MODULATION OF CORTICOSPINAL OUTPUT TO THE HAND VIA SOMATOSENSORY AFFERENT INPUT

By

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A Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree Master of Science in Kinesiology

MASTER OF SCIENCE (2014)

(KINESIOLOGY)

McMaster University, Hamilton, ON, Canada

TITLE: Modulation of Corticospinal Output to the Hand via Somatosensory Afferent Input

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NUMBER OF PAGES: 101

ABSTRACT

The primary somatosensory cortex (SI) and primary motor cortex (M1) receive somatosensory afferent input to influence motor hand circuitry and control. Much of the literature has investigated this relationship extensively using animal models. In contrast, much of these relationships and neural mechanisms are still not well understood in humans. The present work investigated homosynaptic and hetersynaptic protocol's modulatory effects on SI and M1 sensorimotor circuitry. Experiment 1 used the homosynaptic protocol continuous theta-burst stimulation (cTBS) over SI and M1 and measured motor evoked potentials (MEP) and short-latency afferent inhibition (SAI). CTBS over M1 suppressed MEPs and did not alter SAI. In contrast, cTBS over SI facilitated MEPs and decreased median and digital nerve evoked SAI. Experiment 2 used the heterosynaptic protocol rapid-rate paired associative stimulation (rPAS) on SI and M1. SAI and MEPs were measured to investigate the sensorimotor changes following rPAS. Results indicated minimal decreases in SAI but increases in MEPs following SI rPAS. However, M1 rPAS lead to significant reductions in SAI and increased MEPs. The findings from this thesis highlight the selective modulation of sensorimotor circuitry through the use of various stimulation protocols.

ACKNOWLEDGEMENTS

First, I want to thank my supervisor Dr. Aimee Nelson for her patience, guidance and wisdom for the time that I have been with the lab. Thank you for the countless hours and the funding that you have provided to further this research. Your input and feedback have taught me about the rigour, hard work, and passion that good science requires. Further, you have taught me invaluable life lessons as a mentor and friend. Thank you for your words of encouragement, especially when I lacked the confidence to believe in myself. Without your help, I would not have been able to make it this far.

I would also like to thank my committee members for their help in this dissertation process. Thank you Dr. Jim Potvin and Dr. Audrey Hicks. Your input and feedback have strengthened the quality of these research projects.

I would like to thank my fellow colleagues, lab mates, and friends in the neurophysiology lab. Thank you Mark Jacobs, Kevin Lee, Michael Asmussen, Christopher Zapallow, Tanner Mackenzie, Aaron Bailey, Peter Mi, and Christy Jones. Your good company have made the long hours in and outside the lab memorable and enjoyable. Also, thank you for the feedback and debates in regards to science and life.

Finally, I would like to thank my friends and my family for your continual love, support, and prayers for me. Thank you for also reminding me that this research ultimately glorifies Jesus and His kingdom.

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June 2014

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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LIST OF ABBREVIATIONS

AMT	Active Motor Threshold
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of Variance
AP	Anterior to Posterior
APB	Abductor Pollicus Brevis
CS	Conditioning Stimulus
CST	Corticospinal Tract
CTBS	Continuous Theta Burst Stimulation
D-wave	Direct Wave
D2	Dopamine Receptor Subtype 2
DN	Digital Nerve
EEG	Electroencephalography
EMG	Electromyography
F-wave	F-response
FDI	First Dorsal Interosseous
FHD	Focal Hand Dystonia
GABA	Gamma (γ) Amino Butyric Acid
H-reflex	Hoffman Reflex
HSD	Honest Significant Difference
ISI	Interstimulus Interval
ITBS	Intermittent theta-burst stimulation
I-Waves	Indirect Waves
LTD	Long-term Depression
LTP	Long-term Potentiation
M-wave	M-response
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MN	Median Nerve
ms	Milliseconds
mV	Millivolts
MVC	Maximum Voluntary Contraction
NMDA	*N*-methyl-d-aspartate
PA	Posterior to anterior
PD	Parkinson's disease
PAS	Paired Associative Stimulation
PNS	Peripheral Nerve Stimulation
rPAS	Rapid-rate Paired Associative Stimulation
RMT	Resting Motor Threshold
RTMS	Repetitive Transcranial Magnetic Stimulation
SAI	Short Latency Afferent Inhibition
SEP	Somatosensory evoked potentials

SI	Primary Somatosensory Cortex
ST	Sensory Threshold
STDP	Spike-timing dependent plasticity
TBS	Theta-burst stimulation
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
TS	Test Stimulus
VP	Ventral posterior nuclei
μV	Microvolts

CHAPTER 1: GOAL OF THESIS

Introduction

Somatosensory afferent input is important for the control of movement and motor learning. Research in non-human primates have investigated the physiological (Lemon 1981) and behavioural significance (Johansson & Westling 1984) of somatosensory afferent processing in motor behaviour. For example, a lesion to the primary somatosensory cortex (SI) impairs the primate's ability to learn complex motor tasks (Pavlides *et al.,* 1993). However, there is much that remains unknown about somatosensory afferent input and its influence in human hand control. Anatomically SI and the primary motor cortex (M1) separated by the central sulcus, are known to have reciprocal connections (Jones *et al.,* 1978). All areas of SI project to M1 with the exception of Brodmann's Area 3b (Vogt & Pandya 1978). Tetanic stimulation of SI leads to long-term potentiation (LTP) of M1.

Further knowledge of how SI and M1 modulate sensorimotor circuitry may provide insight in somatosensory afferent processing within the context of movement. Short-latency afferent inhibition (SAI) is a sensorimotor neural circuit that involves the pairing of peripheral nerve stimulation and transcranial magnetic stimulation (TMS) (Tokimura *et al.,* 2000). SAI has been investigated in a number of clinical populations (ie. Alzheimer's disease) (Di Lazzaro *et al.,* 2002) and typical healthy population (Classen *et al.,* 2000). Understanding the neural mechanisms and modulation of SAI is one method to explore how somatosensory afferent information influences the neural circuitry within SI and M1.

Sensorimotor cortex may be modulated using homosynaptic and heterosynaptic plasticity protocols. One example of a homosynaptic protocol is continuous theta-burst stimulation (cTBS), which uses bursts of TMS to modulate cortical excitability. In contrast, heterosynaptic protocols like rapid-rate paired associative stimulation (rPAS) modulate cortical excitability using somatosensory peripheral nerve stimulation and cortical stimulation from TMS. The precise timing of the two inputs induces spike-timing dependent plasticity (STDP). The investigation of various protocols like cTBS and rPAS over sensorimotor cortex may allow for selective modulation of neural circuitry.

Goals of Thesis

The goal of the thesis is to investigate how M1 and SI influence early sensorimotor integration as measured via SAI. To achieve this goal, two non-invasive techniques called continuous theta-burst stimulation (cTBS) and rapid-rate paired associative stimulation (rPAS) were used to modulate M1 and SI in order to further understand how these two cortical areas influence SAI. In addition to SAI, motor evoked potentials (MEP) elicited by single-pulse TMS were used as a measure of net corticospinal excitability. Collectively, the studies in this thesis provided new information about the mechanisms by which somatosensory afferent input and cortex influences motor output directed to muscles in the hand. Further, the data obtained from this thesis may be used for developing new therapeutic approaches to alter SAI and/or MEP amplitude in clinical populations. In summary, the research thesis will contribute to the understanding of sensory control of the hand and will provide new neuroscience

techniques for modulating a measure of sensorimotor integration and corticospinal output to muscles of the hand.

Summary of Experiments

Experiment 1: Continuous theta-burst stimulation over primary somatosensory cortex modulates short-latency afferent inhibition

Experiment 1 investigated the effects of cTBS over SI and M1 on MEPs and SAI. MEPs and SAI were recorded from the first dorsal interosseous (FDI) muscle of the right hand before and following 30 Hz cTBS over left-hemisphere SI and M1. CTBS over M1 suppressed MEPs and did not alter SAI. In contrast, cTBS over SI facilitated MEPs and decreased median and digital nerve evoked SAI.

Experiment 2: Effects of Rapid-rate Paired Associative Stimulation (rPAS) on Sensorimotor Circuitry

Experiment 2 investigated effects of rPAS applied over primary somatosensory cortex (SI) and its effects on SAI. This was done by repeated pairing of median nerve stimulation and TMS applied over SI at 5Hz. MEPs and SAI were measured from the abductor pollicus brevis (APB) before and following rPAS over left-hemisphere M1 and SI. Results indicated minimal decreases in SAI following SI rPAS. In contrast, M1 rPAS lead to significant reductions in SAI at 45 minutes following stimulation. MEPs were increased following both M1 and SI rPAS.

CHAPTER 2: LITERATURE REVIEW

Somatosensory Afferent and Motor Efferent Pathways

Anatomy and Physiology of Somatosensory Transmission

Somatosensory processing begins with several nerve endings including mechanoreceptors, golgi tendon organs, muscle spindle fibres, and joint receptors (Kandel *et al.,* 2000). These nerve endings generate potentials that are transmitted to the peripheral nerves which then synapse with the dorsal horn of the spinal cord. These potentials then travel superiorly through the dorsal column of the spine where once they reach the lower medulla and synapse at the nucleus cuneatus. Second order afferent fibres decussate and ascend via medial lemniscus to terminate at the thalamus where it is further processed by the ventral posterior nuclei (VP). However, the superior aspect of the VP primarily relays proprioceptive inputs and the inferior aspect relays tactile information (Kaas 1993). Finally, these thalamocortical projections are the third order afferent fibres that extend to the respective areas of SI.

Pyramidal neurons within SI have elongated structures to form the anatomical boundaries of vertical columns (Kaas 1993). Theses cortical columns contribute to the somatotopic mapping of SI. Representations of the body are arranged inferior to superior with the superior aspects more lateral to the inferior segments. Each cortical area of SI (Brodmann's areas 1, 2, 3a, and 3b) contain distinct somatotopic maps (Kaas *et al.,* 1979). Cutaneous inputs are received in area 3b and project to area 1(Friedman *et al.,* 2008).

Proprioceptive inputs are received in area 3a (Iwamura *et al.*, 1993) and project to areas 1 and 2.

Anatomy and Physiology of Motor Efferent Pathway

Several descending pathways contribute to human motor control (ie. reticulospinal pathway, rubrospinal pathway, vestibulospinal). However, I will primarily focus on the corticospinal tract (CST) as this is the pathway that contributes most to upper extremity control. The CST has been extensively reviewed by several investigators (Dum & Strick 2002; Lemon 2008) and originates from several cortical areas including the premotor cortex, supplementary motor area (SMA), and cingulate motor areas. However, ~40% of the projections from the CST originate from M1 (Dum & Strick 2002). Large betz cells (pyramidal) in cortical layer V form the descending tracts which synapse and interact with spinal interneurons and motoneurons. It is interesting to note that only M1 has tracts which extend to laminae IX of the spinal cord and directly activate spinal motor neurons. Though motor output is the major function of M1 and the CST, these pathways contribute to other functions such as inhibition of sensory fibres (Canedo 1997) and control of spinal reflexes (Pierrot-Deseilligny & Burke 2005).

In contrast to Penfield's original model, we know now that M1 is not simply a somatotopic mapped motor strip. Particularly in the hand and forearm regions, M1 is found to have many overlapping representations of muscles and body parts (Sanes *et al.*, 1995). Further investigation of M1 has revealed its control in movement kinematics (direction and speed) and kinetics (force) (Kandel *et al.*, 2000). Neurons within M1appear

to have a preferred direction as it will discharge to a greater or lesser degree depending on the movement vector. Movement vectors are specific discharge rates that neural populations within M1 output based on a direction. Aside from specific vectors, several intracortical and cortico-cortical mechanisms may also modulate M1 activity as this is a central area to motor learning (Kandel *et al.*, 2000).

SI and M1 Connectivity

Aside from area 3b, all other areas of SI have projections to M1. Inversely, M1 also has projections to the dorsal column nuclei and areas 1 and 2 of SI (Canedo 1997; Jones *et al.*, 1978). These reciprocal projections between M1 and SI have physiological and behavioural implications for the sensorimotor system. In cats, stimulation of area 3a leads to increases in excitatory post-synaptic potentials within M1 (Zarzecki *et al.*, 1978). Similarly, tetanic stimulation of SI leads to long-term potentiation of M1 (Iriki *et al.*, 1989). These physiological mechanisms may be pertinent to motor learning as ablation of SI in non-human primates impairs the acquisition phase of a novel movement but not the retention phase (Pavlides *et al.*, 1993). Also, the cooling of area 2 leads to uncoordinated ataxic-like movements, which dissipate once SI is warmed again (Brinkman *et al.*, 1985). Finally, increased plastic changes within SI areas have been speculated as underlying mechanisms for movement disorders like focal hand dystonia (FHD) (Baumer *et al.*, 2007; Byl *et al.*, 1997; Tamura *et al.*, 2008).

Nerve Stimulation to Investigate Neurophysiological Measurements

Nerve Stimulation

Peripheral nerve stimulation is the stimulation of the axon of a nerve due to the migration of electrons and ions (Rattay 1990). An artificial electrical circuit is created using electrodes with two components: the cathode (electron donor) and the anode (electron acceptor). The flow of electrons from the anode to cathode creates an electrical field within the tissue medium leading to a migration of electrons, which at high enough intensities depolarizes the axon of the neuron. Biphasic electrical stimulations are preferred over monophasic stimulations as monophasic pulses may lead to charge accumulation (Rattay 1990). The initial phase of the bipolar stimulus evokes the action potential whereas the subsequent phase reverses the electrochemical processes rapidly via hyperpolarization.

With suprathreshold stimulation of a peripheral nerve (ie. median nerve), axonal depolarization leads to an action potential which travels both away from and towards the cell body (Lipski 1981). Both motor responses can be recorded through electromyography. The response away from the cell body in a motor neuron can be recorded from the target muscle fibres and is known as the M-response (M-Wave). In contrast, the response travelling back to the soma leads to a second action potential which is propagated away from the cell body along the axon where a later F-response (F-wave) can be recorded. F-waves have been used extensively in neurophysiology to investigate nerve conduction velocities and motor neuron excitability (Fisher 2002). However, F-

wave measurements are not as sensitive to changes in spinal motor neuron excitability as the alternative technique known as the H-reflex (Hultborn & Nielsen 1995). These differences in sensitivity can be experimentally observed when using heteronymous Ia facilitation of the femoral motoneuron. Changes in spinal excitability were much more pronounced when H-reflexes were recorded in the soleus muscle in comparison to the F-waves recorded.

Hoffman Reflex (H-Reflex)

The Hoffman reflex (H-reflex) is evoked by electrically stimulating Ia afferent fibres in the lower or upper limb which synapse to alpha motor neurons activating the associated muscles (Schieppati 1987). H-reflex is measured as a late potential to the M-response. In response to higher nerve stimulation intensities, both M-wave and H-reflex increase. However, the initial increase in H-reflex is followed by a decrease due to the collision of the antidromic volley (Schieppati 1987). H-reflex amplitudes can be normalized to the maximal M-wave, where the ratio is used as an indicator of the excitability of spinal motor neurons (Fisher 2002).

Though H-reflexes are a more sensitive measurement to changes in spinal motor neuron excitability compared to F-waves, there are still several methodological limitations. For instance, H-reflexes are also a measurement of the Ia-afferent synapses and the inhibitory mechanisms modulating it (Hultborn & Nielsen 1995). Also, it is not definite that the H-reflex is purely monosynaptic and does not involve oligosynaptic inhibition via Renshaw cells which may influence the excitability of the motorneurons.

Finally, H-reflexes are commonly evoked in the lower extremity and forearm muscles but are difficult to evoke in the hand muscles (Hultborn & Nielsen 1995).

Somatosensory Evoked Potentials (SEP)

Somatosensory evoked potentials (SEP) are event related potentials recorded using surface electroencephalography (EEG) electrodes. SEPs are generated via somatosensory stimulation (ie. median nerve stimulation) which activates afferent pathways recruiting subcortical structures, regions of the cerebellum, and regions of the cerebral cortex (Allison *et al.,* 1991). Polarity and latency are the factors frequently used in the nomenclature of specific potentials (ie. P25 represents a positive potential at 25 ms). When the median nerve is stimulated and potentials are recorded on the scalp surface overlying SI, the N20 and P25 potentials are generated by the pyramidal neurons of area 3b (Allison *et al.,* 1989). The N20 has been used as reference of the latency required for the afferent volley from the median nerve stimulation to reach the cortex (Allison *et al.,* 1989). The peak-to-peak amplitude of SEPs are recorded to measure the excitability of SI pyramidal neurons (Enomoto *et al.,* 2001).

Similar to short-latency SEP, paired-pulse inhibition can be used to assess cortical changes in excitability. When two median nerve stimulations are applied in succession with ISIs of ~30ms, decreases in second SEP amplitude can be observed in the N20-P25 potentials (Shagass & Schwartz 1964). Paired-pulse SEPs have been used as measures of changes in cortical excitability and cortical plasticity following interventions like 5Hz rTMS (Ragert *et al.,* 2003) and tactile co-activation (Hoffken *et al.,* 2007).

Transcranial Magnetic Stimulation (TMS) Techniques to Investigate Sensorimotor Circuits

Introduction to TMS

Transcranial magnetic stimulation (TMS) is a non-invasive means of stimulating the human cortex and was initially introduced by Barker *et al.* (1985). TMS works through Faraday's principle of electromagnetic induction (Kobayashi & Pascual-Leone 2003). A rapid current is passed through the conductive coil and produces a magnetic field perpendicular to the coil. When the coil is placed tangentially to the scalp the magnetic field can pass the cranium and induce a secondary current parallel to the cranium. With sufficient charge and temporal summation within, the stimulated neurons via TMS create an action potential (Hallett 2007). TMS can be applied over various cortical regions but the area most commonly investigated is the primary motor cortex (M1). When a powerful enough pulse is applied over M1 a number of descending volleys are produced (Di Lazzaro *et al.*, 2004). A summation of descending volleys may depolarize the spinal motoneurons, where an action potential can activate the target muscle and be recorded through electromyography. This is known as a motor evoked potential (MEP) (Di Lazzaro *et al.*, 2004). The peak-to-peak amplitude of MEPs can be a used as indicators of the net excitability of the corticospinal tract (Hallett 2007). MEPs involve several groups of cortical cells and also spinal neurons, therefore increases in MEP amplitude may not be a direct indicator of increased cortical excitability (Petersen *et al.*, 2003).

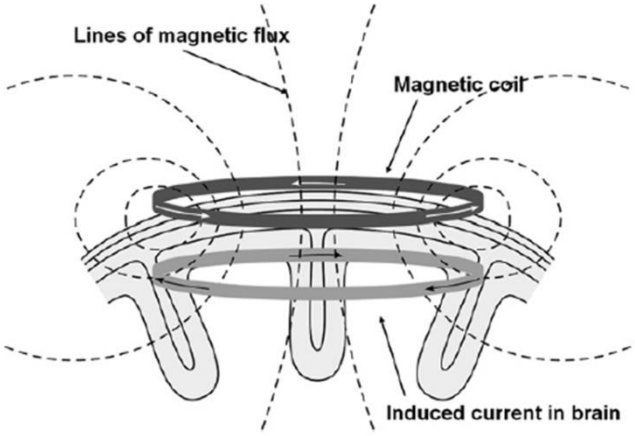

Figure 1. TMS Magnetic Induction

TMS uses Faraday's Principle of Magnetic Induction to depolarize neurons. (Adapted from Hallett, 2007)

TMS is a unique tool for investigating neuronal excitability as the technique targets specific populations of neurons. The recruitment of corticospinal neurons differs between TMS and transcranial electrical stimulation (TES). TES is shown to activate corticospinal tract through direct activation of the pyramidal neurons. However, TMS activates M1 primarily through transsynaptic activation of the pyramidal tract. This was examined in detail by Di Lazzaro et al. (2004) where descending spinal volleys were recorded with spinal cord stimulators in patients with lumbar pain. The latencies of the spinal volleys were compared using TES and also different TMS orientations, intensities and type. Initial descending volleys were observed at 2-2.6 ms latency when applying anodal stimulation to M1 and were termed as the D-wave. The D-wave is believed to be direct activation of the pyramidal tract axons (Di Lazzaro *et al.,* 2004). Following the D-wave, subsequent volleys with periodicity around 1.5 ms afterwards were termed as the indirect wave (I1-wave) generated via interneurons which are believed to synapse and excite the pyramidal neurons. Varying coil orientations produce different descending

volley latencies. Coil orientations which produce a posterior-anterior current, anterior-posterior current, lateral-medial current in the cortex leads to spinal volleys of I1-wave, I3-wave (3 ms after I1), and D-wave, respectively(Di Lazzaro *et al.,* 2004). At higher intensities of TMS stimulation, recruitment of D-wave is observed followed by subsequent I-waves. Though the cortical origins of I-waves are not completely known, it is speculated that these interneurons reside in layers II/III of the motor cortex and transsynaptically active the corticospinal output neurons of M1's layer V (Di, V *et al.*, 2012).

A computerized model of the cortex provides comprehensive understanding of the physical structures stimulated by TMS. Past investigations proposed that the activation of the horizontal fibres is along the crown of the gyrus; these fibres then terminate to vertical fibres which are a few millimetres deep to the crown of the gyrus (Silva *et al.,* 2008). This heterogeneous model concurs with the previously proposed models where horizontal fibres are initially activated by TMS and subsequently activate the pyramidal tracts transsynaptically. It is important that there is continual investigation regarding the neural populations that TMS activates as these findings enable further understanding of neural mechanisms (ie. intracortical circuits, cortico-cortical connections, afferent inhibition).

