# ALTERATIONS TO GABAERGIC INHIBITION IN PRIMARY MOTOR CORTEX

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**By**

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## ABSTRACT

Motor evoked potential (MEP) and short- and long-interval intracortical inhibition (SICI and LICI) are measures for corticospinal output and intracortical inhibition within the primary motor cortex (M1), respectively. These measures are known to be associated with motor output and control and have been implicated within neurophysiological disorders. It is likely that the dysfunction of such motor cortical circuits are associated with the motor deficiencies presented within clinical populations such as spinal cord injury (SCI), amyloid lateral sclerosis, and focal hand dystonia. In Experiment 1, the MEP, SICI, and LICI circuits were characterized in participants with incomplete SCI and compared to uninjured controls to identify differences. MEPs and SICI were reduced while LICI was increased in participants with SCI. In Experiment 2, single and paired continuous theta burst stimulation (cTBS) were delivered over M1 or primary somatosensory cortex (SI) in order to induce plasticity within either cortical areas and produce changes to MEP and SICI. All interventions were able to facilitate MEP, but none of the interventions were sufficient in altering SICI. The findings in this thesis highlight the alterations in motor cortical circuits associated with motor output and control within the SCI population and the potential interventions that can be implemented in reverting those circuit changes.

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Peter Mi

August 2015

## AUTHOR'S DECLARATION

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

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| | |
|---|---|
| ANOVA | Analysis of Variance |
| AMT | Active Motor Threshold |
| CS | Conditioning Stimulus |
| CNS | Central Nervous System |
| CSN | Corticospinal Neuron |
| cTBS | Continuous Theta Burst Stimulation |
| D-wave | Direct Wave |
| EMG | Electromyography |
| FDI | First Dorsal Interosseous |
| FCR | Flexor Carpi Radialis |
| GABA | Gamma-Aminobutyric Acid |
| H-reflex | Hoffman Reflex |
| ISI | Interstimulus Interval |
| I-wave | Indirect Wave |
| LICI | Long-Interval Intracortical Inhibition |
| LTD | Long Term Depression |
| LTP | Long Term Potentiation |
| M1 | Primary Motor Cortex |
| MEP | Motor Evoked Potential |
| MSO | Maximum Stimulator Output |
| MT | Motor Threshold |
| MVC | Maximum Voluntary Contraction |
| PAS | Paired Associative Stimulation |
| RMT | Resting Motor Threshold |
| rTMS | Repetitive Transcranial Magnetic Stimulation |
| SI | Primary Somatosensory Cortex |
| SICI | Short-Interval Intracortical Inhibition |
| TBS | Theta Burst Stimulation |
| TES | Transcranial Electrical Stimulation |
| TMS | Transcranial Magnetic Stimulation |
| TS | Test Stimulus |
| µV | Microvolts |

# Chapter 1: Goals of the thesis

## Alterations to Intracortical Inhibition Following Spinal Cord Injury and Plasticity Intervention

### 1.0 Overview of Thesis

The overarching purpose of the thesis was to advance our understanding of GABAergic inhibitory circuits following injury to the spinal cord and following a plasticity protocol applied over primary motor cortex and primary somatosensory cortex.

Inhibition within the motor cortex in the form of short and long-interval intracortical inhibition (SICI and LICI) has been shown to participate in the control prior to and also during movement (Ridding *et al.*, 1995;Reynolds & Ashby, 1999;Sohn *et al.*, 2002). Therefore, alterations to such inhibitory circuits and impairments in motor function and control are associated. Such alterations may be likely in the upper limb of individuals with SCI according to previous reports of SICI (Roy *et al.*, 2011) and LICI (Barry *et al.*, 2013). This thesis has shown that SICI is reduced and LICI is increased in individuals with SCI.

By reverting potential changes to cortical inhibition and thus motor control in a clinical population, we may observe improvements in motor control. Two sequential continuous theta-burst stimulation (cTBS) protocols over M1 have been shown to induce both increases (Murakami *et al.*, 2012) and decreases (Goldsworthy *et al.*, 2013) to SICI based on varying stimulation parameters. Based on the findings from characterizing SICI and LICI within the SCI population, an appropriate cTBS protocol can be used to potentially restore intracortical function and potentially motor control.

Additionally, SI has also proven to be a promising cortical area in reverting impairments in motor function and control via stimulation or otherwise (Foki *et al.*, 2015;Havrankova *et al.*, 2010;Guggenmos *et al.*, 2013). There are no published reports of SICI alterations following a single cTBS protocol. However, two sequential cTBS protocols over SI may be able to induce changes similar to previous reports (Goldsworthy *et al.*, 2012b).

## 1.1 Goal of Experiments

### *1.1.1 Goal of experiment 1 - Short- and long-interval intracortical inhibition in incomplete spinal cord injury*

The goal of the first project was to characterize the SICI and LICI circuitry in individuals with spinal cord injury. Recruitment curves of SICI and LICI were obtained from participants with SCI and uninjured controls at rest and during an isometric contraction of the flexor carpi radialis (FCR) muscle. This allowed for the ability to observe the changes to not only resting SICI and LICI but also SICI and LICI during the contraction of the FCR muscle in order to reveal potential changes to the modifications of SICI and LICI post injury.

### *1.1.2 Goal of experiment 2 - Effects of continuous theta-burst stimulation on modulation of motor evoked potential and short-interval intracortical inhibition*

The goal of the second project was to investigate the effects of a plasticity-inducing repetitive transcranial magnetic stimulation protocol called cTBS over primary motor and primary somatosensory cortex (M1 and SI) on the modulation of SICI in the first dorsal interosseous (FDI) muscle. Any alterations to MEPs and/or SICI can be applied to the participants with SCI in attempts to produce regular modulation of SICI and improve movement control in SCI.

## 1.2 Significance of the thesis

Motor cortical circuits such as SICI and LICI have been demonstrated to participate during the onset of movement initiation as well as during movement itself (Ridding *et al.*, 1995;Reynolds & Ashby, 1999;Sohn *et al.*, 2002). Both SICI and LICI are reduced during a tonic contraction of agonist muscles (Ridding *et al.*, 1995;Hammond & Vallence, 2007) and appear to be modulated in a task-dependent manner (Paulus *et al.*, 2008).

To date, research characterizing SICI and LICI within the spinal cord injured (SCI) population is lacking. There are only four publications in the literature that have characterized SICI and LICI following an injury to the spinal cord (Saturno *et al.*, 2008;Barry *et al.*, 2013;Bunday & Perez, 2012;Roy *et al.*, 2011). Further, these authors typically focus their attention on one state of the muscle; either only at rest (Saturno *et al.*, 2008) or only during an isometric contraction (Roy *et al.*, 2011). There are however, a couple of authors who have looked at the effects of introducing a tonic contraction to the agonist muscle to LICI specifically in SCI (Barry *et al.*, 2013;Bunday & Perez, 2012). Overall, there remains a distinct lack of research in the characterization of the task-dependent modulation of SICI and LICI within SCI.

With the characterization of the changes to the SICI and LICI circuits and the associated changes to the modulation of the circuits following an injury to the spinal cord, it allows for the exploration of plasticity interventions which can potentially alter and improve the modulation of the circuits. The use of plasticity interventions on intracortical circuits have yet to be investigated and their effects on these circuits during an isometric contraction are unknown.

This thesis acts to not only thoroughly investigate the modulation of SICI and LICI in participants with SCI, but also explores a plasticity intervention and its potential effects on the

modulation of SICI in uninjured controls, both of which are completely novel pieces of research in areas that no one has previously investigated. The changes that occur to the modulation of the inhibitory circuits in the motor cortex following a SCI can be a target for therapeutic interventions which can act to revert those changes occurring in the motor cortex and allow physiological improvements to occur such as recovery of movement and motor control of affected limbs. Not only does this work contribute to the understanding of SCI and its effects on the motor cortex at the fundamental neuroscience level, it also provides a potential new therapy for rehabilitation of motor function in the SCI population at the clinical neuroscience level.

# Chapter 2: Review of Background Literature

## 2.1 Transcranial Magnetic Stimulation (TMS)

Transcranial magnetic stimulation (TMS) is a non-invasive and painless technique of brain stimulation that can be used in humans to investigate the neurophysiology of the cerebral cortex, such as the motor cortex. Based on the principle of electromagnetic induction discovered by Michael Faraday in 1838, TMS allows for the depolarization of populations of neurons by inducing an ionic current in the brain (Kobayashi *et al.*, 2003). Conductive wiring wrapped in a coil placed on the scalp with strong and rapidly-changing electrical current passing through it generates rapidly changing magnetic field pulses perpendicular to the coil and the scalp which lasts up to 100 μs and up to 2 Tesla in intensity (Hallett, 2007). The magnetic pulses penetrate the skull virtually unimpeded and result in the generation of the secondary electrical current, which is parallel but opposite to the current of the coil, within the neurons below the coil (Hallett, 2007).The ability for the current within the brain to depolarize the neurons depends on

the intensity of the current induced. This current is directly related to the intensity of the current within the stimulating coil, and by varying this intensity the generation of pulses both sub- and supra-threshold can be made (Hallett, 2007).

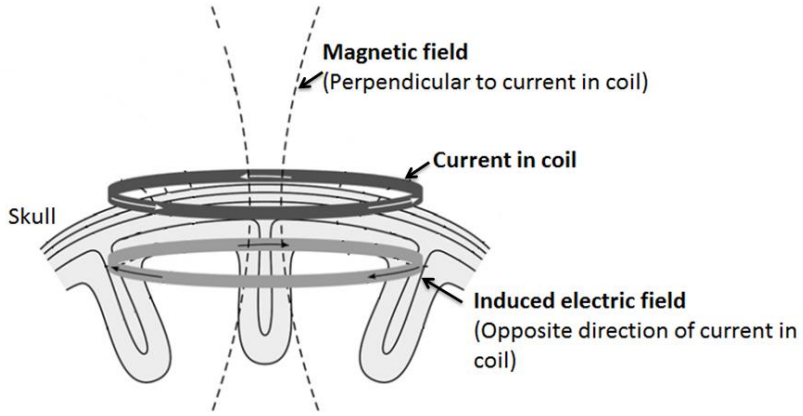


Figure 2.1: In TMS, the current in the coil generates a magnetic field which induces a secondary current in the brain in the opposite direction of the coil.

Single pulse TMS activate corticospinal neurons within the motor cortex in two ways: directly (D-waves) on the axon of the neurons, or indirectly (I1-, I2-, and I3-waves) through trans-synaptic activation of the neurons (PATTON & Amassian, 1954). Direct electrical recordings from the pyramidal tracts of monkeys show the onset of D-waves at approximately 0.5 msec and the I-waves to follow the D-waves at 1.5 msec intervals (Patton and Amassian 1954). Similar epidural recordings in humans also show a number of D- and I-waves occurring at different time intervals following cortical stimulation (Berardelli *et al.*, 1990;Nakamura *et al.*, 1996). The threshold to elicit the D-waves are lower than the threshold to elicit the I-waves. Different

orientations of the TMS coil can selectively recruit different types of descending volleys. By placing the coil to induce a posterior to anterior direction, I1-waves are preferentially recruited. Conversely, an anterior to posterior current allows for I3-waves to be activated first. D-waves can be elicited as well by orientating the coil in a lateral to medial direction (Sakai *et al.*, 1997).

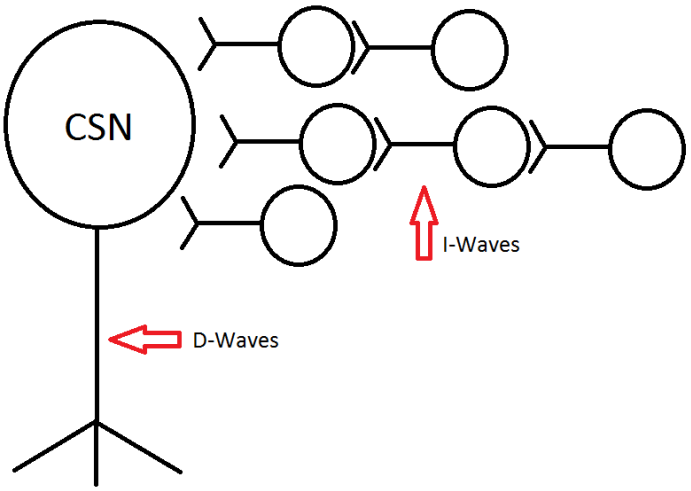


Figure 2.2: Cortical stimulation can act on the corticospinal neuron directly (D-waves) or indirectly (I-waves) through transsynaptic connections.

### *2.1.1 Motor Evoked Potentials (MEPs)*

The motor evoked potential (MEP) is the response of the contralateral muscle when a supra-threshold TMS pulse is applied over the motor cortex of the muscle of interest (Kobayashi *et al.*, 2003). A single TMS pulse with sufficient intensity to depolarize the neurons causes an action potential to travel down the corticospinal tract towards the muscle represented by the motor cortex (Hallett, 2007). Upon traversing the spinal cord and reaching the muscle, the action

potential allows calcium to enter the pre-synaptic neuron and causes a release of acetylcholine into the neuromuscular junction causing contraction of the muscle. This contraction can be recording using electromyography (EMG) and is known as the MEP. MEP peak-to-peak amplitudes are most commonly used as a measure of corticospinal and spinal motoneuron activity (Petersen *et al.*, 2003), where increases in amplitudes reflect increases in excitability and decreases reflect decreases in excitability. As such, MEP amplitudes are larger when the muscle is contracting at baseline compared to rest since the motor neuron pool is at a higher level of activity and therefore easier to provoke an increase of activation (Hallett, 2007). MEP area, measured as the area of the mean rectified EMG, can also be used in place of or in conjunction with amplitude for measuring excitability (Roy *et al.*, 2011). The area of an MEP can be useful in the analysis of polyphasic MEP activity where amplitudes lack the capability to capture all necessary information. MEP responsiveness or latency can be used to provide information regarding muscle function and performance (Choi *et al.*, 2014) and can be a used to characterize function in diseased populations.

### *2.1.2 Motor thresholds*

Motor threshold (MT) is a measure of membrane excitability, specifically the membrane excitability of the corticospinal neurons and spinal neurons (Rossini *et al.*, 1994). The MT is altered and changed in certain disease populations and holds importance in the clinical field (Kobayashi *et al.*, 2003). Resting motor threshold (RMT) is defined as the lowest stimulation intensity required to evoke MEPs of minimum amplitude of 50 μV in 5 out of 10 consecutive trials while the muscle is at rest (Rossini & Rossi, 2007). At rest, the motoneurons in the spinal

cord require more than one descending volley to fire, the RMT is dependent on glutamatergic synaptic excitability of the corticospinal neurons (Day *et al.*, 1989). Alternatively, active motor threshold (AMT) is defined as the lowest stimulation intensity required to evoke MEPs of minimum amplitude 200 μV in 5 out of 10 consecutive trials while the targeted muscle is held at a slight contraction, typically 15 - 20% of the maximum voluntary contraction (MVC) (Rossini & Rossi, 2007). When contracted, many corticospinal neurons as well as spinal cord motoneurons are close to firing threshold, and RMT therefore is dependent on axon threshold of the neurons regulated by sodium channels (Hodgkin & Huxley, 1952). MT can be increased in the presence of drugs which block voltage-gated sodium channels such as carbamazepine (Ziemann *et al.*, 1996a) and oxacarbazepine (Kimiskidis *et al.*, 2005). Ketamine, an NMDA receptor antagonist, which acts to increase glutamatergic activity, causes a decrease in MT (Di Lazzaro *et al.*, 2003). The changes to MT in diseased populations such as SCI when compared to healthy controls can often help to explain the changes in membrane excitability in both the corticospinal as well as the spinal neuron populations.

### *2.1.3 Short-Interval Intracortical Inhibition (SICI)*

SICI is a well characterized inhibitory circuit whereby a test stimulus (TS) gets its amplitude inhibited in the presence of a conditioning stimulus (CS) (Kujirai *et al.*, 1993;Kobayashi *et al.*, 2003;Di Lazzaro *et al.*, 2000). The two stimuli are delivered to the same cortical region through the use of a single stimulating coil separated by an interstimulus interval (ISI) between 1-5 ms (Kujirai *et al.*, 1993). The inhibitory effects are only observed when the first CS is a subthreshold stimulus and the second TS is a suprathreshold stimulus (Kujirai *et al.*, 1993). Recordings on

spinal reflexes showed no changes during SICI actions and suggests a modification of synaptic excitability occurring cortically rather than at the spinal level (Kujirai *et al.*, 1993). Further, direct epidural recordings of descending volleys confirm the cortical origins of SICI and revealed that the CS evokes no descending activity and only the later I-waves are suppressed (Di Lazzaro *et al.*, 1998). The CS produces inhibitory postsynaptic potentials (IPSPs) at the corticospinal neurons which causes a reduction in the number of action potentials that can be elicited by the subsequent TS (Ilic *et al.*, 2002). SICI has been evidenced to be mediated by $GABA_A$ receptors in the brain (Ziemann *et al.*, 1996b;Ilic *et al.*, 2002). SICI in certain patient populations often show reduction of inhibition such as Stroke (Hummel *et al.*, 2009), ALS (Menon *et al.*, 2014;Vucic *et al.*, 2011), and Multiple Sclerosis (Vucic *et al.*, 2012;Conte *et al.*, 2009).

