THETA-BURST STIMULATION ON TACTILE TEMPORAL ORDER JUDGMENT

THE INVESTIGATION OF THETA-BURST STIMULATION OVER PRIMARY SOMATOSENSORY CORTEX ON TACTILE TEMPORAL ORDER JUDGMENT

By

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ABSTRACT

Temporal order judgment (TOJ) refers to one's ability to successively report the temporal order of two tactile stimuli delivered to independent skin sites. The brain regions involved in processing TOJ remain unclear. Research has shown that TOJ performance can be impaired with a conditioning background stimuli and this phenomenon, known as TOJ synchronization (TOJ-S), is suggested to be mediated by inhibitory neural mechanisms within the primary somatosensory cortex (SI) that create perceptual binding across the two skin sites. Continuous theta-burst stimulation (cTBS) over SI impairs tactile spatial and temporal acuity. This dissertation examines the effects of cTBS on TOJ and TOJ-S performance on the hand. In Experiment 1, TOJ and TOJ-S were measured from the right hand before and for up to 34 minutes following 50 Hz cTBS over SI. In Experiment 2, same measurements were obtained bilaterally for up to 42 minutes following 30 Hz cTBS over SI. Compared to pre-cTBS values, TOJ was impaired for up to 42 minutes on the right hand following 30 Hz cTBS. TOJ-S performance was improved for up to 18 minutes on the right hand following 50 Hz cTBS. These experiments reveal two major findings. First, cTBS act upon different inhibitory circuits that are suggested to mediate TOJ and TOJ-S. Second, cTBS parameters may dictate cTBS effects over SI excitability. The findings of this work not only emphasize the significant contributions of SI on tactile temporal perception, it provides novel insight of the underlying neural mechanisms of cTBS effects on SI cortical excitability.

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TABLE OF CONTENTS

LIST OF FIGURES

List of Abbreviation

AMT	Active Motor Threshold
ANOVA	Analysis of Variance
CM	Cortical Metrics
CTBS	Continuous Theta-burst Stimulation
DB	Double-Bouqet Cell
DCML	Dorsal Column Medial Lemniscus
EMG	Electromyography
FDI	First Dorsal Interosseous Muscle
GABA	Gamma (ϒ)-Aminobutyric Acid
HFO	High Frequency Oscillation
ISI	Interstimulus Interval
MSO	Maximum Stimulator Output
MVC	Maximum Voluntary Contraction
OIS	Optimal Intrinsic Signaling
PC	Pacinian Corpuscles
TDT	Temporal Discrimination Threshold
TBS	Theta-Burst Stimulation
TOJ	Temporal Order Judgment
TOJ-S	Temporal Order Judgment Synchronization
RTMS	Repetitive Transcranial Magnetic Stimulation
SEP	Somatosensory Evoked Potential
SI	Primary Somatosensory Cortex
VPL	Ventral Posterior Lateral Nucleus
VPM	Ventral Posterior Medial Nucleus

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.

CHAPTER 1 – Goal of Thesis

1.1 Overview of Thesis

This thesis aims to advance our understanding of underlying cortical processes in the somatosensory system mediating tactile perception. Tactile perception refers to the successful coordination of tactile inputs and motor outputs which is required for optimal performances in a wide range of motor tasks (Goodwin & Wheat, 2004). Somatosensory feedback from the hand plays a significant role for hand function and motor control in the upper limb. However, the primary areas that are involved in processing tactile perception remain unclear. There is common agreement that the primary somatosensory cortex (SI) is an essential candidate for regulating tactile perception (Duncan & Boynton, 2007;Luna *et al.*, 2005;Lenz *et al.*, 2012). Patients with movement disorders and lesions to SI neural pathways demonstrate sensory deficits, such as impaired tactile frequency, two-point and temporal discrimination (Makous *et al.*, 1996;Staines *et al.*, 2002;Tamura *et al.*, 2008). One method to probe the underlying mechanisms that govern tactile perception is via repetitive transcranial magnetic stimulation TMS (rTMS). Numerous evidence suggests that rTMS is capable of altering intracortical excitability that persists after termination of rTMS (Song *et al.*, 2011). There is further support that briefly altering cortical excitability within SI can disrupt or improve tactile perception depending on the protocol used. For instance, previous studies have shown that rTMS delivered over SI at high frequencies (≥ 5 Hz) improved tactile two-point discrimination performance (Tegenthoff *et al.*, 2005). In contrast, low frequency rTMS impairs frequency and two-point discrimination (Knecht *et al.*, 2003;Vidoni *et al.*, 2010). Recently, theta-burst stimulation (TBS), a form of rTMS

delivered at a high frequency and low intensity has also shown to impair tactile temporal acuity (Conte *et al.*, 2012;Rai *et al.*, 2012).

Tactile temporal order judgment (TOJ) offers a new avenue to understand the role of SI in processing tactile temporal acuity. TOJ refers to one's ability to report the temporal order of successive taps delivered over independent skin sites (Tommerdahl *et al.*, 2007;Takahashi *et al.*, 2012). The underlying mechanisms that govern tactile TOJ processing remain unclear despite the abundant knowledge of TOJ in both visual and auditory domains (Hirsh.J., 1961;Allan, 1975;Woo *et al.*, 2009;Bolognini *et al.*, 2010). This Master's thesis work includes novel experiments to address the question of whether SI is involved in tactile temporal processing. TOJ in the presence and absence of additional stimuli were taken as measures of tactile perception before and after cTBS.

1.2 Significance of Thesis Work

Experiment 1, performed at the University of Waterloo, revealed a reduction of the synchronization effect following cTBS, suggesting that the activity of inhibitory interneurons within SI may be altered by cTBS. This experiment adds new understanding of the underlying neural mechanisms within the primary sensory areas involved in processing tactile perception. Future experiments could aim to investigate other cortical or sub-cortical areas that have direct or indirect projections to SI. Understanding such functional connectivity between SI and its neighboring areas may help further understand the involved regions in processing tactile perception and the complexity of sensory

processing. Previous work has demonstrated suppression of tactile temporal and spatial discrimination following the application of cTBS over SI. Experiment 2 further examined a modified cTBS paradigm in search for further understanding of the tactile perceptual effects of cTBS. Experiment 2 which was conducted at McMaster University, revealed significant impairments in temporal order judgment performance following stimulation. This suggests that neural circuitries that mediate TOJ and TOJ-S may respond differently to a modified form of cTBS.

In summary, both experiments provide important contributions to the fundamentals of behavior neuroscience by confirming the cortical regions involved in processing tactile temporal perception. This knowledge can be further applied to clinical populations that demonstrate sensory and motor impairments due to altered cortical excitability such as in autism and Focal hand dystonia (Casanova *et al.*, 2003;Scontrini *et al.*, 2009). Finally, the mechanisms of TBS on suppressing cortical excitability allows for a better understanding of the development of potential therapeutic tools for patients that exhibit altered cortical excitability.

1.3 Outline of Thesis Chapter

The thesis is arranged as follows:

Chapter 1 outlines the motivation and significance of thesis. It also states key findings from Experiment 1 and Experiment 2.

Chapter 2 details the theory of repetitive TMS used in this experiment. Also, the role of the primary somatosensory cortex in processing tactile stimuli and tactile perception will be provided.

Chapter 3 presents the manuscript drafted for Experiment 1.

Chapter 4 presents the manuscript drafted for Experiment 2.

Chapter 5 addresses the goal of this thesis and provides conclusions from both experiments.

1.4 Summary of Contributions

Experiment 1 sought to investigate the modulation of tactile temporal order judgment in the presence and absence of synchronous stimuli on the right hand following 50 Hz cTBS over SI. TOJ and TOJ synchronization (TOJ-S) performance were measured in eight right-handed individuals prior to and following cTBS over left-hemisphere SI.

Experiment 2 investigates the modulation of tactile temporal order judgment in the presence and absence of synchronous stimuli bilaterally following 30 Hz cTBS over left-hemisphere SI. TOJ and TOJ-S performance were obtained from ten right-handed individuals prior to and following the application of cTBS.

CHAPTER 2 – Review of Literature

2.1 Peripheral Response to Tactile Stimuli

The human skin receives tactile stimuli through several low-threshold mechanoreceptors. These receptors are morphologically located throughout different parts of the skin and undergo deformation under the response of light stimulation (Maeno *et al.*, 1998). Mechanoreceptors can be classified into two major categories corresponding to their ability to respond to vibrotactile stimuli: slowly adapting and rapidly adapting. Slowly adapting type I fibers (SAI fibers) innervate the Merkel disc receptors which are located beneath the epidermis of the skin. The abundance of Merkel cells near the surface of the skin allow for sensitive responses to light touch and change in pressure (Hamann, 1995). Specifically, the anatomical arrangements of these cells in the fingertip provide a precise localization to detect tactile stimuli (Haeberle & Lumpkin, 2008). Primary afferent fibers originating from Merkel receptors transmit tactile information to the cortex to its corresponding neural receptive field (Johansson & Vallbo, 1983). Merkel cells typically have smaller defined receptive fields with high sensitivity (Johansson, 1978). Rapidly adapting type I (RAI) receptors, also known as Meissners corpuscles are highly sensitivity to light touch as they fire bursts of action potentials upon receiving initial contact to stimuli (Talbot *et al.*, 1968;Sathian *et al.*, 1989). Similar to SAI, these receptors are highly populated at the fingertips and represent small but highly sensitive receptive fields (Johansson, 1978;LaMotte *et al.*, 1998). Rapidly adapting type II fibers (RAII) terminate at the Pacinian corpuscles (PC) located deep in the subcutaneous tissue and these receptors respond to vibration with a range of frequencies from 100-400 Hz (Talbot

et al., 1968). Due to its large size, it captures a wide area of skin and hence represents a larger receptive field (Johansson, 1978).

Vibrotactile stimulation applied over mechanoreceptors excites a whole population of afferents including both SA and RA fibers. A chain of events occur upon excitation of these peripheral nerve fibers (Mountcastle *et al.*, 1967). First, changes in permeability occur at the axonal endings of each nerve. When local depolarization at the nerve ending reaches the threshold for the activated mechanoreceptor, an action potential (receptor potential) is produced. Further, the action potential travels along the afferent fibers which then synapse at the dorsal root of the spinal cord, eventually reaching the somatosensory cortex. Overall, the initiation of neural transmission at the receptor level allows for neural coding of touch.

2.2 Neural Transmission of Tactile Stimuli

The mechanism of which tactile stimuli is transmitted to the cortex can be explained by the dorsal column medial lemniscuses pathway (DCML) (Mountcastle *et al.*, 1967). Action potentials generated at the nerve endings carry encoded touch information through first-order afferent fibers (dorsal root ganglion) to form synapses at the dorsal horn within the spinal cord. Touch fibers then ascend to the upper laminae through dorsal columns located within the spinal cord. Once at the medulla, they synapse at the dorsal column nuclei: the cuneate nucleus (Mountcastle, 2005). Second-order afferent fibers in the dorsal column nuclei then cross over the midline in the medulla where they ascend in the medial lemniscus to the ventral posterior lateral (VPL) and medial nuclei (VPM) of

the thalamus (Mountcastle, 2005). Finally, third-order afferent fibers from the thalamus then project to specific areas within the primary somatosensory cortex (SI).

2.3 Physiological Structure of SI

The primary somatosensory cortex spans four cytoarchitectureal areas along the rostra-caudal axis of the parietal lobe, forming its anterior and posterior border by the central sulcus and postcentral sulcus, respectively (Jones *et al.*, 1982). These morphologically and functionally distinct regions are commonly referred to as Brodmann areas 3a, 3b, 1 and 2 (Brodmann, 1909). Within SI, Brodmann areas 3b and 1 mainly receive mechanoreceptive inputs from the skin, whereas areas 3a and 2 mainly receive proprioceptive information from receptors in muscles, joints and the skin (Mountcastle, 2005). Thalamocortical afferents terminate upon six different layers of cells exhibiting both excitatory and inhibitory mechanisms (Mountcastle, 2005). Excitatory cells such as the pyramidal neurons are highly populated at the superficial layer whereas inhibitory cells such as the GABAergic, double-bouqet cells (DB) all mainly presented in layers II and III (Mountcastle, 2005). Axons from DB cells project vertically into bundles and terminate upon both inhibitory interneurons and pyramidal cells (Mountcastle, 2005). This arrangement imposes a strong stream of inhibition or dis-inhibition within SI. Other inhibitory cells such as the large and small basket cells and chandelier cells also exert inhibitory control on pyramidal cells (Markram *et al.*, 2004). Local excitatory neurons and interneurons of layers IV and IIIb, which contain the spiny non-pyramidal or stellate cells, receive post-synaptic targets of afferents from the VPL of the thalamus. Tactile processing within SI can be explained by the principle of lateral inhibition, which is

thought to be a delineation of individual cortical columns (minicolumns) from their neighbors (Mountcastle, 2005;Favorov & Kelly, 1994). It is known that the cortex consists of inhibitory DB cells that define minicolumnar organization in the brain. GABAergic neurons play a vital role in lateral inhibition by creating an inhibitory connection between adjacent minicolumns, thereby causing them to become functionally dissimilar. Patient populations with a lack of these inhibitors would be affected in discriminating between competing forms of sensory information (Casanova *et al.*, 2003).

2.4 Neural Processing within SI

How is tactile information perceived in the primary somatosensory cortex? Changing the intensity, frequency or duration of the applied stimuli could change the receptive field and specifically the excitability of neurons in the somatosensory cortex (Tommerdahl *et al.*, 2010;DiCarlo & Johnson, 2002). It has been shown that increasing amplitude of vibrotactile stimuli would increase the ability to discriminate between two stimuli (Francisco *et al.*, 2008). Further, using optical intrinsic signal imaging (OIS) in studying the vibrotactile stimulation response in SI in squirrel monkeys, one study found that increasing the duration of the vibrotactile stimuli would increase absorbances in central regions of activation while suppressing responses (decreased absorbance) in areas surrounding the activated areas, also known as surround inhibition. This increased in absorbance in central regions is believed to be due to an increase in neuronal firing within the area receiving maximal excitation (Tommerdahl *et al.*, 2010;Simons *et al.*, 2007). These studies suggest that the somatosensory cortex is involved in sensory processing and that neurons within receptive fields could be altered via changing the sensory modalities

of the tasks. In fact, much emphasize has been focused on understanding the role of SI in perceiving precisely the spatial location of the applied stimuli. When two stimuli are applied over the skin at different locations simultaneously, SI is capable of extracting the code of the spatial differences between the two stimuli. While spatial aspects have been studied thoroughly, the role of SI in temporal processing, specifically the ability to discriminate two stimuli being temporally different has also received much attention in recent years. Reports on lesions of the dorsal column within the somatosensory system in primates showed impairment in temporal processing. It has been shown that monkeys with lesions to the dorsal column fail to discriminate between cutaneous stimulation frequencies of 10-35 Hz (Makous *et al.*, 1996).

2.5 Somatosensory Evoked Potentials

Stimulation of primary afferent fibers elicits neural activity that travels along the ascending DCML pathway. A portion of this activity can be measured in humans non-invasively from the scalp. Cortical somatosensory evoked potentials (SEPs) can be generated in response to stimulation of the contralateral median nerve at the wrist. These SEPs are defined as short-latency potentials since they appear 40 ms after median nerve stimulation (Allison *et al.*, 1989). Cortical components such as the N20, P25 and N30 potentials are thought to be generated in the contralateral somatosensory cortex in areas 3b & 1, which mainly receives mechanoreceptive inputs from the skin (Allison *et al.*, 1989). Often, SEPs provides neuroscience research with a better understanding of the sensory pathway since changes in amplitude and latencies could relate to impaired sensory processing in patient populations.

2.6 Cortical Metrics Device (CM)

The Cortical Metric device is a four-site vibrotactile stimulator that can be used to provide reliable and accurate quantitative measures of tactile perception through various psychophysical tasks that vary across different sensory modalities. These tasks are meant to provide a further understanding of the cortical areas involved in controlling tactile perception. The device consists of two components: a computer interface and a portable four-site stimulator. A designed interface using C# programming language and Windows Presentation Foundation (WPF) framework allows for control of the stimulator and the administration of psychophysical protocols. The stimulator consists of four rotatory cylindrical disks with a circular probe (5 mm inner diameter) attached individually to the surface of each disk. Previous versions of the CM device have demonstrated changes in spatial and temporal acuity with repetitive tactile stimulation to a maximum of two skin sites (Tommerdahl *et al.*, 2007;Rai *et al.*, 2012). In this current version, the CM device was designed to deliver repetitive stimulation simultaneously or sequentially to a maximum of four fingertips. This device has been used in a study involving healthy control subjects using a finger agnosia tool (Holden *et al.*, 2012). See Figure 2.1 for device and Holden et al. (2012) for full description. In both of our studies temporal order judgment (TOJ) measures were performed using the CM device.

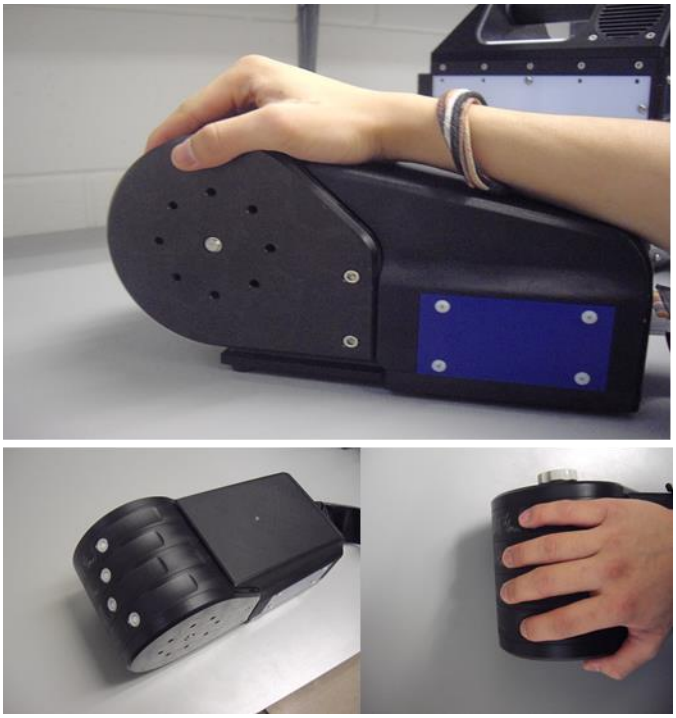

Figure 2.1. Cortical Metrics Device (CM). Participant's hand is placed on the device with arm rested on the extended surface (Top). Portable stimulator device with stimulator probes attached individually on four cylindrical disks (Bottom left). Digits 2 to 4 gently rested on each stimulator probe. Digit 2 and 4 received tactile stimulation during TOJ tasks (Bottom right).

2.7 Temporal Order Judgment

2.7.1 Temporal order Judgement (TOJ)

The application of TOJ involves two probes that vibrate at a certain frequency and amplitude separated by a variable interstimulus interval (ISI). The subject reports which finger they felt received the first stimulus. Responses are measured through a mouse click with the non-stimulated hand. The ISI increases or decreases depending on the accuracy

of the response by the subject. A correct response results in a decrease in ISI by a set value while an incorrect response results in an increase in ISI by the same value. As the task becomes more difficult, the ISI value becomes small. In contrast, a large ISI value makes the task easier. To assess TOJ performance, the lowest ISI value to correctly report the order of the stimulus pair (i.e. TOJ threshold) has been used in both healthy and clinical populations (Tommerdahl *et al.*, 2007;Tommerdahl *et al.*, 2008;Nelson *et al.*, 2012).

2.7.2 TOJ Synchronization

Similar to TOJ, the application of TOJ synchronization involves two probes that vibrate at a certain frequency and amplitude separated by an ISI. However, subjects perceive temporal order of the paired stimulus in the presence of a 25 Hz synchronized conditioning stimuli delivered concurrently and periodically at the two skin sites. The presence of the additional stimuli has been shown to disrupt normal TOJ performance in healthy individuals (Tommerdahl *et al.*, 2007). In contrast, certain patient groups with cortical deficits do not show the impairment of TOJ. To assess TOJ synchronization performance, threshold values have been used in both healthy and patient populations (Tommerdahl *et al.*, 2007;Tommerdahl *et al.*, 2008;Nelson *et al.*, 2012).