Motor Threshold

Each individual subject and target muscle responds differently to TMS. Due to this variability of response to TMS, thresholds are usually measured for each individual (Wassermann *et al.,* 2008). Motor thresholds are believed to reflect the membrane

excitability of corticospinal neurons and interneurons of the cortex (Kobayashi & Pascual-Leone 2003). More importantly, motor thresholds essentially provide an indicator of the efficacy of the chain of activation from the cortical neuronal membrane all the way to the muscle.

Motor threshold can be measured at rest and with tonic contraction. Resting motor threshold (RMT) is defined as the intensity required to evoke a 50 μV MEP in 5 out of 10 trials (Rossini et al. 1994). Motor threshold measured whilst there is tonic contraction of the target muscle is known as active motor threshold (AMT). AMT is usually the intensity required to evoke a 200 μV MEP in 5 out of 10 trials (Rossi *et al.,* 2009). Submaximal activation of target muscles has been speculated to decrease MEP variability (Darling *et al.,* 2006).

Short-latency Afferent Inhibition (SAI)

One common technique to investigate sensorimotor integration via TMS is by pairing it with peripheral nerve stimulation of a mixed (ie. median nerve) or cutaneous (ie. digital nerve) nerve. A single TMS pulse preceded by peripheral nerve stimulation ~20-28 ms may lead to a decrease in MEP amplitude; this phenomenon is known as short-latency afferent inhibition (SAI) (Tokimura *et al.*, 2000). The specific interstimulus interval (ISI) accounts for the amount of time required for the afferent information to reach M1. The greatest degree of inhibition is observed approximately 2-4 ms after the arrival of afferent information to SI (Tokimura *et al.,* 2000). SAI is believed to be cortically mediated by central cholinergic activity (Di Lazzaro *et al.,* 2000) but is also

modulated by other neurotransmitters such as $GABA_A$ (Di, V *et al.*, 2005).The degree of inhibition in SAI is somatotopically dependent. Contiguous digits require lower intensity of stimulation to induce SAI and also retain a longer inhibitory effect in comparison to non-contiguous digits (Tamburin *et al.*, 2001). Also, mixed nerve stimulation of homotopic muscles versus heterotopic muscles leads to stronger inhibition (Classen *et al.*, 2000). However, in contrast to previous findings, similar degrees of inhibition with homotopic and heterotopic muscles in SAI have been observed (Fischer & Orth 2011).

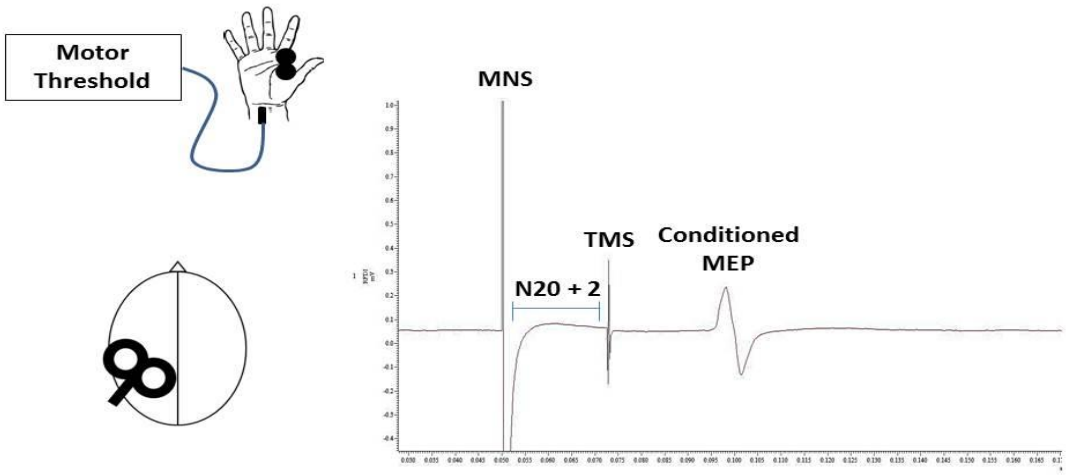

Figure 2. SAI Experimental setup
Experimental setup of SAI induced via TMS and median nerve stimulation

The degree of inhibition observed in SAI can be modulated via motor or sensory tasks. During selective finger movement, SAI is decreased in the muscle that is moving but not in the surrounding muscles (Voller *et al.*, 2006). Similarly, a decrease in SAI is also seen prior to finger movement (Asmussen *et al.*, 2013). Depending on the pre-movement phase, SAI modulation may be cortically or spinally mediated (Asmussen *et*

al., 2013). However, a sensory task that requires spatial attention leads to increases in SAI (Kotb *et al.*, 2005).

Repetitive Transcranial Magnetic Stimulation (rTMS)

Transcranial magnetic stimulation has the ability to cause transient changes in cortical excitability when used in repetitive bouts. With high frequency stimulation (>5Hz) over the primary motor cortex a facilitation effect occurs as measured by MEPs (Pascual-Leone *et al.*, 1994); on the other hand low frequency stimulation (<1Hz) usually causes an inhibitory effect (Chen *et al.*, 1997); these paradigms are termed repetitive TMS (rTMS). Duration of stimulation may also influence the magnitude of modulation and duration of rTMS effects (Peinemann *et al.*, 2004). RTMS can also lead to sensory changes when applied to the sensorimotor cortex. Low frequency rTMS to M1 leads to decreases in SEPs, a measure of sensory cortical activity (Enomoto *et al.*, 2001). RTMS can also lead to behavioural changes as 1 Hz rTMS to SI lead to impairments in a rotary tracking task (Vidoni *et al.*, 2010). In contrast, 5 Hz rTMS lead to increases in sensory cortex activity recorded with paired-pulse stimulation (Ragert *et al.*, 2004).

Theta Burst Stimulation (TBS)

RTMS has led to several novel TMS stimulation paradigms like theta-burst stimulation (TBS) (Huang *et al.*, 2005). Using TBS instead of traditional rTMS allows for shorter, safer, and more efficient methods to modulate corticospinal excitability. TBS was first introduced using a 50 Hz train of stimulation (burst) delivered at a theta frequency

(5Hz) with a total of 600 stimuli, applied over M1 (Huang *et al.*, 2005). If the trains of stimulation are delivered in an intermittent fashion (2s train every 10s) this is known as intermittent theta burst stimulation (iTBS) that leads to a facilitatory effect transiently if applied over M1. If TMS trains are delivered continuously this is known as continuous theta burst stimulation (cTBS) which leads to a transient depression of corticospinal excitability in M1.

The mechanisms by which TBS modulates cortical excitability are still not fully understood. However, synaptic plasticity is one widely proposed and accepted mechanism (Pell *et al.*, 2011). Long term potentiation (LTP) and long term depression (LTD) observed in animal models are a good framework for understanding the synaptic changes that may be occurring in TBS. Similar to LTP and LTD in animals, calcium and N-methyl-D-aspartate (NMDA) channels are crucial to the up and down regulation of AMPA receptors are speculated to be mediators of TBS. Administration of NMDA receptor antagonist abolishes the effects of cTBS and iTBS (Huang *et al.*, 2007). Similarly, dopamine may also be a crucial mediating factor in the plasticity effects of TBS as blockage of D2 receptors abolishes TBS effects (Monte-Silva *et al.*, 2011). TBS may also be involved in the modulation of GABA levels as there are elevated concentrations following cTBS observed via magnetic resonance spectroscopy (Stagg *et al.*, 2009).

Several stimulation parameters can influence the modulatory effects of TBS. The number of pulses given during cTBS relates to the duration of corticospinal modulation as

300 stimuli leads to a shorter period of inhibition than 600 stimuli (Huang *et al.*, 2005). Aside from duration, stimulation intensity may also influence the direction and magnitude of effects. CTBS at a higher intensity may at times lead to facilitation, whereas a lower intensity of cTBS can lead to transient inhibition of corticospinal excitability (Doeltgen & Ridding 2011). Further, the current direction influenced by coil orientation during cTBS may also influence the degree of modulation. Stimulation in the traditional PA-AP or AP-PA orientation both lead to transient inhibition of corticospinal excitability but PA-AP shows a greater degree of inhibition (Talelli *et al.*, 2007). Finally, frequency can influence the variability of the effects of TBS. A modified frequency of 30 Hz cTBS applied at 6Hz frequency (600 stimuli) leads to more robust changes in corticospinal excitability and also less inter-subject variability (Goldsworthy *et al.*, 2012a; Goldsworthy *et al.*, 2012b).

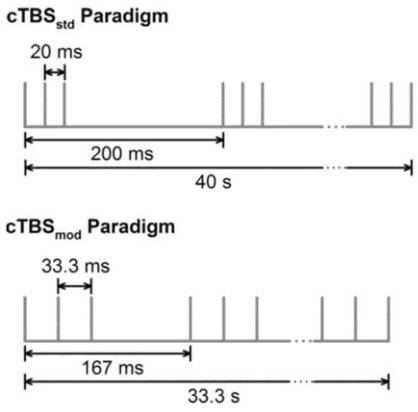

Figure 3. Modified 30 Hz cTBS

Comparison of parameters in standard TBS vs. modified TBS (adapted from Goldsworthy, 2012)

Aside from M1, TBS has also been applied over other neural structures. CTBS over premotor cortex leads similar suppression of MEPs (Huang *et al.*, 2009). CTBS over

Brodmann's Area 5, a higher-order somatosensory area, leads to robust increases in MEP whether it is applied intermittently or continuously (Premji *et al.,* 2011). Similarly, cTBS applied over SI leads to increases in MEPs as well (Jacobs *et al.,* 2013; Jacobs *et al.,* 2012) but not in all cases (Ishikawa *et al.,* 2007).

Paired Associative Stimulation (PAS)

Multiple pairs of peripheral nerve stimulation applied with TMS can lead to associative plasticity of the motor cortex, a protocol known as paired associative plasticity (PAS) (Stefan *et al.,* 2000). A 10 ms or 25 ms ISI between the nerve stimulation and TMS can lead to decreases or increases in MEPs, respectively (Wolters *et al.,* 2003). Aside from ISI, directing the subject's attention is important to the effects and modulation by PAS (Stefan *et al.,* 2004). PAS has also been applied to SI which led to increases in SEP amplitudes (Wolters *et al.,* 2005). Changes in cortical excitability following SI PAS have been correlated to a decrease in tactile discrimination as well (Litvak *et al.,* 2007). However, other reports found no changes in cortical excitability following SI PAS (Krivanekova *et al.,* 2011). Since the introduction of PAS, there have been several modifications of the protocol; factors such as duration, intensity, and frequency have been altered and investigated (Stefan *et al.,* 2004; Ziemann *et al.,* 2004).

PAS was first applied at a high frequency (5 Hz) by Quartarone *et al.* (2006) and is known as rapid-rate paired associative stimulation (rPAS). Previously, PAS protocols were usually applied at low frequencies (ie. 0.1 Hz), using small number of stimulations, and also longer durations (Stefan *et al.,* 2000; Wolters *et al.,* 2005). In contrast, rPAS

delivers 600 pairs of stimuli at 5 Hz with a specific ISI of 25 ms between the median nerve and TMS pulse (Quartarone *et al.*, 2006). Persisting increases in corticospinal excitability are seen for up to an hour following rPAS. Central cholinergic activity may be a mediator of rPAS as patients with Alzheimer's disease had no significant changes in corticospinal excitability following rPAS in comparison to healthy controls (Terranova *et al.*, 2013).

Modulation of SAI Using Non-invasive Stimulation

SAI can be modulated through several non-invasive methods of stimulating the sensorimotor cortex. Low frequency rTMS and iTBS over the motor cortex modulate net corticospinal excitability without altering SAI (Fischer & Orth 2011; Zamir *et al.*, 2012). Similarly, 1 Hz rTMS decreases SAI in patients with focal hand dystonia but not in controls (Baumer *et al.*, 2007). Protocols involving peripheral stimulation with or without TMS have been used to modulate SAI. RPAS transiently decreases SAI for up to an hour (Quartarone *et al.*, 2006; Terranova *et al.*, 2013) and 40 minutes of neuromuscular electrical stimulation temporarily abolishes SAI (Mang *et al.*, 2012).

CHAPTER 3: CONTINUOUS THETA-BURST STIMULATION OVER PRIMARY SOMATOSENSORY CORTEX MODULATES SHORT-LATENCY AFFERENT INHIBITION

Previously published as:

Tsang P, Jacobs MF, Lee KGH, Asmussen MJ, Zapallow CM, Nelson AJ:

"Continuous theta-burst stimulation over primary somatosensory cortex modulates short-latency afferent inhibition**", Clinical Neurophysiology. Accepted February 21, 2014.**

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INTRODUCTION

Motor evoked potentials (MEPs) elicited via transcranial magnetic stimulation (TMS) are reduced when preceded by electrical stimulation of a peripheral nerve approximately 20 to 25 ms earlier (Tokimura *et al.,* 2000). This phenomenon is known as short-latency afferent inhibition (SAI) and is considered to be mediated via acetylcholine (Ach) (Di, V *et al.,* 2000) at the level of the cortex (Tokimura *et al.,* 2000). SAI is modulated by dopamine (Sailer *et al.,* 2003), and $GABA_A$ agonist lorazepam reduces SAI (Di, V *et al.,* 2005). SAI interacts with other neural circuits that are driven by GABAergic interneurons such as short-interval intracortical inhibition (Alle *et al.,* 2009), short-interval interhemispheric inhibition (Tsutsumi *et al.,* 2012) and long-interval intracortical inhibition (Udupa *et al.,* 2009). Further, SAI evoked by mixed (ie. median) and cutaneous (ie. digital) nerves may be somatotopically dependent whereby muscles which are innervated by the stimulated nerve produce a greater degree of SAI than non-

innervated muscles (Classen *et al.*, 2000). For instance, digits in closer proximity to the digital nerve stimulated produce a greater degree of SAI (Tamburin *et al.*, 2001).

The magnitude of SAI is modifiable. SAI is increased with spatial attention controlled by a counting task (Kotb *et al.*, 2005). In contrast, SAI can be selectively reduced in specific movement or pre-movement phases during finger tasks (Asmussen *et al.*, 2013; Voller *et al.*, 2006). Depending on the pre-movement phase, decreases in SAI may be driven via cortical or spinal mechanisms (Asmussen *et al.*, 2013). Repetitive TMS (rTMS) may also alter SAI. Low-frequency rTMS applied over primary somatosensory cortex (SI) reduces SAI in focal dystonia but does not alter SAI in healthy controls (Baumer *et al.*, 2007). Over M1, rTMS decreases MEPs without changing SAI (Fischer & Orth 2011). Intermittent theta burst stimulation (iTBS) applied to M1 increases SAI in Parkinson's disease (PD) patients on medication without altering SAI in controls (Zamir *et al.*, 2012). Identifying a method such as cTBS to modify SAI may provide new therapeutic approaches for modulating abnormal sensorimotor circuitry in specific clinical populations such as stroke and dystonia.

Continuous theta burst stimulation (cTBS) applied over M1 decreases MEPs (Goldsworthy *et al.*, 2012a) but increases MEPs when applied over SI (Jacobs *et al.*, 2013; Jacobs *et al.*, 2012) and higher-order somatosensory area 5 (Premji *et al.*, 2011). The present study investigated the effects of 30 Hz cTBS delivered over left-hemisphere SI and M1 on SAI and MEPs measured from the first dorsal interosseous muscle (FDI) of the right hand. CTBS at 30 Hz delivered over M1 results in less inter-subject variability and longer lasting effects compared to the traditional 50 Hz cTBS (Goldsworthy *et al.*,

2012a). It was hypothesized that MEPs will be decreased for up to 25 minutes and increased for up to 45 minutes following cTBS over M1 and SI, respectively (Goldsworthy *et al.,* 2012a; Jacobs *et al.,* 2013).

METHODS

Participants

Eighteen individuals (7 Males, Mean age = 21 ± 2.0, range of 19 to 25) participated. All participants were right handed as determined by a subset of the Edinburgh Handedness Scale (Oldfield 1971). In Experiment 1, all participants completed two experimental sessions separated by a minimum of one week. In Experiment 2, nine subjects that were in Experiment 1 returned to participate (1 Male, mean age, SD, range = 20.4 ± 1.9, 19-25). This study was approved by the McMaster Research Ethics Board and conformed to the Declaration of Helsinki.

Electromyography (EMG) Recording

Surface electrodes (9mm diameter Ag-AgCl) were used to record electromyography (EMG) from the first dorsal interosseous (FDI) muscle of the right hand in a bipolar montage with the active electrode placed over the muscle belly and the reference electrode placed over the metacarpophalangeal joint of the index finger. Electromyography was band-passed filtered between 20 Hz and 2.5 KHz and amplified x1000 (Intronix Technologies Corporation Model 2024F with Signal Conditioning; Intronix Technologies Corporation, Bolton, Canada) and digitized at 5 KHz by an analog-to-digital interface (Power1404; Cambridge Electronics Design, Cambridge, UK).

Neuronavigation and Transcranial Magnetic Stimulation (TMS)

Single pulse TMS was delivered with a custom-built 50 mm diameter figure-of-eight branding coil connected to a Magstim 200^2 stimulator (Magstim, Whitland, UK). The motor hotspot for the FDI was determined over left-hemisphere M1. The hotspot was identified as the optimal location with the lowest threshold and most consistent responses isolated in relaxed FDI of ~1 mV MEP amplitude. The figure-of-eight coil was positioned over the motor hotspot at ~ 45 degrees to the mid-sagittal plane to induce a posterior-to-anterior monophasic current in the cortex. The motor hotspot was marked by digital registration using a standard MRI template via Brainsight 2 Neuronavigation (Rogue Research, Montreal, Canada). This motor hotspot and the 50 mm figure-of-eight branding coil were used for all measures of MEPs and SAI.

CTBS was applied using a Magstim Super Rapid stimulator (Magstim, Whitland, Dyfed, UK) connected to a figure-of-eight air cooled coil with the handle pointed 45 degrees to the mid-sagittal plane to induce the first current in the cortex in the posterior-to-anterior direction. The cTBS protocol is a modified version of the original protocol (Huang *et al.*, 2005). The modified cTBS consisted of 3 stimuli (bursts) applied at intervals of 33.3 ms (30 Hz) repeated at 16.7 ms intervals (6 Hz) as described by Goldsworthy et al. (2012). Resting motor threshold (RMT) was defined as the minimum stimulus intensity required to evoke MEPs with amplitude $\geq$ 50 μV in 5 out of 10 consecutive trials whilst the subject is quiescent (Siebner & Rothwell 2003). CTBS was delivered at 70% of RMT 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 2 cm posterior to the M1 hotspot

using Brainsight 2 Neuronavigation (Rogue Research Inc., Montreal, Canada). RMT was collected with the biphasic pulses of the Magstim Super Rapid stimulator and air-cooled coil.

Experiment 1: SAI and MEPs following cTBS over M1 or SI

Somatosensory Evoked Potentials (SEPs)

SEPs were used to determine the N20 latency for each subject. The N20 potential represents arrival of somatosensory afference to area 3b (Allison *et al.*, 1991). This latency was then used to adjust the ISI for SAI of each subject for SI and M1 sessions. Subjects were seated in a relaxed position during SEP acquisition. SEPs were recorded over left-hemisphere SI following electrical stimulation of the right median nerve at 3 Hz. The median nerve was stimulated using a surface bar electrode (square wave pulse, 0.2 ms duration) at the right wrist (Grass SD 9, Grass Technologies, West Warwick, USA) with the cathode proximal to the anode. Median nerve stimulation was set to motor threshold defined as the minimum intensity to elicit a slight thumb twitch. The active electrode was placed at C3' located 2 cm posterior to C3 (Nuwer *et al.*, 1994a) and referenced to electrode Fpz (International 10-20 System) with the ground electrode placed on the skin overlying the left clavicle. EEG recordings were amplified 10 K and filtered from 2-2500 Hz (Intronix Technologies Corporation Model 2024F with Signal Conditioning, Bolton, Canada). Electrode impedances were maintained at < 5 kΩ (UFI Checktrode, Model 1089 Mk III, UFI, Morro Bay, USA). Five hundred stimuli were delivered and time-locked averaged off-line.

MEPs and SAI

MEPs were collected by averaging the response to 20 single TMS pulses over M1 at an intensity to evoke MEPs of ~1 mV (0.5-1.5 mV range) peak-to-peak amplitude in FDI. The intensity to evoke ~ 1 mV MEP amplitude was determined prior to the pre-cTBS block and this intensity was held constant throughout the session.

For median nerve SAI (MN-SAI), the interstimulus interval between the median nerve stimulation and the TMS pulse was derived from the N20 component of the SEP plus an additional 2 ms as this latency has been reported to induce the greatest inhibition (Tokimura *et al.,* 2000). The median nerve stimulus parameters were the same as those used to obtain the SEPs. The digital nerve of the 2nd digit was stimulated using ring electrodes with the cathode placed on the proximal phalanx and the anode on the intermediate phalanx (square wave pulse, 0.2 ms). Digital nerve stimulation was applied at 3 times sensory threshold. Sensory threshold was defined as the lowest stimulus intensity at which the subject reports sensation of finger stimulation on half of the trials. For digital nerve SAI (DN-SAI) an additional 5 ms was added to the N20 component to account for the corresponding conduction time of afferent input across the palm of the hand (Kimura 1989). For SAI, nerve stimulation intensities were determined prior to baseline and were re-evaluated before each time block. TMS intensity was set to evoke ~ 1 mV in the unconditioned MEP (i.e. TS alone). For SAI, a block of trials included a random presentation of 20 MN-SAI, 20 DN-SAI and 20 TS alone trials for a total of 60 trials.