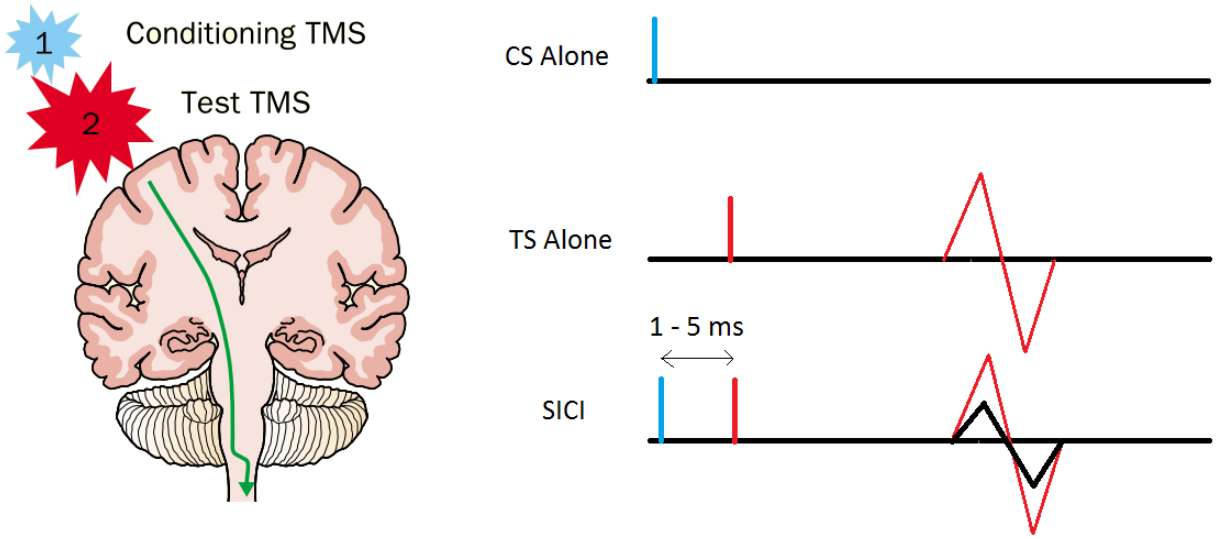


Figure 2.3: SICI involves a subthreshold conditioning stimulus (CS) followed by a suprathreshold test stimulus (TS). Between ISIs of 1 - 5ms, the TS MEP amplitude is observed to be decreased. (Figure modified from Kobayashi et al., 2003).

*2.1.4 Long-Interval Intracortical Inhibition (LICI)*

The LICI circuitry is elicited when a suprathreshold CS acts to inhibit a subsequent suprathreshold TS at ISIs between 50-200 ms using two pulses through one coil (Valls-Sole *et al.*, 1992;Wassermann *et al.*, 1996). Two MEPs are generated as a result of the two suprathreshold stimuli and only the second TS MEP is inhibited. Epidural recordings of descending corticospinal volleys demonstrate the reduction of later I-waves at ISIs of 100-150 ms (Nakamura *et al.*, 1997;Chen *et al.*, 1999b;Di Lazzaro *et al.*, 2002b).

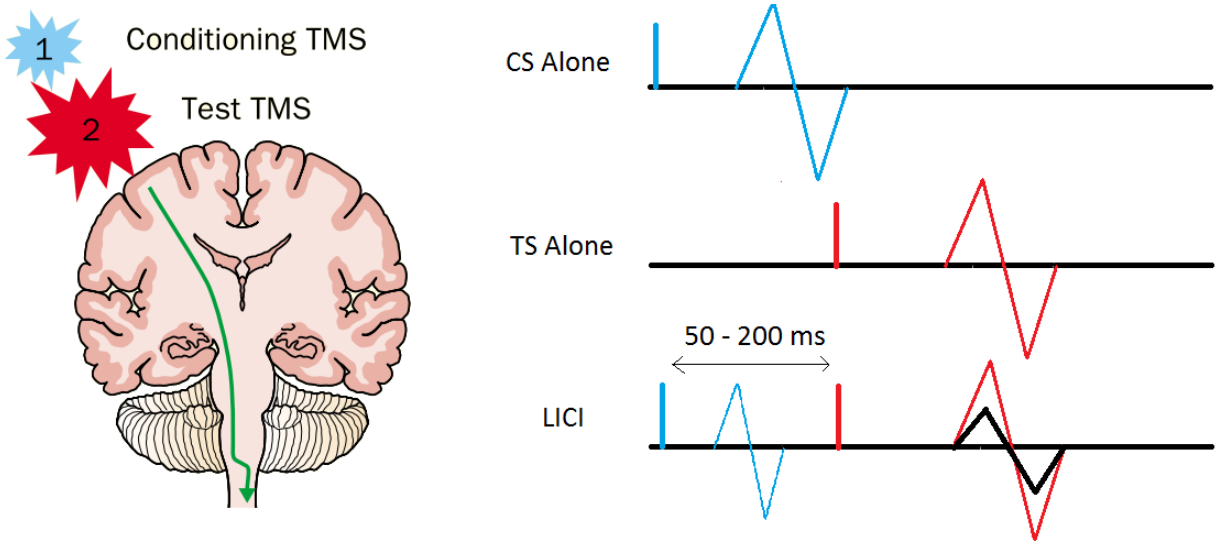


Figure 2.4: LICI involves a suprathreshold conditioning stimulus (CS) followed by a suprathreshold test stimulus (TS). Between ISIs of 50 - 200ms, the TS MEP amplitude is observed to be decreased. (Figure modified from Kobayashi et al., 2003).

*2.1.5 Repetitive Transcranial Magnetic Stimulation*

TMS can be used to cause increases or decreases in synaptic strength known as long-term potentiation (LTP) or depression (LTD) by applying TMS in rapid succession (Hallett,

2007;Thickbroom, 2007). Typically, low rates of rTMS at <1 Hz causes decreases in brain excitability (Chen *et al.*, 1997) and high rates of rTMS above >1 Hz causes increases in brain excitability (Pascual-Leone *et al.*, 1994).

CTBS is when TMS is used repetitively with very short and very high frequency trains of stimuli are delivered at a theta frequency of approximately 5 Hz (Di Lazzaro *et al.*, 2005;Huang *et al.*, 2005). A typical paradigm occurs with three pulses at 50 Hz, repeated at 5 Hz (Hallett, 2007).

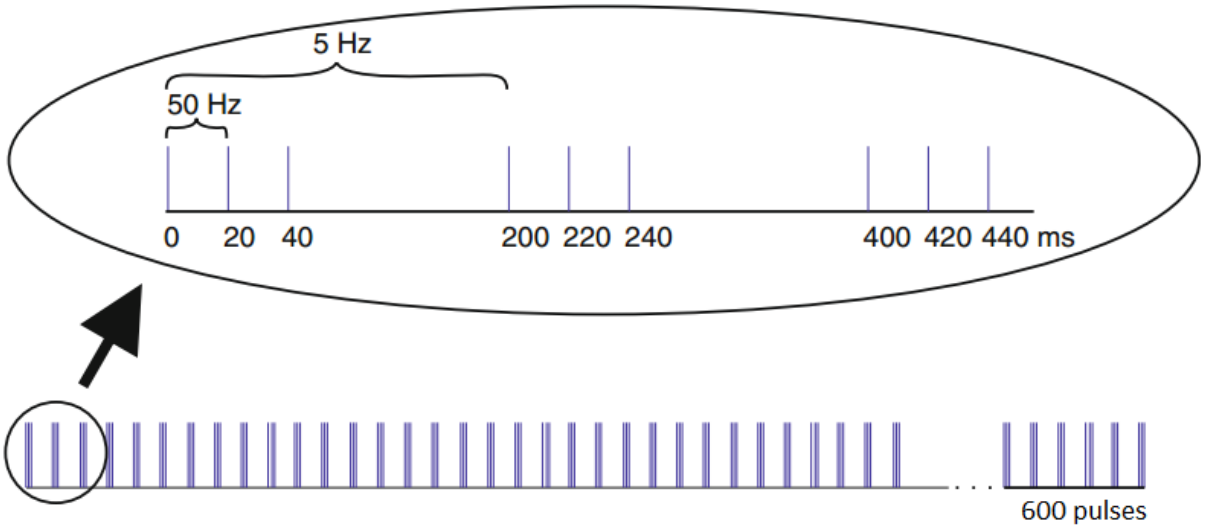


Figure 2.5: Continuous theta burst stimulation consists of three stimuli delivered in bursts at a rate of 50 Hz repeated in a theta like-rhythm of 5 Hz. A total of 200 bursts are delivered without interruption. (Figure modified from Cardenas-Morales, 2010).

The continuous variant of TBS applied over M1 causes decreases in cortical excitability with effects lasting up to one hour (Huang *et al.*, 2005). However, when applied over SI, cortical excitability with cTBS was observed to increase instead (Tsang *et al.*, 2014). The mechanisms why which these rTMS effects are modulated is still unknown, however magnetic resonance

spectroscopy studies suggest increases in $GABA_A$ in the stimulated cortex following cTBS (Stagg *et al.*, 2009). The possibility of glutamatergic and dopaminergic systems being closely associated with cTBS still exists as the effects of cTBS appear to be negated following blockage of NMDA receptors (Huang *et al.*, 2007;Teo *et al.*, 2007) and D2 receptors (Monte-Silva *et al.*, 2011).

### 2.2 Spinal Cord Injury

Cervical spinal cord injury refers to damage to the cervical portion of the spinal cord between C1 - C8 caused by trauma. The complete or partial loss of both muscle function and sensation can occur dependent on severity of injury as the spinal cord relays both sensory and motor information between the brain and the body (Branco *et al.*, 2007).The high cervical nerves C1 - C4 are the most severe of spinal cord injuries and results in the paralysis of arms, hands, trunk, and legs and the individual may not be able to breathe without machine assistance. The lower cervical nerves C5 - C8 are responsible for elbow flexors, wrist extensors, elbow extensors, and finger flexors. Rating of SCI severity can be done through the use of American Spinal Injury Association (ASIA) impairment scale using letters A-E (Kirshblum *et al.*, 2014). An ASIA scale of A represents no preservation of motor or sensory function below the level of the lesion ranging to E, which represents normal motor function and sensation in all segments.

Human corticospinal tract can start to degenerate as early as 12 days following spinal cord injury (Buss *et al.*, 2004). Evidence for reorganization of corticospinal tracts within the spinal cord after injury show a depletion of axons at the injury site and close to normal numbers of axons at a distance from the injury, proposing that degenerated axons were replaced by collateral sprouts of surviving axons (Fishman, 1987).

### *2.2.1 MEP Changes in SCI*

After incomplete spinal cord injury, a number of corticospinal neurons projecting to affected muscles are reduced and voluntary drive is often impaired (Brouwer *et al.*, 1992). When compared to healthy controls, patients with incomplete SCI show significantly smaller (Davey *et al.*, 1999) and delayed (Alexeeva *et al.*, 1997) MEPs. Demyelination of corticospinal tracts (Buss *et al.*, 2004), reduced number of myelinated corticospinal tracts (Fishman, 1987), and retrograde degeneration of injured corticospinal tract axons (Bronson *et al.*, 1978) are thought to be contributing factors in the reduction in MEP latency. The threshold for eliciting an MEP is also increased after SCI (Davey *et al.*, 1999;Smith *et al.*, 2000b).

### *2.2.2 Intracortical Circuit Changes in SCI*

SICI is also observed to be decreased in patients with SCI compared to controls (Roy *et al.*, 2011;Saturno *et al.*, 2008). With LICI, there appears to be an increase in LICI during active tonic contraction of target muscles in patients with incomplete SCI however there does not seem to be any changes to LICI at rest (Barry *et al.*, 2013).

## 2.3 Primary Somatosensory Cortex (SI)

2.3.1 How SI affects Primary Motor Cortex (M1)

The primary somatosensory cortex (SI) has been shown to have direct projections to the primary motor cortex (Jones *et al.*, 1978;Jones & Powell, 1969a;Jones & Powell, 1969b;Vogt & Pandya, 1978) and can directly modify the neural activity within M1 (Iriki *et al.*, 1989;Pavlides *et al.*, 1993;Brinkman *et al.*, 1985;Sakamoto *et al.*, 1987), resulting in changes in motor control and cortical excitability. Through lesioning SI, impairments in motor learning have been observed (Pavlides *et al.*, 1993). Similarly, injection of the $GABA_A$ agonist muscimol into the postcentral gyrus, has shown impairments in fine motor coordination (Hikosaka *et al.*, 1985). Following cooling of the postcentral gyrus, impairments impairments in gross motor control and coordination as well as increases in baseline M1 activity are observed in monkeys (Brinkman *et al.*, 1985). Direct somatosensory input to SI causes decreases in motor output activating the cortical circuits known as short- and long-latency afferent inhibition (SAI and LAI) (Tokimura *et al.*, 2000;Chen *et al.*, 1999a).

## Chapter 3: Experiment 1: Short and long-interval intracortical inhibition in incomplete spinal cord injury

**(Chapter 3 has been accepted into the Canadian Journal of Neurological Sciences and permission has been granted to be published as a part of this Master's Thesis.)**

### 3.1 INTRODUCTION

Extensive modifications to ascending somatosensory pathways, descending motor pathways and sensorimotor cortices follow incomplete spinal cord injury (SCI)(Levy *et al.*, 1990;Curt *et al.*, 2002;Levy *et al.*, 1990). Following experimentally induced deafferentation, inhibitory GABAergic cortical activity in sensorimotor cortices is reduced (Levy *et al.*, 2002;Hendry & Jones, 1986;Welker *et al.*, 1989) which is thought to mediate rapid cortical plasticity (Jacobs & Donoghue, 1991;Levy *et al.*, 2002). In SCI, reductions in GABAergic cortical activity are suggested to promote rapid functional gains (Smith *et al.*, 2000b). However, there is evidence of sustained alterations in cortical inhibition (Smith *et al.*, 2000a;Shimizu *et al.*, 2000) which may lead to alterations in GABA-mediated or modulated motor cortical circuitry.

Short-interval intracortical inhibition (SICI) is an inhibitory motor cortical circuit evoked by a subthreshold transcranial magnetic stimulation (TMS) pulse (i.e. conditioning stimulus (CS)) followed by a suprathreshold TMS pulse (i.e. test stimulus (TS)). When the TS follows the CS by 1-5 ms, the motor evoked potential (MEP) is suppressed (Kujirai *et al.*, 1993). SICI is thought to be cortically mediated (Di Lazzaro *et al.*, 1998) via $GABA_A$ receptor transmission (Ziemann *et al.*, 1996b;Ilic *et al.*, 2002;Ziemann *et al.*, 1996a) since late and not early indirect waves (I-waves) are inhibited (Hanajima *et al.*, 1998;Di Lazzaro *et al.*, 1998). These data suggest that SICI modifies intracortical neuron activity which inhibit the I-3 and later I-waves instead of acting directly on the pyramidal neurons, which results in a net reduction of the corticospinal

output to the spinal cord. In controls, SICI is modulated in advance of and during active muscle contraction and is therefore suggested to participate in the control of movement (Ridding *et al.*, 1995;Reynolds & Ashby, 1999;Sohn *et al.*, 2002).

