INFLUENCE OF PRIMARY SOMATOSENSORY CORTEX ON HAND MOTOR CIRCUITRY AND THE ROLE OF STIMULATION PARAMETERS

By

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Abstract

The primary somatosensory cortex (SI) is important for hand function and influences motor circuitry in the primary motor cortex (M1). Areas 3a, 1 and 2 of SI have direct connectivity with M1. Much of our present knowledge of this connectivity and its relevance to hand function is based on animal research. However, less is known about the neural mechanisms that underpin hand function in humans. The present study investigated the influence of SI on corticospinal excitability as well as inhibitory and excitatory neural circuitry within M1 before and after continuous theta-burst stimulation (cTBS). Additionally, stimulation parameters influence the direction and magnitude of cTBS after-effects. Thus, current direction and frequency of cTBS were manipulated. Two experiments were performed. In Experiment 1, motor-evoked potentials (MEPs) were recorded from the first-dorsal interosseous (FDI) muscle bilaterally before and after 50 Hz cTBS over left SI. In a second condition, the orientation of cTBS was reversed. Experiment 2 measured MEPs, short-latency intracortical inhibition (SICI) and intracortical facilitation (ICF) from the right FDI following a modified 30 Hz cTBS over left SI or M1. The results of Experiment 1 and 2 demonstrate that SI influences M1 circuitry such that MEPs are facilitated following cTBS over SI. However, MEPs are suppressed when the current direction is reversed. CTBS at 30 Hz delivered over M1 suppressed excitatory circuitry that generates MEPs and ICF. The findings from the thesis suggest that SI influences hand motor circuitry and is likely a mechanism by which somatosensory information modulates hand motor function.

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1.0 Goal of Thesis

1.1 Introduction

Research in animal models has revealed that sensory afferent information is important for motor behaviour (Mountcastle, 2005). Cooling or lesioning of the primary somatosensory cortex (SI) of non-human primates result in loss of somatosensation and impairs hand motor control (Brinkman *et al.*, 1985;Carlson, 1981). However, less is known about the interaction of SI and M1 in hand control in humans. It is, therefore, necessary to establish basic neuroscience knowledge in this area. Continuous theta-burst stimulation (cTBS) is a repetitive form of TMS capable of inducing physiological changes in the cortex for up to one hour following stimulation (Huang *et al.*, 2005). This paradigm may be used to suppress targeted cortex in order to test how circuitries in other connected areas of the brain are affected. Given the close integration of sensory input and motor control, cTBS may be one method to explore connectivity between the primary sensory and motor regions that are important in hand function.

1.2 Goals of Thesis

The goal of the thesis was to investigate how the human primary somatosensory cortex (SI) influences motor circuitry that outputs to muscles of the hand. This was investigated using continuous theta-burst stimulation (cTBS), known to supress or enhance cortical activity based on stimulus parameters (Talelli *et al.*, 2007;Suppa *et al.*, 2008;Goldsworthy *et al.*, 2012;Doeltgen & Ridding, 2011). As such, a second element of the thesis involved the adjustment of stimulus parameters in order to maximize the magnitude and duration of cTBS effects over SI. To achieve these goals, several measures of activity in cortical circuitry were recorded using TMS. Single-pulse TMS over M1 provided general measures of corticospinal excitability. Paired-pulse TMS were used to measure changes in specific inhibitory and excitatory interneurons in M1. The measures taken in the experiments may provide novel information on the mechanisms by which SI may influence specific inhibitory and excitatory circuitry of the M1 hand region. This research adds to scientific understanding of sensorimotor integration and its importance for motor behaviour (Abbruzzese & Berardelli, 2003), and presents opportunities for alternate pathways of driving change in motor function and behaviour related to the hand. Second, this circuitry is capable of being modulated by non-invasive rTMS, presenting future opportunities to explore sensorimotor integration in humans. This work may also have clinical significance. Plasticity of the brain is an important marker for recovery following neurological injury, such as stroke (Johansson, 2000) where cTBS may benefit recovery (Meehan *et al.*, 2011). Methods of artificially inducing brain plasticity may be used in conjunction with rehabilitation therapies in order

to maximize gain of motor control. In summary, the research thesis contributes to the understanding of sensory control of hand movement and provides researchers and clinicians in the neuroscience field with an effective protocol to induce changes in hand motor circuitry via the primary somatosensory system.

1.3 Summary of Experiments

Two studies were completed in the thesis. The first study was completed at the University of Waterloo. This study investigated the influence of induced current orientation of cTBS over SI on M1 corticospinal excitability. Motor-evoked potentials were recorded by single pulse TMS over M1 before and after cTBS over SI for up to 45 minutes following cTBS application. In two sessions separated by at least one week, cTBS was applied in either the anterior-to-posterior followed by posterior-to-anterior (AP-PA) or in the opposite direction (i.e. PA-AP). The second proposed study was completed at McMaster University. The main goal of the second study was to more thoroughly explore changes in M1 by recording the output of inhibitory and facilitatory networks and corticospinal excitability following cTBS over SI. Importantly, the stimulation frequency in the cTBS protocol was modified in order to determine if this revised protocol could produce plasticity of a stronger magnitude and longer duration.

Chapter 2: Literature Review

2.1 Primary Somatosensory Cortex

2.1.1 Functional Anatomy and connectivity of SI

The primary somatosensory cortex (SI) is composed of four Brodmann areas including area 1, 2, 3a and 3b in monkeys (Kaas *et al.*, 1979;Merzenich *et al.*, 1978) and humans (Geyer *et al.*, 1997;Nelson & Chen, 2008a). Area 1 and 3b receive cutaneous afferent input (Sur *et al.*, 1980). Area 3a receives proprioceptive input (Iwamura *et al.*, 1993;Huffman & Krubitzer, 2001) and has connections with area 1 and 2 (Huffman & Krubitzer, 2001). Area 2 receives input from both cutaneous and proprioceptive afferents (Iwamura & Tanaka, 1978). Corticocortical projection neurons between SI areas (except area 3b) and primary motor cortex (M1) have been clearly shown in cats (Asanuma *et al.*, 1968;Porter, 1997), and monkeys (Jones *et al.*, 1978a;Huffman & Krubitzer, 2001).

2.1.2 Relevance of SI-M1 connectivity

The significance of the SI-M1 projections may be important with respect to the influence of SI on motor behaviour. Multiple somatosensory areas projecting to M1 may offer multiple forms of sensory feedback which the motor cortex can use to optimize movement (Porter, 1997). Somatosensory input is important for accurate motor tasks such as object gripping and manipulation (Huffman & Krubitzer, 2001;Johansson & Flanagan, 2009). In monkeys, cooling of area 2 led to clumsy and poorly coordinated

movements (Brinkman *et al.*, 1985). When SI is damaged, motor skill acquisition is greatly impaired in monkeys (Pavlides *et al.*, 1993) and cats (Sakamoto *et al.*, 1989). Damage to SI in humans impairs recovery of relearning motor skills (Abela *et al.*, 2012). These findings support the importance and influence of SI on M1 and motor behaviour.

2.2 Transcranial Magnetic Stimulation (TMS)

2.2.1 Introduction to TMS

Transcranial magnetic stimulation (TMS) is a non-invasive technique that uses a time-varying magnetic field to induce an electric current flow in the brain, activating neural circuitry and producing a mixture of excitatory and inhibitory responses (Chen *et al.*, 2008;Terao & Ugawa, 2002;Kobayashi & Pascual-Leone, 2003). Understanding of the mechanisms of TMS is mostly derived from activation of M1 and comparison to electrical stimulation of the cortex. As a function of intensity, electrical stimulation of the motor cortex produces descending volleys that consist of an initial large direct wave (D-wave) followed by several indirect (I-waves) which relate to either direct or indirect activation of the corticospinal neurons in M1 (Terao & Ugawa, 2002). In contrast, TMS produces I-waves initially, followed by direct waves at very high intensities or specific coil position (Di Lazzaro *et al.*, 1998). The preference of TMS to produce I-waves suggests that corticospinal neurons are transynaptically activated via excitatory interneurons that synapse with the corticospinal neuron (Hallett, 2007;Terao & Ugawa, 2002;Pell *et al.*, 2011;Kobayashi & Pascual-Leone, 2003). The descending volleys arrive

at the spinal α-motor neurons that correspond to the upper motor neuron stimulated by TMS. The volleys summate as excitatory post-synaptic potentials (EPSPs) and, if sufficient, depolarize the spinal motoneuron pool leading to an action potential and subsequent contraction of the represented target muscle (Sakai *et al.*, 1997). This contraction in the target muscle can be recorded using electromyography and serve as an index of cortical and corticospinal excitability (Rothwell, 2011).

2.2.2 Repetitive Transcranial Magnetic Stimulation (rTMS)

When TMS pulses are repetitively delivered to the motor cortex, referred to as repetitive TMS (rTMS), the excitability of neuronal populations in this area may be bi-directionally altered for a length of time following rTMS application (Hoogendam *et al.*, 2010). Pulses delivered at a rate of ≤ 1 Hz typically cause cortical inhibition, whereas ≥ 5 Hz stimuli enhance excitability of the cortex (Hallett, 2007). The magnitude and duration of changes are largely dependent on the duration or number of pulses delivered, with more stimuli generally leading to stronger effects (Peinemann *et al.*, 2004). Therefore, effective rTMS may take an extended period of time to deliver.

2.2.3 Continuous Theta-burst Stimulation (cTBS)

CTBS consists of high-frequency, low-intensity bursts of 3 pulses such that 600 pulses may be delivered in as little as 40 seconds (Huang *et al.*, 2005). The inter-pulse

rate is 50 Hz and the inter-burst rate is at 5Hz (Huang *et al.*, 2005). These frequencies were based from animals models whereby high frequency bursts of stimuli led to long-term potentiation (LTP) / depression (LTD) of cortical synapses (Cardenas-Morales *et al.*, 2011). This form of rTMS produces transient after-effects in the motor cortex that last much longer than the period of stimulation. Specifically, cTBS over the motor cortex alters intracortical circuitry and inhibits corticospinal excitability (Huang *et al.*, 2005;Talelli *et al.*, 2007). TBS is an ideal protocol because, unlike rTMS which requires application over lengthy time periods, it requires only a very short application time with long-lasting effects up to an hour (Huang *et al.*, 2005).

2.3 Relevant Circuitry and effects of cTBS

2.3.1 Motor Evoked Potentials

Following from section 2.1 on the mechanisms of TMS, a single pulse over the motor cortex of sufficiently high intensity (i.e. suprathreshold) will cause corticospinal neurons to discharge at specific intervals (generating I-waves) that ultimately result in a brief contraction of the targeted muscle (Di Lazzaro *et al.*, 2003). Muscles in the upper and lower limb may be targeted from M1, but have ranging intensities required to evoke a response. Muscles of the hand, such as the first dorsal interosseous (FDI) and abductor pollicis brevis (APB), are ideal targets as they are easily activated by TMS (Petersen *et al.*, 2003). For this reason, FDI is the target muscle in the thesis. Using surface electromyography (EMG) electrodes placed over the FDI, muscle activation following

suprathreshold TMS is recorded as an electric potential called a motor evoked potential (MEP). The peak-to-peak amplitude of an MEP reflects corticospinal neurons at the cortical and spinal level (Wassermann *et al.*, 2008). Thus, MEPs act as an index of the net excitability in the corticospinal system.

2.3.2 Motor Threshold (MT)

Responsiveness to TMS differs across muscles represented in M1. Specifically, muscles of the hand produce larger MEPs than proximal limb muscles at a given stimulation intensity (Wassermann *et al.*, 2008). In the upper limb, for example, distal hand and finger muscles have greater representation than forearm and proximal arm muscles which is thought to account for differences in neural recruitment by TMS (Wassermann *et al.*, 2008). The first dorsal interosseous (FDI) of the hand is the target muscle in this thesis given its relative ease of activation in hand musculature.

Within the same target muscle, responsiveness to TMS differs greatly between people. It is therefore necessary to standardize TMS protocols to make them comparable across participants and other studies. This is accomplished by setting TMS intensities relative to an individual's activity or motor threshold rather than the absolute stimulation intensity of the equipment. Motor threshold is the lowest stimulator intensity required to evoke a small amplitude MEP (amplitude defined by the experimenter) in at least 5 of 10 consecutive TMS pulses over M1. Resting motor threshold (RMT) is defined as 50 μV and is determined with the participants muscle at rest (Rossini *et al.*, 1994) although other

definitions exist (Alle *et al.*, 2009;Mochizuki *et al.*, 2004). Active motor threshold (AMT) is defined as 200 μV and is determined with the participant maintaining a slight contraction in the FDI (Rossi *et al.*, 2009). Pharmacological evidence suggests that motor threshold indicates the intrinsic membrane excitability of the corticospinal tract neuron (Kobayashi & Pascual-Leone, 2003). Accurate determination of MT is crucial in an experiment because it is used to determine the appropriate intensity for nearly all measures of cortical excitability and rTMS interventions.

2.3.3 Short-interval Intracortical Inhibition (SICI)

When a suprathreshold TMS pulse is preceded by a subthreshold pulse by 1-6 ms, the MEP is suppressed (Kujirai *et al.*, 1993). This effect is known as short-interval intracortical inhibition (SICI). The initial sub-threshold pulse is referred to as the conditioning stimulus (CS), while the subsequent suprathreshold pulse is referred to as the test stimulus (TS). The subthreshold CS pulse fails to evoke descending volleys, offering evidence that SICI occurs at the cortical level (Di Lazzaro *et al.*, 1998). SICI is thought to act on circuitry that generates the MEP during the TS pulse. This is supported by epidural recordings in humans revealing that SICI suppresses late I-waves recruited by the TS pulse (Di Lazzaro *et al.*, 1998). This suggests that the CS pulse activates inhibitory interneurons that supress MEP generating circuitry that is activated by the TS pulse (Kujirai *et al.*, 1993). Specifically, GABAergic inhibitory interneurons activated by the CS pulse produce an inhibitory post-synaptic potential (IPSP) on the excitatory

circuitry synapsing with corticospinal neurons which, in turn, dampens the cortical descending volleys produced by the TS pulse (Ilic *et al.*, 2002). Pharmacological studies have confirmed GABAergic origins of SICI. Benzodiazepines are allosteric agonists for $GABA_A$ receptors and increase SICI (Paulus *et al.*, 2008;Di Lazzaro *et al.*, 2006). These findings support the hypothesis that SICI occurs though the $GABA_A$ receptor (Kujirai *et al.*, 1993;Hanajima *et al.*, 1998). In summary, SICI measures of the state of inhibitory interneuronal networks within M1.

