire M1 map. Additionally, the slight shifts in CoG due to the interventions in the current thesis may also be an explanation as to why changes in the hotspot or central MEP amplitude were not observed. Since, even a slight change in CoG (~1 cm in any direction) could

account for the potential modulations in the hotspot over the scalp (which remained the same before and following all interventions) to be undetectable, especially with subtle changes in central M1 representation excitability.

Several studies using animal models have also demonstrated increases and reorganization of M1 cortical maps due to skilled movement training (Nudo et al., 1996; Kleim et al., 1998; Kleim et al., 2004; Adkins et al., 2006; Ljubisavljevic, 2006; Nudo, 2006), and these do not occur due to simple repetitive movement (Kleim et al., 1998; Nudo, 2006). This has been supported in human research showing that skilled motor movement training and strength training leads to distinct modulations in M1 excitability (Jensen et al., 2005; Adkins et al., 2006). Strength training modulates the excitability of the spinal cord, whereas skilled movement training alone demonstrates M1 map reorganization and increases in cortical excitability. The cortical reorganization and enhancements in spatial excitability observed in this thesis and previous studies could be due to early stages of unmasking pre-existing horizontal connections (Jacobs & Donoghue, 1991; Atwood & Wojtowicz, 1999; Malinow et al., 2000), and increased synaptic transmission through long-term potentiation or depression (LTP/D) in M1 (Woody et al., 1991; Hess & Donoghue, 1996, 1999; Hess et al., 1996; Martin et al., 2000). It is likely that both changes in LTP/D and unmasking pre-existing horizontal connections in M1 occur concurrently during the early stages of the motor adaptation and movement training processes. It is likely that the increases in excitability along the borders of M1 representation due to BMT is primarily due to the unmasking of latent horizontal connections surrounding the target muscle cortical hotspot, without discounting the contribution of a concurrent increased synaptic transmission.

Conversely, the enhanced MEP amplitudes closer to the hotspot due to iTBS to lPMd and cTBS to rM1 may be primarily due to changes in LTP, strengthening the established neural connections within M1. Furthermore, when BMT was preceded by iTBS to lPMd or cTBS to rM1 there were concurrent increases in MEP amplitudes and spatial excitability, which may reflect simultaneous unmasking of horizontal connections and increased synaptic transmission due to LTP.

Another related possibility is that changes in membrane excitability may reflect the rapid modulations in cortical excitability associated with movement training and TBS protocols (Ljubisavljevic, 2006). Changes in membrane excitability could represent another way to modify the connections between neurons. Many studies have demonstrated prolonged increase in the excitability of M1 due to trained movements and other conditions (Sanes & Donoghue, 2000). This suggests that a change in membrane excitability of neuron populations, may be linked to plastic change due to skilled motor training and/or repetitive TMS protocols to related cortical nodes, by increasing the opportunity for multiple neurons to depolarize together and thus, enhancing the probability of strengthening synaptic connections. It is possible that both BMT and TBS of related cortical nodes (iTBS to ipsilateral PMd or cTBS to contralateral M1) may have modulated membrane excitability of neurons that potentially facilitated concurrent, and in some cases greater, enhancements in cortical excitability of the entire spatial representation of target muscles in M1. However, due to the constraints of the methodology of the current thesis this cannot be definitively determined. Indeed, the current studies involved the use of single and paired-pulse TMS as a means to image cortical plasticity and potential underlying neural mechanisms at the population level in

awake human participants (Ljubisavljevic, 2006). Therefore, it is not possible to distinguish the particular cellular and synaptic mechanisms underlying any of the interventions of these studies, other than to indicate that paired-pulse TMS in Study #4 suggested that modulations of GABA-B-related inhibition were involved in the cortical excitability changes observed. Overall, it is possible that the specific enhancements in cortical excitability and reorganization due to short-term BMT, TBS and the combination of these interventions were early markers of rapid functional plasticity involving the entire M1 map of the target muscle representations.