2.8 Transcranial Magnetic Stimulation (TMS)

2.8.1 Mechanisms

Transcranial magnetic stimulation (TMS) is a non-invasive research tool often used to study neural mechanisms and circuitries by inducing neural plasticity through the induction of an electric field within the cortex (Rossi *et al.*, 2009). The principles of electromagnetic field in TMS can be understood based on the concepts of Faraday's Law: rapidly changing magnetic field induces an electric field in the brain (Faraday, 1839). Magnetic fields are generated at a magnetic coil which induces a secondary electric current perpendicular to the direction of the magnetic field (Nollet *et al.*, 2003;Rossi *et al.*, 2009). The intensity of the magnetic field can reach up to 2 Telsa and depending on the coil used, can lasts for about 150 microseconds (Nollet *et al.*, 2003). Due to the low impedance to magnetic fields presented at the skull, the induced electric current can activate neural tissues (Nollet *et al.*, 2003;Hallett, 2007). A figure-of-eight coil which allows stimulation at a depth of 1.5-3.0 cm beneath the scalp is typically employed to obtain a focal stimulation (Rossi *et al.*, 2009). When the coil is positioned on the scalp, the induced electric current reaches the cortex and thereby causes neuronal firing through the depolarization of a targeted neuron membrane (Hallett, 2007). Depending on the stimulation intensity and the positioning of the coil, the activation of cortical neurons generates a descending volley of the pyramidal tract which results in muscle activation that could be measured via surface electromyography (EMG). TMS can be applied in various forms to assess and alter cortical excitability in the form of excitatory and inhibitory neural pathways.

2.8.2 Single-pulse TMS

Applying single-pulse TMS over cortical areas that control for types of muscles such as the primary motor cortex (M1) could induce a motor evoked potential (MEP) often described as a visible muscle twitch. For instance, applying TMS over the hand area of M1 could trigger MEPs at the specific hand muscles. Two hand muscle commonly used for the measurement of muscle activity is the first dorsal interosseous muscle (FDI) and the abductor pollicis brevis (APB). Accurately localizing the cortical area ('motor hotspot') for inducing MEPs depends on both stimulation intensity and coil orientation (Kobayashi & Pascual-Leone, 2003). Motor thresholds represent the lowest intensity for a single-pulse TMS to evoke an MEP in the target muscle during rest or voluntary contraction (Kobayashi & Pascual-Leone, 2003). Resting motor threshold (RMT) is commonly used in TMS studies and it is defined as the minimum intensity to elicit an MEP of $\geq$ 50 μV peak-to-peak amplitude from the muscle in at least 50% of successive trials at rest (Kobayashi & Pascual-Leone, 2003). Subsequently, the active motor threshold (AMT) is defined as the minimum intensity to evoke an MEP of $\geq$ 200 μV during 10% of maximal voluntary contraction in a target muscle (Huang *et al.*, 2005). Motor threshold reflects membrane excitability of the pyramidal neurons and possibly the interneurons projecting onto these neurons. The intensity for different forms of TMS protocols such as repetitive TMS rely on the obtained AMT.

2.8.3 Single-pulse TMS and Tactile Perception

Single-pulse TMS over specific cortical areas have shown to alter tactile perception. For instance, a reduction of behavioral performance for discrimination of congruent multisensory touch than for unisensory touch was observed after application of single-pulse TMS over the posterior parietal cortex (Pasalar *et al.*, 2010). A study investigating the effects of single-pulse and paired-pulse TMS over S1 suggested changes in perception and sensory processing during a sensorimotor task (Meehan *et al.*, 2008). Vibrotactile stimulation discrimination was suppressed after single pulse TMS was applied over S1 (Morley *et al.*, 2007). The influence of TMS over S1 altering tactile perception could be confirmed furthermore by Hannula and colleagues. This group found that monophasic TMS pulses delivered over S1 impaired tactile temporal discrimination (Hannula *et al.*, 2008).

2.8.4 Repetitive TMS (rTMS)

Depending on the form of TMS administered, cortical excitability could outlast the stimulation protocol itself (Ridding & Ziemann, 2010). Repetitive TMS (rTMS) protocols require multiple TMS pulses to be delivered at a constant rate up to 50 Hz (Huang *et al.*, 2005). Previous studies have shown that rTMS at low frequencies (≤ 5 Hz) results in suppression of cortical excitability (Chen *et al.*, 1997;Romero *et al.*, 2002), whereas stimulation at high frequencies (≥ 5 Hz) results in facilitation of cortical excitability at the stimulated area (Ragert *et al.*, 2004). Similar to single-pulse TMS, previous studies suggested that rTMS over SI suppresses tactile perceptual performances.

For instance, low frequency 1-Hz and 0.9-Hz rTMS delivered over SI reduced tactile frequency discrimination (Knecht *et al.*, 2003) and two-point discrimination performances (Satow *et al.*, 2003), respectively. In contrast, high frequency 5-Hz and 15-Hz rTMS over SI improved two-point discrimination performances (Tegenthoff *et al.*, 2005) and tactile perceptual learning (Karim *et al.*, 2006), respectively.

2.8.5 Theta burst TMS (TBS)

Theta burst stimulation, a type of rTMS developed by Huang and colleagues has led to new insights on how TMS could alter cortical excitability at targeted regions. Theta burst stimulation is applied at a lower intensity, higher frequency and at longer durations. This protocol consists of bursts of three pulses given at 50 Hz repeated at every 200 ms, as a result a total of 600 pulses are produced (Huang *et al.*, 2005). Applied at an intensity of 80% active motor threshold (AMT), TBS is known to induce changes in corticospinal excitability that outlasts the stimulation itself (Huang *et al.*, 2005). Two types of TBS, intermittent TBS (iTBS) and continuous TBS (cTBS) have been shown to effectively alter cortical excitability through excitatory or inhibitory neural pathways. ITBS is applied using a 2s train of TBS, repeated every 10 s for up to 190s (Huang *et al.*, 2005). Previous work has proposed that iTBS over the primary motor cortex (M1) generates a facilitatory effect reflected by an increase in motor evoked potentials (MEPs) (Huang *et al.*, 2005). On the contrary, cTBS is recognized for inducing inhibitory effects when delivered over M1 (Cardenas-Morales *et al.*, 2010). During cTBS, a train of pulses are emitted continuously without interruption for 40s (Huang *et al.*, 2005).

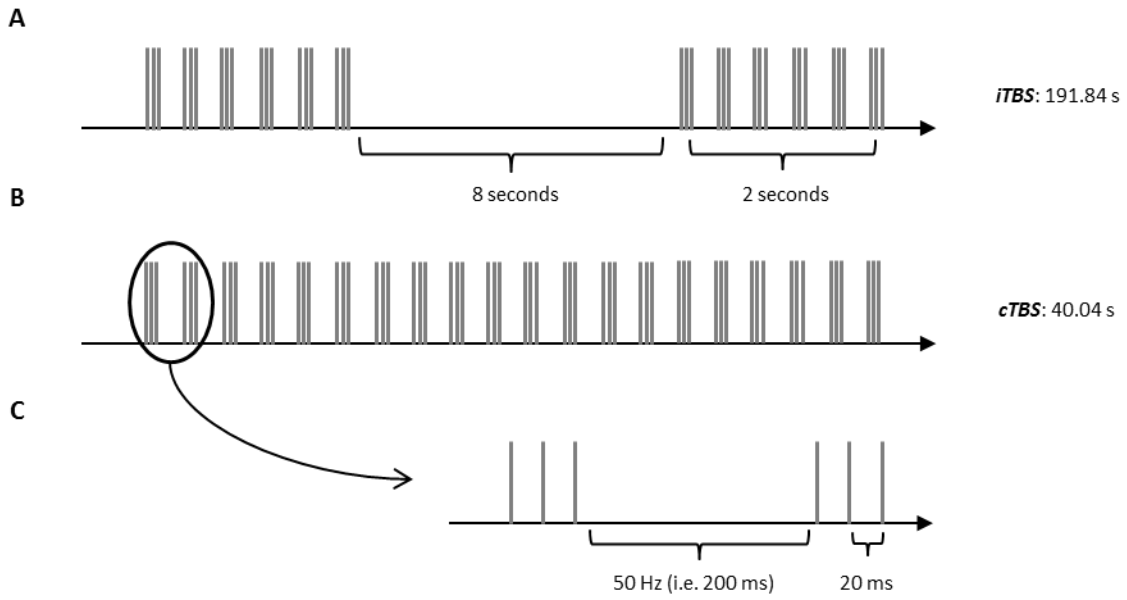

Figure 2.2. Different modalities of theta burst stimulation (TBS). (A) Intermittent TBS (iTBS) paradigm with a 2 second train of TBS repeated every 10 seconds; a total of 600 pulses for a total duration of 191.84s. (B) Continuous TBS (cTBS) paradigm with a train of TBS delivered over a total duration of 40s with 600 pulses. (C) Description of a general theta-burst paradigm with three stimulation pulses delivered at 50 Hz repeated every 200 ms.

2.8.5.1 ITBS

ITBS has been shown to increase excitability at the motor cortex (Cardenas-Morales *et al.*, 2010). Similarly, previous work demonstrated that TBS also exerts similar excitatory effects on the somatosensory cortex in humans (Katayama *et al.*, 2010;Katayama & Rothwell, 2007;Ragert *et al.*, 2008;Premji *et al.*, 2010). For example, somatosensory evoked $N20_{onset}$-$N20_{peak}$ potentials were facilitated following iTBS over S1 (Katayama & Rothwell, 2007). Neural mechanisms within SI can also be examined

through changes in behavioral measurements after TBS. For instance, Ragert and colleagues applied iTBS over SI and showed an improvement in two-point discrimination (Ragert *et al.*, 2008).

2.8.5.2 CTBS

CTBS has been proposed to inhibit SI excitability. One study administered cTBS over left SI and found a decrease in the amplitude of ipsilateral SEPs for at about 13 minutes following stimulation (Ishikawa *et al.*, 2007). CTBS to SI also decreased oxygenated hemoglobin levels in the non-stimulated contralateral SI (Mochizuki *et al.*, 2007). Recent evidence also suggested that cTBS over SI impaired performances of temporal (Rai *et al.*, 2012;Conte *et al.*, 2012) and spatial discrimination (Rai *et al.*, 2012) for about 18 minutes following application of cTBS.

CHAPTER 3: EXPERIMENT 1

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CHAPTER 3: EXPERIMENT 1

Continuous theta-burst stimulation modulates tactile synchronization

Keywords: Temporal order, synchronization, somatosensory cortex

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3.1 Abstract

Background: Temporal order judgement (TOJ) is the ability to detect the order of occurrence of two sequentially delivered stimuli. Previous research has shown that TOJ in the presence of synchronized periodic conditioning stimuli impairs TOJ performance, and this phenomenon is suggested to be mediated by GABAergic interneurons that cause perceptual binding across the two skin sites. Application of continuous theta-burst repetitive TMS (cTBS) over primary somatosensory cortex (SI) alters temporal and spatial tactile perception. The purpose of this study was to examine TOJ perception in the presence and absence of synchronized periodic conditioning stimuli before and after cTBS applied over left-hemisphere SI. A TOJ task was administered on the right index and middle finger (D2 and D3) in two separate sessions in the presence and absence of conditioning stimuli (a background low amplitude sinusoidal vibration). **Results:** CTBS reduced the impact of the conditioning stimuli on TOJ performance for up to 18 minutes following stimulation while sham cTBS did not affect TOJ performance. In contrast, the TOJ task performed in the absence of synchronized conditioning stimulation was unaltered following cTBS. **Conclusion:** We conclude that cTBS suppresses inhibitory networks in SI that mediate perceptual binding during TOJ synchronization. CTBS offers one method to suppress cortical excitability in the cortex and potentially benefit clinical populations with altered inhibitory cortical circuits. Additionally, TOJ measures with conditioning stimuli may provide an avenue to assess sensory processing in neurologically impaired patient populations.

3.2 Introduction

Tactile input is essential for fine motor control of the hand. Patients with impaired hand control often demonstrate abnormalities in touch processing that may contribute to their motor symptoms (Abbruzzese & Berardelli, 2003;Scontrini *et al.*, 2009). Primary somatosensory cortex (SI) is one cortical area that is clearly involved in touch perception (Duncan & Boynton, 2007;Luna *et al.*, 2005;Tremblay *et al.*, 1996) and importantly, has demonstrated plasticity with a number of methods including approaches utilizing repetitive transcranial magnetic stimulation (rTMS) (Conte *et al.*, 2012;Meehan *et al.*, 2008;Ragert *et al.*, 2008;Rai *et al.*, 2012;Song *et al.*, 2011).

Previous studies suggest that SI is involved in temporal processing of tactile information. In Focal hand dystonia, functional (Abbruzzese & Berardelli, 2003) and anatomical abnormalities in SI (Elbert *et al.*, 1998;Nelson *et al.*, 2009) are present. These individuals also demonstrate impaired temporal discrimination threshold (TDT) which is defined as the ability to detect the presence of one versus two stimuli when the pair is delivered over the skin and separated by a varied time interval (Tinazzi *et al.*, 1999;Rai *et al.*, 2012;Pastor *et al.*, 2004;Lacruz *et al.*, 1991). TDT impairments are greatest when lesions affect SI compared to the frontal, temporal and occipital cortex (Lacruz *et al.*, 1991). However, other cortical areas are considered important in TDT processing including the prefrontal cortex, inferior parietal lobe, the basal ganglia, cerebellum, the pre-supplementary motor area and anterior cingulate (Pastor *et al.*, 2004). Temporal order judgment (TOJ) represents another feature of tactile temporal processing in which subjects are required to detect the temporal order of two sequential stimuli delivered

across skin sites. In humans, it remains unclear as to what cortical areas are involved in processing TOJ. There is some evidence in animal studies, however, suggesting the role of SI in TOJ. One study reported an increase in c-Fos expression, a task-relevant neural activation marker in SI of mice following a temporal order judgment task performed with tactile stimuli delivered to the whiskers (Wada *et al.*, 2010). C-Fos was greatly increased in the barrel fields of SI following a TOJ task in which mice were trained to detect the order two tactile air-puff stimuli by orienting their head towards the first or second stimulus (Wada *et al.*, 2010). These results suggest that SI may play a part in TOJ processing.

A perceptual phenomenon called the 'synchronization effect' (TOJ-S) occurs when TOJ is performed in the presence of low amplitude background synchronized vibration (low frequency flutter or 25 Hz) delivered to both skin sites such that TOJ thresholds are impaired in healthy individuals by a factor of 2-4 times (Nelson *et al.*, 2012;Tommerdahl *et al.*, 2007). The impact of TOJ-S is thought to occur by the co-activation of adjacent and/or near-adjacent cortical ensembles in SI that results in conditioning tactile stimuli applied synchronously to adjacent digits. The co-activation of these cortical ensembles perceptually bind adjacent skin sites such that a stimulus presented at one site evokes a response in the adjacent cortical representation, and this leads to impaired TOJ performance (Tommerdahl *et al.*, 2007). Inhibitory interneurons are thought to participate in TOJ-S as it is well documented that inhibition plays a role in cortical synchronization (Singer, 1996;Uhlhaas & Singer, 2010). For example, there is

growing evidence that deficiencies in GABA play a role in autism (Casanova *et al.*, 2003) and the TOJ synchronization effect is abolished in these individuals (Tommerdahl *et al.*, 2008). Dopaminergic neurotransmitter systems may also contribute such that Parkinson's patients on L-dopa do not demonstrate the synchronization effect but show typical impairments when off medication (Nelson *et al.*, 2012). In the present study we investigate the role of SI in TOJ processing in the presence and absence of the synchronization effect.

One method to investigate the role of SI in TOJ processing is via the application of continuous theta burst stimulation (cTBS) (Huang *et al.*, 2005). Previous studies observed impairments in TDT for 5 - 18 minutes following cTBS over SI (Conte *et al.*, 2012;Rai *et al.*, 2012). Such impairment was not observed when cTBS was applied to the dorsal lateral prefrontal cortex and lateral cerebellum (Conte *et al.*, 2012). Similarly, tactile two-point discrimination was also impaired for up to 18 minutes following stimulation over SI (Rai *et al.*, 2012). Previous reports examining SI physiology demonstrated that cTBS over SI suppresses ipsilateral somatosensory evoked potentials (P25/N33) for 13 minutes following stimulation. Further, decreased oxy-hemoglobin concentrations in the contralateral SI and M1 was also observed following stimulation (Mochizuki *et al.*, 2007). In the present study we investigate the influence of cTBS over SI on TOJ and TOJ synchronization. Psychophysical measures were obtained from the right hand before and for up to 34 minutes following real and sham cTBS over left-hemisphere SI.

3.3 Methods

3.3.1 Participants

Sixteen healthy adults participated in one of two experiments (mean age = 23.1 ± 5.2 years, range 19 – 36 years, 5 males). For experiment 1, eight subjects (mean age = 26.5 ± 5.4 years, range 19 – 36 years, 3 males) participated in two sessions separated by a minimum of 1 week. For experiment 2, a different group of eight subjects participated (mean age = 19.7 ± 1.4 years, range = 19 – 23 years, 2 males) in a single session. All participants were right handed determined using a subsection of the Edinburgh Handedness Inventory (Oldfield, 1971). Subjects wore earplugs and headphones to minimize auditory cues during the experiments. All participants provided written consent and the study was approved by the Office of Research Ethics at the University of Waterloo and conformed to the Declaration of Helsinki.

3.3.2 Experimental approach

3.3.2.1 Electromyography (EMG) recording

Measurements of muscle activity were recorded using 9 mm diameter Ag-AgCI surface electrodes. The active electrode was placed over the muscle belly of the right dorsal interosseous muscle and the reference electrode was placed over the metacrapophalangeal joint of the right index finger. EMG was amplified at 1000 gain, bandpass filtered (2Hz – 2.5 kHz, Intronix Technologies Corporation Model 2024F, Bolton, Ontario, Canada), and digitized (5 kHz, Micro 1401, Cambridge Electronics

Design, Cambridge, UK). Signal software (v4.02, Cambridge Electronic Design Limited, Cambridge, UK) was used to acquire and analyze EMG data. Data was stored on a computer for analysis purposes.

3.3.2.2 TMS and Neuronavigation

TMS was delivered through a MagPro stimulator (MCF-B65; Medtronic, Minneapolis, MN, USA) connected to a figure-of-eight coil with a 90 mm diameter with the current flowing away from the handle of the coil. The motor hotspot was defined as the location in the left hemisphere that elicited a MEP in the relaxed right FDI muscle with the TMS coil oriented at 45 degrees to the mid-sagittal line. Active motor threshold (AMT) measurements were performed at this location and defined as the lowest intensity required to evoke MEPs $\geq$ 400 μV in 5 out of 10 consecutive trials during 10% maximum voluntary contraction (MVC). AMT was calculated using biphasic pulses inducing anterior to posterior current followed by posterior to anterior current. MVC was determined by having participants abduct their right index finger against an immovable post with maximal force. Participants maintained 10% MVC using their EMG feedback from the FDI muscle that was displayed on an oscilloscope. Brainsight Neuronavigation software (Rogue Research, Montreal) was used to locate M1 motor hotspot. SI was defined as a point 2 cm posterior to the M1 motor hotspot (Ishikawa *et al.*, 2007). CTBS was applied over SI using the 600 biphasic pulse protocol (Huang *et al.*, 2005;Katayama *et al.*, 2010) at 80% AMT with the handle oriented backwards and laterally at a 45 degree angle to the mid-sagittal line to induce current in the anterior to posterior direction during the initial phase of the pulse (Premji *et al.*, 2010;Rai *et al.*, 2012). The orientation and

position of the coil were marked using the Brainsight software to ensure theta burst stimulation was delivered with minimal variability.

3.3.2.3 Cortical Metrics Device (CMD)

Subjects were seated comfortably in a chair with their left hand rested on a computer touch pad and their right hand placed on the Cortical Metric Device, version CM-4 (Holden *et al.*, 2012). Both the computer laptop and the CM-4 were positioned at a comfortable arm level in front of the participants. The CM-4 device is equipped with 4 circular probes that are located on the surface of each individual rotatory cylindrical disk (Holden *et al.*, 2012). Each disk was rotated independently to adjust for different finger lengths for each participant. Digits 2 through 5 of the right hand were comfortably rested on the surface of the circular probes such that a single probe (5 mm diameter) maintained contact with the glabrous pad of each digit. The finger tips were locked in place prior to each TOJ task. The probes were further indented 500 μm prior to stimulation onset to ensure adequate skin contact across the surface area of the probe. An optical position (force) sensor was attached to each circular probe to provide feedback to the CM-4 device to ensure that the contact force of each fingertip was constant throughout the TOJ task (Holden *et al.*, 2012).