In incomplete SCI, the depth of SICI is reduced in the actively contracted tibialis anterior (TA) muscle when compared to controls, although the circuit is elicited at similar intensities in the two groups (Roy *et al.*, 2011). The same report also indicates the presence of SICI in the contracted first dorsal interosseous (FDI) muscle although it is unknown how this differs from uninjured populations, and specifically in SCI with cervical injury. Collectively, these data contribute to the hypothesis that cortical inhibition is reduced as a result of incomplete SCI and suggest an altered state of $GABA_A$-mediated transmission. However, in SCI, it remains unknown whether the SICI profile is abnormal in a muscle of the upper limb such as the flexor carpi radialis (FCR) muscle. In our experience, chronic incomplete tetraplegia with injury ranging from C3 to C7 retain the ability to perform isometric contraction of the FCR muscle, albeit this ability varies greatly across individuals. This is an important experimental consideration since reliably evoked MEPs of discernable amplitude typically require SCI participants to actively contract the targeted muscle. The present study is focused on FCR, a muscle important in wrist control to achieve grasping, pulling, and writing, (Brorsson *et al.*, 2014) movements that may be lost as a result of cervical SCI (Smith *et al.*, 2000b).

Long-interval intracortical inhibition (LICI) is elicited by a suprathreshold CS depressing the MEP evoked by a subsequent suprathreshold TS delivered 50-200 ms later (Valls-Sole *et al.*, 1992;Wassermann *et al.*, 1996). Cervical epidural recordings of descending corticospinal volleys

suggest a cortical origin for the LICI circuit; later I-waves of the second suprathreshold stimulus are significantly reduced at ISIs of 100-150 ms while the early I-wave is unaltered (Nakamura *et al.*, 1997;Chen *et al.*, 1999b;Di Lazzaro *et al.*, 2002a). LICI appears to be mediated via $GABA_B$ receptors such that baclofen (McDonnell *et al.*, 2006) and other drugs acting on GABA transmission increase LICI (Werhahn *et al.*, 1999;Pierantozzi *et al.*, 2004). One study demonstrated that LICI can be elicited in SCI at rest and continues to persist during active contraction of FDI (Barry *et al.*, 2013). However, in SCI, the LICI recruitment profile remains unknown.

It was hypothesized that uninjured control participants would exhibit SICI at CS intensities of 70, 80, and 90% AMT as described in other studies (Kujirai *et al.*, 1993;Ridding *et al.*, 1995). In accordance with Roy et al., (2011) who studied SICI in the TA muscle in SCI, we hypothesized SICI to exist at CS of 80% AMT, thus representing a narrow range of SICI recruitment. It was further hypothesized that LICI would exist in uninjured controls at suprathreshold intensities (i.e. 100, 110, 120 and 130% AMT (McNeil *et al.*, 2011). In SCI, we hypothesize LICI to exist at a reduced range of only the highest CS intensity (i.e. 130% AMT), in support of the very large intensity required to elicit LICI in SCI elsewhere (Barry *et al.*, 2013).

In this study, we obtained SICI and LICI recruitment curves from the contracted FCR muscle in people with incomplete cervical SCI and age-matched controls. Thorough characterization of the intracortical inhibitory circuits following incomplete SCI is important for determining changes in motor cortical GABAergic transmission. Identifying aberrations within intracortical GABAergic

circuits is fundamental for experimental approaches that promote plasticity in motor cortex to improve arm function in SCI.

## 3.2 METHODS

### *3.2.1 Participants*

Eight individuals with incomplete SCI (mean age = 29.5, SD = 6.7, 7 males, 8 right-handed) and thirteen aged-matched uninjured individuals (mean age = 28.8, SD = 5.6, 8 males, 9 right-handed) participated. Due to the asymmetry of the lesion location and the heterogenic nature of the damage to the spinal cord following SCI, each limb from a SCI participant was treated as an independent limb resulting in the participation of thirteen SCI limbs. Demographic, lesion and medication information for all participants with SCI is found within Table 1. SCI limbs which were unable to perform the required isometric contraction were excluded from analysis. All participants provided written informed consent and the study was approved by the Office of Research Ethics at McMaster University and conformed to the Declaration of Helsinki.

### *3.2.2 Electromyography (EMG)*

Muscle activity was recorded using 9mm diameter Ag-AgCl surface electrodes with the active electrode being placed over the muscle belly of the FCR and the reference electrode placed over the tendon at the wrist. The ground electrode was placed over the medial styloid process at the wrist. EMG was amplified at 1000x gain, bandpass filtered (20 - 2.5kHz, Intronix Technologies Corporation Model 2024F, Bolton, Ontario, Canada) and digitized (5kHz, Micro 1401, Cambridge Electronics Design, Cambridge, UK). Signal software (v5, Cambridge Electronic Design Limited, Cambridge, UK) was used to acquire and analyze EMG data.

*3.2.3 Maximum Voluntary Contraction*

To determine the maximum voluntary contraction (MVC) for FCR, participants were asked to maximally contract the forearm during wrist flexion. Three trials (each 3 s) separated by 18 s of relaxation were performed. This duration of inter-trial rest sufficiently allowed the maximum EMG to be obtained in each subsequent trial (see analysis below). MVC was calculated as the rectified area of a 38 ms window that fell within their maximum contraction period during each of the three trials and was. In cases where participants expressed discomfort due to the high stimulation intensity (i.e. 80%, 90%, or 100% MSO) one trial rather than three would be acquired for the recruitment curves. The duration of 38 ms was chosen to allow direct comparison with background EMG measured during SICI and LICI testing as described below. SICI and LICI recruitment curves were obtained during active isometric contraction of 15 - 20% MVC in FCR in all participants. As reported elsewhere, resting MEPs tend to be small or absent in many individuals with SCI and thus all responses were evoked during a tonic contraction (Roy *et al.*, 2010;Roy *et al.*, 2011). Fatigue within muscles result in reductions to MEP sizes (Kotan *et al.*, 2015), it is therefore important to ensure no effect of fatigue occurs during active contraction of all participants.

*3.2.4 Transcranial Magnetic Stimulation (TMS)*

TMS was delivered using a custom built 50mm diameter figure-of-eight branding coil connected to a Magstim 200 stimulator (Magstim, Whitland, UK). The position and orientation of the coil was monitored throughout the experiment using Brainsight Neuronavigation (Rogue Research, Montreal, Canada) with optical sensors placed on the coil and the participant. The same TMS coil was used for all measures in both experiments. The TMS coil delivered a monophasic pulse

over the optimal location to elicit MEPs in the relaxed FCR at 45° in relation to the parasagittal plane to induce a posterior-lateral to anterior-medial current in the cortex. The motor hotspot was defined at this location where all subsequent measures were taken.

The active motor threshold (AMT) of the SCI group was defined as the stimulator intensity which resulted in MEPs 50 μV larger than the background EMG in 5 out of 10 consecutive trials during a constant contraction of 20% MVC in FCR. The age-matched control participants were then asked to maintain a constant contraction equal to 20% MVC of the SCI match participant (using the voltage of the EMG) and similarly AMT was defined as 50 μV larger than the background EMG in 5 out of 10 consecutive trials. Although this approach equates the magnitude of AMT based on matched EMG contraction, it remains unclear whether perceived exertion to achieve the 20% MVC was similar between groups.

*3.2.5 $MEP_{halfmax}$*

The maximum MEP in mV*ms was defined as the largest rectified area in a 70 ms window following the TMS artifact and was identified by increasing the mean stimulator output (MSO) in 10% increments from 10 – 100%.Area was measured to avoid ambiguities in identifying peaks within polyphasic MEPs that are characteristic of the FCR muscle (Byblow *et al.*, 2012). Three trials were typically obtained at each increment and averaged. $MEP_{halfmax}$ was defined as the MSO required to elicit a rectified area equal to half of the maximum. $MEP_{halfmax}$ was recorded and confirmed in 10 subsequent trials.

*3.2.6 SICI & LICI*

During SICI and LICI testing, the EMG representing 15 - 20% MVC from FCR was displayed as a bright line on an oscilloscope and participants were required to match the position of the FCR EMG controlled line to the 20% MVC target line. Background EMG was calculated as the rectified area of the first 38 ms of each trial window, the length of time from the start of the trial to the onset of TMS stimulation, and expressed as a percent of MVC which was also acquired over a 38 ms window.

SICI in FCR was tested at a CS-TS interstimulus interval (ISI) of 3ms.Thirteen SCI limbs and thirteen control limbs were tested. The CS was delivered at intensities of 60, 70, 80, 90, 100, and 110% AMT. For all participants, the TS intensity was set to elicit $MEP_{halfmax}$ as performed elsewhere (Roy *et al.*, 2011;Roy *et al.*, 2010). This allows the TS to be set at an intensity which enables either suppression or facilitation of the MEP to be observed, a choice pertinent to studies in SCI where MEPs are generally reduced. Fifteen conditioned MEPs (i.e. $MEP_{CS\text{-}TS}$) were collected for each CS intensity and fifteen unconditioned MEPs ($MEP_{TS}$) were collected. The CS intensities were tested in separate blocks and the order of blocks was randomized for each participant. Consecutive stimuli were delivered every 5 s.

LICI in FCR was tested at a CS-TS ISI of 150 ms. Twelve SCI limbs and thirteen control limbs were tested. The CS was delivered at an intensity of 90, 100, 110, 120 and 130% AMT. TS intensity was set at $MEP_{halfmax}$. ISI of 150 ms has been shown to evoke strong LICI for CS intensities between 100 and 120% MT (Valls-Sole *et al.*, 1992). Similar to SICI, fifteen conditioned MEPs were acquired for each CS intensity and fifteen unconditioned MEPs were

acquired. CS intensities were tested in separate blocks and the block order was randomized. Consecutive stimuli were delivered every 5 s.

*3.2.7 Data Analysis*

MEPs were measured as the rectified area of the EMG response in a 70 ms window following the TMS artifact. The assumptions of sphericty were tested for all ANOVAs and Greenhouse-Geisser corrections were used if the assumptions were not met. Normalized MEP areas (i.e. $MEP_{CS\text{-}TS}/MEP_{TS}$) were subjected to a two-way ANOVA with between subject factor GROUP (2 levels; control, SCI) and within subject factor CS intensity (SICI with 6 levels; 60, 70, 80, 90, 100, 110% AMT and LICI with 5 levels; 90, 100, 110, 120, 130% AMT). To test the hypothesis that SICI and LICI exist at CS intensities that typically evoke these circuits (SICI: CS 70, 80, 90%, LICI: 120,130%), Bonferroni corrected *a priori* t-tests compared the un-normalized unconditioned MEP area (i.e. $MEP_{TS}$ ) versus the un-normalized conditioned MEP area (i.e. $MEP_{CS\text{-}TS}$). Further, between group differences were compared using Bonferroni corrected *a priori* t-tests at 90% AMT (SICI) and at 120% and 130% AMT (LICI). Post-hoc Tukey's was used to test for significant main and/or interaction effects following ANOVAs. MVC was analyzed in a two-way repeated measure ANOVA with within factor BLOCK (3 levels; block 1, 2, and 3) and between factor GROUP (2 levels; control, SCI). Background EMG was tested using the above two-way ANOVA statistical model. MEP recruitment curves were analyzed in a two-factor ANOVA with between subject factor GROUP (2 levels; control, SCI) and within subject factor INTENSITY (10 levels; 10, 20, 30….100 MSO). To test whether SCI participants experienced fatigue over the course of the testing session, background EMG was further

analyzed as a function of the factor TIME (5 levels; block 1, 2, 3, 4, and 5). Effect sizes were calculated using Cohen's D. Statistical significance was set at $p < 0.05$.

## 3.3 RESULTS

### *3.3.1 EMG and AMT*

EMG during MVC was not different between groups as revealed by two-way ANOVA ($F_{(1,16)} = 0.415$, $p = 0.529$) (SCI: 30.08 ± 31.9 mV*ms, control: 38.22 ± 18.8 mV*ms). Likewise, there was no difference in MVC between the three blocks ($F_{(2,32)} = 0.144$, $p = 0.866$). One-way repeated measures ANOVA indicated that participants with SCI did not fatigue over the duration of the experiment (TIME, $F_{(2.216,36.146)} = 2.072$, $p = 0.138$). Background EMG was lower in SCI compared to controls (SCI: 2.75 ± 0.56 mV*ms, control: 4.34 ± 0.37 mV*ms, unpaired two-tailed t-test; $p = 0.033$). However, relative EMG, normalized to individual MVCs, were not different between groups (SCI: 24 ± 7.1 %, control: 22 ± 2.9 %, unpaired two-tailed t-test; $p = 0.76$), indicating that both groups of participants were exerting the same percent of their maximum output of their muscles. The group-averaged MSO (with standard deviation) for delivery of the TS (i.e. unconditioned MEP) was 44±6% in controls and 47 ± 7% MSO in SCI. The MSO values were not statistically different (two-tailed, un-paired t-test, $p = 0.33$). A clear peak-to-peak unconditioned MEP was observable in six SCI participants (nine limbs). From these individuals, the peak-to-peak MEP amplitude ranged from ~ 1 to 6 mV, however all analyses were performed on the MEP area.

AMT was significantly greater in SCI compared to controls (SCI: 36 ± 6.8 % MSO, control: 28 ± 1.1 % MSO, unpaired two-tailed t-test; $p = 0.002$). Because the effects of SICI and LICI can be

explained by the higher CS intensity used in participants with SCI, we analyzed SICI and LICI data only from participants in whom the range of AMT overlapped. The AMT matching criterion was designated as any AMT values that fell within 2 standard deviations of the group-averaged mean AMT of the uninjured controls. Any AMT values from either group that fell outside of that range were excluded from further analysis. Therefore, nine SCI limb data (n=6 SCI participants) and nine aged-matched controls with similar AMT values (SCI: 33 ± 4.8 % MSO, control: 30 ± 3.0% MSO, unpaired two-tailed t-test; $p = 0.20$) were subsequently analyzed and the AMT values for all participants pre- and post-AMT matching are shown in Figure 3.1A. This procedure therefore controlled for group differences in CS intensities that could affect the depth of SICI and LICI. Figure 3.1B displays the group-averaged MEP recruitment curve for SCI and controls (AMT matched). Two-way ANOVA revealed an effect of INTENSITY ($F_{(1.396,22.337)} = 41.784$, $p < 0.01$) and an INTENSITY*GROUP interaction ($F_{(1.396,22.337)} = 18.654$, $p < 0.01$). Post-hoc Tukey's revealed greater MEP area in controls versus SCI at all intensities ≥ 50% MSO ($p < 0.05$).

*3.3.2 SICI*

Nine SCI limbs and nine controls were included in the analysis of the SICI experiment. Figure 3.2A displays representative SICI data from an uninjured control and an SCI participant displaying a U-shaped recruitment curve for both groups. $MEP_{halfmax}$ was significantly greater in controls versus SCI (control: 45.12 ± 20.89 mV*ms, SCI: 16.97 ± 10.04 mV*ms, unpaired two-tailed t-test; $p = 0.0036$). The group-averaged recruitment profile of SICI, graphing the normalized MEP data in control and SCI participants during an isometric contraction is shown in Figure 3.2B. Two-way ANOVA revealed no effect of CS ($F_{(1.79,28.71)} = 3.267$, $p = 0.057$) or

GROUP ($F_{(1,16)}$ = 0.039, p = 0.846) or CS*GROUP interactions ($F_{(1.79,28.71)}$ = 1.08, p = 0.347). For uninjured controls, *a priori* Bonferroni corrected t-tests revealed the presence of SICI at all three hypothesized CS intensities of 70% (one-tailed paired t-test, p = 0.01, Cohen's D = 0.5), 80% (one-tailed paired t-test, p = 0.014, Cohen's D = 0.47), and 90% (one-tailed paired t-test, p = 0.013, Cohen's D = 0.5) AMT. For SCI, SICI was not present at 70% (one-tailed paired t-test, p = 0.105) or 80% AMT (one-tailed paired t-test, p = 0.082) but did demonstrate SICI at 90% AMT (one-tailed paired t-test, p = 0.0043, Cohen's D = 0.46). Similar to the analysis performed elsewhere,(Roy *et al.*, 2011) we subsequently tested differences between SCI and controls at 90% AMT and revealed no significant differences between the two groups (two-tailed paired t-test, p = 0.086). Group-averaged background EMG is shown in Figure 3.2C and two-way ANOVA revealed no effect of CS ($F_{(5,120)}$ = 1.017, p = 0.41) or GROUP ($F_{(1,24)}$ = 0.375, p = 0.55) or CS*GROUP interaction ($F_{(5,120)}$ = 2.049, p = 0.08). Figure 3.2D displays the group-averaged recruitment profile of SICI for all thirteen SCI and uninjured control limbs. Two-way ANOVA revealed no effect of CS ($F_{(2.06,49.42)}$ = 2.856, p = 0.066) or GROUP ($F_{(1,24)}$ = 1.197, p = 0.29) or CS*GROUP interactions ($F_{(2.06,49.42)}$ = 0.886, p = 0.42). For uninjured controls, *a priori* Bonferroni corrected t-tests revealed no presence of SICI at any of the three hypothesized CS intensities of 70% (one-tailed paired t-test, p = 0.04), 80% (one-tailed paired t-test, p = 0.04), and 90% (one-tailed paired t-test, p = 0.07) AMT. For SCI, SICI was not present at 70% (one-tailed paired t-test, p = 0.03) or 80% AMT (one-tailed paired t-test, p = 0.04) but did demonstrate SICI at 90% AMT (one-tailed paired t-test, p = 0.0009). There were no differences between SCI and control at 90% AMT (two-tailed paired t-test, p = 0.10).

