**Modulation of inhibitory and excitatory circuits in the primary motor cortex following theta-burst rTMS to area 5**

by

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**AUTHOR'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

Subcortical and cortical loci interact with the primary motor cortex (M1) and influence the neural circuitry mediating hand movement.  Area 5 located within the superior parietal lobule has direct connectivity with M1, is largely dedicated to the representation of the hand, and is considered important for thumb opposition movements.   The present study examined the modulation of inhibitory and excitatory neural circuits within bilateral M1 before and after continuous (cTBS), intermittent (iTBS), and sham theta-burst stimulation (TBS) over left-hemisphere area 5.  Two experiments were performed to address the influence of area 5 on neural circuitry within M1.  Specifically, inhibitory circuitry (short interval intracortical inhibition (SICI)) and excitatory circuitry (motor evoked potentials (MEPs), intracortical facilitation (ICF)), were examined for the representation of the first dorsal interosseous (FDI) muscle within bilateral M1.  MEPs, SICI, and ICF were measured bilaterally before and at 5-20 minutes, 25-40 minutes, and 45-60 minutes after TBS cessation.  The order for right versus left M1 recordings for MEPs, SICI, and ICF recordings were kept constant within subjects across each time block and this order of cortex stimulated (right, left) was randomized across subjects.  The results of Experiment 1 and 2 demonstrate that area 5 selectively influences M1 circuitry such that MEPs are increased bilaterally following area 5 cTBS and increased in the right FDI following area 5 iTBS.  Area 5 TBS does not modulate ICF or SICI.  The novel findings from the Master's thesis suggest area 5 is a cortical loci that influences M1 excitatory circuitry and possibly motor control of the hand.

# ACKNOWLEDGEMENTS

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AMT        Active Motor Threshold
ANOVA     Analysis of Variance
CS          Conditioning Stimulus
CTBS       Continuous Theta-burst Stimulation
CSN        Cortical Spinal Neuron
DT-MRI    Diffusion Tensor Magnetic Resonance Imaging
D-wave     Direct Wave
EMG        Electromyography
FDI         First Dorsal Interosseous
fMRI       Functional Magnetic Resonance Image
GABA      Gamma (γ) Amino Butyric Acid
HRP        Horseradish Peroxidase
ICF         Intracortical Facilitation
IPL         Inferior Parietal Lobule
ISI         Interstimulus Interval
ITBS       Intermittent Theta-burst Stimulation
I-wave     Indirect Wave
LTD        Long Term Potentiation
LTP        Long Term Depression
M1          Primary Motor Cortex
MEP        Motor Evoked Potential
MRI        Magnetic Resonance Imaging
MT         Motor Threshold
NMDA     $N$-methyl-d-aspartate
mV         Millivolts
PPC        Posterior Parietal Cortex
RMT        Resting Motor Threshold
RTMS      Repetitive Transcranial Magnetic Stimulation
SEPs       Somatosensory Evoked Potentials
SI          Primary Somatosensory Cortex
SII         Secondary Somatosensory Cortex
SICI       Short Interval Intracortical Inhibition
SLF        Superior Longitudinal Fasciculus
SMA       Supplementary Motor Area
SPL        Superior Parietal Lobule
TBS        Theta-burst Stimulation
TMS       Transcranial Magnetic Stimulation
TS         Test Stimulus
μV         Microvolts

# Modulation of inhibitory and excitatory circuits in the primary motor cortex following theta-burst rTMS to area 5

## 1.1 Overview of Thesis

The execution of precise control of the hand typically requires a balance in cortical activity between excitatory and inhibitory neural circuits within the primary motor cortex (M1). An imbalance in such neural circuitry is observed in patient groups and symptoms of impaired motor function of the hand are often seen (Focal Hand Dystonia: Ridding et al., 1995a; Chen et al., 1997a; Parkinson's Disease: Ridding et al., 1995b; stroke: Hummel et al., 2009). One method to introduce changes to the neural circuitry within M1 involves applying repetitive Transcranial Magnetic Stimulation (rTMS) over areas that are anatomically connected with M1 (Huang et al., 2009). More specifically, cortical loci that are largely dedicated to the control of hand movement may alter the M1 neural networks specific to hand function. Targeting these remotely connected areas via rTMS may alter the output to the muscles of the hand and could possibly lead to improved hand function in patient groups.

Research in primates has identified several cortical areas involved in somatosensory-guided hand movements, one of which includes area 5 located within the medial superior parietal lobule (SPL). Area 5 exists in species with opposable thumbs with its emergence coinciding with the evolution of skilled hand manipulation (Padberg et al., 2007). Area 5 has a large cortical representation of the hand (Padberg et al., 2005, 2007) and direct projections to M1 (Strick and Kim, 1978). Several studies in non-human primates have described the sensory (Mountcastle et al., 1975; Sakata et al., 1973) and

motor (Kalaska et al., 1990; Mountcastle et al., 1975) functions of area 5 in forelimb and hand movement, however, the function of area 5 within humans is less understood. In humans, activity within the SPL is enhanced during tactile motion discrimination (Nakashita et al., 2008), preparatory signals for upcoming finger-pointing (Astafiev et al., 2003), finger tracking (Grafton et al., 1992), imagined finger movements (Hanakawa et al., 2003) and bilaterally during tactile discrimination of objects (Stoeckel et al., 2004). Damage to this loci results in writing apraxia (Otsuki et al., 1999). Collectively, data in humans suggests the involvement of area 5 in hand movements, but questions remain about how area 5 influences M1 neural networks, the networks that ultimately control the cortical output to the hand. Understanding how area 5 impacts the neural circuitry within M1 may provide insight into its role in hand movement. **The goal of the Master's thesis is to determine the influence of area 5 on the neural circuitry within M1.** Two experiments were performed to address the influence of area 5 on excitatory and inhibitory neural circuitry within M1.

To investigate the influence of area 5 on M1 neural networks, we applied TMS in twenty-five awake, healthy humans. One form of TMS is called theta-burst stimulation (TBS) and when applied intermittently (iTBS) or continuously (cTBS), TBS induces changes in cortical excitability (Huang et al., 2005). By measuring well investigated and clinically relevant neural inhibitory circuitry - short interval intracortical inhibition (SICI) - and excitatory circuitry - motor evoked potential (MEPs) and intracortical facilitation (ICF) - within M1, before and following area 5 TBS, we can further our understanding on the influence area 5 exerts on M1 output. Electromyography (EMG) was recorded from the

first dorsal interosseous (FDI) muscle for the right and left hands in each of the following experiments before and following TBS.

**1.2 Summary of Experiments**

*Experiment 1*

The goal of Experiment 1 was to investigate modulation of inhibitory (SICI) and excitatory (ICF and MEPs) neural circuits within M1 following *cTBS* to area 5. Experiment 1 was conducted in 12 healthy humans.

*Experiment 2*

The goal of Experiment 2 was to investigate modulation of inhibitory (SICI) and excitatory (ICF and MEPs) neural circuitry within M1 following *iTBS* over area 5. Experiment 2 was conducted in 11 healthy humans.

*Sham control*

For comparison with the experimental TBS groups, ICF, MEPs, and SICI were investigated before and following sham TBS in a group of 12 healthy humans naive to TMS stimulation. Data from sham control participants provides a comparison to identify the true effects of real TBS on neural circuits within M1.

**1.3 Significance of Master's Thesis Research**

The data obtained from the Master's thesis studies provides novel neuroscience information that includes identifying an alternate path to modulate M1 cortical output to the hand. Specifically, a bilateral increase in MEPs following cTBS (*Experiment 1)* and an increase in MEPs elicited from the RFDI muscle contralateral to iTBS stimulation

(*Experiment 2)* were observed.  These exciting findings suggest that the output of M1 is influenced bilaterally by left-hemisphere area 5 making this loci a potential candidate for rTMS paradigms applied to clinical populations with impaired hand control.

## 1.4 Outline of Thesis Chapters

The experiments presented in this thesis were performed in healthy adults.  Chapter 2 is a review of the literature and methodology pertinent to the Master's thesis.  Detailed descriptions of the methods employed, specific hypotheses and results from Experiments 1 and 2 will follow in Chapters 3 and 4, respectively.  An overall Discussion in Chapter 5 will provide interpretation of the findings from the Master's thesis experiments.

## Chapter 2: Literature Review

**2.1 Area 5**

*2.1.1 Anatomy and function of area 5 in monkeys*

Electrophysiological and anatomical studies indicate that area 5 within the superior parietal lobule (SPL) resides in the middle of the rostral bank of the intraparietal sulcus (Krubitzer et al., 2005; Pons et al., 1985; Padberg et al., 2010).  Area 5 is dominated by a large representation of the forelimb and hand with minimal territory devoted to other body parts (Padberg et al., 2007).  This loci is organized in an orderly, somatotopic distribution (Hlustik et al., 2001; Padberg et al., 2007) and neurons within area 5 have contralateral, ipsilateral and bilateral receptive fields (Padberg et al., 2007; Iwamura et al., 2000, 2001). In cebus monkeys and macaques with opposable thumbs, area 5 is well developed and is poorly defined or absent in species unable to perform this function.  Further, the emergence of area 5 coincides with skilled manipulation involving digits 1 and 2 (Padberg et al., 2005, 2007).

Neurons within area 5 respond to the stimulation of deep receptors of the skin and joints (Sakata et al. 1973; Mountcastle et al., 1975; Taoka et al., 2000) and are tightly linked to hand movements.  Specifically, area 5 neurons may be involved in initiating reaching and grasping behaviours (Mountcastle et al., 1975; Cohen and Andersen et al., 2002), movement preparation (Snyder et al., 1997), and execution of manual behaviors such as pinch grip and goal-directed tool use.

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*2.1.2 Anatomy and function in humans*

In humans, area 5 is located within the SPL and is comprised of three divisions with distinct cytoarchitecture. These subregions reflect the complexity of area 5 as a higher-order cortical region and suggest its role as both a sensory and motor cortical loci (Scheperjans et al., 2005). In humans, activity within the SPL is enhanced during tactile motion discrimination (Nakashita et al., 2008), reaching and grasping (Grafton et al., 1992), and bilaterally during tactile discrimination of objects (Hanakawa et al., 2003). Stroke encompassing the SPL results in writing apraxia (Otsuki et al., 1999).
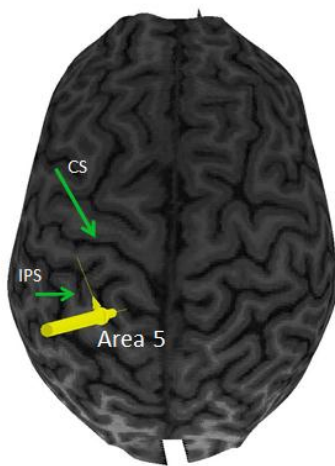


Figure 2.1: Location of area 5 in humans

*2.1.3 Connections between primary motor cortex (M1) and area 5*

Anatomical (Jones et al., 1978, Leichnetz, 1986, Strick and Kim, 1978) and electrophysiological (Zarzecki et al., 1978) studies in monkeys reveal direct projections from area 5 to ipsilateral M1 suggesting a strong coupling between these two loci. In old world monkeys, area 5 neurons that were labeled following horseradish peroxidase

injections into M1, were found within layer III, granular layer IV and in neurons in layer V (Leichnetz, 1986).  In humans and monkeys, the superior longitudinal fasciculus (SLF) is the main fiber tract connecting parietal with frontal regions of the brain (Makris et al., 2005).  The SLF (subdivision 1) is situated within the white matter of the SPL and connects the postcentral and precentral gyri (areas 4 and 5).  Interactions between area 5 and M1 may be mediated via pyramidal cells within area 5 (Strick and Kim, 1978, Leichnetz, 1986) that project via the glutamatergic SLF (Bakiri et al., 2009, Dinglegine et al., 1999, Makris et al., 2005) and terminate on M1 output neurons (Strick and Kim, 1978).

*2.1.4 Spinal contribution of area 5*

Evidence in monkeys suggests a direct axonal projection to the spinal cord from area 5 (Murray and Coulter 1981; Coulter and Jones 1977).  Retrogradely transported axon tracer confined to the dorsal horn of the spinal grey matter revealed labeled neurons within area 5 (Murray and Coulter 1981) while anterograde labeling within area 5 revealed terminal labeling within the lateral half of the dorsal horn (Coulter and Jones 1977).

**2.2 Review of Relevant Methodology**

*2.2.1 Transcranial Magnetic Stimulation (TMS)*

Transcranial magnetic stimulation (TMS) is a non-invasive technique used to examine cortical neural circuits in humans.  Specifically, TMS can be used to investigate inhibitory and excitatory neural circuitry within M1.

TMS is based on the principle of electromagnetic induction as discovered by Michael Faraday in 1838 (Kobayashi et al., 2003).  The primary circuit (the stimulating coil) drives a time varying current that generates a magnetic field that can reach up to 2 Tesla and lasts

for about 100 μs (Hallett 2007).  The magnetic pulses penetrate the scalp and skull to reach

the brain with minimal attenuation.  A secondary current is induced perpendicularly to the

magnetic field and affects the neuron's trans-membrane potential (Pascual-Leone et al.,

2002) (See Figure 2.2).  If the amplitude, spatial characteristics, and duration cause a

depolarization of the neuronal membrane, generation of an action potential will occur.



**Magnetic Field**
(Perpendicular to current in coil)

**Current in coil**

Skull

**Induced electric field**
(Opposite direction of current in the coil)
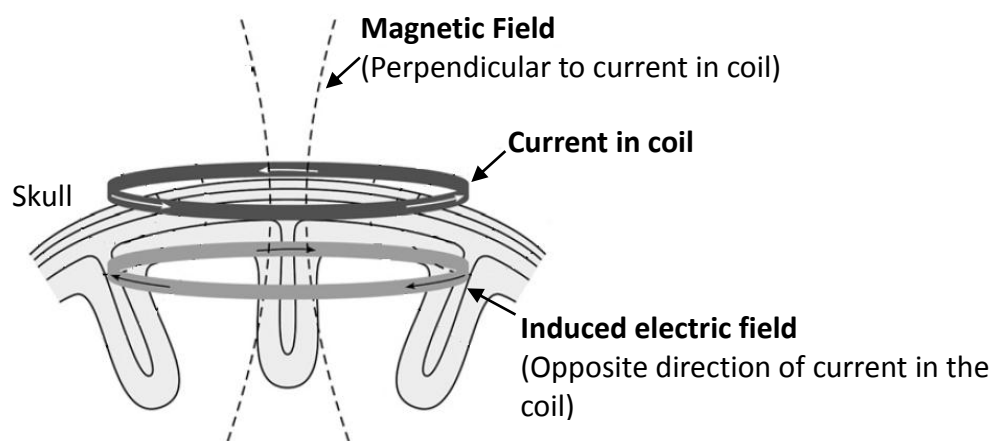
Figure 2.2:  In TMS, the current in the coil generates a magnetic field that induces a
secondary current in the opposite direction.  If the coil is applied over M1, the induced
electric field may activate neuronal elements within M1 to create a motor response in the
target muscle (Figure modified from Hallett 2007).

Previous studies on the exposed monkey cortex showed that a single-pulse of

stimulation can recruit corticospinal neurons in two ways: directly (D-waves) on the axon

of the corticospinal neuron (CSN) or indirectly (I-waves) through trans-synaptic activation

of these output neurons (Rothwell et al., 2005).  In the case of the hand area within M1,

TMS activates CSNs indirectly through the trans-synaptic inputs (Hallett 2000) and

preferentially activates intracortical fibres travelling horizontally with respect to the surface of the cortex (Rothwell 1997).

Different orientations of the TMS coil can recruit different descending volleys (D- versus I-waves). Specifically, if the coil current induces a posterior to anterior direction, I1-waves are recruited first with the lowest threshold intensity. When the direction of stimulation is reversed to induce an anterior to posterior current direction, I3-waves are the first descending volleys to be recruited while lateral to medial currents recruit D-waves at the lowest threshold. This selectivity is thought to be related to the direction of the CSN axons that are being stimulated and the point where the potential difference along the axon is the greatest (Rothwell 2005). The intensity of TMS can be controlled by changing the amount of current flowing into the coil and therefore changing the magnitude of the secondary induced current (Pascual-Leone et al., 2002).

Transcranial magnetic stimulation (TMS) has become a widely used method in neuroscience research and can be used to test several inhibitory and excitatory circuits. Specifically, motor evoked potentials (MEPs), short interval intracortical inhibition (SICI), and intracortical facilitation (ICF) are parameters studied for evaluation of M1 excitability.