3.3.3. Experimental Conditions

3.3.3.1 Experiment 1A: cTBS influences on TOJ and TOJ Synchronization

Temporal order Judgement (TOJ)

TOJ was performed on digit 2 and 3 of the right hand. A single TOJ trial delivered a vibro-tactile stimulus (25 Hz, 40 ms and 200 μm) to the volar surface of the 2nd and 3rd digit tip on the right hand separated by an interstimulus interval (ISI) (see Figure 3.1). The participant was queried to identify which stimulus occurred first (i.e. the 2nd or 3rd digit) and respond as quickly as possible by making a key press with the left hand; left key = 2nd digit, right key = 3rd digit. The digit selected to receive the first stimulus was randomized on a trial-by-trial basis. The ISI was initially set at 150 milleseconds (Nelson *et al.*, 2012;Tommerdahl *et al.*, 2007) and was subsequently altered by a step size of 15% based on the accuracy of the participant's response. TOJ was performed in blocks of 20 trials During the first 10 trials, a 1 up/ 1 down tracking paradigm was used, allowing a single correct answer to cause a 15% reduction of the ISI in the subsequent trial. On the contrary, if an incorrect response was made, the ISI increased by 15% in the following trial. For the last 10 trials, a 2 up/ 1 down tracking algorithm was employed in which two correct responses were required to decrease the ISI by 15%. The combination of these two tracking algorithms enables rapid and reliable determination of each subject's TOJ thresholds (Nelson *et al.*, 2012;Tommerdahl *et al.*, 2007). The inter-trial interval was set at 5 seconds. The threshold for TOJ was defined as the average ISI measured from the

last five trials with each block (trials 16 to 20) as performed elsewhere (Tommerdahl *et al.*, 2007).

TOJ Synchronization (TOJ-S)

TOJ-S was performed on digit 2 and 3 of the right hand. Specifically, a conditioning sinusoidal vibration (25 Hz, 20 μm) was applied to digit 2 and 3 before, concurrently and after the TOJ stimulus pair (Tommerdahl *et al.*, 2007). The task requirements were identical to the TOJ task in that participants were queried to report which stimulus occurred first within the pair. Twenty TOJ-S trials were performed using the identical 1 up/ 1 down and 2 up/ 1 down structure used for the TOJ task. The TOJ-S threshold was taken as the average of the last five trials within a block. A schematic of the TOJ-S task is shown in Figure 3.1.

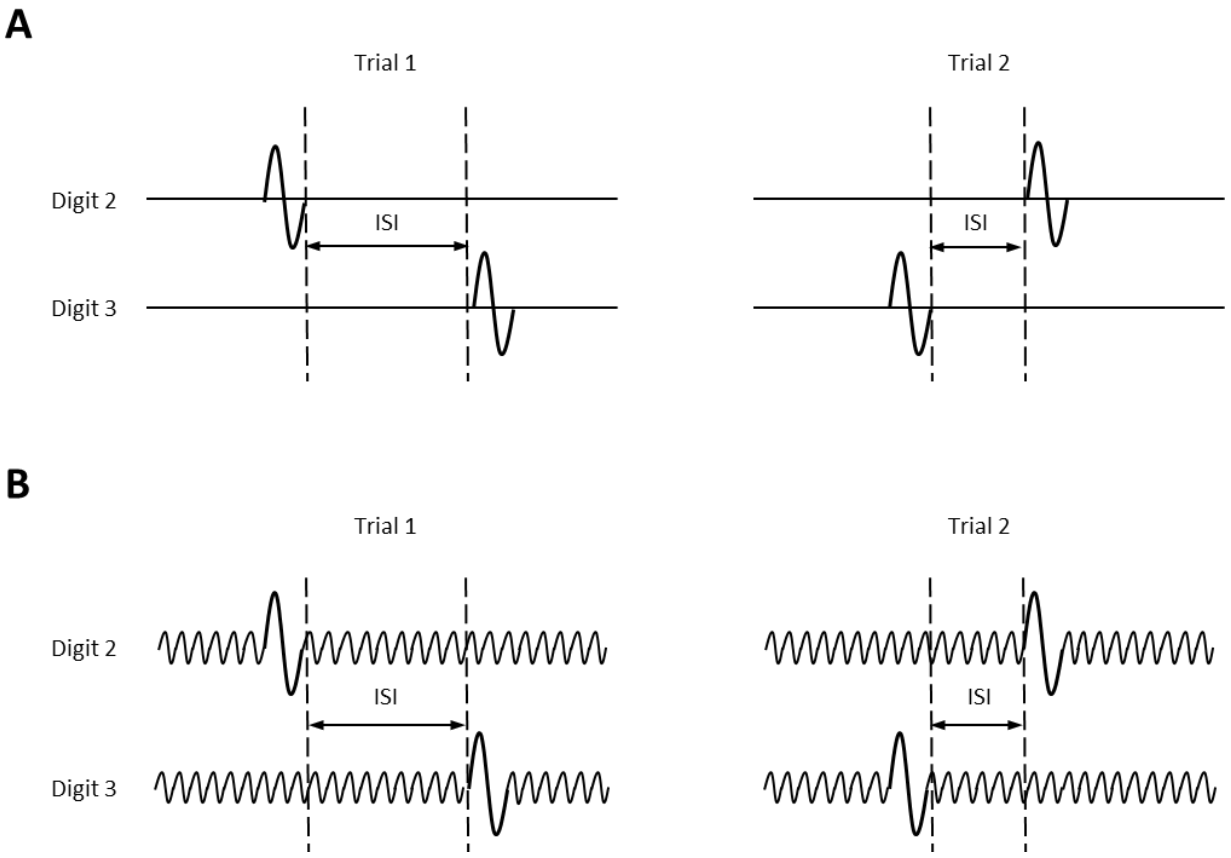


Figure 3.1. (A) Temporal order judgement (TOJ). Two sequential vibrotactile stimuli were delivered in random order to digit two and digit three. Two trials shown with subject response from the first trial resulting in a decrease in the interstimulus interval (ISI). (B) Temporal order judgement with synchronization (TOJ-S). 25 Hz conditioning stimulus delivered concurrently with TOJ task. Two trials shown with subject response from the first trial resulting in a decrease in the interstimulus interval (ISI).

Timeline

TOJ and TOJ-S were measured in different sessions separated by a minimum of one week. 5 participants performed TOJ first while the other 3 participants performed TOJ synchronization first. Within each session, the psychophysical task was performed in 7 blocks (20 trials each) before (T_0) and after cTBS at 3-6 min (T_1), 7-10 min (T_2), 11-14 min (T_3), 15-18 min (T_4), 23-26 min (T_5), and 31-34 min (T_6), in line with our previous report (Rai *et al.*, 2012). The timeline is depicted in Figure 3.2. Prior to performing T_0 participants completed training trials that required five consecutive trials to be performed correctly. During training, visual feedback was displayed on the computer; "Good job" was presented if a correct response was made and "Please try again" was presented if an incorrect response was made. Once performance criteria on the training trials were met, the pre-cTBS block began. No feedback was delivered during the 7 time blocks.

3.3.3.2 Experiment 1B: Sham cTBS and Timeline

Participants performed the TOJ-S task as described above. The protocol was identical to the TOJ-S protocol performed by the real group. The timeline is shown on Figure 3.1. Prior to performing T_0 participants also completed training trials that required five consecutive trials to be performed correctly. Once performance criteria on the training trials were met, T_0 began. No feedback was given during the 7 time blocks. Sham stimulation delivered the real cTBS protocol. The cTBS coil was placed over SI and rotated 90 degrees such that the handle of the coil pointed vertically upward away from the scalp. The coil maintained scalp contact during stimulation.

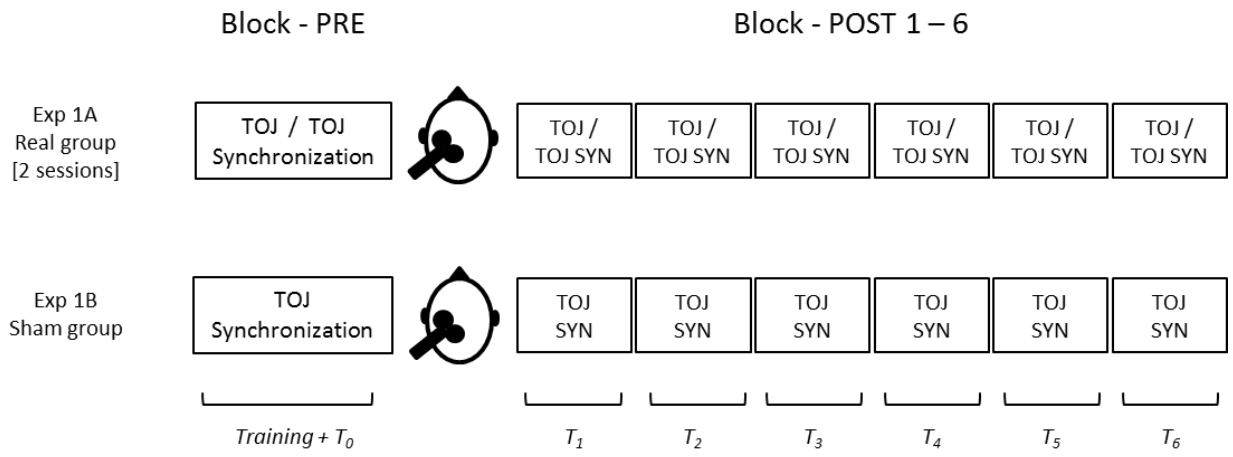


Figure 3.2. Timeline for Experiment 1A and B. Experiment 1A: TOJ and TOJ synchronization performances were obtained before and following real cTBS over left-hemisphere SI. Measurements were taken following cTBS at 3-6 min (T_1), 7-10 min (T_2), 11-14 min (T_3), 15-18 min (T_4), 23-26 min (T_5), and 31-34 min (T_6). Experiment 1B: TOJ synchronization performances were obtained before and following sham cTBS for up to 34 minutes.

3.3.4 Data Analysis

To assess the effects of cTBS on TOJ versus TOJ-S over time, post-cTBS values (T_1, T_2, T_3, T_4, T_5, T_6) were normalized to pre-cTBS values (T_0) for each task, respectively. a two-way repeated measure analyses of variance (ANOVA) with within-subject factors 'TIME' (6 levels: 3-6 min (T_1), 7-10 min (T_2), 11-14 min (T_3), 15-18 min (T_4), 23-26 min (T_5), and 31-34 min (T_6)) and 'TASK' (2 levels: TOJ, TOJ SYN) was performed. Two separate one-way repeated ANOVA with within-subject factor 'TIME' (7 levels: 0 min

(T_0), 3-6 min (T_1), 7-10 min (T_2), 11-14 min (T_3), 15-18 min (T_4), 23-26 min (T_5), and 31-34 min (T_6)) were performed for TOJ and TOJ-S, respectively. A priori hypotheses were tested using contrast estimations and Bonferroni correction for cTBS effects on TOJ (4 comparisons: T_0 vs T_1, T_0 vs T_2, T_0 vs T_3, T_0 vs T_4). No hypothesis was created for TOJ-S. Post-hoc analysis was performed using the Dunnett's test to test for differences following cTBS. To assess the effects of cTBS on TOJ-S (sham group) over time, a one-way repeated measure of ANOVA with within-subject factor 'TIME' (7 levels: T_0, 3-6 min (T_1), 7-10 min (T_2), 11-14 min (T_3), 15-18 min (T_4), 23-26 min (T_5), and 31-34 min (T_6)) was performed. All statistical analysis was performed using SAS 9.2 Windows software (SAS Institute Inc., Cary, North Carolina, US). Significance level was set at $p \leq 0.05$.

3.4 Results

3.4.1 Experiment 1A: cTBS influence on TOJ and TOJ-S

All participants successfully completed the experiment. The group-averaged AMT (with standard deviation) for TOJ was 45.4 ± 7.6% of the maximum stimulator output (MSO) with cTBS delivered at 36.3 ± 6.1% MSO. The mean AMT for TOJ-S was 43.4 ± 8.2% MSO of the stimulator output with cTBS delivered at 34.8 ± 6.6% MSO. A paired t-test (two-tailed) revealed no significant differences between the MSO for TOJ and TOJ-S ($p = 0.18$).

Two-way ANOVA revealed a significant main effect of TASK ($F_{(1, 7)} = 8.12$, $p = 0.0247$), no effect of TIME ($F_{(5, 35)} = 1.16$, $p = 0.35$) or interaction between TASK and TIME ($F_{(5, 35)} = 1.55$, $p = 0.19$). Two separate one-way repeated ANOVAs were

performed for each task (TOJ, TOJ-S) with factor 'TIME' (T_0, T_1, T_2, T_3, T_4, T_5, T_6). For TOJ, ANOVA revealed no significant main effect of TIME ($F_{(6, 42)} = 0.78$, $p = 0.59$). A paired t-test with Bonferroni corrected contrasts (corrected for four comparisons) was performed to compare pre-cTBS values (T_0) to post-cTBS values (T_1, T_2, T_3, T_4) individually for up to 18 minutes following cTBS. Performance was not significantly different between all four blocks versus T_0. One way ANOVA revealed a significant main effect of TIME ($F_{(6, 42)} = 2.27$, $p = 0.05$). Post-hoc analysis using Dunnett's test revealed that TOJ-S values were significantly lower at time blocks T_1 (3-6 min, $p = 0.049$), T_2 (7-10min, $p = 0.022$) and T_4 (11-18 min, $p = 0.05$). The group-averaged data (with standard errors) for TOJ and TOJ-S are shown in Figure 3.3. Group-averaged trial-by-trial TOJ and TOJ-S performance is shown in Figure 3.4. Note that TOJ and TOJ-S performances begins to plateau at ~ trial 10 as shown in previous experiments (Tommerdahl *et al.*, 2007;Rai *et al.*, 2012) and that the effects of cTBS on TOJ-S occur during optimal performance. To investigate whether cTBS significantly altered performance during non-optimal performance (trials 6 through 10) and as threshold values were approached (trials 11 through 15) two one-way ANOVAs with factor TIME were performed. These analyses revealed no significant main effect of TIME for non-optimal performance (trials 6 to 10, $F_{(6, 42)} = 0.57$, $p = 0.75$) and no significant main effect of TIME as threshold values were approached (trials 11 to 15, $F_{(6, 42)} = 0.96$, $p = 0.46$).

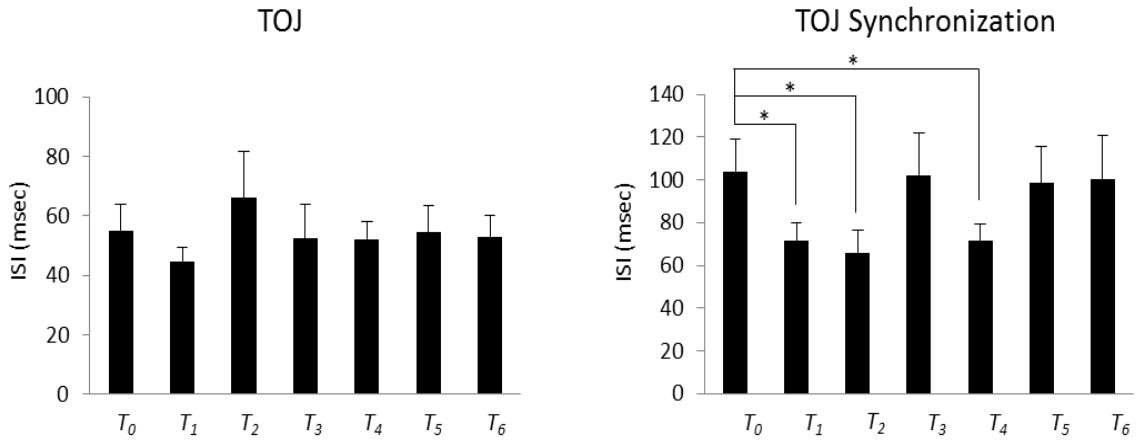


Figure 3.3. Experiment 1A: cTBS influence on TOJ and TOJ synchronization. Left: Group-averaged TOJ (with standard errors) before and at each time block following cTBS. * $p \leq 0.05$. Time blocks measured T_0, 3-6 min (T_1), 7-10 min (T_2), 11-14 min (T_3), 15-18 min (T_4), 23-26 min (T_5), and 31-34 min (T_6). Right: Group-averaged TOJ synchronization (with standard errors) before and at each time block following cTBS. * $p \leq 0.05$.

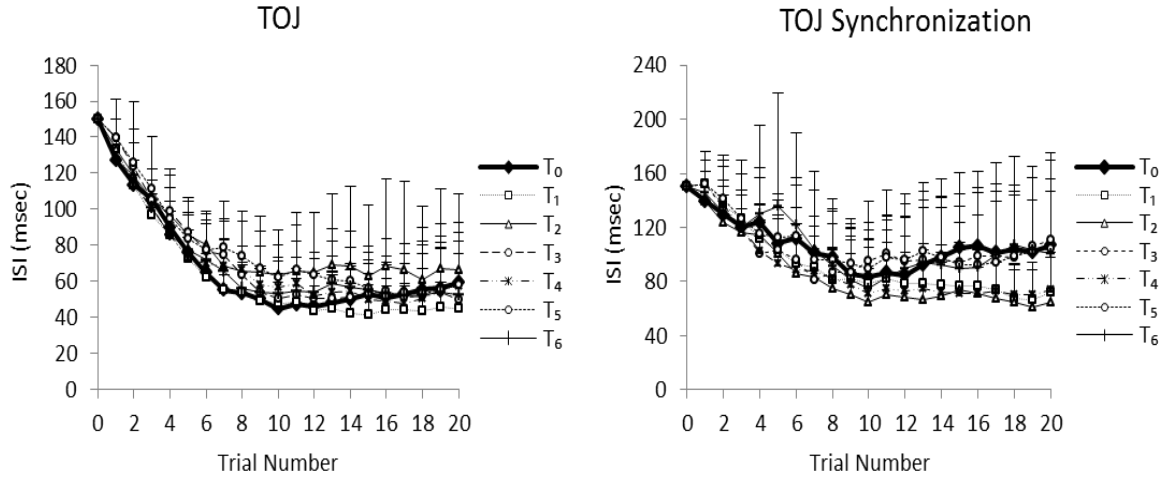


Figure 3.4. Experiment 1A: CTBS influence on TOJ and TOJ synchronization. Left: Group-averaged performance for each trial in each time block for the TOJ condition. Right: Group-averaged performance for each trial in each time block for TOJ synchronization.

3.4.2 Experiment 1B: Sham cTBS on TOJ-S

All participants completed the experiment successfully. The mean AMT for the TOJ-S sham group was 53 ± 7.5% MSO with cTBS delivered at 42 ± 5.8% MSO. The ANOVA revealed no significant effect of TIME ($F_{(6, 42)} = 0.35$, $p = 0.904$). Figure 3.5 displays the group-averaged TOJ-S (with standard errors) before and following sham cTBS. Group-averaged trial-by-trial TOJ-S performance before and following sham cTBS is shown on the right graph of Figure 3.5. Note that sham TOJ-S performance improvement plateaus at ~ trial 10.

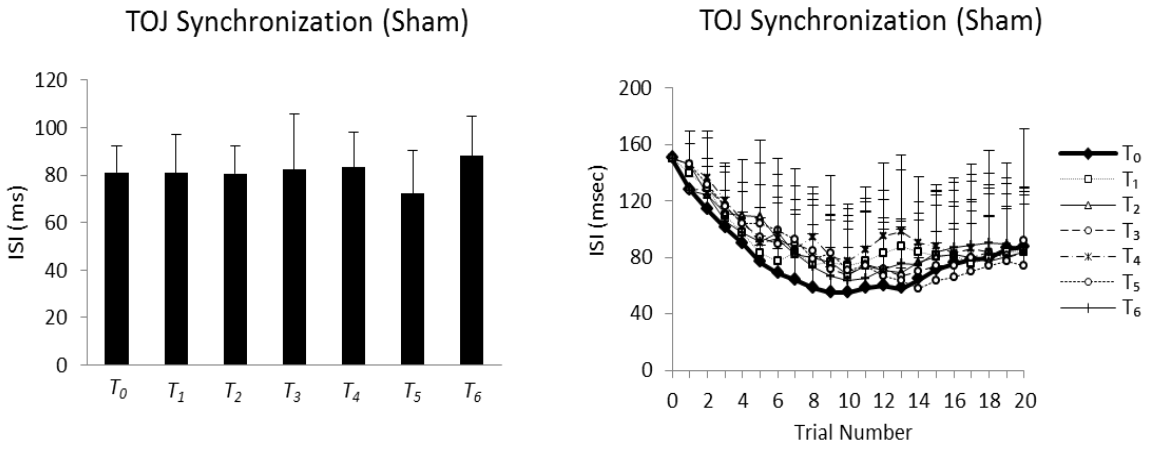


Figure 3.5. Experiment 1B: Sham cTBS on TOJ synchronization. Left: Group-averaged TOJ synchronization (with standard errors) before and at each time block following sham cTBS. * $p \leq 0.05$. Time blocks measured T_0, 3-6 min (T_1), 7-10 min (T_2), 11-14 min (T_3), 15-18 min (T_4), 23-26 min (T_5), and 31-34 min (T_6). Right: Group-averaged performance for each trial in each time block following sham cTBS for the TOJ synchronization condition.