*3.3.3 LICI*

Nine SCI limbs and nine controls were included in the analysis of the LICI experiment. Figure 3.3A displays representative LICI data from an uninjured control and a SCI participant displaying an increase in inhibition with increase in CS intensity for SCI and no LICI in control participants. $MEP_{halfmax}$ was significantly greater in controls than SCI (control: 47.13 ± 20.66 mV*ms, SCI: 16.09 ± 7.47 mV*ms, unpaired two-tailed t-test; p = 0.0017). The group-averaged recruitment profile of LICI graphing the normalized MEP data in control and SCI participants during an isometric contraction is shown in Figure 3.3B. Two-way ANOVA revealed no effect of CS ($F_{(2.46,39.29)}$ = 2.627, p = 0.074), but demonstrated significant effect of GROUP ($F_{(1,16)}$ = 4.353, p = 0.05) and CS*GROUP interaction ($F_{(2.46,39.29)}$ = 3.404, p = 0.035). *A priori* Bonferroni corrected t-tests test revealed LICI in SCI at CS intensities of 120% (one-tailed paired t-test, p = 0.003, Cohen's D = 0.48) and near significance at 130% (one-tailed paired t-test, p = 0.03) AMT. No LICI was observed in uninjured controls at either 120% (one-tailed paired t-test, p = 0.48) or 130% (one-tailed paired t-test, p = 0.44) AMT. Differences between controls and SCI were observed at 120% (one-tailed paired t-test, p = 0.012) and 130% AMT (one-tailed paired t-test, p = 0.012). Figure 3.3C displays the group-averaged background EMG for each CS intensity normalized to the background EMG of the unconditioned MEP. The two-way ANOVA performed for the background EMG revealed no effect of CS ($F_{(4,92)}$ = 1.319, p = 0.27), GROUP ($F_{(1,23)}$ = 0.957, p = 0.34) and no CS*GROUP interaction ($F_{(4,92)}$ = 0.032, p = 0.99). Figure 3.3D displays the group-averaged recruitment profile of LICI for all thirteen SCI and uninjured control limbs, showing the normalized MEP data in control and SCI participants during an isometric contraction. Two-way ANOVA revealed no significance of CS intensity ($F_{(4,92)}$ = 1.347, p = 0.26), but demonstrated significant effect of GROUP ($F_{(1,23)}$ = 5.248, p = 0.031) and significant

CS*GROUP interaction ($F_{(4,92)} = 6.835$, $p < 0.001$). Post-hoc Tukey's test revealed differences between the normalized MEP's of SCI and controls at CS intensities of 120 ($p = 0.001$) and 130% ($p < 0.001$) AMT. Follow-up paired two-tailed t-tests were performed at the different CS intensities to indentify the presence of the LICI circuit within either the control or SCI groups. Controls showed no presence of LICI at either 120 ($p = 0.66$) or 130% ($p = 0.60$) AMT. Conversely, SCI do demonstrate the LICI circuit at both 120 ($p = 0.007$) and 130% ($p = 0.028$) AMT.

### 3.4 DISCUSSION

In this study we observed higher AMT and lower $MEP_{halfmax}$ in the SCI participants compared to the uninjured controls. However, the relative background contraction was not different between the two groups. Due to the differences in AMT between the two groups, a subset of SCI data was used which allowed AMT matching such that group differences in SICI and LICI cannot be attributed to differences resulting from greater CS intensities in one group compared to the other. In SCI, SICI was evoked at a CS intensity of 90% AMT only. Uninjured controls demonstrated SICI at all three hypothesized CS intensities. Our findings confirm previous observations in the tibialis anterior muscle of controls and in chronic tetraplegia, where the range of SICI recruitment appears to be different (Roy *et al.*, 2011). With an isometric contraction of the FCR muscle, LICI remains intact in SCI but is absent within controls. Previous reports have observed LICI to occur in both uninjured and post-spinal cord injured participants at a very high CS intensity during an isometric contraction (Barry *et al.*, 2013). Contrary to these observations, our data demonstrate that none of the hypothesized suprathreshold CS intensities elicited LICI in uninjured controls while LICI was observed in SCI at the highest CS intensity tested. The

persistenceof LICI in SCI alludes to an altered function of this circuit in the presence of muscle activity.

*3.4.1 AMT and MEPs*

The increase in AMT (Davey *et al.*, 1999;Roy *et al.*, 2011;Freund *et al.*, 2011) and decrease in MEPs (Edwards *et al.*, 2013) are consistent with previous literature on the changes to these measurements following an injury to the spinal cord. These changes are likely attributed to the decreases in corticospinal output due to injury.

*3.4.2 SICI*

The magnitude of SICI in the contracted FCR muscle was not different between individuals with SCI and controls. However, we did observe that the range of CS intensities capable of evoking significant SICI within each group were different. In controls, CS intensities of 70, 80 and 90% AMT evoked significant SICI. In SCI, however, the only CS intensity to evoke significant SICI was 90% AMT. These data suggest that SICI in FCR may indeed be observed in individuals with SCI but requires a relatively high CS intensity. This data supports a previous finding whereby SICI was only observed in the TA muscle in individuals with SCI using a CS intensity at 80% AMT while a larger range of CS intensities (60-90% AMT ) evoked SICI in controls (Roy *et al.*, 2011). However, these authors also report greater SICI in controls versus SCI, an effect we did not observe. This difference can be attributed to the specific muscle tested (TA versus FCR). One mechanism to explain the high CS intensity required to evoke SICI in individuals with SCI relates to reductions in GABA concentration and/or $GABA_A$ mediated transmission. As reported elsewhere, damage to ascending pathways leads to reductions in GABA concentration in sensorimotor cortices (Levy *et al.*, 2002;Hendry & Jones, 1986;Welker

*et al.*, 1989) and this may contribute to neural plasticity following injury (Jacobs & Donoghue, 1991;Levy *et al.*, 2002). Such reductions in intracortical GABA concentration are likely to accompany SCI as suggested elsewhere (Smith *et al.*, 2000b;Roy *et al.*, 2011). Further, SICI is reduced in the presence of the $GABA_B$ receptor agonist baclofen (McDonnell *et al.*, 2006) through presynaptic $GABA_B$ receptors inhibiting $GABA_A$ interneurons(Sanger *et al.*, 2001), which is a common medication in our SCI population. Since SICI is reduced due to presynaptic $GABA_B$ autoinhibition (McDonnell *et al.*, 2006;Florian *et al.*, 2008;Werhahn *et al.*, 1999), baclofen may be responsible for our observation that SICI was only observed at 90% AMT CS intensity in participants with SCI. Therefore it is unclear whether the high CS requirement in SCI relates to decreases in GABA concentration and/or the inhibition of $GABA_A$ receptors via baclofen, and both of these sources may contribute to reductions in SICI in our SCI population.

*3.4.3 LICI*

Controls exhibited typical LICI behaviour in the presence of an isometric contraction; little to no inhibition caused by LICI (Sohn *et al.*, 2002) likely via the reduction of the cortical excitability controlling the LICI circuit. Compared to controls, participants with SCI showed greater LICI during contraction at the highest CS intensities used. One study investigated the contraction-related modulation of LICI in controls, in individuals with SCI taking baclofen and also in individuals with SCI not taking baclofen (Barry *et al.*, 2013). Their data demonstrates modulation of LICI such that the SCI group on baclofen showed modulations that parallel those in controls, namely a contraction-related reduction in LICI. In contrast, the SCI group not taking baclofen did not show contraction-related modulation of LICI. In our study, we exposed group differences that appear similar to the control versus non-baclofen group in Barry et al., (2013).

However, it is difficult to make direct comparisons with the latter report since within-group effects of contraction-related modulation are exposed rather than differences between groups.

One possible explanation for the presence of LICI in SCI compared to the absence of LICI in controls might be attributed to the effects of baclofen as almost all of the participants with SCI were taking baclofen. Baclofen is a $GABA_B$ agonist and serves to increase $GABA_B$ transmission within the motor cortex (McDonnell *et al.*, 2006). Increasing $GABA_B$ transmission likely results in an increase in LICI (McDonnell *et al.*, 2006). The mechanism of LICI involves $GABA_B$ receptor-mediated interneurons (McDonnell *et al.*, 2006), which act to inhibit I3- and later I-waves elicited by the TS (Nakamura *et al.*, 1997;Chen *et al.*, 1999b;Di Lazzaro *et al.*, 2002a), resulting in inhibition of the MEP (Paulus *et al.*, 2008). Facilitating these interneurons, by increasing $GABA_B$ transmission, results in a greater inhibition of the I3- and later I-waves, which results in decreased summation of the corticospinal volleys at the level of the spinal cord and a decreased MEP. Alternatively, it is possible that there are abnormalities in the movement-related modulation of LICI that may have arisen following an injury to the spinal cord. As the movement related-modulation of SICI has been hypothesized to be a result of changes to cortical excitability (Ridding *et al.*, 1995;Reynolds & Ashby, 1999), a similar mechanism can also be hypothesized for the movement-related modulation of LICI. Since SCI has been observed to result in reductions in GABA concentration within the sensorimotor cortices (Levy *et al.*, 2002;Hendry & Jones, 1986;Welker *et al.*, 1989), it is a possibility that SCI can also affect the excitability of the primary motor cortex.

*3.4.4 Technical Considerations*

One important issue to consider in TMS studies in SCI is the reduction in the corticospinal output elicited by TMS. We attempted to match the groups' unconditioned MEP by selecting an intensity that fell on the sensitive portion of the recruitment curve (i.e. $MEP_{halfmax}$). However, this resulted in higher absolute MEP values for the control group. Therefore, the effect of the CS on the TS may be different between groups, and may lead to a greater opportunity for conditioning effects to be observed in the group with the lower unconditioned MEP area. Therefore, one limitation is our findings in individuals with SCI may relate to the absolute unconditioned MEP area which was, compared to controls, smaller and therefore may have been more easily conditioned by the CS. Increasing the TS amplitude from 1 to 2 mV reduces LICI (100 ms) but does not alter SICI (Udupa *et al.*, 2010). It is unclear whether such changes occur in LICI using 150 ms ISI. However, the possibility remains that group differences in LICI may relate to larger and smaller TS MEP amplitudes obtained in controls and individuals with SCI, respectively (See Figure 1B). Additionally, other reports indicate that increasing the TS amplitude from 0.2 to 1 mV result in an increase in SICI strength (Daskalakis *et al.*, 2002;Sanger *et al.*, 2001;Wagle-Shukla *et al.*, 2009). However, increasing the TS intensity from 1 to 2 mV decreases SICI strength (Florian *et al.*, 2008;Ilic *et al.*, 2002). Last, one inherent difficulty in studying SCI involves the range of prescribed medications which cannot be withdrawn without caution. Therefore, we have studied individuals in their normally medicated state similar to other studies (Roy *et al.*, 2011;Roy *et al.*, 2010). Further, we did not have access to many participants with SCI who were not taking baclofen and thus were unable to explicitly study the contribution of baclofen to the SICI and LICI circuits. Future studies may consider assessing the relationship between the magnitude of SICI/LICI and baclofen dosage.

### 3.5 CONCL USION

The present study investigated LICI in the contracted FCR muscle in controls and individuals with SCI. Our main finding is that LICI is increased in individuals with SCI compared to controls and we suggest that baclofen and/or impairment in contraction-related modulation may provide plausible explanations for this effect. Further, the present research benefited from the use of a recruitment curve to elucidate the profile of intracortical inhibitory circuits in SCI, and we observed similarities and differences among our groups that may have been missed with the use of a single CS intensity. These findings have advanced our knowledge of the motor cortical changes that accompany chronic SCI and research focused on rehabilitative strategies in SCI populations may consider interventions focused on restoring contraction-related modulation of LICI for the ultimate purpose of improving arm function.

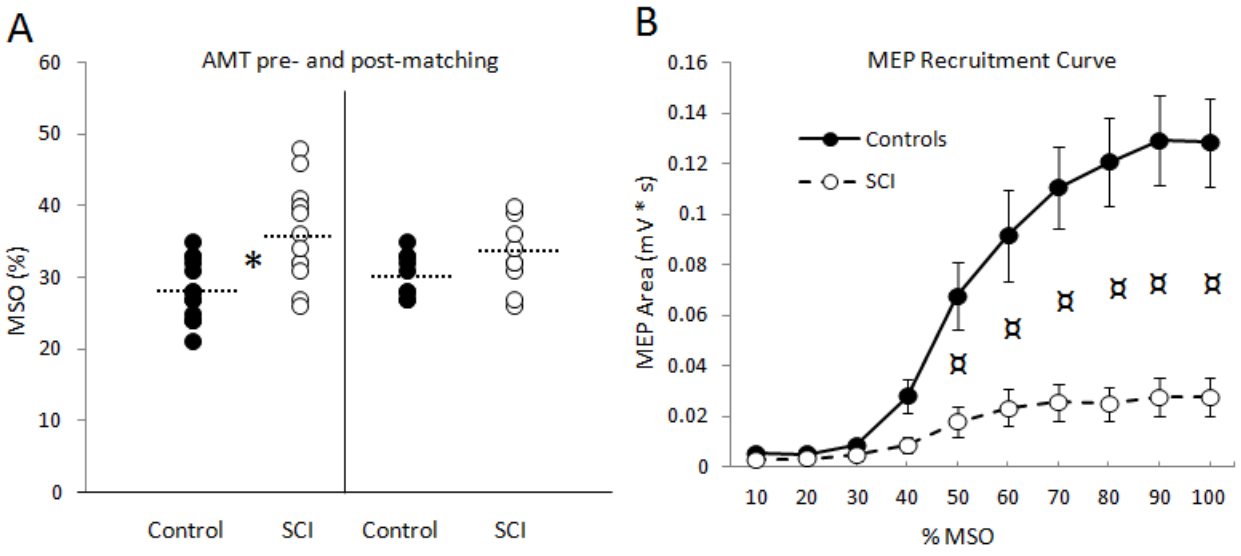


**Figure 3.1. A.** AMT of controls and SCI (*Left)*. Pre-matching showed significantly greater AMT in SCI than controls (*Right)*. Matching the AMT values between the two groups removed any effects caused by differences in CS intensity due to AMT on SICI and LICI. Asterisk represents

significant differences between the two groups. Dashed line represents the average AMT for either controls or SCI. **B.** MEP recruitment curves of AMT matched controls and SCI. Group-averaged MEP area data for all ten stimulator intensities. Asterisk represents differences between the control and SCI group observed at all intensities greater than 50% MSO.

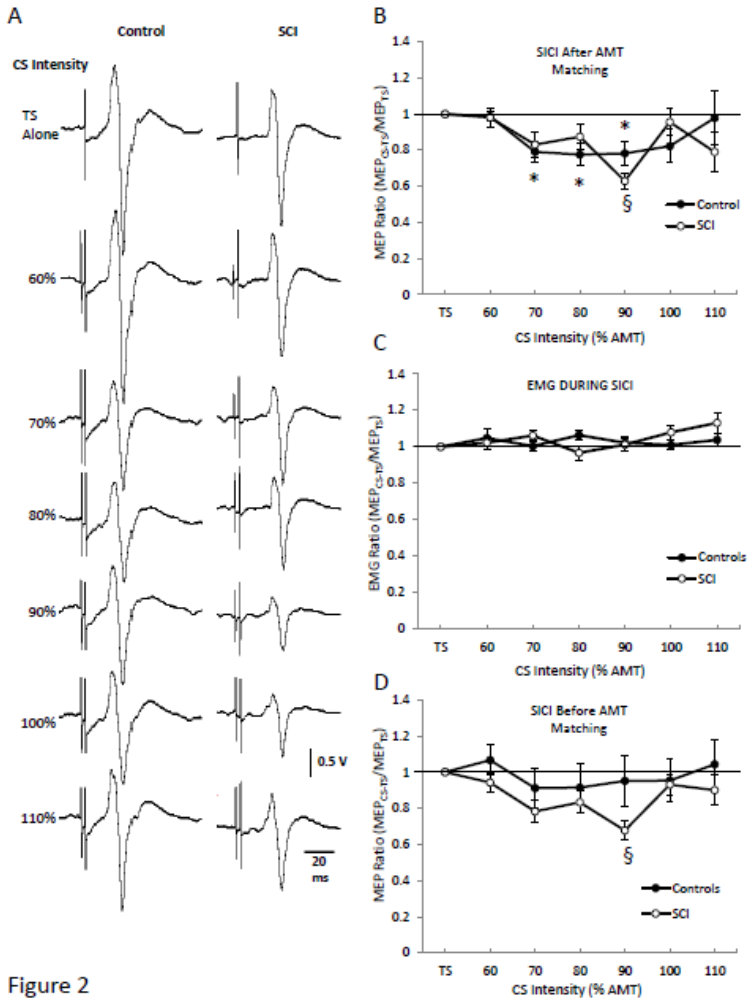


Figure 2

**Figure 3.2. SICI A.** Raw traces of SICI recruitment in controls and SCI. **B.** SICI recruitment curves of controls and SCI following AMT matching between groups. Group-averaged MEP data (with standard error) normalized to the unconditioned MEP for each of the CS intensities.

