UNCOVERING THE ROLE OF THE HUMAN PRIMARY MOTOR CORTEX IN CARDIOVASCULAR CONTROL DURING EXERCISE: A TRANSCRANIAL MAGNETIC STIMULATION INVESTIGATION

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
December 2015

To Roger, for your constant support and uplifting smile.

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Abstract

The possible implication of the human primary motor cortex (M1) in the control of the cardiovascular autonomic system during rest and exercise was assessed in normotensive participants. Participants underwent three conditions using transcranial magnetic stimulation; Sham (control), continuous theta burst stimulation (cTBS) and intermittent theta burst stimulation (iTBS). Heart rate (HR), arterial blood pressure (ABP) and muscle sympathetic nerve activity (MSNA) were recorded post-stimulation during baseline rest, a 2-minute isometric handgrip exercise and a post-exercise ischemia period where blood flow to the previously active forearm and hand is occluded. Results showed that iTBS significantly facilitated M1 compared to sham and baseline ($p \leq 0.05$), but cTBS failed to cause inhibition as expected. However, significant differences between conditions were still found during resting measures. Specifically, systolic BP was significantly greater post-cTBS (134.6 ± 20.1 mmHg) compared to post-iTBS (121.9 ± 21.1 mmHg) ($p \leq 0.05$). Recordings of MSNA burst incidence post-cTBS were significantly great than post-iTBS and sham ($p \leq 0.05$) during minutes three (38 ± 8.8, 29.5 ± 7, 31.8 ± 9.7 bursts/100 beats), four (41.7 ± 12.5, 28 ± 7.4, 33.6 ± 10.5 bursts/100 beats) and five (42.3 ± 13.3, 29.5 ± 11.6, 33.6 ± 10.3 bursts/100 beats). Furthermore, MSNA burst frequency post-cTBS was significantly great than post-iTBS and sham ($p \leq 0.05$) during rest minutes one (25.6 ± 7, 19 ± 9.2, 22.3 ± 6 bursts/minute), three (27.6 ± 6.8, 21 ± 4.8, 23.1 ± 7 bursts/minute), four (29.6 ± 9.7, 20 ± 5.2, 23.8 ± 7.2 bursts/minute), and five (29.6 ± 8.6, 21.1 ± 8.8, 23.9 ± 6.9 bursts/minute). The changes we observed in cardiac autonomic measures suggest M1 may be implicated in the control of the cardiovascular system.

List of Abbreviations Used

ABP - arterial blood pressure
Ach - acetylcholine
ANS - autonomic nervous system
AMT- active motor threshold
CC - central command
CNS - central nervous system
cTBS - continuous theta burst stimulation paradigm
CVLM - caudal ventrolateral medulla
DBP - diastolic blood pressure
EEG - electroencephalogram
EPR - exercise pressor reflex
FDG-PET - fluorodeoxyglucose-positron emission tomography
FDI - first dorsal interosseus
FDS - flexor digitorum superficialis
fMRI - functional magnetic resonance imaging
GABA - gamma aminobutyric acid
HF - high frequency
HR - heart rate
HRV - heart rate variability
IHG - isometric handgrip exercise
IML - intermediolateral nucleus
imTBS - intermediate theta burst stimulation
iTBS - intermittent theta burst stimulation
LF - low frequency
LTP - long-term potentiation
M1 - primary motor cortex
MEP – motor evoked potential
MAP - mean arterial pressure
MRI- magnetic resonance imaging
MSNA- muscle sympathetic nerve activity
NA - nucleus ambiguus
NN - normal-to-normal
NTS - nucleus tractus solitarii
PEI- post-exercise ischemia
PNS - parasympathetic nervous system
PSD - power spectral analysis
Q - cardiac output
RMT - resting motor threshold
rTMS - repetitive transcranial magnetic stimulation
RVLM - rostral ventrolateral medulla
SA Node - sinoatrial node
SBP - systolic blood pressure
S-D - strength-duration

SDNN - standard deviation of the normal-to-normal interval
SNA - sympathetic nerve activity
SNS - sympathetic nervous system
SSNA - skin sympathetic nerve activity
SV - stroke volume
STP - short-term potentiation
TBS - theta burst stimulation
TMS - transcranial magnetic stimulation
TPR - total peripheral resistance

CHAPTER 1: INTRODUCTION

Optimal homeostatic cardiovascular regulation is achieved by a delicate balance of neural innervation between the parasympathetic (heart only) and sympathetic (heart and blood vessels) divisions of the autonomic nervous system. An important regulator of these neural activities is the arterial baroreflex, which originates from stretch-sensitive receptors (i.e., baroreceptors) in the carotid arteries and the aorta. In normotensive individuals there is greater vagal than sympathetic neural innervation to the heart and relatively low levels of muscle sympathetic nerve activity (MSNA), the efferent action potentials generated from the sympathetic nervous system to skeletal muscle arterioles. During exercise, vagal activity is 'withdrawn' and sympathetic activity is augmented to increase arterial blood pressure (ABP), which is necessary to ensure an adequate blood supply to active skeletal muscles.

A normal cardiovascular response to exercise is a rise in ABP. This is also caused, in part, by an increase in sympathetic nerve activity. The blood pressure response to exercise is produced by two separate neural responses. The first response comes from brain activity in regions that are also involved with contracting your muscles and is termed Central Command (CC). The second response, the Exercise Pressor Reflex (EPR), comes from the exercising muscles, which sends information back up to the brain and provides information about how hard they are working (2, 38, 55, 59, 78). The main neural circuitry

involved with the generation of vagal and sympathetic nerve activity resides within the brainstem and spinal cord (24, 53, 54, 91). These medullary nuclei act to control the sympathetic and parasympathetic branches of the autonomic nervous system to adjust HR and ABP as necessary in response to exercise. More recently it has been discovered that higher brain centres above the brainstem are implicated in the effective regulation of these neural outflows to control the cardiovascular system (14, 70, 71, 71, 72, 100, 114). Specifically, the motor cortex has emerged as a cortical region implicated in the control of cardiovascular function (17, 34, 72).

Studies using stimulation techniques to modulate the excitability of the human motor cortex have found promising results that link the motor cortex activity with cardiovascular function (17, 34, 72). In particular, Macefield and colleagues (72), using transcranial magnetic stimulation found that a time-locked cortical stimulus could interfere with the sympathetic vasomotor drive to human skeletal muscle. They discovered that cortical stimulation over the vertex caused a transient inhibition of the sympathetic discharges and decreased skin blood flow, when the stimulus was delivered 200-400 ms after the R-wave of the electrocardiogram, which was then followed by an increase in sympathetic activity. Cortical stimulation over the hand area of the primary motor cortex also caused inhibition of sympathetic bursts and decreased skin blood flow, but to a smaller degree (72). These findings provide compelling evidence that changing the excitability of the motor cortex has a direct impact on cardiovascular control.

Further investigation into the motor cortex using stimulation techniques could provide insight into how the brain controls blood pressure at rest and during exercise. In the current study, transcranial magnetic stimulation (TMS), a non-invasive stimulation technology, was used to change the activity of the motor cortex. Using transcranial magnetic stimulation, to inhibit or facilitate the motor cortex may enable us to gain more knowledge about the central neural control mechanisms of cardiovascular regulation during exercise in humans.

Healthy, normotensive individuals participated in three different TMS stimulation protocols that inhibited (i.e., continuous theta burst stimulation, cTBS), facilitated (intermittent theta burst stimulation, iTBS) or had no effect (Sham TBS) on the excitability of the motor cortex. The participants then underwent an isometric handgrip exercise immediately followed by a post-exercise ischemia period where blood flow is occluded from the previously active forearm and hand. Occlusion of blood flow allows for the separation of the two neural mechanisms that control blood pressure by isolating the metaboreflex component of the EPR. Throughout the experimental protocol, continuous measures of heart rate (HR), blood pressure and MSNA were recorded using an electrocardiograph, a Portapres® blood pressure monitoring device and microneurography, respectively.

The objective of the current proposal was to determine if using TMS to inhibit or facilitate neural activity within the motor cortex would elicit significant changes in HR, MSNA and ABP during rest and isometric handgrip exercise in a population of normotensive men and women. It was hypothesized that facilitating the hand area of the motor cortex would cause a subsequent increase in HR, ABP and MSNA, while inhibiting the motor cortex would cause a decrease in HR, ABP and MSNA at rest. However, during exercise there would be a decrease in HR, ABP and MSNA compared to the control after facilitating the motor cortex. The opposite response, an increase in HR, ABP and MSNA, would occur during exercise after inhibition of the motor cortex as the CC response would need to be increased to maintain the intensity of the isometric handgrip exercise. The sham condition would not produce any effects on resting HR, ABP or MSNA.

The goal of this work was to create new knowledge about how the motor cortex controls heart and blood vessel function in healthy people during resting and exercise conditions. This work aimed to help identify if the primary motor cortex is implicated in the control of blood pressure in humans. Furthermore, this study provided new information that could lead to future studies examining if using non-invasive brain stimulation could be an effective therapeutic method for treating people with cardiovascular disease. The ultimate goal is to help these people achieve a better quality of life and could potentially decrease the high health care costs associated with the long-term treatment of their disease.

CHAPTER 2: LITERATURE REVIEW

2.1 Cardiovascular Regulation & Cardiovascular Response to Exercise

2.1.1 Primer on Autonomic Control of Cardiovascular Function

The purpose of the cardiovascular response to exercise is to increase oxygen and nutrient delivery to and the removal of metabolic waste products from active tissues. The increased mean arterial pressure (MAP) that occurs with exercise is essential for maintaining adequate perfusion pressure and blood flow to the contracting muscles. The cardiovascular system is integrated with various other mechanisms to meet the increased metabolic demands of the body at the onset and continually throughout exercise. This cardiovascular response to exercise varies depending if the exercise is mainly dynamic (rhythmic patterns of contraction and relaxation of muscle) or static (isometric, constant force applied for a period of time without intermittent periods of muscle relaxation). The current study utilizes static or isometric exercise and thus the response to isometric exercise will be the primary focus. Isometric exercise is characterized by an increase in systolic, diastolic and mean ABP (37).

The autonomic nervous system (ANS) is responsible for the balance of sympathetic and vagal or parasympathetic tone that influences HR, blood pressure, and arterial vasoconstriction (sympathetic only). The central

components of the ANS, including cardiac regulation and vasomotor centers are located in the medulla oblongata of the brainstem. The two branches of the ANS, the parasympathetic (PNS) and sympathetic nervous system (SNS), act in opposite manners to modulate blood pressure and HR. Sympathetic nervous system stimulation causes arteriolar constriction (to increase total peripheral resistance), HR and stroke volume (SV) increases and thus the autonomic nervous system has an important role in maintaining a normal blood pressure. It is also important in the mediation of short-term changes in blood pressure in response to stress and physical exercise. Parasympathetic nervous system activity predominates at rest to keep blood pressure and HR lowered by hyperpolarizing the cells of the sinoatrial (SA) node.

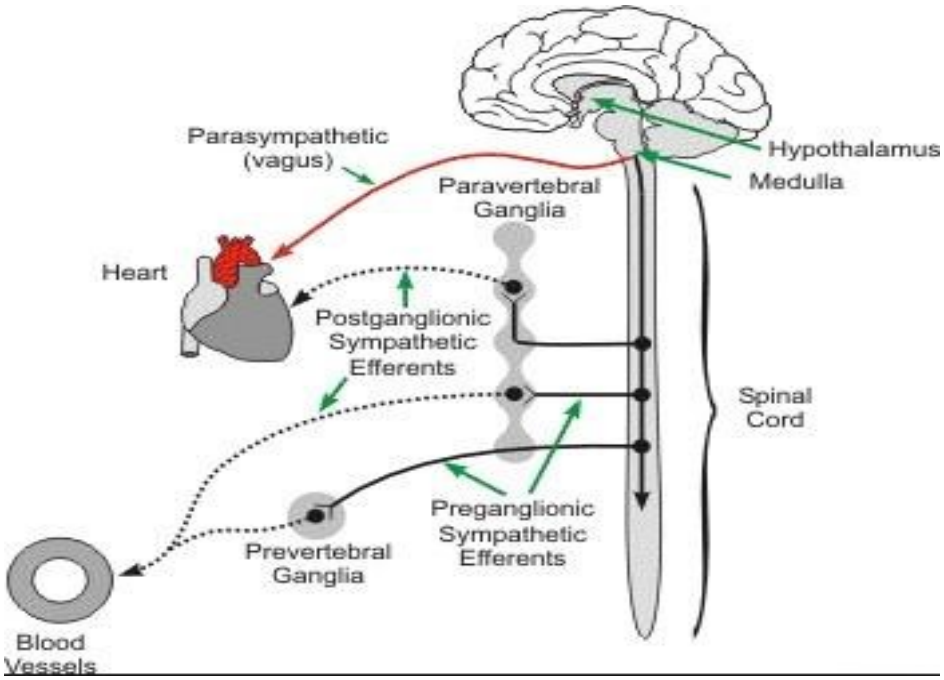

Figure 1. Both Autonomic Nervous System branches, parasympathetic nervous system and sympathetic nervous system efferent pathways to the heart and blood vessels (sympathetic innervation only) (56).

The SA node is a small group of specialized cardiomyocytes within the right atrium of the heart. The SA node allows the heart to have a continuous sinus heart rhythm, acting as a natural pacemaker. Although the heart has its' own intrinsic control of HR, the SA node is highly innervated by the ANS. Specifically, it is affected by the sympathetic nervous system via the spinal nerves (T1-4) and the parasympathetic nervous system via the Vagus nerve (see Figure 1). This configuration of innervation makes the SA node highly susceptible to contrasting actions by both branches of the ANS. Vagus (parasympathetic) stimulation of the SA node causes a decrease in HR. Without any influence by either branch of the ANS, the SA node has an intrinsic HR of approximately 100 beats per minute (122). At rest, the average healthy individual's HR is much less than 100 beats per minute (bpm), this is because at rest the parasympathetic nervous system predominates over the sympathetic system.

Afferent nerves from both divisions of the ANS relay information from the muscles and cardiovascular system to the medulla. From the cardiovascular control centers in the medulla efferent nerves then transmit impulses to the effector organs. The efferent arms of the SNS and PNS are made up of preganglionic neurons located within the central nervous system (CNS) that synapse with peripheral ganglia that innervate target effectors such as the heart and blood vessels (sympathetic innervation only) (see Figure 1).

An important regulator of the activity of the ANS is the arterial baroreflex, which originates from stretch-sensitive receptors (i.e., baroreceptors) in the carotid arteries and the aorta. Baroreceptor afferents travel to the cardiovascular centres in the medulla, beginning with the nucleus tractus solitarii (NTS). The NTS synapses with other cardiovascular medullary nuclei to modulate blood pressure by affecting HR and sympathetic nerve activity (SNA). This modulation of HR and SNA is regulated by the activity of the cardiovascular centers (NTS, rostral ventrolateral medulla, caudal ventrolateral medulla, etc.), which is relayed by the efferent neurons of the PNS and SNS. These efferent arms, more specifically the postganglionic neurons, innervate the effector organs, including: the SA node, smooth muscle of systemic blood vessels and other cardiomyocytes of the heart. The parasympathetic system acts via acetylcholine to cause hyperpolarization of the conductive cells of the SA node, resulting in a decrease in HR. Sympathetic fibers have the opposite effect causing an increase in HR, SV, the force of ventricular contraction and vasoconstriction of the smooth muscle of systemic blood vessels. However, this vasoconstriction of the blood vessels within the exercising skeletal muscle is opposed by metabolic by-product accumulation to cause vasodilation within the exercising musculature.

At the onset of exercise the parasympathetic system withdraws activity to cause the initial rise in HR, with a further increase in HR, SV, and vasoconstriction of non-active tissues caused by the activation of the SNS. During exercise, blood flow throughout the body increases by almost five-fold, with a greater distribution

of oxygenated blood being delivered to exercising muscles. This increased blood flow is affected by the state of the blood vessels, which subsequently affects blood pressure. Blood flow is affected by the viscosity of the blood (which remains relatively stable), the length of the vessel (which stays constant) and the radius of the vessel. This relationship, called Poiseuille's Law, can be described by the equation below:

$$\text{Resistance to Blood Flow} = [nL/r^4]$$

Where n is the viscosity of the blood, L is the length of the vessel and r is the radius of the blood vessel (122). Blood flow is proportional to the pressure difference across the system (highest pressure within arterial circulation minus the lowest pressure within the venous system), and inversely proportional to resistance, as described by the below relationship:

$$\text{Blood flow} = \text{change in pressure}/\text{resistance}$$

While an increase in pressure causes an increase in blood flow, a decrease in resistance (radius of the blood vessel) causes a greater increase in blood flow due to its' fourth-power mathematical relationship. The radius of a blood vessel is determined by the amount of vasodilation or vasoconstriction, in other words, the total peripheral resistance (TPR) of all the vasculature in the systemic circulation. Blood flow to different body parts is primarily determined by the sympathetic nervous system that innervates the smooth muscle that surrounds blood vessels. During periods of rest there is a constant amount of sympathetic activity that slightly constricts the blood vessels, referred to as vasomotor tone. An increase in sympathetic activity, such as that with exercise, increases vasoconstriction to

most areas in the body (122). During exercise vasodilation (a decrease in the resistance and increase in the diameter of the vessel) occurs in the exercising muscles as a result of a direct effect caused by the accumulation of metabolic by-products (e.g. carbon dioxide, lactate, hydrogen ions, etc.). This metabolic vasodilation overrides the SNS-mediated vasoconstriction caused by the EPR in active skeletal muscles. However, the EPR causes SNS vasoconstriction in the less active regions of the body, which helps to increase ABP during exercise. The SNS-mediated vasoconstriction of less or non-active regions allows for a greater percentage of cardiac output (product of HR and SV) to be redistributed to active skeletal muscle.

2.1.2. Cardiovascular Response to Exercise

The neural mechanisms associated with the rise in ABP that occurs during exercise are still not completely understood, but it is known that there are two main mechanisms responsible for the initiation and maintenance of the exercise response. The first, “Central Command” suggests that increases in arterial pressure are due to the direct action of higher brain structures in cortical and subcortical regions. The second is termed the “Exercise Pressor Reflex”, and is responsible for the cardiovascular changes associated with a reflex from active skeletal muscle afferents stimulated by muscle contraction. The EPR is further composed of the Metaboreflex, stimulated by chemical stimuli and the

Mechanoreflex, which is stimulated by mechanical (e.g. stretch receptors) stimuli (59).

Blood pressure is the product of cardiac output (Q) and total peripheral resistance of the systemic vasculature of the body. This relationship is called Ohm's law of the circulation and is described below:

$$MAP = Q \times TPR$$

Changes in Q and TPR are necessary to facilitate the increased needs of exercising muscle metabolism. During exercise, increases in MAP are produced mainly by a rise in Q, which is accomplished by increasing HR and SV.

At the onset of exercise CC acts to remove parasympathetic tone to achieve the necessary rise in HR to meet the metabolic demands of the exercising muscle. With an increase in exercise intensity sympathetic stimulation occurs to further increase HR and BP to meet the increased demands of the muscles. As exercise continues beyond the first minute, a further increase in HR, ABP and MSNA occurs as a result of the EPR. MSNA represents vasoconstrictor nerve activity directed to the blood vessels within skeletal muscle and occurs as pulse-synchronous bursts, with each burst time-locked with the cardiac cycle via the baroreflex (112). This reflex is comprised of two receptor groups that respond to exercise-induced afferent signals arising from within the working muscle. The muscle mechanoreceptors responds to mechanical stimuli such as stretch and pressure (94). More specifically, the Golgi tendon organs, located at the origin and insertion points and within the tendons of skeletal muscle, sense the

changes in muscle tension associated with exercise (94). When the muscle contracts during exercise, the Golgi tendon organs deform causing action potentials to be fired via group IB afferent fibers to the CNS. In contrast, muscle chemoreceptors respond to hypoxia and chemical stimuli, primarily the byproducts of skeletal muscle energy metabolism including carbon dioxide, lactic acid, hydrogen and potassium ions (94). This accumulation arises due to a mismatch between skeletal muscle blood flow and the rate of energy metabolism in the exercising muscles (94). When this accumulation occurs, after approximately a minute of exercise, signals travel to the CNS through group IV fibers to increase blood pressure (79). The resultant increase in ABP initiated by this reflex is designed to facilitate skeletal blood flow and correct this mismatch. Mechanoreceptive afferent signals are transmitted to the brain via type III afferent neurons, while chemoreceptive signals are transmitted via type IV afferent neurons. The delayed increase in ABP during exercise is thus initiated by transmitting these afferent signals up the spinal cord to the brainstem and higher cortical regions resulting in a further increase in sympathetic nerve activity to the heart and blood vessels (94).

2.1.3. Central Command and Exercise Pressor Reflex

CC signals act as a feed-forward neural mechanism either at the onset of exercise, or in response to an increase in exercise intensity. These neural signals innervate autonomic cardiovascular nuclei within the medulla of the

brainstem that produces the initial rise in HR and ABP at the onset of exercise by the withdrawal of vagal activity to the heart and increasing sympathetic nerve activity to the heart and blood vessels (37, 94).

At the onset of exercise, a rise in ABP is observed resulting from decreased parasympathetic activity and increased sympathetic activity. These rapid changes in ANS activity produce an immediate rise in HR and ABP caused by CC. CC is the parallel activation of ventilator, motor and autonomic pathways that occurs at the onset of exercise. CC originates in higher brain structures but the exact location of origin is not fully known. It is believed that these higher brain regions play a role in activating the areas in the medulla of the brainstem that control the cardiovascular response to exercise. The importance of CC influence on the cardiovascular response to exercise is seen in individuals with McArdle's disease (59). In this condition, individuals have a glycogen storage deficiency, and are thus unable to use glucose for exercise metabolism resulting in minimal lactate and acidosis compared to healthy individuals. Without these chemical by-products of exercise to stimulate chemoreceptors, the EPR is greatly blunted resulting in only small increases in blood pressure. Despite this impairment with EPR, McArdle's patients still have increases in ventilation, cardiac output and HR, which are often faster and greater than healthy individuals. This observation of McArdles's disease supports the concept that CC has an important influence on the cardiovascular response to exercise (59).

A study conducted by Goodwin, McCloskey and Mitchell (38) investigated the influence of CC on the cardiovascular control centres of the medulla by conducting a series of experiments on human subjects who were asked to maintain an isometric contraction of either the biceps or triceps while vibration was applied to the biceps tendon to stimulate afferent pathways from muscle mechanoreceptors thus increasing the amount of effort required. When the biceps were contracted, less CC was necessary to reach the given tension because the mechanoreceptors were activated. When the triceps were contracted, the mechanoreceptors were not activated in the bicep muscle (via vibration) and thus more CC was needed to reach the given tension (38). It was found that ABP and HR increased in both conditions, but there was a greater increase in ABP and HR when more effort was required, which stimulated an increase of signals from the CC. From their findings, Goodwin et al. (38) concluded that CC does influence the cardiovascular control centre at the onset of voluntary muscular contraction and in response to an increase in exercise intensity.