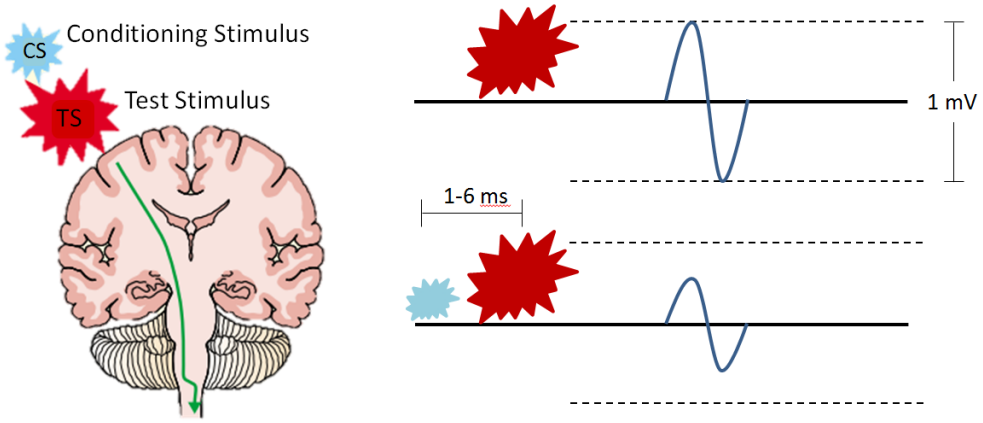

Figure 1. Illustration of SICI. When set at an appropriate intensity and delivered alone, the TS stimulus will produce a ~1 mV MEP. When the subthreshold CS pulse is delivered 1-6 ms before the TS pulse, the resultant MEP is suppressed in amplitude. This is due to inhibitory interneurons acting on the MEP generating circuits generated by the TS pulse. [Reprinted and adapted from The Lancet, Vol. 2, Kobayashi & Pascual-Leone, *Transcranial Magnetic Stimulation in Neurology*, 145-156, 2003, with permission from Elsevier]

2.3.4 Intracortical Facilitation (ICF)

Extending from the SICI protocol, when the interstimulus interval (ISI) is increased to 10-15 ms the MEP evoked by the TS pulse is facilitated compared to the TS alone pulse (Kujirai *et al.*, 1993;Ziemann *et al.*, 1996). One additional difference from SICI is that the ICF effect is maximal at slighter higher CS intensities ~90% AMT (Kujirai *et al.*, 1993). The CS used in ICF does not alter the amplitude of H-reflex of hand musculature, suggesting the facilitation occurs at the cortical level (Ziemann *et al.*, 1996). Although the mechanisms of ICF remain unclear, it is thought that the activated circuitry involves both excitatory and, to a lesser extent, inhibitory networks (Paulus *et al.*, 2008). The main support for dual contributions comes from pharamacological studies implicating inhibitory and excitatory neurotransmitters GABA and glutamate, in respective order (Paulus *et al.*, 2008). Specifically, benzodiazepines decrease ICF, indicating that the $GABA_A$ receptor is involved (Paulus *et al.*, 2008). Glutamatergic NMDA receptor antagonists decrease ICF, implicating the role of excitatory transmission (Paulus *et al.*, 2008). ICF is therefore considered to be a net facilitation of excitatory and inhibitory networks (Paulus *et al.*, 2008). Importantly, however, SICI and ICF represent different cortical interneurons (Ziemann *et al.*, 1996). In summary, ICF measures the state of excitatory interneuronal networks within M1.

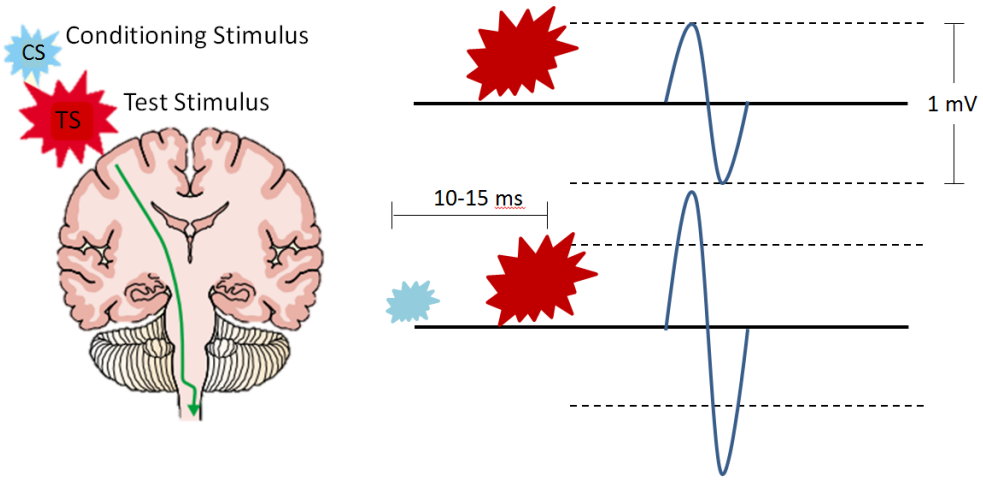

Figure 2. Illustration of ICF. When set at an appropriate intensity and delivered alone, the TS stimulus will produce a ~1 mV MEP. When the subthreshold CS pulse is delivered 10-15ms before the TS pulse, the resultant MEP increases in amplitude. This is thought to be due to facilitatory interneurons acting on the MEP generating circuits generated by the TS pulse. However, the precise mechanisms are unknown. [Reprinted and adapted from The Lancet, Vol. 2, Kobayashi & Pascual-Leone, *Transcranial Magnetic Stimulation in Neurology*, 145-156, 2003, with permission from Elsevier]

2.4 Changes to circuitry following cTBS

2.4.1 Motor Evoked Potentials (MEPs)

Following cTBS over M1 hand area, MEPs amplitudes are suppressed in the contralateral hand for up to 60 minutes (Huang *et al.*, 2005;Talelli *et al.*, 2007;Stefan *et al.*, 2008b;Zafar *et al.*, 2008). Additionally, cTBS alters the homologous muscle representation in the contralateral hemisphere M1. Following cTBS, MEP amplitudes are facilitated in the ipsilateral hand (Suppa *et al.*, 2008;Stefan *et al.*, 2008b). However, suppression of the ipsilateral hand MEPs has also been reported (Ishikawa *et al.*, 2007). These data provide evidence that cTBS is capable of directly or remotely modulating activity in both motor cortices.

2.4.2 Short Interval Intracortical Inhibition (SICI)

Following cTBS over M1, SICI decreases for up to 35 minutes (Huang *et al.*, 2005;Suppa *et al.*, 2008;McAllister *et al.*, 2009). In the ipsilateral hand, SICI decreased for at least 35 minutes (Suppa *et al.*, 2008). In contrast, one study found no changes in SICI following cTBS (Doeltgen & Ridding, 2011). The reasons for this discrepancy are unclear, but may relate to differences in CS intensities used to probe SICI.

2.4.3 Intracortical Facilitation (ICF)

Following cTBS over M1, ICF decreases (Huang *et al.*, 2005). However, some reports indicate no change (Talelli *et al.*, 2007;Suppa *et al.*, 2008;McAllister *et al.*, 2009). In two of these studies, the induced current direction in M1 was opposite that used by Huang (2005), which may contribute to the differences in ICF findings. Further, McAllister (2009) used a lower stimulation intensity of 70% AMT rather than the standard 80% AMT. This intensity may have been too low given that ICF circuitry has a higher intensity threshold for activation (Kujirai *et al.*, 1993).

2.5 Stimulation Parameters that influence TMS/rTMS/cTBS

Although the underlying mechanisms of rTMS and cTBS are not fully understood, it is evident that stimulation parameters play an important role in the characteristics of after-effects (Pell *et al.*, 2011;Cardenas-Morales *et al.*, 2010).

Exploring these factors that influence the after-effects of rTMS may provide insight into these mechanisms and how to optimize protocols for clinical use. Indeed, the magnitude, polarity and duration of after-effects depend on several stimulus parameters including the direction of induced current and the stimulus frequency. It is important to understand how these parameters modulate the outcome of rTMS protocols. This section will review available literature of these parameters as they relate to cTBS. However, due to a limited number of studies on how parameters influence cTBS, it is beneficial to consider how stimulation parameters affect single pulse TMS and rTMS in order to provide rationale for pursuing cTBS parameter optimization.

2.5.1 Current Direction

2.5.1.1 Current direction - TMS

The activation of cortical circuits by TMS is heavily influenced by the direction of current in the brain, most clearly demonstrated using single pulse TMS. When the direction of induced current is perpendicular to the pre-central gyrus, it preferentially activates nerve cells in this spatial orientation that result in a strong MEP response (Niehaus *et al.*, 2000). It is thought that facilitatory neurons are arranged in this orientation, whereas inhibitory circuits are randomly orientated (Ziemann *et al.*, 1996;Pell *et al.*, 2011). Thus, induced currents directed in the posterior-to-anterior (PA) orientation will preferentially activate these facilitatory neurons, eliciting large MEP amplitudes. In contrast, an induced current in the anterior-to-posterior (AP) orientation

elicits a much smaller MEP (Pell *et al.*, 2011). Furthermore, any current direction will activate inhibitory neurons due to their spatial orientation, but not the facilitatory neurons to the extent of a current in the PA orientation. The result is smaller MEP amplitudes in each direction in comparison to PA directed current (Pell *et al.*, 2011).

The waveform of the pulse interacts with current direction. It is, therefore, beneficial to consider both factors. Monophasic TMS in the PA orientation have lower motor thresholds than in the AP direction (Kammer *et al.*, 2001;Niehaus *et al.*, 2000). In contrast, biphasic TMS has lower thresholds in the AP-PA orientation than in the PA-AP direction (Kammer *et al.*, 2001). The fact that monophasic and biphasic pulses are more efficient in opposite orientations suggests that the second, reversing phase in a biphasic pulse may be more effective than the initial rising phase (Kammer *et al.*, 2001;Di Lazzaro *et al.*, 2001a).

There is evidence that induced current directions and pulse waveforms may be activating different populations of cortical circuits or different areas within same population (Sakai *et al.*, 1997;Di Lazzaro *et al.*, 2001b). Investigation into descending volleys evoked from monophasic TMS reveal that pulses in the PA direction tend to activate I-1 waves, whereas AP pulses recruit I-3 waves (Di Lazzaro *et al.*, 2001b;Sakai *et al.*, 1997). It is thought that the interneurons that create I-waves are separate, facilitatory circuits and contribute to the generation of a MEP (Sakai *et al.*, 1997;Di Lazzaro *et al.*, 2001b). These results indicate that monophasic AP induced current in the brain activates different cortical circuits than PA current (Di Lazzaro *et al.*, 2001b).

Concerning biphasic TMS, the recruitment of I-waves is more complex yielded a mixture of I-waves (Di Lazzaro *et al.*, 2001a;Pell *et al.*, 2011). This is in line with the idea that biphasic pulses activate a wider range of excitatory and inhibitory circuits (Pell *et al.*, 2011) and likely plays an important role in cTBS which employs biphasic bursts. Given that these waveform and current direction in TMS differentially influences cortical circuits, it is reasonable to speculate that these parameters may affect the outcome of rTMS and cTBS protocols.

2.5.1.2 Current direction - rTMS

Previous work by Tings et al. (2005) revealed that the polarity of after-effect from monophasic rTMS is dependent on current orientation. Stimulation in PA orientation led to MEP facilitation, whereas stimulation in the AP orientation led to inhibition of MEPs (Tings *et al.*, 2005). This difference was less pronounced with biphasic rTMS. Both PA-AP and AP-PA orientations with biphasic rTMS led to facilitation in the target muscle, but to a lesser degree than PA monophasic facilitation (Tings *et al.*, 2005;Peinemann *et al.*, 2004). Biphasic pulses seem insensitive to orientation and produce weaker facilitation in comparison to monophasic rTMS at high-frequency rates. In high-frequency rTMS, monophasic pulses are ideal to maximize the magnitude of after-effects. Importantly, the desired outcome of facilitation or inhibition can be controlled by current orientation in the PA or AP direction, in respective order.

2.5.1.3 Current direction - cTBS

Few studies have investigated the influence of orientation on the after-effects of TBS. One study by Talelli and colleagues (2007) compared the traditional PA-AP orientation with an AP-PA orientation over the left motor cortex. While both orientations suppressed MEPs, AP-PA orientation suppressed MEPs to a greater degree when stimulation intensities were similar in each orientation (Talelli *et al.*, 2007). One explanation is that biphasic pulses are more efficient at recruiting motor activity with the AP-PA orientation, as seen in TMS (Kammer *et al.*, 2001), and led to greater net effects on MEP amplitude following cTBS. In contrast, a second study found PA-AP led to stronger MEP suppression that AP-PA orientated cTBS (Zafar *et al.*, 2008). However, the authors did not adjust the intensity in the AP-PA orientation which they suggest may have led to differences in findings (Zafar *et al.*, 2008). Thus, the role of induced current direction in the magnitude of cTBS outcome remains unclear. Further, there is conflicting evidence of bilateral effects of cTBS on the stimulated and non-stimulated hemispheres due to orientation. One study found that cTBS in the AP-PA orientation decreased cortical excitability of the stimulated hemisphere and increased excitability in the contralateral, non-stimulated hemisphere (Suppa *et al.*, 2008). A second study used PA-AP orientation found excitability changes only in the stimulated hemisphere (Stefan *et al.*, 2008b). A third study also used PA-AP, but found that excitability in both hemispheres decreased (Ishikawa *et al.*, 2007). In the study by Suppa and colleagues (2008), the traditional PA-AP orientation suppressed MEPs in the stimulated hemisphere only, in agreement with the second study by Stefan and colleagues (2008). While cTBS

consistently suppressed activity in the stimulated hemisphere, the activity of the contralateral hemisphere remains unclear in either orientation. These studies suggest that orientation of the coil during TBS may also influence inter-hemispheric connections, however further investigation is required to resolve conflicting evidence.