Symbols represent differences between the unconditioned and conditioned MEP within the control group (*), and within the SCI group (§) indicating presence of SICI. **C.** SICI background EMG of controls and SCI. Group-averaged EMG ratio data (with standard error) normalized to the EMG of the unconditioned state for all CS intensities. **D.** SICI recruitment curves of controls and SCI. Group-averaged MEP data (with standard error) normalized to the unconditioned MEP for each of the CS intensities. Group-averaged MEP data (with standard error) normalized to the unconditioned MEP for each of the CS intensities. Symbols represent differences between the unconditioned and conditioned MEP within the SCI group (§) indicating presence of SICI.

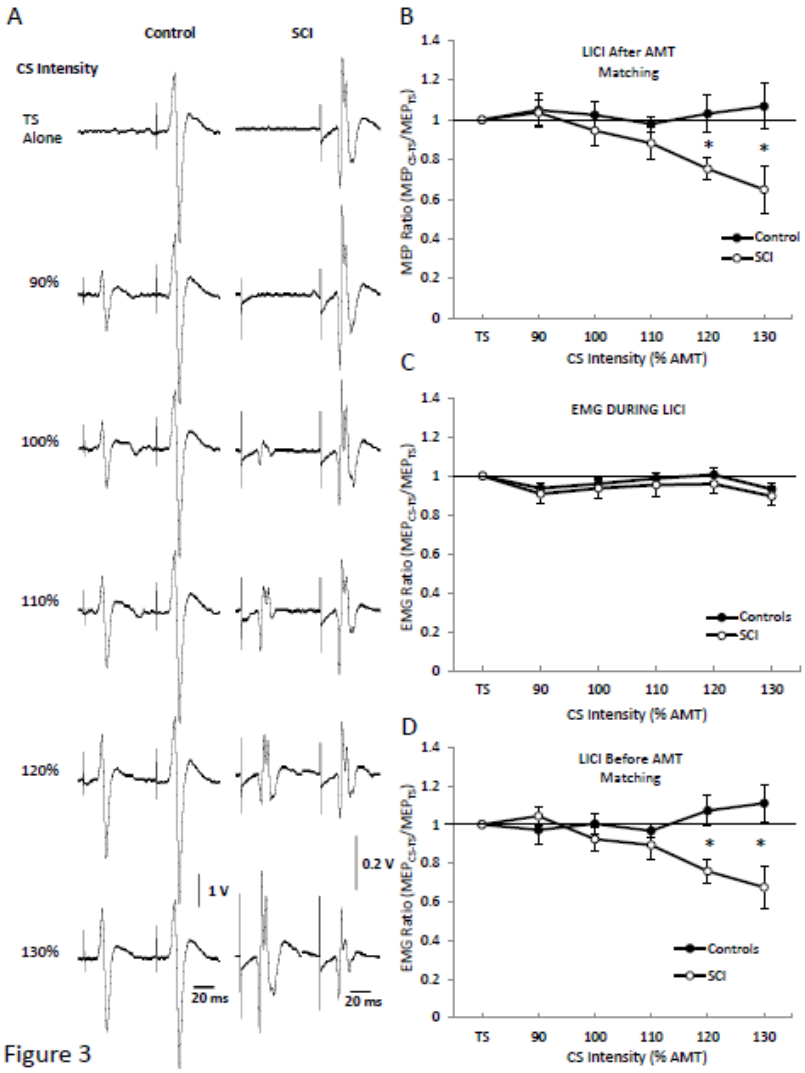


**Figure 3.3. LICI A.** Raw traces of LICI recruitment in controls and SCI. **B.** LICI recruitment curves of controls and SCI following AMT matching between groups. Group-averaged MEP data(with standard error) normalized to the unconditioned MEP for each of the CS intensities. Control MEP ratios are significantly different from SCI MEP ratios at 120% and 130%. Asterisk represents significant differences between the two groups. **C.** Active SICI background EMG of controls and SCI. Group-averaged EMG ratio data (with standard error) normalized to the EMG of the unconditioned state for all CS intensities. **D.** SICI recruitment curves of controls and SCI. Group-averaged MEP data (with standard error) normalized to the unconditioned MEP for each

of the CS intensities. Control MEP ratios are significantly different from SCI MEP ratios at 120% and 130%. Asterisk represents significant differences between the two groups.

Table 3.1

| Participant ID | Age | Injury Level | Years Post Injury | Cause of Injury | ASIA | Medications | AMT (MSO) |
|---|---|---|---|---|---|---|---|
| P001 Right | 27 | C4-C5 | 7 | Traumatic | C | Baclofen | 41% |
| P002 Right | 26 | C3 | 2 | Traumatic | C | Baclofen | 40% |
| P004 Left | 24 | C5-C6 | 2.5 | Traumatic | C | Baclofen, Soflax, Gabapentin, Pantoprazole, Senkot, Detrol | 26% |
| P004 Right | | | | | | | 27% |
| P006 Left | 38 | C5 | 14 | Traumatic | B | Axid, Collase, Ditropan, Baclofen | 39% |
| P006 Right | | | | | | | 36% |
| P007 Right | 27 | C3-C4 | 7 | Traumatic | B | Fentinal Patch, Pregabilin, Baclofen, T.Zanodine, Oxybutin, Hydromorphine | 32% |
| P008 Left | 22 | C5 | 9 | Traumatic | D | Benadryl, Percocet | 34% |
| P008 Right | | | | | | | 31% |

The age of subject, the number of years after the subject sustained a spinal cord injury (SCI) measured at the time of the experiment, and the cause of injury is shown. Sides tested are indicated as left or right FCR. Medications taken by subjects are also listed.

# Chapter 4: Experiment 2: Effects of continuous theta-burst stimulation on modulation of short-interval intracortical inhibition

## 4.1 INTRODUCTION

Plasticity effects at synapses dictate the amplitude and direction of subsequent plasticity at those same synapses (Abraham & Bear, 1996), a process known as metaplasticity. For example, long-term potentiation (LTP) at a synapse favours subsequent long-term depression (LTD) (Abraham, 2008;Abraham & Bear, 1996;Frey *et al.*, 1995) while LTD promotes subsequent LTP (Abraham & Bear, 1996;Abraham, 2008). This mechanism is thought to protect the cells from excess LTP and/or LTD and maintain balance between excitation and inhibition (Abraham, 2008). In humans, metaplasticity may be induced through repetitive transcranial magnetic stimulation (TMS) over the primary motor cortex (M1) by delivering two or more sequential protocols. A single cTBS protocol over M1 induces plasticity over M1 (i.e. LTD) and two sequential cTBS protocols over M1 can lead to metaplasticity (i.e. LTD-LTD leads to LTP) (Muller-Dahlhaus & Ziemann, 2015). The inter-protocol interval between the cTBS protocols is typically 15 minutes (Monte-Silva *et al.*, 2010;Fricke *et al.*, 2011) to elicit the greatest degree of change. A single protocol of cTBS delivered via TMS to human M1 induces a depression on the motor evoked potentials (MEPs) (Huang *et al.*, 2005;Ishikawa *et al.*, 2007;Talelli *et al.*, 2007;Zafar *et al.*, 2008;Murakami *et al.*, 2008;Doeltgen & Ridding, 2011;Goldsworthy *et al.*, 2012a;Wu *et al.*, 2012). While two sequential cTBS protocols facilitate the MEP (Murakami *et al.*, 2012). Using such plasticity interventions to revert altered neurophysiological measures may be a therapeutic approach in restoring proper function.

The effects of a single cTBS protocol can induce plasticity on the cortical inhibitory circuit short-interval intracortical inhibition (SICI) and can decrease (Murakami *et al.*, 2008;Goldsworthy *et al.*, 2013) or not change (Murakami *et al.*, 2012;Doeltgen & Ridding, 2011) this circuit. Similarly, two sequential cTBS protocols aimed to induce metaplasticity in M1 can increase (Murakami *et al.*, 2012), as well as decrease (Goldsworthy *et al.*, 2013) SICI. SICI is associated with movement initiation and control (Reynolds & Ashby, 1999;Sohn *et al.*, 2002;Ridding *et al.*, 1995) and alterations to this circuit may be responsible for motor deficits. Thus by restoring SICI back to regular function, it may also alleviate motor deficits.

CTBS delivered over primary somatosensory cortex (SI) is also effective at altering corticospinal output as measured by changes in the amplitude of MEPs. CTBS over SI facilitates MEPs (Jacobs *et al.*, 2014;Tsang *et al.*, 2014), although this facilitation may be relative to the orientation of the cTBS coil (Ishikawa *et al.*, 2007;Jacobs *et al.*, 2014). SICI, however, appears to be unaffected by cTBS over SI (Jacobs *et al.*, 2014). However, previous reports have shown that a single cTBS protocol over M1 was unable to change MEPs in some cases, but a paired cTBS protocol was able to demonstrate suppression of the MEP (Goldsworthy *et al.*, 2012b). We reason that a single cTBS protocol over SI may be insufficient at altering SICI but perhaps with two sequential cTBS stimulations over SI, the SICI may be more susceptible to change. Current research have been exploring SI as a potential cortical area for stimulation in restoring motor function (Guggenmos *et al.*, 2013) due to its projections to M1 (Rocco-Donovan *et al.*, 2011).

The purpose of the present study was to explore whether cTBS over SI demonstrates metaplasticity effects on neural circuitry within M1 including MEPs and SICI by comparing the

effects of $cTBS_{SI}$ and $cTBS_{SI\text{-}SI}$. Each participant also experienced $cTBS_{M1\text{-}M1}$ to confirm the anticipated metaplastic effects of cTBS when applied over this area (Murakami *et al.*, 2012). We confirm facilitatory effects on MEPs by cTBS-induced plasticity over SI and metaplasticity over M1. However, we observe a similar facilitatory effect on MEPs following the consecutive cTBS protocols over SI. These data indicate that there were no metaplastic-like effects on motor cortical output following $cTBS_{SI\text{-}SI}$. Additionally, we observed a cyclic pattern of change on the MEPs in the case of all three interventions where the largest change occurs between within the 5 – 25 min time range following stimulation.

## 4.2 METHODS

### *4.2.1 Participants*

Twelve right-handed healthy individuals (six females; mean age: 22.3 ± 2.6 years) participated in the study. All participants completed three experimental sessions separated by a minimum of one week. Written informed consent was obtained from all participants prior to testing. None of the participants had a history of neuropsychiatric disease or was on CNS-active drugs at the time of the experiments. All participants gave informed written consent prior to participation. The experiments were approved by the McMaster University Research Ethics Board and conformed to the *Declaration of Helsinki*.

### *4.2.2 Electromyography (EMG)*

Muscle activity was recorded using 9mm diameter Ag-AgCl surface electrodes with the active electrode being placed over the muscle belly of the first dorsal interosseous (FDI) and the reference electrode placed over the metacarpophalangeal joint of the second digit. The ground

electrode was placed over the medial styloid process at the wrist. EMG was amplified (1000 x gain), bandpass filtered (20 - 2.5kHz, Intronix Technologies Corporation Model 2024F, Bolton, Ontario, Canada) and digitized (5kHz, Micro 1401, Cambridge Electronics Design, Cambridge, UK). Signal software (v5, Cambridge Electronic Design Limited, Cambridge, UK) was used to acquire and analyze EMG data.

*4.2.3 Transcranial Magnetic Stimulation (TMS)*

TMS was delivered using a custom built 50 mm diameter figure-of-eight branding coil connected to a Magstim 200 stimulator (Magstim, Whitland, UK). The position and orientation of the coil was monitored throughout the experiment using Brainsight Neuronavigation (Rogue Research, Montreal, Canada) with optical sensors placed on the coil and the participant. The same TMS coil was used for all measures in the experiment. The TMS coil delivered a monophasic pulse over the optimal location to elicit MEPs in the relaxed FDI at 45° in relation to the parasagittal plane to induce a posterior-lateral to anterior-medial current in the cortex. The motor hotspot was defined at this location where all subsequent measures were taken. Resting motor threshold (RMT) was obtained at the motor hotspot and defined as the minimum stimulus intensity to evoke MEPs with amplitudes greater than or equal to 50 µV in 5 out of 10 consecutive trials (Siebner & Rothwell, 2003). Active motor threshold (AMT) was defined as the minimum stimulus intensity required to evoke MEPs with amplitudes greater than or equal to 250 µV in 5 out of 10 consecutive trials whilst the subject is maintaining an index finger abduction of ~10-15% of their maximum voluntary contraction.

*4.2.4 Continuous Theta-Burst Stimulation*

CTBS was applied using a Magstim Super Rapid stimulator (Magstim, Whitland, UK) connected to a figure-of-eight air cooled coil with the handle pointed 45° to the mid-sagittal plane to induce the first current in the cortex in the posterior-lateral to anterior-medial direction. CTBS protocol was delivered with 3 stimuli (bursts) applied at the intervals of 33.3 ms (30 Hz) repeated at 16.7 ms intervals (6 Hz) as described by Goldsworthy et al. (2012) and used elsewhere for stimulation of SI (Jacobs *et al.*, 2014;Tsang *et al.*, 2014).

CTBS was delivered at 80% AMT over the target location within M1 or SI. Within M1, cTBS was delivered over the FDI hotspot. For SI, cTBS was delivered over a position that measured 2 cm posterior to the M1 hotspot. The SI location was digitally measured and marked using Brainsight Neuronavigation.

*4.2.5 Motor evoked potentials (MEPs) and short-interval intracortical inhibition (SICI)*

MEPs were collected by delivering single TMS pulses over M1 at one of three intensities (40, 60, and 80% of the maximum stimulator output). Each intensity was delivered ten times and the orders of the stimulations were randomized. To elicit SICI, the test stimulus (TS) was preceded by the conditioning stimulus (CS) by 2 ms. The CS was set to 70, 80, 90, or 100% AMT and the TS was set to an intensity that evoked a MEP amplitude of ~ 1 mV in the right FDI. Each time block (described below) consisted of 10 conditioned trials (i.e. CS-TS) for each CS intensity and 10 unconditioned trials (i.e. TS alone) for a total of 50 randomized trials per block.

*4.2.6 Experimental Design*

Each session delivered one intervention: cTBS to SI ($cTBS_{SI}$), cTBS then cTBS over SI ($cTBS_{SI\text{-}SI}$), and cTBS then cTBS over M1 ($cTBS_{M1\text{-}M1}$). The inter-protocol interval between cTBS protocols was set at 15 minutes (Murakami et al., 2012). The order of the interventions was counter-balanced across participants. The experimental timeline is shown in Figure 1. RMT, MEPs, and SICI were recorded before ($T_0$) and at 5 - 25 ($T_1$), 30 - 50 ($T_2$), 55 - 75 ($T_3$) minutes following cTBS (Figure 1). RMT was recorded at all time points to observe potential changes in membrane excitability.

*4.2.7 Data Anaylsis*

MEP peak-to-peak amplitudes were averaged for each participant for each intensity at each time block. SICI was calculated as the ratio of the conditioned MEP over the unconditioned MEP (i.e. $MEP_{CS\text{-}TS}/MEP_{TS}$). The assumptions of sphericty were tested for all ANOVAs and Greenhouse-Geisser corrections were used if the assumptions were not met.