*2.2.2 Excitatory Circuitry: Motor Evoked Potentials (MEPs)*

When TMS is applied to M1 at appropriate stimulation intensities, MEPs can be recorded from contralateral extremity muscles (Kobayashi et al., 2003). A single-pulse of TMS stimulates the upper motor neurons within M1 and the impulses are transmitted via the corticospinal tract to the anterior horn cells of the spinal cord (Hess et al., 2005). The resulting D- and I-waves are highly synchronized and when spatially and temporally

summated, they can reach the threshold for firing an action potential (See Figure 2.3). This can subsequently result in the lower motor neuron to fire and send an impulse along the peripheral motor axon. At the level of the muscle, when the action potential is triggered in the pre-synaptic neuron, calcium channels open allowing for the entry of calcium, release of acetylcholine neurotransmitter into the neuromuscular junction, and subsequent muscle contraction. MEPs are usually recorded from the first dorsal interosseous (FDI) muscle (Rothwell 2005), however, they can also be measured in other muscle groups such as abductor pollicis brevis (APB) (Stefan et al., 2008) and abductor digiti minimi (ADM) (Floyd et al., 2009).

Motor threshold (MT) is a measure of membrane excitability of CSNs and spinal motor neurons (Rossini et al., 1994). MT is well investigated and has clinical relevance as it is altered by certain diseases (Kobayashi et al., 2003). Resting motor threshold (RMT) is defined as the lowest intensity required to elicit MEPs that have a peak to peak amplitude of 50 µv or more in a resting target muscle in 5 out of 10 consecutive trials (Orth et al., 2009). When the target muscle is contracted, an increase in excitability of spinal motor neurons is seen. Subsequently, MEPs are larger in amplitude and often shorter in latency during voluntary contraction compared to MEPs obtained from muscles at rest (Rothwell, 2005). Active motor threshold (AMT) is commonly defined as the lowest intensity required to elicit MEPs that have a peak to peak amplitude of 200 µv or more during a 10% maximal voluntary contraction (MVC) (Orth et al., 2009).
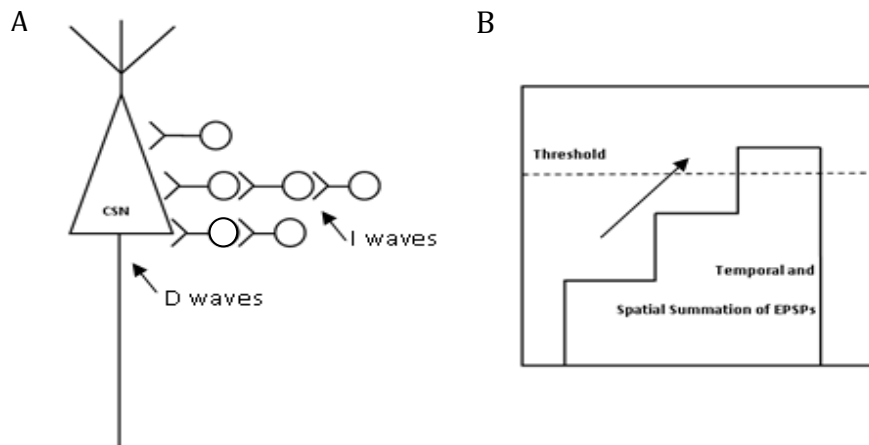
Figure 2.3:  At the upper motor neuron (A), TMS can stimulate the CSN directly (D-waves) or indirectly (I-waves) through trans-synaptic connections.  The temporal and spatial summation of the excitatory post-synaptic potentials at the lower motor neurons, (B), may reach threshold potential leading to firing of the lower motor neuron (Figure modified from Hallett et al., 2005).

Ultimately, MT and MEP amplitude are excitability measures that provide insights into the efficacy of a chain of synapses from pre-synaptic cortical neurons within M1 to the target muscles.

*2.2.3 Local Inhibitory Circuitry: Short Interval Intracortical Inhibition (SICI)*

SICI is considered to be a well characterized inhibitory circuit that is probed using paired-pulse stimulation (Kujirai et al., 1993; Chen et al., 2003; Nakamura et al., 1997; Di Lazzaro et al., 2002; Kobayashi et al., 2003).  Previous studies have found a reduction in SICI in certain patient populations such as Parkinson's disease (Ridding et al., 1995b), Focal Hand Dystonia (Butefisch et al., 2005; Chen et al., 1997), and Stroke (Hummel et al., 2009).

In this technique, two magnetic stimuli are delivered in close sequence to the same cortical region through a single stimulation coil (Kujirai et al. 1993) (See Figure 2.4). The inhibitory effects of the first subthreshold conditioning stimulus (CS) on the size of the MEP elicited by the subsequent suprathreshold test stimulus (TS) are observed when the interstimulus interval (ISI) between the pair of stimuli is between 1-6 ms (Kujirai et al., 1993). A lack of change in spinal reflexes has suggested that SICI is a result of synaptic interactions occurring cortically within M1 rather than spinally (Rothwell 2005; Kujirai et al., 1993). This has been confirmed through direct recordings of descending volleys that have revealed that a subthreshold CS evokes no descending activity, and suppresses later I-waves (Di Lazzaro et al., 1998). The CS activates the low-threshold inhibitory interneurons within M1 and reduces the number of action potentials generated by the suprathreshold TS (Kujirai et al., 1993).
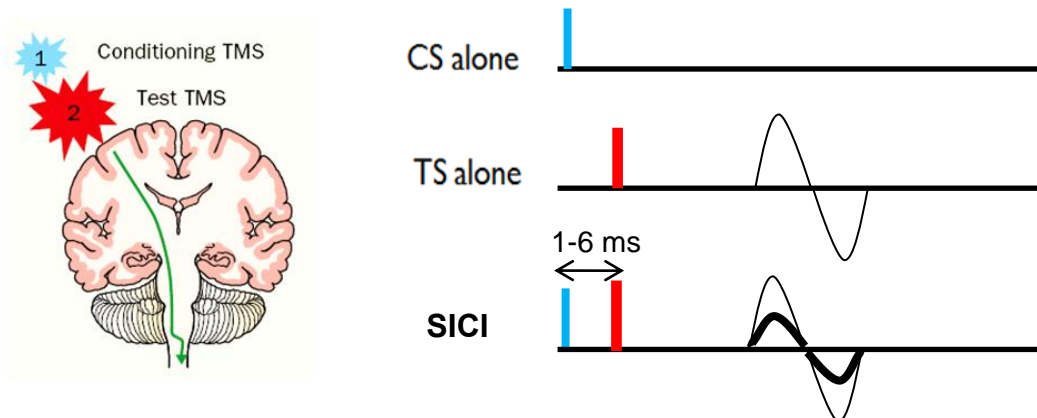


Figure 2.4: SICI involves a subthreshold conditioning stimulus (CS) that is followed by suprathreshold test stimulus (TS). If the ISI between the CS and TS is between 1-6 ms, attenuation of the TS MEP is often observed (Figure modified from Kobayashi et al., 2003).

*2.2.4 Local Excitatory Circuitry: Intracortical Facilitation (ICF)*

Facilitatory interactions occurring locally within M1 can be investigated in a similar fashion to SICI using two TMS pulses through one coil. Specifically, ICF can be probed using ISIs between 10-15 ms and applying a subthreshold CS to influence the response to a subsequent suprathreshold TS (Kujirai et al., 1993). Facilitation becomes stronger with increased CS intensity (Kujirai et al., 1993). It is likely that ICF and SICI act on a separate population of neurons (Ziemann et al., 1996), however the specific pathway of ICF remains unclear.
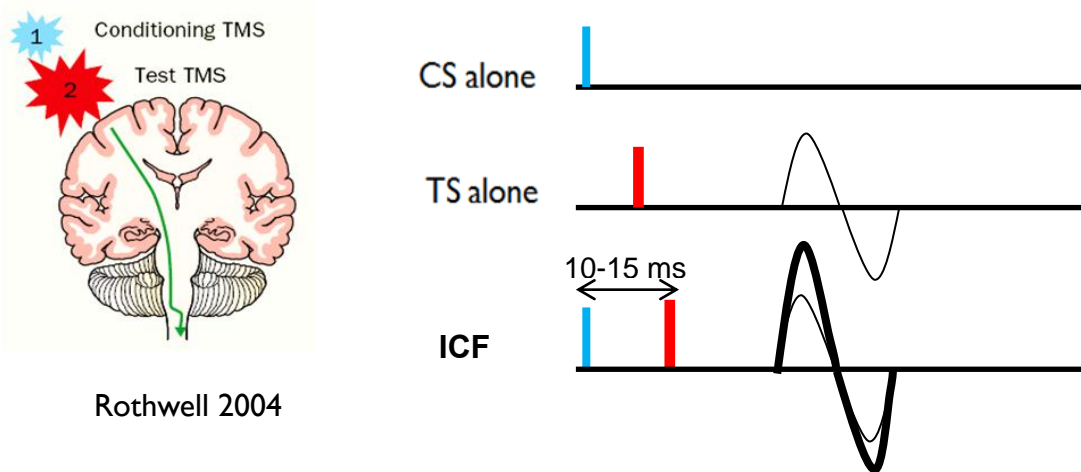


Figure 2.5: ICF involves a subthreshold conditioning stimulus (CS) that is followed by suprathreshold test stimulus (TS). If the ISI between the CS and TS is 10-15 ms, a facilitation of the TS MEP is often observed (Figure modified from Kobayashi et al., 2003).

## 2.3 Repetitive Transcranial Magnetic Stimulation (rTMS)

TMS can be used in a variety of ways to induce excitability changes in the brain. Specifically, when TMS is applied in trains of multiple stimuli to the same cortical area with the appropriate frequency, intensity, and duration of stimulation, excitability at the

stimulation site can be altered (Siebner et al., 2002).  When rTMS is applied over M1, the observed changes are thought to be due to alterations in the synaptic efficacy of excitatory glutamatergic cortical interneurons which project onto CSNs (Huang et al., 2007).  Although the mechanisms of rTMS remain unclear, it is believed that long term potentiation (LTP) and long term depression (LTD)-like effects are similar to those observed in hippocampal rat tissue (Malenka and Bear, 2004).

*2.3.1 Long Term Potentiation/Long Term Depression (LTP/LTD)*

The phenomenon of LTP and LTD of synaptic transmission has been suggested to play a key role in the plastic reorganization changes in the central nervous system (Bliss and Lomo, 1973).  LTP- and LTD-like effects are thought to be related to the modulation of the activity of glutamate receptors (Glu-r), *N*-methyl-d-aspartate receptors (NMDAr), and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPAr) receptors in cortical neurons.  When glutamate binds to NMDA-r and the post- synaptic cell is sufficiently depolarized, $Mg^{2+}$ blocked channels open, and a subsequent influx of calcium ($Ca^{2+}$) occurs. The $Ca^{2+}$ influx is believed to initiate LTP induction (Cooke and Bliss 2006).  Specifically, LTP-like effects are seen if the calcium binds to the carboxyl C-lobe activating calcium-calmodulin-dependant kinase and phosporylating AMPA receptors.  This increases the number of receptors at the post-synaptic membrane and strengthens the synaptic connection.  LTD-like effects are seen when smaller concentrations of calcium bind to the amino N-lobe triggering a phosphatase pathway that causes a decrease in the number of AMPA receptors and permeability (Malenka and Bear 2004).

*2.3.2 Effects of rTMS on MEPs*

Low-frequency rTMS ($\leq$ 1 Hz) decreases MEP amplitude for at least 30 minutes following stimulation (Fitzgerald et al., 2002, Chen et al., 1997b, Maeda et al., 2000, Muellbacher et al., 2000), while high frequency rTMS applied over M1 increases MEP amplitude (Pascual-Leone et al., 1994, Berardelli et al, 1998, Maeda et al., 2000).

*2.3.3 Effects of rTMS on SICI*

In addition to altering MEPs, rTMS may induce changes in SICI. SICI has been shown to be reduced when low and high frequency rTMS is applied over M1 (Modugno et al., 2003; Pascual-Leone et al., 1998; Di Lazzaro et al., 2002; Wu et al., 2000; Peinemann et al., 2000). Other rTMS paradigms have had no effect on SICI (Fitzgerald et al., 2002; Daskalakis et al., 2006). These variations could be due to the intensity, number of stimuli, and frequency of stimulation used (Peinemann et al., 2000, Siebner et al., 2002).

Effects seen with rTMS can alter cortical excitability at both the site of stimulation and remote areas (Hallett 2000). For example, 5 Hz rTMS can increase MEP amplitude for up to 6 minutes in the contralateral M1 (Gorsler et al., 2003). Similarly, 1 Hz rTMS can modulate cortical excitability in the contralateral hemisphere (Wassermann et al., 1998, Schambra et al., 2003).

*2.3.4 Effects of rTMS on ICF*

High frequency rTMS over M1 increases (Wu et al., 2000) or does not alter (Pascual-Leone et al., 1998, Peinemann et al., 2000, Di Lazzaro et al., 2002) excitability in ICF circuitry. Similarly, low frequency rTMS over M1 decreases (Romero et al., 2002) or does not modulate ICF (Modungo et al., 2003).

## 2.4 Theta-burst Stimulation (TBS)

A novel rTMS protocol known as theta-burst stimulation (TBS) requires a shorter stimulation duration and lower stimulation intensity to induce changes in cortical excitability that outlast the period of stimulation for up to 60 minutes (Huang et al., 2005). TBS protocols allow for more comfortable stimulation conditions and may extend to use in the clinical setting. TBS may be an effective method to modulate motor cortex physiology and could serve as a therapeutic tool in some neurological disorders.

TBS consists of single, low-intensity bursts of rTMS at 50 Hz and can target specific populations of neurons (Huang et al., 2005). This protocol is based on animal studies, which showed that bursts of 3-5 pulses at 50-100 Hz, repeated at 5 Hz (theta rhythm) induces LTP/LTD-like effects when applied to M1 or the hippocampus (Larson et al., 1986; Davies et al., 1991). Pyramidal cells in the hippocampus of rats occasionally fire in short, high frequency bursts and the stimulation trains of TBS intend to resemble these patterns. The pattern of delivery of TBS, intermittent versus continuous may determine the direction of excitability changes. The pattern of TBS consists of three pulses delivered at 50 Hz every 200 ms (5 Hz theta). CTBS consists of a 40 second train of uninterrupted TBS (600 pulses) and iTBS consists of a 2 second train of TBS that is repeated every 10 seconds for approximately 192 seconds (600 pulses) (See Figure 2.6).
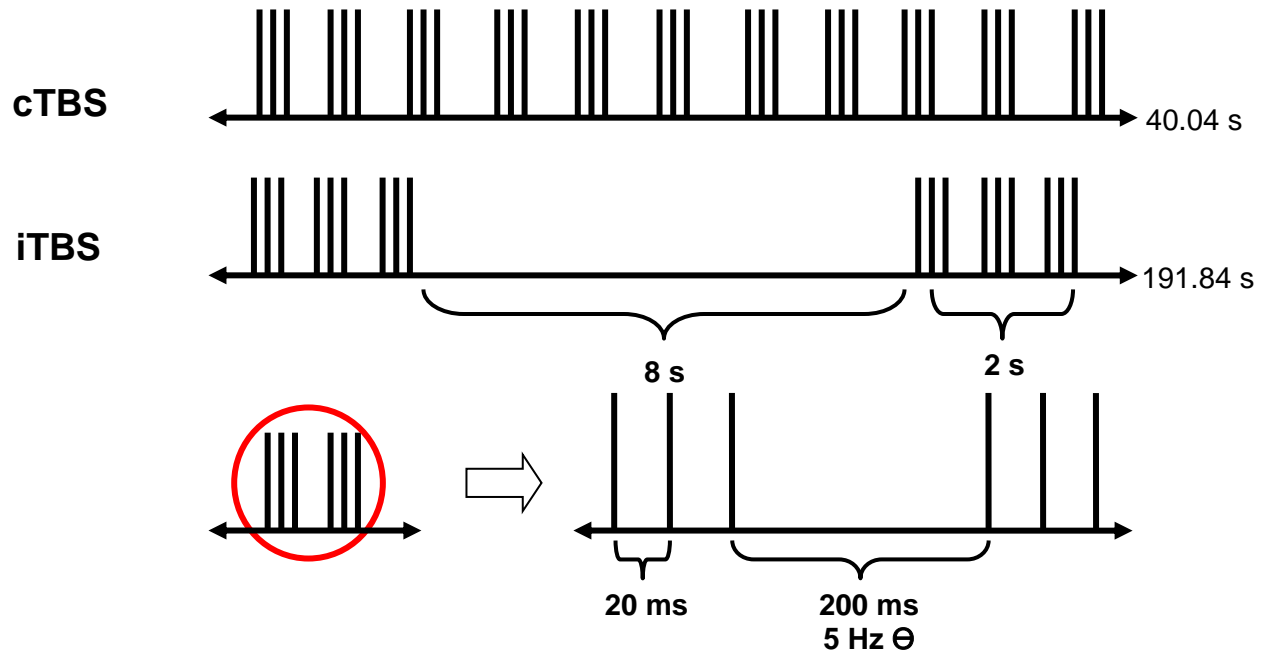
Figure 2.6: CTBS consists of a 40 second train of uninterrupted TBS. ITBS consists of a 2 second train of TBS that is repeated every 10 seconds. Each burst consists of three 50 Hz stimuli (separated by 20 ms) and is repeated every 200 ms (Figure modified from Huang et al., 2005).