2.5.2 Frequency

2.5.2.1 Frequency - rTMS

The rate of stimuli may be the most influential factor in rTMS protocols. The relationship is clear in rTMS such that low frequencies of ≤ 1 Hz inhibit MEP amplitude (Chen *et al.*, 1997), whereas higher frequencies (≥ 5 Hz) facilitate MEP amplitudes (Peinemann *et al.*, 2004). Thus, frequency of stimuli strongly determines the direction of after effects in rTMS.

2.5.2.2 Frequency - cTBS

Alterations in cTBS frequency domains have been done in few studies, mostly in non-M1 areas. The pattern of cTBS frequency may be altered at two points; the timing between pulses (intra-burst frequency) and/or the timing between bursts (inter-burst frequency). Nyffeler and colleagues (2006) developed a modified 30 Hz cTBS protocol based on alterations in both intra- and inter-burst frequencies. The difference in timing from the traditional 50 Hz cTBS protocol is visually displayed in Figure 3. When applied

over the oculomotor cortex, the onset of eye saccades were delayed by 25% after a single bout of cTBS, suggesting a suppressive effect of the 30 Hz paradigm (Nyffeler *et al.*, 2006a). In comparing 1-Hz rTMS and 30 Hz cTBS, both showed inhibitory effects on the oculomotor cortex, yet the effects were longer-lasting in cTBS (Nyffeler *et al.*, 2006b). When applied over M1, 30 Hz cTBS suppresses MEP amplitudes (Wu *et al.*, 2012;Goldsworthy *et al.*, 2012). Further, in comparing the effects of traditional 50 Hz to the modified 30 Hz cTBS paradigm, both significantly suppressed MEPs (Goldsworthy *et al.*, 2012). However, the decreases in MEP amplitude were greater in magnitude and longer lasting (Goldsworthy *et al.*, 2012). Specifically, 50 Hz cTBS significantly suppressed MEPs for only the initial 5 minutes after cTBS was delivered. In comparison, 30 Hz cTBS significantly suppressed MEPs immediately and at every time point up to 30 minutes post-cTBS (Goldsworthy *et al.*, 2012). Additionally, the 30 Hz cTBS effects were consistent across all 12 participants in the study, whereas 50 Hz varied between people such that 3 of 12 participants showed facilitation on MEPs (Goldsworthy *et al.*, 2012). These data suggest that frequency patterns of cTBS influence its efficacy and inter-subject variability.

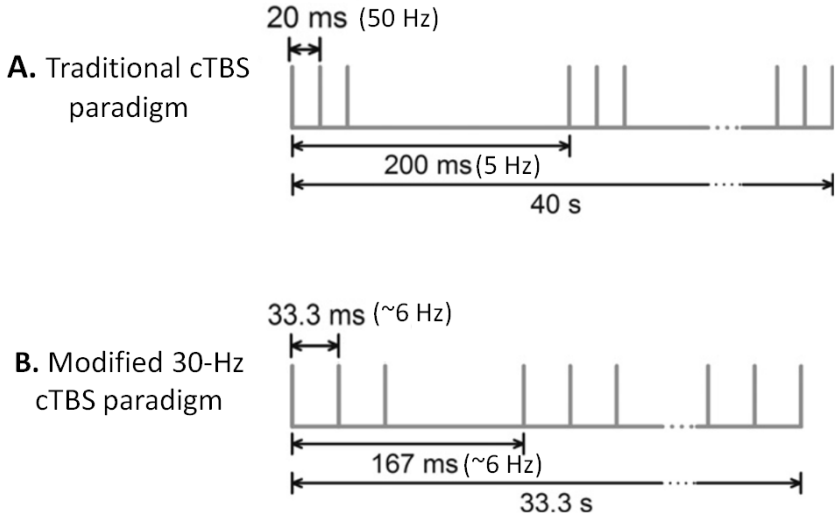

Figure 3. Difference in timing components of A) traditional 50 Hz cTBS protocol and B) the modified 30 Hz cTBS paradigm. In the latter, the inter-pulse stimulus interval is lengthened and the inter-burst interval is shortened. [Reprinted and adapted from Clinical Neurophysiology, 123, Goldsworthy et al., *A comparison of two different continuous theta burst stimulation paradigms applied to the human primary motor cortex*. pg. 2256-2263, 2012, with permission from Elsevier]

Chapter 3: Experiment 1

Current direction specificity of continuous theta-burst stimulation in modulating human motor cortex excitability when applied to somatosensory cortex

3.1 INTRODUCTION

The primary somatosensory cortex (SI) is located in the postcentral gyrus and encompasses Brodmann areas 3a, 3b, 1 and 2 (human:(Geyer *et al.*, 1997;Nelson & Chen, 2008b), monkey:(Kaas *et al.*, 1979)). With the exception of 3b, areas within the postcentral gyrus have direct projections to the primary motor cortex (M1) (Jones *et al.*, 1978b) and are therefore positioned to influence M1 neural activity and potentially modify motor behaviour. In cats, tetanic stimulation of the postcentral gyrus leads to an increase in the responsiveness of M1 neurons (Iriki *et al.*, 1989;Sakamoto *et al.*, 1987) and cooling the postcentral gyrus in monkeys increases the tonic firing of M1 neurons (Brinkman *et al.*, 1985). In humans, several approaches have demonstrated somatosensory influences on M1 excitability. Paired associative stimulation (PAS) involving median nerve stimulation followed 25 ms later by a pulse of transcranial magnetic stimulation (TMS) to M1 leads to an increase in motor evoked potential (MEP) amplitude (Stefan *et al.*, 2000), but a decrease if the two stimuli are separated by only 10 ms (Wolters *et al.*, 2003). Continuous theta-burst stimulation (cTBS) delivers low intensity, high-frequency bursts of TMS (Huang *et al.*, 2005) over a short duration and can alter neural activity within SI for up to ~ 13 minutes following stimulation (Ishikawa *et al.*, 2007).

A previous study has shown that cTBS over SI does not alter M1 excitability, however using the same intensity, cTBS over M1 does indeed alter the excitability within SI (Ishikawa *et al.*, 2007). These data suggest that a cTBS intensity of 80% active motor threshold (defined as ≥ 200μV MEP) derived from M1 excitability may be sufficient to induce changes in the M1 to SI path but insufficient to alter the influence from SI to M1. However, rTMS delivered over SI at higher intensities such as 90% RMT demonstrate changes in SI activation and tactile perception (Tegenthoff *et al.*, 2005) and PAS studies aimed at altering SI excitability have used 150% RMT (Wolters *et al.*, 2005). Further, while motor thresholds are acquired from M1, applying an intensity derived from M1 may not have the same effectiveness in other loci (Stokes *et al.*, 2005). Therefore, in the present study we have chosen to examine the influence of cTBS over SI by using a cTBS intensity slightly higher than that used previously (Ishikawa *et al.*, 2007) by defining a higher AMT (≥ 2000 μV MEP).

Previous studies using cTBS over M1 have revealed that the direction of induced current flow in the cortex determines the amplitude and direction (i.e. increase or decrease) of after-effects (Suppa *et al.*, 2008;Talelli *et al.*, 2007). The effects of current direction may relate to direction-specific preferential recruitment of interneurons that contribute to early and late I-waves (Talelli *et al.*, 2007). It remains unknown whether cTBS over SI will also demonstrate effects that are dependent on current direction and the present study addresses this issue.

We investigate the influence of SI on corticospinal excitability by measuring MEPs before and following 600 pulse cTBS applied over left-hemisphere SI oriented to induce an AP-PA versus PA-AP current in the SI cortex. We previously observed that cTBS in the AP-PA direction over higher-order somatosensory area 5 increases MEP amplitude bilaterally (Premji *et al.*, 2011) and hypothesized that cTBS over SI using the identical direction would yield an increase in MEPs bilaterally in support of the influence of SI on neural circuitry within M1.

3.2 METHODS

Participants

Twenty six right-handed adults (14 males, 12 females; mean age ± SD, range = 23.7 ± 4.9, 18 to 39) were studied. All participants were determined to be healthy using a TMS screening form that queried medical conditions. In the main experiment, the first group of participants enrolled were assigned to the AP-PA group (n=12, 7 males, 25.9 ± 6.2 years) and the second group of participants enrolled were assigned to the PA-AP group (n=8, 5 males, 22.8 ± 2.5 years). In a second series of experiments, eight participants (22.1 ± 3.4 years) were assigned to either the AP-PA group (n=4, 2 males, 22 ± 2.2 years) or PA-AP group (n=4, 2 males, 22 ± 4.8 years) in which cTBS intensity was based on 80% AMT determined in the opposite orientation. Specifically, for the AP-PA group, AMT was determined in the PA-AP orientation. For the PA-AP group, AMT was determined in the AP-PA orientation. Two participants within the second series of

experiments also participated in the main experiment. All participants gave informed written consent prior to participation. The experiments were approved by the Office of Research Ethics at the University of Waterloo and conformed to the *Declaration of Helsinki*.

Electromyographic (EMG) recording

Surface electrodes (9 mm diameter Ag-AgCl) were used to record EMG from the first dorsal interosseous (FDI) muscle of the right (RFDI) and left (LFDI) hand with the active electrode was placed over the muscle belly and the reference electrode was placed over the metacarpophalangeal joint of the index finger. EMG was band-pass filtered between 20 Hz and 2.5 kHz and amplified 1000 x, (Intronix Technologies Corporation Model 2024F, Canada) and digitized at 5 kHz by an analog-to-digital interface (Power1401, Cambridge Electronics Design, Cambridge, UK).

TMS and cTBS

Two custom built 50 mm inner diameter figure-of-eight branding coils connected to two Magstim 200^2 stimulators (Magstim, Whitland, UK) were used to deliver single pulse TMS. Each branding coil was positioned over left and right M1 and oriented 45 degrees to the mid-sagittal line to induce a monophasic pulse in the PA direction. This orientation was used for all MEPs as a measure of corticospinal excitability. The motor

hotspot for the FDI muscle in M1 of each hemisphere was defined as the optimal location for obtaining a MEP in the contralateral, relaxed FDI muscle. Active motor threshold (AMT) was determined at this location and defined as the lowest intensity required to evoke MEPs $\geq$ 2 mV in 5 out of 10 consecutive trials during 10% maximum voluntary contraction of the target FDI muscle. Brainsight Neuronavigation (Rogue Research, Canada) was used to track the location of the TMS coils with respect to cortical targets within M1 and also to measure the distance between the motor hotspot and the SI target using a standard MRI (3T GE scanner, 172 images with 3DFSPGR-IR sequences using a 20 cm FOV). The target in SI was determined as a point 2 cm posterior to the motor hotspot which overlies the post-central gyrus (Ishikawa *et al.*, 2007;Wolters *et al.*, 2005;Rai *et al.*, 2012). For cTBS, a 90 mm outer diameter figure-of-eight coil with a MagPro stimulator (MCF-B65; Medtronic, Minneapolis, MN, USA) was used to deliver biphasic pulses whereby current in the coil flows in a direction away from the handle in the initial phase of the stimulus. The 600 pulse cTBS protocol described elsewhere (Huang *et al.*, 2005) was applied over left-hemisphere SI at 80% AMT. AP-PA was delivered with the coil handle positioned posterior and lateral to induce current flow within the cortex initially in the anterior-to-posterior direction. PA-AP was delivered with the handle pointed anterior and medially to induce current flow within the cortex initially in the posterior-to-anterior direction. AMT was determined for each current direction with the coil oriented in the same direction as the applied cTBS in the main experiment, and in the opposite direction for the second series of experiments.

Motor Evoked Potentials (MEPs)

To assess corticospinal excitability, MEPs were collected by averaging the response from 15 single TMS pulses applied over the left and right M1. The TMS intensity for MEPs was set at a value of percent stimulator output that evoked MEPs of ~1 mV peak-to-peak amplitude in LFDI and RFDI before cTBS and the same value was used throughout the experiment. MEPs were obtained from the left and right FDI before (T_0) and at 5 minutes (T_1), 25 minutes (T_2), and 45 minutes (T_3) following cTBS. The order of testing right versus left M1 was maintained in each participant during the experiment and counterbalanced across participants.

Data Analysis

Two-way Analysis of Variance (ANOVA) with between-subject factor DIRECTION (2 levels; AP-PA and PA-AP) and within-subject factor TIME (4 levels; T_0, T_1, T_2, T_3) was conducted for each dependent measure (MEP_{RFDI}, MEP_{LFDI}) for the main experiment. Sphericity was tested using the Huynh-Feldt tests. Post-hoc Tukey's test was used to identify significant differences among the means. Significance was set at $p \leq 0.05$.

3.3 RESULTS

All participants successfully completed the experiments. For the main experiment, the mean stimulator output (MSO) for cTBS delivery in the AP-PA and PA-AP induced current was 36.3% (± 7.5) and 39.3% (± 5.2). For the second series of experiments, cTBS was delivered at 46% (± 6.4%) for AP-PA and 38% (± 6.9%) MSO for PA-AP.