While the exact neural areas and pathways involved in CC are unknown, many studies have focused on discovering new information on the neural pathways and the descending signals to the cardiovascular autonomic system. Much of this work has used an individual's perception of effort during exercise to assess the level of CC. Since this rating of perceived exertion (RPE) is regulated by feedback and there is some sort of relationship between RPE and CC, CC may

have a feedback component to its' regulation (120). The perception of effort during an exercise task involves a complex interaction of different feedback signals, with some of these being able to affect the cardiovascular system independent of exercise, such as pain. It has been suggested that perhaps some of these feedback signals are integrated with the control of cardiovascular responses related to CC (120). Other studies have focused on determining where CC is located and how it exerts its' effects through the nervous system. Sander and colleagues (98) used blood oxygen level-dependent functional MRI to identify cortical and sub cortical sites involved with CC. Participants performed a submaximal static handgrip exercise followed by a period of PEI. During contraction increases in BOLD signal intensity occurred in the contralateral M1 and cerebellar nuclei and cortex. Bilateral activation of the medial and lateral dorsal medulla was observed during both contraction and PEI, likely representing the NTS and RVLM (98). Williamson et al. (120,121) have summarized the recent knowledge related to central command. They noted that various brain areas have emerged as being implicated in CC including the periaqueductal grey, anterior cingulate cortex, insular cortex and higher cortical areas such as the primary motor cortex. However, the exact role these areas have on CC-mediated cardiovascular responses during exercise (i.e., sympathoexcitatory versus vagal withdrawal) are still unknown. The general neural circuitry of CC is still vastly unknown, however, further investigation into the areas noted is necessary to unveil exactly how CC exerts its' effects. Various researchers have exposed areas including the periaqueductal grey, insular cortex, anterior

cingulate cortex and the primary motor cortex as areas implicated in CC (98, 120, 121). However, the role these areas, especially M1, play in CC is generally unknown. Higher brain structures such as M1 may exert their effects on the cardiovascular system through autonomic medullary centres. The action of M1 in CC may descend to elicit cardiovascular effects similar to the pathways of the corticospinal tracts used to elicit movement, but more research is needed to determine the true role of M1.

The EPR was first demonstrated by Alan and Smirk (2) in 1937, by showing that the rise in arterial pressure could be amplified if blood flow to and from the muscle was cut off (ischemic), and this increase in pressure remained even when the muscle stopped contracting. This led them to believe that the metabolites generated by the exercising skeletal muscle stimulated afferent fibers to increase arterial pressure in an attempt to rid the muscle of the metabolites (2). Later, McCloskey and Mitchell (78) confirmed that this pressor reflex of the cardiovascular system originated from within active skeletal muscle using an animal model (78). In anaesthetized, decerebrate cats isometric exercise of the muscles of the hind limb was stimulated causing a subsequent rise in ABP, with minor increases in HR and pulmonary ventilation. When they occluded the femoral artery and vein, blood pressure remained elevated to almost the same degree as during exercise until occlusion of the hind leg was halted. They came to the conclusion chemical factors within the exercising muscle that accumulate during exercise activate group III and IV muscle afferent fibers within the muscle

to cause the pressor reflex. While a small decrease in arterial pressure was witnessed during the occlusion phase, it was postulated that this was due to mechanoreceptors within the muscle that contribute to the pressor reflex when the skeletal muscle is active, but are no longer activated when exercise stops and occlusion occurs (78). Furthermore, the activation of group III and IV afferent neural fibers results in an increased ABP and HR response by the increased activation of sympathetic nerve activity and decreased parasympathetic activity (94).

Contraction of skeletal muscle leads to increased metabolism, which produces chemical by-products such as potassium, hydrogen ions, lactic acid, arachidonic acid, bradykinin, adenosine and analogues of adenosine triphosphate. These by-products have been shown to mainly activate group IV afferent neurons in muscle (78). These metabolites combine with autonomic factors (CC) to increase sympathetic tone and thus stimulate the increase in blood pressure. Metabolites are seemingly very important to the EPR during periods of ischemia, especially post-exercise ischemia (PEI). It is important to note that neither group III nor group IV are completely activated by mechanical and chemical stimuli respectively. For example, cyclooxygenase products cause an exercise pressor response via both the chemical/metabolic and mechanical afferent nerves. Furthermore, it was shown that work intensity had a direct effect on the magnitude of the mechanoreflex as isometric exercise of higher tensions produced a greater increase in ABP than lower tensions (20).

The importance of metabolites compared to mechanical stimuli for the activation of the EPR is especially important during periods of PEI. Occluding blood flow while a limb is contracting traps all the metabolites within the local circulation of the limb. When contraction ceases there is no longer a mechanical stimulus playing a role in activating the EPR, but the chemical by-products resultant from contraction metabolism are trapped within the local circulation and thus still exert their effects to activate the metaboreflex component of the EPR.

In order to differentiate the physiological responses elicited by CC versus the EPR we utilized isometric handgrip exercise (IHG) followed immediately by a brief period of PEI to the active forearm. Voluntary IHG exercise stimulates CC signals destined for the cardiovascular control centre in the medulla and the active skeletal muscles. When moderate intensity IHG (i.e., 40% of maximal contraction force) begins the mechoreceptors are activated by contracting muscles and when it is sustained for longer than one minute, an accumulation of metabolite by-products ensues, thus activating the EPR (38, 78). Therefore, both CC and the EPR contribute to the increase in HR, ABP and MSNA during the latter stages of IHG exercise (see Figure 2). Immediately following the IHG exercise, blood flow to the previously active forearm will be temporarily occluded, thus “trapping” the chemical by-products formed during exercise within the forearm. Trapping these by-products will keep the EPR active until circulation is

restored. However, since the participant is no longer engaged in voluntary muscle contraction, the CC signals have ceased.

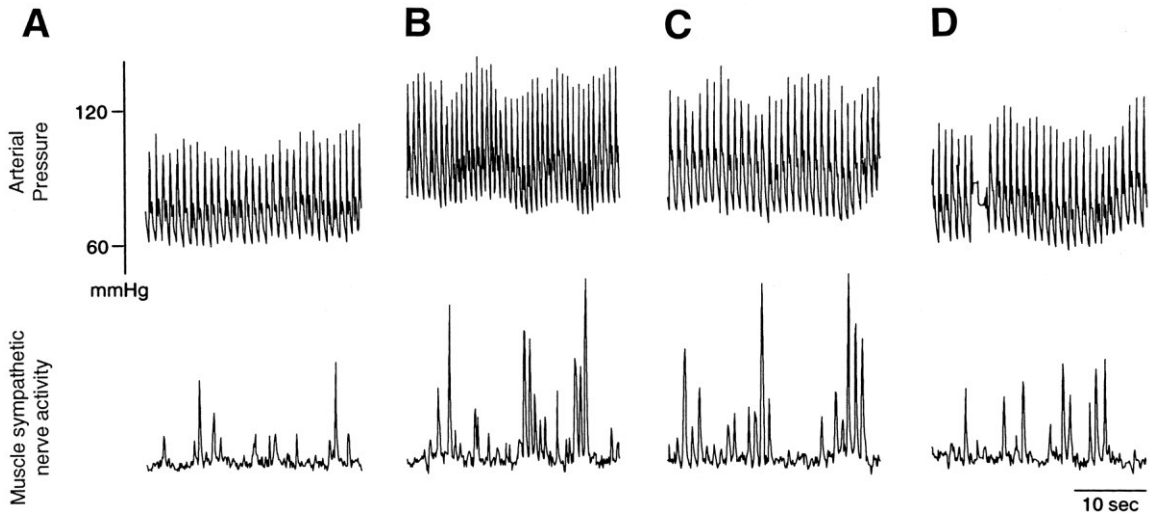

Figure 2. Simultaneous recordings of the blood pressure waveform and MSNA at rest (A) and during handgrip exercise (B), post-exercise ischemia (C), and recovery (D) (57).

2.1.4. Arterial Baroreflex

Baroreceptors are mechanoreceptors and are described as being stretch-sensitive therefore they are stimulated by stretch of the carotid and aortic sinuses that occurs when an increase in blood pressure occurs. When stimulated, the baroreceptors fire action potentials at a rate proportional to the amount of stretch; the greater the stretch related to a higher rise in blood pressure, the greater the rate at which the baroreceptors generate action potentials. The afferent baroreceptor input travels though the glossopharyngeal (from the carotid sinus) and vagal (from the aortic arch) nerves to the nucleus tractus solitarii (NTS) (see Figure 3). The NTS is a vertical column of grey matter within the medulla

oblongata through which a white bundle of nerve fibers called the solitary tract runs. This bundle contains fibers, which synapse with the neurons of the NTS which projects to various other cardiovascular centers in the brainstem (see Figure 4) and higher brain regions forming autonomic relay circuits (53).

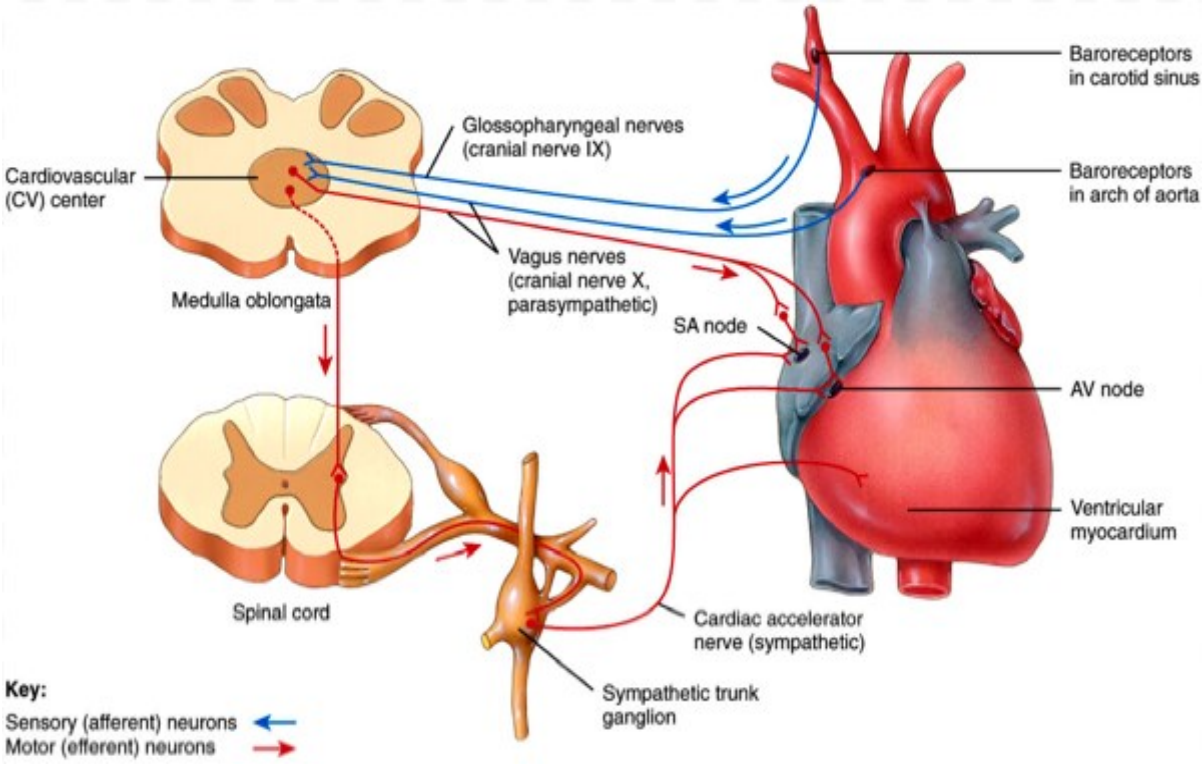

Figure 3. Baroreceptor anatomical location in the carotid sinus and arch of aorta, with sensory afferent neurons (blue) travelling the brainstem nuclei and motor efferent neurons (red) travelling to the sinoatrial node (110).

Higher structures in the brain have been shown to be important for regulating the parasympathetic and sympathetic output from medullary centres that control cardiovascular function (17, 70-72, 100, 123). The NTS can thus be thought of as the main site for the transfer of information and initiating the proper response regarding cardiovascular regulation. Experimental lesions to the NTS in animal

models have shown acute hypertension resulting from the inability to properly regulate blood pressure (31). Beat to beat changes are detected by the firing rate of these baroreceptors, which relay this information to the NTS for continuous regulation of blood pressure. Input from the baroreceptors to the NTS is via excitatory glutamate synapse. The NTS then projects, via output neurons to the caudal ventrolateral medulla (CVLM) and nucleus ambiguus (NA). This network establishes the set point by which HR and sympathetic nerve activity regulate blood pressure (see Figure 4). The CVLM acts as the sympathetic “brake” by directly controlling the rostral ventrolateral medulla (RVLM). The RVLM is the main source of pre-ganglionic sympathetic activity, containing motor neurons that project excitatory fibers to pre-ganglionic nerve fibers in the spinal cord. The RVLM provides the sympathetic drive that causes vasoconstriction of the blood vessels, cardiac contractility and blood pressure increase in response to exercise (35). The CVLM acts to inhibit the RVLM, the sympathetic “gas pedal”, via inhibitory γ-Aminobutyric acid (GABA) synapses when blood pressure needs to be lowered. The NA is a parasympathetic center and thus excitatory synapses from the CVLM increases its function. At rest this keeps parasympathetic tone greater than sympathetic to keep a constant normal blood pressure.

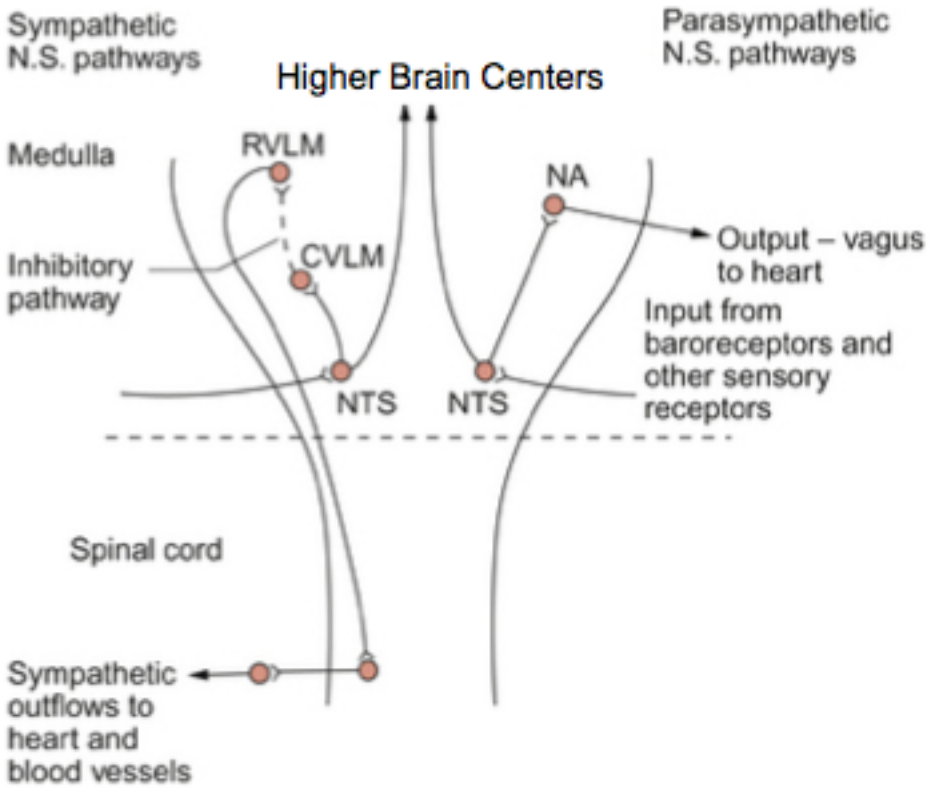

Figure 4. Medullary nuclei and sympathetic and parasympathetic pathways involved in the control of blood pressure regulation in humans. CVLM=caudal ventrolateral medulla; NA=nucleus ambiguous; NTS= nucleus tractus solitarius; RVLM=rostral ventrolateral medulla (18).

The arterial baroreflex is one of the mechanisms of the human body for regulating blood pressure to increase during exercise. This negative feedback loop consists of baroreceptor cells in the aortic arch and carotid sinus that relay information back to medullary cardiovascular centers in the brainstem that exert changes in sympathetic and vagal tone to adjust HR and maintain homeostatic blood pressure. The set point the baroreflex operates at rest and resets during exercise to increase blood pressure. If the baroreflex did not reset to increase blood pressure during exercise the system would increase excitatory activity of the NTS resulting in increased parasympathetic activity from the NA and excites

CVLM to inhibit the sympathetic outputs from the RVLM. This would mean during exercise there would be a reduction in SNA and HR to lower blood pressure, which does not occur (Figure 4). In reality, somatosensory input form the exercising muscles combined with an increased firing rate from the baroreceptors acts to control the amount of sympathetic activation to regulate blood pressure. Increased firing rate from the NTS excites the parasympathetic NA to reset the baroreflex laterally (see Figure 5C i) (91). Then with somatosensory input, the NTS excites the CVLM to decrease sympathetic output from the RVLM. Excitatory synapses from the RVLM project to the intermediolateral nucleus (IML) within the spinal cord, which stimulates pre-ganglionic sympathetic neurons, which then synapse with ganglionic sympathetic efferent neurons to increase HR, and MSNA and thus ABP (see Figure 5C ii) (91). The overall removal of vagal or parasympathetic tone and increase in sympathetic tone results in the increase in HR, MSNA and ABP, as observed during exercise (55, 91)

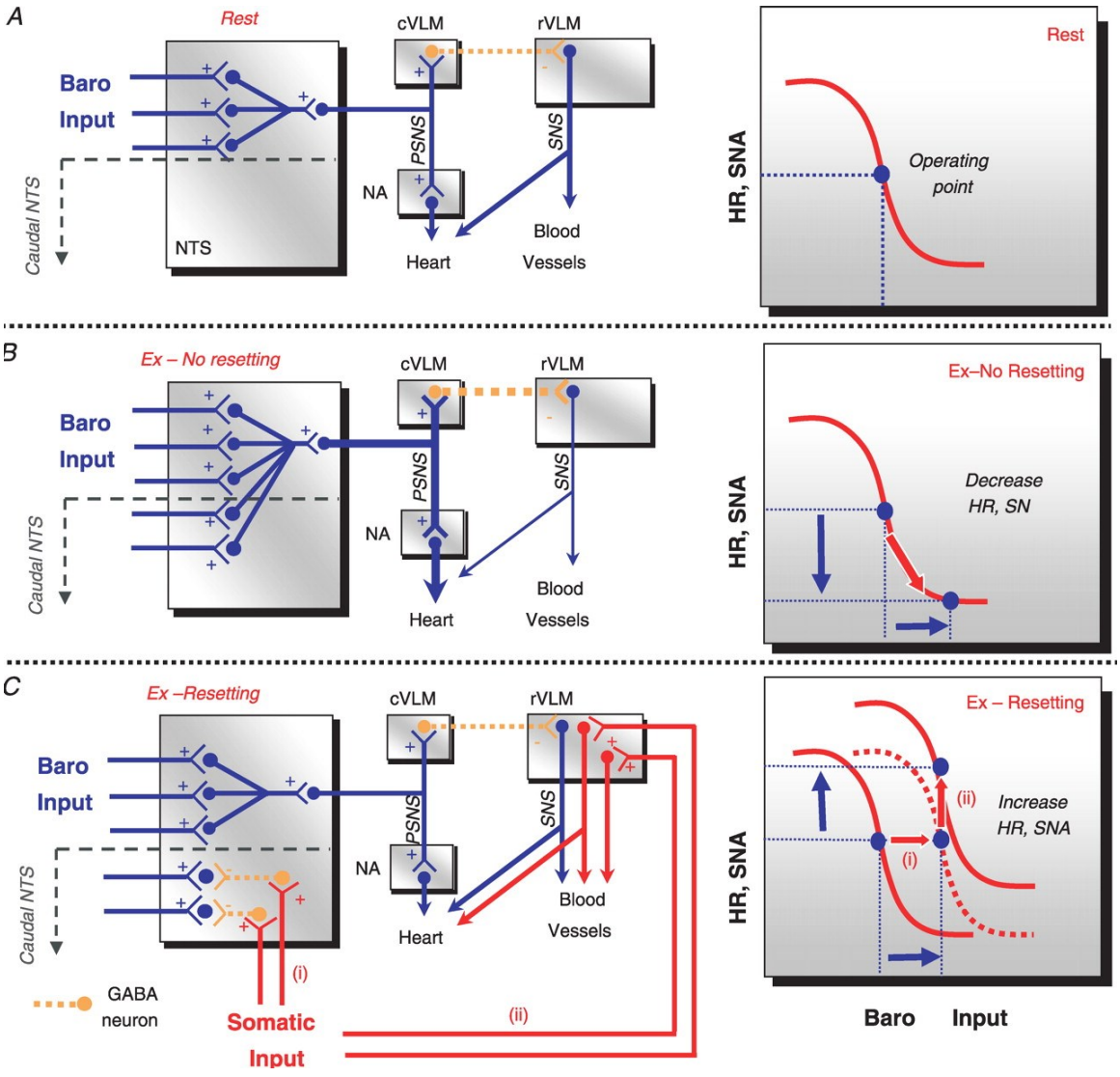

Figure 5. Baroreflex resetting during exercise. The Baroreflex and associated controlling medullary nuclei are shown demonstrating how a set point is achieved during rest (A) in absence of the baroreflex (B) and during exercise (C) (91).

2.2 Background on Experimental Techniques

2.2.1 Heart Rate Variability

Heart rate variability (HRV) is the fluctuation in HR that occurs from beat-to-beat changes in the interval between consecutive heart beats during the normal sinus

rhythm of the heart (108). Heart rate variability is a complex physiological phenomenon that is affected by the inputs to the SA node of the heart. The SA node is a group of pacemaker cells with the wall of the right atrium. The cardiomyocytes of the SA node generate the initial action potentials (electrical activity) that trigger the contraction of the rest of the heart. The SA node is innervated by parasympathetic and sympathetic fibers that influence the rate at which action potentials are generated, thus influencing the rate at which the heart contracts. The SA node can also be affected by circulating hormones such as epinephrine and norepinephrine, as well as serum ion concentrations and cellular hypoxia, but these have little effect on short-term fluctuations in HR. Since these effects are too slow to affect HRV in small windows of time, changes in HRV in the short-term can provide information on the state of the divisions of the autonomic nervous system, the parasympathetic and sympathetic nervous systems (99). The parasympathetic innervation of the SA node originates predominantly from the efferent branches of the right vagal nerves in healthy individuals at rest having a depressor effect to slow down the intrinsic HR down to 60-80 beats per minute. Parasympathetic activity is mediated by a ligand-gating agent, acetylcholine (ACh), acting through a G-protein that activates iK/ACh channels (124). This parasympathetic activity increases the outflow of potassium slowing the SA node by making the resting cardiac membrane potential more negative. Therefore, a greater stimulation and amount of time is needed to reach threshold before the next action potential from the SA node is delivered. Increased sympathetic tone to the SA node increases the rate at which

SA node action potentials are generated resulting in an increased HR during periods of stress and exercise. The dual innervation of the SA node is never balanced or constant resulting in beat-to-beat variations in heart, which in turn can provide information on the modulation of cardiac vagal and sympathetic activity (1, 87, 99).

Heart rate variability can be evaluated using time or frequency domain methods. The more simple time domain methods determine instantaneous HR or the intervals between normal QRS complexes. These intervals, termed normal-to-normal (NN) intervals, are used to calculate the square root of variance or the standard deviation of the NN interval (SDNN). In frequency domain methods, power spectral analysis (PSD) of the tachogram (series of R-R intervals or intervals between the normal QRS complexes) supplies information on how power (variance) is distributed as a function of frequency using mathematical algorithms (74).