Experimental Design

The experiment timeline is depicted in Figure 4. Each session was divided into four blocks: T_0 (pre-cTBS), T_1 (5 - 20 minutes following cTBS), T_2 (25 - 40 minutes following cTBS), T_3 (45 - 60 minutes following cTBS). M1 and SI sessions were identical with the exception of the location of the cTBS target. The order of sessions (M1, SI) was counterbalanced across subjects. SAI and MEP measures were acquired as shown in Figure 4A.

Experiment 2: "Strong" and "Weak" median nerve SAI following cTBS over SI

Nine participants who also participated in Experiment 1 were studied (1 Male, Mean Age = 20.4 ± 1.9). Experiment 2 investigated whether cTBS modulates MN-SAI evoked by two different intensities of nerve stimulation. In Experiment 1, MN-SAI decreased in 8 out of 12 subjects at T_3 minutes following cTBS over SI, a non-significant trend that occurred at a similar time-point as DN-SAI reduction. To investigate whether reduction of SAI via cTBS depends on the depth of MN-SAI (ie. ratio of inhibition), two intensities of MN-SAI were investigated. The median nerve stimulation intensity was set to elicit a ratio of "Weak SAI" (SAI ~ 0.6), which matched the depth of DN-SAI in Experiment 1 and a "Strong SAI" (SAI at motor threshold) which replicates MN-SAI in Experiment 1. These intensities were determined prior to baseline. MN-SAI used the same ISI's that were determined from the subjects in Experiment 1. Measurements of MN-SAI were taken before and after 30 Hz cTBS was applied over SI. The experimental timeline is depicted in Figure 4B. Each time block was divided into two sub-blocks that delivered

either "Strong SAI" or "Weak SAI" trials only. Thirty trials (20 conditioned, 10 unconditioned) were tested within each sub-block. The order of sub-blocks (Strong SAI and Weak SAI) was counterbalanced across subjects.

Experiment 3: Longevity of DN-SAI reduction following cTBS over SI

To test the longevity of changes in SAI following cTBS over SI, three individuals who were tested previously in Experiment 1 participated (3 females, ages 21, 20 and 20). SAI was recorded before and after SI cTBS at intervals used previously (T_1, T_2, T_3) and at additional post-cTBS intervals of T_4 (90 minutes), T_5 (120 minutes) and the following day (T_6) if responses had not returned to baseline.

Data Analysis

For Experiment 1, the MEP peak-to-peak amplitude was averaged for each participant for each time block. Specifically, a *priori* hypotheses were tested using Bonferroni corrected one-tailed paired *t*-tests (see above for hypotheses).

For sensory threshold following SI cTBS, one-way repeated measures analysis of variance (ANOVA) with factors TIME (T_0, T_1, T_2, T_3) was run. Post-hoc paired two-tailed *t*-tests were used to identify significant differences among the means in the event of significance.

For SAI, the averaged conditioned motor-evoked potential amplitude (i.e. TMS in presence of nerve stimulation) was normalized to the averaged unconditioned MEP amplitude (i.e. test stimulus alone) for each participant and for each time block ($SAI =$

$\frac{CS-TS}{TS}$). The presence of SAI was tested with two-tailed paired *t*-tests between the group-averaged conditioned MEP and TS alone (ie. CS-TS vs. TS) for each time block in both experiments. For Experiment 1, subjects were included in subsequent analyses if SAI < 1 for both nerves (DN, MN) and both sessions to allow for a within-subject statistical comparison (ANOVA) with factors NERVE (2 levels; DN, MN), TIME (3 levels; T_1, T_2, T_3), and SITE (2 levels; M1, SI). In the latter analyses SAI obtained from each post-cTBS block was normalized to the T_0 time block to account for the difference in SAI during 'pre' between the two nerves. Post-hoc paired two-tailed *t*-tests were used to identify significant differences among the means in the event of significance.

For Experiment 2, to test for changes in SAI following cTBS, *a priori* hypotheses were tested using Bonferroni corrected one-tailed paired *t*-tests. In order to examine if the two intensities were modulated differently following cTBS, we normalized the post cTBS blocks to the T_0 and performed a two-way repeated measures ANOVA with factors INTENSITY (2 levels; Strong, Weak) and TIME (3 levels; T_1, T_2, T_3). For all ANOVAs, the Greenhouse-Geisser method was used to correct for non-sphericity. All significance levels were set at $p \leq 0.05$.

RESULTS

Experiment 1

Six participants did not meet the criteria for inclusion such that SAI was not observed for both nerves and both sites. Twelve remaining subjects were included in subsequent

analyses (3 Males, Mean age = 20.7 ± 1.9). The average percentage of the maximal stimulator output (MSO) for cTBS delivery was 41.8% (± 7.2) and 40.8% (± 8.0) for the M1 and SI session, respectively, and was not significantly different (two-tailed paired *t*-test, $p = 0.14$).

MEPs

The group-averaged MEP amplitude was not significantly different at T_0 between the M1 and SI session (two-tailed paired *t*-test, $p = 0.12$). *A priori* planned comparisons (Bonferroni corrected *t*-test) revealed a significant decrease in MEP amplitude at T_1 ($p = 0.02$) but not at T_2 following M1 cTBS ($p = 0.16$). Figure 5A displays the group-averaged data (with standard error of the mean) for MEPs following M1 cTBS at each time point. For M1 cTBS, there was no effect of TIME for sensory threshold ($F_{(3, 33)} = 2.344$, $p = 0.09$). *A priori* planned comparisons (Bonferroni corrected *t*-test) revealed a trend for increases in MEP amplitudes at T_1 ($p = 0.03$), T_2 ($p = 0.04$), and T_3 ($p = 0.03$). For SI cTBS, there was no effect of TIME for sensory threshold ($F_{(3, 33)} = 1.936$, $p = 0.143$).

SAI

A three-way repeated measures ANOVA revealed no effect of NERVE ($F_{(1, 11)} = 1.33$, $p = 0.27$), no effect of SITE ($F_{(1, 11)} = 3.34$, $p = 0.09$) and no effect of TIME ($F_{(2, 22)} = 3.17$, $p = 0.06$) and no significant interactions. In this analysis, SAI for each post-cTBS time block was normalized to SAI at T_0 (within nerve and site) and Figure 6 displays the ratio in each respective condition (with standard error of the mean) for each site, nerve and time block. Subsequent analyses examined each nerve and site separately using repeated

measures one-way ANOVA and are shown in Figure 7. The difference in T_0 SAI for DN versus MN was confirmed for the M1 session (two-tailed paired *t*-test, $p = 0.001$) and the SI session ($p = 0.014$) and indicated that SAI was significantly greater for MN compared to DN at T_0 in both sessions. For these analyses, SAI in post-cTBS time blocks were not normalized to SAI in T_0. For M1 cTBS, these analyses revealed no effect of TIME for either nerve (median, $F_{(3, 33)} = 0.49$, $p = 0.69$; digital, $F_{(3, 33)} = 0.55$, $p = 0.65$) (Figure 7A, B). For SI cTBS, with MN-SAI (Figure 7C) there was no significant effect of TIME ($F_{(3, 33)} = 1.42$, $p = 0.25$). However, a trend for reduced SAI was observed at T_3 in 8 of 12 participants. DN-SAI following cTBS over SI (Figure 7D) revealed a significant effect of TIME ($F_{(3, 33)} = 4.72$, $p = 0.007$). Subsequent post hoc two-tailed paired *t*-tests revealed a significant reduction of SAI at T_3 ($p = 0.019$) but not at T_1 ($p = 0.63$) or T_2 ($p = 0.18$) compared to T_0. These data indicate that DN-SAI gradually decreased following SI cTBS until significant reduction was observed at T_3. Although DN-SAI was reduced following SI cTBS, two-tailed paired *t*-tests showed that conditioned MEPs were still significantly reduced in comparison to unconditioned MEPs (T_0: $p < 0.001$; T_1: $p < 0.001$; T_2: $p < 0.01$; T_3: $p = 0.03$). DN-SAI is reduced following SI cTBS but still remains. In summary, it appears that SI cTBS significantly reduces DN-SAI at T_3 although a trend exists for a similar time course of reduced MN-SAI.

Experiment 2

All subjects demonstrated SAI for both MN intensities (n = 9, 1 Male, Mean age = 20.4 ± 1.9). The average MSO for SI cTBS delivery was 40.4 % (± 7.6) which was not significantly different than the cTBS intensity used over SI in Experiment 1 ($p = 0.93$).

Figure 8A and 8B display the group-averaged data (with standard error of mean) for "Strong SAI" and "Weak SAI", respectively, at each time point. For "Strong SAI" , *a priori* planned comparison (Bonferroni corrected *t*-test) revealed a significant decrease in SAI at T_3 ($p = 0.009$) indicating that cTBS significantly reduces MN-SAI at T_3. For "Weak SAI", *a priori t*-test revealed a significant decrease in SAI ($p = 0.015$). Further, two-way repeated ANOVA revealed no effects of INTENSITY ($F_{(1, 8)} = 2.521$, $p = 0.151$), TIME ($F_{(2, 16)} = 1.811$, $p = 0.196$), or their interaction ($F_{(2, 16)} = 0.814$, $p = 0.461$). Although MN-SAI was modulated at both nerve stimulation intensities, SAI was still maintained. Conditioned MEPs were significantly reduced in comparison to unconditioned MEPs in "Strong SAI" (T_0: $p < 0.001$; T_1: $p = 0.001$; T_2: $p < 0.001$; T_3: $p < 0.001$) and "Weak SAI" (T_0: $p < 0.001$; T_1: $p = 0.003$; T_2: $p = 0.05$; T_3: $p = 0.04$). In summary, it appears that cTBS reduces MN-SAI at T_3, similar to the effects on DN-SAI.

Experiment 3

The longevity of the SI cTBS induced reduction is shown in Figure 9 for three participants. The reduction in DN-SAI persisted greater than 2 hours in two individuals and returned to baseline by the following day.

DISCUSSION

In the present study, the modulation of SAI and MEPs via 30 Hz cTBS was investigated in young adults. Novel findings include a reduction in SAI at T_3 following cTBS over SI. In contrast, cTBS over M1 had no significant effect on SAI modulation. In support of previous research, 30 Hz cTBS over M1 suppressed MEPs (Goldsworthy *et al.*,

2012a) with opposite effects when applied over SI (Jacobs *et al.,* 2013; Jacobs *et al.,* 2012). The present data suggests that the modulation of SAI may originate in SI cortex and that cTBS may be modulating the net excitability of corticocortical projections from SI to M1.

In support of previous studies, we observed that MEPs were decreased following M1 cTBS (Goldsworthy *et al.,* 2012a; Huang *et al.,* 2005). Similar to the 30 Hz cTBS effects reported elsewhere (Goldsworthy *et al.,* 2012a), we observed a magnitude reduction of ~23% at T_1 but also noted differences in the duration of suppression between studies which may relate to the stimulation intensity used (i.e. 80% versus 70% RMT as used here). In contrast, SAI was not modulated following M1 cTBS, similar to the findings in healthy controls following iTBS over M1 (Zamir *et al.,* 2012) and low frequency rTMS over M1 (Fischer & Orth 2011).

There was a strong trend for MEP facilitation when cTBS was applied over SI. MEPs increased over time such that at T_3, the averaged amplitude increased by ~63%. Although the effects were not statistically significant these data support previous findings of MEP facilitation when cTBS was applied over SI (Jacobs *et al.,* 2013; Jacobs *et al.,* 2012) and higher-order somatosensory area 5 (Premji *et al.,* 2011), where ~60% and ~50% increases in MEP amplitude were observed following cTBS, respectively. Further, the greatest MEP facilitation in Jacobs et al. (2012) was observed at T_3 following cTBS, in line with the present trend. Our data also indicate that cTBS over SI modulates SAI with a reduction of ~ 40% at T_3. These findings are in contrast to the lack of SAI modulation following low-frequency rTMS over SI in healthy controls (Baumer *et al.,*

2007). However, in the latter report SAI measures were obtained immediately following rTMS (Baumer *et al.*, 2007) while we observed a gradual reduction of SAI that was statistically significant by T_3 following stimulation.

It is interesting to note that following SI cTBS, MEP facilitation and SAI reduction follow a similar time course with maximal effects occurring at T_3 suggesting similarities in the mechanisms by which cTBS acts. SAI causes a reduction of later I-wave generating interneurons that are believed to transsynaptically synapse on corticospinal neurons (Tokimura *et al.*, 2000). We speculate that cTBS over SI creates a net facilitatory effect on the later I-wave circuitry that leads to both facilitation of MEP, as proposed elsewhere (Jacobs *et al.*, 2013) and reduced SAI. Though mechanisms governing the net facilitation remain unclear, it would appear that changes in GABAergic inhibition are likely occurring in SI following cTBS. GABA concentration in sensorimotor cortex increases following cTBS (Stagg *et al.*, 2009) and increases in $GABA_A$ are known to reduce SAI (Di, V *et al.*, 2005). Alternatively, cTBS in SI may target cholinergic neurons and one suggestion is that this may act to alter the SI responsiveness to somatosensory input. In rats, blocking central ACh receptors leads to complex changes in somatosensory evoked potentials such as enhancing some cortical potentials but not all (Dancause *et al.*, 2001). In humans, SAI is reduced and even abolished in the presence of ACh antagonist, Scopolamine (Di, V *et al.*, 2000). In contrast, M1 cTBS does not change SAI and this lends support to the finding that cTBS over M1 acts primarily on I1 as opposed to later I-waves (Di Lazzaro *et al.*, 2005a).

Following SI cTBS, increases in MEPs and decreases in SAI occur gradually over time. Such gradual changes are observed following cortico-cortical paired associative stimulation (PAS) between the posterior parietal cortex and M1 whereby the greatest increases in MEPs occurred at ~ 60 minutes following stimulation (Chao *et al.*, 2013a). Similarly, in traditional PAS, changes in MEPs do not appear immediately but rather occur gradually and are significant at ~ 30 minutes following stimulation (Stefan *et al.*, 2000). Gradual changes in neural activity are also seen in animal models of potentiation or depression of synapses (Bi & Poo 1998; Fino *et al.*, 2005).

Our findings cannot definitively conclude that SAI is mediated via a synaptic relay in SI. CTBS over SI reduced SAI but did not abolish activity in this circuit. Therefore, it remains unclear whether SAI is mediated by a synaptic relay through SI or via direct thalamocortical projections to M1 (Lemon 1981; Tokimura *et al.*, 2000). However, the present findings indicate that SI modulates SAI and this may occur via a path distinct from that which generates SAI. A similar speculation of parallel paths has been made for other transcortical reflexes such as stretch reflexes to perturbations (Kimura *et al.*, 2006). Similar to our findings, whole hand afferent input facilitates MEPs for up to one hour via mechanical (Christova *et al.*, 2011) or electrical stimulation (Golaszewski *et al.*, 2004). Further, rapid-rate paired associative stimulation, which integrates both repetitive TMS over M1 and repetitive nerve stimulation, results in findings very similar to those observed here – MEP facilitation and SAI reduction for up to one hour following stimulation (Quartarone *et al.*, 2006; Terranova *et al.*, 2013). Collectively, the existing data suggest that changes in SI activity originating from either

the periphery or directly via cTBS, yield very similar results on SAI circuitry and corticospinal excitability.

CTBS over SI modulates SAI regardless of the nerve composition. Findings from Experiment 1 indicated that cutaneous evoked SAI was more easily modifiable than mixed nerve SAI, a finding we thought was attributed to the lower magnitude of SAI for the cutaneous versus mixed nerve. The intensity of digital nerve stimulation influences the depth and field of inhibition as greater intensities recruit more afferent fibres (Tamburin *et al.,* 2001). We note that in this study, the strength of median and digital nerve SAI were similar to that seen elsewhere (median; (Alle *et al.,* 2009; Asmussen *et al.,* 2013; Fischer & Orth 2011; Tokimura *et al.,* 2000; Udupa *et al.,* 2009; Young-Bernier *et al.,* 2012; Zamir *et al.,* 2012) ; digital; (Asmussen *et al.,* 2013; Baumer *et al.,* 2007; Richardson *et al.,* 2008; Voller *et al.,* 2006). Experiment 2, however demonstrated that mixed nerve SAI was indeed modifiable at both weak and strong levels of SAI, confirming the trend we observed with strong SAI in 67% of participants in Experiment 1. Despite the reduction of SAI, the inhibitory circuit was maintained (ie. SAI < 1). Our data, therefore, indicate that cTBS over SI is capable of modulating SAI across nerve types and at various initial depths of SAI.

There are two limitations that should be noted. First, it is not clear why median nerve SAI was significantly reduced in Experiment 2 but not in Experiment 1. CTBS intensity does not appear to contribute since the nine participants showed similar RMT values in both experiments. However, one factor that may have influenced the results was the time of the day in which the subjects were tested (ie. morning vs. afternoon). In

Experiment 1, we did not control for the time of day in which participants were tested and data were collected in both the morning and afternoon. However in Experiment 2, participants were tested in the afternoon only. There is evidence that plasticity-inducing protocols are more effective during the afternoon. Increases in MEP amplitude are larger when PAS is delivered in the afternoon compared to the morning, an effect that may relate to cortisol levels (Sale *et al.*, 2007). Second, we cannot eliminate the possibility that current spread from SI to M1 may contribute to the results. However, we have demonstrated that differential effects are observed for following SI versus M1 stimulation. Therefore, when the intensity of stimulation over these two loci is matched, the effect of SI versus M1 cTBS on MEPs and SAI are indeed different.

The present findings have identified a method to alter SAI in young adults. Several patient populations show abnormal SAI and TMS methods may be one avenue for attempting to alter this circuitry. Patient's with PD show normal SAI off medication but a decrease in SAI while on medication (Sailer *et al.*, 2003). Similarly, SAI is reduced in Alzheimer's disease (Di Lazzaro *et al.*, 2002; Nardone *et al.*, 2008) and mild cognitive impairment (Yarnall *et al.*, 2013). SAI is also impaired in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (Palomar *et al.*, 2013a). The present research indicates that cTBS over SI may be one method by which SAI circuitry targeted to the hand may be altered and future studies may test whether these effects also occur in clinical populations.

FIGURES

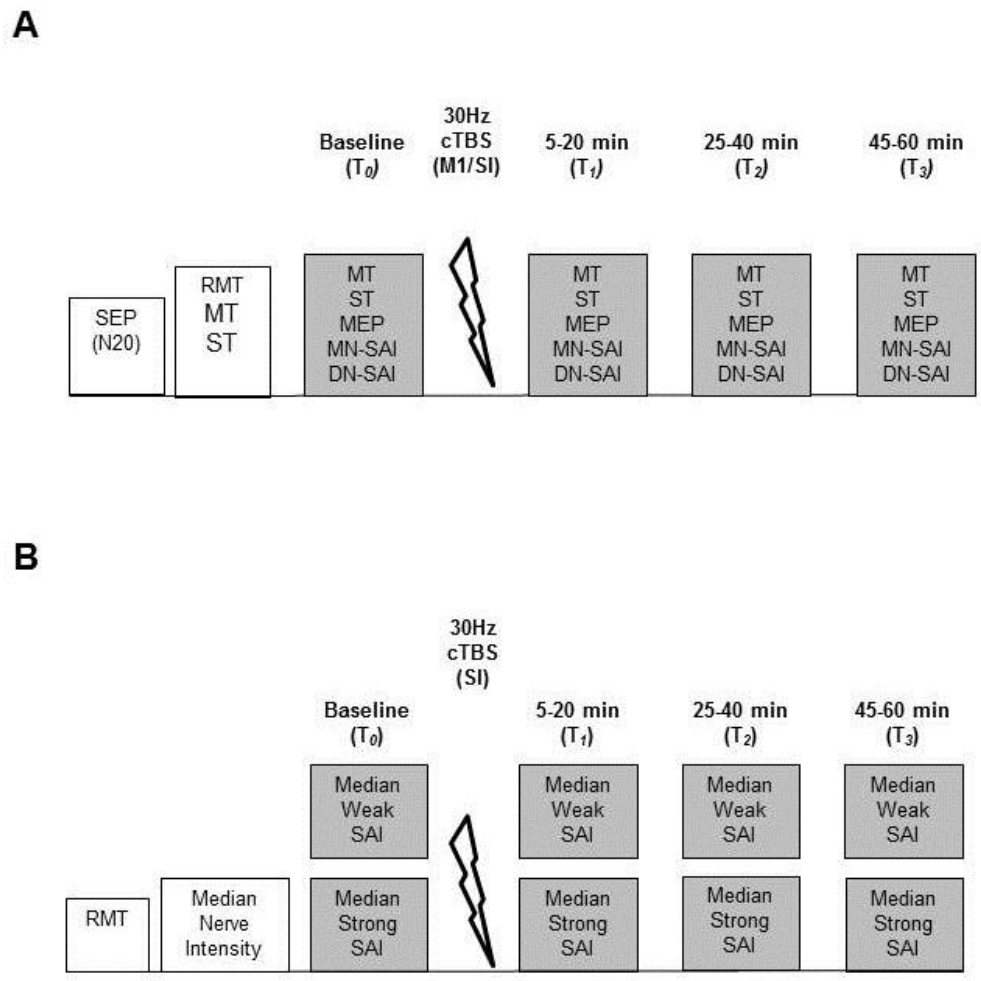

Figure 4: Experimental Timeline

A) Experiment 1. CTBS was delivered to M1 and SI in the same group of participants. Measures of MEPs, median nerve SAI and digital nerve SAI were acquired from right FDI before (T_0) and at 5-20 minutes (T_1), 25-40 minutes (T_2) and 45-60 minutes (T_3) following cTBS delivered over the left-hemisphere. Sensory threshold intensities (ST) were determined prior to baseline and were re-evaluated before each time block. **B)** Experiment 2. CTBS was delivered to SI. Measures of 'Strong' (0.4) and 'Weak' (0.6) median nerve SAI were acquired at the same time blocks as in Experiment 1.