Motor Evoked Potentials (MEPs)

For the main experiment, the two-way ANOVA for MEPs recorded from RFDI, contralateral to SI cTBS revealed a significant effect of DIRECTION ($F_{(1,18)}$=12.08, p=0.002), no effect of TIME ($F_{(3,54)}$=1.02, p=0.38) and a significant interaction ($F_{(3,54)}$=3.41, p=0.02). Post-hoc Tukey's tests revealed that, compared with pre-cTBS amplitudes, cTBS delivered in AP-PA resulted in a significant facilitation of MEP amplitude at 5 (p=0.05) and at 45 minutes (p=0.003) with a near significant facilitation at 25 minutes (p=0.06) (Figure 4, left). These effects were observed in 9 and 11 of 12 participants for the two significant time blocks, respectively. In contrast, cTBS in the PA-AP direction resulted in a suppression of MEP amplitude at 25 minutes (p=0.03). This effect was observed in 7 of 8 participants. For MEPs recorded from LFDI, ipsilateral to SI cTBS, two-way ANOVA revealed a significant effect of DIRECTION ($F_{(1,18)}$=8.38, p=0.009) and no effect of TIME ($F_{(3,54)}$ = 0.19, p = 0.89) and no interaction ($F_{(3,54)}$=2.0, p=0.12). One-way repeated measures ANOVA investigating each direction

separately revealed no significant effect of TIME for either AP-PA ($F_{(3,33)}$=0.57, p=0.63) or PA-AP ($F_{(3,21)}$=1.91, p=0.15). Although the effect of TIME was not statistically significant, there is a trending decrease in MEP amplitude for the PA-AP direction (Figure 4, right). The group-averaged MEP amplitudes (with standard errors) for the second experiment are shown in Figure 5. Despite using a cTBS intensity based in the opposite AMT direction, these data show a similar trend as that observed in the main experiment. This suggests that the mechanism of observed bidirectional MEP changes in M1 are not due to intensity differences between AP-PA and PA-AP determined AMTs but rather the direction of induced current.

3.4 DISCUSSION

The present study investigated the influence of SI on corticospinal excitability related to muscles of the hand within primary motor cortex. Measures from both hands were acquired before and for up to 45 minutes following cTBS over left-hemisphere SI. We observed that cTBS delivered with an induced AP-PA current direction over left SI increased MEPs in the contralateral, right hand while cTBS in the PA-AP direction led to suppressed MEP amplitude. These data provide evidence that cTBS over SI influences corticospinal excitability with effects that are dependent on the applied current direction.

We observed that PA-AP current suppressed MEPs in the contralateral hand while the opposite orientation facilitated MEPs. One explanation for the influence of current direction may relate to stimulation intensity differences used for the two orientations. This difference is derived from the calculation of AMT from each orientation: AMT is typically higher in PA-AP compared to AP-PA (Kammer *et al.*, 2001). To address this concern, we conducted a second experiment in which cTBS was applied in AP-PA or PA-AP over SI based on 80% AMT determined in the PA-AP or AP-PA orientation, respectively. In the AP-PA cTBS group, RFDI MEPs were facilitated, similar to the main experiment. In the PA-AP cTBS group, RFDI MEPs were suppressed, also similar to the main experiment. These data suggest that the effects of cTBS over SI on corticospinal excitability most likely relate to the direction of induced current and not the intensity of stimulation *per se*. In further support of this suggestion, MEPs in the non-stimulated M1 were facilitated following cTBS over M1 in AP-PA, but were not altered following cTBS in PA-AP suggesting orientation-specific effects of cTBS in remote loci (Suppa *et al.*, 2008). Further support is gained from the finding that intermittent TBS over SI also demonstrates orientation specific effects such that PA-AP direction increases SEP components while AP-PA has no effect (Katayama & Rothwell, 2007).

The neural mechanisms that mediate the observed current direction effects are unclear though one speculation we propose involves selective targeting of specific populations of interneurons within SI. In motor cortex, biphasic pulses evoke a mixture of descending waves where opposite current orientations differ in the recruitment order of indirect or I-waves (Di Lazzaro *et al.*, 2001a). Pulses in AP-PA orientation evoke I-1

waves at threshold intensities, while PA-AP pulses preferentially recruit later I-3 waves (Di Lazzaro *et al.*, 2001a). These descending waves are thought to represent excitatory interneurons which synapse with corticospinal neurons (Di Lazzaro *et al.*, 2001a). The differences in recruitment order due to orientation suggest that AP-PA and PA-AP induced current directions may affect different populations of excitatory and inhibitory interneurons within M1 (Di Lazzaro *et al.*, 2001a). Further, monophasic TMS in PA and AP directions have been shown to recruit specific I-waves via different mechanisms or populations of neurons (Ni *et al.*, 2011). CTBS in AP-PA orientation targets interneurons such that I-1 waves are suppressed (Di Lazzaro *et al.*, 2005). Therefore it is possible that our observed cTBS direction specific effects are mediated by preferential activation of distinct populations of interneurons within SI, analogous to the influence of current direction on I-wave recruitment in motor cortex. Inhibitory GABAergic interneurons within SI are thought to mediate the late component of high-frequency oscillations (HFOs) following median nerve stimulation (Osaki & Hashimoto, 2011) and are suppressed following cTBS over SI in the PA-AP direction (Katayama *et al.*, 2010).

There are limitations in this study that could affect the interpretation of these data. First, we did not measure physiological changes within SI. However, tactile perception is changed following 600 pulse cTBS using lower intensities than those we employed (Rai *et al.*, 2012). Second, as it is difficult to ascertain the spatial extent of cTBS current spread, effects may relate to direct changes induced within SI but may also relate to changes induced in M1 excitability through current spread. Third, it is possible that effects observed in M1 are due to cTBS-induced antidromic activation of neurons

projecting from M1 to SI, or due to changes in reciprocal connectivity with M1 or other loci such as secondary somatosensory cortex, all of which could potentially mediate the change observed in corticospinal output.

3.5 CONCLUSION

The presented findings have important clinical implications for studies that aim to alter corticospinal output directed at muscles of the hand such as in focal hand dystonia. Corticospinal output to the hand is modified by changes in the excitability of SI. Such output can be increased or decreased depending on the direction of induced current flow within SI. SI may ultimately be one cortical area that has potential clinical value in changing the corticospinal output to the hand.

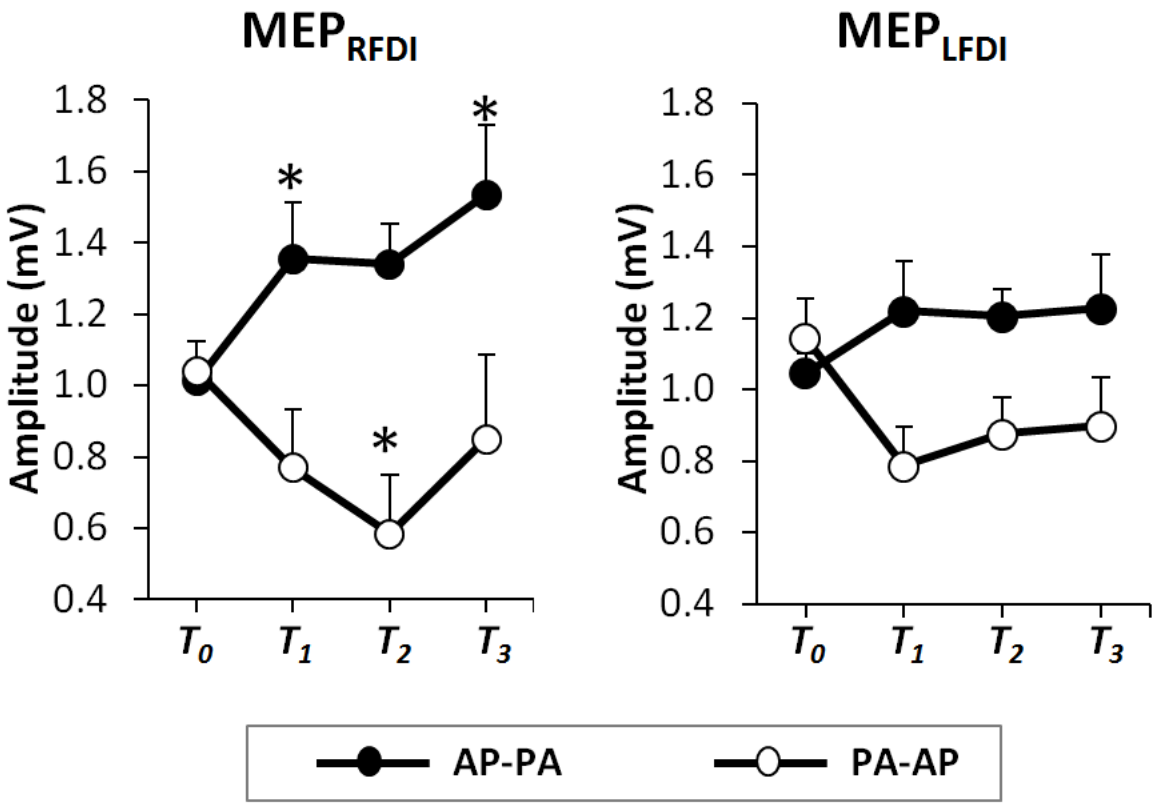

Figure 4. AP-PA versus PA-AP. Group-averaged MEP amplitude (with standard errors) before (T_0) and at 5 minutes (T_1), 25 minutes (T_2) and 45 minutes (T_3) following cTBS over left-hemisphere SI for RFDI (left) and LFDI (right). Asterisks indicate $p \leq 0.05$.

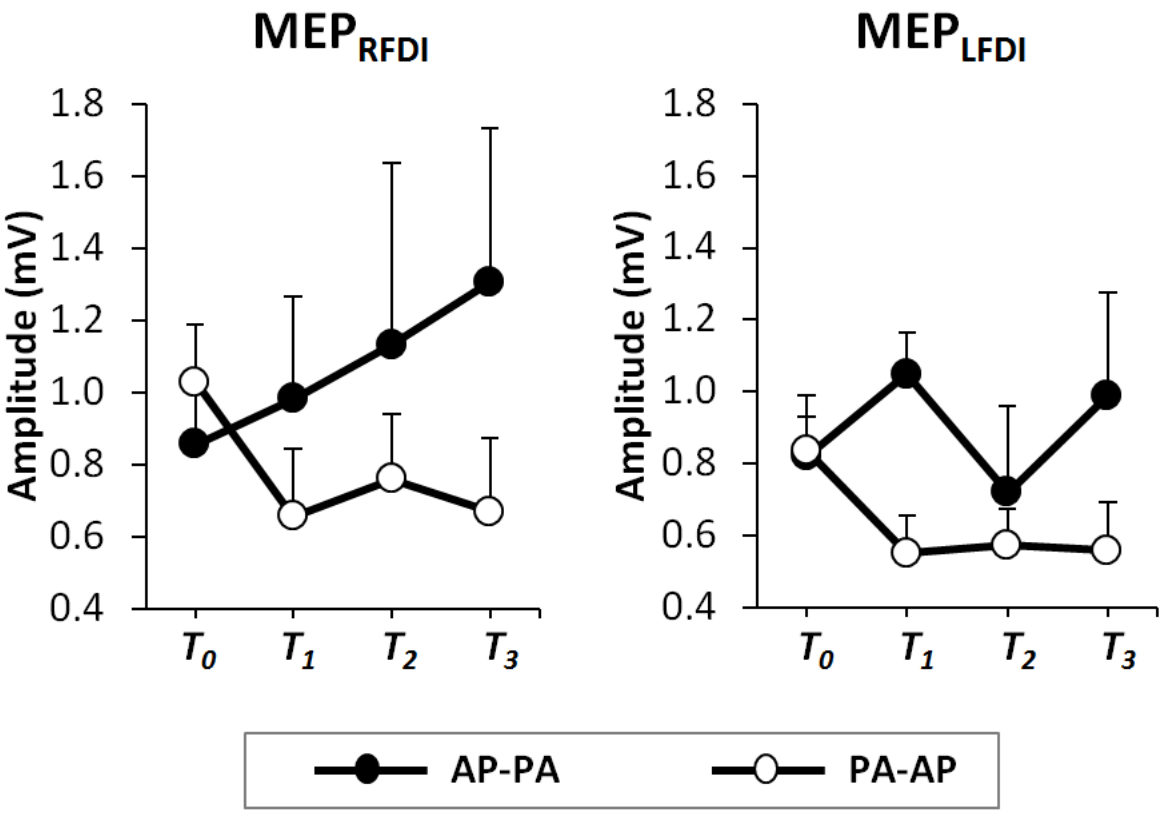

Figure 5. AP-PA and PA-AP with reversed AMT-specific intensity. Group-averaged (n=4 for each orientation) MEP amplitude (with standard errors) before (T_0) and at 5 minutes (T_1), 25 minutes (T_2) and 45 minutes (T_3) following cTBS over left-hemisphere SI for RFDI (left) and LFDI (right).

Chapter 4: Experiment 2

30 Hz continuous theta-burst stimulation alters hand circuitry when applied over the primary motor and sensory cortices.

4.1 INTRODUCTION

The primary somatosensory cortex (SI) participates in the control of finger and hand movement and may act via corticocortical connections to primary motor cortex (M1). Indeed, animal research has clearly shown that SI exerts influence on M1 circuitry (Ghosh & Porter, 1988;Rocco-Donovan *et al.*, 2011) and influences motor learning and behaviour (Pavlides *et al.*, 1993;Brinkman *et al.*, 1985;Hikosaka *et al.*, 1985). In humans, transcranial magnetic stimulation (TMS) offers a non-invasive and painless method to investigate the influence of somatosensory afferent input and cortex on the neural circuitry within M1 that controls the muscles of the hand. A single TMS pulse conditioned by electrical stimulation of a peripheral nerve results in suppression of the corticospinal output (Tokimura *et al.*, 2000) and intracortical circuits within M1 (Aimonetti & Nielsen, 2001). Somatosensory afference evoked by via high-frequency muscle vibration to hand musculature increases corticospinal excitability and decreases short-latency intracortical inhibition (SICI) in the targeted muscle representation (Rosenkranz & Rothwell, 2003) and persists beyond 30 minutes when vibration is applied for 15 minutes (Rosenkranz & Rothwell, 2004). Repetitive TMS over SI impairs motor learning (Vidoni *et al.*, 2010) and when applied in a 50 Hz continuous theta-burst (cTBS) paradigm alters corticospinal excitability (Jacobs *et al.*, 2012). Thus, evidence in

humans indicates that manipulations in somatosensory afference and neural activity within SI modulate hand motor circuitry.