Previous research utilizing certain pharmacological agents that block sympathetic and parasympathetic inputs to the SA node, show that almost all variations in HR greater than 0.03 Hz are due to the fluctuating changes in autonomic activity (1, 87, 99). Furthermore, HR in the high frequency band (HF) of 0.15 to 0.40 Hz are almost exclusively due to efferent parasympathetic or vagal tone as shown by clinical and experimental observations of autonomic procedures such as electrical vagal stimulation and vagotomies where a branch or branches of the

vagus nerve are cut (108). The low frequency (LF) component of 0.06 to 0.10 Hz, is more debated, with some believing it represents solely sympathetic activity (especially when expressed in normalized units) and others arguing it represents both sympathetic and parasympathetic activity (108). The inconsistency with the LF component arose from the observation that during certain conditions that are associated with excitation of the SNS, there was actually a decrease rather than increase in the absolute power of the LF component (108).

The concept of sympathovagal balance originated as a way to quantify the activation of sympathetic or parasympathetic nervous systems because in almost all physiological conditions the activation of one branch of the ANS involves the inhibition of the other (74). An important point regarding the HRV in the HF band is that the main driver of HRV is due to respiration, which produces a vagally-mediated respiratory sinus arrhythmia. The extent of the HF variation is mainly dependent on the depth of respiration, which can vary greatly over time and between individuals, therefore the depth of respiration must be taken into account using a strain gauge device around the thorax.

2.2.2 Portapres® Continuous Blood Pressure Measure System

Blood pressure is a frequently measured physiological parameter that is highly variable depending on blood vessel properties, vessel wall smooth muscle tone, respiration and diurnal rhythm. Blood pressure variability poses an issue as

moment-by-moment changes are important for understanding underlying control mechanisms, but they are also difficult to measure and record accurately. There are two general categories of measuring blood pressure, direct invasive and indirect non-invasive. Both methods have their respective pros and cons. Direct invasive measures of human blood pressure require the use of a hollow needle inserted into an artery connected to a long liquid-filled catheter with a pressure transducer situated at the heart level. While this method can provide a great deal of information (baroreflex sensitivity, SV, cardiac output, systemic vascular resistance), it is highly invasive and has many associated risks and ethical concerns. Furthermore, it requires a highly skilled and trained individual to perform the technique safely and to obtain accurate measurements (64).

There are several non-invasive indirect techniques, but the current study will be utilizing the Portapres® system, based on the Penaz-Wesseling method (64). The Portapres® system provides an absolute measure of blood pressure from the finger as well as the ABP waveform. Since the Portapres® derives continuous estimates of cardiac output from the peripheral pulse, it has the potential to be an extremely valuable physiological and clinical tool. Portapres® Model-2 measures blood pressure from the finger using the arterial volume-clamp method of physiologist J. Penaz, and the “Physiocal” (physiological calibration) criteria method by Wesseling to account for the criteria for the proper unloading for the finger arteries. Any slow movements of the hand are corrected by the height correction unit of the Portapres® (64, 89, 119). The volume-clamp method

involves continuously monitoring changes in finger arterial volume with a photoplethysmograph (a LED light source and receiver) that is inside a finger cuff. The signal from the photoplethysmograph allows finger arterial diameter to be kept constant by adapting the pressure in the cuff air bladder. The pressure needed to keep the artery at a constant diameter is then used to estimate intra-arterial pressure.

2.2.3 Microneurography

Two types of efferent sympathetic nerve activity, MSNA and skin sympathetic nerve activity (SSNA) are commonly recorded as measure of sympathetic function. MSNA is controlled mainly by the arterial baroreflex and is modified by afferent signals from nerves sensitive to skeletal muscle metabolic activity, arterial chemoreceptors and CC. The SSNA targets skin vasculature and sweat glands and responds primarily to CC (66). Microneurography involves directly recording efferent post-ganglionic sympathetic vasoconstrictor nerve traffic directed towards resistance blood vessels within skeletal muscle through the use of a tungsten microelectrode inserted into a superficial nerve, with a reference electrode in nearby non-nerve tissue (38). It is minimally invasive, with little to no side effects (25, 113). Direct recordings of MSNA will be made using sterilized unipolar tungsten microelectrodes (FHC; Bowdoin, Maine) inserted percutaneously into a muscle nerve fascicle (active microelectrode) of the common fibular nerve and 2-3 cm subcutaneously from the active recording site

(reference microelectrode). This type of microelectrode enables the recording of myelinated fibers and has a diameter of about 1 μm at the tip that is inserted intraneurally. Nerve discharges are calculated by the difference in voltage between the recording electrode and the reference electrode. The electrode records the raw signal that is amplified and filtered, which produces a neurogram of the recorded MSNA (see Figure 2). In this study, MSNA was recorded from the common fibular nerve on the lateral side of the popliteal fossa at the head of the fibula. The nerve wraps around the head of the fibula and is palpable at this location. The general nerve site is located by muscle twitches or paresthesia caused by stimulation through the skin and then the recording electrode is inserted. Further small adjustments are needed to locate the sympathetic nerve fibers, which are found while listening and observing the recordings for bursts of sympathetic activity.

2.2.4 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive and painless procedure that uses a series of brief magnetic pulses applied to the outside of the head to excite or inhibit cortical regions in human brain. Its' application and use in a wide variety of disciplines has continued to grow since its' first use in 1985. The device involves a coil which is placed over the intact scalp and when charged induces a magnetic field which transmits through the scalp to produce

an electrical current. This electrical current elicits changes in neural activity by increasing or decreasing neuron excitability depending on stimulation parameters used. TMS can be applied one stimulus at a time, single-pulse TMS, in pairs of stimuli separated by a variable interval, paired-pulse TMS, or in trains, repetitive TMS (rTMS). Repetitive TMS can be used to transiently facilitate (excite) or disrupt (inhibit) neural activity, with the desired outcome (facilitation or inhibition) achieved based on the frequency of stimulation used (93). In general, rTMS performed at low frequencies (≤ 1 Hz) can be used to temporarily disrupt the function of a specific cortical region while rTMS at high frequencies (≥ 1Hz) excites cortical regions.

Repetitive TMS may be more beneficial for theurapeutic purposes due to its longer duration of after-effects compared to single-pulse TMS (49). The longer duration after-effect period usually lasts 30-60 minutes but depends on various parameters including the number of pulses applied, the rate of application and the intensity of each stimulus (80, 88). A more recently developed type of rTMS entitled Theta Burst Stimulation (TBS) is being utilized to induce further cortical changes in the human primary motor cortex and prolong the after-effects of stimulation. TBS stimulation is at a much more rapid rate than traditional rTMS for a much shorter period of time, resulting in consistent, long-lasting effects on motor cortex physiology (49). TBS is being increasingly used in replace of more traditional TMS protocols because it uses fewer pulses and a shorter duration of stimulation to elicit its' results (85). Specifically, TBS follows specific paradigms;

each paradigm is based off the TBS pattern in which trains of 3 pulses of stimulation are given at 50 Hz, so that each pulses is separated by 20 ms. The continuous theta burst stimulation paradigm (cTBS) is a 40-second train of uninterrupted TBS for a total of 600 pulses. The blocks of 3 pulses are applied at 50 Hz so that there is 20 ms between each individual pulse and then these trains of 3 pulses are applied at 5 Hz so that there is 200 ms between each train (see Figure 6 A). With intermittent theta burst stimulation pattern (iTBS), the train of 3 pulses is still applied at 50 Hz, but 10 trains are applied within 2 seconds with an inter-train interval of 200 ms (5 Hz). This block is repeated every 10 seconds for 200 seconds for a total of 600 pulses (see Figure 6 B). Of particular note is the comparison of TBS to more traditional TMS paradigms; one session of cTBS lasting 40 seconds elicited changes in cortical excitability for an hour, compared to it's single pulse TMS counterpart, which involved 20 minutes of stimulation with changes only lasting 20 minutes following stimulation. Furthermore, the shorter duration of stimulation has the added benefits for participants and researchers by being more convenient with lower levels of participant discomfort (49). Huang et al. (49) tested the effects of intermittent and continuous TBS by measuring cortical excitability using single pulse TMS before and after TBS stimulation to evoke a motor evoked potential (MEP) in the right first dorsal interosseous muscle (dominant hand in all subjects). Results determined that after cTBS, MEPs were suppressed for at least 20 minutes up to an hour, while intermittent TBS induced facilitatory effects. The current study utilized the TBS parameters previously found to be facilitative and inhibitory in order to elicit

known effects on cortical excitability. Utilizing TBS protocol over single or paired pulse TMS has the added benefit of decreased stimulation time that assisted the researcher and minimized the participants' involvement time.

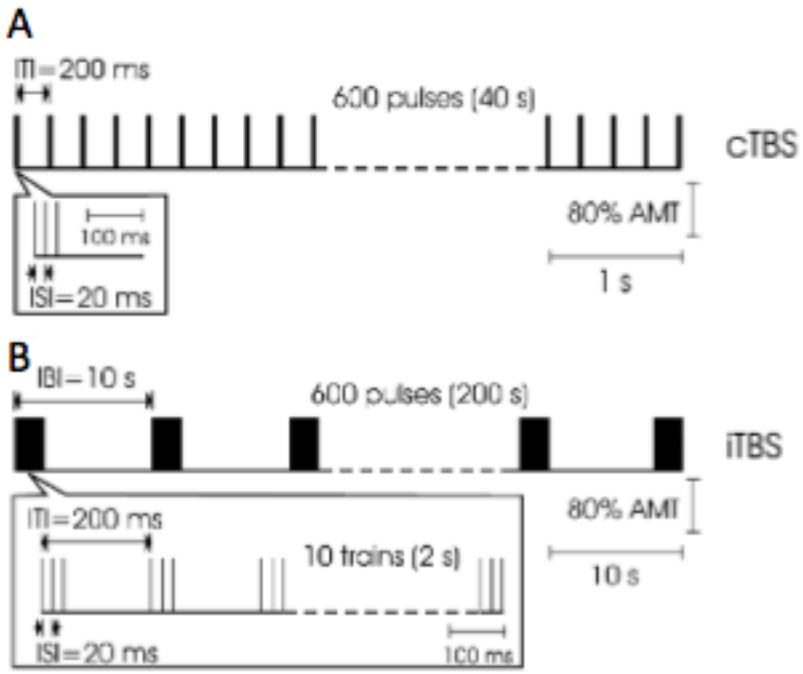

Figure 6. Illustration of the two TBS paradigms used in the current study. (A) Illustration of the cTBS pattern of a train of 3 pulses with an inter-stimulus interval (ISI) of 20 ms (50 Hz), and an inter-train interval (ITI) of 200 ms (5Hz) for a total of 600 pulses and a duration of 40 seconds. (B) Illustration of the iTBS pattern of a train of 3 pulses with an ISI of 20 ms (50 Hz), and an ITI of 200 ms (5 Hz). Blocks of ten trains are applied in 2 seconds, with an inter-block interval of ten seconds, for a total of 20 blocks applied in 200 seconds (600 pulses total) (82)

2.3 Animal Research on the Motor Cortex

Animal research has greatly contributed to our knowledge of how blood pressure is regulated by higher brain structures, but it cannot definitively prove that these findings relate directly to the conscious human model. While it is well understood that the various brain regions that compose the CC work to regulate

cardiovascular control, the details concerning CC are not known. It is still unknown what brain areas control different aspects of cardiovascular regulation, but the motor cortex has emerged as an important area worth more investigation (17, 34, 72, 100).

Research on the medullary nuclei associated with cardiovascular control has been performed on cats, resulting in promising information on how these nuclei receive and send information to control cardiovascular measures including ABP and sympathetic activity. Dampney et al. (24) found that the NTS is the main area to receive inputs from the arterial baroreceptors, and it then sends excitatory projections to the CVLM. The CVLM then employs its' inhibitory control on the RVLM which is the main output nucleus responsible of vasoconstrictor sympathetic activity to control ABP. In terms of activity levels, this means when input to the baroreceptors is low (as such when DBP is low), NTS and CVLM activity is low and RVLM activity is high because the baroreceptor's negative feedback has been removed (24). This work on medullary nuclei provides more information on cardiovascular autonomic control, but also suggest more research on higher brain structures is needed to provide a better picture of how CC exerts its' control and where it is located.

Previous research on animals has revealed that stimulation of areas of the brain associated with movement control illicit affects on the cardiovascular system. Various mammals, including monkeys, cats, dogs, rabbits and rats have all exhibited changes in blood pressure and/or HR upon stimulation of the motor

cortex (44-46, 65). Stimulation parameters and devices varied, especially in earlier studies when stimulation parameters were not known to be inhibitory or facilitative. This resulted in fluctuating changes in HR and ABP depending on how much and how quickly the motor cortex of the animals was stimulated in a particular study (44-46, 65). Hong et al. (45) used rTMS of 10Hz, 20% above resting motor threshold (the minimal stimulus intensity that produces a minimal motor evoked response, RMT) on Wistar-Kyoto rats, finding a significant decrease in ABP and a transient decrease in HR compared to baseline, unstimulated values. They suggest that the decrease in ABP was due to the inhibition of the activity of the SNS, and not the activation of the PNS. The decrease in HR was so transient that it is likely not implicated in the decrease in ABP (45). Research on animals has provided researchers with some knowledge on what areas of the brain may be involved in the control of cardiovascular measures, but they are also restricted by certain limitations. The brains of animals are often smaller and differ in morphology thus the areas being stimulated are often difficult to definitively identify. The work performed on animals has led to research performed on humans as stimulation devices and knowledge of the human cortex evolved.

2.4 Human Research on the Motor Cortex

The motor cortex of the human brain is responsible for the control and execution of any voluntary movements and is located at the posterior frontal lobe

immediately anterior to the central sulcus that separates the frontal and parietal lobes. The motor cortex is composed of several different areas that work together to produce movement, but individually have their own functions. Specifically, the primary motor cortex, in the precentral gyrus, controls the gross movements of the muscles within the human body. Each muscle group is arranged somatotopically and the amount of cortical tissue devoted to controlling a specific body area is proportional to the amount of innervated tissue in the area. This means that body areas that have more complex and fine motor movement patterns have greater representation within M1. For example, the hands and fingers have a large representation in M1 compared to the trunk and legs, which have more simple or gross movement patterns. The hands and fingers have a large area of tissue devoted to its' control, and thus is relatively easily located on the scalp with single electrical stimulations.

Skeletal muscles are controlled by signals conducted by efferent lower motor neurons that can originate from the spinal cord, lower levels of the brain and the motor cortex. With increased movement complexity, for example from a simple reflex to movements that require the process of thought, the level of control moves from just the spinal cord to input from the motor cortex. While motor reflexes, like when you touch a hot stove and pull away immediately, are pre-programmed within the spinal cord, a more complex movement such as an isometric handgrip exercise, is controlled with input from the primary motor cortex. The main pathways involved when an individual decides to perform a

movement include the corticospinal tracts, which connect the motor cortex to the spinal cord. The pre-frontal region of the cortex activates the upper motor neurons of M1 to commence the efferent activity through the spinal cord to cause movement such as with initiation of an isometric handgrip exercise. The axons of these corticospinal tracts originate in layer V (Betz cells) of the motor cortex. The axons of these upper motor neurons pass through the internal capsule of the forebrain to the cerebral peduncle at the base of the midbrain. From there they continue through the pons to the ventral surface of the medulla to form the medullary pyramids. A tract from each hemisphere (left and right) divides into the lateral spinothalamic tract (crosses the midline to the other side of the spinal cord) and the ventral spinothalamic tract. Fibers of the lateral spinothalamic tract connect to interneurons, which project to motor neurons to control the movement of the muscles of the limbs and digits. Fibers of the ventral spinothalamic tract synapse with interneurons, which connect with motor neurons that control the movement of the midline of the body.

Research involving areas of the human cortex has implicated various regions in the control of cardiovascular measures. Many studies have inadvertently found evidence linking cortical activity to cardiovascular control. There is evidence that certain mental states that are cortically controlled such as chronic mental stress and depression, cause hypertension, and increases in MSNA, norepinephrine and cardiovascular morbidity (5, 33). While these studies didn’t link any particular cortical region to the changes in cardiovascular measures, they still provide

convincing evidence that the cardiovascular system is inexplicitly linked to cortical activity. Findings linking the cortex to changes in cardiovascular measures, combined with research investigating CC and EPR have led to researchers seeking the specific regions associated with the control of cardiovascular measures.

Yoshida et al. (123) investigated the effects of rTMS on HRV, a measure of autonomic function. Their results showed a transient increase in LF after 0.2 Hz stimulation at 90% RMT for 350 seconds over the vertex, but not after the sham condition. This finding suggests that rTMS may influence the ANS. Similar results were found by Udupa et al. (112), who found a decrease in the LF:HF ratio in depression patients as demonstrated by measures of HRV after application of rTMS. These studies may suggest that modulating the activity the cortex using external stimulation has a modulatory effect on measures related to cardiovascular control.

Gulli and colleagues (39) investigated the use of low frequency rTMS (8 minutes of 0.7Hz at 100% resting motor threshold) on both hemispheres of the prefrontal cortex. They found that this stimulation induced significant bradycardia and a significant drop in LF:HF ratio of HRV after stimulation of both hemispheres They explained that this is likely due to increased vagal activity as they also observed an increase in normalized HF (with the right hemisphere being significantly higher), which is solely representative of vagal activity (39).

Recent developments in technology and experimental design have allowed researchers to couple the simultaneous use of microneurography and functional magnetic resonance imaging (fMRI) so that baroreflex activity can be seen in real time (71). The time-varied fluctuations in blood oxygen level dependent (BOLD) signal intensity can now be seen in cortical regions and important cardiovascular medullary nuclei such as the NTS, CVLM and RVLM, in combination with the MSNA recordings. A subsequent study by Macefield, James and Henderson (70, 71) used his method of concurrent fMRI and MSNA recordings to determine that there is a direct link between high cortical neuronal activity and high MSNA activity. The results of this study showed that fluctuations in BOLD signals (a marker of neuronal activity) of the cortex, matched the fluctuations in the recorded MSNA signal (70, 71).

Schlindwein and colleagues (100) sought to identify the brain regions associated with sympathetic activity generation at rest using fluorodeoxyglucose-positron emission tomography (FDG-PET). They measured autonomic function parameters including blood pressure, HR, HRV and plasma catecholamines while participants underwent the FDG-PET (100). They determined from their results, that sympathetic activity is positively correlated to the primary motor cortex, with higher motor cortex activity being associated with high sympathetic activity at rest (100). Schlindwein (100) was able to establish a connection between high motor cortex activity and high sympathetic activity in humans at

rest, suggesting the motor cortex may play a role in the modulation of the cardiovascular system

It has been shown that different cortical and subcortical structures innervate autonomic cardiovascular nuclei within the medulla of the brainstem that produce the changes in HR and ABP by modulating sympathetic activity to the heart and blood vessels (39, 70, 71, 100, 112, 123). This research has elucidated that the motor cortex and caudate nuclei influence the control of the cardiovascular system (39, 70, 71, 100, 112, 123). Previous evidence such of that of Schlindwein and colleagues, combined with advances in experimental technologies has allowed many researchers to specifically investigate the motor cortex as an area of interest implicated in cardiovascular control (17, 34, 72, 100).

Anodal transcranial direct current stimulation (tDCS) has been shown to be comparable to iTBS in that it acts on the motor cortex increasing excitability as shown by increased MEP amplitude obtained using TMS (6). Clancy and colleagues (17) investigated the effects of anodal tDCS over the motor cortex while measuring non-invasive autonomic measures including heart rate variability, muscle sympathetic nerve activity and baroreflex sensitivity (17). tDCS involves the use of two electrodes placed on the scalp and the current flows between them so nerve polarization occurs over the target area. Excitatory anodal tDCS over the motor cortex on the non-dominant hemisphere resulted in

an increase in the LF: HF ratio of HRV, which continued post-stimulation. The normalized HF component of HRV decreased during the post-stimulation phase. These changes in heart rate variability suggest that excitatory anodal tDCS stimulation may increase sympathetic activity (17). The decrease in the high frequency power also suggests that vagal activity may be reduced. Despite there being high inter-individual variability between participants when measuring MSNA, it is still highly reproducible within a given individual (40). Despite no significant differences in baroreflex sensitivity, HR and blood pressure, there was a significant increase in MSNA burst incidence during stimulation and increased further post-stimulation. They concluded that anodal tDCS over the primary motor cortex could shift cardiac autonomic balance so that sympathetic activity dominates (17).

A study utilizing TMS on human participants motor cortex found an acceleration of HR and a triphasic ABP response (initial increase followed by a decrease and subsequent increase) following 10 single pulses at a frequency of 20 Hz for a total of 500ms (34). In subsequent study by Macefield et al. (72) TMS was used to determine whether a time-locked cortical stimulus could interfere with the sympathetic vasomotor drive to human skeletal muscle. It was found that cortical stimulation over the vertex caused a transient inhibition of the sympathetic discharges and decreased skin blood flow, when the stimulus was delivered 200-400 ms after the R-wave of the electrocardiogram, which was followed by an increase in sympathetic activity. Cortical stimulation over the hand area of the

primary motor cortex also caused inhibition of sympathetic bursts and decreased skin blood flow, but to a smaller degree (72). This transient inhibition followed by an increase in sympathetic activity is direct evidence that the modulation of cortical areas, mainly the motor cortex, has a direct influence on cardiovascular control (see Figure 7). Specifically, facilitating the motor cortex activity, such as with excitatory iTBS, may cause a subsequent increase in cardiovascular measures such as MSNA.

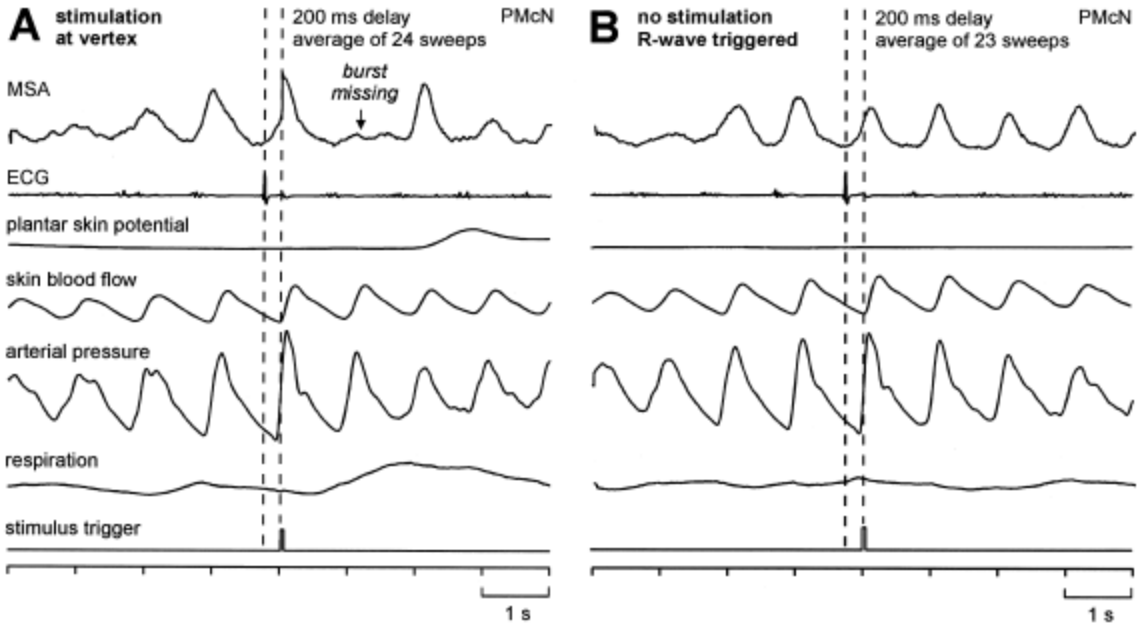

Figure 7. Averaged records of muscle sympathetic nerve activity (MSA), ECG, plantar skin potential, skin blood flow, arterial pressure and respiration from one subject of Macefield et al. (56). (A) Measures after stimulation delivered to the vertex 200 ms after the R-wave show a missing second burst of sympathetic activity compared to control (B) where no stimulation was delivered (72).