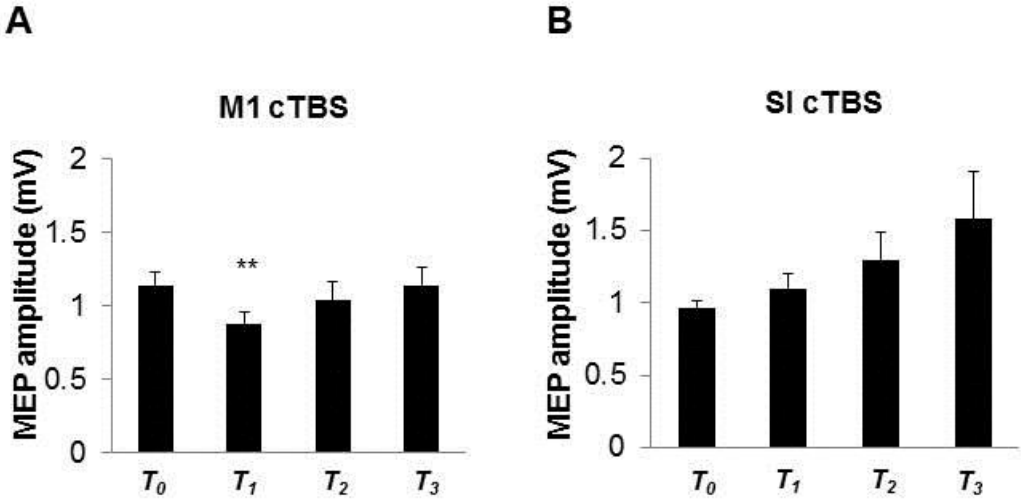

Figure 5: MEP Modulation Following cTBS

Group-averaged MEP amplitude with standard error of the mean following cTBS over **A)** left M1 and **B)** SI at T0 (pre-cTBS), T1 (5-20 minutes), T2 (25-40 minutes), and T3 (45-60 minutes) for the right first dorsal interosseous (RFDI). An asterisk over a single time block indicates it was significantly different than T0. Significant differences were tested at $p \leq 0.05$.

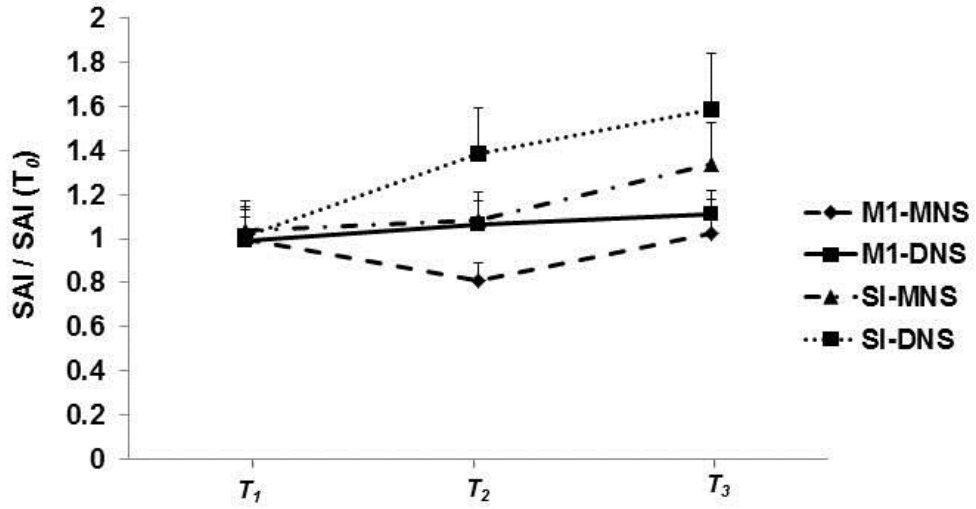

Figure 6: SAI normalized to Pre

The y-axis ratio was calculated by normalizing the each individual's post-cTBS SAI (T1, T2, T3) to their pre-cTBS SAI (T0) SAI with standard error of the mean for each site and nerve condition. Following cTBS, SAI is similar at T1 but diverges at later time blocks T2 and T3 with SI cTBS leading to a decrease in SAI.

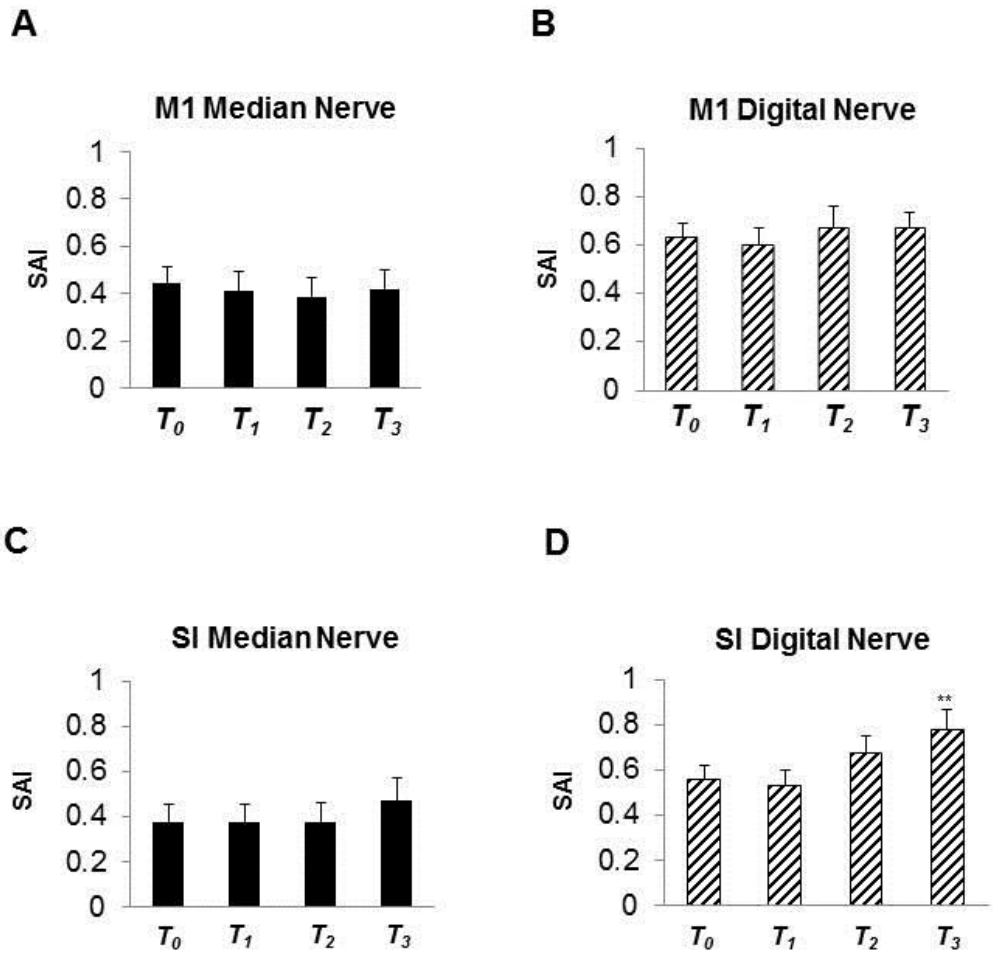

Figure 7: SAI following cTBS

MN-SAI **A)** and DN-SAI **B)** following M1 cTBS and MN-SAI **C)** and DN-SAI **D)** following SI cTBS with standard error of mean. SAI is expressed as a ratio of the conditioned MEP amplitude (CS-TS) normalized to the unconditioned MEP amplitude (TS). Significant reduction in DN-SAI was observed at 45 minutes following SI cTBS. Significant differences were tested at $p \leq 0.05$.

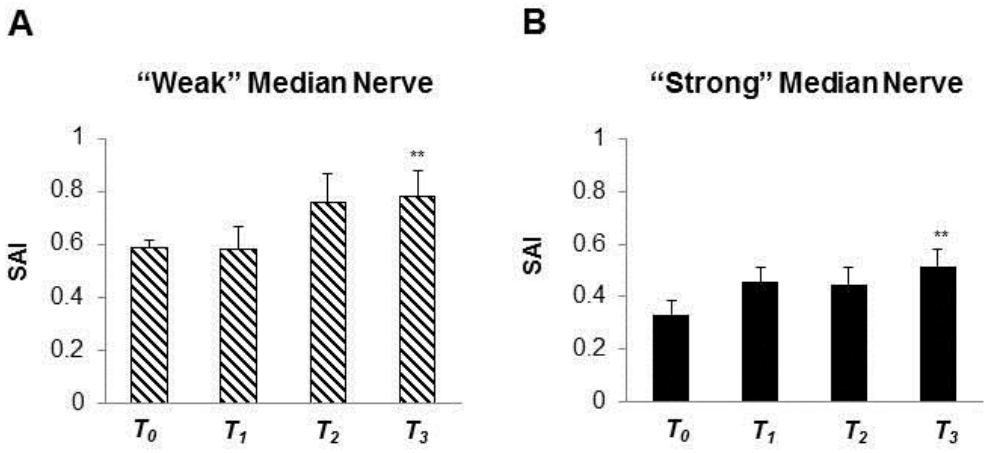

Figure 8: MN-SAI following cTBS

"Weak" MN-SAI (SAI ~ 0.6) **A)** and "Strong" MN-SAI (SAI at motor threshold) **B)** following cTBS over SI. Similar to DN-SAI following SI cTBS in Experiment 1, significant reduction of MN-SAI was observed in both "Strong" and "Weak" MN-SAI at 45 minutes after cTBS. Significant differences were tested at $p \leq 0.05$.

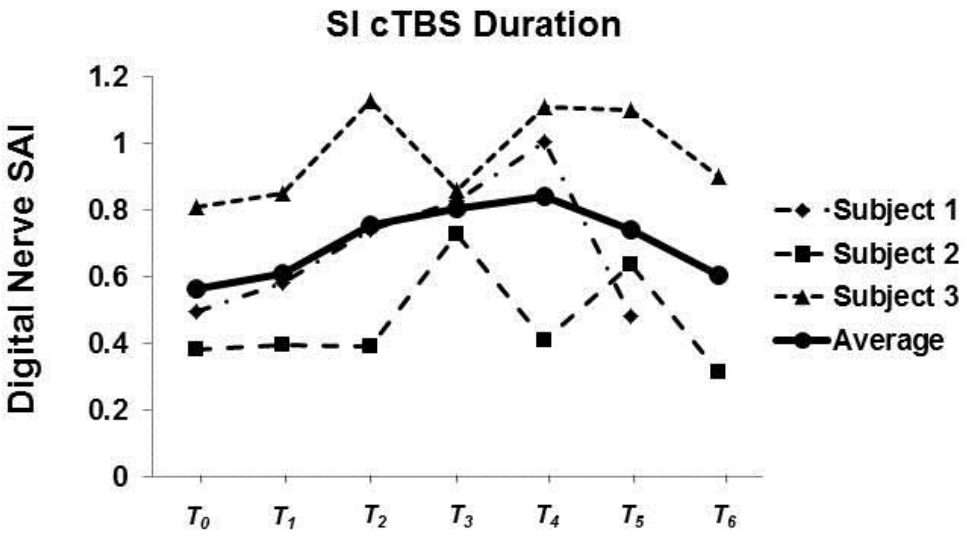

Figure 9: Longevity of SAI Reduction

Longevity of DN-SAI modulation following SI cTBS. Individual participant data for DN-SAI following SI cTBS is shown using dashed lines. Group averaged DN-SAI is shown in the solid line. SAI continually decreases for up to 1.5 hours (T4) following SI cTBS but returns back to baseline the following day (T6).

CHAPTER 4: EFFECTS OF RAPID-RATE PAIRED ASSOCIATIVE STIMULATION ON SENSORIMOTOR CIRCUITRY

INTRODUCTION

In recent years, there have been widespread investigations of several non-invasive techniques that transiently modulate cortical excitability. Repetitive transcranial magnetic stimulation (rTMS) was first introduced to induce long-term potentiation (LTP) like effects when stimulated at higher frequencies (ie. ≥ 5Hz) or long-term depression (LTD) like effects when delivered at low frequencies (< 1 Hz) (Chen *et al.,* 1997; Pascual-Leone *et al.,* 1994). Aside from traditional rTMS, theta burst stimulation (TBS) protocols have been developed based on animal models of plasticity which decrease the duration of the stimulation paradigm but still induce robust LTP or LTD-like effects (Jacobs *et al.,* 2013). Modifications of protocols are continually being pursued to increase the efficiency of rTMS and decrease inter-subject variability (Goldsworthy *et al.,* 2012a; Goldsworthy *et al.,* 2012b).

Paired associative stimulation (PAS) involves repeated pairing of TMS preceded by peripheral nerve stimulation and leads to transient changes in cortical excitability at specific interstimulus intervals. First, the postsynaptic neuron is activated by weak inputs from the presynaptic neuron (ie. afferent input from nerve stimulation), subsequently the postsynaptic neuron is activated by a strong input (ie. TMS at cortex) leading to LTP-like effects (Stefan *et al.,* 2000). On the other hand, when the order of events is reversed, LTD-like effects occur (Wolters *et al.,* 2003). Spike-timing-dependent plasticity (STDP) and changes in synaptic efficacy are what drive these changes in cortical excitability

within the motor cortex. These synaptic changes are believed to be driven by NMDA receptors as bicuculline abolishes effects of PAS (Stefan *et al.,* 2002). PAS applied to the primary somatosensory cortex (SI) may also lead to changes in sensory cortical excitability as measured with median nerve somatosensory evoked potentials (SEP) (Wolters *et al.,* 2005). Specifically there is a modulation of the P25 component believed to be generated in the upper cortical layers of area 3b (Allison *et al.,* 1991). There are several experimental parameters which may influence the efficacy of M1 PAS such as age (Fathi *et al.,* 2010), attention (Stefan *et al.,* 2004) and time of day (Sale *et al.,* 2007). Similarly, SI PAS has shown great variability in its effects, as the same SEP modulations have not been observed across all studies (Krivanekova *et al.,* 2011; Murakami *et al.,* 2008; Tamura *et al.,* 2008). Important for the purpose of the present study, PAS may also be applied at high frequency (5 Hz) and is therefore known as rapid-rate paired associative stimulation (rPAS). RPAS also modulates corticospinal excitability but requires a shorter duration of stimulation and induces longer lasting effects (Quartarone *et al.,* 2006). In contrast to traditional low-frequency PAS, rPAS has the ability to modulate sensorimotor circuitry.

Short-latency afferent inhibition (SAI) is observed when median or digital nerve stimulation precedes a single TMS pulse by ~20-28 ms, leading to a decrease in the motor evoked potential's (MEP) amplitude (Tokimura *et al.,* 2000). SAI is considered to be cortically mediated and is suggested to indicate central cholinergic activity (Di Lazzaro *et al.,* 2000). Reduced SAI is observed in Alzheimer's disease (Di Lazzaro *et al.,* 2002), Parkinson's disease on levodopa medication (Sailer *et al.,* 2003), Parkinson's disease with

mild cognitive impairment (Yarnall *et al.*, 2013), and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy patients (Palomar *et al.*, 2013b). Interventions such as muscarinic antagonist scopolamine (Di Lazzaro *et al.*, 2000) and $GABA_A$ agonist lorazepam (Di Lazzaro *et al.*, 2005c) can reduce SAI in a typical population. To date, there have been few reports of increased SAI. Reduced SAI in Alzheimer's disease can be restored by acetylcholinesterase inhibitor, rivastigmine (Di Lazzaro *et al.*, 2005b). Similarly, a spatial attention task can also increase the degree of SAI (Kotb *et al.*, 2005).

Understanding the mechanisms that underpin SAI and the modulation of this circuit may allow for future intervention approaches in patient populations. In our laboratory we have observed that 30 Hz continuous theta burst stimulation (cTBS) applied over SI decreases SAI induced by the median or digital nerve (Tsang *et al.*, 2014). Similarly, when rPAS is applied to M1 (M1 rPAS) there are decrease in SAI and increases in corticospinal excitability for up to one hour in controls but not in patients with Alzheimer's disease (Quartarone *et al.*, 2006; Terranova *et al.*, 2013). Based on these findings, there is a strong speculation that SI modulation of corticospinal excitability and SAI may share a similar mechanism. This modulatory mechanism may occur via increased net activity of the late indirect waves (I-waves) generated by interneurons that are believed to excite the corticospinal neurons (Tsang *et al.*, 2014).

The present study explores the effects of rPAS over SI and M1 on corticospinal excitability and SAI. To date, there have been no investigations that explore the effects of

rPAS when TMS is applied to SI. No hypotheses were made for SI rPAS as it is a novel paradigm.

METHODS

Participants

Twelve individuals participated in the study (4 Males, Mean age = 20.9 ± 2.9) of which twelve took part in Experiment 1 and eight in Experiment 2. All participants were deemed right handed as determined by a subset of the Edinburgh Handedness Scale (Oldfield 1971). All individuals participated in two experimental sessions separated by a minimum of one week. This study was approved by the McMaster Research Ethics Board and conformed to the Declaration of Helsinki.

Electromyography (EMG) Recording

Surface electrodes (9 mm diameter Ag-AgCl) were used to record electromyography (EMG) from the abductor pollicus brevis (APB) muscle of the right hand with a tendon-belly arrangement. EMG recordings were band-passed filtered between 20 Hz and 2.5 KHz, amplified x1000 (Intronix Technologies Corporation Model 2024F with Signal Conditioning; Intronix Technologies Corporation, Bolton, Ontario, Canada) and digitized at 5 KHz by an analog-to-digital interface (Power1404; Cambridge Electronics Design, Cambridge, UK).

Neuronavigation and Single-Pulse Transcranial Magnetic Stimulation (TMS)

Single-pulse TMS was applied using a custom-built 50 mm diameter figure-of-eight branding coil connected to a Magstim 200^2 stimulator (Magstim, Whitland, UK). The motor hotspot for APB of the right hand was determined within left-hemisphere M1. The motor hotspot was identified as the optimal location that elicited MEPs with the lowest threshold and most consistent responses isolated in relaxed APB. The figure-of-eight coil was placed over the motor hotspot oriented 45 degrees to the mid-sagittal plane to induce a posterior to anterior monophasic current in the cortex. The motor hotspots were marked by digital registration using a standard MRI template via Brainsight 2 Neuronavigation (Rogue Research, Canada). This motor hotspot and the 50 mm figure-of-eight branding coil were used for all measures of MEPs and SAI.

Somatosensory evoked potentials

Subjects were seated in a relaxed position during SEP acquisition. SEPs were recorded over left-hemisphere SI following electrical stimulation of the right median nerve at 3 Hz. The median nerve were stimulated using a surface bar electrode (square wave pulse, 0.2 ms duration) at the right wrist (Grass SD 9, Grass Technologies, West Warwick, USA) with the cathode proximal to the anode. Median nerve stimulation was set to motor threshold defined as the minimum intensity to elicit a slight thumb twitch. The active electrode was placed at C3' located 2 cm posterior to C3 (Nuwer *et al.*, 1994b) and referenced to electrode Fz (International 10-20 System) with the ground electrode placed on the skin overlying the left clavicle. EEG recordings were amplified (x 10 K) and filtered from 2-2500 Hz (Intronix Technologies Corporation Model 2024F with Signal

Conditioning, Bolton, Canada). Electrode impedances were maintained at < 5 kΩ (UFI Checktrode, Model 1089 Mk III, UFI, Morro Bay, USA). Five hundred stimuli were delivered and time-locked averaged off-line. The N20 potential represents arrival of somatosensory afference to area 3b (Allison *et al.*, 1991). These latencies were used to adjust the interstimulus interval (ISI) for SAI on each subject for all experiments. The amplitude of the N20-P25 peak-to-peak potentials were used in Experiment 2 only.

Rapid-Rate Paired Associative Stimulation (rPAS)

RPAS involved repetitive pairing of median nerve stimulation and TMS at 5Hz. The median nerve was stimulated using a surface bar electrode (square wave pulse, 0.5 ms duration) at the right wrist (Grass SD 9, Grass Technologies, West Warwick, USA) with the cathode proximal to the anode. Median nerve stimulation was set to two time's sensory threshold. Sensory threshold was defined as the minimum intensity at which the subject reported sensation of wrist stimulation on half of the trials (Quartarone *et al.*, 2006).