The mechanisms that mediate the SI modulation of M1 corticospinal and intracortical circuits are not fully understood. Within sensorimotor cortex, alterations in GABAergic activity is thought to mediate cortical plasticity after peripheral nerve lesions (Jacobs & Donoghue, 1991), deafferentation by ischemic nerve block (Levy *et al.*, 2002) and prolonged somatosensory stimulation (Kaelin-Lang *et al.*, 2002). GABAergic activity in the motor cortex may be probed using short-latency intracortical inhibition (SICI). Thus, the excitability of GABAergic circuitry can be monitored in M1 following SI-cTBS and may advance our understanding of the mechanism by which SI influences circuitry within M1.

The traditional 50 Hz cTBS has been recently shown to have high subject variability (Hamada *et al.*, 2012). The authors found that after-effects were highly correlated with I-wave recruitment, which may be partially due to stimulation parameters. Alterations in cTBS frequency domains may lead to more effective stimulation cTBS protocols. Nyffeler and colleagues (2006) developed a 30 Hz cTBS protocol that delayed the onset of eye saccades when applied over the oculomotor cortex. When applied over M1, 30 Hz cTBS suppresses corticospinal excitability (Wu *et al.*, 2012;Goldsworthy *et al.*, 2012) for a longer period of time than the traditional 50 Hz paradigm (Goldsworthy *et al.*, 2012). Specifically, 50 Hz cTBS significantly suppressed MEPs for only the initial 5 minutes after cTBS was delivered. In comparison, 30 Hz cTBS significantly suppressed

MEPs immediately and at every time point up to 30 minutes post-cTBS (Goldsworthy *et al.*, 2012). Additionally, the 30 Hz cTBS effects were consistent across all 12 participants studied, whereas 50 Hz effects varied between people such that 3 of 12 participants showed facilitation of MEPs (Goldsworthy *et al.*, 2012). These data suggest that a 30Hz cTBS paradigm may be more effective at supressing cortical excitability and reducing subject variability. However, the effect of 30 Hz cTBS on intracortical M1 circuitry has not yet been explored.

The purpose of the current study was to explore the influence of SI on corticospinal excitability and intracortical circuitry within M1. Characterizing the influence of SI projections to M1 circuitry suggests the complexity of the somatic influence on motor output. MEPs, short-latency intracortical inhibition (SICI) and intracortical facilitation (ICF) were recorded before and after 30Hz cTBS was applied over ipsilateral SI or M1. It was hypothesized that MEP amplitude would decrease at post 5, 25 and 45 minutes compared to baseline following cTBS over left SI (SI-cTBS), similar to previous findings (Jacobs *et al.*, 2012). CTBS over M1 (M1-cTBS) was hypothesized to decrease MEP amplitude and SICI at post 5, 25 and 45 minutes compared to baseline.

4.2 METHODS

Participants

Fourteen healthy, right-handed participants completed this study (9 females, mean age ± SD, range = 21.3 ± 1.6, 18 to 23). All participants were determined to be healthy using a TMS screening form that queried medical conditions. Right-handedness was confirmed using a subset of the Edinburgh Handedness Inventory (Oldfield, 1971). All participants gave informed written consent prior to participation. The experiments were approved by the Ethics Committee of McMaster University and conformed to the *Declaration of Helsinki*.

Electromyographic (EMG) recording

Surface electrodes (9 mm diameter Ag-AgCl) were used to record EMG from the RFDI using the belly-tendon montage. EMG was band-pass filtered between 20 Hz and 2.5 kHz and amplified 1000 x, (Intronix Technologies Corporation Model 2024F, Bolton, Canada) and digitized at 5 kHz by an analog-to-digital interface (Power1401, Cambridge Electronics Design, Cambridge, UK).

TMS and Neuronavigation

Single-pulse, monophasic TMS was delivered with a custom built figure-of-eight branding coil (50 mm inner diameter) connected to a Magstim 200^2 stimulator (Magstim, Whitland, UK). Paired-pulse TMS was delivered with a second custom built figure-of-eight branding coil (50 mm inner diameter) connected to a Bistim unit (Magstim, Whitland, UK) that allowed pairs of TMS pulses to be delivered through one coil in rapid succession. CTBS was applied using a 70 mm inner diameter figure-of-eight coil with a Magstim Super $Rapid^2$ Plus (Magstim, Whitland, UK). To determine the motor hotspot for the RFDI muscle, a branding coil was positioned over the left M1 and oriented 45 degrees to the mid-sagittal line to induce a monophasic pulse in the posterior-to-anterior (PA) direction. The motor hotspot was defined as the optimal location for obtaining a MEP in the contralateral, relaxed FDI muscle. Resting motor threshold (RMT) was determined at the RFDI motor hotspot and defined as the lowest intensity required to evoke MEPs $\geq$ 50 μV in 5 out of 10 consecutive trials (Rossi *et al.*, 2009) while the participant was relaxed. Background EMG was monitored online in both left and right FDI muscles to ensure participant maintained complete relaxation of the target and homologous muscle. RMT was acquired for the monophasic coil used for paired-pulse TMS and separately for the biphasic coil used to deliver cTBS. Brainsight Neuronavigation (Rogue Research, Montreal, Canada) was used to track the location of the TMS coils with respect to the marked motor hotspot. A standard MRI from one individual was obtained using a 3T GE scanner (172 images) with 3DFSPGR-IR

sequences using a 20 cm FOV (256 x 256) and used for all participants to ensure proper placement of the coil with respect to the M1 motor hotspot or marked SI location.

Experimental Design

CTBS over left SI or M1 on MEPs, SICI and ICF

Participants completed two TMS sessions separated by at least one week. Following cTBS over the left SI or left M1 in separate sessions, measures of motor circuitry were assessed from the right first dorsal interosseous (RFDI) muscle. The order of the two sessions was counter-balanced across participants. Motor-evoked potentials (MEPs), short-latency intracortical inhibition (SICI), and intracortical facilitation (ICF) were recorded before (T_0) and at 5 (T_1), 25 (T_2), and 45 (T_3) minutes following cTBS (Fig.7). In both sessions, cTBS was applied at an intensity of 55% RMT using a 30 Hz, 600 pulse protocol (Goldsworthy *et al.*, 2012). The coil was orientated to induce a posterior-to-anterior followed by anterior-to-posterior (PA-AP) current. Twenty MEPs were recorded in each time block at an intensity set to evoke a ~1 mV amplitude response in RFDI muscle determined at baseline (T_0). The intensity for MEPs was held constant throughout the remainder of the study. To measure SICI and ICF, the intensity of the first or conditioning stimulus (CS) was set to 70% RMT and the second or test stimulus (TS) was set to an intensity that evoked a MEP amplitude of ~1 mV in the RFDI. The TS intensity was adjusted as necessary after cTBS to match pre- and post-cTBS TS MEP amplitudes. The interstimulus-interval (ISI) between the CS and TS was 2 ms and 10 ms

for SICI and ICF, respectively. For intracortical circuitry, each time block consisted of 10 SICI trials, 10 ICF trials and 10 unconditioned trials (TS alone) presented randomly for a total of 30 trials. The interval between individual trials of MEPs, SICI and ICF was set to 7 ms with 20% variance.

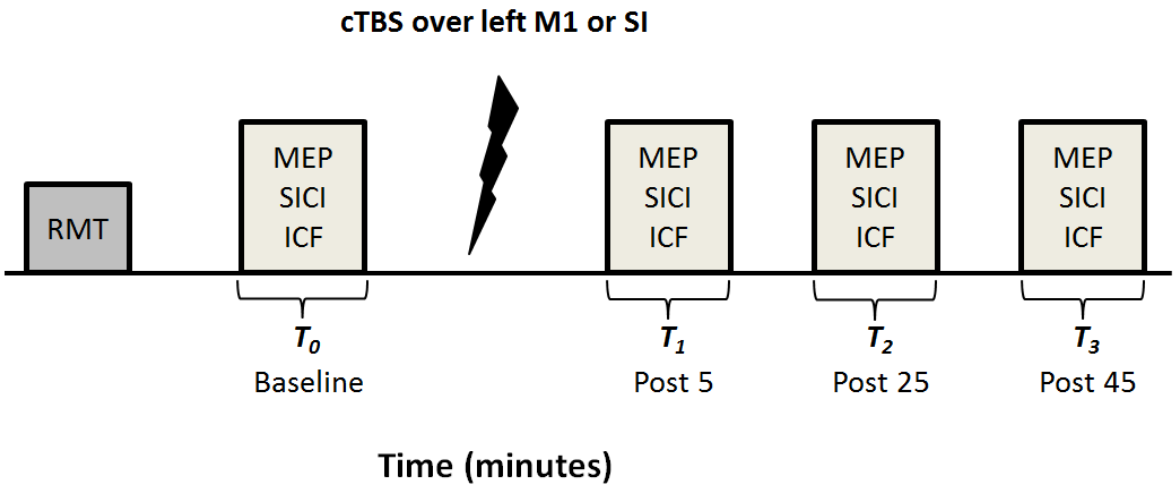

Figure 6. Timeline of experiment. A) After determination of RMT values, MEPs, SICI and ICF were recorded before and after cTBS over left SI or M1

Data Analysis

To investigate changes in motor circuitry following cTBS over left M1 or SI, peak-to-peak amplitudes of MEPs, SICI and ICF measures from the RFDI were analysed in separate one-way repeated measures ANOVA with factor TIME (4 levels; pre, post 5, post 25, post 45 mins). Post-hoc Tukey's test were conducted following a significant effect of TIME in the ANOVA. *A priori* hypotheses were tested with *t*-test and Bonferonni corrected. Statistical significance for all tests set to a p-value of < 0.05.

4.3 Results

M1-cTBS

The one-way ANOVA for MEPs revealed a significant effect of TIME ($F_{(3,39)}$=8.75, p=0.0001). Post-hoc Tukey's test revealed that, compared with pre-cTBS amplitudes, MEPs were significantly suppressed at 5 (p=0.0002) and 25 (p=0.001) minutes (Fig. 7A). One-way ANOVA for SICI revealed no significant effect of TIME ($F_{(3,39)}$=0.73, p=0.54). *A priori* Bonferonni corrected *t*-tests revealed no significant differences at 5 (p=0.44), 25 (p=0.11), or 45 (p=0.25) minutes compared with baseline amplitudes (Fig. 8A). Two individuals were excluded from ICF analysis because they did not show ICF at baseline. One-way ANOVA for ICF revealed a significant effect of TIME ($F_{(3,33)}$=5.56, p=0.003). Post-hoc Tukey's test revealed that ICF was significantly suppressed at 5 (p=0.02), 25 (p=0.002) and 45 (p=0.02) minutes following M1-cTBS (Fig. 8B).

SI-cTBS

The one-way ANOVA for MEPs revealed a significant effect of TIME ($F_{(3,39)}$=4.31, p=0.01). Post-hoc Tukey's test revealed that, compared with pre-cTBS amplitudes, MEPs were significantly increased at 25 (p=0.005) minutes (Fig. 7B). One individual was excluded from SICI and another from ICF because they did not show either measure in baseline. One-way ANOVA revealed no significant effect of TIME for SICI ($F_{(3,36)}$=0.87, p=0.46) or ICF ($F_{(3,36)}$=0.31, p=0.81) (Fig. 9).

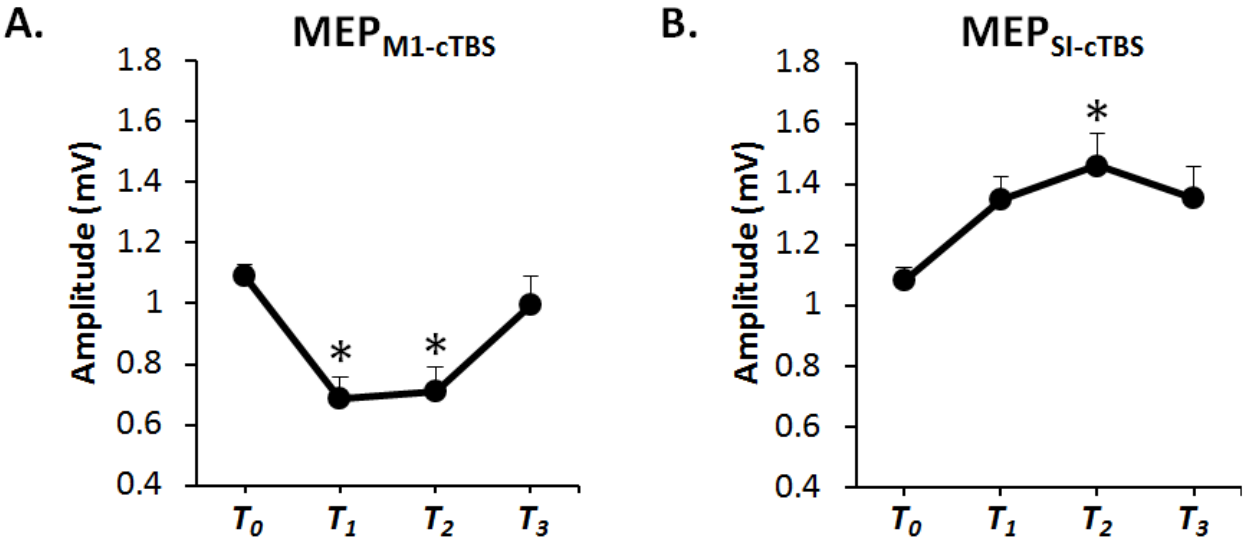

Figure 7. 30 Hz cTBS on MEP amplitude. A) Following cTBS over M1, MEP amplitudes were significantly decreased at 5 minutes (T_1) and 25 minutes (T_2). B) In contrast, cTBS over SI increased MEP amplitudes significantly at 25 minutes (T_2) compared to baseline (T_0).