While the research available on the motor cortex's potential implication on cardiovascular autonomic control is limited, there is even less research investigated the effects of stimulation over the motor cortex on cardiovascular

measures. Though limited, the studies that have been completed using stimulation devices such as tDCS and TMS provide evidence that modulating motor cortex excitability has resulted in changes in cardiovascular measures. More specifically, studies utilizing excitatory stimulation paradigms have observed increases in cardiovascular autonomic measures (17, 34, 72)

Researchers have also used individuals who have experienced a stroke in the motor cortex to observe changes in cardiovascular measures. When an individual experiences a stroke, there is lasting damage to the affected area that can affect neuronal processes. In the case of a stroke in the motor cortex, the individual often experience motor deficits that impact movement. These deficits extend beyond the outwardly visible impairment of motor function. The research on stroke patient populations has shown that they also experience disturbed regulation of blood pressure, HR and MSNA during rest, exercise and PEI periods (61, 80, 116). Specifically most stroke patients experience a drop in ABP within the initial ten days after stroke, with a mean SBP drop of 20 mmHg and DBP drop of 10 mmHg (116). The impact the lesions caused by stroke can have on the cardiovascular system provides further evidence that the motor cortex is linked to cardiovascular control. Specifically, lesions in the motor cortex caused by stroke are comparable to the inhibitory effects of cTBS, as motor cortex stroke patients experience a drop in ABP (116). Stroke patients also have low HRV, which may suggest that causing temporary inhibition such as with cTBS, may lead to a decrease in HRV (61).

Furthermore, in a study of stroke patients compared to age-matched, healthy controls, the stroke patients experienced a significant increase in blood pressure, HR and MSNA during a 2-minute 35% IHG exercise. However, when compared to the control group, the magnitude of these cardiovascular responses was attenuated (81). These results suggest that isometric exercises performed by those who have experienced a stroke may successfully activate the EPR, but the attenuated response may be related to brain lesions affecting the activation of CC.

The evidence from these studies targeting the motor cortex have revealed that it may play an important role in the regulation of the cardiovascular system. While it is still not understood what extent or what role it plays, the motor cortex is an area of interest to help explain the autonomic control of the cardiovascular system. Studies targeting the human motor cortex have the potential to reveal new information regarding the control of HR, blood pressure and sympathetic tone at rest and during exercise. Specifically, using stimulation devices such as TMS, to facilitate or inhibit the excitability of M1 may lead to increases or decreases, respectively, in resting cardiovascular measures.

2.5 Knowledge Gaps

There currently is research that has implicated CC in the control and regulation of cardiovascular function (2, 38). Despite researchers discovering various medullary, subcortical and cortical brain regions that could potentially play a role in CC, the motor cortex has been neglected despite being a promising area for understanding the neural mechanisms associated with cardiovascular control during exercise. Furthermore, no previous studies have attempted to uncover whether or not the cardiovascular response to exercise can be altered following changes in motor cortex excitability using TMS. The current study will attempt to minimize this gap in the research. Transcranial magnetic stimulation has not been widely used in cardiovascular function studies; yet has great potential to help further our understanding of how cortical regions in the brain affect cardiovascular function.

2.6 Objective and Hypothesis

Non-invasive brain stimulation was used to determine if the forearm region of M1 controls the regulation of blood pressure in humans. The current study used continuous measurements of non-invasive blood measure, HR and MSNA during a baseline rest period, while performing an isometric handgrip exercise and PEI protocol in efforts to separate the CV responses elicited by CC and the Metaboreflex component of the EPR. These measurements will be performed on

healthy men and women during the exercise task after three conditions of brain stimulation; 1) inhibitory stimulation (cTBS) 2) excitatory stimulation (iTBS) 3) control (Sham TBS).

The objective of the current proposal was to determine if using TMS to inhibit or facilitate neural activity within the motor cortex would elicit significant changes in HR, MSNA and ABP during rest and isometric handgrip exercise in a population of normotensive men and women. Specifically it was hypothesized that facilitating the forearm area of the motor cortex would cause a subsequent increase in HR, ABP and MSNA, while inhibiting the forearm area of the motor cortex would cause a decrease in HR, ABP and MSNA during periods of rest. However, during exercise, compared to Sham, the excitatory effects of iTBS would lessen the cardiovascular response to isometric handgrip exercise as the magnitude of CC activation would be lessened compared to the target exercise intensity. Similarly, during exercise, compared to Sham, the inhibitory effects of cTBS would exaggerate the cardiovascular response to the isometric handgrip exercise as the level of CC activation would be heightened in effort to maintain the exercise intensity. The Sham condition was hypothesized to produce any effect on HR, ABP or MSNA due to the lack of actual stimulation being applied to the brain.

CHAPTER 3: METHODOLOGY

3.1 Participants

Participants were recruited via posters displayed at Dalhousie University, IWK Children's Hospital and the Queen Elizabeth II Health Science Centre (Appendix B). Posters were displayed in high traffic areas of the above buildings in order to gain the greatest amount of exposure.

To estimate sample size, we considered published mean values ± standard deviations of MAP responses to IHG and PEI in a population of normotensive individuals (99). Based on a resting MAP of 83 ± 8 mmHg and MAP increases of 105 ± 11mmHg (IHG) and 98 ± 11mmHg (PEI), we calculated values for Cohen's "d" (2.29 and 1.56) and effect size (0.75 and 0.62) (http://www.uccs.edu/~faculty/lbecker/). These values were then inputted into a power calculator (G Power v3.1.3, cicimelli post-stroke) using a repeated measures ANOVA within factors model with a power (beta) of 95%. An alpha value of 0.05 resulted in an estimated sample size of 8 and 11 respectively (IHG vs. PEI MAP values). To account for potential participant dropout and difficulties finding a stable sympathetic nerve signal (~75% success rate), it was estimated that a total of 15 participants would be recruited.

3.1.1 Inclusion Criteria

All participants were normotensive (systolic ≤139 mmHg, diastolic ≤89 mmHg), non-smokers and not obese (body mass index ≤30 kg/m^2), pregnant or taking medications for overt cardiovascular, metabolic, pulmonary or neurological disorders as determined by a health history questionnaire (Appendix C). To avoid the confounding influence of circulating ovarian hormones on resting and reflex-mediated cardiovascular function, females using oral contraceptives were all tested within 4 days from the onset of menstruation. Due to the need for participants to view the visual feedback regarding the amount of force applied to the handgrip dynamometer, all participants had normal or corrected-to-normal vision. All participants were instructed to arrive well hydrated, ~3 hours following a light meal. Participants were provided with a written set of instructions to remind them of these pre-study requests (Appendix D).

3.1.2. Exclusion Criteria

All participants were screened for contraindications to exercise using the Physical Activity Readiness Questionnaire (PAR-Q, Appendix E) and handedness was determined by the Edinburgh Handedness Inventory (Appendix F) (86). Any question checked "yes" on the PAR-Q excluded the participant from the study. Individuals with syndromes characterized by autonomic and/or cardiovascular effects (e.g. diabetes mellitus, Raynaud’s disease) were also

excluded from participation. Repetitive stimulation of cortical tissue presents the very rare occurrence for an epileptic event, among other less severe health concerns such as syncope, headache and local pain or discomfort (93). However, strict safety and training guidelines were followed to minimize the potential for any adverse events. Any participants who answered, “yes” to any of the Health Questionnaire (Appendix C) or TMS Screening Form (Appendix G) questions were excluded from the study. Furthermore, because of the magnetic field generated by TMS, additional exclusion criteria included:

- Previous surgery involving metal clips, rods, screws, pins, or wires.
- Participants with a heart pacemaker.
- Presence of any metallic foreign objects (i.e. shrapnel).
- Implanted electrodes, pumps or electrical devices.
- Cochlear (inner ear) implants.

As TMS induces an electrical current in the brain that alters the excitability of the cortical tissue, there are additional contraindications for participants undergoing TMS. These include history of or predisposing factors for epilepsy, previous injury to the head, or prior neurosurgery as well as any major medical or psychiatric conditions or medications that could lower the seizure threshold. To assist in ensuring participant safety; participants completed a Transcranial Magnetic Stimulation screening form (Appendix G), which indicates any contraindications to undergoing TMS. Furthermore, safety guidelines and

operating procedures to be followed are outlined in the TMS Standard Operating Procedure (Appendix H) and TMS Safety Document (Appendix I).

3.2 Experimental Protocol

Participants who met the above-stated inclusion criteria, did not meet any of the exclusion criteria and provided informed consent arrived at The Dalplex after avoiding the following for the previous 24 hours:

- Intense physical activity that requires a large amount of effort and causes a substantial increase in HR (running, bicycling, weight training, etc.)
- Alcoholic beverages
- Caffeinated products (coffee, tea, chocolate, etc.)
- Nicotine containing products (cigarettes, Nicorette gums, etc.)

Participants were asked to eat a light meal approximately 3 hours prior to the experimental session. Participants were also instructed to stay well hydrated the night before (approximately 5 hours before bed) and the morning of the session (~250ml or 1 cup of water per hour). Participants were also asked to wear a shirt that does not restrict access to the bicep muscle, as an arm cuff was wrapped around the bicep muscle for use during the PEI phase of the experiment. Furthermore, participants were asked to wear shorts that do not cover the calf

below the knee in order to minimize interference with the set up of equipment used to measure MSNA.

Prior to arriving to the Human Exercise and Cardiovascular Physiology Laboratory in the Kinesiology Suite of The Dalplex (Room 218), participants completed all inclusion/exclusion questionnaires, and provided informed consent. Upon arrival, participants' height (m), weight (kg), were recorded and BMI calculated to ensure they were below the exclusion criteria limit of 30 kg/m^2. Their resting brachial ABP was then measured (Carescape V100, General Electric Healthcare) to ensure confirm they were normotensive. If not, they were excluded from the study. Participants were then familiarized with all testing equipment and procedures before commencing the experiment.

The current study consisted mainly of a single test day that lasted approximately three hours. Some participants participated in two study sessions; the first test day consisted of familiarizing the participant with the study and associated equipment, as well as determining if the individuals' AMT was below the stipulated stimulator output maximum of 70%. This maximum output was set because the maximum stimulator output of the TMS device during the application of the experimental TBS protocols are 80% of the AMT. Individuals whose AMT was above 70% stimulator output did not come participate in the second testing session as they exceeded the maximum stimulator output necessary for the TBS protocols. These participants had too high of a stimulation threshold to have TBS

performed on them within the confines of ethical safety and the device's power output ability, and thus were discontinued from the study. Individuals who were below the 70% stimulator output were deemed as 'reactive' to TMS and were invited to return back for the full testing session. This upper limit of 70% of stimulator output led to us experiencing greater participant dropout than what was initially estimated. On the main test day, theta-burst stimulation was always applied to the non-dominant flexor digitorum superficialis (FDS) region of the primary motor cortex as determined by the Edinburgh Handedness Inventory (Appendix F). During the testing session, the participant underwent three theta burst stimulation conditions. Participants always underwent the sham stimulation condition first, followed by either the facilitative or inhibitory theta burst stimulation condition. The inhibitory pattern consisted of *continuous* theta burst stimulation [50 Hz @ 80% of active motor threshold (AMT), with a total stimulation time of 40 seconds for a total of 600 pulses] and the facilitative pattern was *intermittent* theta burst stimulation (50 Hz @ 80% AMT, with 2 seconds of stimulation repeated every 10 seconds for 200 seconds for a total of 600 pulses). The order of the two stimulation conditions, iTBS and cTBS, were randomly determined by a coin flip.

3.3 Data Collection and Analysis

3.3.1 Electromyography

Participants were outfitted with electrodes to for electromyographic (EMG) recordings of the flexor digitorum superficialis. The electrodes were placed longitudinally on the belly of the muscle (see Figure 8). The flexor digitorum superficialis originates from the medial epicondyle of the humerus and inserts on the anterior margins on the bases of the middle phalanges of the four fingers. While supporting the participant's arm, the medial aspect of the ventral surface was palpated near the elbow while the participant flexed their fingers by creating a fist. The electrodes (reference and recording) were placed approximately 1-2 cm apart on the area palpated with the greatest muscle mass. The investigator then marked the placement of the EMG electrodes with a washable marker on the muscle belly of the non-dominant flexor digitorum superficialis. The skin area was cleaned with an alcohol wipe to ensure adherence and conductance, prior to applying the EMG electrodes. After the application of the electrodes, the participant was asked to squeeze the handgrip dynomometer several times to confirm they were working correctly. Prior to any data collection participants performed two, five-second maximal voluntary contractions using the IHG dynamometer. Each MVC was separated by 30 seconds of rest and the voltage change produced by each maximal contraction was recorded. The maximum voltage signal generated between the two trials was designated as the MVC.

This MVC value was then used to calibrate the handgrip dynamometer to represent 100%, with the baseline value (no contraction) periods representing 0%. The absolute value MVC was recorded in Newtons.

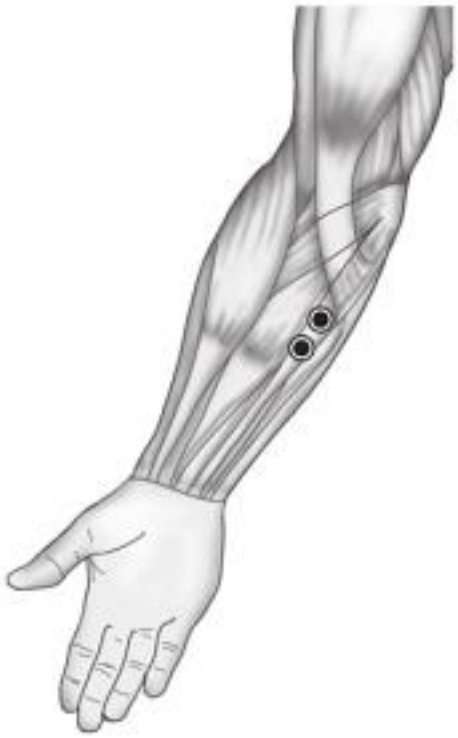

Figure 8. Flexor digitorum superficialis muscle with placement of the electromyography electrodes indicated by the circular markings (21).

3.3.2 Electrocardiogram

Participants were outfitted with adhesive silver-silver chloride electrodes for electrocardiogram (ECG, 3-lead, bipolar configuration) to record HR.

3.3.3 Respiration

The Pneumotrace II, strain-gauge band (UFI, California, USA) was secured around the participants' thorax at the level of the xyphoid process to track respiratory movements.

3.3.4 Arterial Blood Pressure

Arterial blood pressure was measured from the non-dominant upper arm (brachial artery) using a non-invasive semi-automated patient monitor (Carescape™ v100, General Electric Healthcare). In addition, a Portapres® (Finapres Medical Systems, B.V., The Netherlands) provided continuous non-invasive measurements of ABP from the middle and index fingers of the dominant hand. The values obtained using the Carescape™ automated patient monitor were used to calibrate the Portapres® blood pressure waveform. The finger from which ABP recordings were collected was switched if the participant expressed discomfort at any point and between conditions to prevent pooling of venous blood within the fingers and to minimize participant discomfort. Furthermore, the Portapres® was turned off during recovery periods to minimize any further discomfort for the participants.

3.3.5 Microneurography

Direct recordings of MSNA were collected using the microneurography technique. Microneurography is a common procedure used to examine sympathetic nerve activity in superficial nerves. A pre-amplifier (Nerve Traffic Analyzer system 6624C, University of Iowa: Bioengineering) with a gain of 1000 was secured above the participant's right knee. Sterilized unipolar tungsten microelectrodes (FHC; Bowdoin, Maine) were inserted percutaneously into a muscle nerve fascicle (active microelectrode) of the common fibular nerve and 2-3cm subcutaneously from the active recording site (reference microelectrode). The recording site was confirmed by manual manipulation until the characteristic pulse-synchronous burst pattern was observed. This pattern had to continue without any paresthesia of the skin, any response to skin afferents, elicited by a gentle stroking of the lower leg skin or any loud noises. Furthermore, it did have to respond with an increase in frequency to an end-expiratory apnea and to the hypotensive phase experienced while performing a Valsalva's Manoeuvre.

An inflatable pressure cuff connected in series to a rapid cuff inflator (Hokanson® E20 Rapid Cuff Inflator) was secured around the participant's non-dominant upper arm so that blood flow to the forearm could be completely occluded. Finally, a strain-gauge based isometric handgrip dynamometer (ADInstruments) was placed within the participant's non-dominant hand.

All signals were recorded using dedicated data acquisition (PowerLab, ADInstruments) and analysis software (Lab Chart, ADInstruments). The ECG was sampled at 1000 Hz. The Pneumotrace II was sampled at 40 Hz, the Portapres® at 200 Hz, the integrated mean MSNA neurogram at 200 Hz, (raw signal band-pass filtered: 200-5000 Hz), and the handgrip dynamometer at 20 Hz.

3.3.6 Transcranial Magnetic Stimulation

Upon arrival to the lab the participants were asked to remove all jewellery and remove any outer clothing that restricts access to the arm up to the bicep muscle so that the arm cuff could be secured around the upper arm. Participants were asked if they wanted earplugs to minimize the effects of the loud “click” that occurs when a stimulus is discharged. Participants were also asked if they wanted a personal mouth guard to wear during the experiment to minimize any jaw movement, jaw clenching or teeth grinding that may occur during the application of TMS. Participants were instructed to sit upright with their head straight, jaw parallel to the floor with their back against the back of the chair and were continually instructed to minimize head movements during TMS application. Participants were given pillows to sit on and behind their back for comfort, as well as foam padding to increase comfort around their feet and to support the leg being used to measure MSNA.

An air-cooled figure-of-eight magnetic stimulation coil connected to a Magstim Rapid^2 stimulator (Magstim, Whitland, UK) was used for all applications. The figure-8 coil was positioned tangentially to the skull with the handle pointed backwards and laterally at an angle of 45° to the sagittal plane. Thus, TMS induced current flow in the brain is in the posterior–anterior direction. Stimuli were applied over the non-dominant FDS muscle area, (as determined by the Edinburgh Handedness Inventory, Appendix F) of the primary motor cortex via the figure-8 coil (Magstim, Whitland, UK). For the sham condition, the figure-8 coil was turned 90 degrees away from the participant's scalp, which produces the auditory stimulus (loud click) but does not cause any stimulation of brain networks. This also provides the weight of the coil still being felt on the participants scalp.

3.3.7 Stimulus Location Determination

After participant set-up, determination of the optimal stimulus location was performed. The objective was to locate the motor ‘hotspot’ for the flexor digitorum superficialis. This motor ‘hotspot’ is the location that yields the largest MEP amplitude using the minimal necessary stimulator intensity over the non-dominant flexor digitorum superficialis muscle area of the motor cortex. A maximal MEP was defined as the highest amplitude recorded while systematically applying single pulses over the target area. Amplitudes of the MEPs were calculated automatically by the LabChart software. The MEP peak-

to-peak amplitudes (the distance from the highest to the lowest point) were calculated and recorded. When a maximal MEP was found, the location was recorded by tracing the outline of the figure-8 coil on the scalp cap. The size of the maximal MEP was recorded in LabChart.

Participants were first assisted with putting on a silicon scalp cap that would help the researcher find the FDS region of the motor cortex. The dimensions of their scalp were measured from the nasion to the inion and the left to right pre-auricular points of the ears. The intersection of these measures was marked as the vertex. The investigator then explained to the participant that “We will be starting brain stimulation on and off for approximately the next 30 minutes. You will likely feel a pinching or tapping sensation on your scalp and may experience movement in your hand and forearm. Please try to keep still as possible and let us know if you feel uncomfortable at any time”. The participant was instructed to gently squeeze (~5% maximal voluntary contraction, MVC) the non-dominant hand to help facilitate a maximal MEP. Systematic stimulation was applied over a 5 cm by 5 cm marked grid around the C3 position on the international 10-20 system for an electroencephalogram (EEG) (i.e., 50% of the measured distance between the vertex of the head and the left preauricular point). The grid consisted of intersecting lines every 0.5 cm and acted as a guide so that stimulation could be systematically performed without missing any potential areas or repeating stimulation in the same spot. TMS stimulus intensity was adjusted to a level that did not produce large movements of the arm, but was

high enough to elicit EMG activity within the flexor digitorum superficialis. Stimulation intensity began at 40% of the stimulator output. If the single TMS pulse did not elicit an MEP the stimulation coil was repositioned 0.5 cm anterior; if the MEP was still absent, the coil was moved 0.5 cm to the left or right as required, as marked by the grid on the skull cap. The effect of stimulation, as determined by the peak-to-peak amplitude of the MEP, was re-checked at each new position using the same stimulus intensity. If no suitable location elicited a response then the process was repeated using greater stimulus intensity. The stimulus intensities were increased in 5% increments until a maximal MEP was observed. Maximal MEPs varied between individuals, but generally ranged between 150-200 μv. When the maximal MEP occurred, the site was confirmed using several more single stimulation pulses, then the area was marked on the scalp cap by tracing the over edge of the coil. Mapping of this area was done at the beginning of every experimental session, and the marked scalp cap was used throughout the experimental protocol to assist in the systematic determination of the optimal stimulus location.

3.3.8 Active Motor Threshold Determination

After the optimal stimulation site was found, the active motor threshold was determined. To determine AMT, single TMS pulses were applied on the determined optimal scalp location while monitoring for: (a) participant sensation of the stimulus of the FDS muscle, and (b) the occurrence of a MEP in the FDS

muscle with an amplitude of at least ≥ 50 μV [detected via surface electromyography (EMG) overlying the FDS muscle. The AMT was defined as the minimal stimulation intensity that elicited the above responses in 5 out of 10 trials after stimulation of the corresponding cortical area when the muscles are contracted to 5% of maximal voluntary contraction force (101). Once this intensity was determined it was recorded in LabChart and used to determine the stimulation intensity of the experimental TBS conditions. The 5 of 10 trials that elicited an MEP were recorded and acted as a baseline measurement.

For muscles of the hand and forearm, AMT is usually in the range of 45-60% of the maximum stimulator output with a 70 mm typical figure-of-eight coil; if AMT was not evident before increasing the stimulus intensity to 70% of the maximum stimulator output, the researcher repeated the stimulus location protocol to possibly find a better location (101). Adjustments to find a better location included re-measuring the participant's scalp, extending the grid area and increasing stimulation intensity. The experimental stimulation output for iTBS and cTBS was set at 80% of the stimulation output found during the AMT process.

After the optimal stimulation location and AMT was determined, the participant underwent the experimental protocol of seated rest, isometric handgrip exercise, PEI and recovery with no stimulation (see Figure 9). This served as a baseline control for the sham, inhibitory and facilitative conditions.

Figure 9. Pictorial depiction of the optimal stimulation site, AMT, MVC and baseline recording of rest, IHG exercise, PEI and recovery periods of the experimental protocol.