For $cTBS_{M1\text{-}M1}$, a one-way analysis of variance (ANOVA) using the within-subject factor TIME (4 levels; $T_0$, $T_1$, $T_2$, $T_3$) was performed for variables RMT and MEP. For MEPs, each intensity (40, 60 and 80% MSO) was analyzed independently. SICI was analyzed in a two-way ANOVA using within subject factors TIME (4 levels; $T_0$, $T_1$, $T_2$, $T_3$) and INTENSITY (4 levels; 70%, 80%, 90%, and 100% AMT). Subsequent one-way ANOVAs were performed separating the SICI data by intensity using within factor TIME (4 levels; $T_0$, $T_1$, $T_2$, $T_3$).

For the investigation of SI, a two-way ANOVA using the within-subject factors TIME (4 levels; $T_0$, $T_1$, $T_2$, $T_3$) and INTERVENTION (2 levels; $cTBS_{SI}$ and $cTBS_{SI\text{-}SI}$) was performed on dependent measures RMT and MEP. Similar to above, the three intensities were investigated. However, for each intensity, MEPs at $T_0$ were first compared between the two interventions to confirm they are not different. SICI was in a three-way ANOVA using within-subject factors TIME (4 levels; $T_0$, $T_1$, $T_2$, $T_3$), INTENSITY (4 levels; $T_0$, $T_1$, $T_2$, $T_3$), and INTERVENTION (2 levels; $cTBS_{SI}$ and $cTBS_{SI\text{-}SI}$).

To further examine the time course of change of each intervention, the average percent change in MEP size elicited by 80% MSO between each time point was investigated. The percent change in the MEP amplitude was calculated for each individual for each of the three intervals of time (i.e. % change from $T_0$ to $T_1$, % change from $T_1$ to $T_2$, and % change from T2 to $T_3$) for each intervention

A two-way ANOVA was performed using within-subject factors INTERVAL (3 levels; $T_0$ to $T_1$, $T_1$ to $T_2$, $T_2$ to $T_3$) and INTERVENTION (3 levels; $cTBS_{SI}$, $cTBS_{SI\text{-}SI}$, $cTBSs_{M1\text{-}M1}$).

Post-hoc Tukey's tests were conducted following significant effects in the ANOVA. Statistical significance for all tests was set at $p \leq 0.05$.

## 4.3 RESULTS

### $cTBS_{M1\text{-}M1}$

#### *4.3.1 RMT and MEPs*

RMT was unaltered following $cTBS_{M1\text{-}M1}$ ($F_{(3,24)} = 1.075$, $p = 0.378$) (see means in Table 1). Figure 4.2A displays the group-averaged MEP amplitudes of the $cTBS_{M1\text{-}M1}$ intervention at 40, 60, and 80% MSO. For 60% and 80% MSO, one-way ANOVA revealed a significant effect of TIME (60%; $F_{(1.579,12.631)} = 6.546$, $p = 0.015$, 80%; $F_{(3,24)} = 7.812$, $p = 0.001$) with greater MEPs at $T_1$, $T_2$ and $T_3$ compared to T0 (tukey's, $p < 0.05$). MEPs were unchanged by cTBS-cTBS at 40% MSO ($F_{(3,24)} = 1.114$, $p = 0.363$).

*SICI*

Figure 4.2B displays the group-averaged SICI of the $cTBS_{M1\text{-}M1}$ intervention with all CS intensities at each time block. Two-way ANOVA revealed an effect of INTENSITY ($F_{(1.64,13.119)} = 7.51$, $p = 0.009$) but no effect of TIME ($F_{(3,24)} = 0.453$, $p = 0.718$) or TIME * INTENSITY interaction ($F_{(9,72)} = 0.567$, $p = 0.820$). Subsequent one-way ANOVAs separating SICI by intensity revealed no effect of TIME at any of the four CS intensities (70%; $F_{(3,24)} = 0.166$, $p = 0.918$, 80%; $F_{(3,24)} = 0.727$, $p = 0.546$, 90%; $F_{(3,24)} = 0.818$, $p = 0.497$, 100%; $F_{(3,24)} = 0.986$, $p = 0.416$). These data indicate that SICI remains unchanged following $cTBS_{M1\text{-}M1}$.

**$cTBS_{SI\text{-}SI}$ and $cTBS_{SI}$**

*RMT and MEPs*

RMT was not significantly different between the $cTBS_{SI\text{-}SI}$ and $cTBS_{SI}$ interventions at $T_0$ (SI-SI: 39 ± 6.5 % MSO, SI: 40 ± 8.3 % MSO, paired two-tailed t-test; p = 0.141). Two-way ANOVA on RMT data revealed no effects (INTERVENTION: $F_{(1,8)} = 0$, p = 1.00; TIME:$F_{(3,24)} = 0.243$, p = 0.866; INTERVENTION*TIME:$F_{(3,24)} = 2.023$, p = 0.137) (see means in Table 1). MEPs were not different at $T_0$ between $cTBS_{SI\text{-}SI}$ and $cTBS_{SI}$ (40%; SI-SI: 0.90 ± 1.14 mV, SI: 1.09 ± 1.56 mV, two-tailed paired t-test; p = 0.567, 60%; SI-SI: 3.18 ± 1.95 mV, SI: 3.24 ± 2.33 mV, two-tailed paired t-test; p = 0.899, 80%; SI-SI: 3.52 ± 1.47 mV, SI: 4.16 ± 1.92 mV, two-tailed paired t-test; p = 0.100). Figure 4.3A displays the group-averaged MEP amplitudes of the $cTBS_{SI\text{-}SI}$ and $cTBS_{SI}$ interventions elicited by 40, 60, and 80% MSO. For MEPs at 80% MSO, two-way ANOVA revealed an effect of TIME ($F_{(1.331,10.648)} = 7.506$, p = 0.015) but no INTERVENTION ($F_{(1,8)} = 0.686$, p = 0.432) or INTERVENTION*TIME effects ($F_{(3,24)} = 0.316$, p = 0.814). Post-hoc Tukey's revealed greater MEPs at time points $T_2$ and $T_3$ compared to $T_0$ ($p < 0.05$) indicating that MEPs are facilitated by both $cTBS_{SI}$ and $cTBS_{SI\text{-}SI}$ and there were no differences between the two interventions. No effects were present for MEPs at 40% (INTERVENTION; $F_{(1,8)} = 0.189$, p = 0.675, TIME; $F_{(3,24)} = 0.35$, p = 0.79, INTERVENTION*TIME; $F_{(1.171,9.365)} = 0.591$, p = 0.487) or 60% MSO (INTERVENTION, $F_{(1,8)} = 0.018$, p = 0.896, TIME; $F_{(1.649,13.189)} = 1.827$, p = 0.201, INTERVENTION*TIME; $F_{(3,24)} = 0.403$, p = 0.752).

*SICI*

Figure 4.3B displays the group-averaged SICI of the SI-SI and SI interventions with all CS intensities. The unconditioned MEPs were not significantly different between $cTBS_{SI\text{-}SI}$ and $cTBS_{SI}$ (SI-SI: 1.54 ± 0.42 mV, SI: 1.37 ± 0.53 mV, paired two-tailed t-test, p = 0.433) (see means in Table 2). Three-way ANOVA revealed no significant effects (INTERVENTION; $F_{(1,8)}$ = 0.859, p = 0.381; TIME: $F_{(3,24)}$ = 0.515, p = 0.676; INTENSITY: $F_{(1.36,10.88)}$ = 2.037, p = 0.182; INTERVENTION*TIME: $F_{(3,24)}$ = 0.833, p = 0.489; INTERVENION*INTENSITY: $F_{(1.496,11.965)}$ = 0.264, p = 0.71; TIME*INTENSITY: $F_{(9,72)}$ = 0.905, p = 0.526; INTERVENTION*TIME*INTENSITY: $F_{(9,72)}$ = 1.046, p = 0.413).

**Time Course of Change**

All three interventions showed increases in MEP sizes elicited at 80% MSO. Therefore, subsequent analyses was performed to further examine the timecourse of MEPs changes elicited at 80% MSO. Figure 4.4A displays the group averaged percent change of MEPs elicited by 80% MSO for all three interventions for each time interval. Two-way ANOVA revealed an effect of INTERVAL ($F_{(2,16)}$ = 5.465, p = 0.016) but no INTERVENTION ($F_{(2,16)}$ = 0.747, p = 0.49) or INTERVENTION*INTERVAL interaction ($F_{(4,32)}$ = 1.338, p = 0.277). Post-hoc Tukey's revealed significantly greater percent changes during the $T_0$-$T_1$ inter than between $T_1$-$T_2$. These data indicate that increases in MEPs occur predominantly during the first 25 minutes following the intervention and that these increases subside significantly between 30 and 50 minutes.

To further examine the timecourse of changes on an individual basis, the individual MEP data at 80% MSO was depicted in Figure 4.4B. In the case of $cTBS_{M1\text{-}M1,}$ all participants show increases in MEP amplitude from $T_0$ to $T_{1.}$ Then the participants are split; half show further increases and the other half show decreases from $T_1$ to $T_2$. From there, most participants continue show increases in MEP amplitudes from $T_2$ to $T_3$. Similarly, $cTBS_{SI\text{-}SI}$ show most participants increase in MEP amplitude from $T_0$ to $T_{1.}$ Followed by half the participants showing increases and the other half showing decreases from $T_1$ to $T_2$. Then a majority of the participants increased in amplitude from $T_2$ to $T_3$. In the case of SI plasticity, most participants show minimal increases or even decreases to MEP amplitude from $T_0$ to $T_1$. The MEP amplitudes do not appear to show many changes from $T_1$ to $T_2$. From $T_2$ to $T_3$, the participants appear to be divided with half showing further increases and the other half showing decreases in MEP amplitudes. In summary, it appears that the $cTBS_{M1\text{-}M1}$ resulted in large increases in MEP sizes primarily within the $T_0 - T_1$ interval, $cTBS_{SI\text{-}SI}$ resulted in large increases within both the $T_0 - T_1$ and $T_2 - T_3$ interval, and $cTBS_{SI}$ demonstrated the largest increases within the $T_2 - T_3$ interval.

## 4.4 DISCUSSION

Our study confirmed previous findings of $cTBS_{M1\text{-}M1}$ and $cTBS_{SI}$ demonstrating increases in MEP amplitude in both instances. However, we did not observe changes in intracortical inhibition as measured through SICI in either intervention platform. It also revealed two novel findings regarding $cTBS_{SI\text{-}SI}$. First, there were no metaplastic-like effects on corticospinal output and SICI following the intervention protocol. Second, the majority of changes for all three interventions predominantly occur between within the 5 – 25 minute time range following the

final cTBS protocol. These data suggest plasticity on a remote area known to have influence on M1 produces changes to motor cortical output but not intracortical inhibition and that the cortical outputs do not demonstrate metaplasticity as seen in the case of $cTBS_{M1\text{-}M1}$. Additionally, cTBS effects appear to follow a cyclic trend of change whereby 5 – 25 minutes post-intervention shows large changes while 30 – 50 minutes post-intervention show very small changes to MEP amplitude.

### *4.4.1 $cTBS_{M1\text{-}M1}$*

The observed increase in MEP amplitudes following $cTBS_{M1\text{-}M1}$ support previous findings (Murakami *et al.*, 2012) but conflicts with other reports of inhibition of MEPs with a similar inter-intervention interval (Gamboa *et al.*, 2011). Similar to the MEP changes reported by Murakami et al. (2012), our study demonstrated increases in MEPs elicited by larger stimulator intensities (i.e. 60 and 80% MSO) facilitation while they showed also facilitation with larger intensities needed to elicit 1 mV or larger. The degree of increase is also similar with the two studies (i.e. approximately 40% increase in amplitude).

Additionally, we observed no changes to SICI following $cTBS_{M1\text{-}M1}$ partially in agreement with the results observed from Murakami et al., (2012), the same $cTBS_{M1\text{-}M1}$ protocol parameter as the current study (i.e. 80% AMT cTBS intensity) demonstrated no changes to SICI but a low intensity priming cTBS (i.e. 70% AMT cTBS intensity) demonstrated increases (Murakami *et al.*, 2012) to SICI. Murakami et al., (2012) observed no changes to SICI following the same protocol as the one used in the present study. This is in contrast to the results reported by Goldsworthy et al., (2013), demonstrating decreases to SICI following the use 70% RMT as the stimulation intensity in the cTBS protocol. Our results along with previous findings reveal a

large degree of variability regarding the effects of cTBS plasticity and metaplasticity on M1 and explain the different observations seen in these cases. Other sources demonstrate the limited test-retest reproducibility of $cTBS_{M1}$ on changes in MEPs (Vernet *et al.*, 2014). Similarly, our qualitative analysis on individual participants' responses to $cTBS_{M1\text{-}M1}$ confirms the variability of the protocol.

### *4.4.2 $cTBS_{SI}$ and $cTBS_{SI\text{-}SI}$*

The purpose of the study was to investigate the differential effects of $cTBS_{SI}$ and $cTBS_{SI\text{-}SI}$ on M1 output and intracortical inhibition within M1. We demonstrated that both protocols resulted in increases in MEP amplitudes in similar manners and neither protocol demonstrated any changes to SICI. The results of single cTBS stimulation over SI is in accordance with previous findings demonstrating increases in MEP amplitude (Ishikawa *et al.*, 2007;Jacobs *et al.*, 2014;Tsang *et al.*, 2014) which show trends towards continual increase over time and no effects on SICI (Jacobs *et al.*, 2014). It was hypothesized that $cTBS_{SI\text{-}SI}$ would demonstrate metaplastic effects (i.e. a decrease in MEP amplitude). We suspect that regardless of metaplasticity occurring within SI following $cTBS_{SI\text{-}SI}$, the effects do not translate to motor cortical output but rather continue to produce facilitatory effects in M1.

### *4.4.3 Percent Change*

Our results revealed that the largest degree of change in MEPs occur within the 5 – 25 min interval following the delivery of the last cTBS protocol in all three interventions. Conversely, the 30 – 50 min interval shows a minimal degree of change. This demonstrates the immediate effects of the cTBS protocol as observed previously (Gamboa *et al.*, 2011;Goldsworthy *et al.*,

2012a). Additionally, there appears to be a recline in MEP facilitation at the last time interval (i.e. 55 – 75 min), alluding to a cyclic cTBS effect which diminishes after each cycle. This cyclic effect has also been observed elsewhere (Vernet *et al.*, 2014) and also observed in tactile perception of the hand following cTBS to SI (Rai *et al.*, 2012).

### *4.4.4 Limitations*

The primary confound of this work would be the possibility of current spread from $cTBS_{SI\text{-}SI}$ or $cTBS_{SI}$ to M1 thus producing the effects observed in MEP changes. However, the focality of the Magstim 70 mm double coil used to induce cTBS in the study indicate a current induced with a radius of 1. 6 cm (calculated by $A = \pi r^2 s$, A = area, r = radius) (Thielscher & Kammer, 2004) and these data were obtained using an intensity of 120% of resting motor threshold. Our stimulation was delivered at an intensity of 80% AMT and it is unlikely that spatial spread of current can account for the observed changes.

### *4.4.5 Significance*

The use of cTBS protocols to stimulate over SI has been able to produce facilitatory effects on the MEP size with no significant difference between $cTBS_{SI\text{-}SI}$ and $cTBS_{SI}$. With the reduction of MEPs in clinical populations such as SCI (Smith *et al.*, 2000b) and amyothrophic lateral sclerosis (Khedr *et al.*, 2011) and the association between MEPs and motor cortical output, an intervention capable of increasing MEPs in these individuals may be able to improve the poor motor output. In particular, SI in clinical populations known to be associated with poor motor output such as Parkinson's disease have shown reductions in SI activation (Foki *et al.*, 2015).

Additionally, stimulation over SI (Havrankova *et al.*, 2010) and restoring connections between M1 and SI (Guggenmos *et al.*, 2013) have shown benefits on regaining motor function.