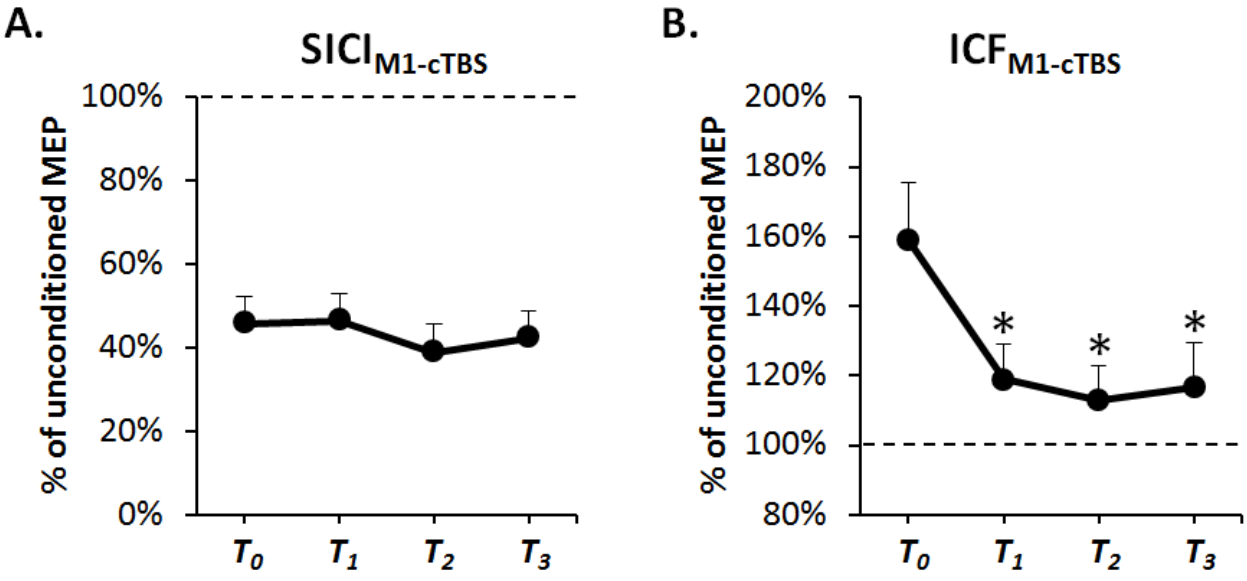

Figure 8. 30 Hz cTBS over M1 on intracortical circuitry. A) SICI was not significantly altered compared to baseline (T_0). B) ICF was significantly decreased at 5 (T_1), 25 (T_2), and 45 (T_3) minutes compared to baseline (T_0).

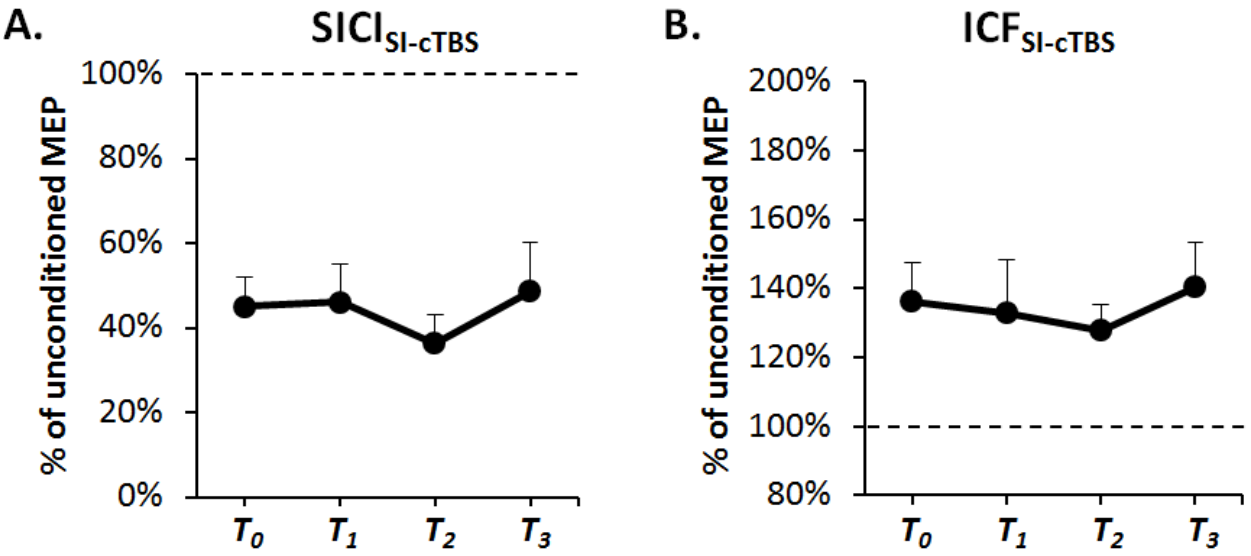

Figure 9. 30 Hz cTBS over SI on intracortical circuitry. A) SICI and B) ICF were not significantly altered compared to baseline (T_0).

4.4 Discussion

The present study demonstrated that cTBS delivered over SI significantly increases corticospinal excitability without altering intracortical M1 circuitry of SICI or ICF. In contrast, cTBS over M1 reduces corticospinal excitability and intracortical facilitation and does not alter SICI.

The importance of SI-M1 connections in motor skill acquisition (Pavlides *et al.*, 1993) suggests that somatosensory information may be relayed at the cortical level in order to modulate motor output in humans. To characterize this relationship, cTBS was applied over SI to determine whether this cortical area modulates corticospinal excitability and intracortical circuitry in M1. The present study indicates that SI-cTBS

facilitates corticospinal excitability, confirming a previous study (Jacobs *et al.*, 2012). In contrast, M1-cTBS suppressed corticospinal excitability in the same group of participants, supporting previous reports following 30 Hz cTBS over M1 (Wu *et al.*, 2012;Goldsworthy *et al.*, 2012).

No significant changes in SICI occurred following 30 Hz cTBS over SI or M1. Decreases in SICI following 50 Hz cTBS are reported in several studies (Huang *et al.*, 2005;Talelli *et al.*, 2007;Suppa *et al.*, 2008) while other studies report no change (Doeltgen & Ridding, 2011). In the present study, the lack of effects from stimulation to either SI or M1 suggests that 30 Hz cTBS was ineffective at modulating SICI. Alternatively, changes in SICI may have been subtle and were not detected by using a single CS intensity. It has been suggested that a range of CS intensities may be more appropriate for measuring the integrity of SICI circuitry (Orth *et al.*, 2003;Doeltgen & Ridding, 2011). Intra- and inter-subject variability of SICI obtained with a single CS intensity (Orth *et al.*, 2003) may contribute to the discrepancies in literature with respect to the after-effects of cTBS. Thus, it is possible that any observed changes in intracortical circuitry in the present study may have been limited by the use of a single CS intensity.

ICF was unaltered following SI-cTBS, but was significantly suppressed following M1-cTBS. One reason for absence of changes following SI-cTBS was subject variability such that ICF increased in six participants, decreased in five participants and no change in one participant. Although cTBS was originally shown to supress ICF following M1-

cTBS (Huang *et al.*, 2005) it has been resistant to modulation in subsequent studies using the same 50 Hz protocol (Suppa *et al.*, 2008;Talelli *et al.*, 2007;McAllister *et al.*, 2009). Traditional 50 Hz cTBS over sensory area 5 also failed to produce any significant changes in ICF (Premji *et al.*, 2011). The current findings demonstrate that 30 Hz cTBS over M1 is effective in reducing ICF. Further, it suggests 30 Hz cTBS may act to decrease in activity of local excitatory interneurons that generate ICF (Reis *et al.*, 2008). CTBS has been shown to be NMDA receptor-dependant (Huang *et al.*, 2007) and thus thought to induce long-term depression (LTD)-like effects on excitatory circuitry. The overall decrease in corticospinal excitability and ICF with no changes in SICI suggests that 30 Hz M1-cTBS may preferentially induce LTD-like changes in excitatory circuits.

In the present study, it was observed that cTBS over SI modulates corticospinal excitability 25 minutes following stimulation. There is a clear trend of increasing excitability at 5 minutes, peaking at 25 minutes and beginning to descend towards baseline by 45 minutes. These data are in agreement with previous work in which traditional 50 Hz cTBS delivered over SI modulated MEPs with effects that depend on coil orientation (Jacobs *et al.*, 2012). However, two studies found no significant change in MEP amplitudes following 50 Hz cTBS over left SI (Ishikawa *et al.*, 2007;Katayama *et al.*, 2010). One explanation for different findings between studies may be due to stimulation intensity, current direction and frequency of cTBS paradigms. Stimulation parameters are suspected to play a role in TBS after-effects, but no general consensus for optimal stimulation parameters currently exists (Cardenas-Morales *et al.*, 2010;Pell *et al.*, 2011). Jacobs et al (2012) reasoned that other rTMS protocols that successfully induced

SI plasticity used a higher stimulation intensity (Tegenthoff *et al.*, 2005), suggesting that changes in SI neural circuitry may require higher intensity than used in previous cTBS studies (Ishikawa *et al.*, 2007;Katayama *et al.*, 2010). While the latter two studies investigating SI-cTBS applied the protocol at 80% AMT, Jacobs et al (2012) defined a higher threshold level producing a greater sub-threshold stimulation intensity of cTBS compared to previous studies (Ishikawa *et al.*, 2007;Katayama *et al.*, 2010) and reported significant changes in MEP amplitudes. Specifically, MEP amplitudes were facilitated or suppressed in AP-PA or PA-AP current directions, respectively. These changes were independent of intensity, but taking the higher cTBS intensity in account compared to other studies using low intensity (Ishikawa *et al.*, 2007;Katayama *et al.*, 2010) suggests that both current direction and stimulation intensity modulate after-effects of cTBS. This is supported by direction-specific (Talelli *et al.*, 2007) and intensity-dependent (Doeltgen & Ridding, 2011) after-effects on corticospinal excitability following 50 Hz M1-cTBS. In the present work, frequency characteristics of intra- and inter-train pulses were modified to form a 30 Hz paradigm (Nyffeler *et al.*, 2006b) delivered over SI at low-intensity of 55% RMT. Previously, 30 Hz M1-cTBS has shown consistent suppression of MEP amplitude delivered at 90% (Wu *et al.*, 2012), 80% (Goldsworthy *et al.*, 2012), and here 55% of RMT, suggesting the frequency component of cTBS may be the one of the most influential parameters. A future study might confirm this by investigating the effect of current direction of the after-effects of 30 Hz cTBS. Taken together, this suggests that stimulation parameters play an important role and may explain differences between present and previous reports of SI-cTBS on corticospinal excitability.

Corticocortical projections from SI have been postulated to play a role in motor learning and may represent an anatomical route of somatosensory influence on motor circuitry (Pavlides *et al.*, 1993). Evidence suggests that cTBS may act on corticocortical afferents from other cortical areas to M1 and remotely create long-term plasticity in MEP-generating circuitry. CTBS has been shown to facilitate MEP amplitudes in the non-stimulated hemisphere M1 likely via transcallosal projections (Suppa *et al.*, 2008;Stefan *et al.*, 2008a). In the ipsilateral hemisphere, cTBS over premotor cortex decreases MEPs (Huang *et al.*, 2009). Previously, cTBS over SI increased or decreased MEP amplitude depending on cTBS current orientation (Jacobs *et al.*, 2012). Similarly, cTBS to higher order sensory area 5 facilitates MEP amplitudes for up to an hour (Premji *et al.*, 2011). These data support the influence of corticocortical projections to M1 following cTBS over remote, connected areas. In the present study, facilitation of MEPs following SI-cTBS suggests that SI may directly influence corticospinal neurons or interneurons that mediate corticospinal excitability. Further support is gained in animal studies. Stimulation of corticocortical afferents in SI produces both excitatory (EPSP) and inhibitory post-synaptic potentials (IPSP) in layer III and V M1 pyramidal neurons suggesting that SI afferent fibres project to excitatory and inhibitory circuitry (Ghosh & Porter, 1988). Tetanic stimulation of SI produces long-term potentiation of neurons in superficial layers of M1 (Sakamoto *et al.*, 1987) which transmit information to deeper, output pyramidal neurons in layer 5 (Kaneko *et al.*, 1994b). The net outcome of SI-cTBS in the present findings is increased corticospinal excitability. However, it is unclear if SI-cTBS acts to facilitate or disinhibit motor output. We propose that SI-cTBS may act

through a direct corticocortical route to potentiate excitatory I-wave interneurons that generate MEPs leading to a prolonged increase in corticospinal output from M1.

4.5 Conclusion

The present research supports the role of SI in mediating cortical hand motor output and may be important in understanding movement disorders such as focal hand dystonia, in which the integration of sensory and motor information is impaired (Abbruzzese *et al.*, 2001). Future work may involve a combination of peripheral and central somatosensory manipulation in order to further elucidate the role of SI and somatic influence on motor circuitry.

Chapter 5: General Discussion

5.1 Summary of experiments

The purpose of Experiment 1 was to investigate the influence of SI on hand motor circuitry as measured by MEPs recorded from an index finger muscle of both hands. In order to investigate the influence of current orientation, cTBS was delivered in AP-PA or PA-AP orientation in two separate groups of participants. In AP-PA orientation, MEPs were facilitated in the hand contralateral to stimulation at 5 and 45 minutes following cTBS to left SI, and a near significant increase at 25 minutes. In PA-AP orientation, MEPs were significantly suppressed in the same hand at 25 minutes following cTBS to left SI. Although MEPs in the ipsilateral hand were not significantly different from baseline, there appears to be a bilateral trend for excitability changes such that AP-PA facilitated and PA-AP suppressed MEPs of both hands. The results of Experiment 1 indicate that SI alters corticospinal excitability related to muscles of the hand. Further, the direction of excitability changes following 50 Hz cTBS over SI is dependent on the orientation of induced current flow. Influence of current direction on corticospinal excitability following 50 Hz cTBS has been reported previously (Talelli *et al.*, 2007;Suppa *et al.*, 2008).