3.3.9 Experimental Stimulation

After the stimulation condition (Sham, inhibitory or facilitative) was administered to the participant, the effectiveness of the stimulation was confirmed by using single pulse TMS while the muscles were weakly contracted (~5%). The MEPs' amplitude post-stimulation were recorded and used to determine the stimulation effects on cortical excitability. After the sham stimulation it was expected that there would be no changes in the maximum peak-to-peak amplitude of the MEP because no stimulation was applied. It was anticipated that the inhibitory TBS condition (i.e., cTBS) would decrease the MEP (compared to baseline) after a single pulse of stimulation, while following the facilitative stimulation (i.e., iTBS), a single pulse would result in an increased MEP amplitude. The baseline average was used after experimental conditions to determine if the cortical tissue had returned to a "resting" or baseline state. This process was completed following each recovery period to ensure there were no lasting effects on cortical excitability before the next stimulation parameter was performed. The stimulation intensity of the experimental TBS conditions was determined as 80% of the AMT. The stimulation output maximum during AMT was set as 70% because of the

limitations of the Magstim. The TBS protocols heat up the coil at a faster rate, and requires a greater amount of power, than the single pulse protocol used to find AMT. This means the Magstim can only be set at 56% of stimulator output during TBS before it shuts down prior to completing the full TBS protocol. Since we determined 80% of AMT to be the optimal stimulation intensity for the TBS protocols, any participant with an AMT of greater than 70% had to be excluded because the TBS protocols could not be successfully completed.

3.3.10 Experimental Task

The submaximal isometric handgrip (IHG) exercise task involved the participants continuously contracting the handgrip dynamometer using their hand and forearm muscles (such as squeezing an object in your hand) at the percentage instructed for the time indicated by the researcher. Participants were instructed to try to squeeze at 35% of their MVC for a total of two minutes. Feedback of how hard to contract or squeeze the dynamometer during the experimental trials was presented on a screen in front of the participant. The researcher instructed the participant when to start and stop contracting during the trials. The IHG exercise was immediately followed by a 2-minute period of blood flow occlusion to the exercising forearm to isolate the Metaboreflex component of the IHG exercise. This blood flow occlusion was initiated by inflating the upper arm cuff during the last 5 seconds of the IHG exercise. The upper arm cuff was deflated at the end of the 2-minute PEI period, which marked the beginning of the recovery period.

The recovery period lasted approximately 5-10 minutes (depending on how long the after-effects of the TBS last, therefore it varied between participants and conditions) to ensure that there were no lasting-effects on cortical excitability from the preceding TMS stimulation condition before the subsequent experimental stimulation condition was applied. To ensure there were no lasting changes, the amplitude of several MEPs (evoked by a single pulse of TMS) was used to compare to the values obtained before the experimental protocol (baseline). If the single pulse test revealed that MEP amplitudes were much higher (i.e., intermittent TBS) or lower (continuous TBS) than baseline recorded values, then the recovery time was lengthened by five minutes. After a further five minutes the single pulse test was performed again, if MEP values had reached baseline values then the next TBS condition could be applied, if not, this process was repeated until baseline values were reached. The investigator then ensured the participant was still as comfortable as possible before checking the optimal stimulus location and then the protocol was repeated again starting at the TMS condition phase, which was the randomly determined facilitative or inhibitory paradigm. This process was repeated for a third and final time for the second stimulus paradigm (see Figure 10).

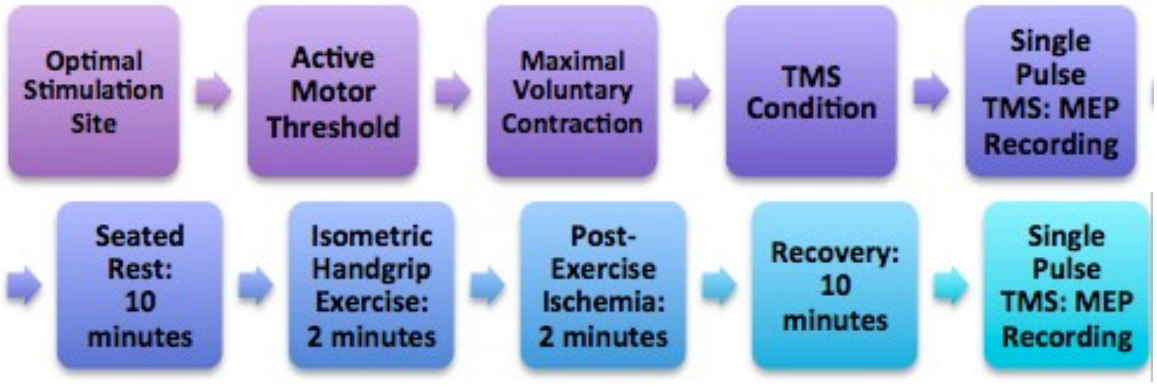

Figure 10. Pictorial depiction of the study design with the three TMS conditions performed in the order of: sham followed in a randomly selected order of inhibitory and facilitative.

3.4 Neurophysiologic Data Analysis

3.4.1 Heart Rate Variability Analysis

Electrocardiogram-derived measurements of HR (beats per minute) were quantified by determining the time between successive cardiac intervals (i.e. the time between successive R-waves of the ECG). In addition, fast-Fourier transform spectral analysis of HRV was used to estimate relative parasympathetic innervation to the sinoatrial node (high frequency component, 0.15-0.40 Hz). The low frequency (0.05-0.15 Hz) and high frequency powers were presented in absolute and relative units, which is the absolute power divided by the total power (0.04-0.4 Hz). The ratio between the low frequency and high frequency components was used to describe sympathovagal balance, which is the ratio of parasympathetic to sympathetic activity neural contributions to the modulation of HR (108).

3.4.2 Respiration Rate

Respiratory rate was determined by calculating the time between successive peak inspirations and converted to breaths per minute.

3.4.3 Muscle Sympathetic Nervous Activity Analysis

Bursts of MSNA were detected from the mean voltage neurogram and used for all quantitative analysis. To generate the mean voltage neurogram, the raw analogue signal was amplified (approximately 75 000 times), integrated (0.1 second time constant), full-wave rectified and band-pass filtered (500-2000 Hz). MSNA was quantified as burst frequency (bursts per minute), and burst incidence (bursts per 100 heart beats).

3.4.4 Portapres® Continuous Blood Pressure Analysis

The finger arterial pressure waveform generated by the Portapres® was calibrated to brachial artery blood pressure using the Carescape semi-automated blood pressure monitor. The equation: ⅓ systolic pressure + ⅔ diastolic pressure was used to calculate mean arterial pressure. Using the blood pressure module for LabChart software, cardiovascular parameters from arterial pressure signals were detected and then analyzed after recording using classifier and analysis

view. Using classifier view the section of time to be analyzed was highlighted. Then using analysis view any pressure cycles that were contaminated by artifacts or abnormal cycle heights or durations were manually selected and removed from analysis. New channels were then created that automatically calculated diastolic and systolic pressure based on the highlighted selection of the blood pressure waveform. Diastolic pressure was determined as the minimum pressure recorded for each R-wave, while systolic was the highest pressure recorded.

For all neurophysiologic data, an average of the initial five-minute baseline rest period, as well as, one-minute averages during the post-TBS five-minute baseline rest periods, and during the first and second minutes of IHG and PEI time points were determined.

3.5 Statistical Analyses

All neurophysiologic data were expressed as means ± standard deviations and statistical analysis of within-group differences were assessed by a two-way (Condition × Time) analysis of variance for repeated measures, where Condition refers to baseline and type of TMS protocol (Sham, cTBS, and iTBS) and Time refers to the one-minute averaged Rest, IHG, and PEI periods. Furthermore, a one-way analysis of variance was performed on all neurophysiologic data to determine differences between conditions during the five-minute averaged rest

period. A one-way analysis of variance was also conducted on maximal evoked potential data to determine differences between conditions (Baseline, Sham, cTBS and iTBS). The level of significance was set at $p<0.05$ and adjusted using Bonferonni's method as required. All statistical analyses were performed in SPSS (Version 21.0. Armonk, NY: IBM Corp). Mauchly's test of sphericity was performed and if the assumption of sphericity was been violated, the Greenhouse-Geisser correction was performed to adjust the degrees of freedom accordingly. A distribution plot was constructed using mean MEP amplitudes from each participant (each represented by a different symbol) for each condition to show spread of data to determine 'cTBS responders' from 'cTBS non-responders' and 'iTBS responders' from 'iTBS non-responders'. This plot was also completed again but used the MEP difference from baseline for each participant, instead of their original MEP amplitudes. Each graph shows the dispersion of each participant's MEP amplitudes for each condition. Participants that were clustered together with the highest increase from baseline following iTBS were considered 'iTBS Responders', while those with the smallest difference were considered 'iTBS Non-responders'. For cTBS, the participants clustered together with the lowest values were considered 'cTBS Responders' and those with the highest positive values were considered 'cTBS non-Responders'. Participants that were between both clusters of groups were not classified as either responders or non-responders.

CHAPTER 4: RESULTS

4.1 Participant Descriptive Statistics

A total of 34 participants were recruited for the present project. However, 6 were excluded for health conditions including previous diagnoses of: single or multiple concussions, depression, and hearing loss that were contraindications for TMS. Six more were excluded after the first visit because the motor 'hot spot' could not be found or the stimulator output threshold was too high. Another 10 decided to forgo participating in the second visit due to various reasons including scheduling or time conflicts, disinterest, or discomfort experienced during the first visit. Twelve (5 men, 7 women) normotensive and recreationally active young adults met all inclusion/exclusion criteria and provided informed consent to participate in the study (Table 1).

Table 1. Descriptive statistics for the 12 participants.

Variable	Mean ± S.D.	Range
Age (years)	22.7 ± 3.1	19-30
Height (cm)	172.4 ± 9.2	157.5-189.1
Weight (kg)	73.9 ± 8.5	62.7-90.2
BMI (kg/m^2)	24.9 ± 2.5	21.9-29.1
AMT (%S.O)	53.3 ± 12.6	27-64
MVC (N)	317 ± 122	145-559

AMT, active motor threshold; %S.O., percentage of maximum stimulator output; MVC, maximal voluntary contraction; N, Newtons

4.2 Motor Evoked Potential Amplitudes

The ability of TBS to alter the excitability of the motor cortex was assessed by comparing the changes in MEP amplitude during baseline (i.e., no TMS), post-sham, post-cTBS and post-iTBS. Motor evoked potentials measured post-sham did not significantly differ ($p = 1.0$) from baseline. Motor evoked potentials were not significantly depressed ($p = 1.0$) post-cTBS compared to baseline. Compared to baseline, and sham, MEP amplitudes measured post-iTBS were significantly (both, $p \leq 0.02$) greater. There was also a trend towards post-iTBS MEP amplitudes being significantly greater than those measured post-cTBS ($p = 0.06$) (See Figure 11).

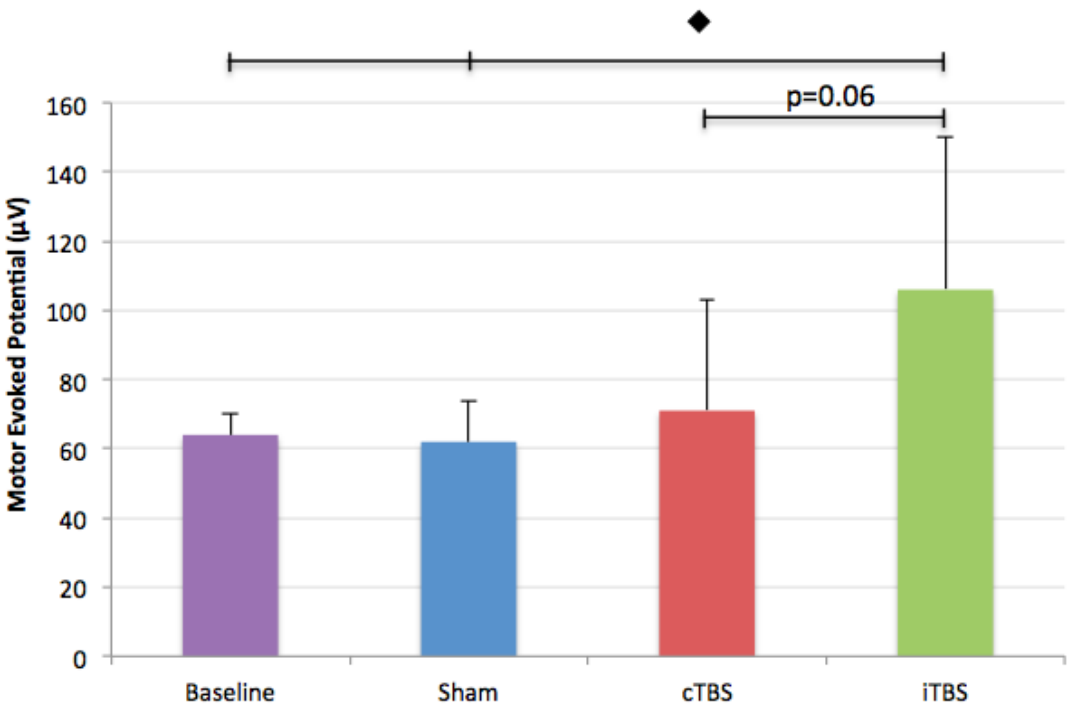

Figure 11. Averaged baseline and post-TBS condition (Sham, cTBS, and iTBS) single-pulse MEP amplitude responses. Data are presented as Means ± Standard Deviations. ◆, $p \leq 0.02$ between iTBS and Baseline, and Sham. MEP, motor evoked potential; cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

Individual averaged MEPs were plotted for each of the four conditions (See Figure 12). The difference (from Baseline) in MEP amplitudes for Sham, iTBS and cTBS conditions were also plotted for each participant (See Figure 13). Each symbol represents a different participant for each TBS condition and baseline. Each graph shows the dispersion of each participant's MEP amplitudes to each condition. Baseline and Sham results showed little variation, with little variation between participants. iTBS and cTBS were loosely dispersed and highly varied between participants, thus demonstrating no clear, consistent effect of either TBS protocol. Participants with the highest increase from baseline following iTBS were considered 'iTBS Responders', while those with the smallest difference were considered 'iTBS Non-responders'. For cTBS, the participants with the lowest grouping of values were considered 'cTBS Responders' and those with the highest grouping of positive values were considered 'cTBS non-Responders'. A total of three participants each were classified as 'iTBS Responders', 'iTBS Non-Responders' and 'cTBS Responders', with two participants classified as 'cTBS Non-Responders'. Participants that fell between both clusters of groups were not classified as either responders or non-responders and were excluded from further analysis of the responder and non-responder data.

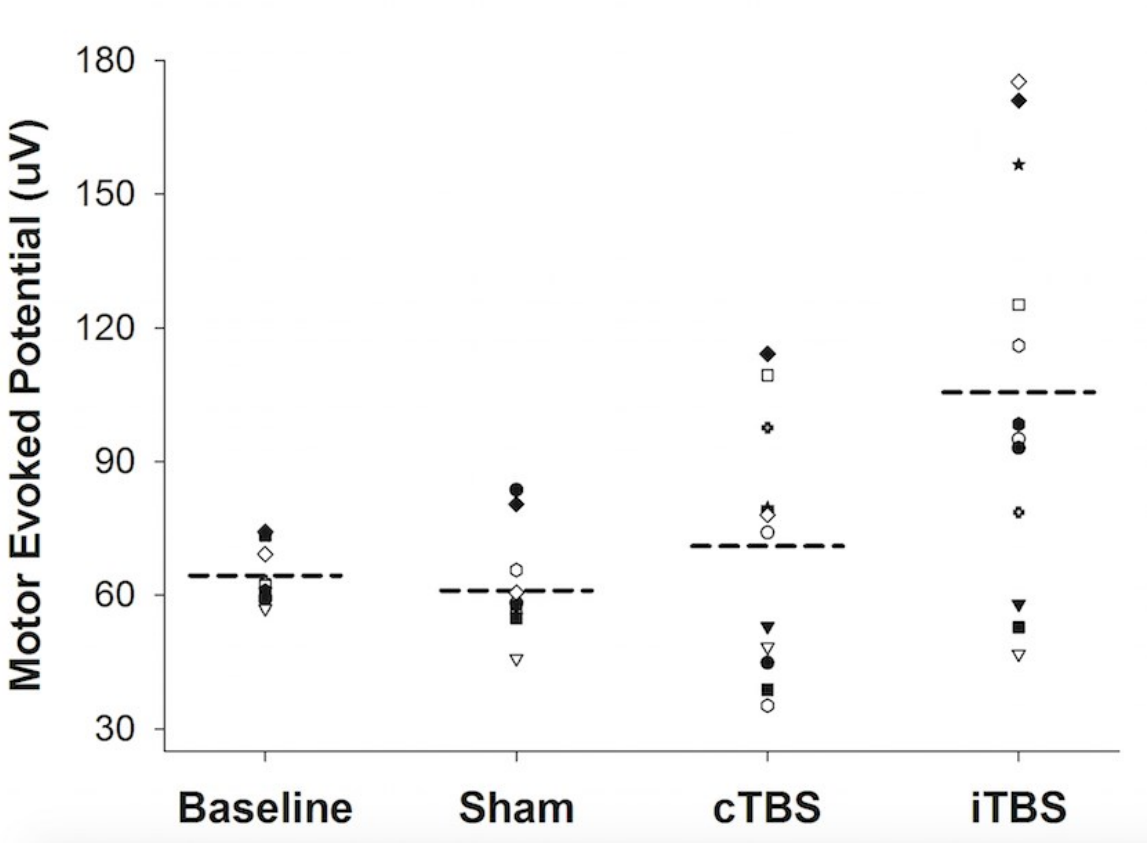

Figure 12. Individual Motor Evoked Potentials measured post-Baseline (i.e., no TBS), Sham, iTBS and cTBS. Each participant is represented by a different symbol, horizontal dashed lines represent the mean MEP value for each condition. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

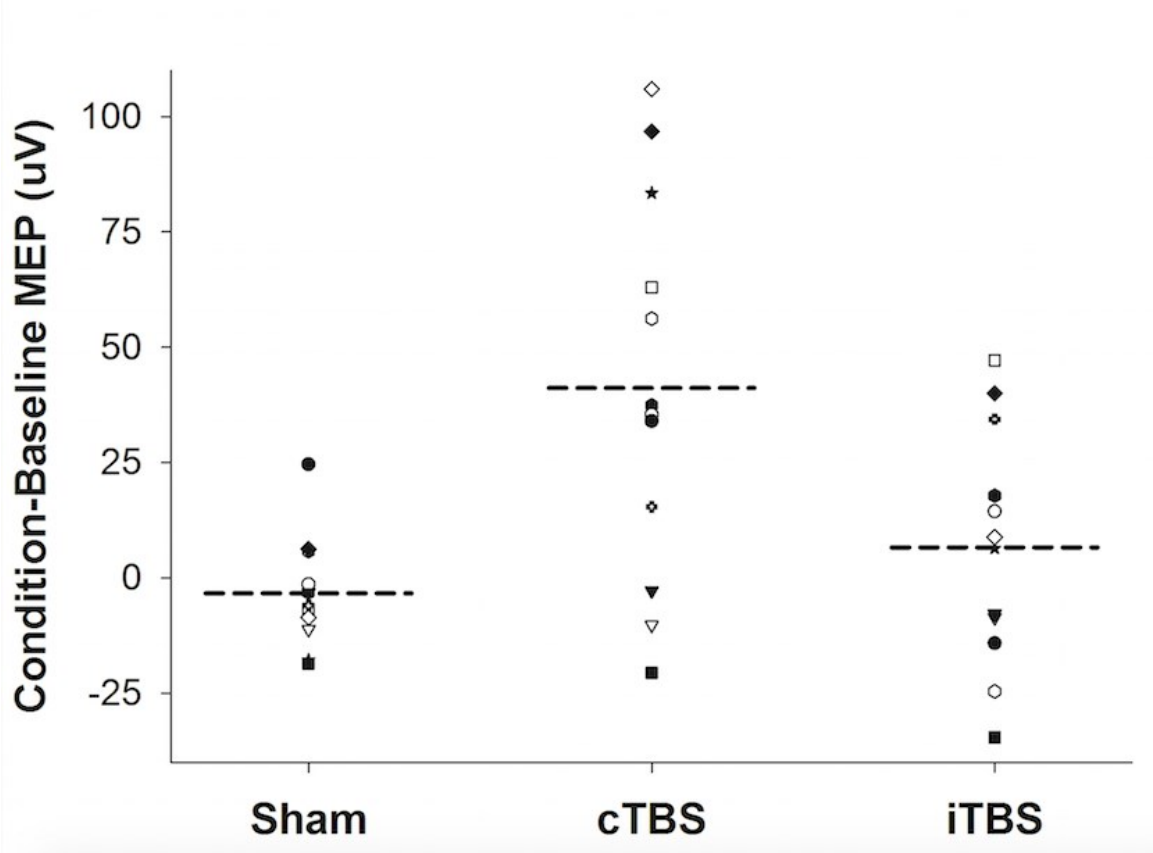

Figure 13. Individual Motor Evoked Potential differences (from Baseline) for Sham, iTBS and cTBS conditions. Each participant is represented by a different symbol, horizontal dashed lines represent the mean MEP value for each condition. The three highest participants for iTBS are classified as 'iTBS Responders', while the opposite three lowest are 'iTBS Non-responders'. For cTBS, the highest three are 'cTBS Non-responders' and the lowest two are 'cTBS Responders'. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

4.3 Resting Data

4.3.1. Heart Rate Variability

There were no differences between conditions in HRV absolute low frequency (p=1.00), HRV absolute high frequency (p=1.00), HRV relative low frequency (p=1.00), relative high frequency (p=1.00), or the ratio of low to high frequency HRV (p=1.00). (Refer to Table 2)

Table 2. Baseline and post-TMS resting heart variability data.

		Baseline	Sham	cTBS	iTBS
HRV					
Low Frequency	**Absolute (μs^2)**	1563 ±1398	2095 ± 2074	2704 ± 2608	2235 ± 1783
	Relative (nu)	59 ± 22	61 ± 21	68 ±15	66 ±15
High Frequency	**Absolute (μs^2)**	1735 ± 2945	2142 ± 3097	1703 ± 2631	1603 ± 2438
	Relative (nu)	40 ± 21	39 ± 21	32 ±14	34 ±14
LF/HF Ratio		2.3 ± 2.0	2.7 ± 2.5	2.8 ± 1.9	2.6 ± 1.7

Values are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation; HRV, heart rate variability; LF, low frequency; HF, high frequency

4.3.2 Heart Rate

There was no interaction effect ($F_{8,80}$= 0.65, p = 0.73) for minute-averaged recordings of heart rate collected during rest. There was also no main effect of TBS condition on the HR response to exercise ($F_{2,20}$= 0.98, p = 0.39). Finally, there was no main effect of time on the HR response to exercise ($F_{4, 40}$= 1.6, p = 0.19). There was a trend towards significance (p = 0.054) between iTBS and cTBS at time point Rest 3 (See Figure 14). Heart rate recordings averaged for the entire five-minute rest period also did not significantly differ (all, p > 0.7) between conditions (See Figure 15).