3.5 Discussion

The present study investigated the influence of cTBS over left-hemisphere SI on TOJ performance and the TOJ synchronization effect in the contralateral hand. Novel findings indicate that cTBS reduced the TOJ synchronization effect for up to 18 minutes while sham cTBS had no such effect. We attribute cTBS effects to changes in the excitability of neural activity within SI. We discuss these findings and their neural mechanisms below

In the present study, TOJ performance was unaltered following cTBS which questions the role of SI in TOJ processing. This finding was unexpected as previous studies showed changes in tactile perception after suppression-inducing protocols such as low frequency repetitive TMS (Satow *et al.*, 2003;Knecht *et al.*, 2003) and cTBS (Rai *et al.*, 2012;Conte *et al.*, 2012). However, it should be noted that TDT and TOJ tasks are not identical. Therefore, cTBS may act differently on the populations of neurons that mediate each of these percepts (Conte *et al.*, 2012;Rai *et al.*, 2012). Alternatively, the lack of change in TOJ may relate to cTBS technical parameters such as intensity and the direction of induced current flow, which are known to determine cTBS effects (Jacobs *et al.*, 2012;Doeltgen & Ridding, 2011;Siebner *et al.*, 2009). For instance, cTBS delivered over the primary motor cortex (M1) at 80% AMT yields different results in MEP amplitudes when delivered at 70% RMT (McAllister *et al.*, 2009). Another explanation may be that other cortical areas may be dominant in the TOJ task, including the secondary somatosensory cortex (Pons *et al.*, 1992;Romo *et al.*, 2002), parietal cortex (Seyal *et al.*, 1995;Nager *et al.*, 2004), anterior cingulate, supplementary motor areas

(Lacruz *et al.*, 1991;Pastor *et al.*, 2004) and the cerebellum (Manganelli *et al.*, 2013), which may compensate for changes in SI excitability induced by cTBS. There is also growing evidence for the specialized role of the superior temporal gyrus in tactile temporal perception (Bolognini *et al.*, 2010). Most recently, functional magnetic resonance imaging data indicate that prefrontal and parietal cortices may play an integral part in TOJ (Takahashi *et al.*, 2012). Hence, contributions from different cortical or subcortical areas may suggest the complexity of tactile TOJ.

Following cTBS, we observed a reduction of the TOJ-S effect. TOJ-S thresholds were reduced for up to 18 minutes. Significant reduction of the TOJ-S effect occurred from 3 to 10 minutes and again from 15-18 minutes following cTBS. The maximum effect was observed from 7-10 min following cTBS, which is the timeframe for maximal physiological effects of cTBS seen elsewhere (Ishikawa *et al.*, 2007;Katayama *et al.*, 2010;Di Lazzaro *et al.*, 2005). We observed that the TOJ-S effect is abolished from 7 to 10 minutes following cTBS such that thresholds were not different from TOJ pre-cTBS values (paired t-test, TOJ baseline versus TOJ-S at T_2, $p = 0.21$). The time varying effect of cTBS on TOJ is also similar to the effects on TDT (Rai *et al.*, 2012). Specifically, both studies observed significant impairments immediately following cTBS, followed by no significant change from 11 to 14 minutes and followed again by significant perceptual impairments from 15 to 18 minutes (Rai *et al.*, 2012). Further, both studies indicate that cTBS effects persist for up to 18 minutes and not at later time blocks. Exposing such variability in the time course of cTBS effects may be a result of the frequency sampling intervals used in our study (i.e. every 3 minutes without inter-block breaks).

The mechanisms that underpin TOJ and TOJ-S are not fully understood although GABAergic activity via lateral inhibition across cortical columns and in-field inhibition within cortical columns likely mediates these percepts. For the TOJ task, the somatosensory cortex provides information about the loci of the two tactile stimuli, and in the absence of the synchronized conditioning stimulus, this information is robustly delivered. In the presence of periodic and synchronous conditioning stimuli to D2 and D3, it has been proposed that the evoked response of the cortical representations of D2 and D3 become functionally linked in a manner that a tap to one digit results in a response at both sites and a consequent degradation of spatial resolution between digit representations (Tommerdahl *et al.*, 2007;Tommerdahl *et al.*, 2008). Recent observations from in vivo non-human primate studies support that idea (T.M.Forshey *et al.*, 2012), and although the mechanisms of this synchronization effect are not fully understood, GABAergic mediated activity (e.g., lateral inhibition) is a necessary component. Stimulation of afferent fibers creates excitation in corresponding cortical columns that evoke lateral inhibition between the excited columns. The amount of lateral inhibition depends on the magnitude and duration of the initial excitation within the cortical columns (Chen *et al.*, 2003;Friedman *et al.*, 2008). Lateral inhibition dissipates over time, resulting in decreased lateral inhibition received from neighbouring columns (Gardner & Costanzo, 1980). We speculate that correct TOJ performance occurs when lateral inhibition dissipates to allow the cortical columns receiving the second stimulus in the TOJ pair to be excited. There is some evidence that lateral inhibition is also fundamental for the TOJ-S effect. Patients with autism demonstrate a narrowing of neuropil space between minicolumns, an effect

associated with a reduction in GABAergic interneurons (Casanova *et al.*, 2002) that mediate lateral inhibition. In contrast to control subjects, autistic patients do not demonstrate the TOJ-S effect (Tommerdahl *et al.*, 2008). Further, the absence of the TOJ-S effect in migraineurs and concussed individuals has been postulated to be the result of an imbalance between cortical excitation and GABA mediated inhibition (Nguyen *et al.*, 2013;De *et al.*, 2012). In addition to lateral inhibitory mechanisms that function across the columns, in-field inhibition occurs within cortical columns whereby the period of initial excitation is followed by a period of inhibition that persists from ~ 60 to 100 ms (Gardner & Costanzo, 1980). We speculate that this type of inhibition may be particularly relevant to the TOJ-S task whereby the low-amplitude background vibration creates synchronous excitation in adjacent cortical columns. For TOJ to be performed in the presence of such synchronous vibration, the excitation of the cortical columns evoked by the second stimulus in the TOJ pair must exceed both in-field inhibition created by the low-amplitude vibration and the lateral inhibition.

Although the mechanisms by which cTBS alters neural activity are not fully understood, there is evidence to indicate that inhibitory networks within SI are suppressed. Previous work demonstrates that late sub-components of high frequency oscillations (HFO) evoked potentials from SI, which are associated with GABA inhibitory interneurons in superficial layers within SI (Hashimoto *et al.*, 1999;Hashimoto *et al.*, 1996), are suppressed by cTBS over SI at 15 min (Katayama *et al.*, 2010). In the present study, cTBS is likely to have suppressed lateral and/or in-field inhibitory circuits that

mediate tactile perceptual binding across cortical columns, thereby reducing the synchronization effect for up to 18 minutes following stimulation.

The present research demonstrates that cTBS alters TOJ synchronization performance and we believe that these changes are not attributed to cTBS altering learning processes. CTBS affects motor learning in healthy individuals (Clerget *et al.*, 2012;Iezzi *et al.*, 2010) and in post-stroke patients (Meehan *et al.*, 2011). Further, cTBS has shown to degrade timing accuracy of a sensorimotor synchronization task (Bijsterbosch *et al.*, 2011). However, in rats, cTBS does not alter the learning of a tactile discrimination task (Mix *et al.*, 2010). We implemented approaches to minimize such learning in the present study. First, training trials were presented in advance of the testing trials. Such training trials required subjects to correctly complete 3 blocks of 5 consecutive correct trials prior to data acquisition. Second, thresholds were calculated as the average of the last five trials within each block, that is, from trials 16 through 20. Performance during TOJ plateaus at ~ trials 10 and beyond (Tommerdahl *et al.*, 2007;Tommerdahl *et al.*, 2008). Therefore, we are using data only from trials in which there is no further change in performance.

In summary, we found that continuous theta-burst stimulation over the primary somatosensory cortex reduced the synchronization effect that led to an improvement in TOJ performance. There were no significant changes to TOJ performance when cTBS

was delivered over SI. This study adds direct evidence that cTBS induces temporal changes in the SI that lead to altered tactile perception (Rai *et al.*, 2012;Conte *et al.*, 2012). It has provided a more refined hypothesis regarding the underlying mechanisms of tactile perception that can be tested in future studies.

CHAPTER 4 – Experiment 2

Investigation of tactile temporal order judgment on the hand following 30 Hz continuous theta-burst stimulation over the primary somatosensory cortex

4.1 Introduction

The human brain is equipped to process tactile inputs from the hand at temporal resolutions (Johansson & Vallbo, 1979) essential for precise hand control. Impairments in tactile temporal acuity are found in neurological disorders that exhibit compromised hand function (Artieda *et al.*, 1992;Tinazzi *et al.*, 1999;Sanger *et al.*, 2001;Abbruzzese & Berardelli, 2003;Scontrini *et al.*, 2009). Temporal order judgment (TOJ) and temporal discrimination (TD) are two psychophysical tools used to assess tactile temporal acuity. TOJ is the ability to distinguish the order of sequential stimuli delivered over distinct skin sites (Shore *et al.*, 2005;Tommerdahl *et al.*, 2007;Nelson *et al.*, 2012) while TD represents the ability to report the presence of one or two stimuli delivered over the same or distinct skin surfaces (Lacruz *et al.*, 1991;Hoshiyama *et al.*, 2004). Although the cortical regions involved in tactile temporal perception remain unclear, numerous lines of evidence from non-human primates, humans and mice suggest that the primary somatosensory cortex (SI) participates in both TD and TOJ (Lacruz *et al.*, 1991;Recanzone *et al.*, 1992;Pastor *et al.*, 2004;Wada *et al.*, 2010).

The use of non-invasive repetitive trancranial magnetic stimulation (rTMS) presents an ideal opportunity to investigate the role of SI in tactile perception. Low-frequency 0.9 and 1-Hz rTMS increases 2-pt spatial and vibrotactile frequency discrimination thresholds, respectively (Satow *et al.*, 2003;Knecht *et al.*, 2003). RTMS

delivered continuously at 50 Hz (Huang *et al.*, 2005), known as continuous theta-burst stimulation (cTBS), has not only shown to impair TD thresholds (Rai *et al.*, 2012;Conte *et al.*, 2012) but also suppress SI cortical physiology as indicated by reduced dipole peak-to-peak N20-P25 amplitudes and SI-hemoglobin levels (Ishikawa *et al.*, 2007;Mochizuki *et al.*, 2007).

TOJ thresholds in healthy individuals are typically between 30 and 50 ms indicating that the order of the two stimuli is indistinguishable at lower values. An interesting phenomenon called TOJ synchronization (TOJ-S) occurs when TOJ is performed in the presence of a synchronous low-amplitude vibration such that TOJ thresholds are impaired and thresholds may be increased up to 100 ms in healthy subjects (Tommerdahl *et al.*, 2008). The difficulty in detecting the order during TOJ-S has been attributed to perceptual binding caused by the low-amplitude vibration that occurs between two adjacent cortical columns that receive inputs from the two stimulated digits. Further, the perceptual binding is thought to be mediated by GABA inhibitory interneurons (Tommerdahl *et al.*, 2008). In a previous study, we demonstrated that TOJ-S effects were largely reduced (i.e. performance improved) following cTBS over SI. In contrast, TOJ performance was not significantly altered. We previously speculated that cTBS suppresses GABA-mediated inhibitory interneurons between the two digits such that subjects were no longer impaired by the binding of the two digits during the presence of additional vibration.

The lack of cTBS effects on TOJ performance observed in Experiment 1 may relate to the specific cTBS stimulation parameters. Specifically, stimulation frequency

may dictate cTBS effects. Unlike 50 Hz cTBS, which was employed previously in Experiment 1 and identical to that used in other reports (Huang *et al.*, 2005;Rai *et al.*, 2012), a modified version consisting of a burst of three stimuli repeated at 30 Hz over primary motor cortex (M1) has been suggested to induce stronger and more reliable inhibitory effects. When comparing 50 Hz and 30 Hz cTBS on corticospinal excitability, a previous study suggested that 30 Hz cTBS over M1 produced a greater magnitude of MEP suppression and resulted in lower inter-subject variability (Goldsworthy *et al.*, 2012). It is previously known that 50 Hz cTBS over M1 produces long-term depression (LTD)-like effects that causes modification of synaptic connectivity of neurons within M1, yet it is unknown whether 30 Hz cTBS contributes to the same or different stimulation pattern that leads to more robust neuroplastic changes in corticospinal excitability. When delivered over SI, 50 Hz cTBS has been associated with reduced excitability of GABA inhibitory interneurons, as indicated by removal of the TOJ-S effect (Experiment 1). Hence, it is reasonable to suggest that the stronger net inhibition induced by 30 Hz cTBS may be due to greater LTD-like effects on either excitatory or inhibitory neurons.

Experiment 1 revealed changes of TOJ-S performance on the hand contralateral to the hemisphere receiving cTBS. Studies have revealed that unilateral tactile stimuli activate bilateral receptive fields within SI. Such activation in both hemispheres is potentially due to transcollsal connections projecting from one hemisphere (contralateral to stimuli) to the opposite hemisphere (ipsilateral to stimuli). It remains unclear whether cTBS effects may extend to the ipsilateral hand via transcallosal connections. One study

suggests this possibility by demonstrating an increase in inter-hemispheric inhibition following cTBS over left-SI (Zapallow, 2012).

To clarify whether SI is involved in processing TOJ we delivered 30 Hz cTBS over left-hemisphere SI. TOJ and TOJ-S performance was assessed bilaterally prior to and up to 42 minutes following stimulation. We hypothesized impairment in TOJ performance on the hand contralateral to 30 Hz cTBS. Further, in line with our previous study we hypothesized an improvement in TOJ-S performance on the contralateral hand (Experiment 1).

4.2 Methods

4.2.1 Participants

20 healthy young adults participated in one of two experiments (mean age = 20.9 ± 2.1, 13 females). For Experiment 2A, 10 subjects (mean age = 20.2 ± 2.2 years, range 18 – 25 years, 8 females) participated in two sessions separated by a minimum of 1 week. For experiment 2B, 10 different subjects (mean age = 21.7 ± 1.8, 5 females) participated in a single session. All participants were right handed determined using a subsection of the Edinburgh Handedness Inventory (Oldfield, 1971). Subjects wore earplugs covered by earmuffs to minimize auditory cues during the psychophysical sessions. All participants provided written consent and the study was approved by the Office of Research Ethics at McMaster University and conformed to the Declaration of Helsinki.

4.2.2. Experimental Approach

Electromyography Recordings

Electromyographic (EMG) recordings of the right dorsal interosseous muscle (rFDI) in Experiment 2 followed that for Experiment 1 (Chapter 3, methods).

TMS and Neuronavigation

TMS was delivered through a Magstim Super Rapid^2 stimulator (Magstim Company, Whitland, Wales, UK) connected to a figure-of-eight coil with an air-cooled double 70 mm diameter with the current flowing in a direction away from the handle of the coil. The motor hotspot was defined as the location in the left hemisphere that elicited a MEP in the relaxed right FDI muscle with the TMS coil oriented at 45 degrees to the mid-sagittal line. Active motor threshold (AMT) measurements were performed at this location and defined as the lowest intensity required to evoke MEPs $\geq$ 200 μV in 5 out of 10 consecutive trials during 20% maximum voluntary contraction (MVC) (Goldsworthy, 2012). AMT was calculated using biphasic pulses inducing posterior to anterior current followed by anterior to posterior current. MVC was determined by having participants abduct their right index finger against an immovable post with maximal force. Participants maintained 20% MVC using their EMG feedback from the FDI muscle that was displayed on an oscilloscope. Brainsight Neuronavigation software (Rogue Research, Montreal) was used to mark the location of the M1 motor hotspot. SI was defined as a point 2 cm posterior to the motor hotspot and digitally marked using Brainsight. The paradigm for 30 Hz cTBS consisted of a total of 600 pulses applied in burst of three TMS

pulses delivered repetitively at 33.3 ms (Figure 4.1) (Nyffeler, 2006; Goldsworthy, 2012). CTBS was applied over SI at 80% AMT with the handle oriented backwards and laterally at a 45 degree angle to the mid-sagittal line to induce current in the anterior to posterior direction during the initial phase of the pulse. The orientation and position of the coil were marked using the Brainsight software to ensure theta burst stimulation was delivered with minimal variability over the cortex. . In all experiments, cTBS was applied over left-hemisphere SI.

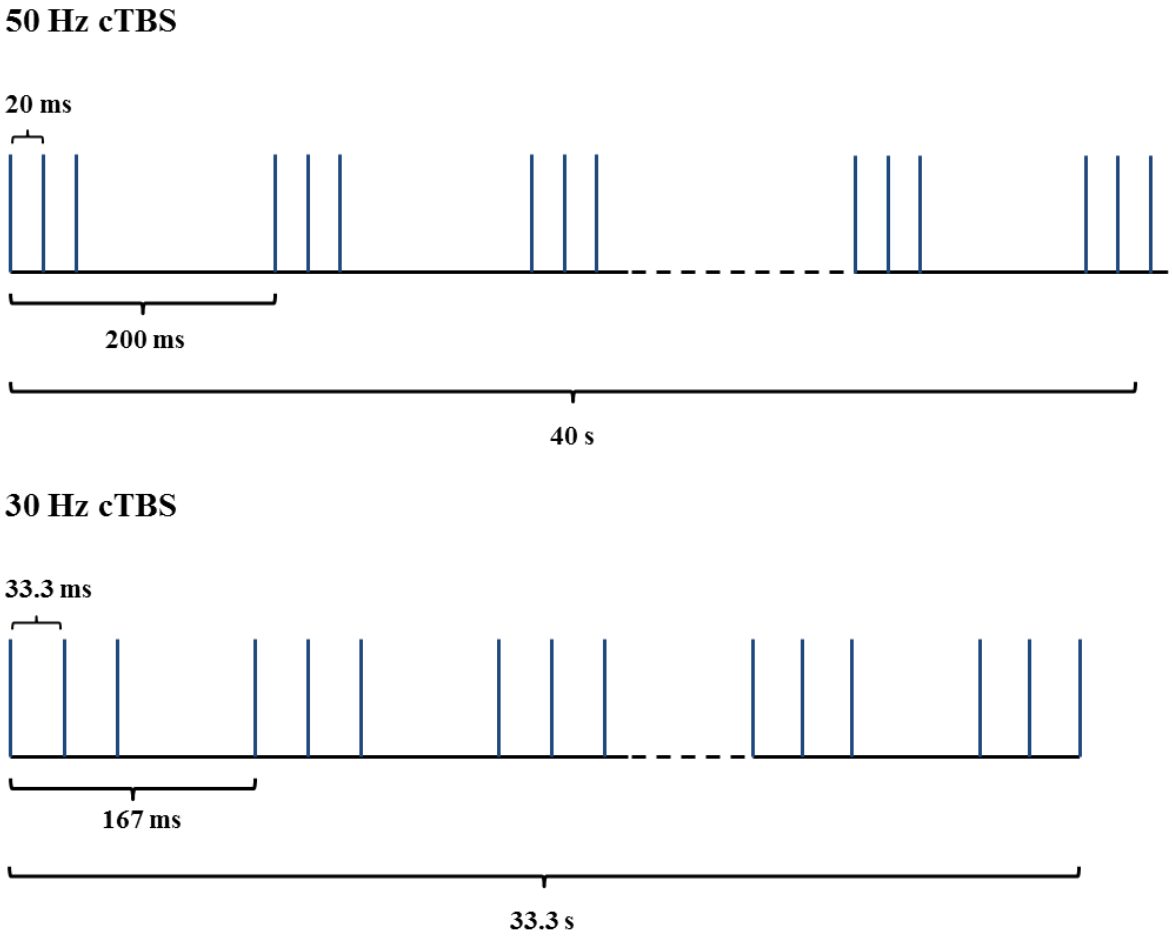


Figure 4.1 A comparison of two cTBS paradigms. Above: cTBS delivered at 50 Hz, with repeated bursts at 200 ms. Below: cTBS delivered at 30 Hz, with repeated bursts at 167 ms. Figure adapted from (Goldsworthy *et al.*, 2012).