6.2 Thesis Limitations

There are limitations that could affect the interpretation of the work. In all the studies of the thesis, there were participants that took part in more than one experiment/intervention, with no individuals performing the BMT twice, except for Study #1, where six individuals performed the in-phase and anti-phase training. However, it is likely that in-phase and anti-phase training types were sufficiently different so that one training session did not affect the other. The purpose for no individuals repeating the in-phase BMT was so that behavioural performance and therefore the potential cortical excitability changes were not confounded. Second, a control group was not tested in any specific study. Therefore we did not test the placebo effects of training and stimulation. However, a small group of participants were collected with M1 cortical mapping before and following a rest period for approximately the amount of time that BMT (and/or TBS protocol) would take and found no changes in M1 excitability (See Appendix 1). Third, for the M1 mapping studies (Studies #1, #2, and #3) the total number of TMS pulses across the entire M1 map for the ECR representation may have caused a modulation in

cortical excitability itself. However, at each individual grid position 10 TMS pulses occurred with an ISI of ~2 seconds, with a time delay of 30-45 seconds with no stimulation when acquiring each following grid position. Therefore, it is unlikely that the frequency and total number of single-pulse stimulations led to a modulation of cortical excitability. Also, although training involved wrist flexion movements, as well as wrist extension, MEPs were not recorded from the flexor wrist muscles to observe the possibility of similar changes as observed in the wrist extensor muscles. For Study #2 and #4, the localization of the left hemisphere PMd was based on previous studies indicating that PMd is 2.5 cm anterior to the M1 hotspot (Picard & Strick, 2001; Huang et al., 2009; Ortu et al., 2009; Stinear et al., 2009). However, these studies based the location of PMd on the averages among individuals, which places PMd at a range of 0.88 to 2.29 cm anterior to the central sulcus (Picard & Strick, 2001). Therefore, there is variability among individual locations of left PMd relative to M1, and it is possible that the location of PMd was not always stimulated (or solely stimulated) by iTBS. It would have been a benefit to have each individuals MRI in order to locate PMd. Also, for Study #3, single-pulse MEPs were not acquired from rM1 before or after application of cTBS to rM1 in either group that experienced cTBS. Therefore, it cannot be verified that cTBS suppressed the stimulated M1 as previous work has shown (Huang et al., 2005). Finally, as previous mentioned above, Study #4 only acquired all TMS measures from the hotspot in M1 bilaterally. Therefore, it is possible that the lack of effects in the hypothesized measures may be due to the fact that TMS measures were not acquired in the cortical territory surrounding the hotspot, where modulations in excitability were observed in the

previous studies (Studies #1, #2, and #3). Further research will control for and investigate these potential confounding variables.

6.3 Future Directions

There are many different studies that could evolve from the work of the current thesis. Four potential experiments will be presented.

Study #1 found increased excitability of M1 due to in-phase, and not anti-phase bimanual movement training. Also, Study #4 found an asymmetrical reduction of inhibition from the left to right M1, which could be due to the slightly different type of in-phase BMT implemented, one that required varying degrees of simultaneous co-contraction. Therefore, it is of interest to investigate further varying the degrees of co-contraction during a visuomotor bimanual movement task to understand the kinematic parameters required for potentially inducing modulations in intracortical and interhemispheric inhibition. Therefore, this study will compare three conditions: 1) complete in-phase BMT (consistent simultaneous co-contraction of wrist extensors), 2) complete anti-phase BMT (consistent simultaneous antagonist muscle co-contraction) and a task which requires a 3) varying amount of co-contraction ("in-anti-phase movements"). This study would follow similar pre and post measures as in Study #4.

The second proposed study involves the investigation of the non-dominant M1 cortical plasticity, since these studies entail the use of both upper-limbs simultaneously. Study #4 found asymmetrical effects of intracortical and interhemispheric inhibition (much in the right M1), and Studies #1-#3 recorded MEP spatial distribution from the dominant upper-limb hemisphere (left M1). Therefore, it would be very useful to understand the excitability modulations in the non-dominant hemisphere (right) M1.