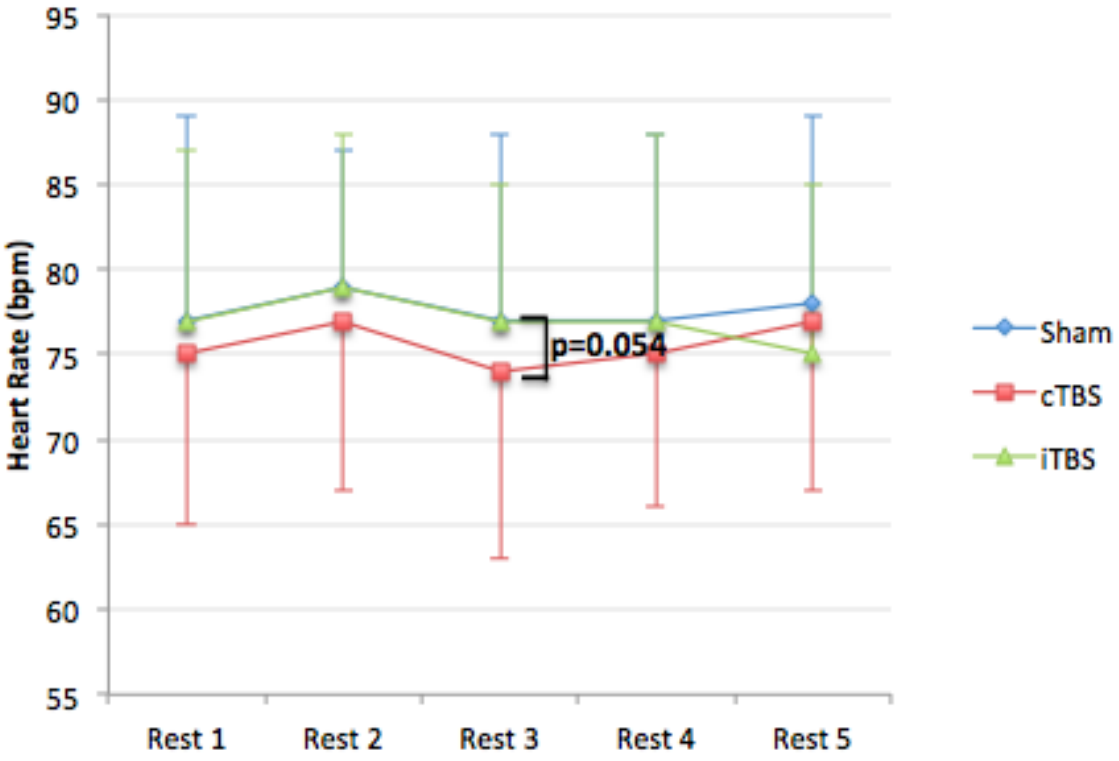

Figure 14. Averaged one-minute resting heart rate recordings post-TBS condition (Sham, cTBS, and iTBS). No significant differences between conditions or time points ($p > 0.7$), although there was a trend towards significance between cTBS and iTBS at Rest 3 ($p = 0.054$). Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

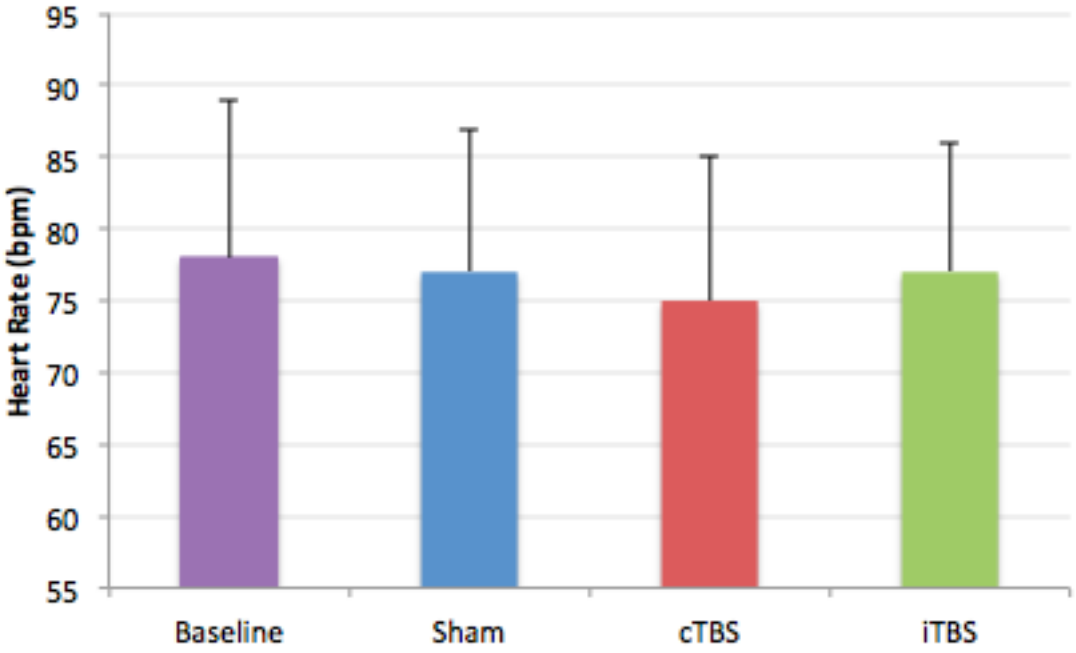

Figure 15. Averaged five-minute resting heart rate recordings post-TBS condition (Sham, cTBS, and iTBS). No significant difference between conditions, p = 1.0. Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

4.3.3 Mean Arterial Pressure

There was no interaction effect ($F_{2, 18.3}$= 0.65, p = 0.54) for minute-averaged recordings of mean arterial pressure collected during rest. There was also no main effect of TBS condition on the MAP response to exercise ($F_{2,18}$= 0.21, p = 0.81). Finally, there was no main effect of time on the MAP response to exercise ($F_{2.1, 18.8}$= 0.34, p = 0.73). There was a trend towards significance (p = 0.07) between iTBS and cTBS at time point Rest 1 (See Figure 16). MAP recordings averaged for the entire five-minute rest period also did not significantly differ (p ≥ 0.2) between conditions (See Figure 17).

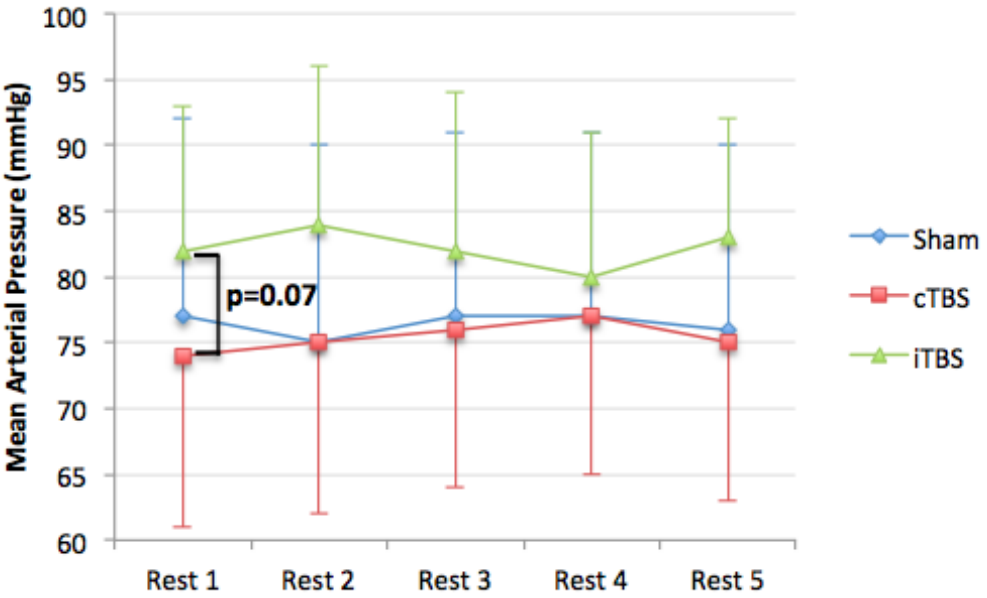

Figure 16. Averaged one-minute resting mean arterial pressure recordings post-TBS condition (Sham, cTBS, and iTBS). There were no significant differences between conditions or time points ($p = 1.0$), although there was a trend towards significance between conditions iTBS and cTBS at Rest 1 ($p = 0.07$). Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

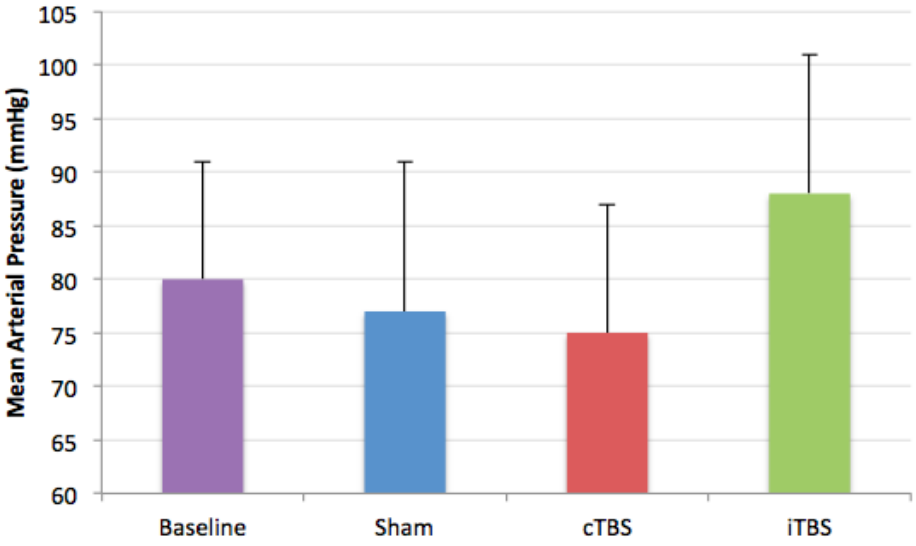

Figure 17. Averaged five-minute resting mean arterial pressure recordings post-TBS condition (Sham, cTBS, and iTBS). There were no significant differences between conditions, ($p > 0.2$). Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

4.3.4 Systolic Blood Pressure

There was no interaction effect ($F_{3.2, 31.9}$= 1.4, p = 0.27) for minute-averaged recordings of systolic blood pressure collected during rest. There was a main effect of TBS condition on the SBP response to exercise ($F_{2,20}$= 0.5, p = 0.6). Finally, there was no main effect of time on the SBP response to exercise ($F_{1.8, 17.6}$= 0.35, p = 0.7).

Minute-averaged recordings of SBP collected during the five-minute rest period post-TBS significantly differed between conditions iTBS and cTBS (p = 0.04) during the second minute of rest (See Figure 18). Also, systolic blood pressure recordings averaged for the entire five-minute rest period did not significantly differ (p = 1.0) between conditions (See Figure 19).

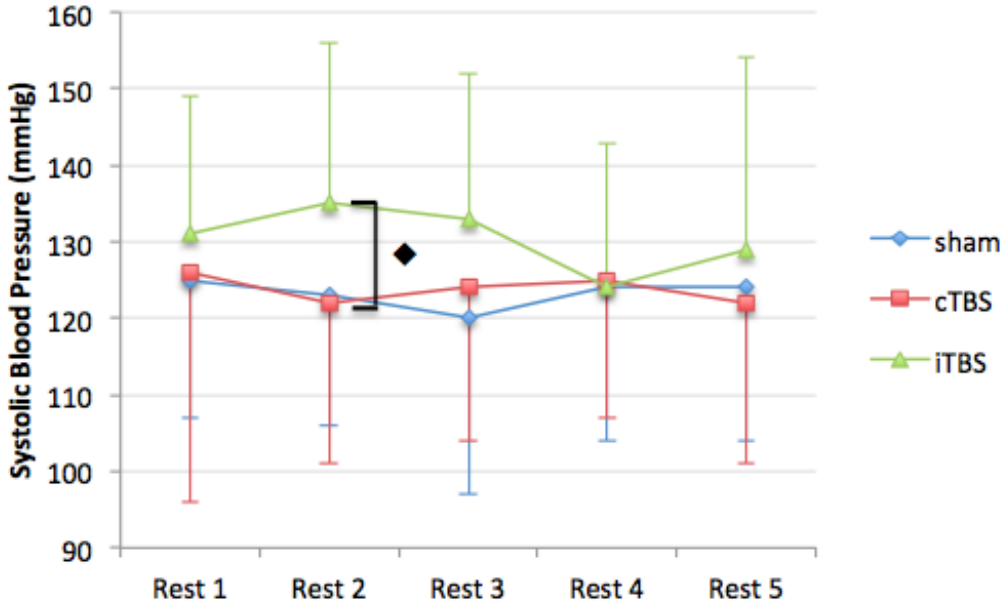

Figure 18. Averaged one-minute resting systolic blood pressure recordings post-stimulation (Sham, cTBS, and iTBS). Data are presented as Means ± Standard Deviations. ◆, p = 0.04 between iTBS and cTBS. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

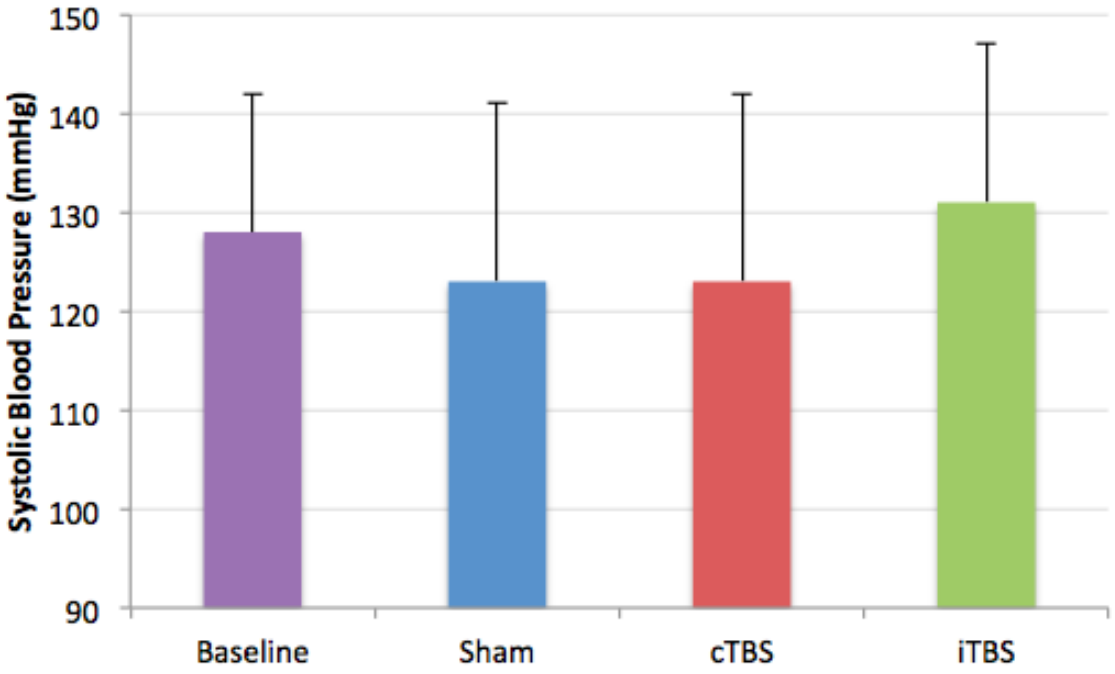

Figure 19. Averaged five-minute resting systolic blood pressure recordings post-TBS condition (Sham, cTBS, and iTBS). No significant difference between conditions, p = 1.0. Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

4.3.5 Diastolic Blood Pressure

There was no interaction effect ($F_{2.1, 20.7}$= 0.71, p = 0.51) for minute-averaged recordings of diastolic blood pressure collected during rest. There was also no main effect of TBS condition on the HR response to exercise ($F_{2,20}$= 0.34, p = 0.7). Finally, there was no main effect of time on the MAP response to exercise ($F_{4, 40}$= 0.7, p = 0.6). There was a trend towards significance (p = 0.09) between iTBS and cTBS at time point Rest 1 (See Figure 20). Diastolic blood pressure

recordings averaged for the entire five-minute rest period also did not significantly differ (p = 1.00) between conditions (See Figure 21).

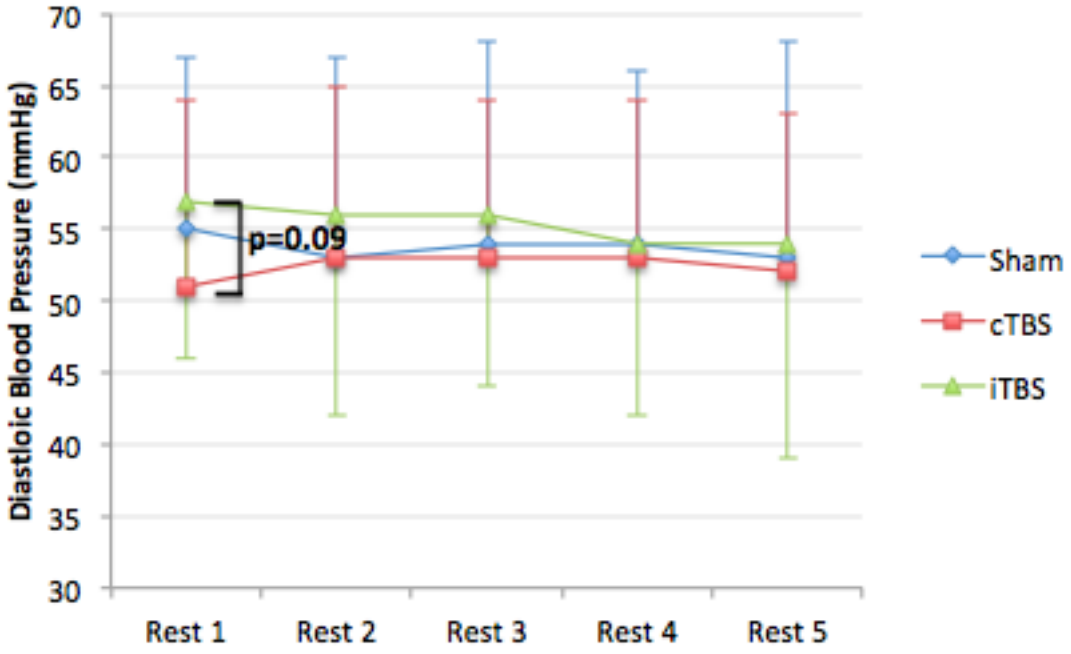

Figure 20. Averaged one-minute resting diastolic blood pressure recordings post-TBS condition (Sham, cTBS, and iTBS). No significant difference between conditions or time points, although there was a trend towards significance (p = 0.09) between iTBS and cTBS at Rest 1. Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

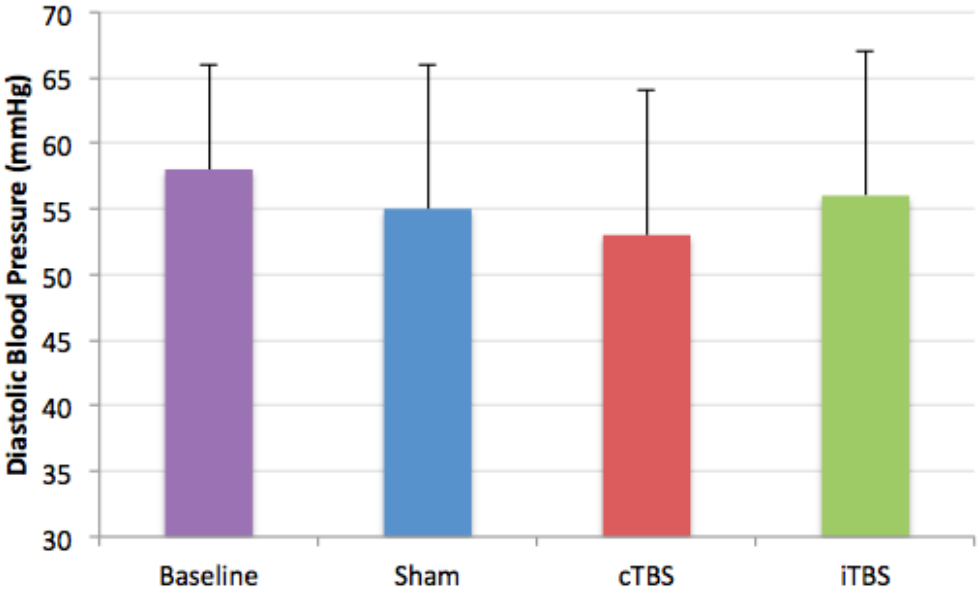

Figure 21. Averaged five-minute resting diastolic blood pressure recordings post-TBS condition (Sham, cTBS, and iTBS). No significant difference between conditions, p = 1.0. Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

4.3.6 Muscle Sympathetic Nerve Activity (Burst Frequency)

There was no interaction effect ($F_{8, 56}$= 0.89, p = 0.5) for minute-averaged recordings of muscle sympathetic nerve activity burst frequency collected during rest. There was a main effect of TBS condition on the MSNA burst frequency during rest ($F_{2, 14}$= 6.4, p = 0.01). One minute-averaged recordings of muscle sympathetic nerve activity burst frequency collected during the five-minute rest period post-TBS significantly differed between conditions iTBS and cTBS ($p \leq 0.01$) during minutes 3, 4 and 5, with cTBS being greater than iTBS. Recordings post-Sham were also significantly lower than cTBS ($p \leq 0.04$) during minutes 1, 3, 4 and 5 (See Figure 22). However, muscle sympathetic nerve activity burst frequency recordings averaged for the entire five-minute rest period did not

significantly differ (p = 1.0) between conditions (See Figure 23). Finally, there was no main effect of time on the MSNA burst frequency during rest ($F_{1.5, 10.8}$= 1.9, p = 0.2).