The repetitive TMS for rPAS was applied using a Magstim Super Rapid stimulator (Magstim, Whitland, Dyfed, UK) connected to a figure of eight air cooled coil with the handle pointed 45 degrees to the mid-sagittal plane to induce the first current in the cortex in the posterior to anterior direction. The rTMS was applied at intervals of 200 ms (5 Hz) repeated at as described by Quartarone et al. (2006). Resting motor threshold (RMT) was determined with the same coil and was defined as the minimum stimulus intensity required to evoke MEPs with amplitude ≥ 50 μV in 5 out of 10 consecutive

trials whilst the subject is quiescent (Siebner & Rothwell 2003). RTMS was delivered at 70% of resting motor threshold (RMT) over the target location. SI location was defined at a position 2 cm posterior to the M1 APB hotspot using Brainsight 2 Neuronavigation as indicated by a previous study using near-infrared spectroscopy (Okamoto *et al.*, 2004). RMT were calculated using the biphasic pulses of the Magstim Super Rapid stimulator and air-cooled coil.

Motor Evoked Potentials and Short-Latency Afferent Inhibition

MEPs were collected by averaging the response to 15 single TMS pulses over M1 at an intensity to evoke MEPs of ~1 mV peak-to-peak amplitude in APB. The intensity to evoke ~ 1 mV MEP amplitude was determined prior to the pre-rPAS block and this intensity was held constant throughout the session.

For SAI, the ISI between the median nerve stimulation and the TMS pulse were derived from the N20 component of the SEP (Tokimura *et al.*, 2000). The median nerve stimulation was set at motor threshold (see above). These intensities were determined prior to T_0 and were adjusted if necessary prior to each time block. For each time block, fifteen trials were presented randomly for each of conditioned MEP (CS-TS) and unconditioned test stimulus (TS alone) for a total of thirty trials.

Experiment 1: MEPs and SAI following M1 rPAS and SI rPAS

Individuals participated in two sessions, one for M1 rPAS and the other for SI rPAS separated by a minimum of one week. For the M1 session, rPAS was applied at 5 Hz with

600 pairs of stimuli continuously delivered to the left M1 for ~2 minutes. Each stimulus consisted of a peripheral nerve stimulation applied to the right median nerve followed by a biphasic TMS pulse given to the left M1. There was an interstimulus interval (ISI) of N20 + 5 ms between the conditioning stimulus (CS) and the test stimulus (TS). An ISI of N20 + 5 ms was chosen to replicate the 25 ms ISI reported (Quartarone *et al.*, 2006). The N20 latency represents the arrival of afferent information to the area 3b for each individual and 5 ms accounts for the time required for the afferent input to influence the sensorimotor cortices (Goldring *et al.*, 1970). For the SI session, rPAS were set to an ISI of N20 as this allows for the median nerve afferent information to converge with the TMS pulse at SI. The two sessions of rPAS were separated by a minimum of one week apart.

At the beginning of Experiment 1(M1 and SI), five subjects in the M1 rPAS session (2 males, Mean age = 20.4 ± 1.3) and ten subjects in the SI rPAS session (3 males, Mean age = 21.3 ± 3.0) were instructed to use his/her first and second digit to pinch a load cell (Trandsucer Techniques, model THA-50-Q load cell) for approximately three seconds. Maximal pinch force (amplitude) was measured three times at the beginning of the experimental session and three times at the very end of the experimental session following the collection of the MEP and SAI data.

Experimental Timeline

The experiment timeline for Experiment 1 is depicted in Figure 10A. Each session was divided into four time blocks: T_0 (pre-rPAS), T_1 (5 - 20 minutes), T_2 (25 - 40 minutes), T_3

(45 - 60 minutes). For Experiment 1, Max Pinch Force was measured at the start of the experiment and immediately after the 45 minute test session.

Experiment 2: SI rPAS at N20 + 2.5 ms

Experiment 2 investigated whether SI rPAS, with a longer ISI (N20 + 2.5 ms), would modulate SAI. In Experiment 1, SI rPAS the ISI was set to each individual's N20 and facilitated MEPs but did not modulate SAI. The N20 latency represents the arrival of afferent information to area 3b which does not have direction projections to M1. We speculated that SI rPAS, set at an ISI of N20, may not be inducing STDP in the population of interneurons that may modulate SAI. Since ISI is crucial to the effects of PAS and rPAS (Quartarone *et al.*, 2006; Wolters *et al.*, 2003), we wanted to increase the length of the ISI by 2.5 ms to target the population of neurons later in the afferent pathway, which may be responsible for modulating SAI. Eight individuals who also participated in Experiment 1 were studied (2 Males, Mean age = 21.6 ± 3.3). Similar to Experiment 1, MEPs and SAI were measured at baseline and at 5 minutes (T_1), 25 minutes (T_2) and 45 minutes (T_3) after SI rPAS (N20 + 2.5ms).

SEPs and paired-pulse inhibition were recorded for each subject at the beginning of the experiment and 45 minutes following rPAS. Both measurements were recorded over left-hemisphere SI following electrical with the same montage mentioned above. For SEPs, stimulation of the right median nerve was set to motor threshold (0.2 ms duration) at a frequency of 3 Hz (Ishikawa *et al.*, 2007). To assess changes in SI intracortical inhibition, paired-pulse inhibition was elicited by paired stimulation of the median nerve with an ISI

of 30 ms (Ragert *et al.*, 2003). MNS was applied at motor threshold (0.2 ms duration) and paired stimuli were applied at 2Hz. Five hundred epochs of SEPs and paired-pulse inhibitions were recorded, time-locked, and averaged off-line.

Experimental Timeline

The experiment timeline for Experiment 2 is depicted in Figure 10B. Each session was divided into four time blocks: T_0 (pre-rPAS), T_1 (5 - 20 minutes), T_2 (25 - 40 minutes), T_3 (45 - 60 minutes). For Experiment 2, SEPs and paired-pulse inhibition were measured at the start of the experiment and after the 45 minute test session.

Data Analysis

The area of the MEP was calculated for each participant and each time block. MEP area was measured instead of peak-to-peak amplitude because several polyphasic MEPs were observed when recording from right APB. For SAI, the averaged conditioned MEP areas (CS-TS) were normalized to the averaged unconditioned MEP areas (TS alone) for each participant and for each time block (ie. $SAI = \frac{CS-TS}{TS\ Alone}$). Two-tailed paired t-tests were run to test for significant reduction of conditioned MEP area (CS-TS) in comparison to unconditioned MEP area (TS alone).

For Experiment 1, MEPs and SAI were analyzed with a two-way repeated measures analysis of variance (ANOVA) using within subject factors TIME (4 levels; T_0, T_1, T_2, T_3) and SITE (2 levels; M1, SI). Post hoc Tukey's honest significant difference (HSD)

test was used to identify significant differences among the means in the event of significance. Significance was set at $p \leq 0.05$.

Maximal pinch force (amplitude) was averaged across three trials. Two-tailed paired *t*-tests were run to compare Max Force before and after rPAS was applied over M1 and SI.

For Experiment 2, MEPs and SAI were analyzed with one-way repeated measures ANOVAs using within subject factors TIME (4 levels; T_0, T_1, T_2, T_3). Post hoc Tukey's honest significant difference (HSD) test was used to identify differences among the means in the event of significance. For all ANOVAs, the Greenhouse-Geisser method was used to correct for non-sphericity. Significance was set at $p \leq 0.05$.

For SEP analysis, peak-to-peak amplitudes of the cortical N20-P25 potentials component were analysed and compared before and 45 minutes after SI rPAS (N20 + 2.5). Paired-pulse inhibition was expressed as a ratio (A2/A1) of the amplitudes of the second (A2) and the first (A1) N20-P25 peaks (see Figure 11 for example of SEP potentials and paired-pulse inhibition).

Two-tailed paired *t*-tests were run to compare SEP amplitude and paired-pulse inhibition before and 45 minutes after SI rPAS (N20 + 2.5ms).

RESULTS

Experiment 1: MEPs and SAI following M1 rPAS and SI rPAS

All 12 participants successfully completed the two sessions (4 Males, Mean age = 20.9 ± 2.9). The average maximal TMS output for rPAS delivery was 40% (± 6.1) and 38.8% (± 5.5) for the M1 and SI session, respectively, and were not significantly different (two-tailed paired *t*-test, $p = 0.39$). Similarly, the average nerve stimulation for M1 rPAS and SI rPAS was 14.7V (± 8.9) and 16.3V (± 8.0), respectively, and were not significantly different (two-tailed paired *t*-test, $p = 0.30$).

MEPs

The group-averaged MEP amplitude was not significantly different at T_0 between the M1 and SI session (two-tailed paired *t*-test, $p = 0.28$). Two-way ANOVA revealed a significant main effect of TIME ($F_{(3, 33)} = 3.32$, $p = 0.03$) without SITE ($F_{(3, 33)} = 0.65$, $p = 0.44$) or interaction effects ($F_{(3, 33)} = 2.47$, $p = 0.08$). Post hoc Tukey's HSD revealed significantly greater MEPs at T_3 compared to T_0 ($p < 0.05$) indicating that MEPs were significantly facilitated following M1 rPAS, but only after 45 minutes following stimulation. Figure 12 displays the group-averaged data (with standard error of the mean) for MEPs following M1 rPAS and SI rPAS at each time point.

SAI

Two-way repeated measures ANOVA revealed significant effects of TIME ($F_{(2, 22)} = 4.64$, $p = 0.008$) and TIME x SITE ($F_{(3, 33)} = 3.99$, $p = 0.016$) without a main effect of SITE ($F_{(1, 11)} = 3.33$, $p = 0.095$). Post hoc Tukey's HSD revealed a significant interaction at T_3 where SAI was reduced following M1 compared to SI rPAS ($p < 0.05$). At T_3, SAI was substantially decreased in the M1 rPAS session whereas following SI rPAS, SAI not

significantly changed. For M1 rPAS, SAI was reduced to an extent where the conditioned MEPs were not significantly different than the unconditioned MEPs at T_3. Two-tailed paired *t*-tests showed that conditioned MEPs were not significantly different in comparison to unconditioned MEPs ($p = 0.07$). In summary, it appears that M1 rPAS decreases SAI, but SI rPAS does not significantly modulate SAI. Figure 13 displays the group-averaged data (with standard error of the mean) for SAI following M1 rPAS and SI rPAS at each time point.

Maximal Pinch Force

In M1 rPAS Max Pinch Force was measured in five participants (2 Males, Mean age = 20.4 ± 1.3). Mean for Max Pinch Force was 11.6N before and 24.6N following M1 rPAS. Ten participants (3 males, Mean age = 21.3 ± 3.0) completed the Max Pinch Force measurement following SI rPAS. Mean for Max Pinch Force was 16.5N before and 19.8N following SI rPAS. Two-tailed paired t-test comparing Max Pinch Force before and after rPAS revealed no significant changes when applied to M1 ($p = 0.11$) or SI ($p = 0.32$). These data indicate that M1 rPAS and SI rPAS did not significantly change Maximal Pinch Force.

Experiment 2: SI rPAS at N20 + 2.5 ms

All subjects successfully completed the session (n = 8, 2 Males, Mean age = 21.6 ± 3.3). The average maximal TMS output for rPAS delivery was 38% (± 6.1) for the session, similar to Experiment 1 (38.8%). Also similar to Experiment 1 (16.3V), the average nerve stimulation for rPAS was 13.6V (± 9.6).

MEPs

One-way repeated measures ANOVA revealed a significant main effect of TIME ($F_{(3, 21)} = 3.97$, $p = 0.02$). Post hoc Tukey's HSD revealed a similar increase as Experiment1, where MEP amplitudes were greater at T_3 compared to T_0 ($p < 0.05$). Figure 14 displays the group-averaged data (with standard error of the mean) for MEPs following SI rPAS (N20 + 2.5 ms) at each time point.

SAI

One-way repeated measures ANOVA revealed a non-significant main effect of TIME ($F_{(1.9, 13)} = 0.55$, $p = 0.58$). Post hoc Tukey's HSD revealed significantly greater MEPs at T_3 compared to T_0 ($p < 0.05$). Figure 15 displays the group-averaged data (with standard error of the mean) for SAI following SI rPAS (N20 + 2.5 ms) at each time point.

SEP and Paired-pulse Inhibition

Two-tailed paired t-tests comparing the N20-P25 amplitude at baseline (T_0) and at 45 minutes after rPAS (T_3) revealed no significant change ($p = 0.48$). Similarly, two-tailed paired t-tests comparing paired-pulse inhibition at baseline (T_0) and 45 minutes after rPAS (T_3) revealed no significant change ($p = 0.47$). These data indicate that there were no measurable changes in SI cortical excitability following SI rPAS (N20 + 2.5 ms)

DISCUSSION

Novel findings of this study include the effects of rPAS applied to SI. When rPAS is directed to SI, there was a differential effect on MEPs and SAI. MEPs increased while

there was no effect on SAI. When the ISI of SI rPAS was increased by 2.5 ms, to encourage STDP in the upper layers of SI, there were no changes in SAI and the facilitation of MEPs was very similar to Experiment 1. These results indicate that rPAS over SI can modulate corticospinal excitability from M1, but does not significantly modify SAI circuitry. These results further support SI having multiple pathways that may influence activity in motor cortex. Further, in support of the literature, rPAS directed to M1 increased MEP amplitude and decreased SAI (Quartarone *et al.,* 2006).

These data have shown that SI rPAS is capable of modulating M1 circuits. Although the precise mechanisms for the modulation are unclear, it is likely that STDP is occurring in SI and this plasticity is specific to the projection that influences the corticospinal output neurons in M1. SI is known to have direct excitatory projections to pyramidal output neurons residing in layer V of M1 (Ghosh & Porter 1988) and the STDP may be happening within this subset of SI to M1 projection neurons. Conversely, we note that rPAS to SI did not create STDP in the circuitry that projects to the motor cortical circuitry of SAI. This suggests that the effects of SI rPAS may be specific to discrete neuronal populations that mediate separate influences on distinct circuits in motor cortex.

We also note that rPAS over SI does not alter N20-P25 potentials or paired-pulse evoked potential inhibition. The fact that evoked potentials are not changed by SI rPAS does not rule out that plasticity is not occurring in SI. Plasticity must be present in the sensorimotor cortex for corticospinal excitability to have increased. Therefore, these data further support that SI rPAS may be selectively potentiating a discrete population of

neurons that do not contribute to observable changes in SEP amplitude and do not contribute to the SAI circuitry.

In support of the literature, we note that MEP increases and SAI is reduced following M1 rPAS. The specific timing of rPAS was defined at N20 + 5ms, to allow for afferent information to traverse to M1 through SI (Goldring *et al.,* 1970; Quartarone *et al.*, 2006). Therefore, these data do not rule out the importance of SI in the SAI circuitry, but they do indicate that STDP must occur within motor cortex in order to change SAI. The mechanisms that may mediate such changes are likely to include the later I3 generating interneurons (Tokimura *et al.*, 2000; Tsang *et al.*, 2014) that may be recipient to superficial projections from SI to M1 (Kaneko *et al.,* 1994a; Kosar *et al.,* 1985). We speculate that M1 rPAS is likely causing LTP at these specific excitatory projections leading to a net increase in corticospinal excitability and decreases in SAI. However, it is possible that M1 rPAS may potentially act on two different neuronal circuits where one modulates a net increase in corticospinal excitability whilst the other modulated SAI circuitry.

Though PAS and rPAS both use heterosynaptic stimulation to induce STDP, some differences should be addressed. Differential plasticity effects of PAS in comparison to rPAS should not be a surprise as these two protocols likely induce STDP differently. For example, SAI is relatively unchanged following low-frequency PAS (Stefan *et al.,* 2002), whereas M1 rPAS induces decreases in SAI in our present findings and other reports (Quartarone *et al.*, 2006; Terranova *et al.,* 2013).Similar to previous reports of PAS to SI, we observed no changes in motor circuitry including MEPs (Krivanekova *et al.,* 2011),

whereas SI rPAS induced increases in MEPs. Another contrast between PAS and rPAS are changes observed in sensory cortical excitability. SI rPAS did not induce any measureable changes to SEPs or paired-pulse inhibition, whereas low-frequency PAS to SI facilitated the N20-P25 potential (Litvak *et al.,* 2007; Wolters *et al.,* 2005), though not all reports have found the same modulation (Krivanekova *et al.,* 2011).

There are limitations which must be considered for this present experiment. First, SI rPAS may be at the wrong ISI to induce the correct STDP, as past literature indicates that a shorter ISI creates such plasticity in PAS (Litvak *et al.,* 2007; Wolters *et al.,* 2005). Second, the N20-P25 potentials and paired-pulse inhibition, measured in Experiment 2, may not be sensitive to SI physiological changes induced via SI rPAS. These measurements are strongly driven by the activation of area 3b pyramidal neurons, which were unaltered via STDP (Allison *et al.,* 1991; Ragert *et al.,* 2003). Future studies may use measurements such as high frequency oscillations that are more sensitive to changes in inhibitory interneurons (Katayama *et al.,* 2010) to detect changes following SI rPAS.

FIGURES

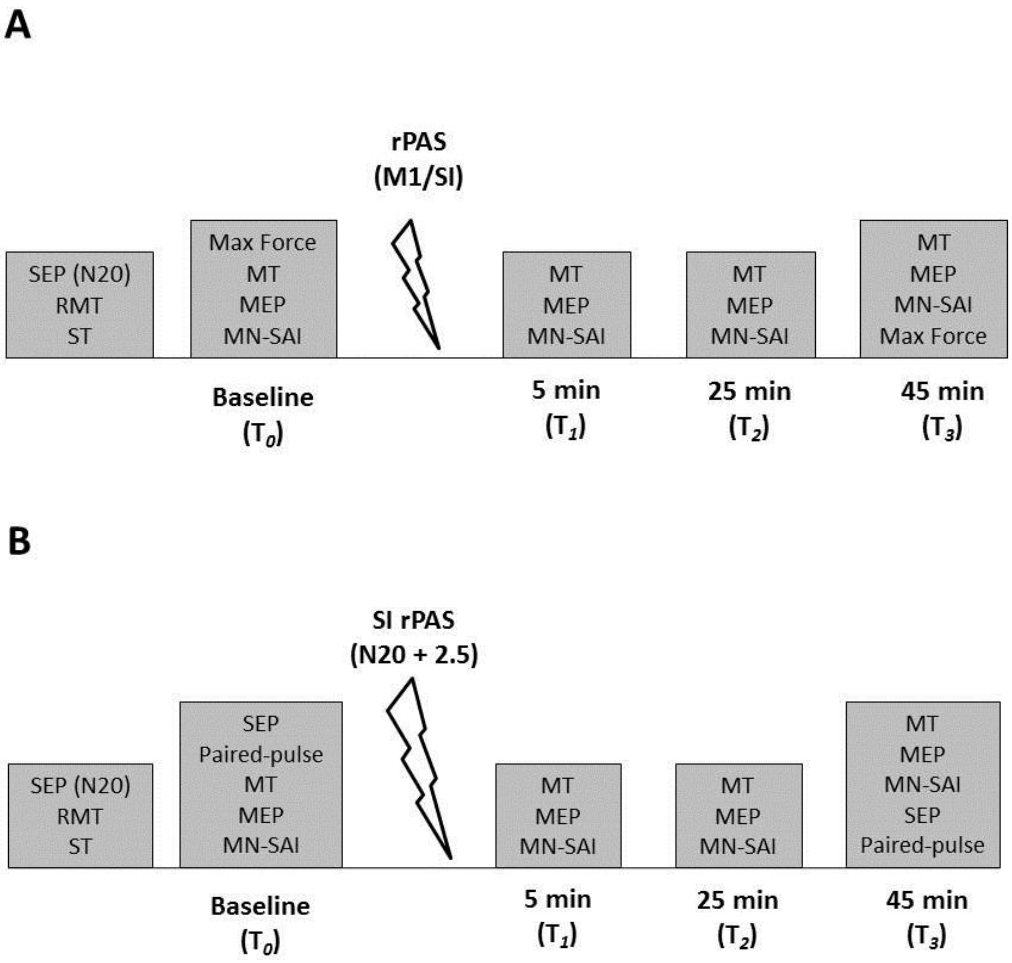

Figure 10: rPAS Experimental Timeline

A) Experiment Timeline for Experiment 1: RPAS was delivered to M1 and SI in the same group of participants. Measures of MEPs and SAI were acquired from right APB before (T0) and at 5-20 minutes (T1), 25-40 minutes (T2) and 45-60 minutes (T3) following rPAS delivery. Motor threshold intensities (MT) were determined prior to baseline and were re-evaluated before each time block **B)** Experimental Timeline for Experiment 2: RPAS was delivered to SI in eight of the participants from Experiment 1. Measures of MEPs and SAI were acquired from right APB before (T0) and at 5-20 minutes (T1), 25-40 minutes (T2) and 45-60 minutes (T3) following rPAS delivery. SEPs and paired-pulse inhibition were acquired at the start and very end of the experiment as well.