Therefore, this study will compare two conditions: 1) short-term in-phase BMT, and 2) anti-phase BMT. This study would follow similar pre and post measures as in Study #2, mapping the extents of the right M1 representations off the wrist flexors and extensors, as well as the APB and adductor digiti minimi muscles. This study will complement the results of Study #1 (Neva et al., 2012), furthering the understanding of the excitability modulations of the entire M1 representation in both hemispheres.

The third study uses fMRI to localize the cortical nodes involved in short-term in-phase versus anti-phase BMT. These studies could confirm the findings of Study #2 and #3, demonstrating the vital contribution of PMd and contralateral M1. It is possible that a single session of training would not be evident using fMRI, therefore several training sessions would be required. Further, once specific cortical nodes are identified due to in-phase or anti-phase BMT, these cortical loci could be targeted using iTBS to enhance the effects of short-term BMT. Conversely, these same cortical loci could be targeted using cTBS to investigate the necessity of these particular cortical nodes during cortical and behavioural adaptations from BMT.

Finally, the fourth proposed study would target the stroke patient population. Previous studies have shown that cTBS applied to the contralesional M1, or somatosensory cortex, followed by skilled motor training leads to enhanced cortical excitability in the ipsilesional cortex and improved motor performance in the affected upper-limb (Meehan et al., 2011). The objective of this study would be to determine the short-term intrahemispheric and interhemispheric cortical M1 adaptations that are affected by altering sensorimotor-related cortical nodes with rTMS paired with short-term in-phase BMT in individuals with stroke. This study will firstly determine whether

individual stroke patients will be able to perform a single short-term training session of BMT. Secondly, these patients will be then perform short-term BMT following the application of TBS protocols to motor-related areas in order to enhance ipsilesional excitability. Single, paired and dual-coil TMS will be measured at M1 ECR hotspot bilaterally and 1 cm in each anatomical direction surrounding the hotspot, in order to assess the overall M1 map. The Wolf Motor Function Test (WMFT) will assess motor function before and after the interventions. Participants will perform simultaneous in-phase wrist flexion/extension movements that move a cursor to targets displayed on a computer screen (Neva et al., 2012). One BMT session will consist of 5 blocks of 160 movements (800 movements total). Response time and kinematic measures will be recorded. Participants will be split up into four groups of 10 (both individuals with stroke and matched controls) 1) BMT (Neva et al., 2012; Smith & Staines, 2012), 2) cTBS over contralesional S1, 3) M1 and, 4) intermittent TBS over ipsilesional PMd, with all groups performing BMT immediately following stimulation. The proposed research has both clinical and theoretical significance as it provides insight into whether excitability changes in motor-related areas of the stroke-injured and uninjured brain may enhance the effectiveness of use-dependent cortical adaptations. This information will advance understanding of the factors that stimulate neuroplasticity and inform the development of novel therapeutic interventions for individuals living with the aftereffects of stroke.

6.4 Conclusion

The current thesis demonstrates the advantage of bimanual visuomotor movement training (BMT), theta burst stimulation (TBS) protocols to remote but related cortical nodes and a combination of these interventions to enhance M1 excitability. This is the

first series of studies that have shown BMT-induced cortical adaptation due to a specific training type (in-phase), and that repetitive stimulation of related cortical areas applied previously may enhance this BMT-induced cortical excitability in unique ways, and at times a greater extent, than any intervention alone. These results reveal that M1 excitability may be modulated by enhancement of PM areas and down-regulation of contralateral homologous M1. Furthermore, these results demonstrate that modulation of these cortical nodes may be advantageous in furthering cortical excitability changes in distinct ways, which could be useful in adapting more efficient rehabilitation of cortical areas affecting upper-limb function. These data will guide training and stimulation techniques that modulate cortical plasticity in the healthy population and in clinical settings. It may be that the modulation of remote cortical areas to M1 (i.e. lPMd and contralateral M1) in combination with rehabilitation training could be advantageous in enhancing short-term plasticity in damaged M1.