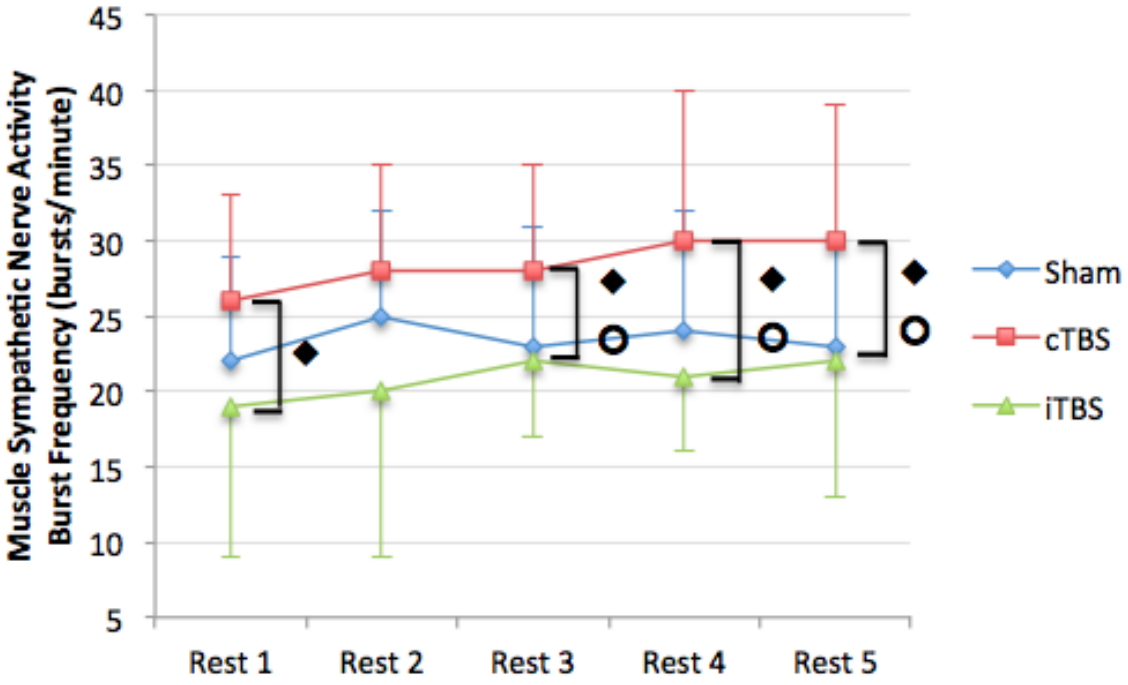

Figure 22. Averaged one-minute resting muscle sympathetic nerve activity burst frequency recordings post-stimulation (Sham, cTBS, and iTBS). Data are presented as Means ± Standard Deviations. ◆ , p ≤ 0.03 between iTBS and cTBS. O , p ≤ 0.02 between cTBS and Sham. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

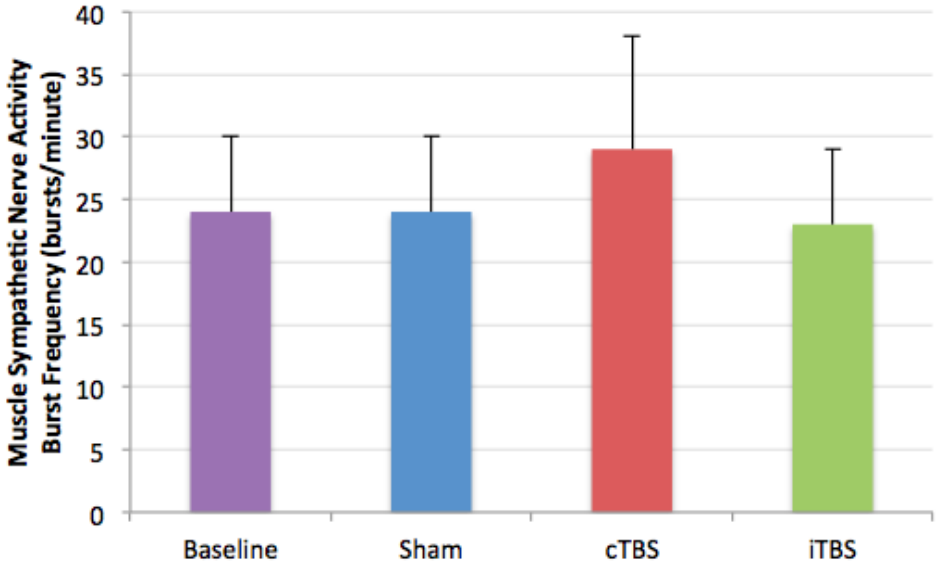

Figure 23. Averaged five-minute resting muscle sympathetic nerve activity burst frequency recordings post-TBS condition (Sham, cTBS, and iTBS). No significant difference between conditions, $p > 0.4$. Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

For the group results, most differences were observed in the collection of muscle sympathetic nerve activity, therefore responder and non-responder data were plotted for both MSNA burst frequency and burst incidence (See Figures 24, 25, 28, 29, 37, 38, 39 and 40). However, one participant who was classified both as an 'iTBS Responder' and 'cTBS Non-Responder', experienced technical difficulties collecting MSNA data, and thus there are only two and one participant in each of those respective groups. Due to the small group sizes of these responders and non-responders, there were no significant differences.

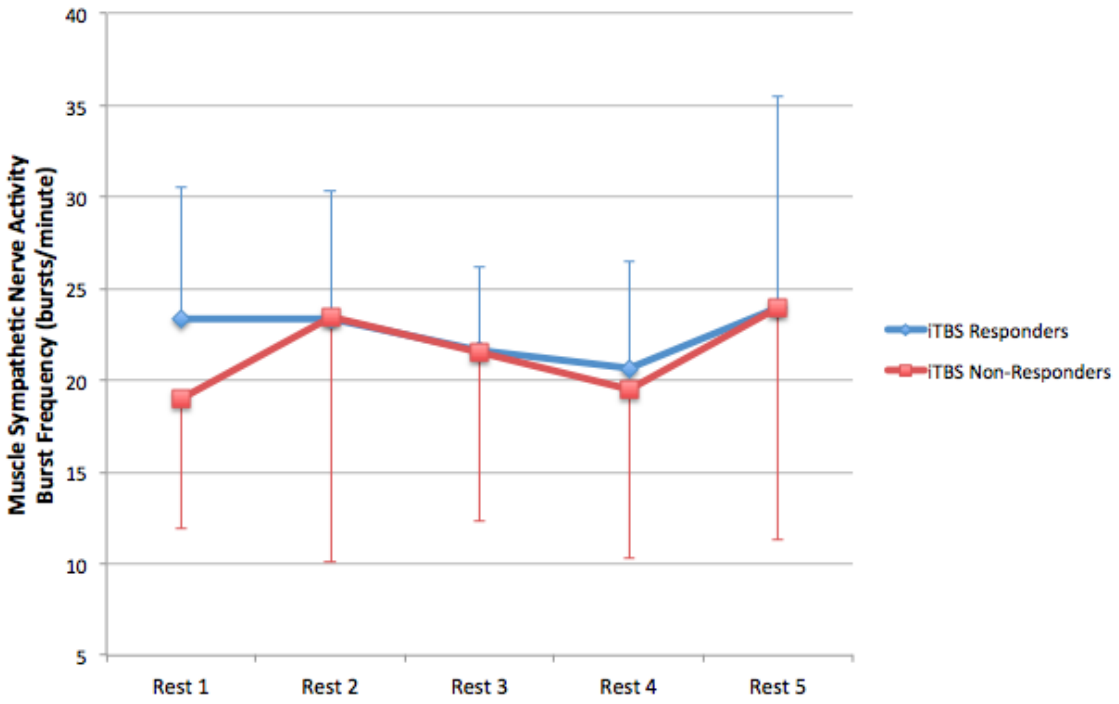

Figure 24. iTBS Responder and Non-responder averaged one-minute resting muscle sympathetic nerve activity burst frequency recordings post-iTBS stimulation. iTBS, intermittent theta burst stimulation.

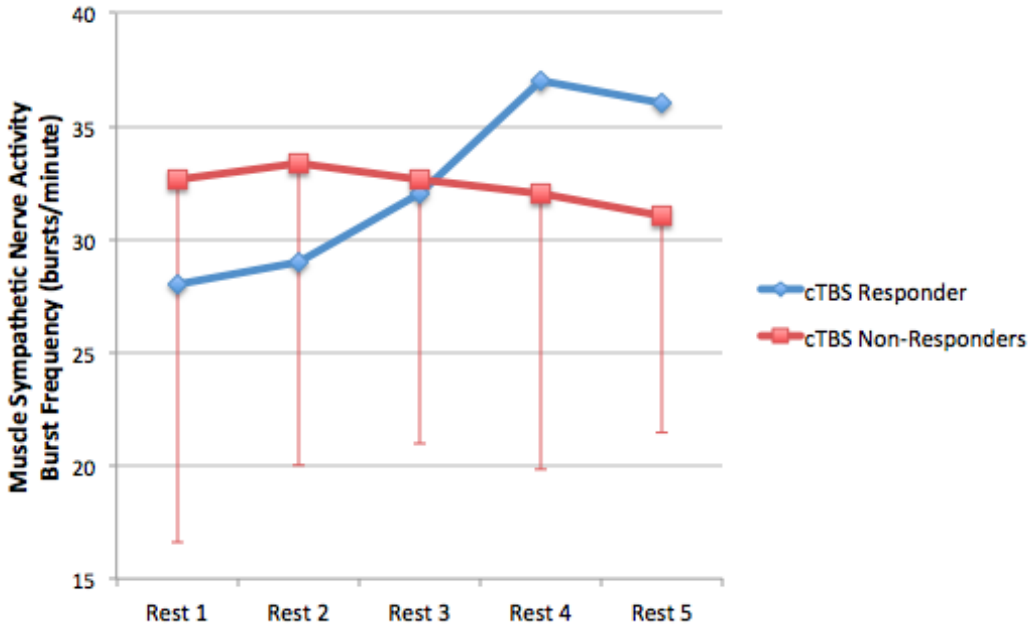

Figure 25. cTBS Responder and Non-responder averaged one-minute resting muscle sympathetic nerve activity burst frequency recordings post-cTBS stimulation. cTBS, continuous theta burst stimulation

4.3.7 Muscle Sympathetic Nerve Activity (Burst Incidence)

There was no interaction effect ($F_{8, 56}$= 0.85, p = 0.49) for minute-averaged recordings of muscle sympathetic nerve activity burst incidence collected during rest. There was a main effect of TBS condition on the MSNA burst incidence response to exercise ($F_{2, 14}$= 6.5, p = 0.01). Finally, there was no main effect of time on the MSNA burst incidence response to exercise ($F_{1.5, 10.8}$= 2.2, p = 0.16).

Minute-averaged recordings of muscle sympathetic nerve activity burst incidence collected during the five-minute rest period post-TBS significantly differed between iTBS and cTBS conditions ($p \leq 0.03$) during minutes 3, 4 and 5. Recordings post-Sham were also significantly lower than cTBS ($p \leq 0.02$) during minutes 3, 4 and 5 (See Figure 26). Also, muscle sympathetic nerve activity burst incidence averaged for the entire five-minute rest period did not significantly differ (p = 1.0) between conditions (See Figure 27).

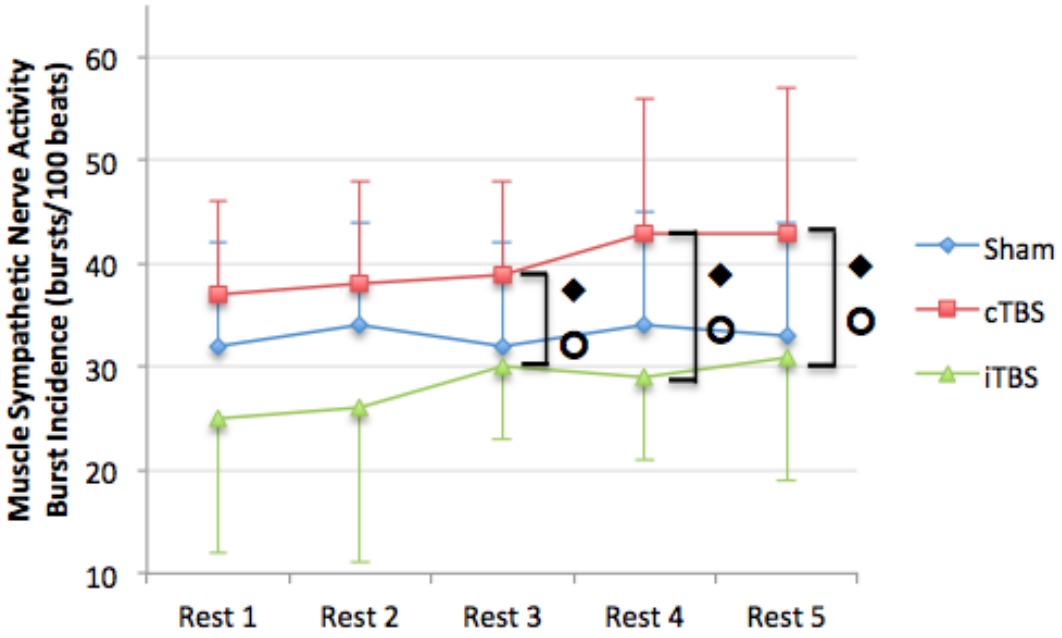

Figure 26. Averaged one-minute resting muscle sympathetic nerve activity burst incidence recordings post-stimulation (Sham, cTBS, and iTBS). Data are presented as Means ± Standard Deviations. ◆ , $p \leq 0.04$ between iTBS and cTBS. **O** , $p \leq 0.02$ between Sham and cTBS. There was also a trend toward significance between sham and cTBS at Rest 2, $p = 0.08$. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

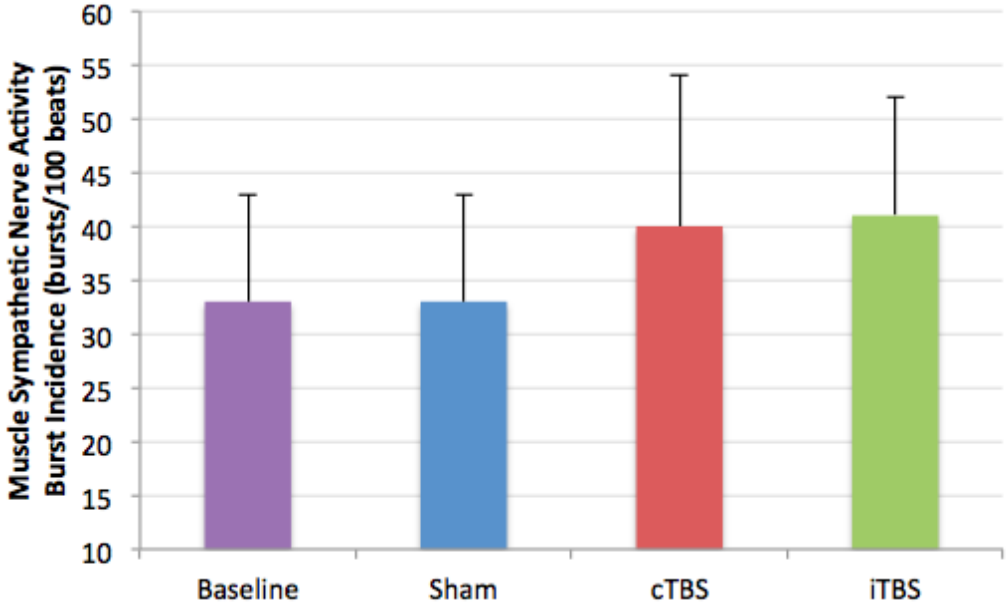

Figure 27. Averaged five-minute resting muscle sympathetic nerve activity burst incidence recordings post-TBS condition (Sham, cTBS, and iTBS). No significant difference between conditions, all, $p > 0.6$. Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

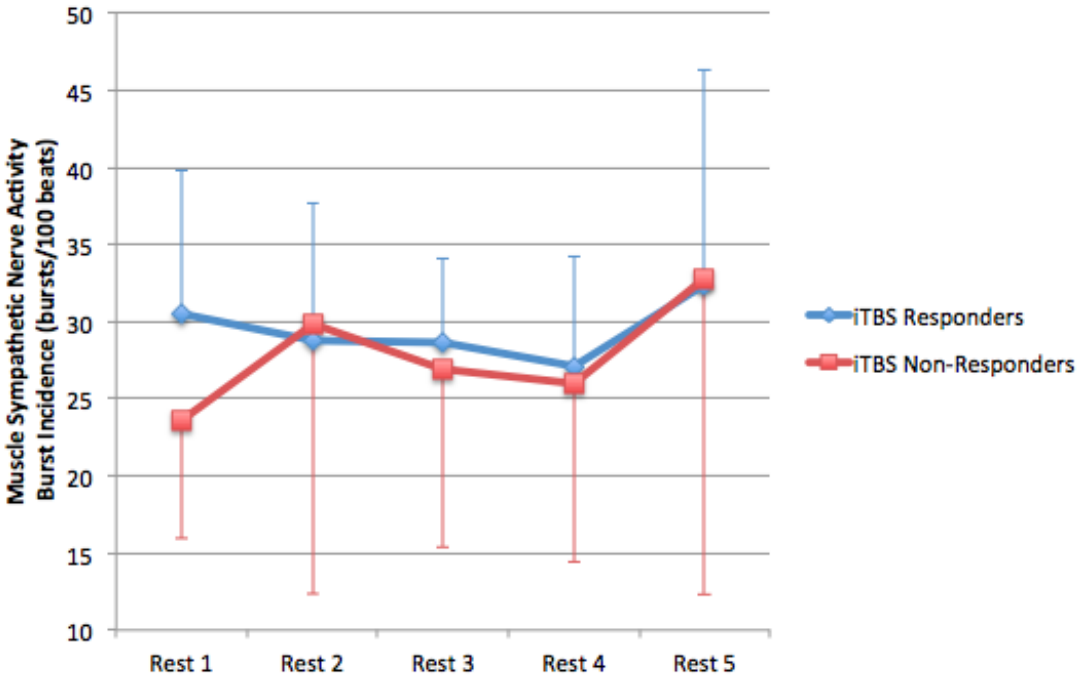

Figure 28. iTBS Responder and Non-responder averaged one-minute resting muscle sympathetic nerve activity burst incidence recordings post-iTBS stimulation. iTBS, intermittent theta burst stimulation.

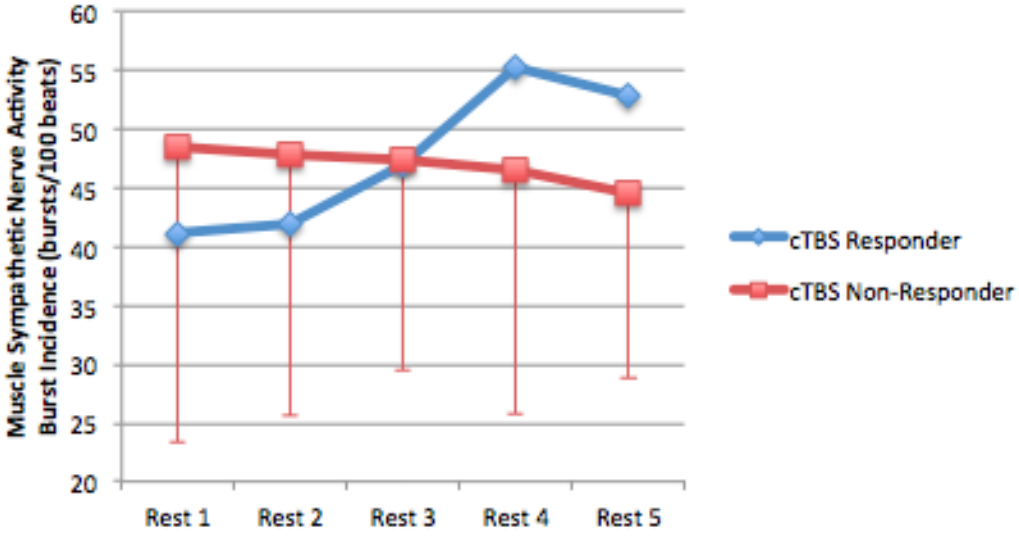

Figure 29. cTBS Responder and Non-responder averaged one-minute resting muscle sympathetic nerve activity burst incidence responses post-cTBS stimulation. cTBS, continuous theta burst stimulation.

4.3.8 Respiration Rate

There was no interaction effect ($F_{8, 88}$= 0.85, p = 0.56) for minute-averaged recordings of respiration rate collected during rest. There was also no main effect of TBS condition on the respiration rate response to exercise ($F_{2,22}$= 0.09, p = 0.9). Finally, there was no main effect of time on the respiration rate response to exercise ($F_{4, 44}$= 0.6, p = 0.67) (See Figure 30). Respiration rate averaged for the entire five-minute rest period also did not significantly differ (p = 1.0) between conditions (See Figure 31).

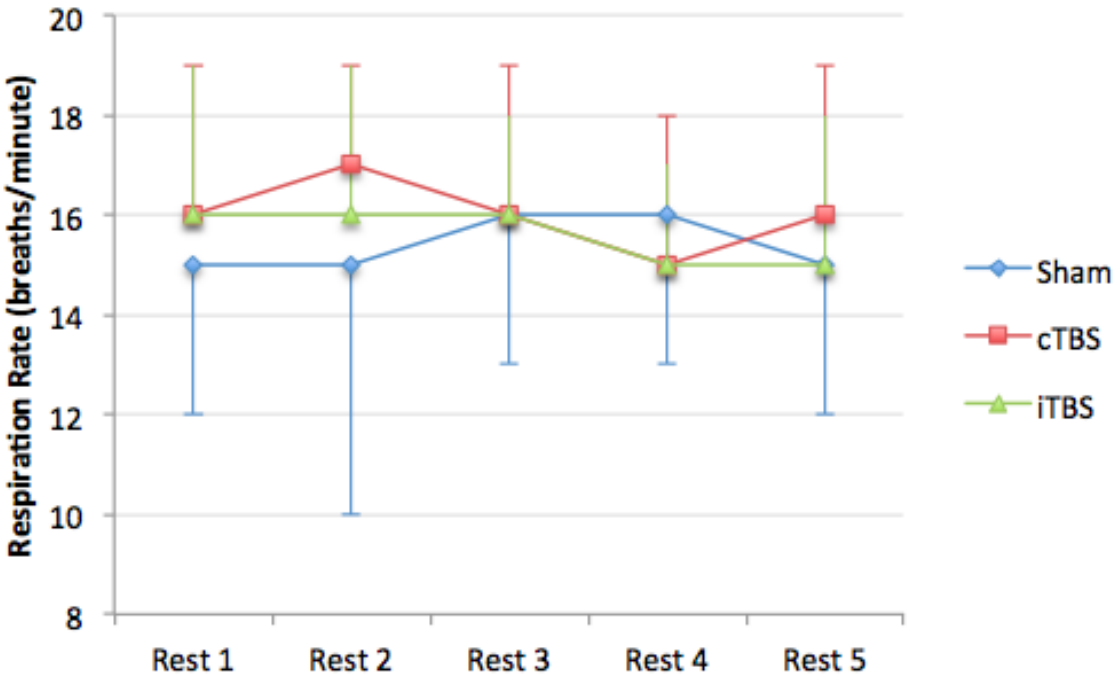

Figure 30. A) Averaged one-minute resting respiration rate recordings post-TBS condition (Sham, cTBS, and iTBS). No significant difference between conditions or time points, p > 0.2. No significant difference between conditions, p = 1.0. Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

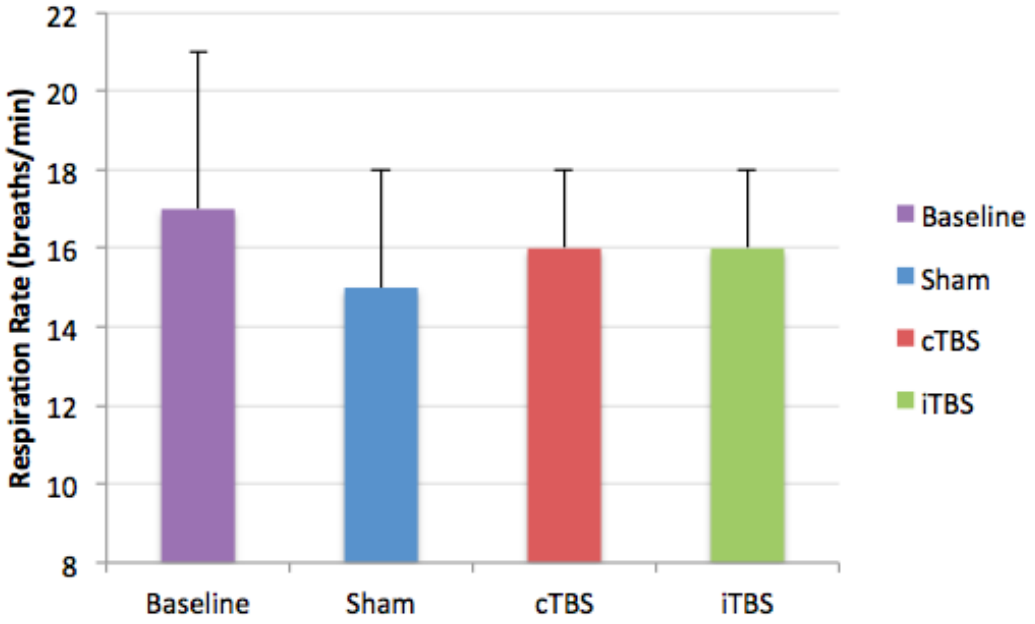

Figure 31. Averaged five-minute resting respiration rate recordings post-TBS condition (Sham, cTBS, and iTBS). No significant difference between conditions, p = 1.0. Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

4.4 Isometric Handgrip Exercise and Post-Exercise Ischemia Data

4.4.1 Heart Rate

There was no interaction effect ($F_{3.2,32.2}$= 0.36, p = 0.80) for minute-averaged recordings of HR collected during IHG and PEI compared to rest . There was also no main effect of TBS condition on the HR response to exercise ($F_{2,20}$=0.8, p = 0.93). However, there was a main effect of time ($F_{1.8, 17.9}$= 23.3, p < 0.000). Specifically, HR was higher during the second minute of isometric handgrip exercise (IHG 2) than all other time points (all, p < 0.008). HR was also higher during the first minute of handgrip exercise (IHG 1) than Rest and the first minute of post-exercise ischemia (PEI 1) (all, p < 0.02) (See Figure 32).