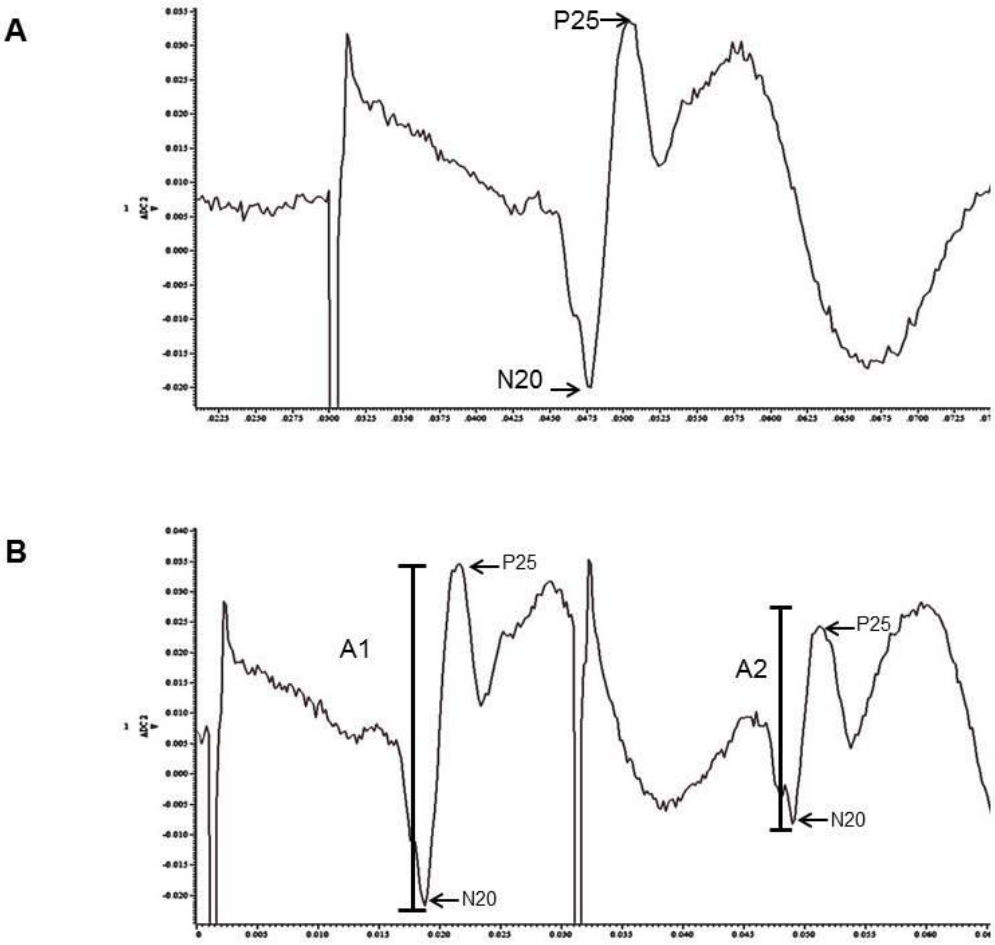

Figure 11: Sample SEP Traces

Example of SEP and paired-pulse inhibition traces from one participant before the application of rPAS: **A)** N20-P25 potential recorded from C3' **B)** Paired-pulse inhibition measured by normalizing the later N20-P25 potential (A2) by the earlier N20-P25 potential (A1).

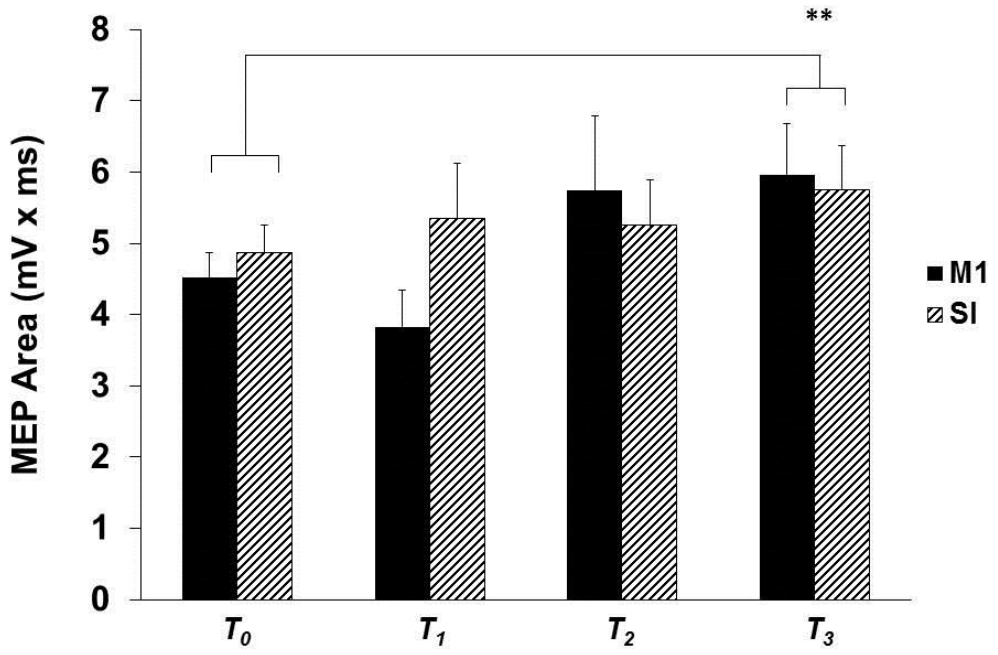

Figure 12: MEP Modulation following rPAS

Group-averaged (n = 12) MEP area with standard error of the mean following M1 rPAS (solid) and SI rPAS (diagonal lines) at pre-rPAS, 5-20 minutes, 25-40 minutes, and 45-60 minutes for the right abductor pollicus brevis (RAPB). An asterisk over a single time block indicates that MEP area was significantly greater at T3 compared to pre-rPAS. Significant differences were tested at $p \leq 0.05$.

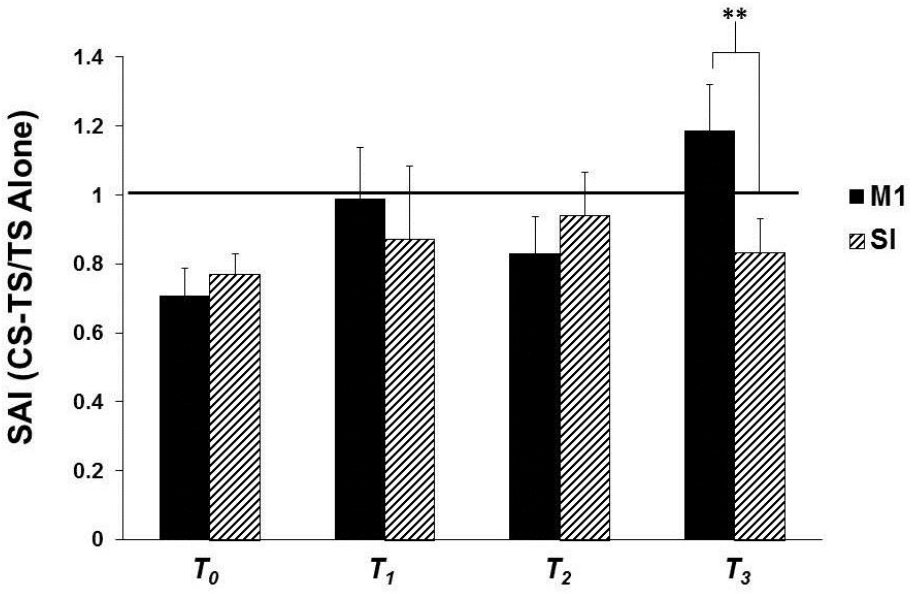

Figure 13: Modulation of SAI following rPAS

Group-averaged SAI with standard error of the mean following M1 rPAS (solid) and SI rPAS (diagonal lines) at pre-rPAS, 5-20 minutes, 25-40 minutes, and 45-60 minutes for the right abductor pollicus brevis (RAPB). An asterisk over a single time block indicates where SAI was significantly reduced following M1 rPAS in comparison to SI rPAS. Significant differences were tested at $p \leq 0.05$.

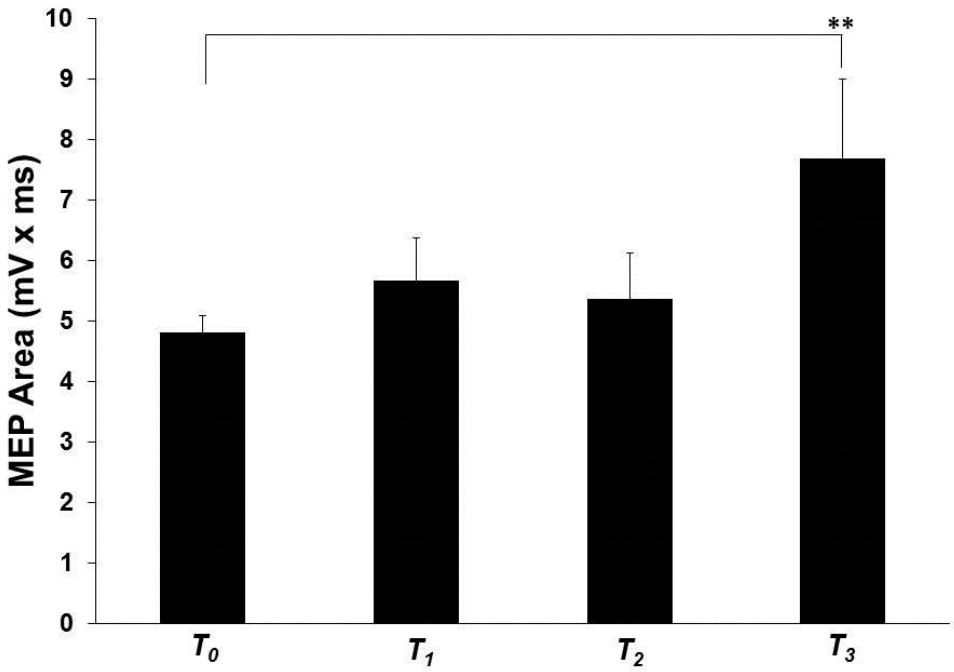

Figure 14: MEP Modulation by Modified SI rPAS

Group-averaged MEP with standard error of the mean following SI rPAS (N20 + 2.5ms) at pre-rPAS, 5-20 minutes, 25-40 minutes, and 45-60 minutes for the right abductor pollicus brevis (RAPB). An asterisk over a single time block indicates it was significantly greater than pre-rPAS. Significant differences were tested at $p \leq 0.05$.

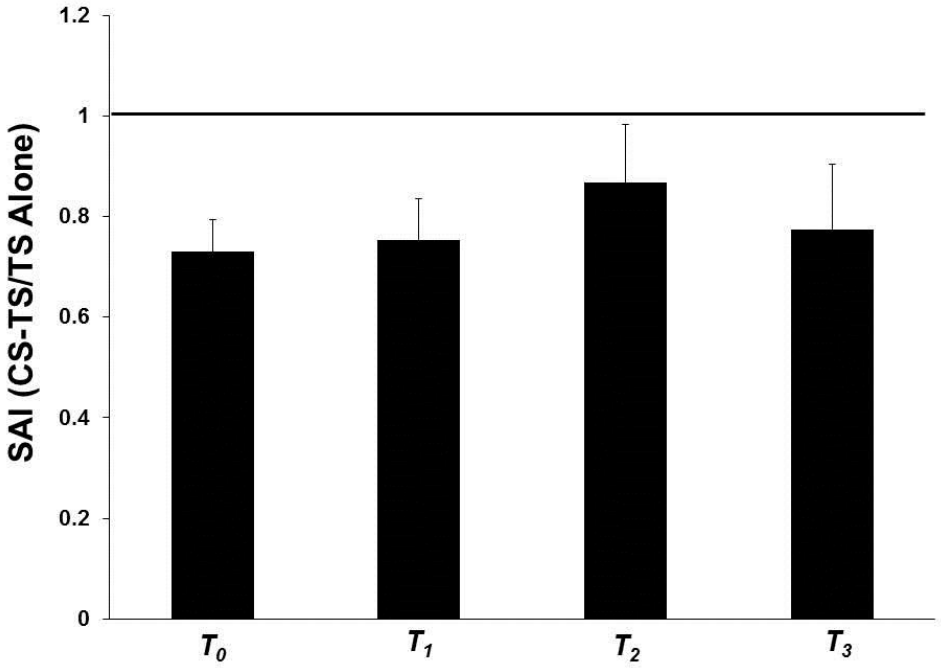

Figure 15: SAI After Modified SI rPAS

Group-averaged SAI with standard error of the mean following SI rPAS (N20 + 2.5ms) at pre-rPAS, 5-20 minutes, 25-40 minutes, and 45-60 minutes for the right abductor pollicus brevis (RAPB). No significant changes were observed following SI rPAS (N20 + 2.5 ms). Significant differences were tested at $p \leq 0.05$.

GENERAL DISCUSSION

Summary of Experiments

Experiment 1 investigated the effects of homosynaptic plasticity stimulation applied to cortical areas M1 and SI on the sensorimotor SAI circuitry. To investigate these effects, MEPs and SAI were recorded from right FDI and cTBS was delivered to M1 and SI in the same individuals. When cTBS was applied to M1, MEPs were decreased at 5-20 minutes while SAI remain unchanged. For SI cTBS, MEPs were trending towards an increase at 45 minutes and SAI was significantly reduced at 45 minutes. These results indicate that cTBS over SI and M1 increases and decreases corticospinal excitability, respectively. Further, a homosynaptic plasticity protocol over SI is capable of modulating SAI; a finding which has never been demonstrated previously with other repetitive TMS protocols (Baumer *et al.*, 2007; Fischer & Orth 2011; Zamir *et al.*, 2012).

Experiment 2 investigated the effects of heterosynaptic plasticity stimulation applied to cortical areas M1 and SI on the sensorimotor SAI circuitry. Measures of MEPs and SAI were recorded in APB before and after M1 and SI rPAS. It is important to note, that the location and timing of rPAS was modified to promote STDP in SI. MEPs were facilitated at 45 minutes following M1 and SI rPAS while SAI was decreased at 45 minutes following M1 rPAS only.

Experiments 1 and 2 present evidence that neural activity in M1 and SI modulate corticospinal excitability and sensorimotor circuitry that outputs to the hand. In these two experiments, MEPs were facilitated following cTBS and rPAS, with the exception of cTBS applied to M1 which is well-known to cause suppression (Goldsworthy *et al.*, 2012a; Jacobs *et al.*, 2013). However SI cTBS and M1 rPAS both decrease SAI in comparison to baseline. The summary of modulatory effects on MEP and SAI are shown in Figure 16 below. The following discussion will explore the effects of cTBS and rPAS of sensorimotor areas on MEPs and SAI, potential neural mechanisms, possible implications of these findings, and limitations of this work.

A

MEPs	**Over M1**	**Over SI**
cTBS	↓	↑
rPAS	↑	↑

B

SAI	**Over M1**	**Over SI**
cTBS	○	↑
rPAS	↑	○

↑ = Increases ↓ = Decreases ○ = No Change

Figure 16: Differential Modulation of MEPs and SAI

Homosynaptic and heterosynaptic plasticity stimulation over M1 and SI and the resultant effects on **A)** MEP **B)** SAI.

Different effects of cTBS and rPAS on MEP amplitude

In Experiment 1, 30 Hz cTBS applied to M1 led to MEP suppression in right first dorsal interroseous (RFDI) for up to 5 minutes. However, in contrast, rPAS was applied to M1 (Experiment 2) lead to MEP facilitation of APB at 45 minutes. The contrast in direction and timing of effects is likely due to the different approaches of these plasticity protocols. 30 Hz cTBS uses homosynaptic stimulation to induce LTD-like effects on the corticospinal tract. High frequency bursts of TMS are applied in order to induce suppression in cortical excitability (Goldsworthy *et al.,* 2012a). In contrast, rPAS is a heterosynaptic stimulation, which incorporates Hebbian principles of association to induce LTP-like effects. When the afferent input and postsynaptic action potential are timed to promote the coincident activity at the postsynaptic neuron with the right latencies, STDP can increase the synaptic efficacy of the corticospinal neurons (Quartarone *et al.,* 2006; Stefan *et al.,* 2000; Stefan *et al.,* 2002). CTBS is known to produce MEP suppression whether it is applied at 50 Hz (Huang *et al.,* 2005) or 30 Hz (Goldsworthy *et al.,* 2012a). Though past reports of 50 Hz cTBS have observed MEP increases and inter-subject variability (Hamada *et al.,* 2013), 30 Hz cTBS has been reported by investigators to induce consistent suppression of MEPs (Goldsworthy *et al.,* 2012a; Jacobs *et al.,* 2013). In contrast, PAS and rPAS applied at an ISI of ~25 ms, is known to induce MEP increases when applied to M1 (Quartarone *et al.,* 2006; Stefan *et al.,* 2000; Wolters *et al.,* 2003). It is important to note that suppression of MEPs following rPAS was not observed in Experiment 2 or elsewhere (Quartarone *et al.,* 2006). Taken together, these data suggest that an increase of MEPs via M1 modulation seems to be

most effective through heterosynaptic stimulation (rPAS) while decreases in MEPs are most effectively driven through homosynaptic stimulation (cTBS).

There have been few investigations where MEPs have been measured following modulation of SI. Previous reports of cTBS applied to SI have observed no changes in MEPs (Ishikawa *et al.,* 2007), but MEP increases were observed when current direction (Jacobs *et al.,* 2012) and frequency (Jacobs *et al.*, 2013) of cTBS were modified. Similar to past reports, Experiment 1 showed trends for increased MEPs at 45 minutes following the homosynaptic cTBS plasticity protocol to SI. Experiment 2 applied the heterosynaptic plasticity protocol, rPAS, which evoked an increase in MEPs at 45 minutes following SI stimulation. It is also interesting to note that MEP increases were very similar in SI rPAS whether an ISI of N20 or N20 + 2.5 ms was used. This may suggest that ISI and timing may not be as important of a factor for changes in MEPs but, instead, emphasizes the importance of STDP location. Together, these data suggest that SI plasticity by homosynaptic and heterosynaptic stimulation increases corticospinal excitability, but in a delayed manner. This is consistent with previous reports where plasticity induced in somatosensory cortex (Jacobs *et al.,* 2013; Jacobs *et al.,* 2012) or higher order somatosensory cortex (Chao *et al.,* 2013b; Premji *et al.*, 2011), had maximal increases in MEPs after a delay of at least 25 minutes.

Different effects of cTBS and rPAS on SAI

SAI is believed to be cortically mediated by muscarinic receptors (Di Lazzaro *et al.*, 2000) and is also modulated by GABA-A receptors (Di Lazzaro *et al.,* 2005c; Di, V *et*

al., 2006). In Experiment 1, 30 Hz cTBS over M1 led to no changes on SAI. In contrast, for Experiment 2, rPAS over M1 lead to decreases in SAI at 45 minutes. Unaltered SAI following 30 Hz cTBS on M1 is in line with previous reports where other forms of homosynaptic stimulation like 1 Hz rTMS (Fischer & Orth 2011) and iTBS (Zamir *et al.*, 2012) yielded no change in SAI. In Experiment 2, M1 rPAS was set to the specific ISI of N20 + 5 ms to allow for STDP to occur in M1. This specific STDP, led to decreases in SAI at 45 minutes. These results support previous reports where M1 rPAS at ISI 25ms leads to reductions in SAI (Quartarone *et al.,* 2006; Terranova *et al.,* 2013). The data in this thesis indicate that heterosynaptic and not homosynaptic protocols, may be better suited for targeting the interneuronal population involved in modulating SAI when delivered over M1.

30 Hz cTBS applied to SI decreased SAI for more than an hour. Regardless of whether SAI was induced via median nerve or digital nerve stimulation, 30 Hz cTBS decreased the level of inhibition. This is in contrast to past reports using homosynaptic stimulation, where low-frequency rTMS applied to SI did not induce changes to SAI (Baumer *et al.*, 2007). Homosynaptic plasticity (ie. rTMS and cTBS) of SI has frequently been used to induce changes in SEPs but not sensorimotor circuitry (Enomoto *et al.*, 2001; Ishikawa *et al.*, 2007; Ragert *et al.*, 2003). Similarly, heterosynaptic plasticity of SI has frequently been used to modulate SEP (Litvak *et al.*, 2007; Wolters *et al.*, 2005) but not motor circuitry except for one investigation (Krivanekova *et al.,* 2011). To our knowledge, there have been no previous attempts or reports of using heterosynaptic plasticity to modulate SAI. SI rPAS however, induced no changes in SAI regardless of

ISI. It is also interesting to note that in Experiment 2 there were no measurable changes in SEPs or intracortical inhibition observed following SI rPAS. Therefore the data from this thesis suggests that homosynaptic and not heterosynaptic stimulation of SI changes SAI circuitry.