*2.4.1 Differences between cTBS and iTBS stimulation protocols*

Di Lazzaro et al., (2005) demonstrated that cTBS leads to a pronounced decrease in

the excitability of cortical circuits generating the I1-wave and iTBS alters later I-waves with

minimal influences on I1- and D-waves (Di Lazzaro et al., 2008). These differences could be

due to the different population of neurons that may be sensitive to the effects of iTBS and

cTBS. At present, the physiological mechanisms of TBS are not fully understood. Two

possible mechanisms for the influence of TBS on cortical excitability include a role for

NMDA and GABA (Cardenas-Morales et al., 2008).

*2.4.2 Effect of TBS on MEPs*

When applied over M1, cTBS decreases the amplitude of MEPs for 20-60 minutes (Huang et. al, 2005, Talelli et al., 2007a, Zafar, et al., 2008), while iTBS increases MEP amplitude for 15-20 minutes (Huang et. al, 2005, Zafar et. al, 2008).  Similar to rTMS, TBS can alter cortical excitability at both the site of stimulation and remote areas.  Ishikawa et al. (2007) has shown the effects of cTBS applied at 80% AMT over M1 reduces MEP amplitude elicited in right and left FDI muscles.  In contrast, Suppa et al. (2008) applied cTBS over right M1 and found a reduction in MEP amplitude from the right and left FDI muscles when using an anterior to posterior current flow direction.  In the same study, a change of direction to a posterior to anterior flow of current resulted in a suppression in MEPs elicited from the stimulated hemisphere, with no effect in the contralateral hemisphere.  Similar to the latter finding, Stefan et al. (2008) found cTBS applied at 70% RMT facilitated MEP amplitude in the contralateral hemisphere.  When iTBS is applied over M1, an increase in MEP amplitude in the stimulated hemisphere (Huang et al., 2005, Suppa et al., 2008) and a decrease in MEP amplitude in the non-stimulated hemisphere (Suppa et al. 2008) was observed.  TBS protocols may also alter M1 excitability when applied to loci that are anatomically and functionally connected to M1.  For example, when cTBS is applied over the left premotor cortex, MEP amplitude is decreased in the right FDI muscle (Huang 2009).

*2.4.3 Effects of TBS on SICI*

In addition to modulating MEPs in M1, TBS may have an effect on the SICI.  Changes in SICI induced by TBS protocols appear to be more consistent (Huang et al., 2005; Talelli et

al., 2007; Suppa et al., 2008) than those observed when rTMS is applied over M1 (Pascual-Leone et al., 1998; Wu et al., 2000; Modungo et al., 2003 Fitzgerald et al., 2002; Daskalakis et al., 2006).  Huang et al. (2005) found an increase in SICI after application of iTBS over M1 and a reduction in SICI after cTBS that lasted up to 20 minutes.  When Talleli et al. (2007) applied cTBS over M1, a reduction in SICI lasted up to 20 minutes after stimulation.  These findings were again replicated in a study done by Suppa et al. (2008) where the facilitatory effects of iTBS and the suppressive effects of cTBS on SICI were observed and persisted for up to 35 and 45 minutes, respectively.

*2.4.4 Effects of TBS on ICF*

Following M1 cTBS, ICF can be decreased (Huang et al., 2005) or remain unaltered (Suppa et al., 2008; Talleli et al., 2007).  ITBS applied over M1 has no effect in modulating ICF circuitry (Huang et al., 2005; Suppa et al., 2008).  From the studies where no changes in ICF were reported following cTBS to M1, Talleli and Suppa's groups applied TBS in an anterior to posterior current direction while Huang et al., (2005) used a posterior to anterior direction and a 300 pulse cTBS paradigm which may account for the different findings.

**Chapter 3: Experiment 1**
**Modulation of neural circuitry within primary motor cortex following**
**continuous theta-burst rTMS over area 5**


**3.1 Rationale**

Neural circuitry within the primary motor cortex (M1) influences the control of

hand muscles (Ridding et al., 1995a; Chen, 2004).  Typically, in healthy individuals there

exists a balance of excitatory and inhibitory neural circuits within M1.  An imbalance in M1

circuitry may be observed in patient groups who present with impaired control of hand

movement.  For example, individuals with Focal Hand Dystonia (Ridding et al., 1995a, Chen

et al., 1997), Stroke (Hummel et al., 2009), and Parkinson's Disease (Ridding et al., 1995b)

reveal alterations in the normal inhibitory and excitatory circuitry within M1.  Attempts to

alter the M1 neural circuitry within such patients using repetitive Transcranial Magnetic

Stimulation (rTMS) have revealed short-lasting and modest changes to cortical excitability

(Siebner et al., 1999a, 1999b).

One form of rTMS called theta-burst stimulation (TBS) requires shorter stimulation

times and is applied with lower intensities making this paradigm better suited for clinical

applications.  TBS applied directly over M1 can facilitate (iTBS) (Huang et al., 2005; Zafar et

al., 2008) or depress (cTBS) (Huang et al., 2005; Stefan et al., 2008; Zafar et al., 2008; Suppa

et al., 2008) cortical excitability in the targeted cortex for a short period of time with effects

that are comparable to that of conventional rTMS protocols (Cardenas-Morales et al.,

2009).  In addition, cTBS can modulate behaviour such that when applied over premotor

cortex and anterior intraparietal sulcus, cTBS disrupts the predictive scaling of isometric

finger forces (Nowak et al., 2009) and decreases muscle activity related to the index finger

(Davare et al., 2010), respectively.  Although TBS protocols, when applied over M1's hand

representation, have the potential to modulate physiology and hand movement, they are

also limited by the short duration and modest after-effects.  Identifying other cortical loci

involved in hand movement that can influence the M1 output to the hand may provide an

alternative target for rTMS therapies.  Such therapies may yield long-lasting and robust

changes in motor output to the muscles controlling movement of the hand.

　　　Area 5, located within the medial superior parietal lobule (SPL) in monkeys and

humans (Darian-Smith et al., 1996), is responsive to somatosensory input (Mountcastle et

al., 1975), is linked to skilled hand manipulation (Padberg et al., 2007) and has a large

cortical representation of the hand (Padberg et al., 2005, 2007).  Anatomical (Jones et al.,

1978; Strick and Kim, 1978) and electrophysiological (Zarzecki et al., 1978) studies in

monkeys reveal direct projections from area 5 to M1 with the magnitude of input as

substantial (Strick and Kim, 1978) or greater (Leichnetz 1986) than that originating in the

primary somatosensory cortex (SI) suggesting area 5 may provide input critical to M1

function.  In humans, the superior longitudinal fasciculus (SLF) association fiber pathway

likely mediates the connectivity between area 5 and ipsilateral M1 (Makris et al., 2005).

Area 5 interacts with M1 during the processing of somatosensory input applied to the

thumb and index finger compared to rest (Ziluk et al., 2010) and cTBS over area 5 increases

pinch forces bilaterally (Premji et al., 2010a).  However, there remain several uncertainties

about the influence of area 5 on M1 circuitry with respect to the muscles of the hand.

Identifying area 5's influence on M1 clinically-relevant inhibitory and excitatory networks

may reveal area 5 as a target loci for rTMS paradigms.

The goal of the present experiment was to investigate the influence of area 5 on inhibitory and facilitatory networks within M1 serving the first dorsal interosseous (FDI) muscle of the hand. Several TMS studies have revealed distinct networks of facilitation- *motor evoked potentials (MEP)* and *intracortical facilitation (ICF),* and inhibition- *short interval intracortical inhibition (SICI).* SICI is observed when pairs of TMS pulses are delivered over M1 with an inter-stimulus interval (ISI) between 1-6 ms (Kujirai et al., 1993; Roshan et al., 2003) and ICF is revealed at inter-pulse intervals of 10-15 ms (Kujirai et al., 1993). CTBS over M1 reduces MEPs (Huang et al., 2005; Suppa et al., 2008; Stefan et al., 2008; Zafar et al., 2008), ICF (Huang et al., 2005) and SICI (Huang et al., 2005, Suppa et al., 2008), and alters the neural circuitry within contralateral M1 although the direction of contralateral changes is variable (Ishikawa et al., 2007; Suppa et al., 2008; Stefan et al., 2008). Circuitry within M1 may also be modulated following cTBS over remote loci. CTBS to left premotor cortex decreases MEP amplitude from the right FDI muscle but has no effect on SICI or ICF (Huang et al., 2009) suggesting that specific neural circuitry may be selectively modulated from loci outside of M1. To address the goal of Experiment 1, excitatory circuitry (ICF and MEPs) and inhibitory circuitry (SICI) were measured before and following cTBS over left-hemisphere area 5. Area 5, with its large cortical representation of the hand (Padberg et al., 2005, 2007), connectivity to M1 (Jones et al., 1978; Strick and Kim, 1978) and role in skilled hand movement (Padberg et al., 2007), may modulate M1 output and could be a primate candidate for rTMS therapies in the control of hand movement.

**3.2 Hypotheses**

The present study investigated bilateral neural circuitry within M1 following cTBS over left-hemisphere area 5. Specifically, the inhibitory circuit, SICI, and excitatory circuits, MEPs and ICF, were measured before and after real and sham cTBS to area 5. The hypotheses are as follows;

Excitatory Neural Circuitry:

Motor Evoked Potentials (MEPs) and Intracortical Facilitation (ICF)

MEPs elicited from the left and right M1 cortices will increase for up to 60 minutes (post 1, 2, 3) following cTBS cessation, similar to the longevity of after-effects following cTBS over M1 (Huang et al. 2005) and premotor cortices (Huang et al., 2009). It is hypothesized that ICF will increase bilaterally following cessation of cTBS to area 5. These effects are hypothesized to persist for up to 20 minutes (post 1), similar to the time course whereby ICF was facilitated following cTBS directly over M1 (Huang et al., 2005). No changes following sham TBS were expected for MEPs and ICF.

Inhibitory Neural Circuitry:

Short interval intracortical inhibition (SICI)

SICI will decrease bilaterally following area 5 cTBS. Based on an increase in net excitability in M1 following cTBS over area 5 (Premji et al., 2010a), we expect a reduction in M1 inhibitory circuitry bilaterally with effects lasting up to 45 minutes (post 1, 2) (Suppa et al., 2008). No changes following sham TBS were expected for SICI.

## 3.3 Methods

### 3.3.1 Participants

Twelve healthy participants (7 females, mean age years, SD 26 ± 3.7) were studied with cTBS to area 5 and 12 participants (6 females, mean age 22.1 years, SD ± 2.56) were studied with sham (placebo) TBS.  Right-handedness was confirmed using a subset of the Edinburgh Handedness Inventory (EHI) (Oldfield, 1971).  All subjects gave informed written consent.  This study was approved by the Office of Research Ethics at the University of Waterloo.

### 3.3.2 Experimental approach

### Neuronavigation and Transcranial magnetic stimulation

Single and paired-pulse magnetic stimulation was delivered using two custom-built 50 mm inner diameter figure-of-eight branding coils connected to two Magstim 200² stimulators (Magstim, Whitland, UK).  CTBS was applied using a 90 mm outer diameter figure of eight coil with a MagPro stimulator (MCF-B65; Medtronic, Minneapolis, MN, USA).  Figure 3.1 displays an example of the location for TBS over area 5 in one participant.  Area 5 was identified as the medial part of the superior parietal lobule using the anatomical MRI for each participant.
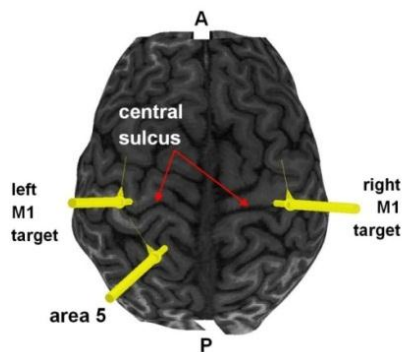


Figure 3.1:  Example of the location for TBS over area 5

To determine the motor hotspot for the FDI muscle within M1 of each hemisphere, the branding coil was positioned over left or right M1 and oriented 45 degrees to the mid-sagittal line to induce a posterior to anterior current direction. The motor hotspot was defined as the M1 location optimal for eliciting a MEP in the contralateral relaxed FDI muscle. The motor hotspot was deemed within the precentral gyrus for each participant as determined by the digital registration of each coil with each individual's MRI using Brainsight Neuronavigation (Rogue Research, Canada). MRI was conducted on a 3T GE scanner (172 images) with 3DFSPGR-IR sequences using a 20 cm FOV (256 x 256). Active motor threshold (AMT) was determined at the motor hotspot and defined as the lowest intensity required to evoke MEPs 200 μV amplitude in 5 out of 10 consecutive trials during 10% MVC of FDI (Orth et al., 2009).

*EMG recording*

Surface EMG was recorded from the FDI muscle on the right and left hand using 9 mm diameter Ag-AgCl surface electrodes. The active electrodes were placed over the muscle belly and the reference electrode was placed over the metacarpophalangeal joint of the index finger and thumb for FDI. EMG was amplified 1000 x, band-pass filtered between 2 Hz to 2.5 kHz (Intronix Technologies Corporation Model 2024F, Canada), digitized at 5 kHz by an analog-to-digital interface (Micro1401, Cambridge Electronics Design, Cambridge, UK) and stored on a computer for off-line analysis.

*Dependent Measures*

*MEPs (excitatory circuitry)*

To evoke MEPs, fifteen single TMS pulses were applied over the left and right FDI

motor hotspot within M1.  TMS intensity was set at a value that evoked MEPs of ~1 mV

amplitude in left and right FDI muscles before cTBS and the identical intensity (same value)

was used following stimulation as done elsewhere (Huang et al., 2009; Suppa et al., 2008).

*SICI (inhibitory circuitry) and ICF (excitatory circuitry)*

For paired-pulse stimulation, both the CS and TS were applied over the M1 through

the same coil connected to a Magstim $200^2$ stimulator operating via a Bistim module.

Paired-pulse paradigms, SICI and ICF, were performed using a subthreshold CS followed by

a suprathreshold TS to the FDI motor hotspot (Kujirai et al., 1993).  The ISI for SICI and ICF

was 3 and 10 ms, respectively, to achieve intracortical inhibition (Kujirai et al., 1993) and

facilitation (Di Lazzaro et al., 2006).  The CS was set at 80% AMT for SICI and ICF as

determined before cTBS stimulation and this value was kept constant throughout the

experiment (Huang et al., 2009; Suppa et al., 2008).  The TS intensity was adjusted to evoke

MEPs in contralateral FDI of ~ 1 mV before and after cTBS (Huang et al., 2009; Huang et al.,

2005; Suppa et al., 2008).  Stimulation intensities for the CS and TS were adjusted to

accommodate the reduced output of the Bistim module.  Fifteen trials with an inter-trial

interval of 5 seconds were collected for left and right SICI and ICF.

*3.3.3 Sham control*

For the group receiving the sham cTBS, AMT was collected to determine CS

intensities for SICI and ICF as described above.  The TBS coil was turned off and the sound

associated with cTBS was audio recorded and played to participants during sham

stimulation.  The origin of the sound was out of sight for participants and adjacent to the

TMS machine.  Participants were positioned in the Brainsight apparatus with their surface

skull anatomy aligned with a standard MRI.  An approximate location within the SPL and

the TMS coil was placed over this target.  No subject reported knowing that the stimulation

was a sham placebo.  MEPs and SICI/ICF were recorded at the same intervals as real cTBS.

*3.3.4 Experimental Timeline*

MEPs, SICI, and ICF were measured bilaterally before and at 5-20 minutes, 25-40

minutes, and 45-60 minutes after cTBS cessation.  The order for right versus left M1

recordings for MEPs, SICI, and ICF recordings were kept constant within subjects across

each time block and this order of hand stimulated (right hand, left hand) was randomized

across subjects.  Figure 3.2 depicts the experimental timeline.