Experiment 2 also investigated SI-mediated changes in motor circuitry. In the right index finger only, measures of corticospinal excitability and intracortical circuitry were collected before and after cTBS over left SI. Importantly, a modified 30 Hz cTBS paradigm was used in Experiment 2. In order to confirm the local suppressive effect of

the 30 Hz protocol on cortical excitability, cTBS was applied over left M1. Corticospinal excitability was suppressed for up to 25 minutes following M1-cTBS, similar to previous reports (Wu *et al.*, 2012;Goldsworthy *et al.*, 2012). ICF was suppressed for at least 45 minutes following M1-cTBS, but no significant changes occurred in SICI. When cTBS was applied over SI, corticospinal excitability was facilitated 25 minutes following stimulation. However, changes in intracortical circuitry measuring by paired-pulse TMS were less clear. Group-level measures for SICI and ICF did not reveal any significant effect or direction following cTBS to SI due to inter-subject variability.

Experiments 1 and 2 present evidence that changing the neural activity in SI modulates motor circuitry of the hand. In both experiments, MEPs recorded from the RFDI are facilitated for a period of time beyond cTBS. These data indicate that SI modulates motor output to the hand muscles and may play a role in motor function of the hand. CTBS may be used to modulate these areas and is influenced by parameters such as current direction and frequency of stimulation. The following sections will discuss potential mechanisms, stimulation parameters and limitations of the work.

5.2 Neural Mechanisms of SI-M1 Modulation of Corticospinal Excitability

This research is the first to explore changes in intracortical inhibition and facilitation following SI-cTBS in humans. Previous work investigating the influence of somatosensory afference on motor circuitry and output has focused on peripheral nerve stimulation applied with or without TMS in combination. Repetitive afferent input alone

(Ridding *et al.*, 2001) or paired with a TMS pulse over M1 (Stefan *et al.*, 2002;Ridding & Taylor, 2001) results in long-lasting facilitation of corticospinal excitability, but no changes in SICI. However, activation of muscle spindle afferents by FDI muscle vibration increases MEP amplitudes and decreases SICI in the target muscle (Rosenkranz & Rothwell, 2003). In the present work MEP amplitudes were increased, but changes in SICI and ICF were seen within individuals but were highly variable across participants following 30 Hz SI-cTBS and no group-level effect emerged. Although the effects vary between participants, these data collectively support the modulation of hand motor circuitry via somatosensory input from both peripheral and cortical levels. However, somatosensory information from the periphery may reach M1 directly via thalamocortical projections as well as through SI corticocortical connections (Ghosh *et al.*, 1987). The current work investigated SI-mediated changes in corticospinal excitability which may act directly via corticocortical connections between SI. Therefore, only neural mechanisms of SI to M1 projections are discussed.

Subareas 1, 2 and 3a of SI project to M1, the majority of which originate in area 2 (Ghosh *et al.*, 1987). Afferent projections from SI reach interneurons and pyramidal cells located within layers III and V of the motor cortex (Ghosh & Porter, 1988;Porter, 1996). The corticospinal tract originates from large pyramidal neurons in layer V of M1 and gives rise to descending motor output (Kaneko *et al.*, 2000). Altered synaptic activation of the pyramidal cells in in layer V will therefore alter motor output. Within M1, stimulation of neurons in superficial layer III produces potentials in layer V pyramidal neurons (Kaneko *et al.*, 1994b;Kaneko *et al.*, 1994a). This suggests that SI

corticocortical projections may modulate corticospinal excitability via two routes; from SI directly to layer V pyramidal output neurons, or indirectly by a relay through superficial layers II/III (Kaneko *et al.*, 2000). It has been suggested that these SI corticocortical projections serve as a source of sensory modulation of motor circuitry and behaviour, which may occur in the superficial layers II/III of M1 (Keller *et al.*, 1990;Kaneko *et al.*, 2000). Indeed, SI projections to M1 neurons are important for learning new motor skills (Pavlides *et al.*, 1993) and tetanic stimulation of SI produces long-term potentiation in M1 layer II/III pyramidal and non-pyramidal neurons, but not in deeper layers (Iriki *et al.*, 1989;Keller *et al.*, 1990). Electrical surface stimulation of SI produces an EPSP following by IPSP or IPSP alone on superficial and deep layer pyramidal neurons of M1 suggesting that somatosensory afferents target both excitatory pyramidal neurons and inhibitory interneurons in multiple layers (Ghosh & Porter, 1988). Thus, evidence suggests that SI is capable of modulating cortical circuitry in multiple layers of M1 and presents a possible mechanism for SI-cTBS mediated changes in the present work.

One model suggests that I-wave circuitry which generates a MEP is located in superficial layers II/III of the motor cortex (Di, V *et al.*, 2012). Activation of excitatory pyramidal neurons in layers II/III by TMS transynaptically activate large pyramidal tract neurons (PTNs) in layer V, leading ultimately to a MEP in the representative hand muscle (Di, V *et al.*, 2012). Based on the above evidence, one possible mechanism for MEP facilitation following SI-cTBS is long-term potentiation of superficial layers II/III of M1 via SI corticocortical projections resulting in greater depolarization of descending

output PTNs in layer V, thus leading to a prolonged increase in corticospinal excitability. Alternatively, SI afferents may potentiate layer V PTNs via a direct corticocortical route also increasing corticospinal excitability. Experiment 1 demonstrated that corticospinal excitability could be suppressed by reversing the direct of cTBS current flow. It seems probable that current direction has differential effects on SI neural circuitry. While it is difficult to confirm this in humans, it can be speculated that excitatory and inhibitory cells in the superficial layers of SI are sensitive to current direction as they are in M1. Thus, reversing the current direction may activate a population of SI interneurons that act to depress the excitability of M1 circuitry. Experiment 2 demonstrated that a modified cTBS frequency facilitated corticospinal excitability despite using a current direction that suppressed MEPs in Experiment 1. This suggests that frequency may be a more influential component than current direction on SI neural components that influence M1.

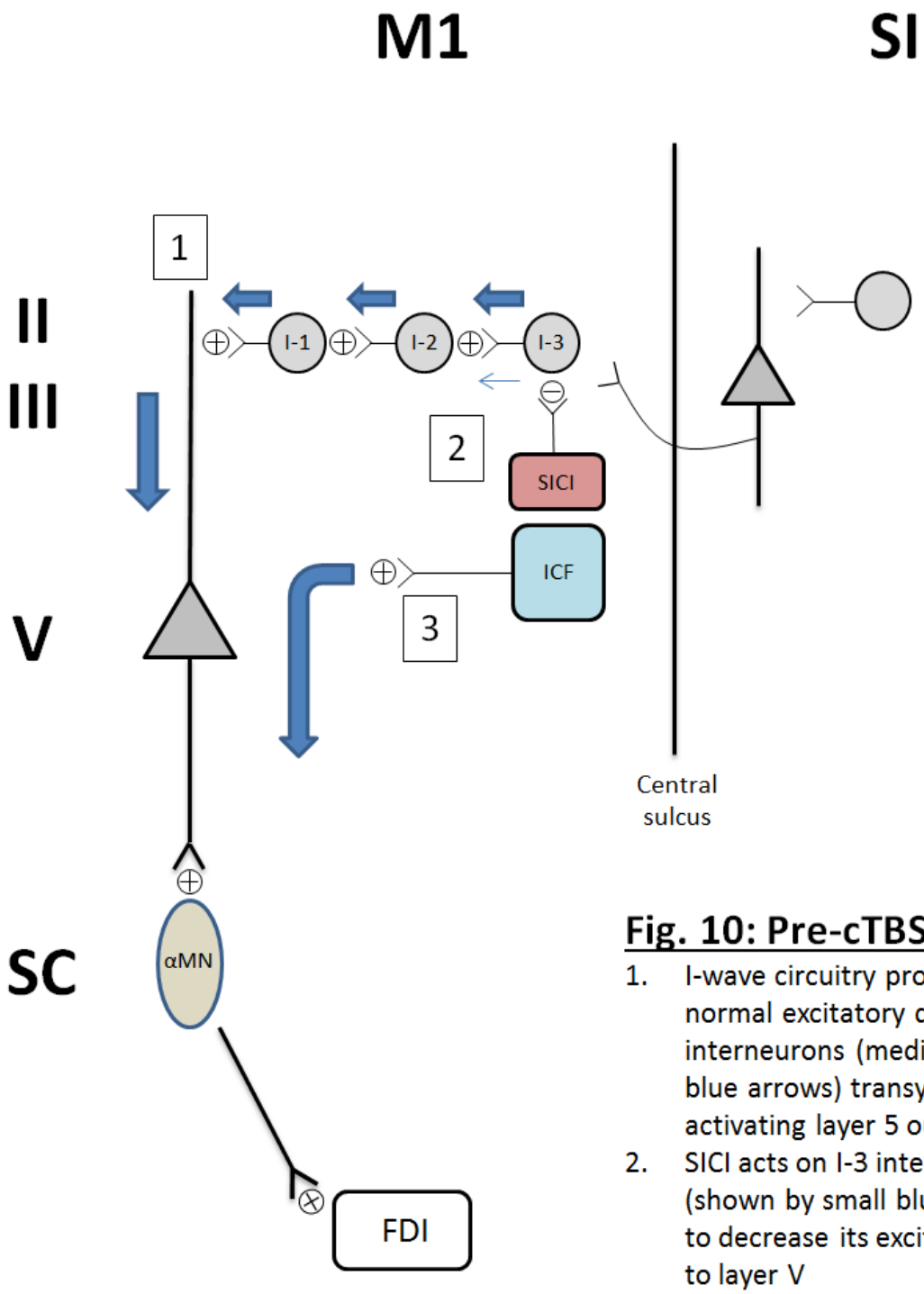

Fig. 10: Pre-cTBS

1. I-wave circuitry produces normal excitatory drive of interneurons (medium sized blue arrows) transynaptically activating layer 5 output cell
2. SICI acts on I-3 interneuron (shown by small blue arrow) to decrease its excitatory drive to layer V
3. ICF site of interaction unknown, but net effect is facilitation (large blue arrow)

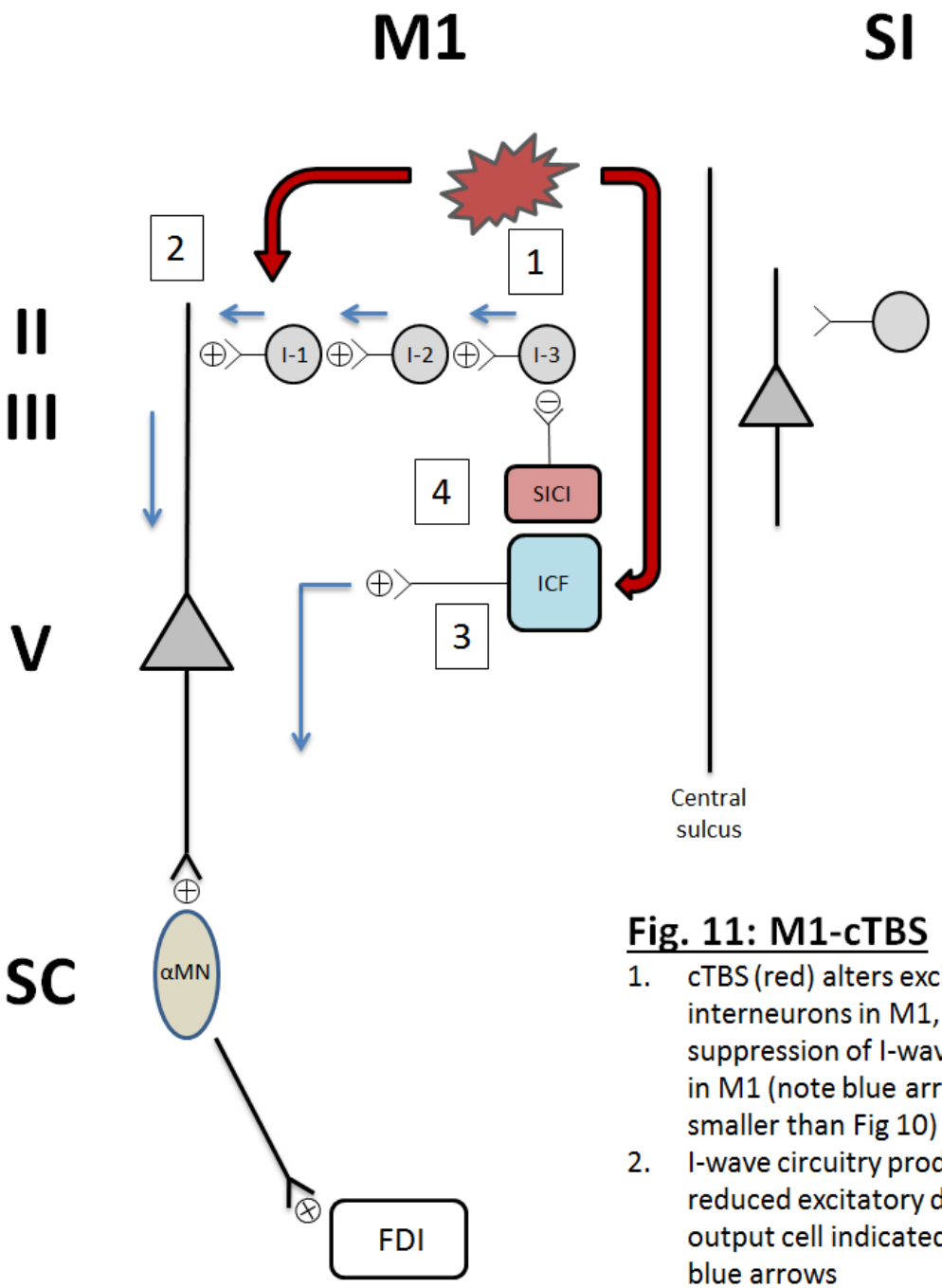

Fig. 11: M1-cTBS

1. cTBS (red) alters excitatory interneurons in M1, net effect is suppression of I-wave circuitry in M1 (note blue arrows are smaller than Fig 10)
2. I-wave circuitry produces reduced excitatory drive to output cell indicated by small blue arrows
3. cTBS alters excitatory interneurons that generate ICF, results in decreased output shown by small blue arrow
4. SICI not affected