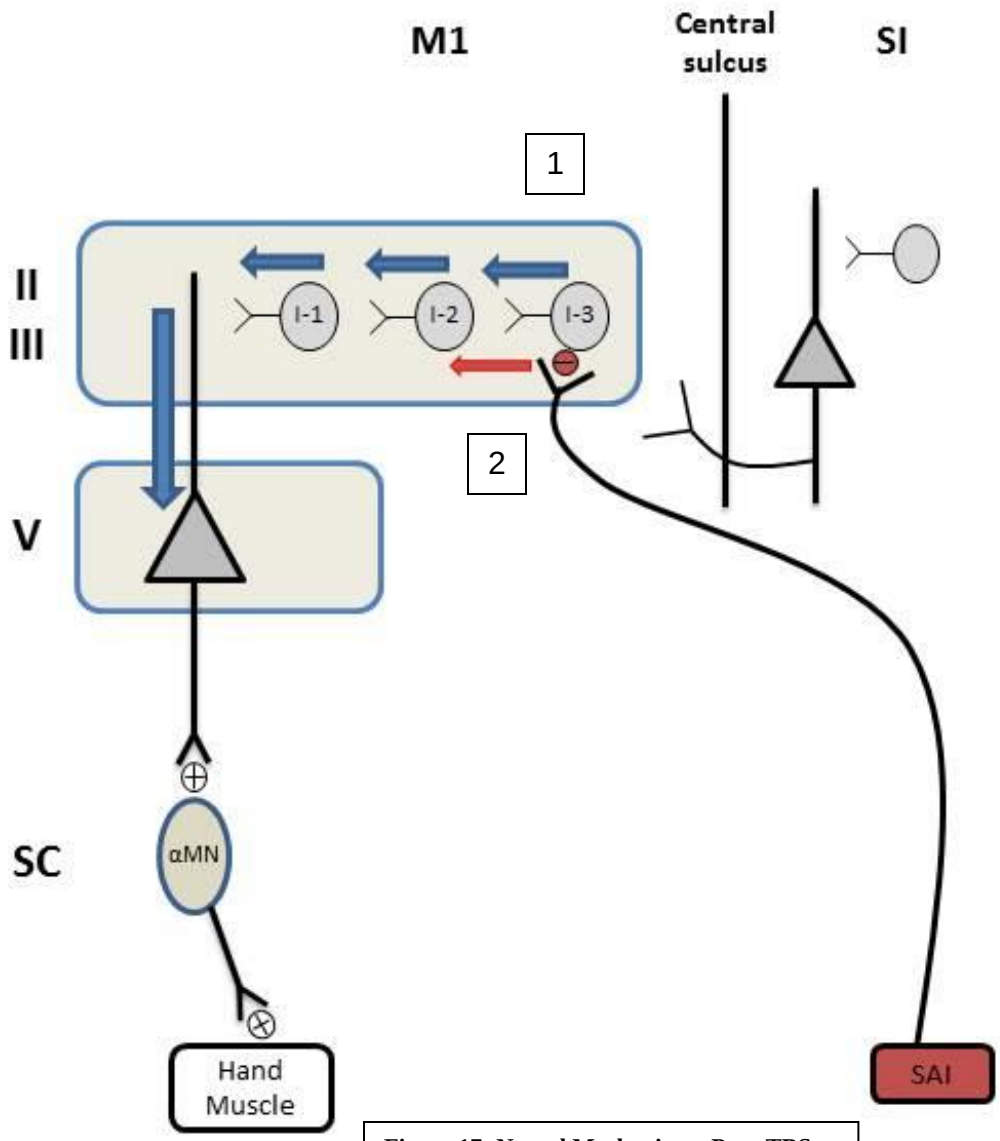

Figure 17: Neural Mechanism - Pre-cTBS or rPAS

(Adapted from Jacobs et al. 2013)

1. Interneuron circuitry in layer II/III produces I-wave excitatory drive (blue arrows) and synapse to activate layer V output neurons
2. SAI acts on I3 interneuron (red arrow) to decrease the excitatory drive and decrease corticospinal output

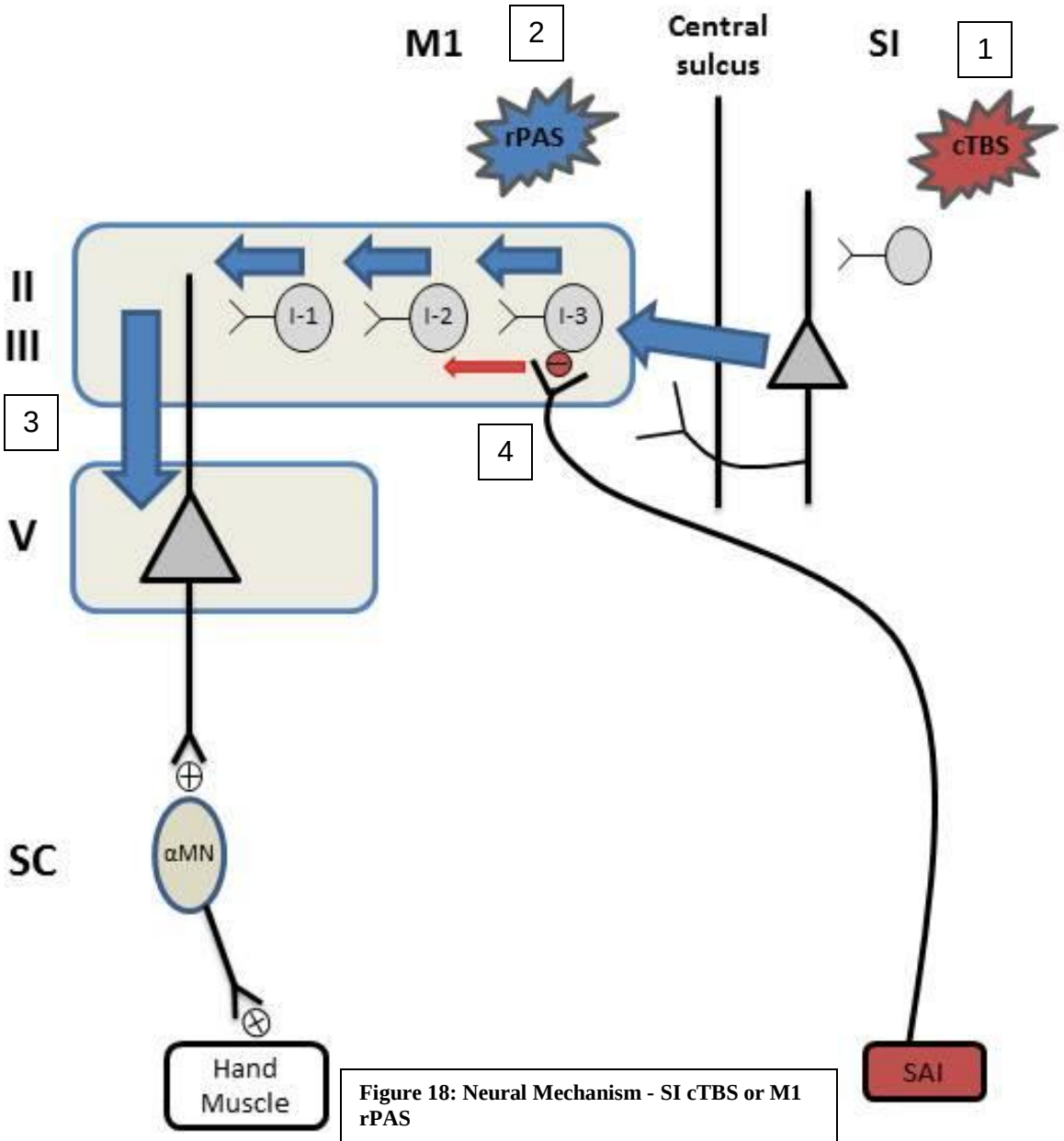

Figure 18: Neural Mechanism - SI cTBS or M1 rPAS

(Adapted from Jacobs et al. 2013)

1. cTBS alters interneurons in SI which drives a net facilitation of M1 interneurons (large blue arrows)
2. Similarly, rPAS facilitates the M1 interneurons (large blue arrows)
3. Facilitated I-wave excitatory drive leads to an increased corticospinal output
4. Increased drive from I3 interneuron also decreases the degree of inhibition from SAI

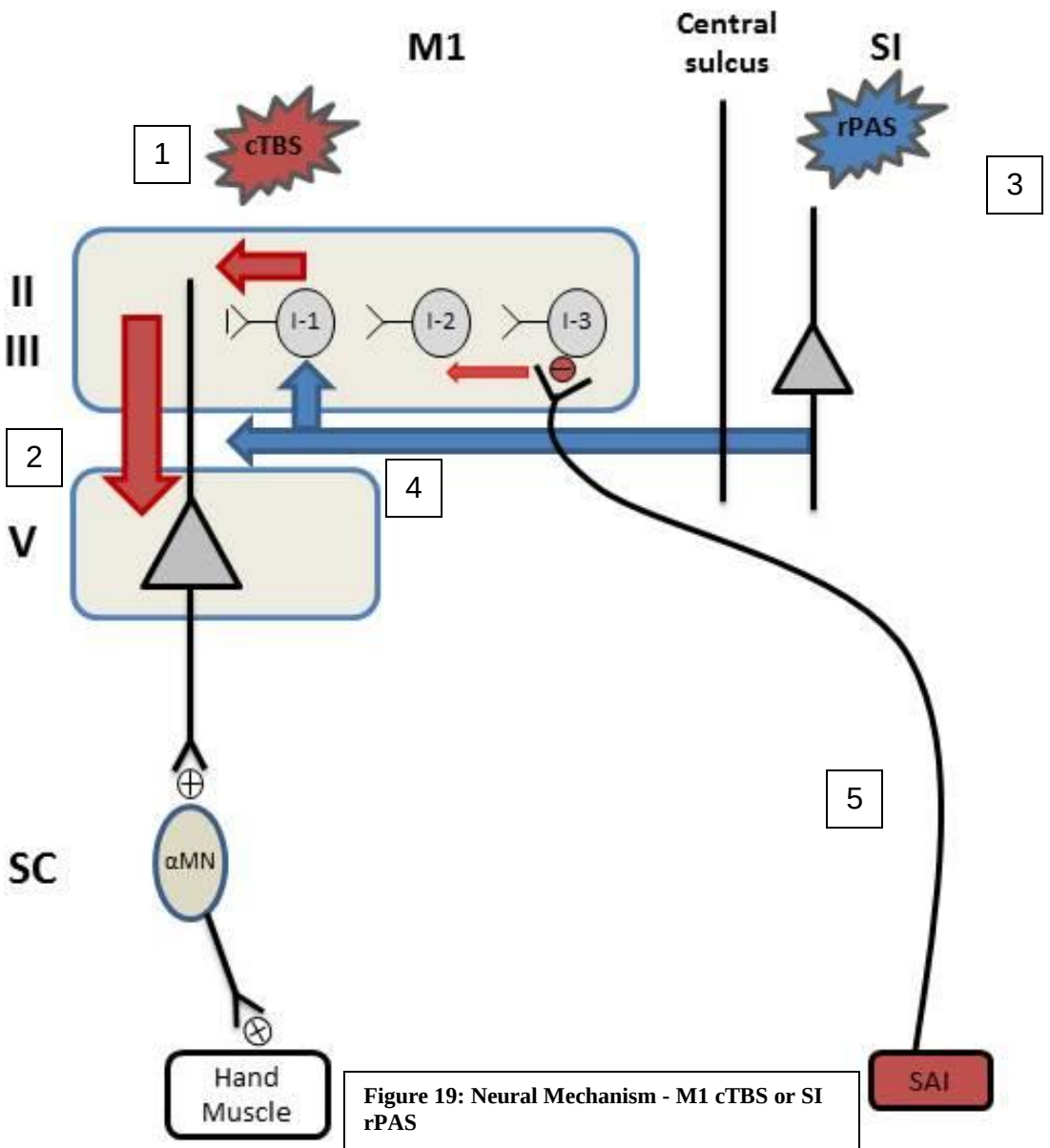

Figure 19: Neural Mechanism - M1 cTBS or SI rPAS

(Adapted from Jacobs et al. 2013)

1. cTBS has an inhibitory effect on the early interneurons of M1 (medium red arrow)
2. This creates a net inhibitory drive to the corticospinal output neurons (large red arrow)
3. SI rPAS potentiates neurons in SI
4. SI neurons generate net facilitation of either interneurons or corticospinal output neurons in M1 (large blue arrow)
5. SAI is unaffected by SI facilitation

Neural Substrates and Mechanisms

This research is the first to explore changes in SAI following SI cTBS, and also the first to investigate the effects of rPAS applied to SI. It is known that M1 may receive somatosensory afference via direct thalamocortical projections, as well as through SI-M1 projections (Lemon & Porter 1976a). However, SAI is believed to be a cortically mediated sensorimotor circuit (Tokimura *et al.,* 2000). Therefore, neural mechanisms of SI and M1 will be primarily discussed.

MEPs and SAI are mediated by interneurons and corticospinal output neurons of the motor cortex. These output neurons primarily reside within layers III and V of the motor cortex (Ghosh & Porter 1988). The vertical output neurons project to form a large portion of the descending corticospinal tract (Kaneko *et al.,* 2000). In contrast, the interneurons and horizontal output neurons reside primarily in layers II/III of M1(Kosar *et al.,* 1985). These horizontal neurons have excitatory projections to the corticospinal output neurons residing in layer V. MEPs measured via TMS are primarily activated through these horizontal fibres and interneurons (Di Lazzaro *et al.*, 2004). Increased excitatory drive from I-wave interneurons leads to net increased corticospinal output as reviewed by previous reports (Di, V *et al.,* 2012; Rusu *et al.,* 2014) and Chapter 2 of the thesis (Literature Review). Similarly, SAI decreases corticospinal output by decreasing the excitatory drive from late I-wave generating interneurons (Tokimura *et al.,* 2000). Figure 17 shows the neural mechanisms of the interneurons, the corticospinal output neurons, and SAI.

Facilitation of late I-wave generating interneurons of M1 is a possible mechanism of SI cTBS and M1 rPAS effects. Increased activity of late interneurons leads to decreases in SAI and increases in MEPs. It is known that SI projects to layers III and V of M1 (Zarzecki *et al.,* 1978).

However, area 2, which sends the densest projections to M1 (Yumiya & Ghez 1984) terminates mostly in layers II/III (Kaneko *et al.,* 1994b; Kosar *et al.,* 1985; Porter & Sakamoto 1988). Similar to a model proposed by Jacobs et al. (2013), I speculate that SI cTBS may modulate corticospinal excitability and SAI indirectly through a SI-M1 relay which ultimately facilitates the interneurons in layers II/III of M1 (Kaneko *et al.,* 2000). It is well-known that SI projections to M1 are largely excitatory (Kosar *et al.,* 1985) and that tetanic stimulation of SI may lead to LTP in M1 layer II/III neurons (Iriki *et al.,* 1989). Likewise in rodent models, homosynaptic theta burst stimulation induces LTP in layers II/III neurons when GABA receptors are modulated via bicuculine (Hess *et al.,* 1996). CTBS, which is known to modulate GABA levels in the human cortex (Stagg *et al.,* 2009), was used in Experiment 1 to induce these LTP-like effects. In contrast, for Experiment 2, facilitation of M1 interneurons was induced via heterosynaptic stimulation (rPAS) without alterations to GABA levels (Quartarone *et al.,* 2006; Stefan *et al.,* 2002). Hess *et al.* (1996) suggests a mechanism where vertical afferent input neurons gate GABAergic inhibitory neurons that normally generate a tonic inhibition of LTP in the horizontal output motor neurons residing in layers II/III. Similarly, I speculate that M1 rPAS in Experiment 2 used low intensity TMS to activate the horizontal fibres of M1 and median nerve stimulation to provide vertical afferent input to M1, ultimately leading to the facilitation of layer II/III interneurons within M1. The collective data suggest that homosynaptic SI stimulation and heterosynaptic M1 stimulation may modulate corticospinal excitability and SAI through the late interneurons of M1. Figure 18 presents the possible mechanism for the modulation of SAI and MEPs via SI cTBS and M1 rPAS in the present work.

M1 cTBS and SI rPAS may be modulating the early I-wave interneurons or corticospinal neurons directly. M1 cTBS has widely been reported to induce LTD-like effects (Goldsworthy *et*

al., 2012a; Jacobs *et al.*, 2013) by modulating earlier I1 waves (Di Lazzaro *et al.*, 2005a). If SAI is primarily mediated by later interneurons (ie. I3-waves), as mentioned previously, then M1 cTBS modulation of I1-waves would change corticospinal excitability (MEPs), but not SAI. With the present data, it is not clear whether SI rPAS directly modulates corticospinal neurons or early I-wave interneurons. However, SI rPAS may facilitate a more direct SI-M1 projection (ie. to layers III and V of motor cortex). SI rPAS modulation of MEPs and not SAI seems to suggest that late inhibitory interneurons (I3-wave) are not the mechanism by which corticospinal excitability is increased. Figure 19 presents the possible mechanisms for the modulation of MEPs via M1 cTBS and SI rPAS in the present work.

Limitations

A few limitations must be considered in this work. First, somatosensory afferent input via median nerve stimulation may relay to M1 directly through thalamocortical projections (Lemon & Porter 1976b). If this was the case then the ISI for M1 rPAS should be modified (ie. ISI of N20) in order to induce STDP. However, pilot data from eight participants (who were from Experiment 1) revealed no significant changes in MEPs or SAI, suggesting that somatosensory afference needs to traverse from SI to M1 for LTP-like effects to occur. Second, MEPs are not only modulated via cortical neuronal excitability but also spinal motoneuron excitability as well. In order to further investigate whether rPAS or cTBS induced changes in spinal motoneurons, H-reflexes should be measured following these protocols. However, H-reflexes are extremely difficult to induce in intrinsic muscles of the hand (Hultborn & Nielsen 1995).

Conclusions

In summary, this research demonstrates that different approaches of plasticity stimulation to the primary somatosensory cortex and primary motor cortex may influence sensorimotor circuitry of the hand. Sensorimotor integration is integral to motor control of the hand (Johansson & Westling 1984) and motor learning (Pavlides *et al.,* 1993). Although many investigators explore the influence of somatosensory plasticity on cortical sensory changes, few have investigated sensorimotor circuitry following the modulation of SI. Using non-invasive cTBS and rPAS, we have shown that these stimulation protocols are able to produce selective facilitation or suppression of neural circuitry. These differential modulations of select sensorimotor circuitry can be manipulated by the appropriate protocols of rPAS and cTBS. Aside from contributing to our fundamental understanding of sensorimotor circuitry of the human hand, selective modulation may have significant clinical relevance in populations with altered hand control. Motor and sensorimotor circuitry may be manipulated by targeting cortical areas. Sensory re-training induces plasticity and improves motor function in patients with movement disorders and peripheral nerve lesions, suggesting that plasticity may drive cortical reorganization and functional motor recovery (Byl *et al.,* 2008; Miller *et al.,* 2012). Using CTBS and rPAS, to increase or decrease neural activity in hand representations may be used to further explore somatic influence on motor circuitry in humans or as part of a functional rehabilitation therapy to improve hand function.

REFERENCE

Alle H, Heidegger T, Krivanekova L, Ziemann U. Interactions between short-interval intracortical inhibition and short-latency afferent inhibition in human motor cortex. J Physiol 2009; 587: 5163-5176.

Allison T, McCarthy G, Wood CC, Darcey TM, Spencer DD, Williamson PD. Human cortical potentials evoked by stimulation of the median nerve. II. Cytoarchitectonic areas generating short-latency activity. Journal of Neurophysiology 1989; 62: 694-710.

Allison T, McCarthy G, Wood C, JONES SJ. Potentials Evoked In Human and Monkey Cerebral Cortex By Stimulation Of The Median Nerve: A Review of Scalp And Intracranial Recordings. Brain 1991; 114: 2465-2503.

Asmussen MJ, Jacobs MF, Lee KGH, Zapallow CM, Nelson AJ. Short-Latency Afferent Inhibition Modulation during Finger Movement. PLoS One 2013; 8: e60496.

Baumer T, Demiralay C, Hidding U, Bikmullina R, Helmich RC, Wunderlich S, et al. Abnormal plasticity of the sensorimotor cortex to slow repetitive transcranial magnetic stimulation in patients with writer's cramp. Mov Disord 2007; 22: 81-90.

Bi GQ, Poo MM. Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. J Neurosci 1998; 18: 10464-10472.

Brinkman J, Colebatch JG, Porter R, York DH. Responses of precentral cells during cooling of post-central cortex in conscious monkeys. J Physiol 1985; 368: 611-625.

Byl NN, Merzenich MM, Cheung S, Bedenbaugh P, Nagarajan SS, Jenkins WM. A primate model for studying focal dystonia and repetitive strain injury: effects on the primary somatosensory cortex. Phys Ther 1997; 77: 269-284.

Byl NN, Pitsch EA, Abrams GM. Functional outcomes can vary by dose: learning-based sensorimotor training for patients stable poststroke. Neurorehabil Neural Repair 2008; 22: 494-504.

Canedo A. Primary motor cortex influences on the descending and ascending systems. Prog Neurobiol 1997; 51: 287-335.

Chao CC, Karabanov AN, Paine R, Carolina de CA, Kukke SN, Wu T, et al. Induction of Motor Associative Plasticity in the Posterior Parietal Cortex-Primary Motor Network. Cereb Cortex 2013a.

Chao CC, Karabanov AN, Paine R, Carolina de CA, Kukke SN, Wu T, et al. Induction of Motor Associative Plasticity in the Posterior Parietal Cortex-Primary Motor Network. Cereb Cortex 2013b.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, et al. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 1997; 48: 1398-1403.

Christova M, Rafolt D, Golaszewski S, Gallasch E. Outlasting corticomotor excitability changes induced by 25 Hz whole-hand mechanical stimulation. Eur J Appl Physiol 2011; 111: 3051-3059.

Classen J, Steinfelder B, Liepert J, Stefan K, Celnik P, Cohen LG, et al. Cutaneomotor integration in humans is somatotopically organized at various levels of the nervous system and is task dependent. Exp Brain Res 2000; 130: 48-59.

Dancause N, Dykes RW, Miasnikov AA, Agueev V. Atropine-sensitive and -insensitive components of the somatosensory evoked potential. Brain Res 2001; 910: 67-73.

Darling WG, Wolf SL, Butler AJ. Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. Experimental brain research 2006; 174: 376-385.

Di Lazzaro V, Pilato F, Saturno E, Oliviero A, Dileone M, Mazzone P, et al. Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. J Physiol 2005a; 565: 945-950.

Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Marra C, et al. Neurophysiological predictors of long term response to AChE inhibitors in AD patients. Journal of Neurology, Neurosurgery & Psychiatry 2005b; 76: 1064-1069.