**3.4 Data Analysis**

The paired-pulse MEP amplitude was expressed as a ratio to the mean

unconditioned MEP amplitude (TS alone) for each participant.  Ratios below one represent

inhibition and ratios above one represent facilitation.  Individuals showing greater than

10% inhibition for SICI and 10% facilitation for ICF were included in the analysis for each

group.  Two-way ANOVA with between-subject factor 'INTERVENTION' (cTBS, sham) and

within-subject factor 'TIME' (pre, post1, post 2, post 3) were performed for MEPs, SICI, and

ICF for the right and left FDI muscles.  Post hoc Tukey's test was used to identify any

differences from pre-TBS.  Statistical significance was set at $p \leq 0.05$.

## 3.5 Results

All participants successfully completed the experiment. The mean stimulator output used for delivery of cTBS was 38.17% ($\pm$ 7.8). Table 3.1 is a summary of number of participants included for the results that follow and Tables 3.2, 3.3, and 3.4 provide overall trends for MEPs, SICI, and ICF, respectively.

### 3.5.1 Motor Evoked Potentials (MEPs)

For MEPs recorded over right FDI, the hand contralateral to area 5 cTBS, the two-way ANOVA revealed a significant main effect of INTERVENTION ($F_{(1,66)}$ = 4.93 p=0.037) and TIME ($F_{(3,66)}$ = 7.76 p = 0.0002), and a significant interaction between INTERVENTION and TIME ($F_{(3, 66)}$ = 7.96, p = 0.0001). Post-hoc Tukey's test revealed that MEP amplitude was significantly greater at 5 (p = 0.0001), 25 (p = 0.0001), and 45 (p = 0.0159) minutes following cTBS compared to pre-cTBS values. There were no differences amongst MEP amplitudes in the sham intervention. Figure 3.3A displays the group-averaged MEPs (with standard errors) for right FDI before (pre) and after (post 1, 2, 3) cTBS and sham control.

For MEPs recorded over left FDI, ipsilateral to area 5 cTBS, the two-way ANOVA revealed no effect of INTERVENTION ($F_{(1,66)}$ = 1.04 p= 0.3185), a significant main effect of TIME ($F_{(3,66)}$ = 3.05 p= 0.0344), and a borderline significant interaction effect between INTERVENTION and TIME ($F_{(3,66)}$ = 2.62 p= 0.0583) suggesting that the TIME effect may be dependent on INTERVENTION membership. Subsequently, we are considering this interaction to be significant. Post-hoc Tukey's test revealed that compared to pre-TBS, MEP amplitude was significantly greater at 5 (p = 0.0026), 25 (p = 0.0003), and 45 (p = 0.0308)

minutes following cTBS.  Figure 3.3B displays the group-averaged MEPs (with standard errors) for left FDI before and after cTBS and sham TBS.

*3.5.2 Short Interval Intracortical Inhibition (SICI)*

For SICI recorded over right FDI, contralateral to area 5 cTBS, the two-way ANOVA revealed no effects of INTERVENTION ($F_{(1,57)}$ = 0.26 p= 0.6163), TIME ($F_{(3,57)}$ = 2.30 p= 0.0868), or between the INTERVENTION and TIME interaction ($F_{(3,57)}$ = 0.31 p= 0.8210). Figure 3.4A displays the group-averaged MEPs (with standard errors) for right FDI before and after cTBS and sham TBS.

Similarly, for SICI recorded over left FDI, ipsilateral to area 5 cTBS, the two-way ANOVA revealed no effects of INTERVENTION ($F_{(1,60)}$ = 2.24 p= 0.1502), TIME ($F_{(3,60)}$ = 2.67 p= 0.0554), or INTERVENTION and TIME ($F_{(3,60)}$ = 0.99 p= 0.4043).  Two one-way ANOVAs for the main effect of TIME revealed no changes following stimulation for the cTBS and sham TBS groups.  Figure 3.4B displays the group-averaged MEPs (with standard errors) for left FDI before and after cTBS and sham TBS.

*3.5.3 Intracortical Facilitation (ICF)*

No effects following cTBS were observed for ICF measured from the right (INTERVENTION ($F_{(1,57)}$= 3.79 p= 0.0664), TIME ($F_{(3,57)}$ = 0.14 p= 0.9356) or INTERVENTION and TIME ($F_{(3,57)}$ = 1.27 p= 0.2922)) or left (INTERVENTION ($F_{(1,54)}$ = 2.69 p= 0.1183), TIME ($F_{(3,54)}$ = 0.40 p= 0.7526), or INTERVENTION and TIME ($F_{(3,54)}$ = 0.16 p= 0.9199)) hands.  Figure 3.5A and 3.5B displays the group-averaged MEPs (with standard errors) for right and left FDI, respectively, before and after cTBS and sham TBS.

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**3.6 Discussion**

The present experiment investigated the influence of area 5 on neural circuitry within M1. Neural excitatory and inhibitory circuitry within bilateral M1 was assessed before and following cTBS over left-hemisphere area 5. Excitatory circuitry was assessed using both single-pulse MEPs and paired-pulse ICF and inhibitory circuitry was assessed using paired-pulse SICI. To assess the longevity of the after-effects, MEPs, ICF, and SICI were measured at three time points following cTBS cessation. A sham control group with participants naive to TMS was collected with the same conditions as the cTBS group. Novel findings from the present study include the observation that MEPs are increased bilaterally with amplitude changes that exceed those of cTBS applied directly over M1 (Huang et al., 2005). SICI and ICF remain unaltered following area 5 cTBS.

The direction of changes in cortical excitability following left area 5 cTBS was in line with the predictions for MEPs based on the bilateral increases in pinch forces following cTBS to left-hemisphere area 5 (Premji et al., 2010a). In that study, pinch forces were increased for 25 minutes following area 5 cTBS (Premji et al., 2010a). The present findings deviate from this behavioural data since the modulation in M1 excitability persists up to 60 minutes in stimulated and non-stimulated hemispheres. These findings are comparable to the longevity of after-effects on MEP amplitude following cTBS applied directly over M1 (Huang et al., 2008, Suppa et al., 2008) and the premotor cortex (Huang et al., 2009). Interestingly, the maximum change in MEP amplitude in the present study (132%) exceeds the maximum change following cTBS over M1 (49%) (Huang et al., 2005). In the latter study where cTBS is applied directly over M1, changes in descending output are thought to

reflect a depression in excitatory synapses in the I1-wave circuit, a pathway generating a MEP (Di Lazzaro et al., 2005, 2008). Subsequent to area 5 cTBS, a greater modulation in the I1-wave circuitry may contribute to a greater overall facilitation of the corticospinal neuron output.

In addition to effects in the FDI muscle contralateral to the site of cTBS, MEPs were also increased in the ipsilateral hand with changes occurring immediately and persisting up to 1 hour. Previous cTBS protocols applied over M1 have reported alterations in neural activity within contralateral M1 for up to 30 minutes (Suppa et al,. 2008). The increased MEPs in the ipsilateral hand may be mediated by transcallosal projections between homologous area 5 (Padberg et al., 2005). It is unlikely the bilateral increase in MEPs is mediated via inter-hemispheric trancallosal connections between homologous M1 where MEP amplitude increases in one hemisphere and decreases in the other following TBS (Suppa et al., 2008).

SICI recorded from both hands remained unaltered following cTBS to left-hemisphere area 5, in contrast to the hypotheses. This finding is incongruent to the observations following cTBS applied directly over M1 where SICI was decreased (i.e. reduced inhibition) (Huang et al., 2005, Suppa et al., 2008). However, in line with our observation, cTBS over the premotor cortex did not alter SICI (Huang et al., 2009). One explanation for the differing results following cTBS directly over M1 compared to remotely connected loci such as area 5 or the premotor cortex may relate to the cTBS intensity. It is possible that cTBS at a lower intensity is required to recruit inhibitory neurons involved in SICI through the area 5 to M1 interaction. In a study performed by McAllister et al., (2009), SICI following cTBS at 70% AMT was reduced from 44% to 78%; it may be that lower

intensities for TBS would preferentially recruit inhibitory pathways within M1 and lead to a net decrease SICI.

In the present study, cTBS over area 5 did not modulate the excitability of circuitry mediating ICF. These findings are similar to those following cTBS over the premotor cortex (Huang et al., 2009) and over M1 (Suppa et al., 2008). However, in another study where cTBS was also applied over M1 using a 300 pulse (20 second) paradigm, a short-lasting decrease in ICF at 10 minutes following stimulation was observed in the stimulated hemisphere (Huang et al., 2005). ICF is recruited at intensities higher than SICI (McAllister et al, 2009) and this provides one explanation why ICF remained unaltered in the present study. Further, ICF may depend on more than one circuit (Hanajima et al., 1998) and changes in excitability may require alterations in several populations of neurons that ultimately target ICF output neurons. CTBS over area 5 may not modulate these populations of neurons which mediate ICF circuitry.

In summary, an increase in bilateral MEPs and no change in SICI or ICF following cTBS over left-hemisphere area 5 was observed. These results suggest the influence of area 5 on M1 may be specific to corticospinal neurons and may not alter the excitability of excitatory or inhibitory interneurons involved in ICF and SICI, respectively. Although speculative, one mechanism through which area 5 may influence MEPs is through direct projections to M1 (Jones et al., 1978; Strick and Kim 1978; Leichnetz, 1986); pyramidal cells within area 5 project (Leichnetz, 1986) via glutamatergic SLF fibers (Makris et al., 2005; Bakiri et al., 2009; Dingledine et al., 1999) and terminate on output neurons within M1 (Strick and Kim, 1978). Via this pathway we expect a net facilitation in the SLF fibers leading to an increased M1 corticospinal output. In turn, an increase in MEPs, ICF, and

reduction in SICI would be expected.  An alternative mechanism may relate to changes in

the excitability of the spinal motor neuronal pool.  Evidence in monkeys suggests a direct

axonal projection from area 5 to the dorsal horn of the spinal cord (Murray and Coulter,

1981; Coulter and Jones, 1977).  However, these projections terminate within intra-laminar

regions and not to the anterior horn which is the modulating influence of the spinal circuits

to the muscles of the hand.

　　　The present study demonstrated area 5 influences M1 output.  Specifically, cTBS

over area 5 increases MEPs bilaterally.  In contrast, cTBS does not modulate excitability in

circuitry mediating SICI and ICF.  These exciting findings provide novel information to

understand the potential role of area 5 in hand movement.  Targeting area 5 using rTMS

paradigms in clinical populations with impaired hand control may restore imbalances in

facilitatory circuitry within M1 and may ultimately modify the control of muscles of the

hand.

**A**



TBS over
left area 5

| MEP<br>SICI<br>ICF<br>RFDI | MEP<br>SICI<br>ICF<br>LFDI |
|---|---|

Pre - TBS

| MEP<br>SICI<br>ICF<br>RFDI | MEP<br>SICI<br>ICF<br>LFDI |
|---|---|

Post 5-20

| MEP<br>SICI<br>ICF<br>RFDI | MEP<br>SICI<br>ICF<br>LFDI |
|---|---|

Post 25-40

| MEP<br>SICI<br>ICF<br>RFDI | MEP<br>SICI<br>ICF<br>LFDI |
|---|---|

Post 45-60
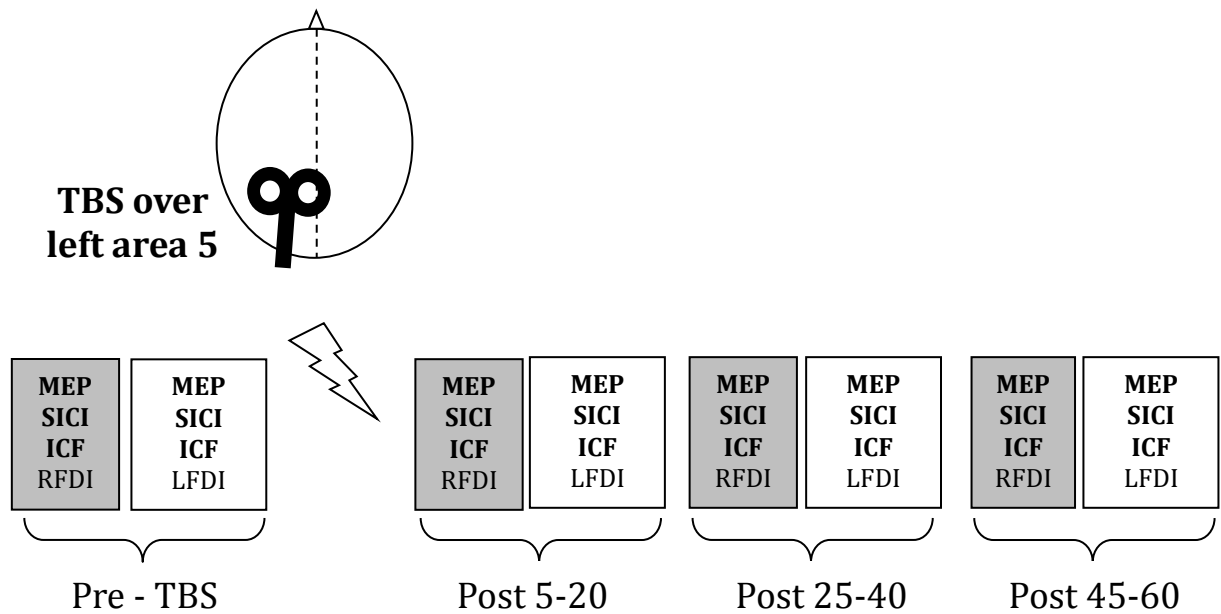
**B**

| Post Time Blocks | MEPs | SICI/ICF | MEPs | SICI/ICF |
|---|---|---|---|---|
| **Post 1** (5 to 20 min) | 5-7 min | 7-11 min | 11-13 min | 13-17 min |
| **Post 2** (25 to 40 min) | 25-27 min | 27-31 min | 31-33 min | 33-37 min |
| **Post 3** (45 to 60 min) | 45-47 min | 47-51 min | 51-53 min | 53-57 min |

**Figure 3.2** Experimental timeline. A. MEPs, SICI, and ICF were measured bilaterally before and at 5-20 minutes, 25- 40 minutes, and 45-60 minutes after cTBS (Experiment 1), iTBS (Experiment 2) and sham TBS cessation. The order for right versus left M1 recordings for MEPs, SICI, and ICF recordings were kept constant within subjects across each time block and this order of hand stimulated (right hand, left hand) was randomized across subjects. B. MEPs and SICI/ICF were always collected at the same time points between subjects and across cTBS, iTBS, and sham interventions. For example, if the right side was collected first, MEPs were collected between 5 to 7 minutes and SICI/ICF between 7 to 11 minutes. The left side was then collected; MEPs were collected between 11 to 13 minutes and SICI/ICF between 13 to 17 minutes). If the left side was first, MEPs and SICI/ICF were collected at the identical time points above.

**A**                           **MEPs from RFDI**

**B**                           **MEPs from LFDI**
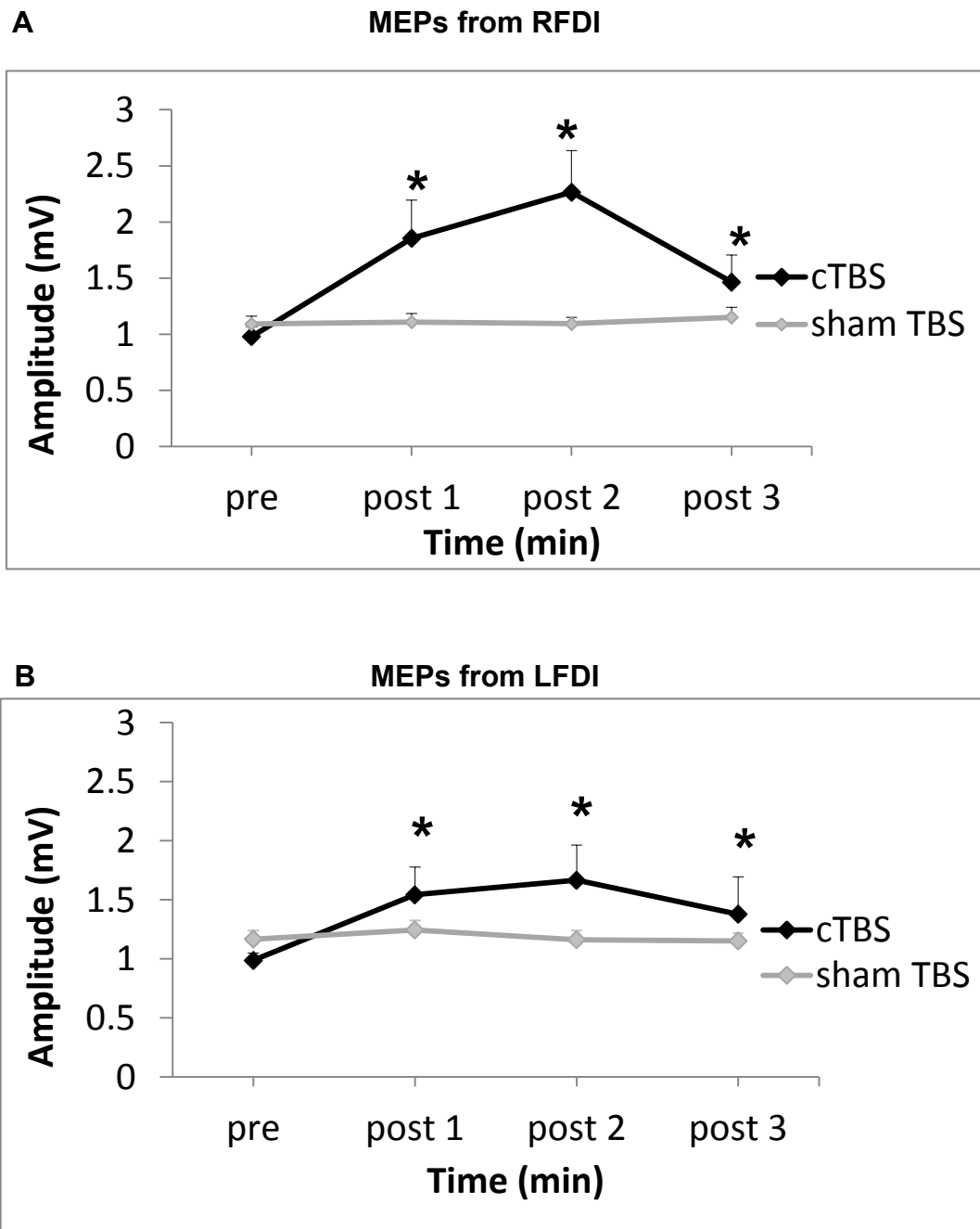
**Figure 3.3** Motor evoked potentials (MEPs) following left-hemisphere cTBS. A. MEPs recorded from the right FDI increased at 5 (p=0.0001), 25 (p=0.0001), and 45 (p=0.0159) minutes following cTBS. B. MEPs recorded from the left FDI revealed an increase at at 5 (p = 0.0026), 25 (p = 0.0003), and 45 (p = 0.0308) minutes following cTBS. Error bars represent standard error of the means. * p ≤ 0.05.