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Appendix 1: Primary motor cortex (M1) cortical mapping of extensor muscle representation before and following rest

Rationale

The purpose of this study was to investigate the potential modulations in cortical excitability by acquiring the extents of M1 cortical map through single-pulse TMS as in Studies #1-#3 of the current thesis. There is a potential that the previous studies collected the M1 maps at a frequency of ~ 0.5 Hz (~2 seconds) between single-pulse stimulations at each individual position on the scalp could have induced a modulation in M1 cortical excitability. However, as previously mentioned in the limitations of the studies of this thesis, there was a period of time in between stimulation at each grid position of 45-60 seconds. In addition, the number of single-pulse stimulations during individual experiments were variable, yet usually involved an average total of 280-320 stimulations. There is a potential the mere total number of single-pulse stimulations may have modulated M1 excitability. Therefore, it was hypothesized that collection of the extents of M1 cortical map will not cause a modulation of the cortical excitable area of left M1.

Materials & Methods

Participants

Eight healthy participants took part in the study. All gave written consent to participate in the study which has been approved by the Office of Research Ethics at the University of Waterloo.

Electromyographic (EMG) recording

Surface EMG was recorded in the same way as previous studies.

TMS & Neuronavigation

Focal TMS was performed as in Studies #1-#3. All participants performed no behavioural task. Instead, the single-pulse TMS mapping measurements of the ECR

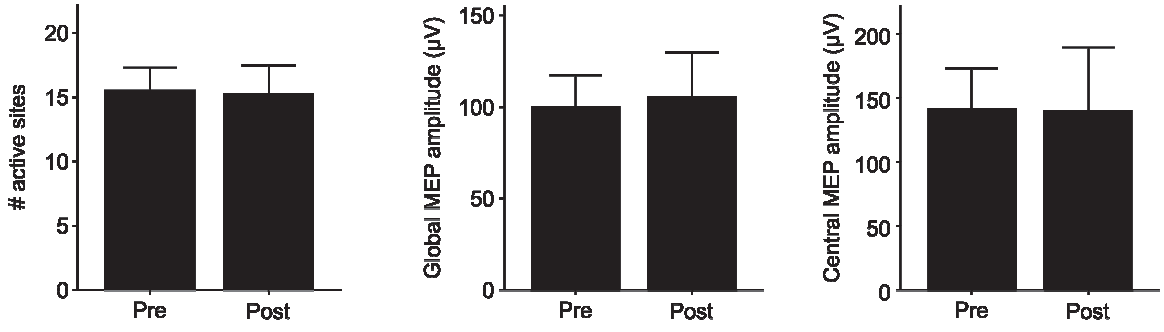
representation were performed before and following a rest period of approximately 30 min (the amount of time required on average to complete the BMT tasks performed in the previous studies).

Statistical analysis

To investigate the potential effects of cortical mapping, analysis was performed between the pre and post time points with the dependent measures of spatial extent, global, and central MEP amplitude. Therefore, a repeated measures ANOVA was performed with TIME as a factor (group 1: rest – pre and 30 min post acquisition of pre mapping).

Results

Appendix figure 1 displays the spatial map, global, and central MEP amplitude before and after the intervention of rest. There was no change in spatial extent of ECR in M1, as a one-way repeated measures ANOVA revealed no increase in active sites between before and after rest alone ($F(1, 7)=0.026$, $p=0.876$). There was also no change in global ($F(1, 7)=0.067$, $p=0.803$) or central ($F(1, 7)=0.001$, $p=0.973$) MEP amplitudes before and after rest alone.