Di Lazzaro V, Oliviero A, Pilato F, Saturno E, Dileone M, Mazzone P, et al. The physiological basis of transcranial motor cortex stimulation in conscious humans. Clinical Neurophysiology 2004; 115: 255-266.

Di Lazzaro V, Oliviero A, Profice P, Pennisi MA, Di Giovanni S, Zito G, et al. Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. Experimental brain research 2000; 135: 455-461.

Di Lazzaro V, Oliviero A, Saturno E, Dileone M, Pilato F, Nardone R, et al. Effects of lorazepam on short latency afferent inhibition and short latency intracortical inhibition in humans. The Journal of physiology 2005c; 564: 661-668.

Di Lazzaro V, Oliviero A, Tonali PA, Marra C, Daniele A, Profice P, et al. Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. Neurology 2002; 59: 392-397.

Di L, V, Oliviero A, Profice P, Pennisi MA, Di GS, Zito G, et al. Muscarinic receptor blockade has differential effects on the excitability of intracortical circuits in the human motor cortex. Exp Brain Res 2000; 135: 455-461.

Di L, V, Oliviero A, Saturno E, Dileone M, Pilato F, Nardone R, et al. Effects of lorazepam on short latency afferent inhibition and short latency intracortical inhibition in humans. J Physiol 2005; 564: 661-668.

Di L, V, Pilato F, Dileone M, Ranieri F, Ricci V, Profice P, et al. GABAA receptor subtype specific enhancement of inhibition in human motor cortex. J Physiol 2006; 575: 721-726.

Di L, V, Profice P, Ranieri F, Capone F, Dileone M, Oliviero A, et al. I-wave origin and modulation. Brain Stimul 2012; 5: 512-525.

Doeltgen SH, Ridding MC. Low-intensity, short-interval theta burst stimulation modulates excitatory but not inhibitory motor networks. Clinical Neurophysiology 2011; 122: 1411-1416.

Dum RP, Strick PL. Motor areas in the frontal lobe of the primate. Physiology & behavior 2002; 77: 677-682.

Enomoto H, Ugawa Y, Hanajima R, Yuasa K, Mochizuki H, Terao Y, et al. Decreased sensory cortical excitability after 1 Hz rTMS over the ipsilateral primary motor cortex. Clinical Neurophysiology 2001; 112: 2154-2158.

Fathi D, Ueki Y, Mima T, Koganemaru S, Nagamine T, Tawfik A, et al. Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. Clin Neurophysiol 2010; 121: 90-93.

Fino E, Glowinski J, Venance L. Bidirectional activity-dependent plasticity at corticostriatal synapses. J Neurosci 2005; 25: 11279-11287.

Fischer M, Orth M. Short-latency sensory afferent inhibition: conditioning stimulus intensity, recording site, and effects of 1 Hz repetitive TMS. Brain Stimul 2011; 4: 202-209.

Fisher MA. H reflexes and f waves fundamentals, normal and abnormal patterns. Neurologic clinics 2002; 20: 339-360.

Friedman RM, Chen LM, Roe AW. Responses of areas 3b and 1 in anesthetized squirrel monkeys to single-and dual-site stimulation of the digits. Journal of Neurophysiology 2008; 100: 3185-3196.

Ghosh S, Porter R. Morphology of pyramidal neurones in monkey motor cortex and the synaptic actions of their intracortical axon collaterals. The Journal of physiology 1988; 400: 593-615.

Golaszewski SM, Siedentopf CM, Koppelstaetter F, Rhomberg P, Guendisch GM, Schlager A, et al. Modulatory effects on human sensorimotor cortex by whole-hand afferent electrical stimulation. Neurology 2004; 62: 2262-2269.

Goldring S, Aras E, Weber PC. Comparative study of sensory input to motor cortex in animals and man. Electroencephalogr Clin Neurophysiol 1970; 29: 537-550.

Goldsworthy MR, Pitcher JB, Ridding MC. A comparison of two different continuous theta burst stimulation paradigms applied to the human primary motor cortex. Clin Neurophysiol 2012a; 123: 2256-2263.

Goldsworthy MR, Pitcher JB, Ridding MC. The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. Eur J Neurosci 2012b; 35: 125-134.

Hallett M. Transcranial magnetic stimulation: a primer. Neuron 2007; 55: 187-199.

Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC. The role of interneuron networks in driving human motor cortical plasticity. Cereb Cortex 2013; 23: 1593-1605.

Hess G, Aizenman CD, Donoghue JP. Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex. J Neurophysiol 1996; 75: 1765-1778.

Hoffken O, Veit M, Knossalla F, Lissek S, Bliem B, Ragert P, et al. Sustained increase of somatosensory cortex excitability by tactile coactivation studied by paired median nerve stimulation in humans correlates with perceptual gain. J Physiol 2007; 584: 463-471.

Huang YZ, Chen RS, Rothwell JC, Wen HY. The after-effect of human theta burst stimulation is NMDA receptor dependent. Clin Neurophysiol 2007; 118: 1028-1032.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005; 45: 201-206.

Huang YZ, Rothwell JC, Lu CS, Wang J, Weng YH, Lai SC, et al. The effect of continuous theta burst stimulation over premotor cortex on circuits in primary motor cortex and spinal cord. Clinical Neurophysiology 2009; 120: 796-801.

Hultborn H, Nielsen JB. H–reflexes and F–responses are not equally sensitive to changes in motoneuronal excitability. Muscle Nerve 1995; 18: 1471-1474.

Iriki A, Pavlides C, Keller A, ASANUMA H. Long-term potentiation in the motor cortex. Science 1989; 245: 1385-1387.

Ishikawa S, Matsunaga K, Nakanishi R, Kawahira K, Murayama N, Tsuji S, et al. Effect of theta burst stimulation over the human sensorimotor cortex on motor and somatosensory evoked potentials. Clin Neurophysiol 2007; 118: 1033-1043.

Iwamura Y, Tanaka M, Sakamoto M, Hikosaka O. Rostrocaudal gradients in the neuronal receptive field complexity in the finger region of the alert monkey's postcentral gyrus. Exp Brain Res 1993; 92: 360-368.

Jacobs MF, Tsang P, Lee KG, Asmussen MJ, Zapallow CM, Nelson AJ. 30 Hz Theta-burst Stimulation Over Primary Somatosensory Cortex Modulates Corticospinal Output to the Hand. Brain Stimul 2013.

Jacobs MF, Zapallow CM, Tsang P, Lee KG, Asmussen MJ, Nelson AJ. Current direction specificity of continuous theta-burst stimulation in modulating human motor cortex excitability when applied to somatosensory cortex. Neuroreport 2012; 23: 927-931.

Johansson RS, Westling G. Roles of glabrous skin receptors and sensorimotor memory in automatic control of precision grip when lifting rougher or more slippery objects. Experimental brain research 1984; 56: 550-564.

Jones EG, Coulter JD, Hendry SH. Intracortical connectivity of architectonic fields in the somatic sensory, motor and parietal cortex of monkeys. J Comp Neurol 1978; 181: 291-347.

Kaas JH, Nelson RJ, Sur M, Lin CS, Merzenich MM. Multiple representations of the body within the primary somatosensory cortex of primates. Science 1979; 204: 521-523.

Kaas JH. The functional organization of somatosensory cortex in primates. Annals of Anatomy-Anatomischer Anzeiger 1993; 175: 509-518.

Kandel, E. R., Schwartz, J. H., & Jessell, T. M. (2000). *Principles of neural science*, 4 ed. McGraw-Hill New York.

Kaneko T, Caria MA, Asanuma H. Information processing within the motor cortex. II. Intracortical connections between neurons receiving somatosensory cortical input and motor output neurons of the cortex. J Comp Neurol 1994a; 345: 172-184.

Kaneko T, Caria MA, ASANUMA H. Information processing within the motor cortex. II. Intracortical connections between neurons receiving somatosensory cortical input and motor output neurons of the cortex. J Comp Neurol 1994b; 345: 172-184.

Kaneko T, Cho R, Li Y, Nomura S, Mizuno N. Predominant information transfer from layer III pyramidal neurons to corticospinal neurons. J Comp Neurol 2000; 423: 52-65.

Katayama T, Suppa A, Rothwell JC. Somatosensory evoked potentials and high frequency oscillations are differently modulated by theta burst stimulation over primary somatosensory cortex in humans. Clin Neurophysiol 2010; 121: 2097-2103.

Kimura J. Assessment of individual nerves. Electrodiagnosis in Diseases of Nerve and Muscle: Principles and Practice, ed 1989; 2: 103-138.

Kimura T, Haggard P, Gomi H. Transcranial magnetic stimulation over sensorimotor cortex disrupts anticipatory reflex gain modulation for skilled action. J Neurosci 2006; 26: 9272-9281.

Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. The Lancet Neurology 2003; 2: 145-156.

Kosar E, Waters RS, Tsukahara N, ASANUMA H. Anatomical and physiological properties of the projection from the sensory cortex to the motor cortex in normal cats: the difference between corticocortical and thalamocortical projections. Brain Res 1985; 345: 68-78.

Kotb MA, Mima T, Ueki Y, Begum T, Khafagi AT, Fukuyama H, et al. Effect of spatial attention on human sensorimotor integration studied by transcranial magnetic stimulation. Clin Neurophysiol 2005; 116: 1195-1200.

Krivanekova L, Lu MK, Bliem B, Ziemann U. Modulation of excitability in human primary somatosensory and motor cortex by paired associative stimulation targeting the primary somatosensory cortex. Eur J Neurosci 2011; 34: 1292-1300.

Lemon RN. Functional properties of monkey motor cortex neurones receiving afferent input from the hand and fingers. J Physiol 1981; 311: 497-519.

Lemon RN, Porter R. Afferent input to movement-related precentral neurones in conscious monkeys. Proc R Soc Lond B Biol Sci 1976a; 194: 313-339.

Lemon RN, Porter R. Afferent input to movement-related precentral neurones in conscious monkeys. Proc R Soc Lond B Biol Sci 1976b; 194: 313-339.

Lemon RN. Descending pathways in motor control. Annu Rev Neurosci 2008; 31: 195-218.

Lipski J. Antidromic activation of neurones as an analytic tool in the study of the central nervous system. Journal of neuroscience methods 1981; 4: 1-32.

Litvak V, Zeller D, Oostenveld R, Maris E, Cohen A, Schramm A, et al. LTP-like changes induced by paired associative stimulation of the primary somatosensory cortex in humans: source analysis and associated changes in behaviour. Eur J Neurosci 2007; 25: 2862-2874.

Mang CS, Bergquist AJ, Roshko SM, Collins DF. Loss of short-latency afferent inhibition and emergence of afferent facilitation following neuromuscular electrical stimulation. Neuroscience Letters 2012.

Miller LK, Chester R, Jerosch-Herold C. Effects of sensory reeducation programs on functional hand sensibility after median and ulnar repair: a systematic review. J Hand Ther 2012; 25: 297-306.

Monte-Silva K, Ruge D, Teo JT, Paulus W, Rothwell JC, Nitsche MA. D2 receptor block abolishes theta burst stimulation-induced neuroplasticity in the human motor cortex. Neuropsychopharmacology 2011; 36: 2097-2102.

Murakami T, Sakuma K, Nomura T, Uemura Y, Hashimoto I, Nakashima K. Changes in somatosensory-evoked potentials and high-frequency oscillations after paired-associative stimulation. Exp Brain Res 2008; 184: 339-347.

Nardone R, Bergmann J, Kronbichler M, Kunz A, Klein S, Caleri F, et al. Abnormal short latency afferent inhibition in early Alzheimers disease: a transcranial magnetic demonstration. J Neural Transm 2008; 115: 1557-1562.

Nuwer MR, Lehmann D, Lopes da SF, Matsuoka S, Sutherling W, Vibert JF. IFCN guidelines for topographic and frequency analysis of EEGs and EPs. Report of an IFCN committee. International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol 1994a; 91: 1-5.

Nuwer MR, Lehmann D, Lopes da Silva F, Matsuoka S, Sutherling W, Vibert JF. IFCN guidelines for topographic and frequency analysis of EEGs and EPs. Report of an IFCN committee. Electroencephalography and clinical Neurophysiology 1994b; 91: 1-5.

Okamoto M, Dan H, Sakamoto K, Takeo K, Shimizu K, Kohno S, et al. Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10ΓÇô20 system oriented for transcranial functional brain mapping. NeuroImage 2004; 21: 99-111.

Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971; 9: 97-113.

Palomar FJ, Suarez A, Franco E, Carrillo F, Gil-Neciga E, Mir P. Abnormal sensorimotor plasticity in CADASIL correlates with neuropsychological impairment. J Neurol Neurosurg Psychiatry 2013a; 84: 329-336.

Palomar FJ, Suárez A, Franco E, Carrillo F, Gil-Neciga E, Mir P. Abnormal sensorimotor plasticity in CADASIL correlates with neuropsychological impairment. Journal of Neurology, Neurosurgery & Psychiatry 2013b; 84: 329-336.

Pascual-Leone A, Valls-Sol+® J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain 1994; 117: 847-858.

Pavlides C, Miyashita E, Asanuma H. Projection from the sensory to the motor cortex is important in learning motor skills in the monkey. Journal of Neurophysiology 1993; 70: 733-741.

Peinemann A, Reimer B, L+Âer C, Quartarone A, M++nchau A, Conrad B, et al. Long-lasting increase in corticospinal excitability after 1800 pulses of subthreshold 5 Hz repetitive TMS to the primary motor cortex. Clinical Neurophysiology 2004; 115: 1519-1526.

Pell GS, Roth Y, Zangen A. Modulation of cortical excitability induced by repetitive transcranial magnetic stimulation: influence of timing and geometrical parameters and underlying mechanisms. Progress in neurobiology 2011; 93: 59-98.

Petersen NT, Pyndt HS, Nielsen JB. Investigating human motor control by transcranial magnetic stimulation. Experimental brain research 2003; 152: 1-16.

Pierrot-Deseilligny, E. & Burke, D. (2005). *The circuitry of the human spinal cord: its role in motor control and movement disorders* Cambridge University Press.

Porter LL, Sakamoto K. Organization and synaptic relationships of the projection from the primary sensory to the primary motor cortex in the cat. J Comp Neurol 1988; 271: 387-396.

Premji A, Rai N, Nelson A. Area 5 influences excitability within the primary motor cortex in humans. PLoS One 2011; 6: e20023.

Quartarone A, Rizzo V, Bagnato S, Morgante F, Sant'angelo A, Girlanda P, et al. Rapid-rate paired associative stimulation of the median nerve and motor cortex can produce long-lasting changes in motor cortical excitability in humans. J Physiol 2006; 575: 657-670.

Ragert P, Becker M, Tegenthoff M, Pleger B, Dinse HR. Sustained increase of somatosensory cortex excitability by 5 Hz repetitive transcranial magnetic stimulation studied by paired median nerve stimulation in humans. Neurosci Lett 2004; 356: 91-94.

Ragert P, Dinse HR, Pleger B, Wilimzig C, Frombach E, Schwenkreis P, et al. Combination of 5 Hz repetitive transcranial magnetic stimulation (rTMS) and tactile coactivation boosts tactile discrimination in humans. Neurosci Lett 2003; 348: 105-108.

Rattay, F. (1990). *Electrical nerve stimulation: theory, experiments and applications* Springer Verlag.

Richardson SP, Bliem B, Lomarev M, Shamim E, Dang N, Hallett M. Changes in short afferent inhibition during phasic movement in focal dystonia. Muscle Nerve 2008; 37: 358-363.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clinical Neurophysiology 2009; 120: 2008-2039.

Rusu CV, Murakami M, Ziemann U, Triesch J. A model of TMS-induced I-waves in motor cortex. Brain Stimul 2014; 7: 401-414.

Sailer A, Molnar GF, Paradiso G, Gunraj CA, Lang AE, Chen R. Short and long latency afferent inhibition in Parkinson's disease. Brain 2003; 126: 1883-1894.

Sale MV, Ridding MC, Nordstrom MA. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp Brain Res 2007; 181: 615-626.

Sanes JN, Donoghue JP, Thangaraj V, Edelman RR, Warach S. Shared neural substrates controlling hand movements in human motor cortex. Science 1995; 268: 1775-1777.

Schieppati M. The Hoffmann reflex: a means of assessing spinal reflex excitability and its descending control in man. Progress in neurobiology 1987; 28: 345-376.

Shagass C, Schwartz M. Recovery functions of somatosensory peripheral nerve and cerebral evoked responses in man. Electroencephalography and clinical Neurophysiology 1964; 17: 126-135.

Siebner H, Rothwell J. Transcranial magnetic stimulation: new insights into representational cortical plasticity. Exp Brain Res 2003; 148: 1-16.

Silva S, Basser PJ, Miranda PC. Elucidating the mechanisms and loci of neuronal excitation by transcranial magnetic stimulation using a finite element model of a cortical sulcus. Clinical Neurophysiology 2008; 119: 2405-2413.

Stagg CJ, Wylezinska M, Matthews PM, Johansen-Berg H, Jezzard P, Rothwell JC, et al. Neurochemical effects of theta burst stimulation as assessed by magnetic resonance spectroscopy. J Neurophysiol 2009; 101: 2872-2877.

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 2000; 123 Pt 3: 572-584.

Stefan K, Kunesch E, Benecke R, Cohen LG, Classen J. Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. The Journal of physiology 2002; 543: 699-708.

Stefan K, Wycislo M, Classen J. Modulation of associative human motor cortical plasticity by attention. Journal of Neurophysiology 2004; 92: 66-72.

Talelli P, Cheeran BJ, Teo JT, Rothwell JC. Pattern-specific role of the current orientation used to deliver Theta Burst Stimulation. Clin Neurophysiol 2007; 118: 1815-1823.

Tamburin S, Manganotti P, Zanette G, Fiaschi A. Cutaneomotor integration in human hand motor areas: somatotopic effect and interaction of afferents. Exp Brain Res 2001; 141: 232-241.

Tamura Y, Matsuhashi M, Lin P, Ou B, Vorbach S, Kakigi R, et al. Impaired intracortical inhibition in the primary somatosensory cortex in focal hand dystonia. Mov Disord 2008; 23: 558-565.

Terranova C, SantAngelo A, Morgante F, Rizzo V, Allegra R, Arena MG, et al. Impairment of sensory-motor plasticity in mild Alzheimer's disease. Brain Stimul 2013; 6: 62-66.

Tokimura H, Di L, V, Tokimura Y, Oliviero A, Profice P, Insola A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol 2000; 523 Pt 2: 503-513.

Tsang P, Jacobs MF, Lee KG, Asmussen MJ, Zapallow CM, Nelson AJ. Continuous theta-burst stimulation over primary somatosensory cortex modulates short-latency afferent inhibition. Clin Neurophysiol 2014.

Tsutsumi R, Shirota Y, Ohminami S, Terao Y, Ugawa Y, Hanajima R. Conditioning intensity-dependent interaction between short-latency interhemispheric inhibition and short-latency afferent inhibition. J Neurophysiol 2012; 108: 1130-1137.

Udupa K, Ni Z, Gunraj C, Chen R. Interactions between short latency afferent inhibition and long interval intracortical inhibition. Exp Brain Res 2009; 199: 177-183.

Vidoni ED, Acerra NE, Dao E, Meehan SK, Boyd LA. Role of the primary somatosensory cortex in motor learning: An rTMS study. Neurobiol Learn Mem 2010; 93: 532-539.

Vogt BA, Pandya DN. Cortico-cortical connections of somatic sensory cortex (areas 3, 1 and 2) in the rhesus monkey. J Comp Neurol 1978; 177: 179-191.

Voller B, St Clair Gibson A, Dambrosia J, Pirio Richardson S, Lomarev M, Dang N, et al. Short-latency afferent inhibition during selective finger movement. Exp Brain Res 2006; 169: 226-231.

Wassermann, E., Epstein, C., & Ziemann, U. (2008). *Oxford handbook of transcranial stimulation* Oxford University Press.

Wolters A, Sandbrink F, Schlottmann A, Kunesch E, Stefan K, Cohen LG, et al. A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. J Neurophysiol 2003; 89: 2339-2345.

Wolters A, Schmidt A, Schramm A, Zeller D, Naumann M, Kunesch E, et al. Timing-dependent plasticity in human primary somatosensory cortex. J Physiol 2005; 565: 1039-1052.

Yarnall AJ, Rochester L, Baker MR, David R, Khoo TK, Duncan GW, et al. Short latency afferent inhibition: a biomarker for mild cognitive impairment in Parkinson's disease? Mov Disord 2013; 28: 1285-1288.

Young-Bernier M, Kamil Y, Tremblay F, Davidson PS. Associations between a neurophysiological marker of central cholinergic activity and cognitive functions in young and older adults. Behav Brain Funct 2012; 8: 17.

Yumiya H, Ghez C. Specialized subregions in the cat motor cortex: anatomical demonstration of differential projections to rostral and caudal sectors. Exp Brain Res 1984; 53: 259-276.

Zamir O, Gunraj C, Ni Z, Mazzella F, Chen R. Effects of theta burst stimulation on motor cortex excitability in Parkinson's disease. Clin Neurophysiol 2012; 123: 815-821.

Zarzecki P, Shinoda Y, ASANUMA H. Projection from area 3a to the motor cortex by neurons activated from group I muscle afferents. Exp Brain Res 1978; 33: 269-282.

Ziemann U, Ilia-ç TV, Pauli C, Meintzschel F, Ruge D. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. The Journal of neuroscience 2004; 24: 1666-1672.