**A**                  SICI from RFDI



**B**                  SICI from LFDI
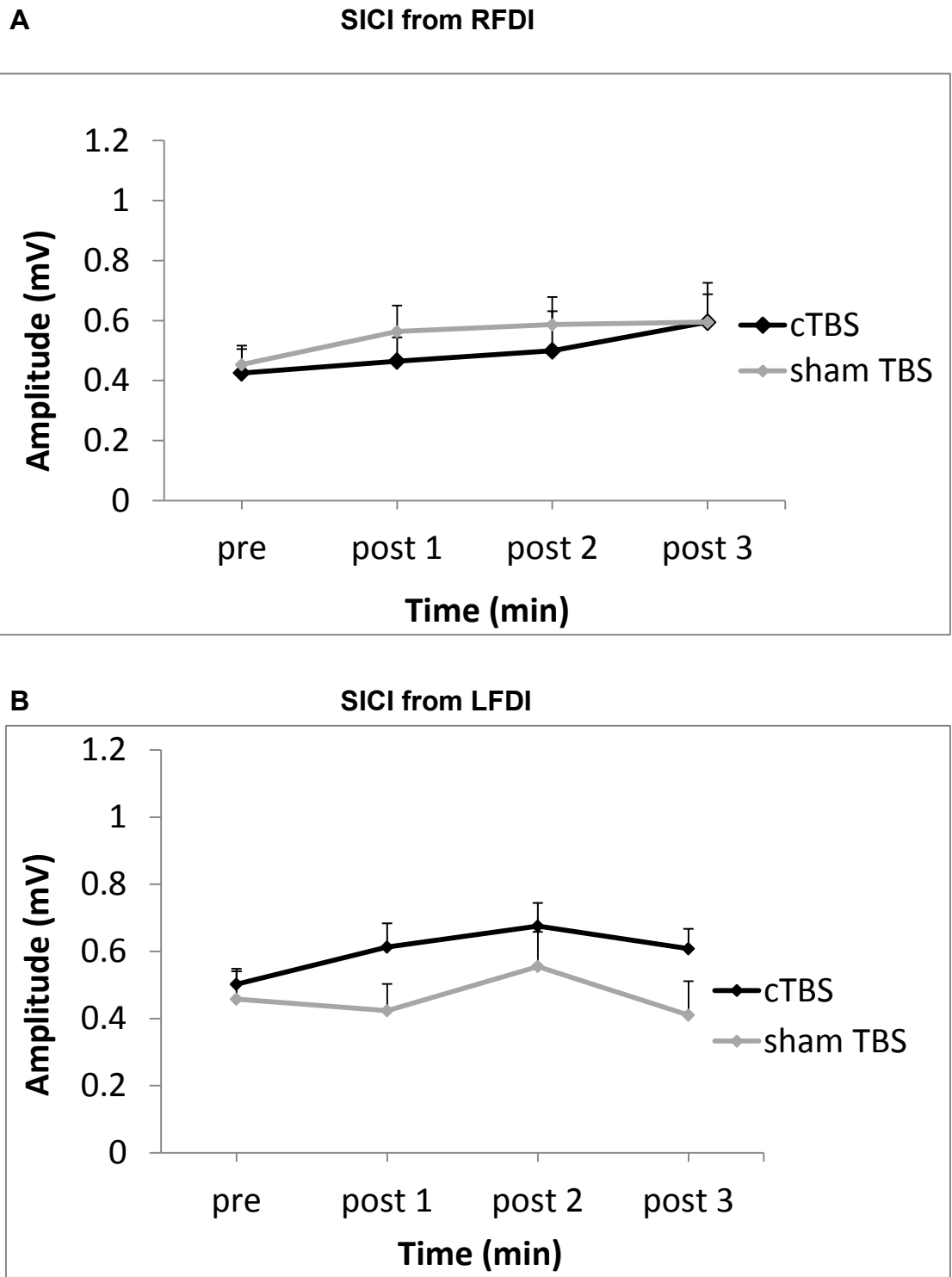
**Figure 3.4** Short interval intracortical inhibition (SICI) following left-hemisphere cTBS. SICI recorded from the right (A) and left FDI (B) remained unaltered following cTBS. Error bars represent standard error of the means.

**A** ICF from RFDI



**B** ICF from LFDI
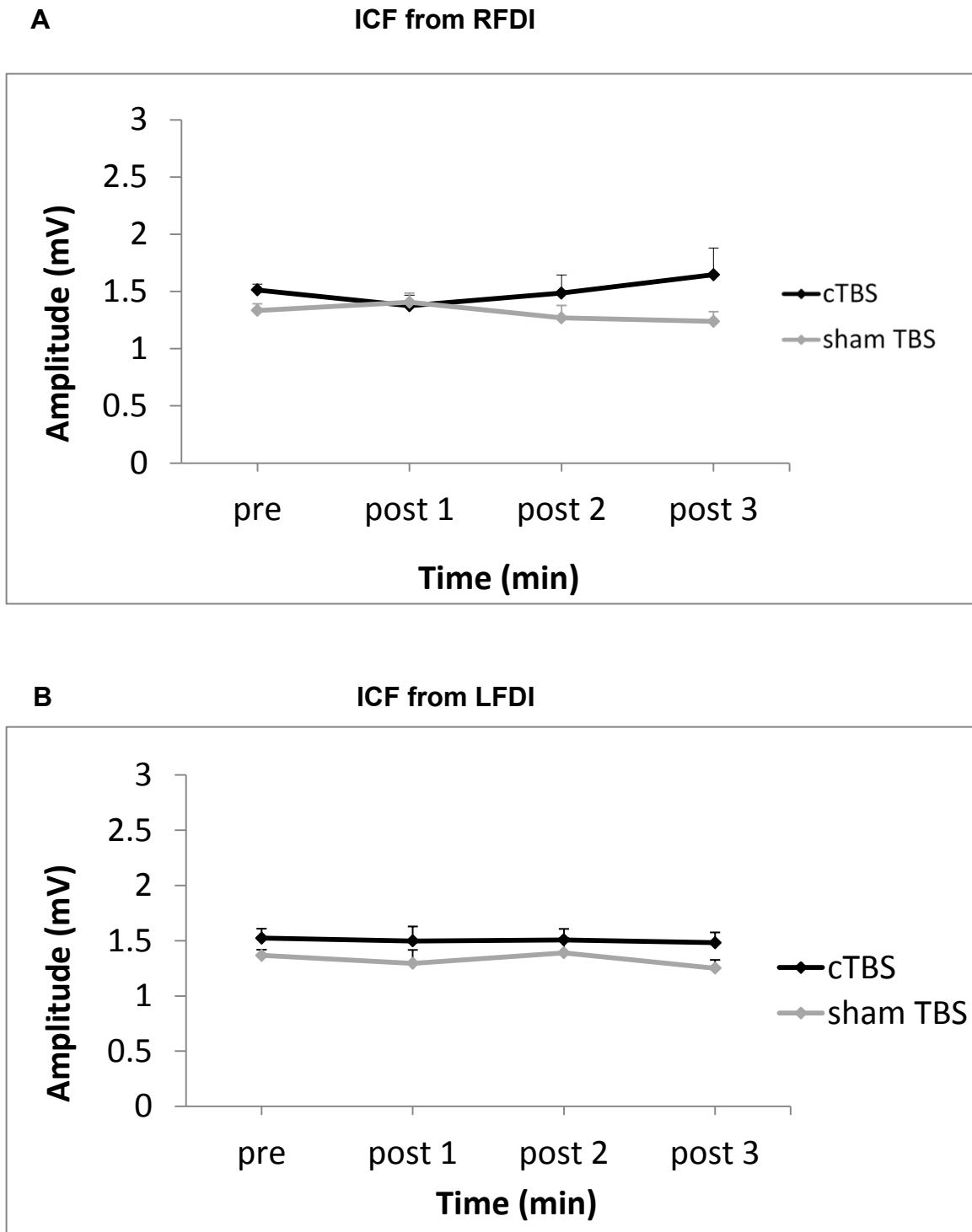


**Figure 3.5** Intracortical facilitation (ICF) following left-hemisphere cTBS. ICF recorded from the right (A) and left FDI (B) remained unaltered following cTBS. Error bars represent standard error of the means.

**Summary of Participants**

|  | MEPs (RFDI) | MEPs (LFDI) | SICI (RFDI) | SICI (LFDI) | ICF (RFDI) | ICF (LFDI) |
|---|---|---|---|---|---|---|
| **cTBS** | n=12 | n=12 | n=9 | n=11 | n=10 | n=12 |
| **Sham TBS** | n= 12 | n= 12 | n= 12 | n= 11 | n= 11 | n= 8 |

**Table 3.1** Summary of participants. Individuals showing greater than 10% inhibition for SICI and 10% facilitation for ICF were included in the analysis for each group.

**MEPs**

RFDI (n=12)

|  | Post 1 | Post 2 | Post 3 |
|---|---|---|---|
| Up | 8 | 10 | 8 |
| Down | 2 | 2 | 4 |
| No change | 2 | 0 | 0 |

LFDI (n=12)

|  | Post 1 | Post 2 | Post 3 |
|---|---|---|---|
| Up | 9 | 10 | 8 |
| Down | 3 | 2 | 4 |
| No change | 0 | 0 | 0 |

**Table 3.2** Summary of trends for MEPs following cTBS. Individuals showing greater than a 10% change from pre-TBS were included in the Up/Down groups.

RFDI (n=9)                              **SICI**

|            | Post 1 | Post 2 | Post 3 |
|------------|--------|--------|--------|
| Up         | 7      | 5      | 5      |
| Down       | 2      | 3      | 2      |
| No change  | 0      | 1      | 2      |

LFDI (n=11)

|            | Post 1 | Post 2 | Post 3 |
|------------|--------|--------|--------|
| Up         | 6      | 7      | 6      |
| Down       | 2      | 2      | 3      |
| No change  | 3      | 2      | 2      |

**Table 3.3**  Summary of trends for SICI following cTBS.  Individuals showing greater than a 10% change from pre-TBS were included in the Up/Down groups.

**ICF**

RFDI (n=10)

|            | Post 1 | Post 2 | Post 3 |
|------------|--------|--------|--------|
| Up         | 2      | 4      | 4      |
| Down       | 8      | 5      | 6      |
| No change  | 0      | 1      | 0      |

LFDI (n=12)

|            | Post 1 | Post 2 | Post 3 |
|------------|--------|--------|--------|
| Up         | 5      | 4      | 3      |
| Down       | 4      | 8      | 7      |
| No change  | 3      | 0      | 2      |

**Table 3.4**  Summary of trends for ICF following cTBS.  Individuals showing greater than a 10% change from pre-TBS were included in the Up/Down groups.

**Chapter 4: Experiment 2**
**Modulation of neural circuitry within primary motor cortex following intermittent theta-burst rTMS over area 5**

## 4.1 Rationale

Neural circuitry within the primary motor cortex (M1) mediates precise control of the hand and is often imbalanced in patient groups with impaired hand function. Theta-burst stimulation (TBS) is one form of repetitive transcranial magnetic stimulation (rTMS) and may alter the excitability of neural networks. When applied over M1 using a specific protocol called intermittent TBS (iTBS), excitability within the targeted cortex (Suppa et al., 2008, Huang et al., 2005, Ishikawa et al., 2008, Katayama et al., 2008) and anatomically connected loci (Suppa et al., 2008) may increase. However, the effects of TBS last up to 1 hour at most. Further, little is known about the clinical application of iTBS in individuals with imbalanced neural circuitry and impaired motor control of the hand. Targeting other cortical areas, such as area 5 that are involved in skilled hand movement and have large cortical territory representing the hand (Padberg et al., 2005, 2007) may develop our understanding on the loci that underpin hand movements.

Experiment 1 demonstrated that cTBS to area 5 has the potential to selectively modulate excitatory circuits within M1. Reports indicate that cTBS and iTBS have differential effects when applied directly over M1; cTBS decreases and iTBS increases the amplitude of MEPs and SICI (Huang et al., 2005, Suppa et al., 2008). Further, the former paradigm reduces ICF while iTBS has no effect (Huang et al, 2005). Studies examining the after-effects of TBS on the non-stimulated M1 have found cTBS may increase (Stefan et al., 2008, Suppa et al., 2008) or decrease (Ishikawa et al., 2007) MEPs, while iTBS has the

opposite effect (Suppa et al., 2008).  Recordings from cervical descending volleys suggest

the two TBS paradigms, when applied over M1, differentially modulate I-wave circuitry.  I-

waves are thought to reflect the trans-synaptic activation of the corticospinal neurons (Di

Lazzaro et al. 2008).  CTBS preferentially affects the I1-wave (Di Lazzaro et al., 2005) while

iTBS modulates the later I-waves (Di Lazzaro et al., 2005).  Similarly, cTBS and iTBS applied

over the left lateral cerebellum decrease and increase MEP amplitude, respectively, in the

left FDI muscle (Koch et al., 2008).  These effects can translate to non-motor loci.  TBS

applied over the primary somatosensory cortex (SI) reduces the amplitude of

somatosensory evoked potentials (SEPs) following cTBS (Ishikawa et al, 2007) and

increases SEPs after iTBS (Premji et al., 2010b).  Collectively, these findings suggest cTBS

and iTBS may have opposite and differential effects on cortical excitability.  However, when

cTBS and iTBS are applied to SI, both paradigms decrease laser evoked potentials recorded

from the secondary somatosensory cortex (SII) (Poreisz et al., 2008).  It may be that TBS

paradigms applied to other remote loci do not produce such differential effects when

recordings are taking from a non-stimulated area.

The goal of the present experiment was to investigate the influence of iTBS over

area 5 on inhibitory and excitatory networks within M1.  To address the goal of Experiment

2, excitatory circuitry (MEPs and ICF) and inhibitory circuitry (SICI) were measured before

and following iTBS over left-hemisphere area 5 at three time points, identical to those in

Experiment 1 and the sham control group.  Neural circuitry within bilateral M1 were

probed using single (MEPs) and paired-pulse (ICF and SICI) TMS.  Applying iTBS over area

5, a loci with large representations of the thumb and index finger and direct projections to

M1, will provide understanding on how this loci influences M1 circuitry with respect to the

first dorsal interosseous (FDI) muscle.  Further, taken together with Experiment 1, the present findings will further our understanding on the differential effects of TBS paradigms on area 5.

*4.2 Hypotheses*

The present study investigated bilateral neural circuitry within M1 following iTBS over left-hemisphere area 5.  Inhibitory circuitry, SICI, and excitatory circuits, MEPs and ICF, were measured before and following iTBS.  The hypotheses were based on the direction of excitability changes following cTBS over area 5 (Experiment 1).