4.2.3 Psychophysical Tasks

4.2.3.1 Experiment 2A: Temporal Order Judgment (TOJ)

TOJ was performed on digit 2 and 3 of one hand. A single TOJ trial delivered a vibro-tactile stimulus (25 Hz, 40 ms and 200 μm) to the volar surface of the 2nd and 3rd

digit tip separated by an interstimulus interval (ISI) using the Cortical Metrics Device (Holden, 2011). The participant was queried to identify which stimulus occurred first (i.e. the 2nd or 3rd digit) and respond as quickly as possible by making a key press with the opposite hand; left key = 2nd digit, right key = 3rd digit. The ISI was initially set at 150 ms (Nelson *et al.*, 2012;Tommerdahl *et al.*, 2007) and was subsequently altered by a step size of 15% based on the accuracy of the participant's response. TOJ was performed in blocks of 20 trials. During the first 10 trials, a 1 up/ 1 down tracking paradigm was used, allowing a single correct answer to cause a 15% reduction of the ISI in the subsequent trial. If an incorrect response was made, the ISI increased by 15% in the following trial. For the last 10 trials, a 2 up/ 1 down tracking algorithm was employed in which two correct responses were required to decrease the ISI by 15%. The combination of these two tracking algorithms enables rapid and reliable determination of each subject's TOJ thresholds (Nelson *et al.*, 2012;Tommerdahl *et al.*, 2007). The digit selected to receive the first stimulus was randomized on a trial-by-trial basis. The inter-trial interval was set at 5 seconds. The threshold for TOJ was defined as the average ISI measured from the last five trials with each block (trials 16 to 20) as performed elsewhere (Tommerdahl *et al.*, 2007;Tommerdahl *et al.*, 2008;Nelson *et al.*, 2012). TOJ measures were obtained from both right and left hand. The order of obtaining right versus left hand TOJ measurements was counter balanced across participants. A schematic of the TOJ synchronization task is shown in Figure 4.2A.

TOJ Synchronization (TOJ-S)

The TOJ-S task was identical to TOJ task. However, a conditioning sinusoidal vibration (25 Hz, 20 µm) was applied to digit 2 and 3 before, and after the TOJ stimulus pair (Tommerdahl *et al.*, 2007). Participants were queried to report which stimulus occurred first within the TOJ pair. Twenty TOJ synchronization trials were performed using the identical 1 up/ 1 down and 2 up/ 1 down structure used for the TOJ task. The TOJ-S threshold was taken as the average of the last five trials within a block (trials 16-20). TOJ synchronization performances were obtained from both right and left hand. The order of obtaining right versus left hand measurements was counter balanced across participants. A schematic of the TOJ synchronization task is shown in Figure 4.2B.

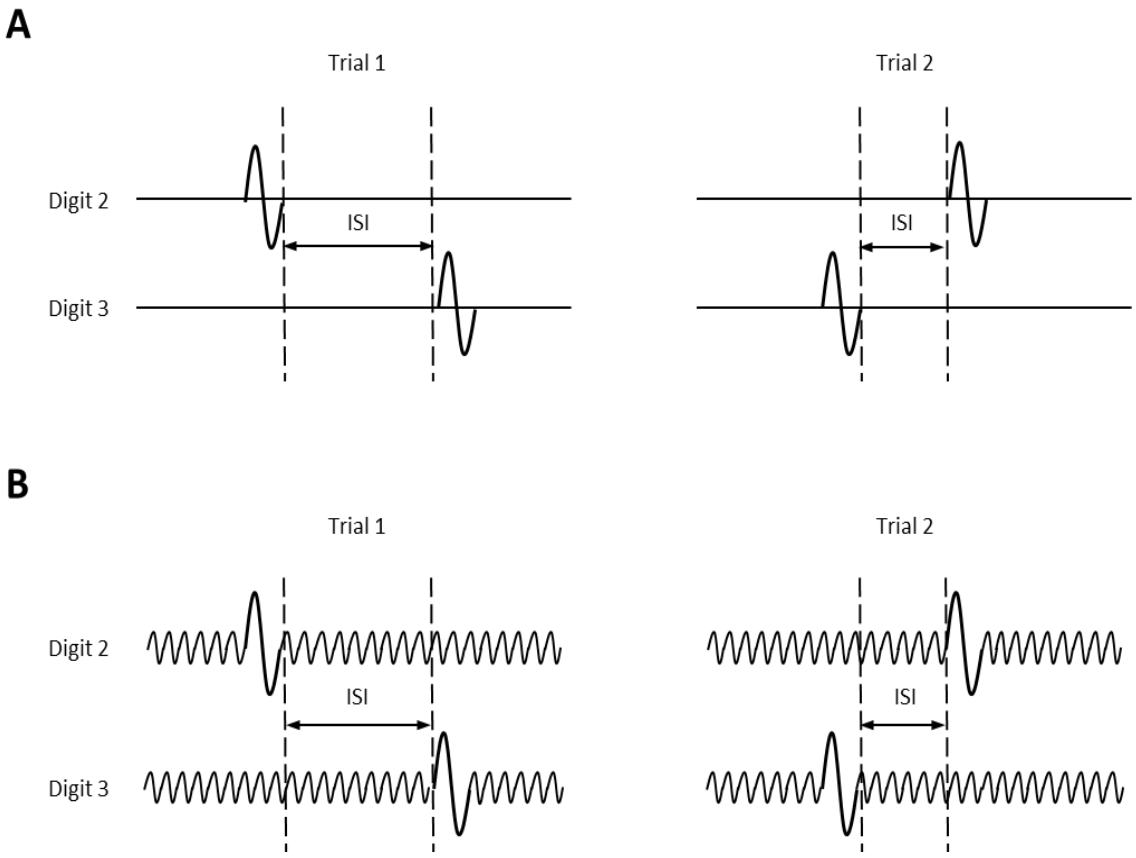


Figure 4.2. (A) Temporal order judgement (TOJ). Two sequential vibrotactile stimuli delivered in random order to digit two and digit three. Two trials shown with subject response from the first trial resulting in a decrease in the interstimulus interval (ISI). (B) Temporal order judgement with synchronization (TOJ-S). 25 Hz conditioning stimulus delivered concurrently with TOJ task. Two trials shown with subject response from the first trial resulting in a decrease in the interstimulus interval (ISI).

Experiment 2A Timeline

In two separate sessions, TOJ and TOJ-S performance were assessed following 30 Hz cTBS. In one of two sessions, TOJ performances were recorded from both hands before and after application of cTBS at 3-10 minutes (T_1), 11-18 minutes (T_2), 19-26

minutes (T_3), 27-34 minutes (T_4), and 35-42 minutes (T_5) minutes. TOJ-S performances on both hands were also measured following the same timeline as in the TOJ session. The order of task administration was counterbalanced across subjects such that 5 subjects started with TOJ while the other half started with TOJ-S. Figure 4.3 displays the timeline for Experiment 2A.

4.2.3.2 Experiment 2B: Sham cTBS on TOJ and timeline

Participants performed the TOJ task as described above. The protocol was identical to the TOJ protocol performed by the real group. TOJ was assessed bilaterally following sham cTBS. The timeline used to assess TOJ follows that used for the real cTBS session. Prior to performing TOJ baseline (T_0) participants completed training trials that required five consecutive trials to be performed correctly. T_0 was measured once performance criteria on the training trials were met. No feedback was given during the 6 testing blocks. For sham cTBS, a figure-of-eight sham coil with a double 70 mm diameter sharing an identical appearance with the real cTBS coil was placed over left-SI to deliver sham stimulation. A continuous 'clicking' sound equivalent to that produced during real cTBS was generated from the coil during sham stimulation. It is important to note that participants received no real cTBS during this session. TOJ performances were recorded from both hands before and after application of sham cTBS at 3-10 minutes (T_1), 11-18 minutes (T_2), 19-26 minutes (T_3), 27-34 minutes (T_4), and 35-42 minutes (T_5) minutes. The order of obtaining right versus left hand TOJ measurements was counter balanced across participants. Figure 4.3 displays the timeline for Experiment 2B.

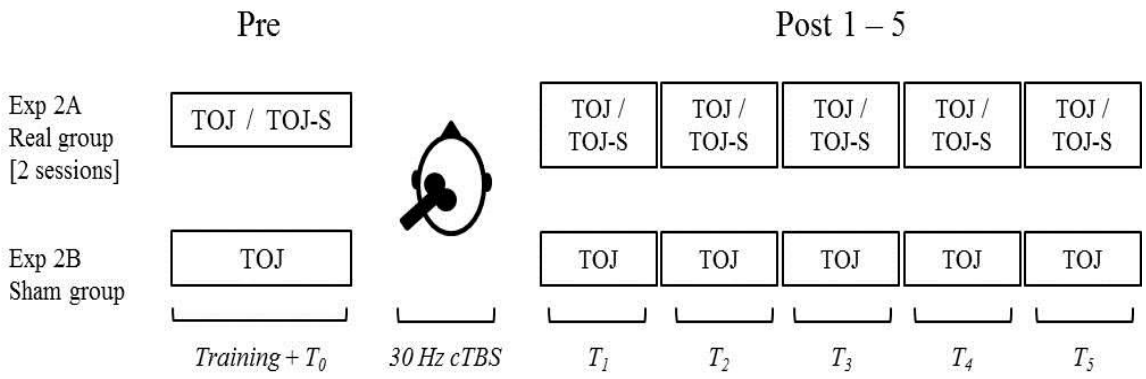


Figure 4.3 Timeline for Experiment 2A and 2B. Experiment 2A: TOJ performances were obtained bilaterally before and following real 30 Hz cTBS over left-hemisphere SI. Measurements were taken at 3-10 min (T_1), 11-18 min (T_2), 19-26 min (T_3), 27-34 min (T_4) and 35-42 min (T_5) following application of cTBS. TOJ-S performances were obtained bilaterally following the same timeline as in the TOJ session. Experiment 2B: TOJ performances were obtained bilaterally before and following sham cTBS following the same timeline as in Experiment 2A.

4.3. Data Analyses

The goal of Experiment 2 is to report whether SI is involved in processing tactile temporal perception by examining the influence of cTBS on TOJ and TOJ-S performance. An impairment of TOJ performance is hypothesized for both hands for up to 18 minutes (Rai *et al.*, 2012) following stimulation. Further, TOJ-S performance is hypothesized to improve for up to 18 minutes (Experiment 1) on both hands. Four separate one-way repeated ANOVAs with within-subject factor 'TIME' (6 levels: T_0, 3-10 min (T_1), 11-18 min (T_2), 19-26 min (T_3), 27-34 min (T_4), 35-42 min (T_5)) were performed for TOJ (right hand), TOJ (left hand), TOJ-S (right hand) and TOJ-S (left hand), respectively. To test

whether the data violated the assumptions of ANOVA, sphercitiy was tested using the Huynh-Feldt (H-F) estimate for each analysis. A priori hypotheses were tested using contrast estimations and Bonferroni corrected for cTBS effects on TOJ on the right hand (2 comparisons: T_0 vs T_1 and T_0 vs T_2). Post-hoc analysis was performed using the Dunnett's test to test for differences in performance following cTBS application. To assess the effects of cTBS on TOJ (sham group) over time, a one-way repeated measure of ANOVA with within-subject factor 'TIME' (6 levels: T_0, 3-10 min (T_1), 11-18 min (T_2), 19-26 min (T_3), 27-34 min (T_4), 35-42 min (T_5)) was performed. All statistical analyses were performed using SAS 9.2 Windows software (SAS Institute Inc., Cary, North Carolina, US). Significance level was set at $p \leq 0.05$.

4.4. Results

4.4.1 Experiment 2A: The influence of 30 Hz cTBS on TOJ and TOJ-S

All participants successfully completed the experiment. The group-averaged AMT (with standard deviation) for TOJ was 43 ± 8 % of the maximum stimulator output (MSO) with cTBS delivered at 34.6 ± 6.5% MSO. The mean AMT for TOJ-S was 43 ± 8.2% MSO of the stimulator output with cTBS delivered at 34.2 ± 6.6% MSO. A paired t-test (two-tailed) revealed no significant differences between the MSO for TOJ and TOJ-S ($p = 0.67$).

One-way ANOVA revealed no significant effects of TIME for TOJ on the left hand ($F_{(5, 59)} = 1.56$, $p = 0.19$). There was a significant effect of TIME ($F_{(5, 59)} = 3.21$, $p = 0.01$) for TOJ on the right hand. A priori contrasts (Bonferroni corrected) revealed significant impairments of TOJ performance (right hand) at 3-10 minutes ($p = 0.013$) and at 11-18 minutes ($p = 0.005$) following stimulation. Dunnett's post hoc analyses further revealed impairments of TOJ at 19-26 minutes ($p = 0.007$), 27-34 minutes ($p = 0.0003$) and 35-42 minutes ($p = 0.012$) compared to pre-cTBS values ($p \leq 0.05$). Figure 4.4 displays the group averaged TOJ data (with standard error) at each time block tested and the group-averaged trial-by-trial TOJ performances on both hands. There were no significant effects of TIME for TOJ-S on the right ($F_{(5, 59)} = 0.89$, $p = 0.49$) or the left hand ($F_{(5, 59)} = 1.45$, $p = 0.22$). Figure 4.5 displays the group averaged TOJ-S data (with standard error) at each time block tested and the group-averaged trial-by-trial TOJ-S performances on both hands.

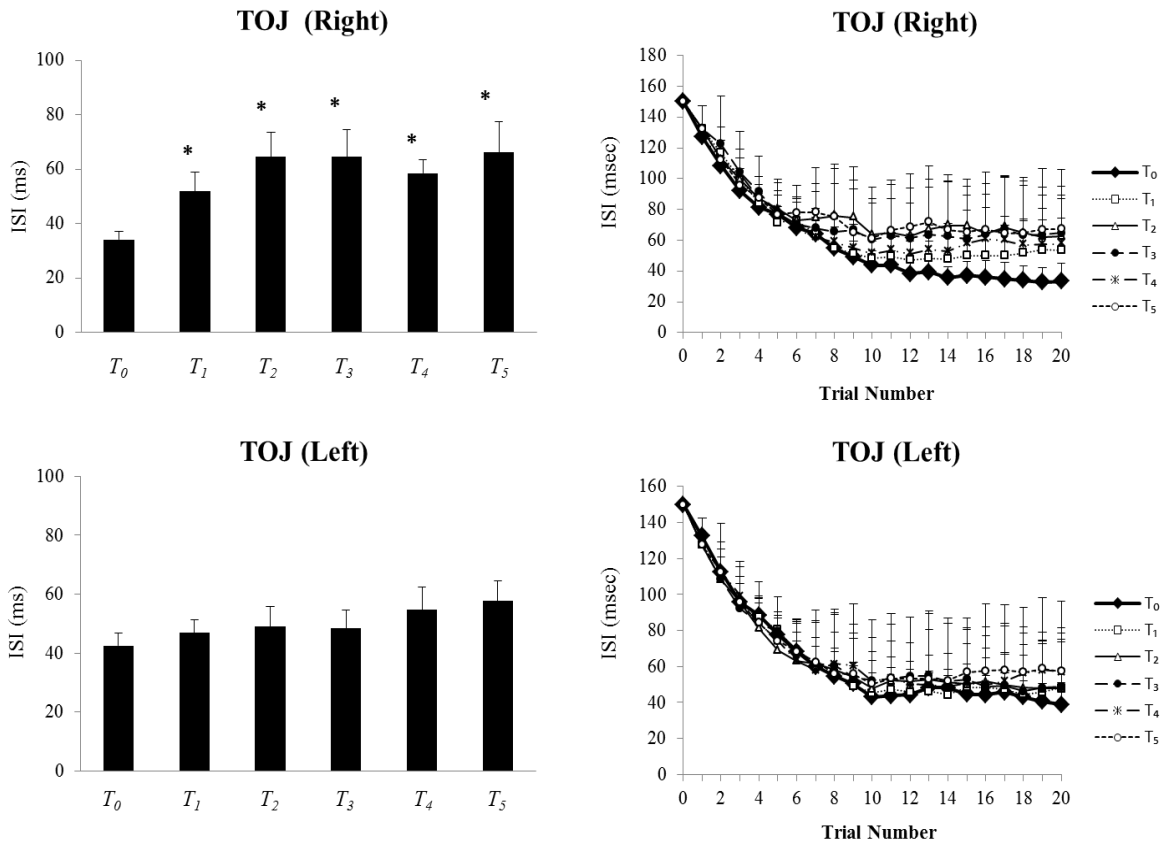


Figure 4.4. Experiment 2A: cTBS influence on TOJ. Group-averaged TOJ (with standard errors) on right hand (top left) and left hand (bottom left) before and at each time block following cTBS. Significance value was set at $p \leq 0.05$. Time blocks measured at baseline (T_0), 3-10 min (T_1), 11-18 min (T_2), 19-26 min (T_3), 27-34 min (T_4) and 35-42 min (T_5). TOJ performance on the right hand were significantly impaired (*) at time blocks T_1, T_2, T_3, T_4 and T_5 compared to baseline values. Group-averaged performance for each trial in each time block for the TOJ on right hand (top right) and left hand (bottom right). Note that impaired TOJ performance (right) by cTBS occurs at subjects' best performance (threshold level).

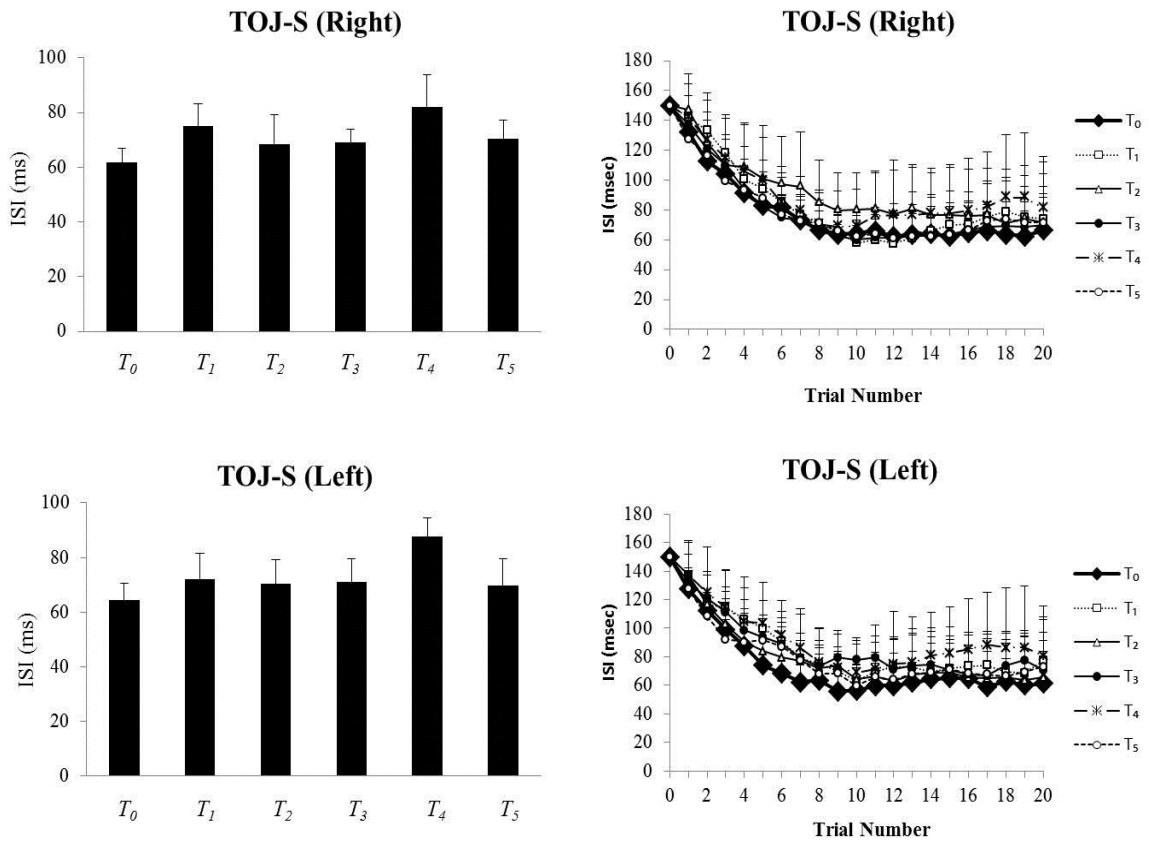


Figure 4.5. Experiment 2A: cTBS influence on TOJ synchronization (TOJ-S). Group-averaged TOJ-S (with standard errors) on right hand (top left) and left hand (bottom left) before and at each time block following cTBS. Time blocks measured at baseline T_0, 3-10 min (T_1), 11-18 min (T_2), 19-26 min (T_3), 27-34 min (T_4) and 35-42 min (T_5). Group-averaged performance for each trial in each time block for the TOJ on right hand (top right) and left hand (bottom right).