Appendix figure 1. Means of all dependent measures for left M1 ECR excitability for all participants before and after rest. *Left* Spatial maps displayed by number of active sites. *Middle* Global MEP amplitude. *Right* Central MEP amplitude. All bars represent SEM. Asterisk indicates significance, $p < 0.05$.

Discussion

M1 cortical excitability of the ECR in the left hemisphere is not modulated by the collection of single-pulse TMS over the extents of the target muscle representation. The results indicated that neither the frequency of single-pulse stimulations, nor the total number of stimulations changed the excitability of the ECR muscle representation in M1. Therefore, it is unlikely that this particular limitation in Studies #1-#3 affected the results observed.

Appendix 2: Behavioural performance parameters during bimanual training (BMT)

Rationale

The purpose of this analysis is to further elucidate the contribution of both upper-limbs to the performance of the bimanual training tasks (BMT) used in the current thesis. The contribution of each individual limb to the behavioural performance of the BMT tasks is of particular interest since all participants were right hand dominant and Studies #1-#3 primarily recorded TMS measures from the left hemisphere M1. The performance of each limb during BMT could be indicative of the particular cortical excitability changes observed in the studies of the current thesis. It was hypothesized that the behavioural of each upper-limb equally contributed to kinematic dependent measurements quantified in the current studies.

Statistical analysis

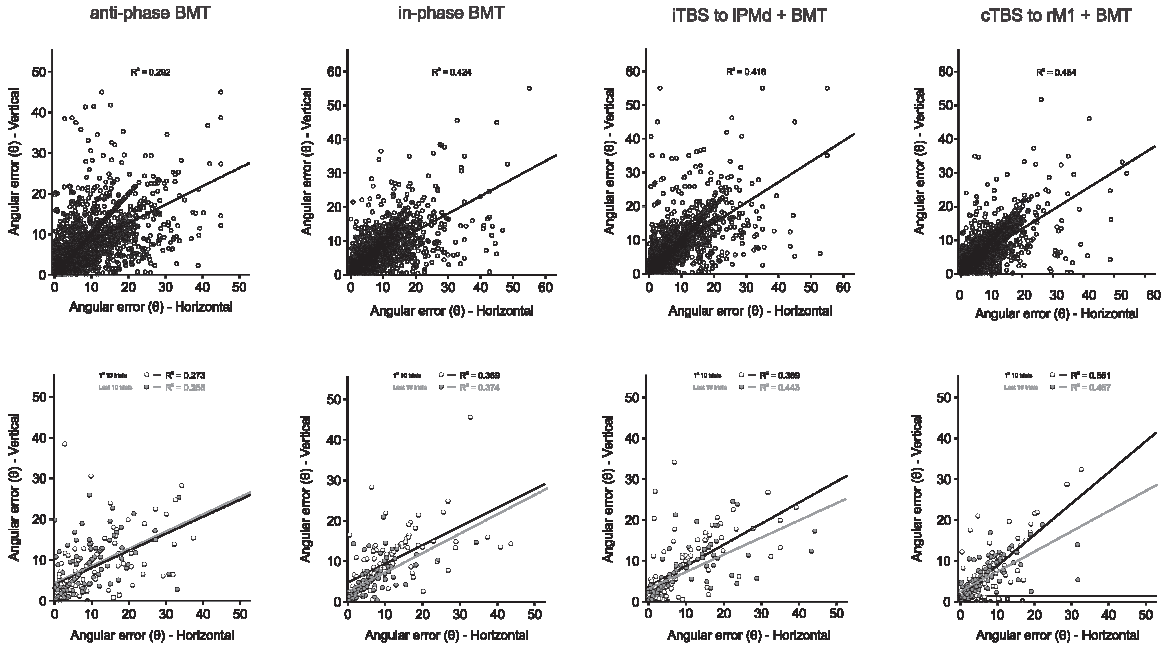
This analysis specifically focused on comparing the horizontal (left upper-limb) and vertical (right upper-limb) peak velocities from each upper-limb during the first 10 trials and last 10 trials of BMT for each group (anti-phase BMT, in-phase BMT, iTBS to lPMd + BMT, cTBS to rM1 + BMT). Therefore, a 2-way ANOVA was performed with

UPPER-LIMB (2 levels: left and right upper-limb) and GROUP (4 levels: anti-phase BMT, in-phase BMT, iTBS to lPMd + BMT, cTBS to rM1 + BMT) as a between subjects factors. *Post hoc* analyses were performed with a Tukey correction method to investigate any further differences between factor GROUP.