Excitatory Neural Circuitry:

Motor Evoked Potentials (MEPs) & Intracortical Facilitation (ICF)

MEPs elicited from the left and right M1 cortices will decrease for up to 45 minutes following iTBS cessation, similar to the longevity of after-effects following iTBS over M1 (Suppa et al., 2008).  Similarly, ICF will increase bilaterally following cessation of iTBS to area 5.  These effects will persist for up to 20 minutes (post 1), similar to the time course whereby ICF was modulated when cTBS was applied directly over M1 (Huang et al., 2005). No changes following sham TBS were expected for MEPs and SICI.

Inhibitory Neural Circuitry:

Short-interval intracortical inhibition (SICI)

SICI will increase bilaterally following iTBS over area 5.  Based on a decrease in net excitability in M1 (MEPs and ICF), we expect an increase in M1 inhibitory circuitry with

effects lasting up to 35 minutes (post 1, 2) (Suppa et al., 2008).  No changes following sham

TBS were expected for circuitry involved in SICI.

**4.3 Methods**

Experimental procedures were similar to those employed in Experiment 1 (Chapter 2) with

the following exceptions;

*4.3.1 Participants*

      Eleven healthy participants (6 females, mean age 27.3 years, SD $\pm$ 3.66) were

studied with iTBS to left-hemisphere area 5.  The same group of participants [twelve

subjects (6 females, mean age 22.1 years, SD $\pm$ 2.56)] with sham (placebo) TBS were used

for comparison against the real iTBS group.  Right-handedness was confirmed using a

subset of the Edinburgh Handedness Inventory (EHI) (Oldfield, 1971).  All subjects gave

informed written consent and this study was approved by the Office of Research Ethics at

the University of Waterloo.

**4.3.2 Experimental approach**

*Neuronavigation and Transcranial magnetic stimulation*

      ITBS was applied using a 90 mm outer diameter figure of eight coil with a MagPro

stimulator (MCF-B65; Medtronic, Minneapolis, MN, USA).

*4.3.3 Experimental Timeline*

      MEPs, SICI, and ICF were measured bilaterally before and at 5-20 minutes (post 1),

25-40 minutes (post 2), and 45-60 minutes (post 3) after iTBS cessation.  The order for

right versus left M1 recordings for MEPs, SICI, and ICF recordings were kept constant

within subjects across each time block and this order of hand stimulated (right hand, left hand) was randomized across subjects.  Figure 3.2 depicts the experimental timeline.

*4.3.4 Data Analysis*

Paired-pulse MEP amplitude was expressed as a ratio to the mean unconditioned MEP amplitude (TS alone) for each participant.  Ratios below one represent inhibition and ratios above one represent facilitation.  Individuals showing greater than 10% inhibition for SICI and 10% facilitation for ICF were included in the analysis for each group.  Two-way ANOVA with between-subject factor 'INTERVENTION' (iTBS, sham) and within-subject factor 'TIME' (pre, post 1, post 2, post 3) were performed for MEPs, SICI, and ICF for the right and left FDI muscles.  Post hoc Tukey's tests were used to identify any differences from pre-TBS.  Statistical significance was set at $p \leq 0.05$.

**4.4 Results**

All participants successfully completed the experiment.  The mean stimulator output used for delivery of iTBS was 36% ($\pm$ 6.9).  Table 4.1 is a summary of the number of participants included for the results that follow and Tables 4.2, 4.3, and 4.4 provide overall trends for each of the dependent measures.

*4.4.1 Motor Evoked Potentials (MEPs)*

For MEPs recorded over right FDI, contralateral to area 5 iTBS, the two-way ANOVA revealed a significant effect of factor TIME ($F_{(3,63)}$ = 5.86 p = 0.0014) and a significant interaction between factors INTERVENTION and TIME ($F_{(3, 63)}$ = 4.86, p = 0.0042).  There was no main effect of INTERVENTION ($F_{(1, 63)}$ = 2.74, p = 0.1128).  Post-hoc Tukey's test

revealed that compared to pre-theta-burst, MEP amplitude was significantly greater at 25 (p =0.0073) and 45 (p = 0.0013) minutes following iTBS.  There was no difference compared to pre-TBS at 5 minutes (p = 0.1703) or amongst MEP amplitudes in the sham intervention.  Figure 4.1A displays the group-averaged MEPs (with standard errors) for right FDI before (pre) and after (post 1, 2, 3) iTBS and sham TBS.

For MEPs recorded over left FDI, ipsilateral to area 5 iTBS, the two-way ANOVA revealed no effects of INTERVENTION ($F_{(1, 63)}$ = 0.60, p = 0.4475), TIME ($F_{(3, 63)}$ = 0.58, p = 0.6276), or the interaction between INTERVENTION and TIME ($F_{(3, 63)}$ = 0.45, p = 0.7156). Figure 4.1B displays the group-averaged MEPs (with standard errors) for left FDI before and after iTBS and sham TBS.

*4.4.2 Short Interval Intracortical Inhibition (SICI)*

For MEPs recorded over right FDI, contralateral to area 5 iTBS, the two-way ANOVA revealed no significant main effects of factor INTERVENTION ($F_{(1, 54)}$ = 0.00, p = 0.9843), TIME ($F_{(3, 54)}$ = 1.39, p = 0.2555) or the interaction between INTERVENTION and TIME ($F_{(3, 54)}$ = 0.43, p = 0.7300).  Similarly, for MEPs recorded over left FDI, ipsilateral area 5 iTBS, the two-way ANOVA revealed no effects of INTERVENTION ($F_{(1, 57)}$ = 0.02, p = 0.8904), TIME ($F_{(3, 57)}$ = 2.73, p = 0.0524), or the interaction between INTERVENTION and TIME ($F_{(3, 57)}$ = 0.26, p = 0.8533).  Figure 4.2A and 4.2B display the group-averaged MEPs (with standard errors) for right FDI and left FDI muscles, respectively, before and after iTBS and sham TBS.

*4.4.3 Intracortical Facilitation (ICF)*

For MEPs recorded over right FDI, contralateral to area 5 iTBS, the two-way ANOVA revealed no significant main effects of factors INTERVENTION (F $_{(1, 51)}$ = 0.22, p = 0.6416), TIME (F $_{(3, 51)}$ = 0.32, p = 0.8140), or the interaction between INTERVENTION and TIME (F $_{(3, 51)}$ = 0.98, p = 0.4093).  Similarly, there were no main effects for INTERVENTION (F $_{(1, 45)}$ = 0.00, p = 0.9628), TIME (F $_{(3, 45)}$ = 0.48, p = 0.6978), or the interaction between INTERVENTION and TIME (F $_{(3, 45)}$ = 0.41, p = 0.7493) for MEPs recorded over left FDI. Figure 4.3A and 4.3B displays the group-averaged MEPs (with standard errors) for right and left FDI muscles, respectively, before and after iTBS and sham TBS.

## 4.5 Discussion

Experiment 2 investigated the influence of area 5 on excitatory and inhibitory neural circuitry within M1 following iTBS over left-hemisphere area 5.  Excitatory circuitry was assessed using both single-pulse MEPs and paired-pulse ICF and inhibitory circuitry was assessed using paired-pulse SICI.  Dependent measures (MEPs, ICF, SICI) were investigated at three time points following iTBS cessation.  A group receiving sham TBS was compared with the group receiving real iTBS.  Novel findings from the present study include an increase in MEPs in the right FDI muscle, contralateral to left-hemisphere area 5 TBS, with onset of effects observed at 25 minutes and persisting for up to one hour.  No changes in the MEP amplitude in the left FDI muscle, ipsilateral to the hemisphere stimulated were observed.  Further, iTBS over area 5 did not alter SICI and ICF.

The increase in M1 cortical excitability following left-hemisphere area 5 iTBS was incongruent with the hypotheses.  The hypotheses for a decrease in M1 excitability following area 5 iTBS was based on the increase in M1 excitability following cTBS over area 5 (Experiment 1).  TBS paradigms when applied directly over M1 have been reported to have differential effects such that cTBS depresses while iTBS increases cortical excitability (Huang et al., 2005, Suppa et al., 2008).  However, Experiment 2 revealed that iTBS over area 5 actually increases cortical excitability as evidenced by the increase in MEP amplitude.  Although, the results were not in line with the hypotheses, this finding is comparable to the changes observed when iTBS is applied directly over M1 whereby MEPs are increased (Huang et al. 2005, Suppa et al., 2008).  In addition, the duration of after-effects in the present experiment is similar to when iTBS is applied directly over M1 (Suppa et al., 2008).  The increase in the MEP amplitude recorded from the right FDI was not observed immediately following iTBS but rather at 20 minutes.  The delayed onset is similar to that when iTBS is applied directly over M1 (Suppa et al., 2008), however these findings are not unanimous (Huang et al., 2005).  Further, iTBS over non-motor loci such as SI facilitates SEPs in the stimulated hemisphere, with effects that are not observed immediately, but rather at 15 minutes following stimulation (Premji et al., 2010).  Collectively, iTBS over area 5 increases MEP amplitude with a similar duration and delay as that observed with iTBS directly over M1 suggesting that similar mechanisms may mediate the effects of iTBS delivered over different loci.

Following iTBS over M1, changes in the excitability of cortical circuits that generate the later I-waves is observed (Di Lazzaro et al., 2008).  Although the exact origin of early and late I-waves remains unclear, I-waves are thought to reflect trans-synaptic inputs to

corticospinal neurons.  The later I-waves likely have an independent cortical mechanism

than that of the earlier I1-wave, which is modulated following cTBS (Di Lazzaro et al.,

2008).  Similar to these changes following M1 TBS, area 5 iTBS may increase the excitability

of later I-wave inputs resulting in a net facilitation of the MEP.  The delayed onset of MEP

increase following area 5 iTBS may be due to a preferential recruitment of neurons

projecting to other anatomically connected loci first and then to M1.  Anatomical studies in

monkeys have identified connections from area 5 that are not just confined to M1, but also

project to the premotor cortex, SII, supplementary motor area, area 7, and the cingulate

cortex (Padberg et al., 2005, Krubitzer and Disbrow, 2005).  In humans and monkeys, the

superior longitudinal fasciculus is a white matter tract extending through several cortical

regions including area 5, the premotor cortex and the supplementary motor area (SMA)

(Makris et al., 2005, Petrides and Pandya 2002).  Further, the premotor cortex, SMA, area 7,

and SII have projections directly to M1 (Leichnetz et al. 1986).  Collectively, it is possible

that area 5 projects to these loci first and then to M1, and may explain delayed onset of

iTBS after-effects.

MEPs from the ipsilateral FDI muscle were unaltered following iTBS, in contrast to

the contralateral effect.  When applied directly over M1, iTBS decreases MEPs from the

ipsilateral FDI muscle with effects lasting up to 45 minutes (Suppa et al. 2008).  Similarly,

iTBS to SI modulates SEPs in the non-stimulated hemisphere however the effects are

observed immediately at 5 minutes and are short-lived (Premji et al,. 2010b).  The data in

Experiment 2 did not reveal such ipsilateral influences of iTBS and it may be that area 5

iTBS does not modulate excitability of corticospinal neurons in the non-stimulated

hemisphere.  In line with the present findings is a study that showed laser evoked

potentials following iTBS were decreased within the ipsilateral SII region and unaltered in SII contralateral to iTBS (Poreisz et al., 2008).
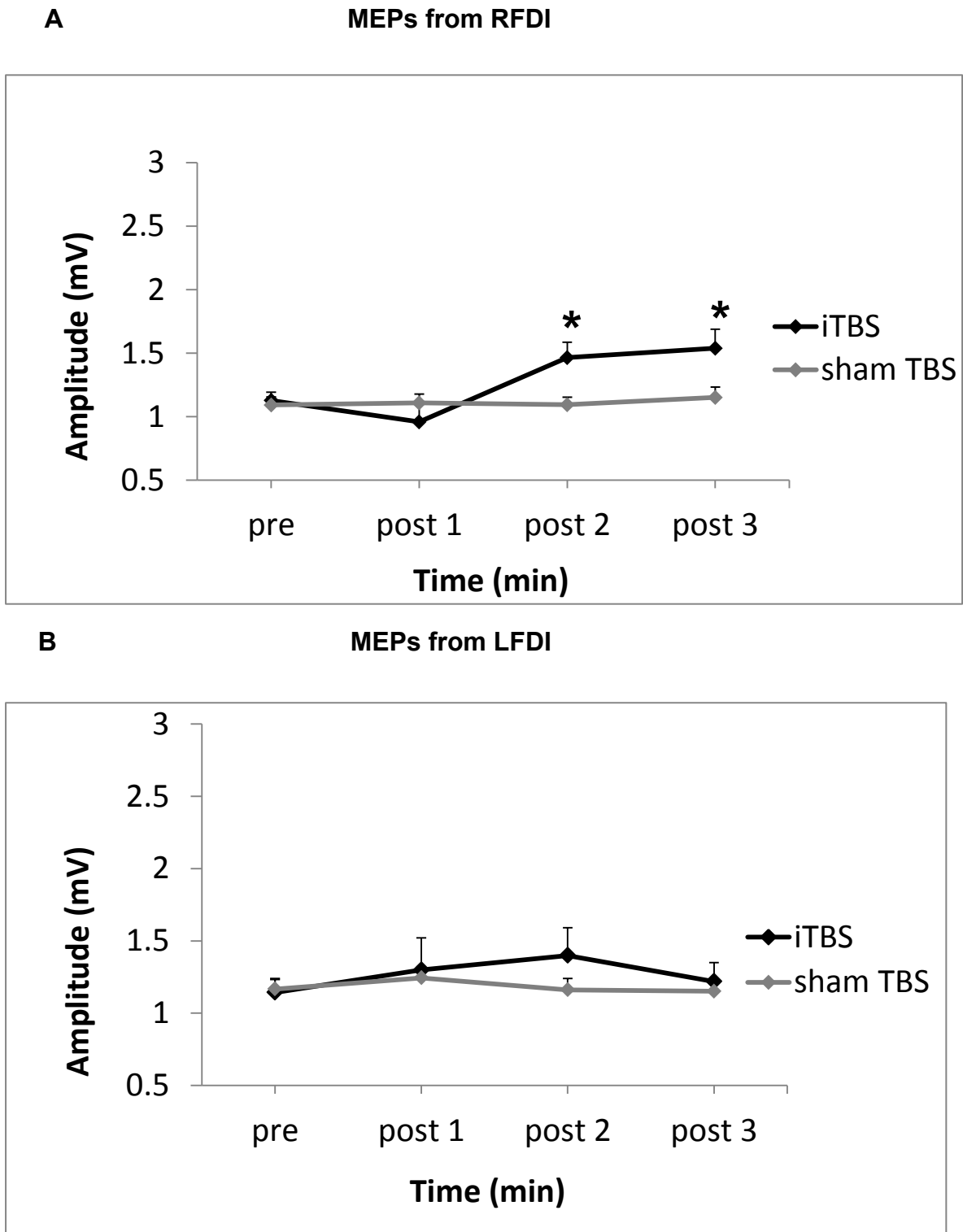
SICI remained unaltered following iTBS to left-hemisphere area 5.  The lack of change in SICI was observed bilaterally and is in contrast to the increase in SICI observed following iTBS directly over M1 (Suppa et al., 2008, Huang et al., 2005).  Unlike cTBS over M1, iTBS using a low-intensity for iTBS (70% AMT) results in no changes in SICI (McAllister et al., 2008).  The authors of the latter study suggest that iTBS patterns may not be optimal for inducing LTP-like changes in inhibitory interneurons or that the lack of effect following iTBS on SICI is intensity-dependent; an intensity of 70 % AMT may not be ideal for modulation of SICI using iTBS.  Similarly, area 5 iTBS may be intensity-dependent and an intensity lower than 70 % AMT may recruit lower threshold circuits involved in SICI.

ITBS over area 5 did not modulate the excitability of circuitry mediating ICF, in contrast to our hypotheses.  These findings are similar to those following iTBS over M1 in the simulated (Huang et al., 2005, Suppa et al., 2008) and non-stimulated (Suppa et al., 2008) hemispheres.  Circuitry mediating ICF may depend on several populations of neurons (Hanajima et al., 1998) and iTBS over M1 and/or area 5 may not modify such circuits.  Further, ICF pathways are recruited at higher intensities and it may be that a higher intensity than 80% AMT for iTBS over area 5 is necessary for alterations in ICF circuitry.

The present study demonstrated iTBS over area 5 increases MEPs from the right FDI muscle, contralateral to the TBS stimulated hemisphere.  In contrast, iTBS does not modulate excitability in the left FDI muscle or in the circuitry mediating SICI and ICF. Interestingly, the results from Experiment 2 suggest iTBS may not have differential effects

in non-motor loci or may be acting on a separate population of neurons.  Further, in line with findings from Experiment 1, area 5 provides an opportunity to alter M1 circuitry and may be a target for rTMS paradigms in clinical populations with altered inhibitory and excitatory networks.