4.4.2 Experiment 2B: The influence of sham cTBS on TOJ

All participants completed the experiment successfully. The mean AMT for the TOJ sham group was 47.8 ± 5.2% MSO with cTBS delivered at 38.3 ± 4.1% MSO. One-way ANOVA revealed no significant effects of TIME for TOJ-S on the right hand ($F_{(5, 59)}$

= 0.89, p = 0.49). Figure 4.6 displays the group averaged TOJ data (with standard error) for the right hand at each time block tested and the group-averaged trial-by-trial TOJ performances following sham 30 Hz cTBS.

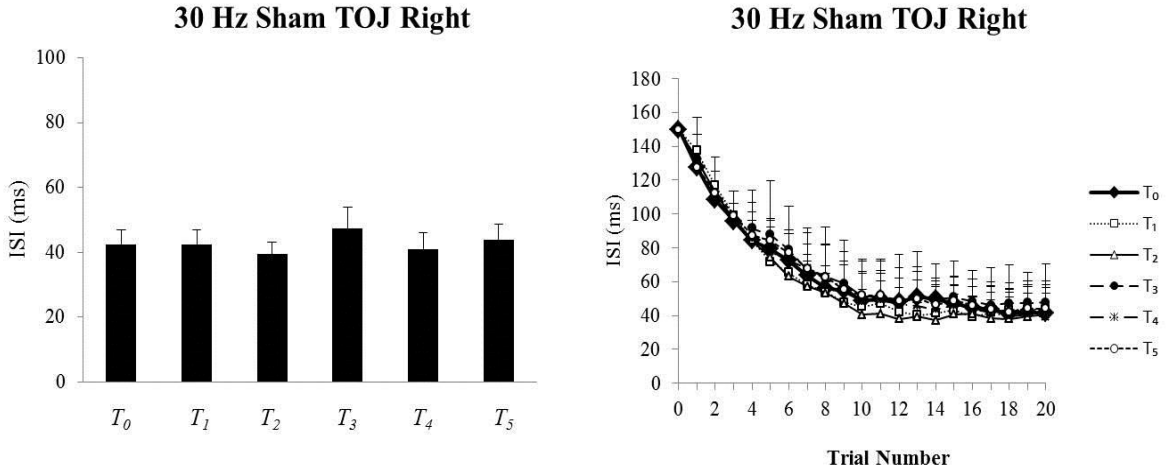


Figure 4.6. Experiment 2B: Sham cTBS on TOJ of the right hand. Left: Group-averaged TOJ (with standard errors) before and at each time block following cTBS. Time blocks measured at baseline T_0, 3-10 min (T_1), 11-18 min (T_2), 19-26 min (T_3), 27-34 min (T_4) and 35-42 min (T_5). Right: Group-averaged performance for each trial in each time block for the TOJ on the right hand.

4.5 Discussion

The present study demonstrated that 30 Hz cTBS over left-hemisphere SI impaired TOJ performance in the contralateral but not the ipsilateral hand for up to 42 minutes following stimulation. TOJ-S performance remained unchanged bilaterally following cTBS. The mechanisms of 30 Hz cTBS on TOJ and TOJ-S are discussed below.

In contrast to a previous report that demonstrated no change in TOJ performance following 50 Hz cTBS, results in this study suggests that TOJ performance was significantly impaired following 30 Hz cTBS. To explain the different results seen on TOJ performance, the mechanisms of cTBS effects using different stimulation frequencies should be highlighted. In a previous study, switching to a different stimulation protocol revealed suppression of cortical excitability in the motor cortex (Goldsworthy *et al.*, 2012). Interestingly, when compared to 50 Hz cTBS, the inter-subject response variability to this modified 30 Hz cTBS paradigm was much lower. One explanation for the robust changes in M1 cortical excitability has been associated with specific frequency bands within the theta range. 30 Hz cTBS consists of intra-burst frequencies of 6 Hz, which is at a higher end of the theta-band compared to 5 Hz from the 50 Hz protocol. The authors who developed 30 Hz cTBS attributed the stronger and long-lasting MEP suppression following motor cortex stimulation to be due to the intra-burst frequency being more close to the end of the theta band which has been associated with voluntary motor behavior (Vanderwolf, 1969). Based on these results, it remains unclear whether changes of cTBS intra-burst frequency on M1 would contribute to changes in tactile perception if cTBS is delivered over SI.

The stronger and less variable corticospinal responses to 30 Hz cTBS may be attributed to greater long term depression (LTD-like) effects on motor circuitry induced by cTBS (Chen *et al.*, 1997;Cardenas-Morales *et al.*, 2010;Goldsworthy *et al.*, 2012). Hence, it is possible that changes in cortical excitability in other cortical areas following cTBS may also involve similar LTD-like effects on neural circuitries. SI is comprised of highly populated GABA inhibitory circuits and cTBS has been shown to change alter the activity of these inhibitory pathways. For instance, 50 Hz cTBS suppresses high-frequency oscillations that are thought to be generated by GABAergic interneurons (Katayama *et al.*, 2010). It is reasonable to suggest that 30 Hz cTBS exhibits stronger LTD-like effects on GABAergic inhibitory interneurons within SI that are associated with mediating lateral inhibition in TOJ and TOJ-S (Tommerdahl *et al.*, 2007;Tommerdahl *et al.*, 2008). Although the mechanisms of TOJ remain unclear, two main processes are required for TOJ to occur. First, lateral inhibition must be projected to neighboring columns by the initial excitation to the columns representing the first digit. Second, lateral inhibition must dissipate over time to allow for the columns representing the third digit to excite. Therefore, TOJ relies on sequential and successful excitation of columns for both digits. The suppression of lateral inhibition by cTBS would prevent the initial spatial contrast for detecting the first tactile stimulus of the TOJ pair. The fact the subjects were still able to identify the order but at longer inter-stimulus intervals suggests that there is dis-inhibition of lateral inhibition over time such that the initial suppression of lateral inhibition returns over time to allow for detection of the first tactile stimulus.

The impairment of TOJ on the right hand which lasts for up to 42 minutes following cTBS represents a novel finding as no previous study has recorded changes in tactile discrimination performances at such time course following 30 Hz cTBS over SI. Previous experiments showed that the temporal effects of cTBS typically last for up to 15 to 34 minutes (Ishikawa *et al.*, 2007;Rai *et al.*, 2012;Conte *et al.*, 2012). Our data suggests that TOJ was impaired at 3-10 min and 11-18 min. This time course of tactile changes aligns with previous experiments (Rai *et al.*, 2012;Conte *et al.*, 2012). TOJ was further impaired at 19-26 min, 27-34 min and for up to 35-42 min which was not reported elsewhere. It is interesting to highlight a further decline in TOJ performance at block 5 (42 min). This prolonged impairment of TOJ performance suggests that 30 Hz cTBS may not only act upon inhibitory neurons that mediate lateral inhibition, but also produce tactile perceptual changes that outlast the stimulation itself for up to 42 min. It should be noted that impaired TOJ performance only occurs on the contralateral hand following stimulation. Sham 30 Hz cTBS revealed no change in TOJ performance on either hand suggesting that the impairment occurred only at the hand contralateral to receiving cTBS. To examine the ipsilateral effects of 30 Hz cTBS on SI, TOJ and TOJ-S performance were also assessed on the ipsilateral hand. However, there were no significant changes to both tasks on the left hand. We conclude that cTBS does not affect TOJ and TOJ-S performance on the hand ipsilateral to receiving stimulation.

This study demonstrated no significant changes in TOJ-S on bilateral hands. This was unexpected since we hypothesized 30 Hz cTBS would suppress GABA inhibitory neurons, in line with speculations from a previous experiment (Experiment 1), thereby

removing the synchronization effect that leads to improvement of TOJ-S task on the contralateral hand. It is worth noting that the baseline measurements obtained from both studies were significantly different (unpaired t.test, $p = 0.01$), the subjects in the present study revealed thresholds of 61.8 ms. This value is below the typical thresholds of 100 ms previously shown in other studies (Tommerdahl *et al.*, 2008). The subjects from the present study also demonstrated better TOJ performance than the previous experiment suggesting that the sample population pool had overall better tactile temporal perception. We suggest that the level of TOJ-S depends on the amount of lateral inhibition created by the first cortical column. It is possible that better TOJ-S performances from the present group are due to less in-field inhibition created within the cortical columns at baseline and therefore participants may not be as affected by the synchronization effect as compared to previous studies. In the present study, 30 Hz cTBS did not alter TOJ-S while 50 Hz improved TOJ-S. One possibility is that 30 Hz and 50 Hz cTBS act on separate inhibitory circuits. Nevertheless, future studies should look to investigate the effects of 30 Hz cTBS over SI on TOJ-S by using subjects that demonstrate similar impairments as shown in previous studies.

In summary, we found that continuous theta-burst stimulation delivered at 30 Hz over the primary somatosensory cortex impaired TOJ performance on the hand contralateral to stimulation. There were no significant changes to TOJ-S performance bilaterally when 30 Hz cTBS was delivered over SI. This study adds direct evidence that cTBS induces temporal changes in the SI that lead to altered tactile perception (Rai *et al.*, 2012;Conte *et al.*, 2012). It has provided a more refined hypothesis regarding the

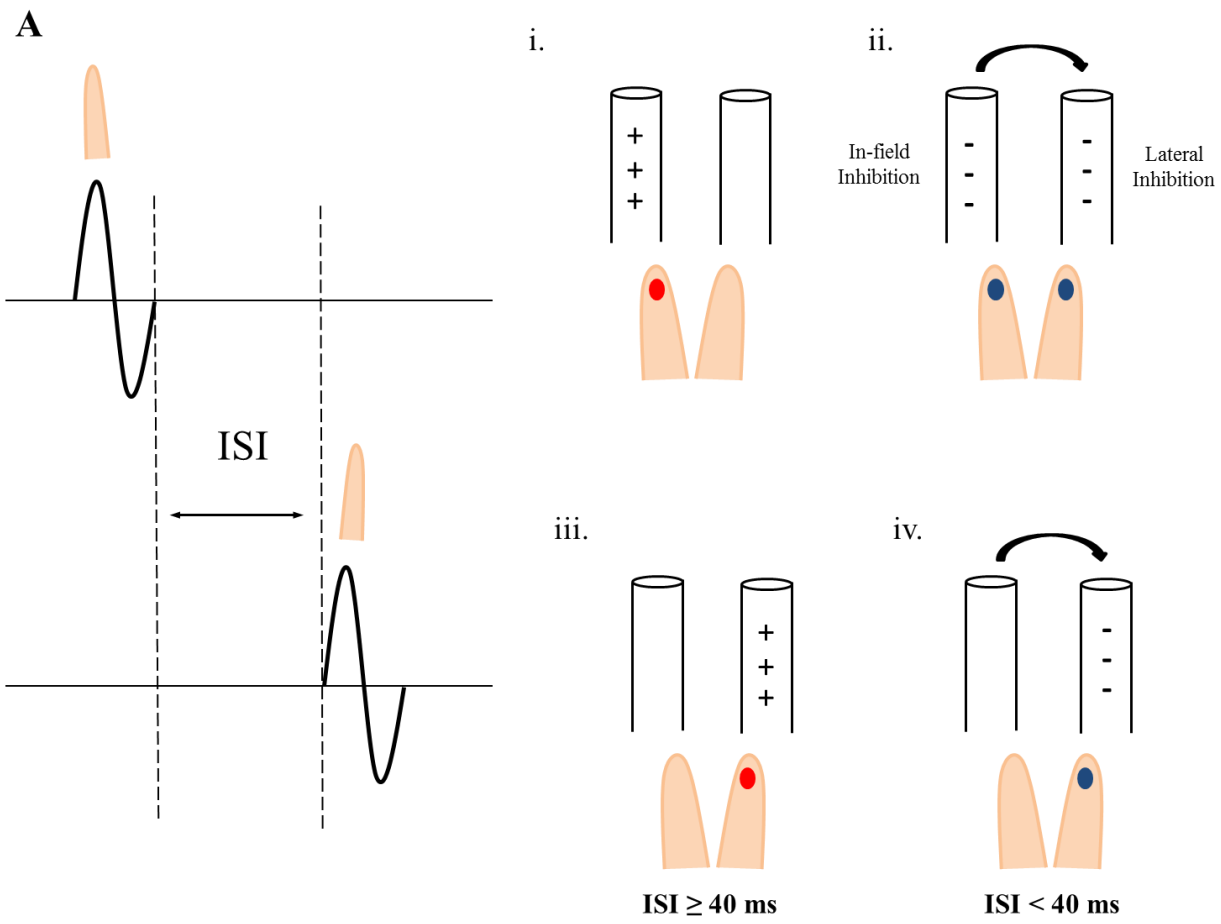
underlying mechanisms of tactile temporal perception and the temporal dynamics of cTBS effects that can be tested in future studies.

CHAPTER 5 – General Discussion

The goal of the Master's thesis was to address the question of whether the primary somatosensory cortex is involved in processing tactile temporal order judgment. Two studies were conducted to investigate whether temporal order judgment alone or in the presence of low-amplitude background vibration (synchronization) maybe modulated following two forms of cTBS delivered over left-hemisphere SI. In the first of two studies, changes in TOJ and TOJ synchronization (TOJ-S) on the right hand were recorded before and following traditional 50 Hz cTBS. Results from the first study demonstrated that 50 Hz cTBS over SI improves TOJ synchronization performance, as indicated by reduced TOJ-S thresholds that persisted for up to 18 minutes follow stimulation. However, TOJ performance was left unaltered following 50 Hz cTBS. In the second study, TOJ and TOJ-S performance were assessed bilaterally before and following 30 Hz cTBS and revealed impairments of TOJ that persists for up to 42 minutes following stimulation while TOJ-S performance remained unaltered. It is interpreted that changes in performance of either task, as seen in both experiments, would suggest that SI is involved in processing temporal perception. Recent findings have also shown that TOJ and TOJ-S may be mediated by different neural circuits. It is therefore possible that changes in TOJ or TOJ-S performance following cTBS may indicate that such changes are due to cTBS acting upon different mechanisms. This work opens the possibility of determining whether SI plays a part in temporal perception, and will allow further elucidation of the temporal dynamics of cTBS effects on SI. The novel research findings and postulated neural correlates involved in processing TOJ and TOJ-S will be discussed below.

TOJ and TOJ-S are mediated by inhibitory pathways

The potential mechanisms for TOJ and TOJ-S are summarized in Figure 5.1. It is important to note that a single TOJ or TOJ-S trial must include two main components. First, lateral inhibition must be present to allow for sufficient spatial contrast between cortical ensembles. FMRI studies have shown that the temporal order of tactile inputs rely on the processing of spatial representations of stimuli in the parietal cortices (Takahashi *et al.*, 2012) suggesting the importance of initial spatial discrimination for correct order judgment. In contrast, a lack of initial spatial contrast between the two receptive fields representing adjacent skin sites would disrupt the ability to detect the presence of two distinguished tactile stimuli. For instance, the presence of synchronizing stimuli delivered simultaneously over the two digits during a TOJ-S trial may reduce initial spatial contrast between cortical columns that represent each digit and thereby impair overall TOJ-S performance (Tommerdahl *et al.*, 2007). Second, lateral inhibition created by the first group of cortical columns must dissipate over time to allow for excitation of cortical columns receiving the second stimulus. In addition to lateral inhibition, it is reasonable to suggest that initial excitation of cortical columns induced by low synchronous vibrotactile stimuli creates in-field inhibition within those cortical columns (Gardner & Costanzo, 1980) thereby also preventing excitation of cortical columns that receive the second stimulus in the TOJ pair. In summary, any source that prevents initial spatial contrast between cortical units or hinders the excitation of the cortical columns representing the second digit may eventually disrupt TOJ and TOJ-S performance.


A
ISI
i.
ii.
In-field Inhibition
Lateral Inhibition
iii.
iv.
ISI ≥ 40 ms
ISI < 40 ms

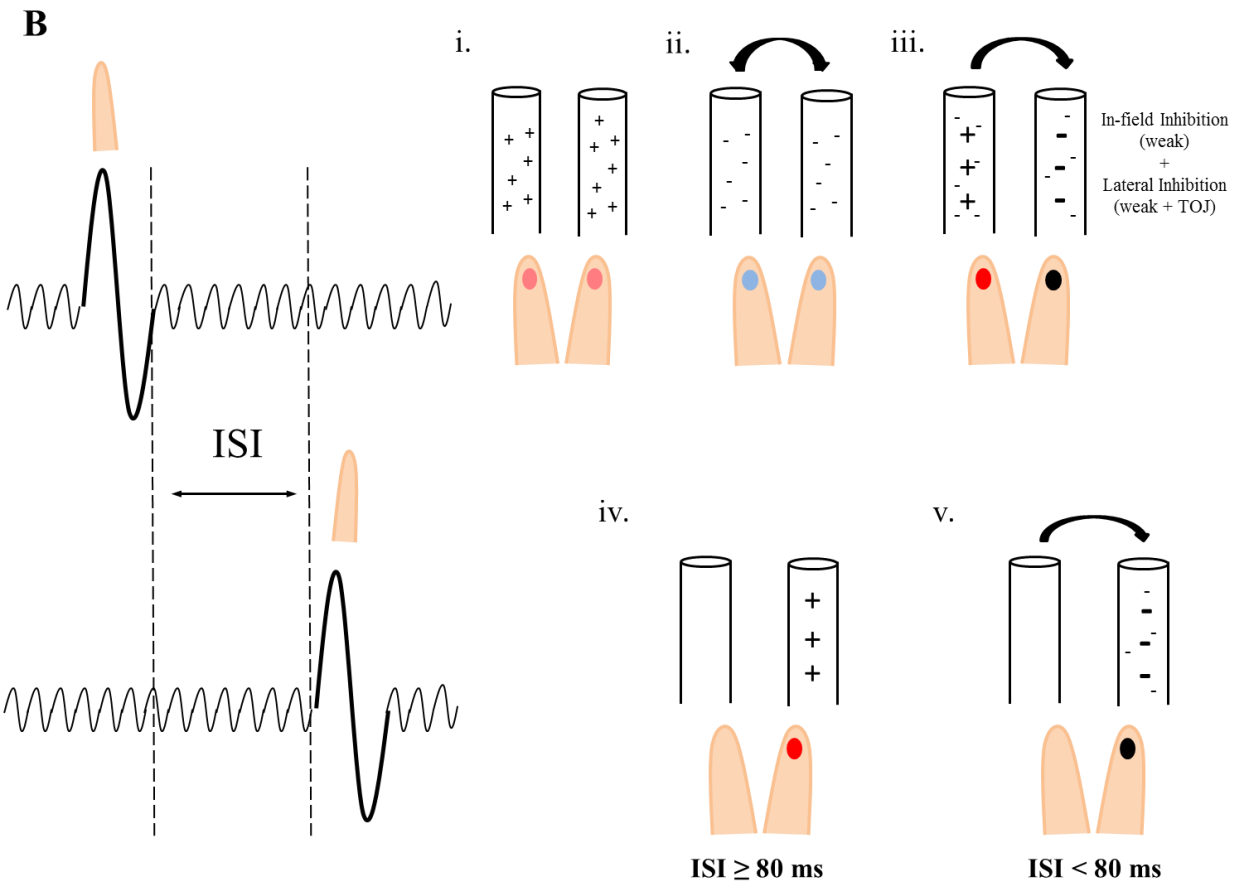


Figure 5.1. Schematic describing neural processes mediating TOJ and TOJ-S. (A) **i.** In this example of a TOJ trial, the delivery of a vibrotactile stimulus to the 2nd digit excites its corresponding cortical column (red; positive signs on digit 2). **ii**. Such excitation creates two types of inhibition. First, in-field inhibition occurs within the cortical columns representing digit 2 (blue; negative signs on digit 2). This type of inhibition prevents further excitation of neurons within its own cortical columns. Second, lateral inhibition (shown as arrow from columns of digit 2 to 3) is created by the initial excitation of digit 2 and acts to inhibit adjacent cortical columns (blue; negative signs on digit 3). TOJ percept depends on sequential excitation of columns in digit 2 and 3. Lateral

inhibition imposed on columns representing digit 3 must dissipate over time before columns in digit 3 may be excited. **iii**. The inter-stimulus interval (ISI) may reflect the time it takes for the excitation of columns in digit 3 to exceed the lateral inhibition within these same columns. If the ISI exceeds 40 ms, the cortical columns in digit 3 are allowed to excite and therefore TOJ can be performed correctly. **iv**. In contrast, if the ISI is below 40 ms, the cortical columns in digit are not allowed to excite due to the existing lateral inhibition, and therefore TOJ cannot be performed correctly. (B) **i**. In this example of a TOJ-S trial, simultaneous vibrotactile stimuli to digits 2 and 3 create weak simultaneous excitation in cortical columns representing both digits (pink; small positive signs). **ii**. Such excitation is followed by simultaneous in-field inhibition within both cortical columns and also lateral inhibition (bi-directional arrow) that occurs on adjacent columns (light blue; note that small negative signs represent both in-field and lateral inhibition created by weak synchronous stimuli). **iii**. The first stimulus from the TOJ pair excites cortical columns representing digit 2 (red, big positive signs) and evokes lateral inhibition in columns representing digit 3 (arrow from digit 2 to 3; large negative signs in digit 3). The second stimulus from the TOJ pair will only excite columns in digit 3 if excitation exceeds existing inhibition caused by lateral inhibition (weak synchronous stimulus from digit 2 and 1st pair of TOJ stimulus) and also existing in-field inhibition created by weak excitation of columns in digit 3 itself (black). **iv**. The inter-stimulus interval (ISI) may reflect the time it takes for the excitation of columns in digit 3 to exceed the inhibition (lateral and in-field) within these same columns. If the ISI exceeds 80 ms, the cortical columns in digit 3 are allowed to excite and therefore TOJ-S can be performed correctly.

v. In contrast, if the ISI is below 80 ms, the cortical columns in digit 3 are not allowed to excite due to existing inhibition (lateral and in-field), therefore TOJ-S cannot be performed correctly.