Results

Appendix figure 2 displays scatterplots of all groups that performed BMT, displaying the angle at peak velocity for both the left (horizontal component) and right (vertical component) upper-limbs, with the top panel showing all training trials for all participants and the bottom panel showing the first and last 10 trials for all participants. Linear regression line is shown. For the first 10 trials of BMT, a 2-way ANOVA revealed no difference in angle at peak velocity between UPPER-LIMB ($F(1, 76)=0.002$, $p=0.968$), no GROUP X UPPER-LIMB interaction ($F(3, 76)=1.016$, $p=0.390$), with an effect of GROUP ($F(3, 76)=4.813$, $p=0.004$). *Post Hoc* analysis revealed a reduced angle at peak velocity (i.e. enhanced performance) in the first 10 trials in the cTBS to rM1 + BMT group compared to those that performed in-phase BMT alone ($p=0.003$) and a close to significant difference with anti-phase BMT alone ($p=0.056$). For the last 10 trials of BMT, a 2-way ANOVA revealed no difference in angle at peak velocity between UPPER-LIMB ($F(1, 74)=1.196$, $p=0.278$) no GROUP X UPPER-LIMB interaction ($F(3, 74)=0.795$, $p=0.501$), with an effect of GROUP ($F(3, 74)=4.163$, $p=0.009$). *Post Hoc* analysis revealed a reduced angle at peak velocity (i.e. enhanced performance) in the last 10 trials in the cTBS to rM1 + BMT group compared to the anti-phase BMT group ($p=0.027$) and a near significant difference with the iTBS to lPMd + BMT group

(p=0.056). These analyses reveal that at the beginning of training (first 10 trials) and the end of training (last 10 trials) there were no differences in the contribution of each upper-limb to the overall performance bimanual movement task. There was a slight difference at the beginning of training (first 10 trials) in the overall performance of the in-phase and anti-phase BMT between the cTBS to rM1 with BMT and BMT alone groups, and between the cTBS to rM1 with BMT and iTBS to lPMd with BMT and anti-phase BMT at the end of training (last 10 trials). This indicated that there was a slight enhancement in performance at the start and finish when BMT was preceded by cTBS to rM1. However, these performance enhancements are very slight and occur only with the dependent measure of angle at peak velocity.



Appendix figure 2. *Top* Scatterplots of the horizontal (x-axis) and vertical (y-axis) contributions to the angular error at peak velocity of the resultant cursor path for anti-phase BMT, in-phase BMT, iTBS to lPMd + BMT and cTBS to rM1 + BMT groups (from left to right in the figure). *Bottom* Scatterplots of the horizontal (x-axis) and vertical (y-axis) contributions to the angular error at peak velocity of the resultant cursor path for the same groups for the first and last 10 trials of BMT. Linear regression fit shown, with R-squared correlation values.

Discussion

Overall, both hands were active simultaneously and were similarly contributing to the resultant cursor movement across training trials in all groups that performed BMT. As confirmed by analysis comparing the angular error of each upper-limb in this appendix, there are equal contributions of each upper-limb to the performance of the BMT task. From this, it is reasonable to assume that generally speaking, participants were performing the task appropriately, with both upper-limbs simultaneously active with the goal of moving the cursor to the particular visual targets displayed. Therefore, the cortical excitability effects observed in the current thesis are likely due to the specific parameters of our BMT training tasks.