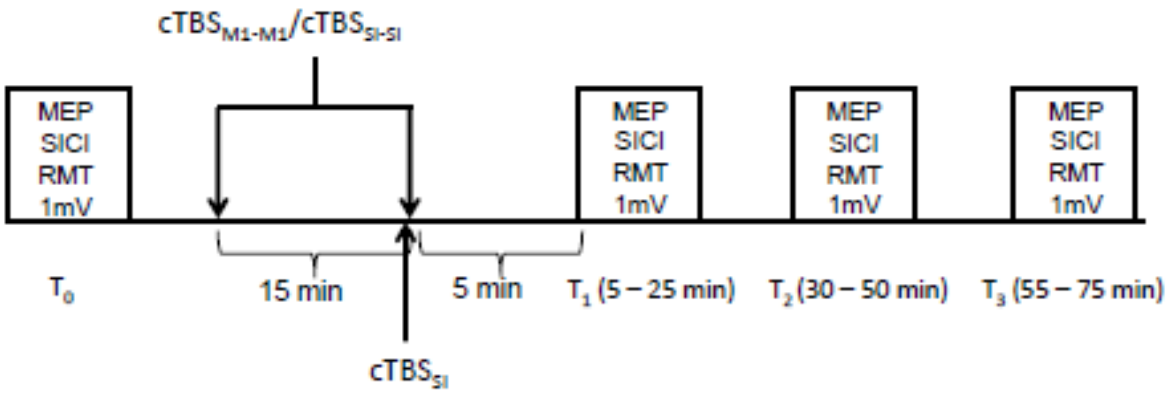


**Figure 4.1.** Experiment Timeline for experiment, cTBS$_{M1-M1}$, cTBS$_{SI-SI}$, or cTBS$_{SI}$ was delivered in the same group of participants. Measures of MEP, RMT, and SICI were acquired from the right FDI before ($T_0$) and at 5 – 25 minutes (T=), 30 – 50 minutes ($T_2$), and 55 – 75 minutes ($T_3$) following final cTBS protocol.

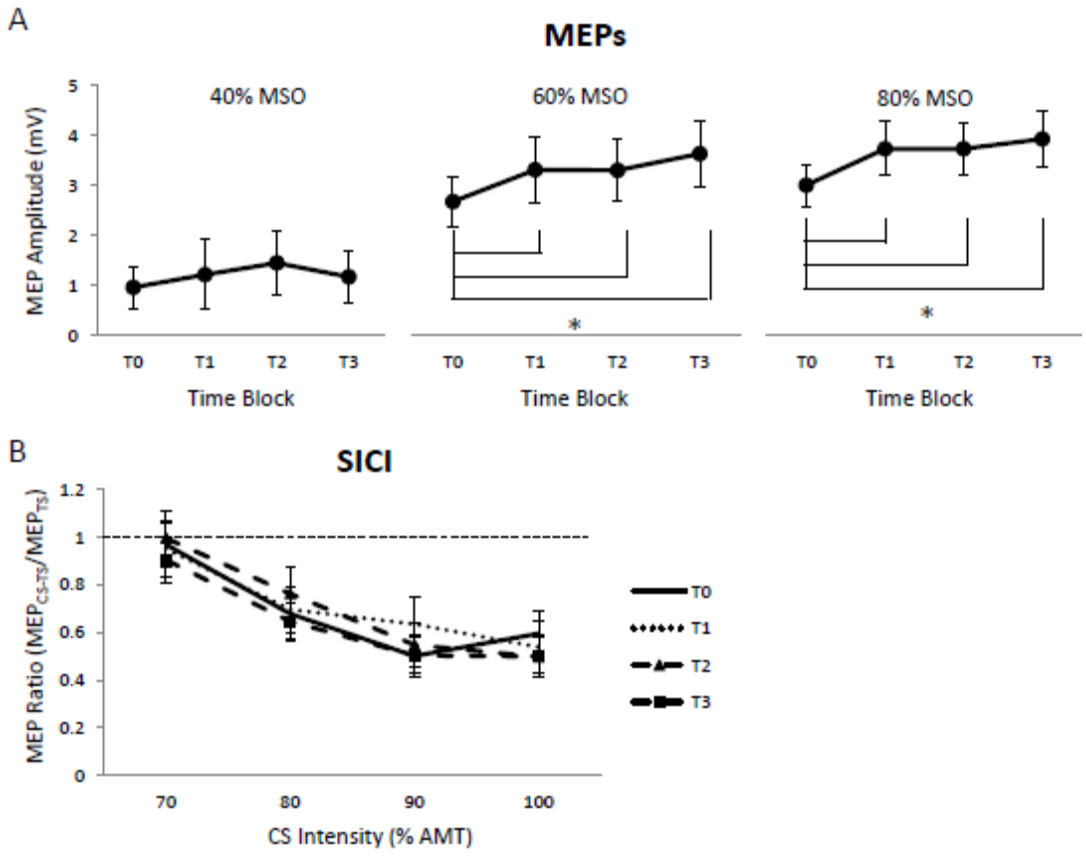


**Figure 4.2 A.** Group-averaged MEP amplitude elicited from all three stimulation intensities following $cTBS_{M1\text{-}M1}$ at pre-cTBS, 5-25 minutes, 30-50 minutes and 55-75 minutes for the right first dorsal interosseus muscle. An asterisk between time blocks signifies significantly greater MEP sizes at later time blocks compared to pre-cTBS. Significant differences were tested at $p < 0.05$. **B.** Group-averaged SICI recruitment curves measured using 4 different conditioning stimulus intensities normalized to the unconditioned MEP.

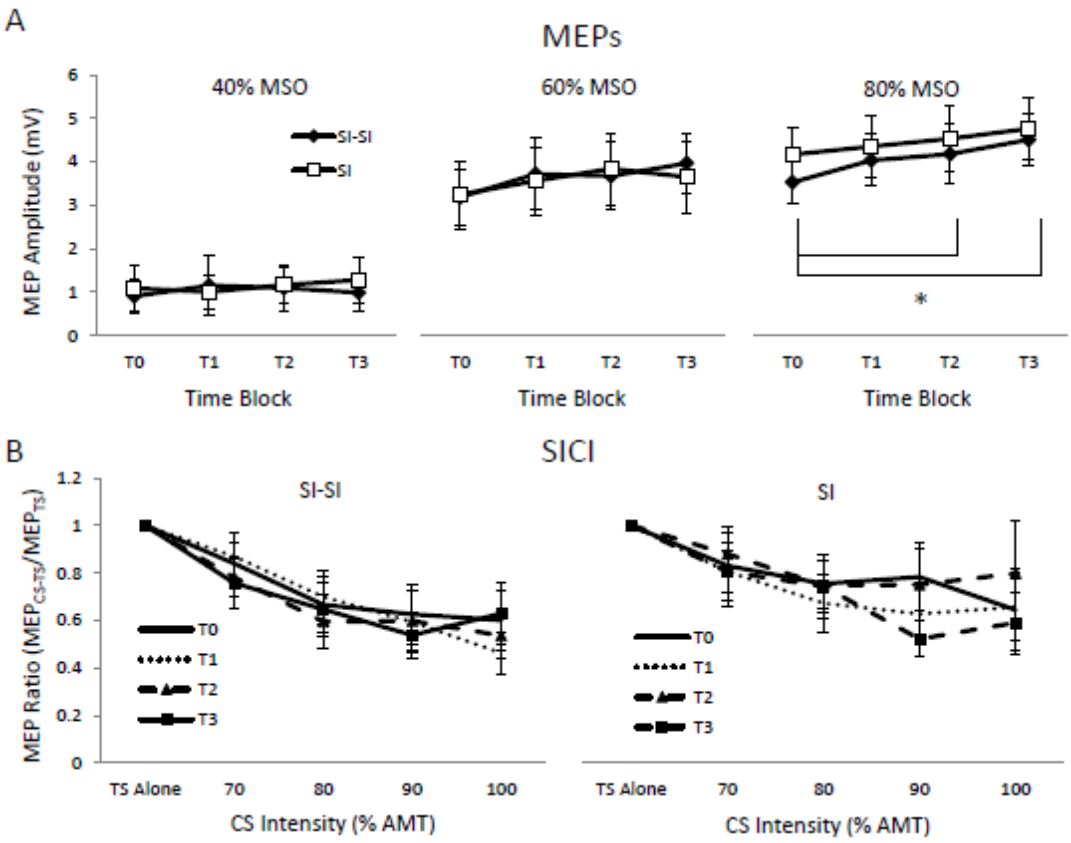


**Figure 4.3.A.** Group-averaged MEP amplitude elicited from all three stimulation intensities following $cTBS_{SI\text{-}SI}$ and $cTBS_{SI}$ at pre-cTBS, 5-25 minutes, 30-50 minutes and 55-75 minutes for the right first dorsal interosseus muscle. An asterisk between time blocks signifies significantly greater MEP sizes at later time blocks compared to pre-cTBS. Significant differences were tested at $p < 0.05$. **B.** Group-averaged SICI recruitment curves measured using 4 different conditioning stimulus intensities normalized to the unconditioned MEP. *Left* data from $cTBS_{SI\text{-}SI}$ and *Right* data from $cTBS_{SI}$.

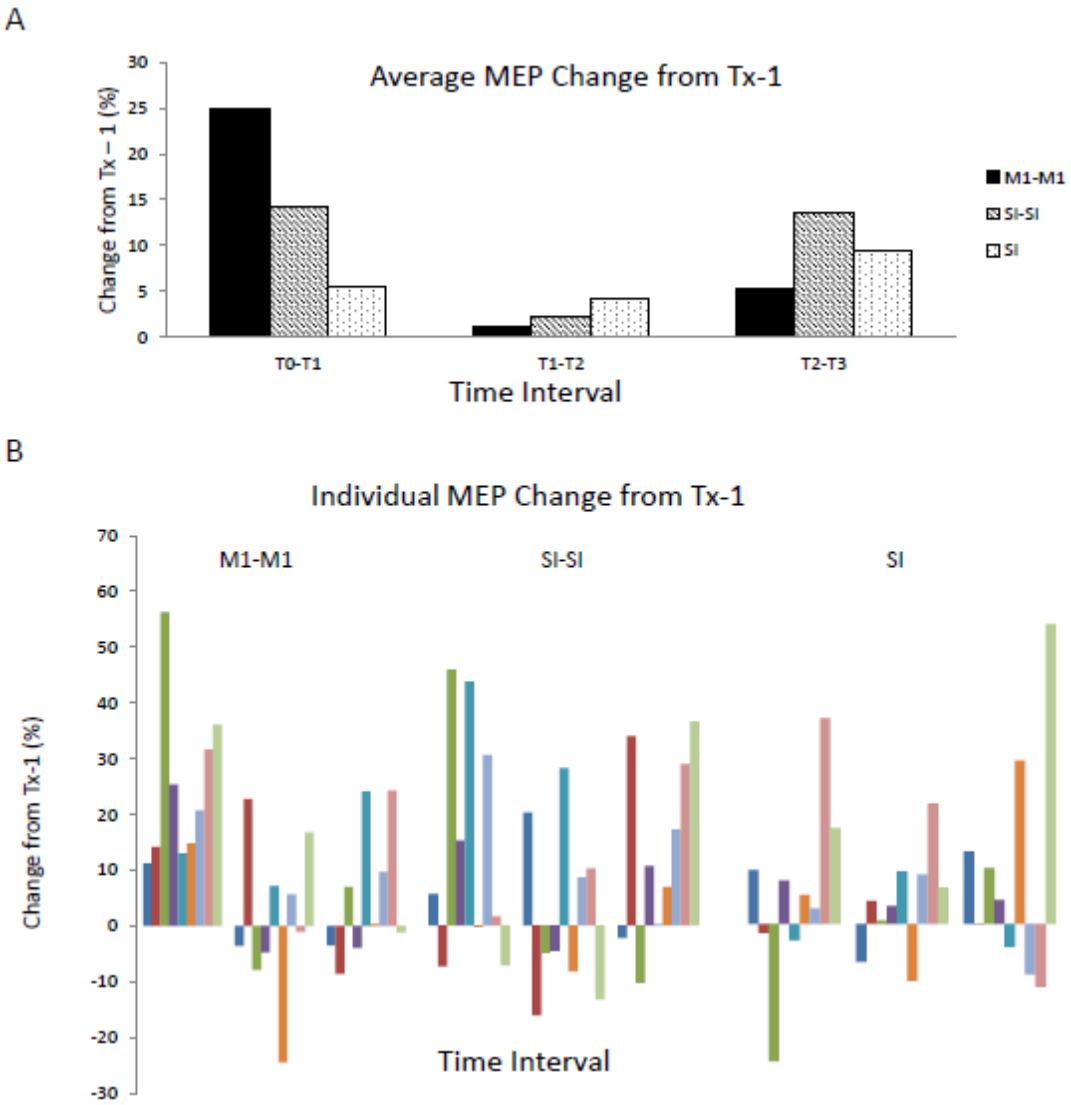


**Figure 4.4.A.** Group-averaged MEP change for MEPs elicited by 80% MSO for all three interventions between time intervals $T_0$-$T_1$, $T_1$-$T_2$, and $T_2$-$T_3$. Asterisk signifies significantly greater MEP change from $T_0$-$T_1$ than from $T_1$-$T_2$. **B.** Individual MEP change for MEPs elicited by 80% MSO for $cTBS_{M1\text{-}M1}$ *Left*, $cTBS_{SI\text{-}SI}$ *Middle*, and $cTBS_{SI}$ *Right*,

Table 4.1 - RMT

| Time | T0 | | | T1 | | | T2 | | | T3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Protocol | M1-M1 | SI-SI | SI | M1-M1 | SI-SI | SI | M1-M1 | SI-SI | SI | M1-M1 | SI-SI | SI |
| P1 | 33 | 34 | 34 | 34 | 32 | 31 | 34 | 32 | 32 | 33 | 33 | 33 |
| P2 | 35 | 34 | 34 | 36 | 38 | 34 | 34 | 40 | 34 | 37 | 41 | 33 |
| P3 | 32 | 34 | 33 | 30 | 33 | 31 | 30 | 33 | 29 | 30 | 33 | 31 |
| P4 | 40 | 41 | 47 | 38 | 41 | 47 | 38 | 41 | 48 | 40 | 42 | 47 |
| P5 | 42 | 42 | 42 | 41 | 42 | 40 | 40 | 43 | 41 | 41 | 40 | 40 |
| P6 | 34 | 34 | 35 | 35 | 33 | 38 | 36 | 35 | 36 | 35 | 36 | 34 |
| P7 | 50 | 46 | 47 | 47 | 50 | 45 | 43 | 51 | 46 | 47 | 50 | 46 |
| P8 | 34 | 33 | 33 | 31 | 32 | 43 | 32 | 30 | 34 | 33 | 31 | 34 |
| P9 | 50 | 51 | 56 | 53 | 51 | 70 | 54 | 52 | 52 | 55 | 52 | 53 |

Table 4.2 - 1 MV

| Time | T0 | | | T1 | | | T2 | | | T3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Protocol | M1-M1 | SI-SI | SI | M1-M1 | SI-SI | SI | M1-M1 | SI-SI | SI | M1-M1 | SI-SI | SI |
| P1 | 40 | 39 | 40 | 42 | 38 | 39 | 40 | 38 | 38 | 40 | 38 | 38 |
| P2 | 45 | 47 | 43 | 45 | 50 | 41 | 43 | 49 | 40 | 46 | 50 | 39 |
| P3 | 35 | 40 | 35 | 35 | 38 | 33 | 34 | 34 | 34 | 35 | 34 | 34 |
| P4 | 53 | 54 | 59 | 53 | 55 | 59 | 54 | 54 | 59 | 52 | 54 | 59 |
| P5 | 47 | 51 | 51 | 47 | 50 | 46 | 49 | 50 | 49 | 49 | 49 | 49 |
| P6 | 43 | 41 | 43 | 44 | 42 | 43 | 44 | 42 | 45 | 44 | 44 | 42 |
| P7 | 74 | 56 | 59 | 67 | 55 | 59 | 67 | 61 | 59 | 66 | 64 | 59 |
| P8 | 41 | 42 | 41 | 41 | 42 | 43 | 41 | 42 | 42 | 41 | 42 | 44 |
| P9 | 67 | 69 | 68 | 67 | 67 | 70 | 67 | 67 | 70 | 67 | 67 | 70 |

# Chapter 5: General Discussion

## 5.1 Summary of Experiments

Experiment 1 aimed to probe changes in GABAergic function by characterizing the recruitment curves of MEPs, SICI, and LICI in the FCR muscle in participants with chronic SCI with injuries between C3 – C7. AMT was greater in, and MEP sizes were lower in individuals with SCI compared to uninjured controls. SICI magnitude was not different between groups although the range of CS intensities to evoke SICI was unique to each group. LICI was reduced in the control group during active contraction and remained present in participants with SCI. These results indicate that there exists an alteration to cortical spinal output as well as intracortical inhibition associated with motor output and control and in people with cervical SCI.