Mechanisms of cTBS on TOJ and TOJ-S

In Experiment 1, 50 Hz cTBS improved TOJ-S performance without affecting TOJ. One possible explanation for the improvement in TOJ-S performance is that cTBS may be suppressing inhibitory neurons that mediate in-field inhibition. Specifically, cTBS suppresses in-field inhibition that is evoked by the initial excitation caused by the low-amplitude vibration. This has the effect of reducing the overall magnitude of inhibition within those cortical columns and allows them to be excited at an earlier point in time (i.e. a shorter inter-stimulus interval which equates to improved TOJ-S performance). The lack of impairment in TOJ performance suggests that lateral inhibition remains unaltered in this study. One possibility for the lack of 50 Hz cTBS effects on lateral inhibition could be due to the higher resistance of inhibitory neurons mediating lateral inhibition. It is possible that these neurons contain high-threshold receptors and are therefore less susceptible to generating receptor potentials following 50 Hz cTBS. In summary, since TOJ-S performance was not impaired but rather was improved, one speculation is that 50 Hz cTBS acts by suppressing in-field inhibition.

In Experiment 2, 30 Hz cTBS over SI revealed impairments of TOJ without significantly altering TOJ-S performance. One possible explanation for the impaired TOJ

performance may be due to 30 Hz cTBS suppressing lateral inhibition. By suppressing inhibitory neurons that mediate lateral inhibition, a decrease in spatial contrast would disrupt the ability to report the presence of one or two stimuli, which is an essential component for TOJ. In contrast, TOJ-S performance remains unchanged following 30 Hz cTBS which suggests that this protocol does not alter in-field inhibition. An alternative explanation for the lack of improvement on TOJ-S by 30 Hz cTBS may be related to an initial reduction of in-field inhibition during TOJ-S baseline measurements. It is clear that reduced lateral and in-field inhibition would decrease the total amount of inhibition within the cortical columns that receives the second stimulus. This may reduce the time it takes for the total amount of inhibition to dissipate which allows the cortical column to excite in a shorter time (i.e. shorter ISI). In Experiment 2, the average baseline performance of TOJ-S (ISI ~ 62 ms) was lower than the average baseline TOJ-S performance from Experiment 1 (ISI ~ 104 ms). This reduction of ISI at baseline observed in Experiment 2 may suggest that the sampled population were less affected by the weak synchronous vibration and therefore experienced less in-field inhibition created by the weak stimuli. It should be carefully noted that the lower ISI in TOJ-S might be due to reduction in lateral inhibition since the performance of TOJ in Experiment 1 (ISI ~ 55 ms) was still greater than the performance of TOJ (ISI ~ 35 ms). The difference in TOJ and TOJ-S performance between Experiment 1 and Experiment 2 is about ~ 20 ms and 40 ms, respectively. Although this is a relative comparison between the two sampled populations in Experiment 1 and 2, it should be noted that a 40 ms difference in TOJ-S baseline values suggests that in-field inhibition may contribute to a greater extent to the

reduction of the total amount of inhibition on cortical columns that receive the second stimulus. In Experiment 1, it is suggested that 50 Hz cTBS suppresses in-field inhibition as demonstrated by an improved TOJ-S performance. It is possible that 30 Hz cTBS exhibits the same mechanism in Experiment 2. However, if subjects in Experiment 2 experienced less in-field inhibition, it could be due to an overall reduced excitability of inhibitory neurons that mediate in-field inhibition. In this respect, 30 Hz cTBS may not target these inhibitory neurons thereby the performance of TOJ-S remains unaltered.

There are several alternative explanations for the obtained differences in TOJ-S baseline performance between Experiment 1 and 2. First, an unpaired t-test (two-tailed) revealed significant age differences ($p = 0.01$) between Experiment 1 (26.5 ± 5.43, range 19-32) and Experiment 2 (20.2 ± 2.18, range 18-25). Previous studies have suggested that an increase in age may contribute to the decline in tactile temporal acuity (Craig, 2010). Therefore, the younger sampled population from Experiment 2 may account for the better performance in TOJ-S, as indicated by lower average baseline ISIs. Second, gender may also play a role in tactile acuity. In Experiment 1, 5 out of 8 subjects (62.5%) recruited were females as opposed from 8 of 10 subjects (80%) that participated in Experiment 2. Studies have revealed females are more sensitive to vibrotactile stimuli than men (Peters *et al*., 2009). Hence it is reasonable to suggest that the larger proportion of females may also explain for the lower ISI obtained in Experiment 2.

How can 30 versus 50 Hz cTBS yield different effects on inhibitory circuits within SI?

The results from both experiments suggest that 50 Hz and 30 Hz cTBS induce neuroplastic changes via different neural mechanisms. It appears that 50 Hz cTBS acts upon lateral inhibition as indicated by impairment of TOJ performance while 30 Hz cTBS acts upon in-field inhibition as demonstrated by improvement of TOJ-S performance. Understanding neural circuits that mediate in-field inhibition and lateral inhibition may help explain the different results from Experiment 1 and 2. Although the specific neurotransmitters involved in both in-field and lateral inhibition remains unclear, GABAergic inhibitory interneurons are suggested to play a role in mediating both types of inhibition. Therefore, it is reasonable to suggest that excitation or suppression of GABAergic circuitry would lead to changes in TOJ and TOJ-S performances.

Current understanding of the mechanisms of 50 Hz cTBS stems from studies applying cTBS over the motor cortex in rats and humans. In rats, 50 Hz cTBS reduces calbindin D-28k expressions in fast-spiking neurons that affect control of dendritic integration of synaptic inputs to pyramidal cells which ultimately alters excitability of inhibitory interneurons (Benali *et al.*, 2011). Further, strengthening of GABAergic synapses can be observed via increased GAD65 expressions, a marker of GABA inhibitory activity, following cTBS (Trippe *et al.*, 2009). In humans, using magnetic resonance spectroscopy, one group demonstrated an increase in GABA levels of the motor cortex following cTBS stimulation (Stagg *et al.*, 2009). These results show that 50 Hz cTBS over motor cortex may increase or decrease the excitability of inhibitory circuits.

In SI, 50 Hz cTBS suppresses inhibitory interneurons in superficial layers of SI as indicated by reduced amplitudes of late components of high frequency oscillations (Katayama *et al.*, 2010). However, evidence suggests that inter-subject variability exists in the responses to 50 Hz cTBS (Ridding & Ziemann, 2010). More importantly in Experiment 1, this variability of cTBS can also be found in both TOJ and TOJ-S task. Although TOJ was not significantly altered following stimulation, an initial improvement was followed by impairment that lasts up to 10 minutes post stimulation. During TOJ-S, improvement of performance was followed by impairment then again followed by improvement that lasts up to 18 minutes. This plasticity-inducing response variability was diminished during the TOJ task in Experiment 2 but remained present in the TOJ-S task. TOJ was significantly impaired following 30 Hz cTBS on the right hand and this impairment was shown to worsen as time progresses. Less variable responses to 30 Hz cTBS may suggest that such protocol produces stronger behavioral effects compared to 50 Hz cTBS. Studies that have applied 30 Hz cTBS over various sensory areas including the oculomotor cortex (Nyffeler *et al.*, 2006) and the posterior parietal cortex (Nyffeler *et al.*, 2008) revealed robust behavioral changes following stimulation. Stronger impacts of 30 Hz cTBS may be more effective for inducing neuroplastic cortical changes, thus the application of 30 Hz cTBS over cortices that control sensory processing such as SI should be investigated further.

Temporal dynamics of cTBS effects on TOJ and TOJ-S

One interesting finding from Experiment 1 revealed improved TOJ-S performance relative to baseline for up to 15-18 minutes following 50 Hz cTBS. Previous studies have

also shown 50 Hz cTBS effects on tactile perception follow a similar time course (Rai *et al.*, 2012;Conte *et al.*, 2012). The findings of both experiments add to the understanding of how neurons react to brief burst of theta frequency stimulation and also the time it takes to recover from imbalance of inhibitory interneuronal substrates that may contribute to the interplay between both feedback of feed-forward inhibitory mechanisms. One notable finding from Experiment 2 revealed impairments of TOJ performance following 30 Hz cTBS for over 42 minutes which outlasts the expected temporal changes induced by 50 Hz cTBS on SI physiology and perception. These findings may suggest that inhibitory neurons mediating both lateral and in-field inhibition can be suppressed following cTBS stimulation depending on the frequency of cTBS parameters. Although it remains unclear whether the extended periods of suppression on TOJ performance reflect greater strength of 30 Hz cTBS over 50 Hz cTBs on inhibitory neurons, it is reasonable to suggest that altering cTBS parameters such as frequency could elicit different temporal effects on tactile temporal perception. This piece of information constitutes a solid step forward to the understanding of temporal aspects of cTBS effects that could assist in identifying optimal plasticity-inducing protocols for further clinical applications.

Is SI involved in tactile temporal perception?

Although neural mechanisms of cTBS remain unclear, ample evidence suggests that cTBS acts on SI physiology, particularly somatosensory evoked potentials (SEP) and high frequency oscillations (HFOs). Previous work has demonstrated a suppression of

peak-to-peak amplitudes of both SEPs and HFOs (Ishikawa *et al.*, 2007;Katayama *et al.*, 2010). It is considered that the origins of SEP's stems from depolarization of apical dendrites of pyramidal cells (Allison *et al.*, 1991) while late subcomponents of HFOs are thought to reflect excitability of GABAergic inhibitory interneurons and cholinergic transmission in superficial layers in SI (Hashimoto *et al.*, 1996). The observed changes of cTBS on both SEP's and HFO's suggests that excitability of pyramidal neurons and inhibitory interneurons underlying SI cortex could be suppressed following stimulation.

Studies have demonstrated that modifying SI physiology via cTBS paradigms disrupts temporal discrimination performances for up to 15-18 minutes following stimulation (Rai *et al.*, 2012;Conte *et al.*, 2012), suggesting the importance of SI not only in processing sensory information but also performing higher-order discriminating abilities. The results from Experiment 1 and 2 explicate the process of modifying temporal order judgment performances following cTBS over SI. Interestingly, changes in tactile discrimination behavior have been shown to parallel changes in SI cortical physiology (Pleger *et al.*, 2001;Duncan & Boynton, 2007), therefore changes in SI physiology may reflect upon changes in tactile temporal perception. Besides human behavioral studies, animal studies have demonstrated an increase in c-fos expression, a neuronal activation marker, in SI of mice after performing tactile TOJ (Wada *et al.*, 2010). Combining the interpretations from both cTBS and animal studies from SI behavioral and physiological markers, it is reasonable to highlight the importance of SI in processing tactile temporal perception.

Other cortical and subcortical areas may contribute to tactile temporal perception

It is naïve to suggest that one cortical area processes or controls tactile temporal order judgment without appreciating the complexity of temporal processing of sensory signals that may rely on coding of firing patterns of individual neurons from multiple cortices (Luna *et al.,* 2005). Neuroimaging studies reveal activation of bilateral premotor cortices, the bilateral middle frontal gyri, bilateral inferior parietal cortices and supramarginal gyri as well as the superior and middle temporal gyri during a tactile TOJ task (Takahashi *et al.*, 2012). It is important to consider cortical and sub-cortical areas that are widely accepted to be involved in timing processes such as the basal ganglia, the cerebellum and temporal parietal junction which may also be candidates for mediating temporal perception (Pastor *et al.,* 2004;Davis *et al.,* 2009). One interesting area suggested to be involved in processing touch information but has been underexplored is the secondary somatosensory cortex (SII). Studies have demonstrated that SII neurons encode stimulus frequency and may play a role in decision-making of sensory information (Romo *et al.*, 2002). Nevertheless, although the focus of this thesis was solely on SI, it is possible that cortices that are anatomically connected via direct or indirect projections to SI may also experience changes in excitability induced by cTBS on SI (Ishikawa *et al.,* 2007).

Importance of tactile temporal perception

Tactile perception relies on spatiotemporal patterns of neural activity processed at multiple sensory layers. The spatial aspects are important for helping detect or localize

the skin sites that receive the afferent stimuli. The coherence of temporal information is much more complex as the arrival of sequential tactile stimuli to the skin must be processed in a temporal manner such that the sequence of tactile events would make sense for cognitive processing. One possible view of temporal processing of touch can be related to the neuronal firing rates during temporal perception. It's been shown that neurons code firing patterns during tactile discrimination (Salinas *et al.,* 2000;Luna *et al.,* 2005). The importance of this feature becomes more prominent in populations that exhibit sensory deficits in vision while touch remains intact. For instance, it's been shown that congenitally blind individuals have quicker processing of touch as demonstrated by quicker ability to read braille than the normal sighted population (Bhattacharjee *et al.,* 2010). Further, inability to process temporally segregated tactile events may also affect one's comprehension. There is evidence that dyslexic individuals show difficulties in temporal order judgment and temporal processing of tactile information (Laasonen *et al.,* 2001).Therefore it is reasonable to suggest that the ability to process sequential tactile patterns may help aid in cognition, memory and learning.

The sensory areas of the brain receive a rich array of somatosensory information from activation of sensory afferent nerves in the glabrous skin, joints and tendons. Fine hand control optimizes the extraction of this information upon contact with an object or a surface. A number of studies have highlighted the importance of tactile perception on hand function. These studies have showed that somatosensory discrimination changes corticospinal excitability (Rosenkranz & Rothwell, 2004). Also, corticomotor excitability was enhanced as indicated by an increase in MEPs in the hand engaged in performing the

tactile discrimination task (Master & Tremblay, 2009). Specifically, sensory information coded temporally in the fingers may be important for providing online sensory feedback during manipulation of objects. One study showed that when monkeys picked up pellet with their fingers, the grasping of the pellet led to increased activity of excitatory neurons followed by increased activity in inhibitory neurons (Iwamura *et al.*, 1985). These data suggests that tactile temporal acuity also plays an important role in manipulation of objects and exploration of the environment.

Future avenues and significance of work

This thesis has shown cTBS over left SI can modulate temporal order judgment performance on the fingertips of the right hand in the presence and absence of weak vibrotactile stimuli. To emphasize the importance of SI in processing tactile temporal perception, tactile TOJ performances across multiple digits should be investigated. This will be important because the manipulation of tools and active exploration of the environment rely heavily on somatosensory feedback from skin surfaces on multiple digits. In this respect, detection of the temporal order of three or four sequential tactile stimuli delivered over adjacent fingers would increase the complexity of TOJ. Understanding TOJ in such context may reveal the extent of the role of SI and its neural constraints on tactile temporal processing. This thesis has focused on cTBS and demonstrated changes in TOJ and TOJ-S performance. Identifying ways to alter perceptual behavior indirectly by inducing physiological changes within SI may allow for better understanding of the neural substrates involved in tactile temporal perception. Results from Experiment 1 and 2 suggest that 30 Hz and 50 Hz cTBS acts upon different

inhibitory mechanisms. Future studies are needed to investigate the different cTBS parameters such as duration, current direction and intensity of stimulation on SI cortical excitability and behavioral changes. Alternatively, the application of non-cTBS methods to study TOJ percepts may also help to confirm the contributions of inhibitory neurons in mediating TOJ and TOJ-S percepts. Future studies may examine this by administrating a GABAergic antagonist such as bicuculline to assess the level of inhibitory activity during TOJ or TOJ-S. It is worth noting that TOJ or TOJ-S can also be employed as a neurosensory assessment tool to evaluate tactile temporal acuity and SI function. This could be particularly interesting in the context of patient groups that demonstrate tactile temporal deficits. Abnormal values of TOJ or TOJ-S performance may reveal impairments of inhibitory mechanisms within SI. Identifying these impairments may help understand the manifestation of certain diseases, specifically those with altered inhibitory mechanisms within SI such as in Autism and stroke.

In conclusion, this Master's thesis work provides a unique contribution to the field of human tactile perception and the usage of non-invasive brain stimulation techniques in showing that perceptual changes following cTBS over SI may depend on the application of different cTBS parameters. Also, the present findings provide support for two types of inhibitory mechanisms that mediate TOJ and TOJ-S percepts. Finally, this work emphasizes the importance of SI in processing tactile temporal perception. Identifying such brain areas and its underlying neural mechanisms that control tactile perception may provide better understanding of fine motor control of the hand.

Reference List

Abbruzzese G & Berardelli A (2003). Sensorimotor integration in movement disorders. *Mov Disord* **18**, 231-240.

Allan L (1975). The relationship between judgments of successiveness and judgment order. *Attention, Perception & Psychophysics* **18**, 29-36.

Allison T, McCarthy G, Wood CC, Darcey TM, Spencer DD, & Williamson PD (1989). Human cortical potentials evoked by stimulation of the median nerve. I. Cytoarchitectonic areas generating short-latency activity. *J Neurophysiol* **62**, 694-710.

Allison T, McCarthy G, Wood CC, & Jones SJ (1991). Potentials evoked in human and monkey cerebral cortex by stimulation of the median nerve. A review of scalp and intracranial recordings. *Brain* **114 (Pt 6)**, 2465-2503.

Artieda J, Pastor MA, Lacruz F, & Obeso JA (1992). Temporal discrimination is abnormal in Parkinson's disease. *Brain* **115 Pt 1**, 199-210.

Benali A, Trippe J, Weiler E, Mix A, Petrasch-Parwez E, Girzalsky W, Eysel UT, Erdmann R, & Funke K (2011). Theta-burst transcranial magnetic stimulation alters cortical inhibition. *J Neurosci* **31**, 1193-1203.

Bhattacharjee A, Ye AJ, Lisak JA, Vargas MG, & Goldreich D (2010). Vibrotactile masking experiments reveal accelerated somatosensory processing in congenitally blind braille readers. *J Neurosci* **30**, 14288-14298.

Bijsterbosch JD, Lee KH, Dyson-Sutton W, Barker AT, & Woodruff PW (2011). Continuous theta burst stimulation over the left pre-motor cortex affects sensorimotor timing accuracy and supraliminal error correction. *Brain Res* **1410**, 101-111.

Bolognini N, Papagno C, Moroni D, & Maravita A (2010). Tactile temporal processing in the auditory cortex. *J Cogn Neurosci* **22**, 1201-1211.

Cardenas-Morales L, Nowak DA, Kammer T, Wolf RC, & Schonfeldt-Lecuona C (2010). Mechanisms and applications of theta-burst rTMS on the human motor cortex. *Brain Topogr* **22**, 294-306.

Casanova MF, Buxhoeveden D, & Gomez J (2003). Disruption in the Inhibitory Architecture of the Cell Minicolumn: Implications for Autisim. *The Neuroscientist* **9**, 496-507.

Casanova MF, Buxhoeveden DP, Switala AE, & Roy E (2002). Minicolumnar pathology in autism. *Neurology* **58**, 428-432.

Chen LM, Friedman RM, & Roe AW (2003). Optical imaging of a tactile illusion in area 3b of the primary somatosensory cortex. *Science* **302**, 881-885.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, & Cohen LG (1997). Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology* **48**, 1398-1403.

Clerget E, Poncin W, Fadiga L, & Olivier E (2012). Role of Broca's area in implicit motor skill learning: evidence from continuous theta-burst magnetic stimulation. *J Cogn Neurosci* **24**, 80-92.

Conte A, Rocchi L, Nardella A, Dispenza S, Scontrini A, Khan N, & Berardelli A (2012). Theta-Burst Stimulation-Induced Plasticity over Primary Somatosensory Cortex Changes Somatosensory Temporal Discrimination in Healthy Humans. *PLoS ONE* **7**, e32979.