**A**                        MEPs from RFDI

**B**                        MEPs from LFDI

**Figure 4.1** Motor evoked potentials following left-hemisphere iTBS.  A.  MEPs recorded from the right FDI increased at 25 (p =0.0073) and 45 (p = 0.0013) minutes following iTBS. B.  MEPs recorded from the left FDI remained unaltered following iTBS.  Error bars represent standard error of the means.  * p ≤ 0.05.

**A**                                                SICI from RFDI



**B**                                                SICI from LFDI
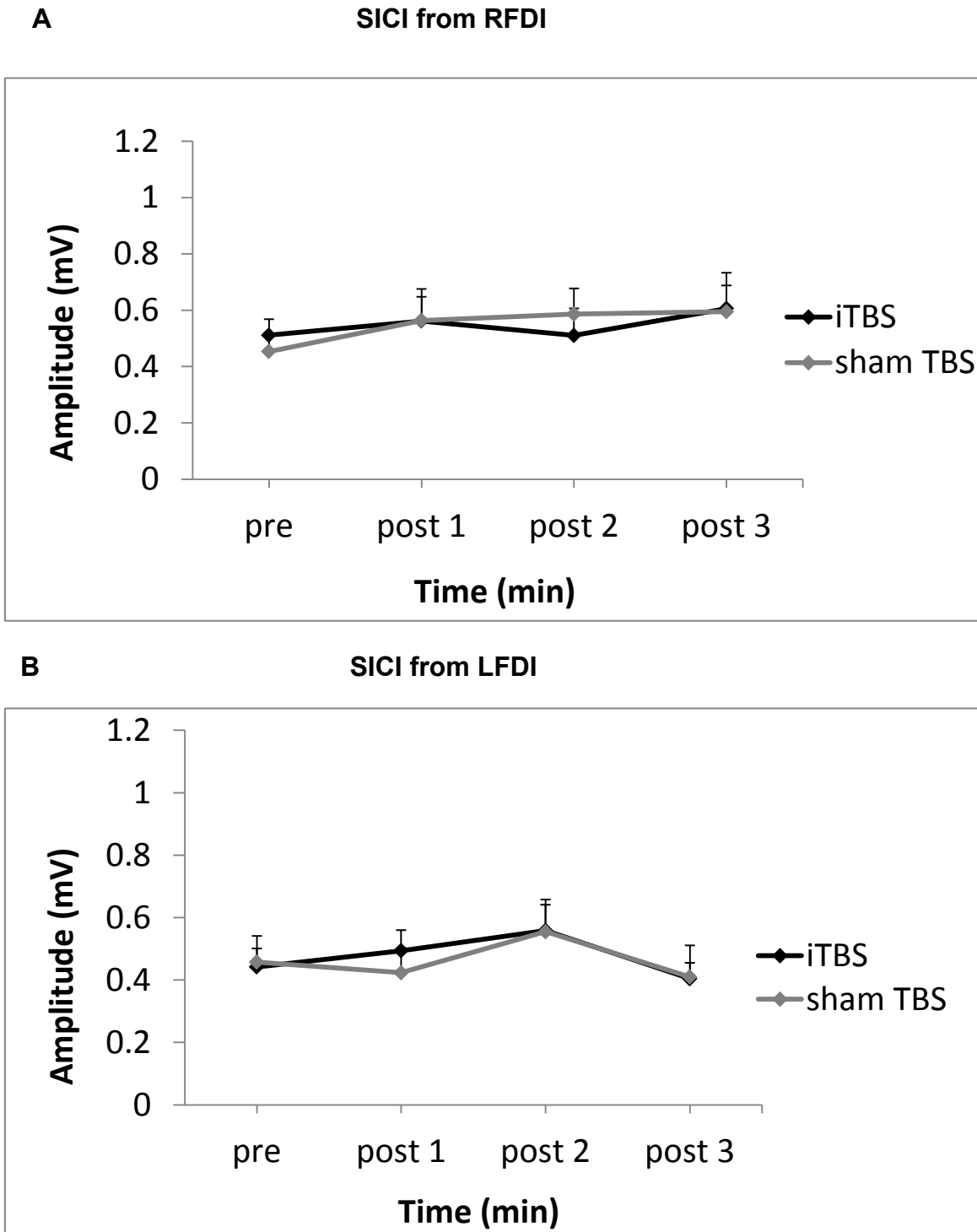


**Figure 4.2** Short interval intracortical inhibition following left-hemisphere iTBS.  SICI recorded from the right (A) and left FDI (B) remained unaltered following iTBS.  Error bars represent standard error of the means.

**A**                              **ICF from RFDI**



**B**                              **ICF from LFDI**
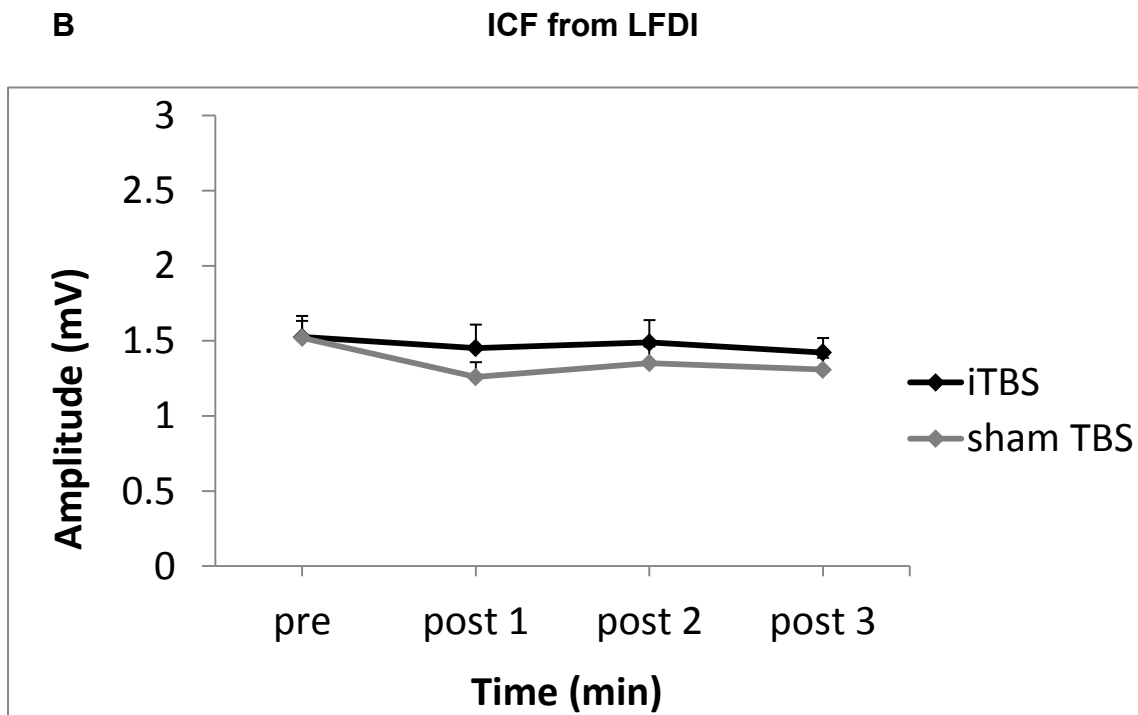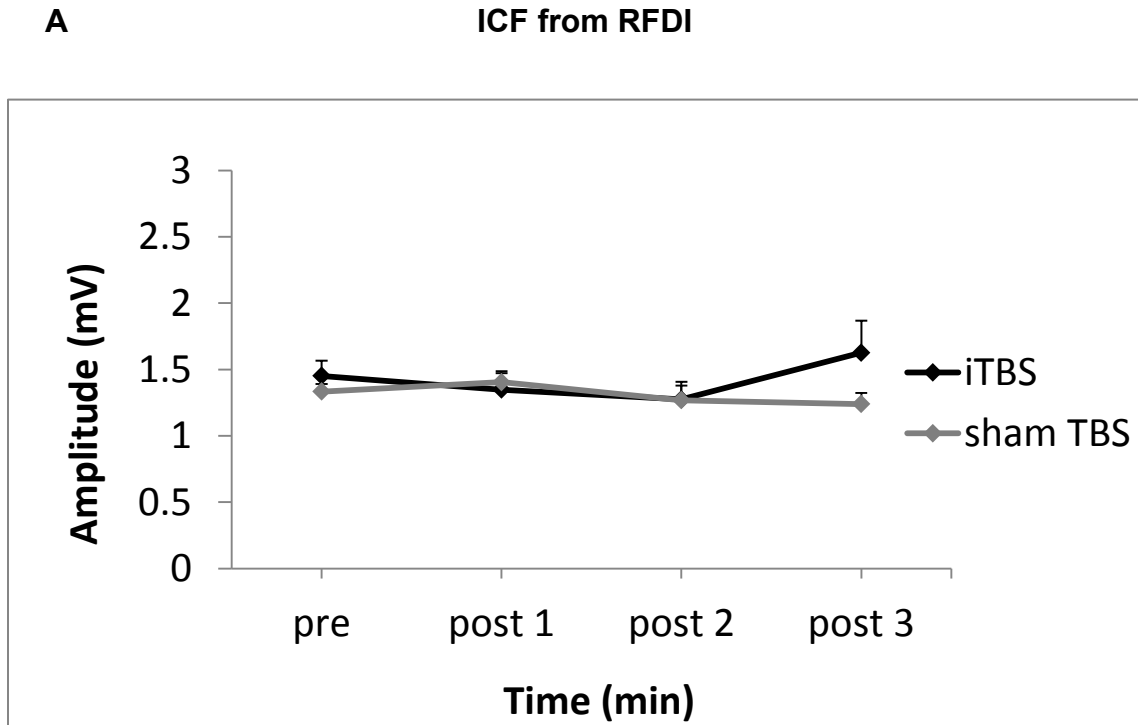


**Figure 4.3** Intracortical facilitation following left-hemisphere iTBS.  ICF recorded from the right (A) and left FDI (B) remained unaltered following iTBS.  Error bars represent standard error of the means.

**Summary of Participants**

|  | MEPs (RFDI) | MEPs (LFDI) | SICI (RFDI) | SICI (LFDI) | ICF (RFDI) | ICF (LFDI) |
|---|---|---|---|---|---|---|
| **iTBS** | n = 11 | n = 11 | n= 8 | n= 10 | n= 8 | n= 9 |
| **Sham TBS** | n= 12 | n= 12 | n= 12 | n= 11 | n= 11 | n= 8 |

**Table 4.1** Summary of participants. **I**ndividuals showing greater than 10% inhibition for SICI and 10% facilitation for ICF were included in the analysis for each group.

**MEPs**

RFDI (n=11)

|  | Post 1 | Post 2 | Post 3 |
|---|---|---|---|
| Up | 3 | 9 | 8 |
| Down | 7 | 2 | 2 |
| No change | 1 | 0 | 1 |

LFDI (n=11)

|  | Post 1 | Post 2 | Post 3 |
|---|---|---|---|
| Up | 7 | 6 | 6 |
| Down | 4 | 3 | 4 |
| No change | 0 | 2 | 1 |

**Table 4.2** Summary of trends for MEPs following iTBS. Individuals showing greater than a 10% change from pre-TBS were included in the Up/Down groups.

**SICI**

RFDI (n=8)

|  | Post 1 | Post 2 | Post 3 |
|---|---|---|---|
| Up | 3 | 2 | 4 |
| Down | 4 | 4 | 3 |
| No change | 1 | 2 | 1 |

LFDI (n=10)

|  | Post 1 | Post 2 | Post 3 |
|---|---|---|---|
| Up | 4 | 6 | 3 |
| Down | 2 | 2 | 5 |
| No change | 4 | 2 | 2 |

**Table 4.3** Summary of trends for SICI following iTBS. Individuals showing greater than a 10% change from pre-TBS were included in the Up/Down groups.

**ICF**

RFDI (n=8)

|  | Post 1 | Post 2 | Post 3 |
|---|---|---|---|
| Up | 3 | 5 | 3 |
| Down | 5 | 2 | 4 |
| No change | 0 | 1 | 1 |

LFDI (n = 9)

|  | Post 1 | Post 2 | Post 3 |
|---|---|---|---|
| Up | 2 | 3 | 4 |
| Down | 4 | 6 | 4 |
| No change | 3 | 0 | 1 |

**Table 4.4** Summary of trends for ICF following iTBS. Individuals showing greater than a 10% change from pre-TBS were included in the Up/Down groups.

**Chapter 5: General Discussion**

The goal of this thesis was to determine the influence of area 5 on the neural circuitry within the primary motor cortex (M1).  To address this goal, two experiments were performed that investigated the excitatory (motor evoked potentials (MEPs) and intracortical facilitation (ICF)) and inhibitory (short-interval intracortical inhibition (SICI)) circuitry within M1.  Experiment 1 investigated the influence of area 5 on ICF, MEPs, and SICI before and following continuous theta-burst stimulation (cTBS).  Experiment 2 investigated the influence of area 5 on ICF, MEPs, and SICI before and following intermittent theta-burst stimulation (iTBS).  Both experiments were focused on the hand; area 5 has a large representation of the hand and may be important in modulating  the balance of excitatory and inhibitory circuitry within M1.  CTBS and iTBS were used since they are paradigms that alter cortical excitability in the targeted cortex and anatomically connected loci.  The findings from both experiments reveal that area 5 selectively influences one component of the excitatory circuitry within M1 (MEPs).  Area 5 did not appear to influence the excitatory circuitry underpinning ICF or the inhibitory circuitry underpinning SICI.  Results from both experiments have provided novel neuroscience information and have applications to patient groups with impaired hand function.  A neural model (Figure 5.1 and 5.2) has been developed to assist with explanation of the results from both experiments and will be discussed in detail in the following discussion.

To our knowledge, this thesis is the first documented attempt to investigate the influence of area 5 on neural circuitry within M1 in humans.  Previous work in humans has identified an interaction between area 5 and ipsilateral M1 (Ziluk et al., 2010), however

questions remain on the specific influence of area 5 on excitatory and inhibitory circuits within M1.  Experiment 1 revealed that cTBS over left-hemisphere area 5 increases MEPs in the contralateral and ipsilateral FDI muscles for up to 1 hour, with a change in amplitude that exceeds the alterations following cTBS applied directly over M1.  CTBS did not modulate the excitability of circuitry involved in SICI and ICF.  These results suggest cTBS over area 5 is selective in modulating M1 circuitry.  Based on these results, iTBS was applied over area 5 in Experiment 2.  An increase in MEPs in the contralateral FDI muscle with no changes in MEP amplitude in the ipsilateral hand was observed.  ITBS did not modulate circuitry involved in SICI or ICF.

*How do these findings inform basic neuroscience*?  The data from both studies suggests area 5 is one cortical loci that influences M1 output to the hand.  It is possible that these effects may translate to other cortical areas such as the premotor cortex and secondary somatosensory cortex (SII), both with representations of the hand and dense projections with M1.  However, area 5 was selected as a target for TBS in the present experiment since it is clearly dominated by the representation of the hand and exists in species with opposable thumbs (Padberg et al., 2005) with its emergence coinciding with the evolution of skilled hand manipulation.  It remains unclear how area 5 modulates hand control since the present work is focused solely on physiological changes within the motor cortex.  However, data in monkeys suggest that area 5 may influence hand movement and future research is needed to probe whether the physiological changes observed in the present thesis parallel changes in motor behaviour of the hand.

*What are the clinical applications of the thesis results?* Area 5 appears to be one cortical loci that may underpin the control of hand movements and may be a source of the imbalance in neural circuitry observed in patient groups with impaired hand function. The present findings shed light on potential clinical applications in individuals with imbalances in M1 circuitry and output to the hand muscles. For example, Focal Hand Dystonia (FHD) is a movement disorder where patients often have excessive muscle contraction leading to abnormal posturing of the hand. The source of FHD remains unclear, however, several studies show imbalances in the inhibitory and excitatory circuits within M1. In particular, reduced SICI leads to hyper-excitable output to the hand. Subsequently, rTMS studies directed over the hyper-excitable motor cortex have documented improvements in hand function, however these changes last for a few days to a few weeks at most. In such patients, understanding the source of the imbalance in circuitry leading to impaired hand function may be critical for developing long-term therapies. In the present study, area 5 cTBS increased MEPs in healthy controls and it is possible that a differential effect in MEPs may be observed in FHD groups as reported elsewhere (Siebner et al., 1999). In the latter study, 1 Hz rTMS delivered to M1 resulted in a decrease in MEPs in the control group and an increase in MEPs in the FHD group. Application of area 5 cTBS in FHD groups may restore imbalances in neural networks involved in the control of hand movements. Similarly, individuals with Stroke show impaired inhibition within M1. For example, individuals with Chronic Stroke display a reduction in inhibition at rest and increased SICI persistence during paretic hand movement (Hummel et al., 2009). In such patient groups, enhanced resting motor cortical excitability may contribute to the recovery process and may be influenced by secondary areas. Based on results from the present experiment, area

5 provides an opportunity to alter downstream targets such as M1 and may be a source of imbalance in M1 circuitry.