Experiment 2 aimed to explore the potential changes to SICI and MEPs with cTBS stimulation over M1 and SI and whether those changes are subject to metaplasticity through the use of two sequential cTBS protocols over cortical areas. Single and double cTBS protocols over SI increased MEPs evoked at high intensities (80% of maximum stimulator output). Similarly, double cTBS protocol over M1 increased MEPs evoked at moderate and high intensities (60 and 80% of maximum stimulator output). There were no changes to SICI following any of the three interventions at any of the conditioning stimulus intensities. These findings indicate that MEP amplitude is influenced by cTBS over SI and that two sequential cTBS protocols over SI did not produce metaplastic effects on MEPs but rather led to similar increases as that observed with a single protocol of cTBS. The degree of change on MEPs as a result of all three interventions appear to follow a cyclic pattern with the largest increases occurring in the first 25 minutes and the smallest increase occur between 30 and 50 minutes.

### 5.2 MEP alterations following SCI and cTBS

In Experiment 1, MEP areas within our participants with SCI were significantly reduced compared to age-matched uninjured controls at all intensities greater than 50% MSO. This reduction is consistent with previous reports of MEP alterations (Edwards *et al.*, 2013) and is likely associated with the decreases in corticospinal output and the integrity of the corticospinal tract (Kobayashi *et al.*, 2003) following injury. Experiment 2 demonstrated increases in MEP amplitude following all three cTBS interventions: $cTBS_{M1\text{-}M1}$, $cTBS_{SI\text{-}SI}$, and $cTBS_{SI}$. Both the $cTBS_{M1\text{-}M1}$ and $cTBS_{SI}$ effects on MEP amplitudes are consistent with previous reports (Murakami *et al.*, 2012;Ishikawa *et al.*, 2007;Jacobs *et al.*, 2014;Tsang *et al.*, 2014). A single protocol of cTBS is thought to induce LTD-like plasticity while two sequential cTBS protocols leads to metaplasticity driving LTP-like plasticity in the opposite direction.

No studies have performed any cTBS protocols over M1 or SI in order to characterize potential changes to MEP sizes in individuals with SCI. By applying any of the three cTBS protocols from Experiment 2, any increases in MEP sizes may directly alter corticospinal output and potentially enhance motor output in individuals with SCI. Not all three interventions produce the same degrees of change in MEPs (i.e. $cTBS_{M1\text{-}M1}$: ~31%; $cTBS_{SI\text{-}SI}$: ~28%; $cTBS_{SI}$: ~14%). Two of the protocols, $cTBS_{M1\text{-}M1}$ and $cTBS_{SI\text{-}SI}$, produce large increases in MEP sizes while $cTBS_{SI}$ increases are slightly less. The stronger interventions involving two sequential protocols may be more suitable for potential application in a clinical population since a larger in increase in MEP may be more likely to increase motor cortical output.

### 5.3 SICI Alterations following SCI and cTBS

SICI is a motor cortical circuit (Di Lazzaro *et al.,* 1998) thought to be mediated through $GABA_A$ receptors (Ziemann *et al.*, 1996b;Ziemann *et al.,* 1996a;Ilic *et al.*, 2002). In Experiment 1, it was revealed that the magnitude of SICI did not decrease but rather the window to elicit SICI was reduced in individuals with SCI compared to uninjured controls, thereby signifying a reduction in the degree of cortical inhibition within these participants. I hypothesize this reduction in inhibition to be associated with muscle spasticity, common in incomplete spinal cord injury (Skold *et al.*, 1999). In Experiment 2, I sought to demonstrate increases in SICI in uninjured control participants using $cTBS_{M1\text{-}M1}$ and explore the potential $cTBS_{SI\text{-}SI}$ has on SICI although $cTBS_{SI}$ has been shown to produce no changes to SICI (Jacobs *et al.*, 2014). In all three protocols, there were no observable changes to SICI at any measured time points. Previous research has demonstrated both increases (Murakami *et al.,* 2012) and decreases (Goldsworthy *et al.*, 2013) to SICI with $cTBS_{M1\text{-}M1}$.

An increase in SICI through the plasticity and/or metaplasticity within individuals with SCI may increase intracortical inhibition and potentially reduce muscle spasticity in these individuals. However, we were unable to reproduce any changes to SICI with the protocol used in Experiment 2 with any of our protocols. One reason for this could be the protocol parameters used in Experiment 2 compared to others who have demonstrated changes to the SICI circuit. In both instances, a burst frequency of 50 Hz was used as opposed to 30 Hz from Experiment 2. Perhaps this frequency enables for a more susceptible change to SICI over M1 and SI.

### 5.4 Model for alterations to motor cortical output and inhibition after SCI

Figure 6.1 depicts the circuitry involved in the observed result of SICI, LICI, and MEP changes following SCI. Interneurons which act to inhibit the late I-waves (Di Lazzaro *et al.*, 1998) act through $GABA_A$ receptors (Ziemann *et al.*, 1996b). The reductions in SICI seen in individuals with SCI compared to uninjured controls to be the result of a reduction in the neurotransmitter GABA and reducing the ability for the interneurons responsible for SICI to act on the late I-waves. This removes the suppressive effects on the I3 interneuron and thus reducing SICI. Similarly, LICI occurs as a result of inhibitory interneurons acting on the later I-waves (Chen *et al.*, 1999b;Di Lazzaro *et al.*, 2002a) mediated through $GABA_B$ receptors (McDonnell *et al.*, 2006). The increase seen in LICI is likely due to the drug baclofen, which a majority of the participants were taking at the time of the experiment. Baclofen is a $GABA_B$ agonist and acts to increase $GABA_B$ transmission (McDonnell *et al.*, 2006) thus increasing LICI. MEPs were decreased due to the reductions in corticospinal output as a result of injury.

### 5.5 Model for alterations to motor cortical output by cTBS.

Figure 6.2 depicts the circuitry involved in the observed result of SICI and MEP changes following three cTBS interventions stimulated over M1or SI. None of the three interventions observed changes to SICI at any time point and were not sufficient at altering the GABA activity by the inhibitiory interneuron acting on the later I-waves (Di Lazzaro *et al.*, 1998). All three interventions were able to increase the size of the MEP. All three interventions were able to increase the excitability of the motor cortical output.

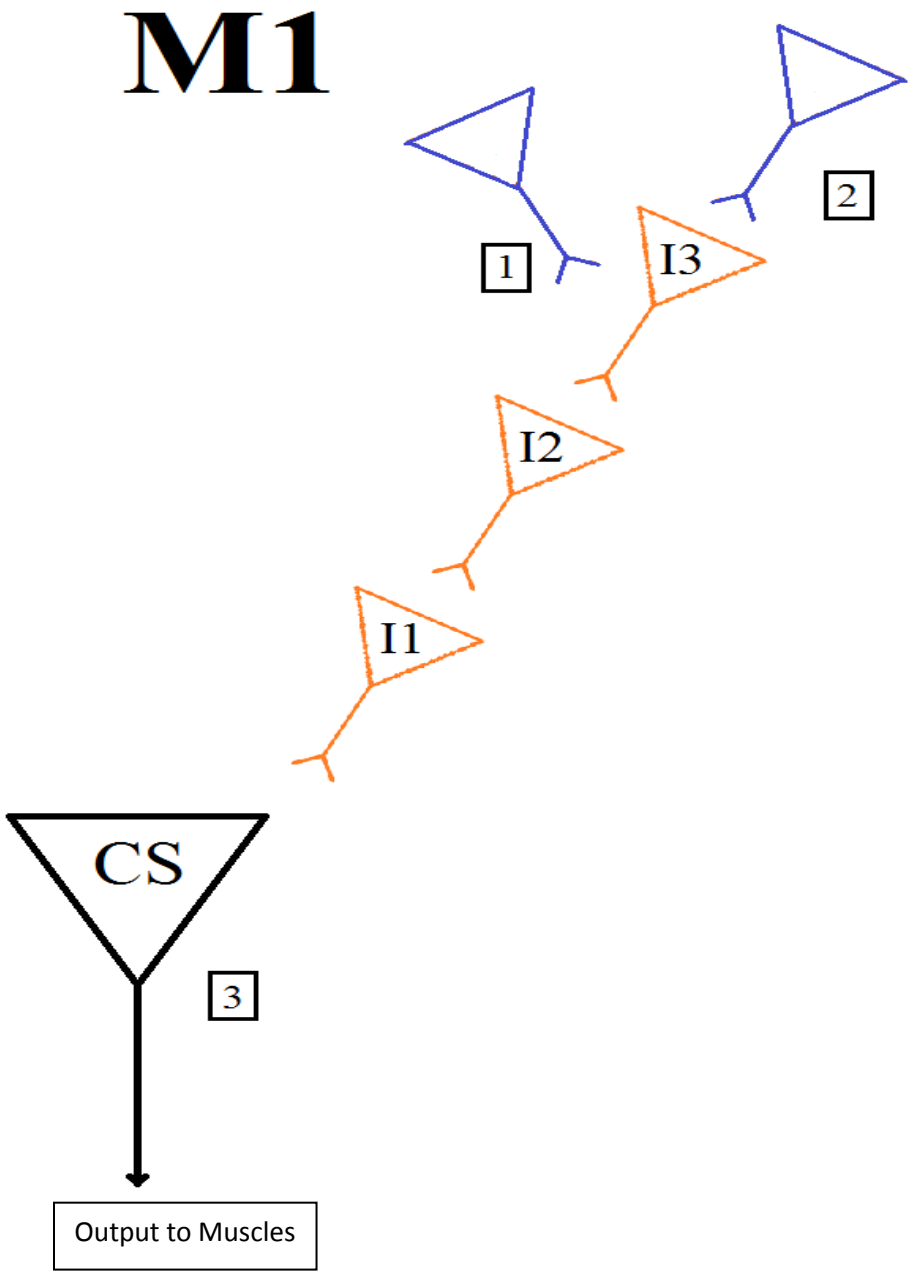


**Figure 6.1: Model for alterations to motor cortical output and inhibition after SCI.**

1) Reductions in $GABA_A$ transmission on inhibitory interneurons responsible for SICI at later I-waves in individuals with SCI on baclofen.
2) Increases in $GABA_B$ transmission on inhibitory interneurons responsible for LICI at later I-waves in individuals with SCI on baclofen.
3) Reductions in corticospinal output in individuals with SCI.

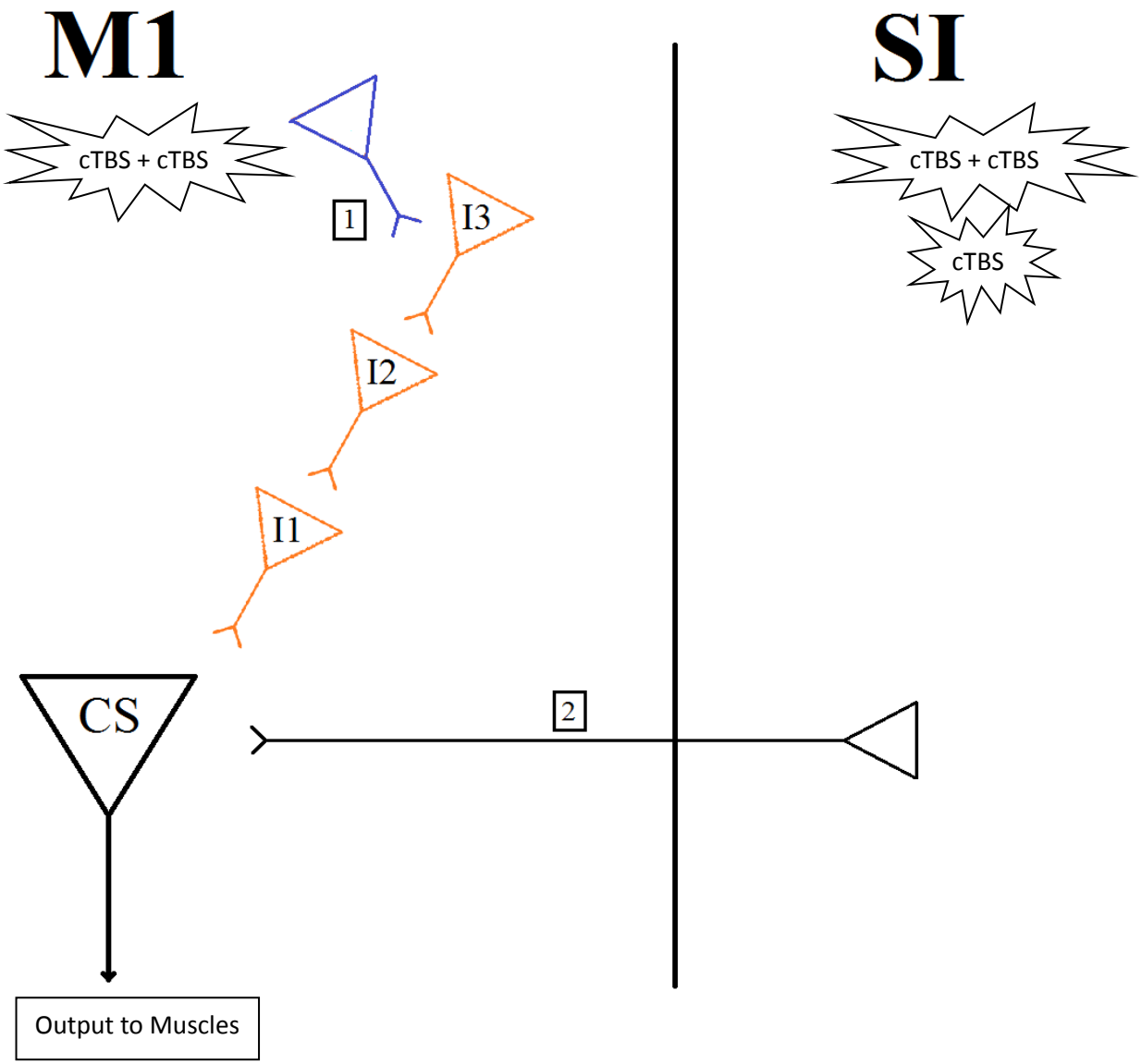


**Figure 6.2: Model for alterations to motor cortical output by cTBS.**

1) None of the three cTBS interventions led to changes to SICI in uninjured controls.
2) All three cTBS interventions led to increases in MEP sizes from increased corticospinal output in uninjured controls.

### 5.6 Future Advancement to SCI Therapy

Current literature primarily focuses on inducing changes to M1 by applying rTMS (Belci *et al.*, 2004;Kumru *et al.*, 2010;Kuppuswamy *et al.*, 2011;Alexeeva & Calancie, 2014) and more recently PAS (Ellaway *et al.*, 2014) to produce changes to cortical excitability and ultimately functional improvements in humans. There are limitations to using rTMS in individuals with SCI over this brain area. One key issue appear to be the need for relatively high strength of TMS required to obtain sustained changes to motor cortical excitability (Ellaway *et al.*, 2014). Such high intensities coupled with such high frequencies can lead to discomfort, pain and distress all of which are transient but can limit the duration of stimulation and effectiveness of the protocol (Nardone *et al.*, 2014).

Other remote brain areas such as SI have been targets of rTMS stimulation and have demonstrated changes in cortical excitability (Ishikawa *et al.*, 2007;Jacobs *et al.*, 2014;Tsang *et al.*, 2014). SI has been a target of therapeutic approaches for disorders presenting with motor control impairments due to its direct projections to M1 (Rocco-Donovan *et al.*, 2011). For example, finger dexterity in Parkinson's disease appears to be reduced with reductions in SI activation (Foki *et al.*, 2015). RTMS over SI improves handwriting in individuals with focal hand dystonia affecting the ability to write for upwards of three weeks following stimulation (Havrankova *et al.*, 2010). Following brain damage in rats, a neural prosthesthetic aimed at re-establishing connections between M1 and SI restores reaching and grasping functions which were impaired following injury (Guggenmos *et al.*, 2013). It is clear that SI is a cortical area involved in motor function, particularly in disorders associated with motor deficits. Therefore, investigating plasticity and metaplasticity within SI in the SCI population can offer a novel therapeutic approach for improving motor deficits seen in this population.

### 5.7 Limitations

Some limitations should be considered in my dissertation research. First, MEPs are not the sole result of cortical neuronal outputs but are also has contributed by spinal motorneurons. To fully investigate whether alterations to MEPs were due to cortical and/or spinal changes, Hoffman-reflexes (H-reflex) could be used. H-reflex is a method to measure changes in spinal motor neuron excitability (Fisher, 2002). However, H-reflexes were not performed as they are difficult to induce in intrinsic muscles of the hand (i.e. FDI) (Hultborn & Nielsen, 1995). Second, in Experiment 1 both SICI and LICI were characterized within all participants however in Experiment 2 only changes in SICI was measured. This is an additional circuit that can be explored further in the future. The reason for not exploring this circuit was to avoid overstimulation of the cortex by adding large a number of TMS trials, which may interfere with the effects of the cTBS protocol.

# Chapter 6: Reference List

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