Davis B, Christie J, & Rorden C (2009). Temporal order judgments activate temporal parietal junction. *J Neurosci* **29**, 3182-3188.

De BL, Henry LC, & Gosselin N (2012). Long-term functional alterations in sports concussion. *Neurosurg Focus* **33**, E8.

Di Lazzaro V, Pilato F, Saturno E, Oliviero A, Dileone M, Mazzone P, Insola A, Tonali PA, Ranieri F, Huang YZ, & Rothwell JC (2005). Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. *J Physiol* **565**, 945-950.

DiCarlo JJ & Johnson KO (2002). Receptive field structure in cortical area 3b of the alert monkey. *Behav Brain Res* **135**, 167-178.

Doeltgen SH & Ridding MC (2011). Low-intensity, short-interval theta burst stimulation modulates excitatory but not inhibitory motor networks. *Clin Neurophysiol* **122**, 1411-1416.

Duncan RO & Boynton GM (2007). Tactile Hyperacuity Thresholds Correlate with Finger Maps in Primary Somatosensory Cortex (S1). *Cerebral Cortex* **17**, 2878-2891.

Elbert T, Candia V, Altenmuller E, Rau H, Sterr A, Rockstroh B, Pantev C, & Taub E (1998). Alteration of digital representations in somatosensory cortex in focal hand dystonia. *Neuroreport* **9**, 3571-3575.

Favorov OV & Kelly DG (1994). Minicolumnar organization within somatosensory cortical segregates: I. Development of afferent connections. *Cereb Cortex* **4**, 408-427.

Francisco E, Tannan V, Zhang Z, Holden J, & Tommerdahl M (2008). Vibrotactile amplitude discrimination capacity parallels magnitude changes in somatosensory cortex and follows Weber's Law. *Exp Brain Res* **191**, 49-56.

Friedman RM, Chen LM, & Roe AW (2008). Responses of areas 3b and 1 in anesthetized squirrel monkeys to single- and dual-site stimulation of the digits. *J Neurophysiol* **100**, 3185-3196.

Gardner EP & Costanzo RM (1980). Temporal integration of multiple-point stimuli in primary somatosensory cortical receptive fields of alert monkeys. *J Neurophysiol* **43**, 444-468.

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

Goodwin AW & Wheat HE (2004). Sensory signals in neural populations underlying tactile perception and manipulation. *Annu Rev Neurosci* **27**, 53-77.

Haeberle H & Lumpkin EA (2008). Merkel Cells in Somatosensation. *Chemosens Percept* **1**, 110-118.

Hallett M (2007). Transcranial magnetic stimulation: a primer. *Neuron* **55**, 187-199.

Hamann W (1995). Mammalian cutaneous mechanoreceptors. *Prog Biophys Mol Biol* **64**, 81-104.

Hannula H, Neuvonen T, Savolainen P, Tukiainen T, Salonen O, Carlson S, & Pertovaara A (2008). Navigated transcranial magnetic stimulation of the primary somatosensory cortex impairs perceptual processing of tactile temporal discrimination. *Neurosci Lett* **437**, 144-147.

Hashimoto I, Kimura T, Fukushima T, Iguchi Y, Saito Y, Terasaki O, & Sakuma K (1999). Reciprocal modulation of somatosensory evoked N20m primary response and high-frequency oscillations by interference stimulation. *Clin Neurophysiol* **110**, 1445-1451.

Hashimoto I, Mashiko T, & Imada T (1996). High-frequency magnetic signals in the human somatosensory cortex. *Electroencephalogr Clin Neurophysiol Suppl* **47**, 67-80.

Hirsh.J. (1961). Perceived order in different sense modalities. *J Exp Psychol* **62**, 423-432.

Holden JK, Nguyen RH, Francisco EM, Zhang Z, Dennis RG, & Tommerdahl M (2012). A novel device for the study of somatosensory information processing. *J Neurosci Methods* **204**, 215-220.

Hoshiyama M, Kakigi R, & Tamura Y (2004). Temporal discrimination threshold on various parts of the body. *Muscle Nerve* **29**, 243-247.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, & Rothwell JC (2005). Theta Burst Stimulation of the Human Motor Cortex. *Neuron* **45**, 201-206.

Iezzi E, Suppa A, Conte A, Agostino R, Nardella A, & Berardelli A (2010). Theta-burst stimulation over primary motor cortex degrades early motor learning. *Eur J Neurosci* **31**, 585-592.

Ishikawa S, Matsunaga K, Nakanishi R, Kawahira K, Murayama N, Tsuji S, Huang YZ, & Rothwell JC (2007). Effect of theta burst stimulation over the human sensorimotor cortex on motor and somatosensory evoked potentials. *Clinical Neurophysiology* **118**, 1033-1043.

Iwamura Y, Tanaka M, Sakamoto M, & Hikosaka O (1985). Vertical neuronal arrays in the postcentral gyrus signaling active touch: a receptive field study in the conscious monkey. *Exp Brain Res* **58**, 412-420.

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

Johansson RS (1978). Tactile sensibility in the human hand: receptive field characteristics of mechanoreceptive units in the glabrous skin area. *J Physiol* **281**, 101-125.

Johansson RS & Vallbo AB (1979). Detection of tactile stimuli. Thresholds of afferent units related to psychophysical thresholds in the human hand. *J Physiol* **297**, 405-422.

Johansson RS & Vallbo +B. Tactile sensory coding in the glabrous skin of the human hand. Trends in neurosciences 6, 27-32. 1983.
Ref Type: Abstract

Jones EG, Friedman DP, & Hendry SH (1982). Thalamic basis of place- and modality-specific columns in monkey somatosensory cortex: a correlative anatomical and physiological study. *J Neurophysiol* **48**, 545-568.

Karim AA, Schuler A, Hegner YL, Friedel E, & Godde B (2006). Facilitating effect of 15-Hz repetitive transcranial magnetic stimulation on tactile perceptual learning. *J Cogn Neurosci* **18**, 1577-1585.

Katayama T & Rothwell JC (2007). Modulation of somatosensory evoked potentials using transcranial magnetic intermittent theta burst stimulation. *Clin Neurophysiol* **118**, 2506-2511.

Katayama T, Suppa A, & Rothwell JC (2010). Somatosensory evoked potentials and high frequency oscillations are differently modulated by theta burst stimulation over primary somatosensory cortex in humans. *Clinical Neurophysiology* **121**, 2097-2103.

Knecht S, Ellger T, Breitenstein C, Bernd RE, & Henningsen H (2003). Changing cortical excitability with low-frequency transcranial magnetic stimulation can induce sustained disruption of tactile perception. *Biol Psychiatry* **53**, 175-179.

Kobayashi M & Pascual-Leone A (2003). Transcranial magnetic stimulation in neurology. *Lancet Neurol* **2**, 145-156.

Laasonen M, Service E, & Virsu V (2001). Temporal order and processing acuity of visual, auditory, and tactile perception in developmentally dyslexic young adults. *Cogn Affect Behav Neurosci* **1**, 394-410.

Lacruz F, Artieda J, Pastor MA, & Obeso JA (1991). The anatomical basis of somaesthetic temporal discrimination in humans. *J Neurol Neurosurg Psychiatry* **54**, 1077-1081.

LaMotte RH, Friedman RM, Lu C, Khalsa PS, & Srinivasan MA (1998). Raised object on a planar surface stroked across the fingerpad: responses of cutaneous mechanoreceptors to shape and orientation. *J Neurophysiol* **80**, 2446-2466.

Lenz M, Tegenthoff M, Kohlhaas K, Stude P, Hoffken O, Gatica Tossi MA, Kalisch T, Kowalewski R, & Dinse HR (2012). Increased excitability of somatosensory cortex in aged humans is associated with impaired tactile acuity. *J Neurosci* **32**, 1811-1816.

Luna R, Hernandez A, Brody CD, & Romo R (2005). Neural codes for perceptual discrimination in primary somatosensory cortex. *Nat Neurosci* **8**, 1210-1219.

Maeno T, Kobayashi K, & Yamazaki N (1998). Relationship between the structure of human finger tissue and the location of tactile receptors. *JSME International Journal-Series C-Dynamics Control Robotics Design and Manufacturing* **41**, 94-100.

Makous JC, Friedman RM, & Vierck CJ, Jr. (1996). Effects of a dorsal column lesion on temporal processing within the somatosensory system of primates. *Exp Brain Res* **112**, 253-267.

Manganelli F, Dubbioso R, Pisciotta C, Antenora A, Nolano M, De MG, Filla A, Berardelli A, & Santoro L (2013). Somatosensory Temporal Discrimination Threshold Is Increased in Patients with Cerebellar Atrophy. *Cerebellum*.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, & Wu C (2004). Interneurons of the neocortical inhibitory system. *Nat Rev Neurosci* **5**, 793-807.

Master S & Tremblay F (2009). Task-specific increase in corticomotor excitability during tactile discrimination. *Exp Brain Res* **194**, 163-172.

McAllister SM, Rothwell JC, & Ridding MC (2009). Selective modulation of intracortical inhibition by low-intensity Theta Burst Stimulation. *Clin Neurophysiol* **120**, 820-826.

Meehan SK, Dao E, Linsdell MA, & Boyd LA (2011). Continuous theta burst stimulation over the contralesional sensory and motor cortex enhances motor learning post-stroke. *Neurosci Lett* **500**, 26-30.

Meehan SK, Legon W, & Staines WR (2008). Paired-pulse transcranial magnetic stimulation of primary somatosensory cortex differentially modulates perception and sensorimotor transformations. *Neuroscience* **157**, 424-431.

Mix A, Benali A, Eysel UT, & Funke K (2010). Continuous and intermittent transcranial magnetic theta burst stimulation modify tactile learning performance and cortical protein expression in the rat differently. *Eur J Neurosci* **32**, 1575-1586.

Mochizuki H, Furubayashi T, Hanajima R, Terao Y, Mizuno Y, Okabe S, & Ugawa Y (2007). Hemoglobin concentration changes in the contralateral hemisphere during and after theta burst stimulation of the human sensorimotor cortices. *Experimental Brain Research* **180**, 667-675.

Morley JW, Vickery RM, Stuart M, & Turman AB (2007). Suppression of vibrotactile discrimination by transcranial magnetic stimulation of primary somatosensory cortex. *Eur J Neurosci* **26**, 1007-1010.

Mountcastle VB (2005). *The sensory hand: neural mechanisms of somatic sensation* Harvard University Press.

Mountcastle VB, Talbot WH, Darian-Smith I, & Kornhuber HH (1967). Neural basis of the sense of flutter-vibration. *Science* **155**, 597-600.

Nager W, Wolters C, Munte TF, & Johannes S (2004). Transcranial magnetic stimulation to the parietal lobes reduces detection of contralateral somatosensory stimuli. *Acta Neurol Scand* **109**, 146-150.

Nelson AJ, Premji A, Rai N, Hoque T, Tommerdahl M, & Chen R (2012). Dopamine alters tactile perception in Parkinson's disease. *Can J Neurol Sci* **39**, 52-57.

Nelson AJ, Blake DT, & Chen R (2009). Digit-specific aberrations in the primary somatosensory cortex in Writer's cramp. *Ann Neurol* **66**, 146-154.

Nguyen R, Ford S, Calhoun A, Gracely R, & Tommerdahl M (2013). Neurosensory assessments of migraine. *Brain Research* **Accepted with revision**.

Nollet H, Van HL, Deprez P, & Vanderstraeten G (2003). Transcranial magnetic stimulation: review of the technique, basic principles and applications. *Vet J* **166**, 28-42.

Nyffeler T, Cazzoli D, Wurtz P, Luthi M, von WR, Chaves S, Deruaz A, Hess CW, & Muri RM (2008). Neglect-like visual exploration behaviour after theta burst transcranial magnetic stimulation of the right posterior parietal cortex. *Eur J Neurosci* **27**, 1809-1813.

Nyffeler T, Wurtz P, Luscher HR, Hess CW, Senn W, Pflugshaupt T, von WR, Luthi M, & Muri RM (2006). Repetitive TMS over the human oculomotor cortex: comparison of 1-Hz and theta burst stimulation. *Neurosci Lett* **409**, 57-60.

Oldfield RC (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* **9**, 97-113.

Pasalar S, Ro T, & Beauchamp MS (2010). TMS of posterior parietal cortex disrupts visual tactile multisensory integration. *Eur J Neurosci* **31**, 1783-1790.

Pastor MA, Day BL, Macaluso E, Friston KJ, & Frackowiak RS (2004). The functional neuroanatomy of temporal discrimination. *J Neurosci* **24**, 2585-2591.

Peters RM, Hackeman E, Goldreich D (2009). Diminutive Digits Discern Delicate Details: Fingertip Size and the Sex Difference in Tactile Spatial Acuity. *The Journal of Neuroscience* **29**, 15756-15761.

Pleger B, Dinse HR, Ragert P, Schwenkreis P, Malin JP, & Tegenthoff M (2001). Shifts in cortical representations predict human discrimination improvement. *Proc Natl Acad Sci U S A* **98**, 12255-12260.

Pons TP, Garraghty PE, & Mishkin M (1992). Serial and parallel processing of tactual information in somatosensory cortex of rhesus monkeys. *J Neurophysiol* **68**, 518-527.

Premji A, Ziluk A, & Nelson A (2010). Bilateral somatosensory evoked potentials following intermittent theta-burst repetitive transcranial magnetic stimulation. *BMC Neuroscience* **11**, 91.

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

Ragert P, Franzkowiak S, Schwenkreis P, Tegenthoff M, & Dinse H (2008). Improvement of tactile perception and enhancement of cortical excitability through intermittent theta burst rTMS over human primary somatosensory cortex. *Experimental Brain Research* **184**, 1-11.

Rai N, Premji A, Tommerdahl M, & Nelson AJ (2012). Continuous theta-burst rTMS over primary somatosensory cortex modulates tactile perception on the hand. *Clin Neurophysiol* **123**, 1226-1233.

Recanzone GH, Merzenich MM, Jenkins WM, Grajski KA, & Dinse HR (1992). Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task. *J Neurophysiol* **67**, 1031-1056.

Ridding MC & Ziemann U (2010). Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J Physiol* **588**, 2291-2304.

Romero JR, Anschel D, Sparing R, Gangitano M, & Pascual-Leone A (2002). Subthreshold low frequency repetitive transcranial magnetic stimulation selectively decreases facilitation in the motor cortex. *Clin Neurophysiol* **113**, 101-107.

Romo R, Hernandez A, Zainos A, Lemus L, & Brody CD (2002). Neuronal correlates of decision-making in secondary somatosensory cortex. *Nat Neurosci* **5**, 1217-1225.

Rosenkranz K & Rothwell JC (2004). The effect of sensory input and attention on the sensorimotor organization of the hand area of the human motor cortex. *J Physiol* **561**, 307-320.

Rossi S, Hallett M, Rossini PM, & Pascual-Leone A (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol* **120**, 2008-2039.

Salinas E, Hernandez A, Zainos A, & Romo R (2000). Periodicity and firing rate as candidate neural codes for the frequency of vibrotactile stimuli. *J Neurosci* **20**, 5503-5515.

Sanger TD, Tarsy D, & Pascual-Leone A (2001). Abnormalities of spatial and temporal sensory discrimination in writer's cramp. *Mov Disord* **16**, 94-99.

Sathian K, Goodwin AW, John KT, & Darian-Smith I (1989). Perceived roughness of a grating: correlation with responses of mechanoreceptive afferents innervating the monkey's fingerpad. *J Neurosci* **9**, 1273-1279.

Satow T, Mima T, Yamamoto J, Oga T, Begum T, Aso T, Hashimoto N, Rothwell JC, & Shibasaki H (2003). Short-lasting impairment of tactile perception by 0.9Hz-rTMS of the sensorimotor cortex. *Neurology* **60**, 1045-1047.

Scontrini A, Conte A, Defazio G, Fiorio M, Fabbrini G, Suppa A, Tinazzi M, & Berardelli A (2009). Somatosensory temporal discrimination in patients with primary focal dystonia. *J Neurol Neurosurg Psychiatry* **80**, 1315-1319.

Seyal M, Ro T, & Rafal R (1995). Increased sensitivity to ipsilateral cutaneous stimuli following transcranial magnetic stimulation of the parietal lobe. *Ann Neurol* **38**, 264-267.

Shore DI, Gray K, Spry E, & Spence C (2005). Spatial modulation of tactile temporal-order judgments. *Perception* **34**, 1251-1262.

Siebner HR, Hartwigsen G, Kassuba T, & Rothwell JC (2009). How does transcranial magnetic stimulation modify neuronal activity in the brain? Implications for studies of cognition. *Cortex* **45**, 1035-1042.

Simons SB, Chiu J, Favorov OV, Whitsel BL, & Tommerdahl M (2007). Duration-dependent response of SI to vibrotactile stimulation in squirrel monkey. *J Neurophysiol* **97**, 2121-2129.

Singer W (1996). Neurophysiology: the changing face of inhibition. *Curr Biol* **6**, 395-397.

Song S, Sandrini M, & Cohen LG (2011). Modifying somatosensory processing with non-invasive brain stimulation. *Restor Neurol Neurosci* **29**, 427-437.

Stagg CJ, Wylezinska M, Matthews PM, Johansen-Berg H, Jezzard P, Rothwell JC, & Bestmann S (2009). Neurochemical effects of theta burst stimulation as assessed by magnetic resonance spectroscopy. *J Neurophysiol* **101**, 2872-2877.

Staines WR, Black SE, Graham SJ, & McIlroy WE (2002). Somatosensory gating and recovery from stroke involving the thalamus. *Stroke* **33**, 2642-2651.

T.M.Forshey, Favorov OV, Tommerdahl M, & Whitsel BL. Flexibility of the SI representation of skin flutter stimulation: A temporally evolving coarse to fine-grain continuum. Society for Neuroscience, New Orleans, U.S.A. 2012. 13-10-2012.
Ref Type: Conference Proceeding

Takahashi T, Kansaku K, Wada M, Shibuya S, & Kitazawa S (2012). Neural Correlates of Tactile Temporal-Order Judgment in Humans: an fMRI Study. *Cereb Cortex*.

Talbot WH, Darian-Smith I, Kornhuber HH, & Mountcastle VB (1968). The sense of flutter-vibration: comparison of the human capacity with response patterns of mechanoreceptive afferents from the monkey hand. *J Neurophysiol* **31**, 301-334.

Tamura Y, Matsuhashi M, Lin P, Ou B, Vorbach S, Kakigi R, & Hallett M (2008). Impaired intracortical inhibition in the primary somatosensory cortex in focal hand dystonia. *Mov Disord* **23**, 558-565.

Tegenthoff M, Ragert P, Pleger B, Schwenkreis P, Forster AF, Nicolas V, & Dinse HR (2005). Improvement of tactile discrimination performance and enlargement of cortical somatosensory maps after 5 Hz rTMS. *PLoS Biol* **3**, e362.

Tinazzi M, Frasson E, Bertolasi L, Fiaschi A, & Aglioti S (1999). Temporal discrimination of somesthetic stimuli is impaired in dystonic patients. *Neuroreport* **10**, 1547-1550.

Tommerdahl M, Favorov OV, & Whitsel BL (2010). Dynamic representations of the somatosensory cortex. *Neurosci Biobehav Rev* **34**, 160-170.

Tommerdahl M, Tannan V, Holden JK, & Baranek GT (2008). Absence of stimulus-driven synchronization effects on sensory perception in autism: Evidence for local underconnectivity? *Behav Brain Funct* **4**, 19.

Tommerdahl M, Tannan V, Zachek M, Holden JK, & Favorov OV (2007). Effects of stimulus-driven synchronization on sensory perception. *Behav Brain Funct* **3**, 61.

Tremblay F, Ageranioti-Belanger SA, & Chapman CE (1996). Cortical mechanisms underlying tactile discrimination in the monkey. I. Role of primary somatosensory cortex in passive texture discrimination. *Journal of Neurophysiology* **76**, 3382-3403.

Trippe J, Mix A, Aydin-Abidin S, Funke K, & Benali A (2009). theta burst and conventional low-frequency rTMS differentially affect GABAergic neurotransmission in the rat cortex. *Exp Brain Res* **199**, 411-421.

Uhlhaas PJ & Singer W (2010). Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci* **11**, 100-113.

Vanderwolf CH (1969). Hippocampal electrical activity and voluntary movement in the rat. *Electroencephalogr Clin Neurophysiol* **26**, 407-418.

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

Wada M, Higo N, Moizumi S, & Kitazawa S (2010). c-Fos Expression during Temporal Order Judgment in Mice. *PLoS ONE* **5**, e10483.

Woo SH, Kim KH, & Lee KM (2009). The role of the right posterior parietal cortex in temporal order judgment. *Brain Cogn* **69**, 337-343.