　　*How does iTBS and cTBS affect neural processing within area 5?* In the proposed model in Figure 5.1, TBS paradigms alter neural activity within area 5 such that the result is a net facilitation in the SLF excitatory projections to the M1 corticospinal neurons. To achieve the net effect, both cTBS and iTBS paradigms may 1) target a different set of neurons within area 5 (Figure 5.1) or 2) each TBS protocol exerts the same effect on identical populations of neurons and ultimately has the same net result (an increase in MEPs). In Figure 5.1A, as hypothesized, cTBS may act on inhibitory interneurons (mono- or tri-synaptic) which in turn project to SLF neurons. A reduction in excitability of the inhibitory interneurons leads to a net facilitation of the SLF and a subsequent increase in MEP amplitude. In Figure 5.2B, iTBS may not act to increase the excitability of the area 5 inhibitory interneuron to lead to a net attenuation of the SLF fibers as predicted. Instead, depicted in Figure 5.1C is a di-synaptic chain of interneurons that may act to facilitate the SLF output to M1, leading to an increase in MEPs.

　　*How does the neural output from area 5 affect M1 circuitry?* The area 5 to M1 influence on excitatory circuitry (MEPs) may be cortically mediated or spinally mediated. The spinal projections terminate within the dorsal horn and not within the ventral horn suggesting the area 5 influence on M1 is likely cortically mediated. However, we cannot exclude the possibility that area 5 has an indirect (di- or polysynaptic) influence on spinal motor neuronal output. Area 5 has direct anatomical connections to M1 through the glutamatergic SLF fibers and these projections synapse on corticospinal neurons within M1. Alternatively, it is possible that area 5 is first projecting to other cortical loci such as

the premotor cortex, SII, supplementary motor area, cingulate cortex, and then to M1

through anatomical connections.  Subsequently, the delayed onset of effects following area

5 iTBS may reflect alterations in a population of neurons that first project to other cortical

loci before influencing M1 circuitry.  In both experiments, SICI and ICF remained unaltered.

It may be that area 5's influence on M1 is confined to corticospinal neurons, leaving the M1

interneurons that mediate SICI and ICF are unaltered.

*Are the effects of TBS over area 5 similar to the effects of TBS directly over M1?*  The

onset and longevity of TBS after-effects in both experiments are in line with previous

literature where TBS is applied directly over M1 (Huang et al., 2005; n=9).  In Experiment

1, cTBS increased MEP amplitude bilaterally immediately following stimulation and these

effects lasted up to 1 hour (n=12).  Following cTBS over M1, MEP amplitude decreases

immediately and persists for up to 1 hour after stimulation in both the stimulated and non-

stimulated hemispheres.  In Experiment 2, iTBS increased MEP amplitude for up to 60

minutes in the contralateral FDI muscle with the onset of effects at 25 minutes (n=11).

Following iTBS over M1, changes in MEP amplitude may be observed at 20 minutes and last

up to 45 minutes (Suppa et al., 2008) or may be observed immediately and persist for 15

minutes (Huang et al., 2005).   In Experiment 2, the delayed onset of MEP amplitude

increase following iTBS may reflect an alternative neural path between area 5 to M1 via

other anatomically connected loci.

The change in MEP amplitude in the present study exceeds the alterations following

cTBS over M1.  MEPs are maximal at between 25-40 minutes (mean change 132.1%) and

between 15-40 min (mean change 42.4 %) following cTBS applied to area 5 (Experiment 1)

and M1 (Huang et al., 2005), respectively.  This surprising and interesting finding may

reflect a more robust modulation (compared to M1 cTBS) in the I1-wave circuitry responsible for generation of MEPs.  In turn, this may contribute to a greater overall facilitation of the corticospinal neuron output following area 5 cTBS.  Following area 5 iTBS, the maximal increase in MEPs is observed between 45-60 minutes (mean change 38.3%) and when iTBS is applied over M1, MEP change is maximal between 1-10 minutes (mean change 75.7%).  ITBS over area 5 does not alter MEP amplitude to the same extent as the cTBS paradigm, a similar observation in a previous study applying iTBS to M1 (McAllister et al. 2009).

*Why did cTBS and iTBS increase MEPs?*  Diverging from common thinking that cTBS depresses and iTBS increases excitability, the present results suggest the differential effects of these paradigms may not be transferable to non-motor loci.  The results in this thesis indicate that cTBS and iTBS to area 5 increase MEP amplitude.  The present finding is supported by the findings that all TBS paradigms- cTBS, iTBS, and intermediate TBS (imTBS)- reduce the amplitude of laser evoked potential, N2, following stimulation over SI (Poreisz et al., 2008).  Despite these findings, the latter paradigm (imTBS) when applied directly over M1 has no effects on MEPs, SICI, or ICF (Huang et al., 2005).  When cTBS is applied over the visual cortex, an increase in phosphene thresholds is observed while iTBS has no effect (Franca et al., 2006).  Another study using infrared spectroscopy identified iTBS (300 pulses) over left SI decreases oxy-hemoglobin in the contralateral hemisphere (Mochizuki et al., 2007).  Collectively, these data suggest TBS paradigms may have variable effects.  When TBS is applied directly over M1, the direction of MEP changes from the contralateral hemisphere is also variable; MEPs may decrease (Ishikawa et al., 2007) or increase (Stefan et al., 2008, Suppa et al. 2008) following cTBS.  These differences could be

due to intensity differences (Ishikawa et al., 2007, Suppa et al., 2008 - 80% AMT; Stefan et al., 2008 - 70% RMT), paradigm differences (Ishikawa et al., 2007, Suppa et al., 2008- 600 pulses; Stefan et al., 2008 – 300 pulses), muscle recordings (Ishikawa et al., 2007, Suppa et al., 2008- FDI muscles; Stefan et al., 2008 – APB muscles) or inter-individual differences. The findings from Experiment 1 and 2 provide another example that iTBS and cTBS protocols may not alter excitability the same way in different cortical loci. As a result, caution must be taken when optimizing on the differential effects of cTBS and iTBS paradigms over non-motor, and in some instances, motor loci (Ishikawa et al., 2007, Suppa et al., 2008, Stefan et al., 2008).

*What are the limitations of the thesis experiments and their interpretation?* The present observations may relate specifically to the direction of TBS current used. TBS was delivered with the induced current flowing in the posterior to anterior direction and such effects may be specific to current direction (Suppa et al., 2008), however these findings are not unanimous (Zafar et al., 2008). In addition, the sham TBS whereby a TBS paradigm recording was played in the background while the TMS coil was placed over the scalp may not be ideal. Utilizing a sham coil or positioning a real TMS coil at an angle over the scalp may produce both acoustic artifact and scalp muscle stimulation (Lisanby et al., 2001) and in turn, may be more suitable for sham stimulation. Further, we cannot identify exactly how iTBS and cTBS act at the neuronal level and we therefore had to make inferences about the neural pathway. Finally, areas 5 and 7 in humans cannot be exactly demarcated due to the close proximity and unclear border between these cortical loci. Subsequently, without a clear boundary on the lateral side of area 5, it is difficult to confine the TMS target to area 5.

*What is the subsequent research direction as a result of the thesis experiments?* The goal of this thesis was to examine the influence of area 5 on M1 neural networks. One avenue that may be addressed is stimulation intensity and direction of TBS over area 5. To date, this is the first investigation that has applied TBS over area 5 and it may be that a lower or higher TBS intensity when applied over area 5 may modulate SICI and ICF circuitry, respectively. It is also possible that anterior to posterior or lateral to medial current directions for area 5 TBS may modulate neural circuitry in a way that deviates from the present findings. Further, although spinal projections from area 5 are to the dorsal horn, the literature on this topic is somewhat sparse and we cannot exclude the possibility that area 5 has an indirect influence on spinal motor neuronal output. To test the possible spinal contribution of area 5 following TBS, investigations on spinal neuron excitability can be probed. Looking at other neural networks such as inter-hemispheric inhibition before and following TBS may help delineate the neural path by which area 5 influences M1. Another avenue is application of the findings of increased MEPs following area 5 cTBS and iTBS in patient groups exhibiting imbalances in M1 neural circuitry. Identifying behavioural effects, and not just physiology, following TBS paradigms may allow for examining motor control of the hand in both healthy controls and patient groups. Finally, while TBS in the present study was applied over left-hemisphere area 5, TBS over right-hemisphere area 5 may have differing effects. Functional MRI reveals homologous areas of the right and left superior parietal lobule (SPL) are involved in different stages of tactile object discrimination (Stoeckel et al., 2004) and it remains unclear whether the observed effects from the presentxperiment are specific for the left-hemisphere or can be seen following TBS to area 5 in the right-hemisphere.

This thesis is the first investigation examining the influence of area 5 on M1 neural circuitry and output to the hand.  TBS and paired-pulse TMS allowed for investigation of the influence of area 5 on excitatory and inhibitory circuitry within M1.  Although the experiments in the Master's thesis were performed in twenty-five healthy humans, the results are directly applicable to certain clinical populations.  The neural model in Figures 5.1 and 5.2 were created to assist with interpretation of the findings and provide some explanation to help delineate the neural pathway by which area 5 influences M1 circuitry.
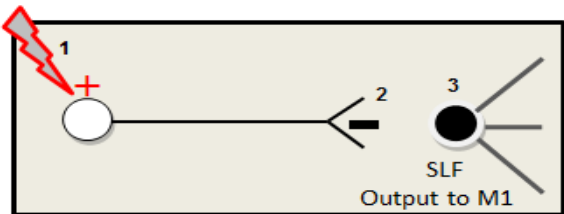
**Predicted and Actual Neural Mechanisms within Area 5**
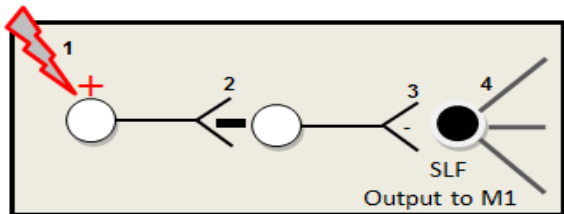
**A  CTBS Hypothesis & Actual**
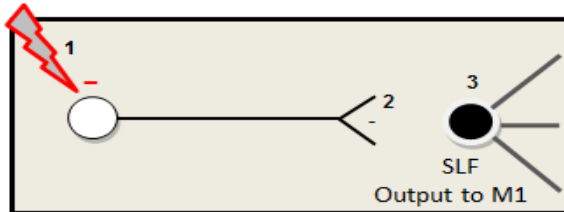
1. CTBS *decreases* excitability in target interneurons (mono- or tri-synaptic) within area 5
2. Decreased inhibition
3. SLF neurons are disinhibited and therefore MEP amplitude increases

**B  ITBS Hypothesis**

1. ITBS *increases* excitability in target interneurons (mono- or tri-synaptic) within area 5
2. Increased inhibition
3. SLF neurons are inhibited and therefore MEP amplitude decreases

**C  ITBS Actual**

1. ITBS *increases* excitability in target interneurons (di- or polysynaptic) within area 5
2. Increased inhibition
3. Subsequent interneuron is inhibited
4. SLF neurons are disinhibited and therefore MEP amplitude increases

*The above models are true if iTBS excites and cTBS depresses targeted cortex. Caveat of model is it does not account for the possibility that both TBS paradigms may affect the same neurons in a similar manner and lead to a net increase in MEP amplitude.*

**Figure 5.1** Neural mechanisms within area 5 following cTBS and iTBS if each produces differential effects.  A.  As hypothesized, cTBS increased MEPs and may be acting on mono- or tri-synaptic populations of neurons to create a net facilitation.  B.  Following iTBS, a decrease in MEP amplitude was hypothesized in a neural network similar to cTBS.  C. Following iTBS, MEP amplitude increased and may be mediated through di- or polu-synaptic neuronal networks.  We cannot rule out that cTBS and iTBS are acting on a different set of neurons within area 5 with the same net result of increased MEPs.
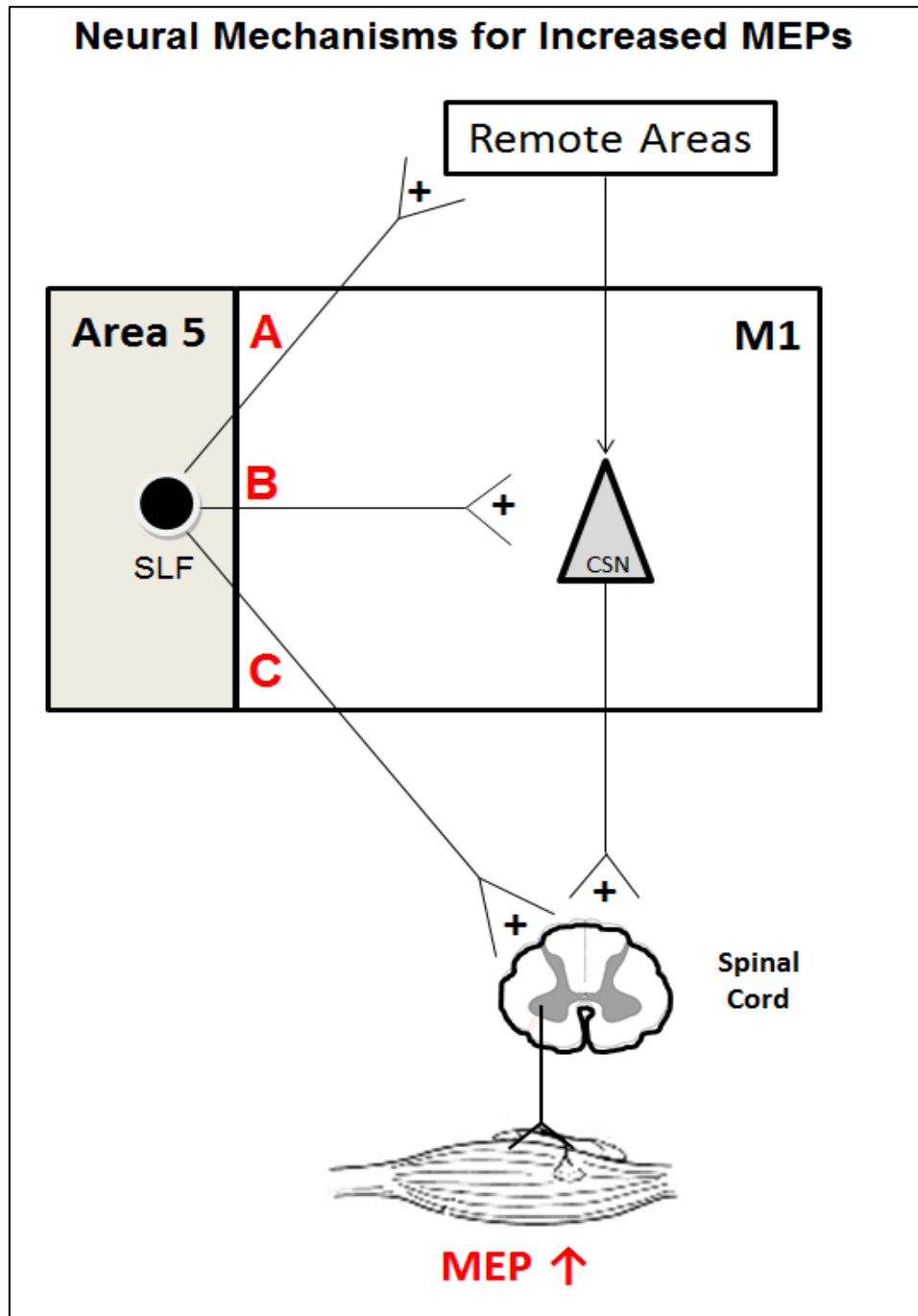
**Figure 5.2** Neural mechanisms for increased MEPs. The area 5 to M1 influence on excitatory circuitry (MEPs) may be cortically mediated or spinally mediated (Figure 5.2). A. Area 5 is first projecting to other cortical loci such as the premotor cortex and supplementary motor area via the SLF and then to M1. B. Area 5 is projecting directly to M1 through the glutamatergic SLF fibers and these projections are synapsing on corticospinal neurons within M1. C. Spinal projections directly from area 5 terminate within intra-laminar regions of the dorsal horn.

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