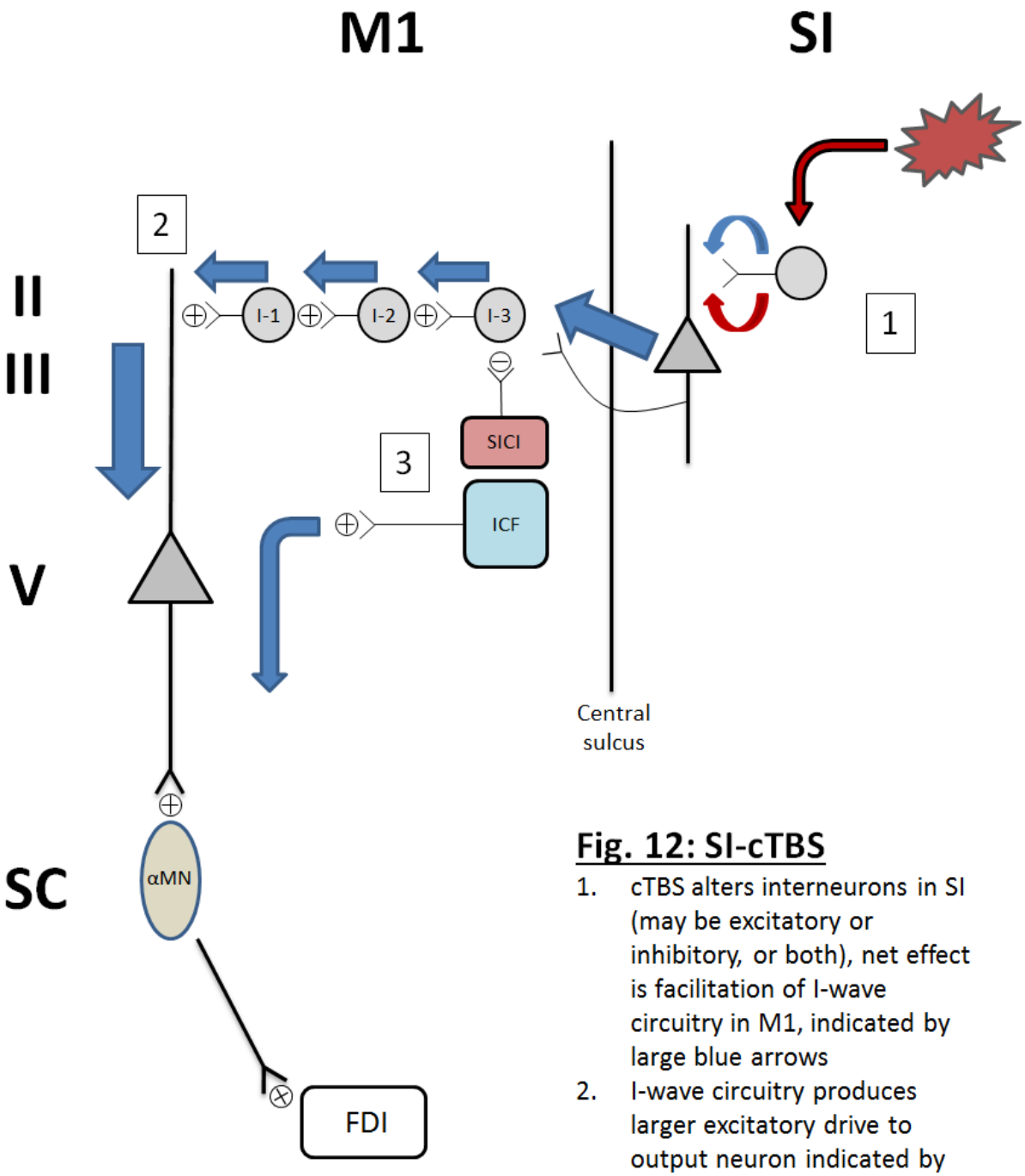

Fig. 12: SI-cTBS

1. cTBS alters interneurons in SI (may be excitatory or inhibitory, or both), net effect is facilitation of I-wave circuitry in M1, indicated by large blue arrows
2. I-wave circuitry produces larger excitatory drive to output neuron indicated by large blue arrows, transynaptically activating layer 5 output cell
3. SICI and ICF not affected

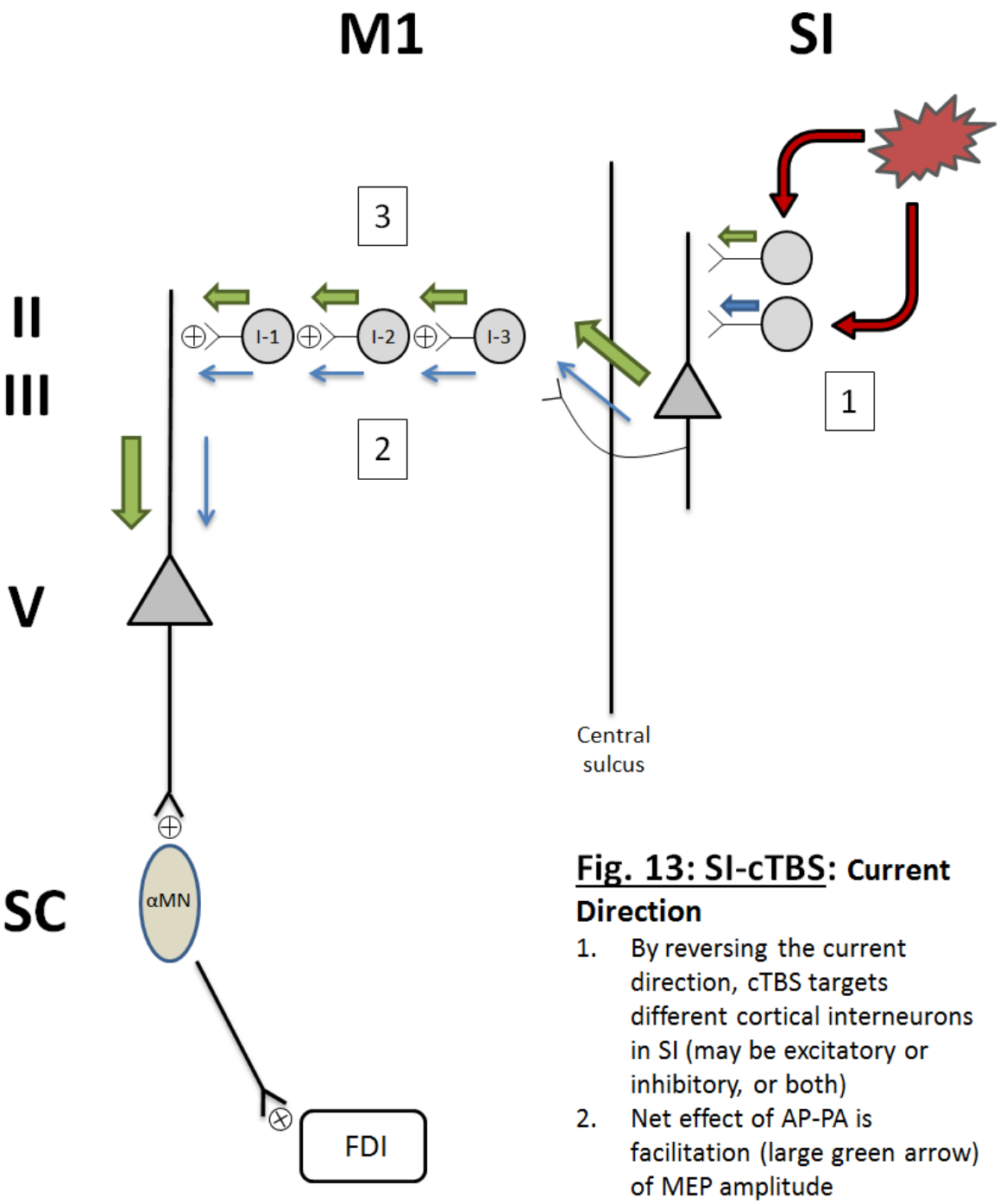

Fig. 13: SI-cTBS: Current Direction

1. By reversing the current direction, cTBS targets different cortical interneurons in SI (may be excitatory or inhibitory, or both)
2. Net effect of AP-PA is facilitation (large green arrow) of MEP amplitude
3. Net effect of PA-AP is suppression (small blue arrow) of MEP amplitude

5.3 Stimulation parameters

In Experiment 1, cTBS over SI in PA-AP orientation led to MEP suppression in the RFDI for up to 25 minutes. The same orientation used in Experiment 2 facilitated MEPs for up to 25 minutes. The contrast is likely due to differences in cTBS stimulation parameters. CTBS in Experiment 1 was applied at a higher intensity, relative to individual motor threshold, than cTBS in Experiment 2. Pilot data suggests that the intensities were applied at 75% RMT and 55% RMT in Experiment 1 and 2, respectively. When applied over M1, 50 Hz cTBS can have enhancing or suppressive effects on corticospinal excitability with small difference in stimulation intensity (Doeltgen & Ridding, 2011). However, 30 Hz cTBS produces consistent suppression of motor circuitry with intensities of 90% RMT (Nyffeler *et al.*, 2006b) 80% RMT (Goldsworthy *et al.*, 2012) and 55% shown in the present work. Thus, 50 Hz cTBS seems to be sensitive to stimulation intensity, whereas 30 Hz cTBS produces reliable effects across a range of intensities. This suggests that cTBS frequency may be the most important consideration of stimulation parameters, similar to rTMS (Pell *et al.*, 2011). However, the effect of current direction on 30 Hz cTBS was not directly tested in the present work.

Current Direction

Previous work over M1 reveals that I-wave recruitment order changes with current direction (Di Lazzaro *et al.*, 2001b), activating distinct populations of interneurons (Ni *et al.*, 2011). The influence of current orientation on SI circuitry,

however, is difficult to ascertain. From Experiment 1, cTBS current direction clearly shows a bidirectional effect on corticospinal excitability when applied over SI. This suggests that AP-PA and PA-AP current directions differentially mediate SI projecting fibres to M1 that are involved in generating the MEP. Although speculative, a possible mechanism may involve preferential activation of distinct populations of interneurons in SI, similar to direction-dependent activation of I-wave circuitry in M1. In a recent study involving 56 participants, high inter-subject variability of cTBS after-effects was strongly correlated with I-wave recruitability (Hamada *et al.*, 2012). The authors applied cTBS in one direction only. However, given the specificity of I-wave recruitment in opposite directions, it is plausible that cTBS current orientation determines after-effects. Indeed, previous work has reported the influence of current direction on cTBS after-effects and proposed that I-wave recruitment was the probable cause (Talelli *et al.*, 2007).

Frequency

30 Hz M1-cTBS suppressed MEP amplitudes in the RFDI for up to 25 minutes before returning to near baseline values at 45 minutes post-stimulation. These findings are in agreement with previous reports of MEP suppression for 30 minutes following 30 Hz M1-cTBS (Goldsworthy *et al.*, 2012). Of interest, MEPs were suppressed even at a very low stimulation intensity of 55% RMT in the present work, compared to 80% RMT by Goldsworthy and colleagues (2012). The authors also found that applying cTBS at 80% AMT, equivalent to ~65-70% RMT also led to long-lasting suppression. In contrast,

a difference of as little as 5% RMT produced opposite after-effects of 50 Hz cTBS (Doeltgen & Ridding, 2011). Together, these data suggest that modified 30 Hz is less sensitive to stimulation intensity and perhaps more reliable than traditional 50 Hz cTBS.

5.4 Limitations

There are limitations in this work. First, a suppressive cortical effect of cTBS on SI local circuitry was not directly confirmed in the two experiments. However, a pilot study of six participants who participated in Experiment 2 revealed no significant changes in N20-P25 or P25-N33 components of somatosensory evoked potentials evoked by electrical stimulation to the contralateral median nerve. There appeared to be a trend for a decrease and increased amplitude in the N20-P25 and P25-N33 components, respectively. However, these results were difficult to interpret due to inter-subject variability. A previous study reported decreased N20-P25 amplitudes following 50 Hz cTBS (Ishikawa *et al.*, 2007). However, confirmation that 30 Hz cTBS alters SI could be a future direction of research to explore intrinsic SI circuitry changes. Second, the effect of current direction on 30 Hz cTBS was not specifically investigated. This knowledge would contribute to the understanding of how stimulation parameters influence cTBS after-effects.

5.5 Conclusion

In summary, this research demonstrates that cortical motor circuitry of the hand is influenced by the primary somatosensory cortex. It is well known that sensory input modulates motor output. Decades of research in mammals has clearly shown that somatosensory input modulates motor cortex circuitry via direct projections at the cortical level. In humans, this relationship has been difficult to prove, but assumed to exist. Although many researchers investigate the influence of somatosensory input from the peripheral level, few have investigated directly at the central level between the primary motor and sensory areas in humans. Using non-invasive cTBS, shown to supress local cortical circuitry, we have shown that stimulation of SI produces long-lasting facilitation or suppression of corticospinal output to the hand. The bidirectional after-effects on motor circuitry can be manipulated by the appropriate selection of cTBS stimulation parameters, namely current direction and frequency of stimulation. These findings are an important contribution to our understanding of motor control of intrinsic hand musculature in humans. Corticocortical afferents from premotor and somatosensory regions suggest a highly dynamic process in the cortical control of hand function (Di, V *et al.*, 2012). This may have significant clinical relevance in populations with impaired hand control in that motor function may be manipulated by targeting various cortical areas. Somatosensory stimulation-induced plasticity improves motor function in stroke patients, suggesting that plasticity may drive cortical reorganization (Peurala *et al.*, 2002) and functional motor recovery (Fraser *et al.*, 2002;Schaechter *et al.*, 2012). SI-cTBS to increase or decrease neural activity in hand representations may be used to further

explore somatic influence on motor circuitry in humans or as part of a functional rehabilitation therapy to improve hand function.

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