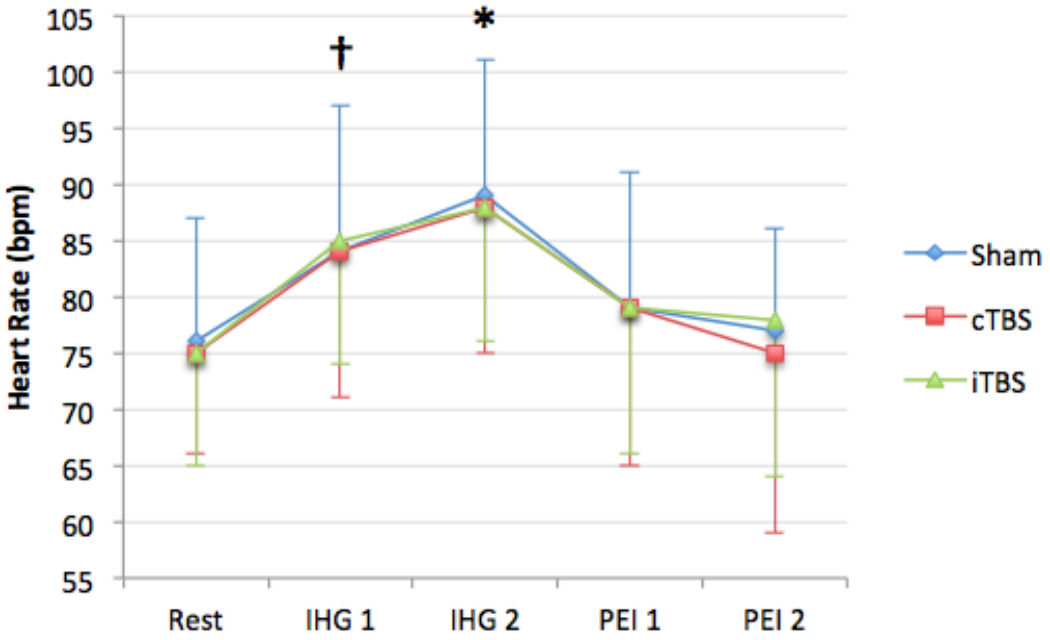

Figure 32. Averaged one-minute heart rate recordings post-TBS condition (Sham, cTBS, and iTBS) during IHG and PEI compared to Rest. No significant difference between conditions, $p > 0.1$. †, The first minute of IHG exercise was significantly greater than Rest and PEI 2 ($p \leq 0.02$). *, The second minute of IHG exercise was significantly greater than Rest, IHG 1, PEI 1 and PEI 2 ($p \leq 0.01$). Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation; IHG, isometric handgrip exercise; PEI, post-exercise ischemia.

4.4.2 Mean Arterial Pressure

There was an interaction effect ($F_{8,80}$= 3.4, p = 0.02) for minute-averaged recordings of MAP collected during IHG and PEI compared to rest. Specifically, iTBS was greater than cTBS during the Rest period. There was no main effect of TBS condition on the MAP response to exercise ($F_{1.2,12.4}$=0.12, p = 0.79). However, there was a main effect of Time ($F_{4, 40}$= 15.8, p < 0.000). Specifically, MAP was lower during Rest than all other time points (all, p ≤ 0.009). MAP was also higher during IHG 2 than PEI 1 (p = 0.04) (See Figure

33). Furthermore, five-minute averaged MAP recorded during rest post-iTBS was significantly higher than post-cTBS (p = 0.001).

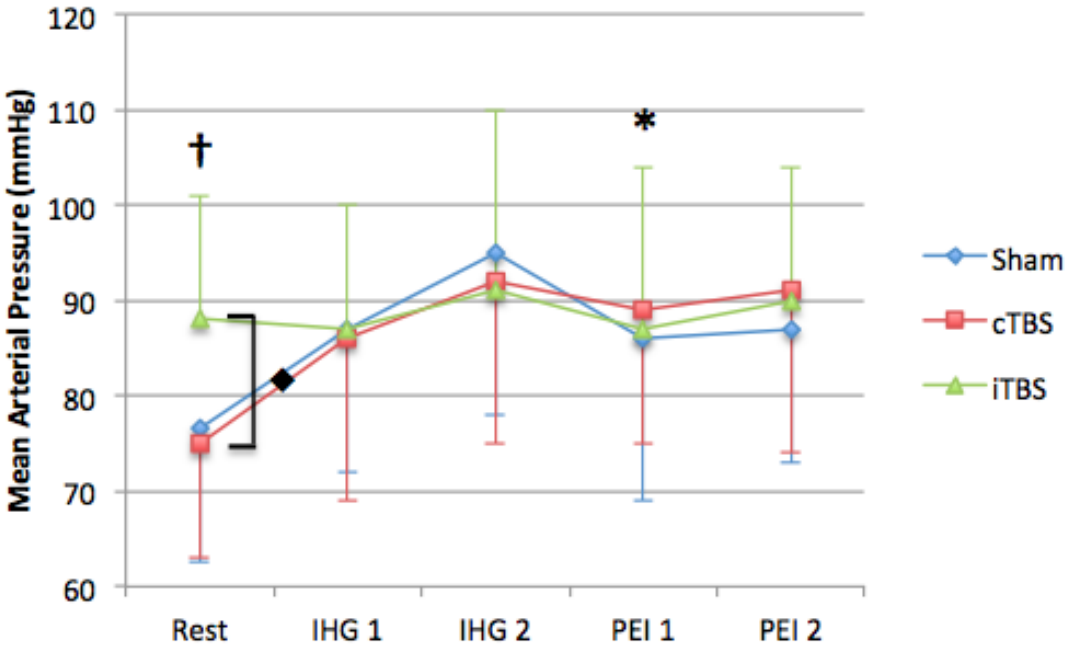

Figure 33. Averaged one-minute mean arterial pressure recordings post-TBS condition (Sham, cTBS, and iTBS) during IHG and PEI compared to Rest. Data are presented as Means ± Standard Deviations. ◆, p = 0.001 between iTBS and cTBS. †, Rest was significantly less than all other time points (p ≤ 0.009). *, the second minute of IHG exercise (IHG 2) was significantly greater than the first minute of PEI (PEI 1, p ≤ 0.04). cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation; IHG, isometric handgrip exercise; PEI, post-exercise ischemia.

4.4.3 Systolic Blood Pressure

There was no interaction effect ($F_{1.7,16.6}$= 0.6, p = 0.61) for minute-averaged recordings of SBP collected during IHG and PEI compared to Rest . There was also no main effect of TBS condition on the SBP response to exercise ($F_{2,20}$=0.45, p = 0.65). However, there was a main effect of Time ($F_{4, 40}$= 10.8, p < 0.000). Specifically, the second minute of IHG exercise was greater than the

first minute (p = 0.009). The second minute of IHG exercise and both PEI time points were also significantly greater than Rest (p < 0.03) (See Figure 34).

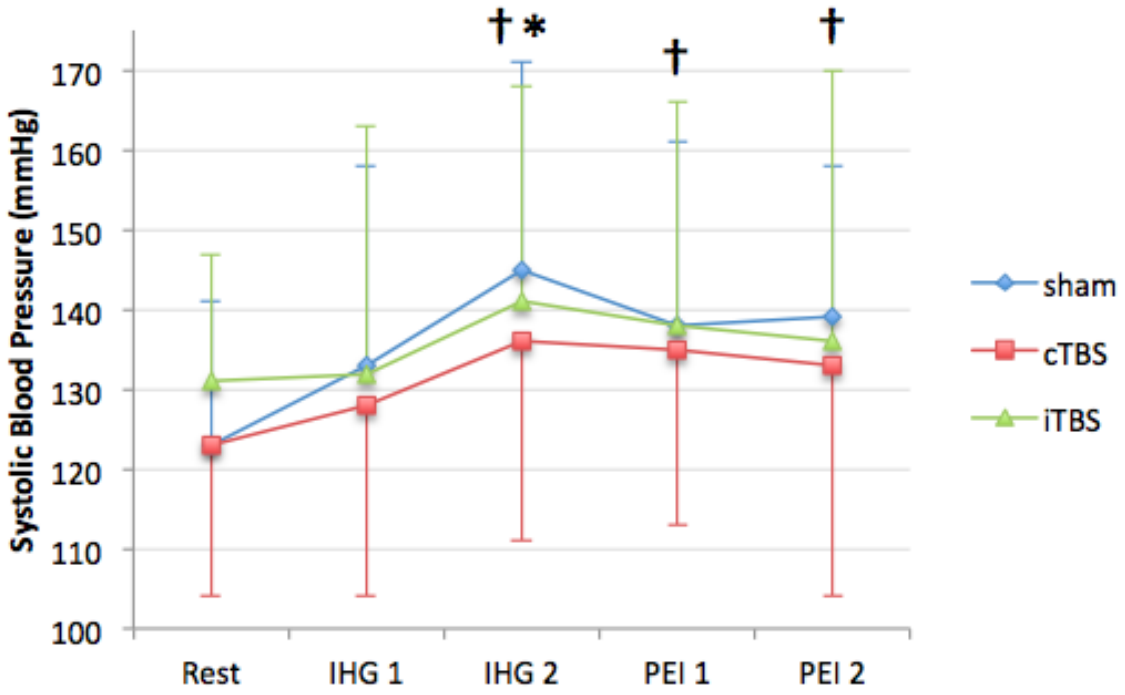

Figure 34. Averaged one-minute systolic blood pressure recordings post-TBS condition (Sham, cTBS, and iTBS) during IHG and PEI compared Rest. No significant difference between conditions, p = 0.2. *, There was a significant difference in time, with the second minute of IHG (IHG 2) exercise being greater than the first minute (IHG 1, p = 0.009). †, IHG 2, PEI 1 and PEI 2 were significantly greater than Rest (p < 0.03). Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation; IHG, isometric handgrip exercise; PEI, post-exercise ischemia.

4.4.4 Diastolic Blood Pressure

There was no interaction effect ($F_{8, 80}$= 1.04, p = 0.41) for minute-averaged recordings of DBP collected during IHG and PEI compared to rest. There was also no main effect of TBS condition on the DBP response to exercise

($F_{2,20}$=0.51, p = 0.61). However, there was a main effect of Time ($F_{2.3, 23.1}$= 18.8, p < 0.000). Specifically, both time points of IHG and PEI were greater than Rest (p ≤ 0.01). The second minute of IHG was also significantly greater than the first minute of IHG and the first minute of PEI (p = 0.02). (See Figure 35).

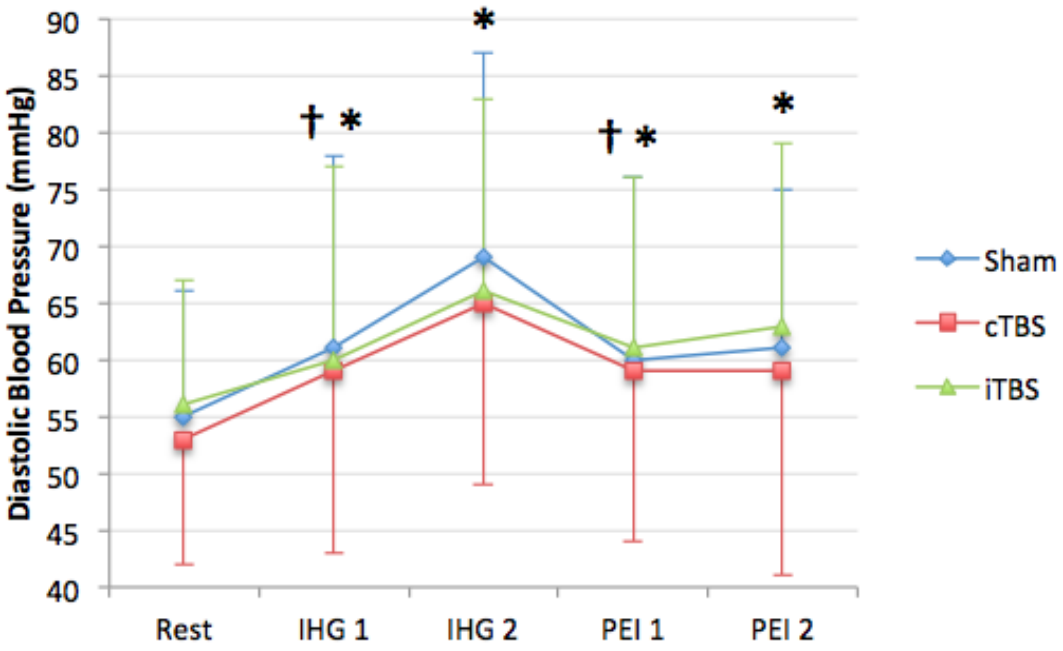

Figure 35. Averaged one-minute diastolic blood pressure recordings post-TBS condition (Sham, cTBS, and iTBS) during IHG and PEI compared to Rest. No significant difference between conditions or time points, p > 0.2. There was a significant difference in time; *, Rest was significantly less than both time points of IHG and PEI (p ≤ 0.01). †, the second minute of IHG was also significantly greater than the first minute of IHG and the first minute of PEI (p = 0.02). Data are presented as Means ± Standard Deviations. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation; IHG, isometric handgrip exercise; PEI, post-exercise ischemia.

4.4.5 Muscle Sympathetic Nerve Activity (Burst Frequency)

There was no interaction effect ($F_{8, 56}$= 2.0, p = 0.07) for minute-averaged recordings of MSNA burst frequency collected during IHG and PEI compared to rest. There was also no main effect of TBS condition on the MSNA burst

frequency response to exercise ($F_{2,14}$= 2.1, p = 0.16). However, there was a main effect of Time ($F_{1.9, 13.8}$= 13.8, p < 0.001). Specifically, there was a significant difference in time IHG 2 and PEI 2 being greater than the first minute of IHG exercise (p ≤ 0.03). The second minute of PEI is also greater than Rest (p = 0.015). There was also a trend toward significance in time differences with IHG 2 and PEI 1 being greater than Rest (p ≤ 0.06) (See Figure 36).

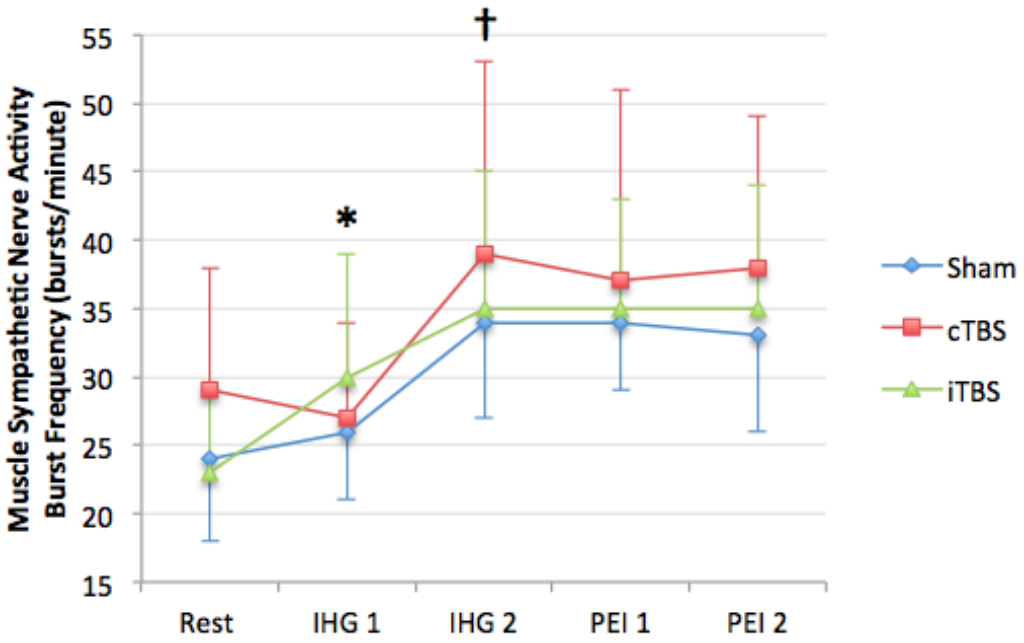

Figure 36. Averaged one-minute muscle sympathetic nerve activity burst frequency recordings post-TBS condition (Sham, cTBS, and iTBS) during IHG and PEI compared Rest. Data are presented as Means ± Standard Deviations. *, there was a significant difference in time with IHG 2 and PEI 2 being greater than the first minute of IHG exercise (p ≤ 0.03). †, the second minute of PEI is also greater than rest (p = 0.015). cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

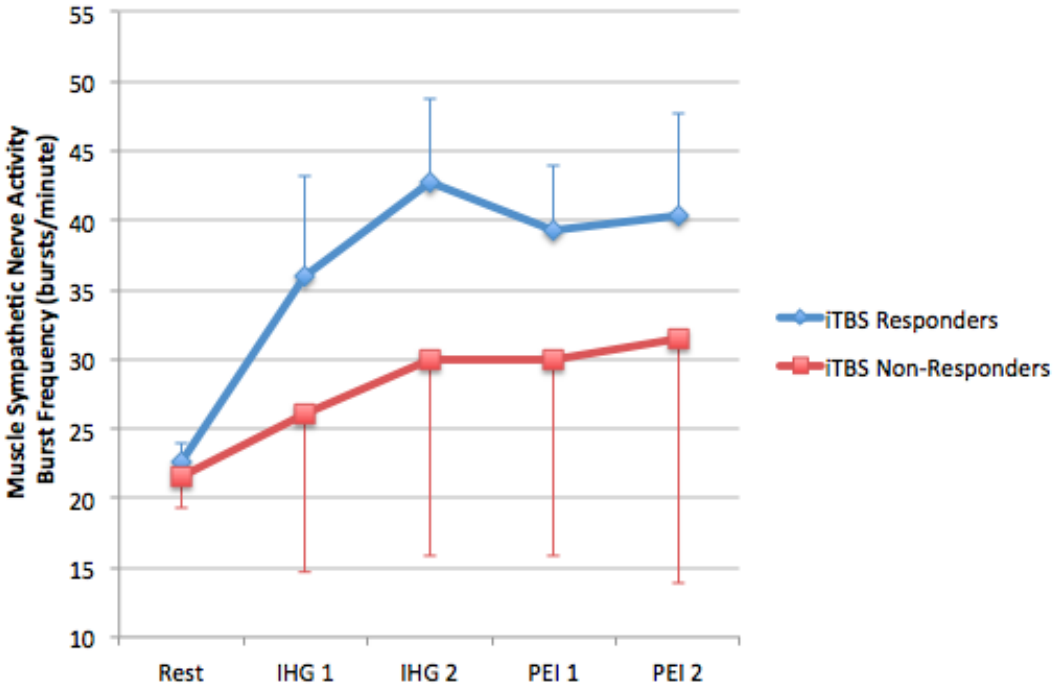

Figure 37. iTBS Responder and Non-responder averaged one-minute muscle sympathetic nerve activity burst frequency responses during Rest, IHG and PEI. iTBS, intermittent theta burst stimulation.

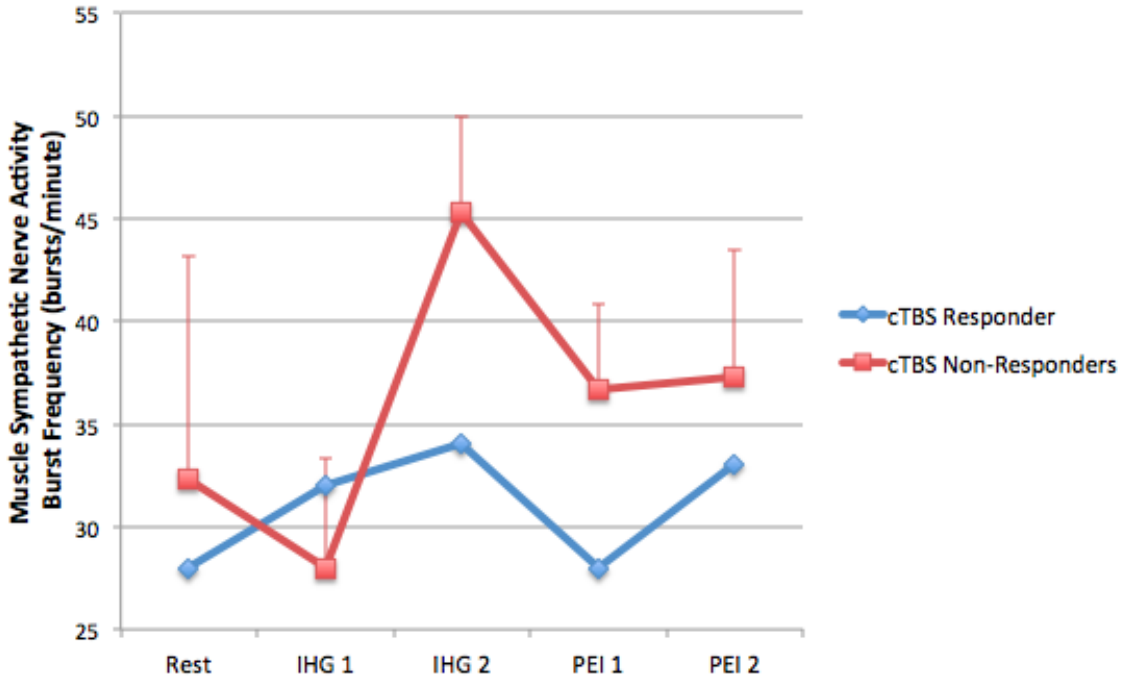

Figure 38. cTBS Responder and Non-responder averaged one-minute muscle sympathetic nerve activity burst frequency recordings during IHG and PEI compared Rest. cTBS, continuous theta burst stimulation

4.4.6 Muscle Sympathetic Nerve Activity (Burst Incidence)

There was no interaction effect ($F_{8, 56}$= 1.2, p = 0.31) for minute-averaged recordings of MSNA burst incidence collected during IHG and PEI compared to rest. There was also no main effect of TBS condition on the MSNA burst incidence response to exercise ($F_{2,14}$=0.78, p = 0.48). However, there was a main effect of Time ($F_{1.8, 12.9}$= 13.4, p = 0.001). Specifically, PEI 2 was significantly greater than Rest, IHG 1 and IHG 2. (p ≤ 0.03) (See Figure 39).

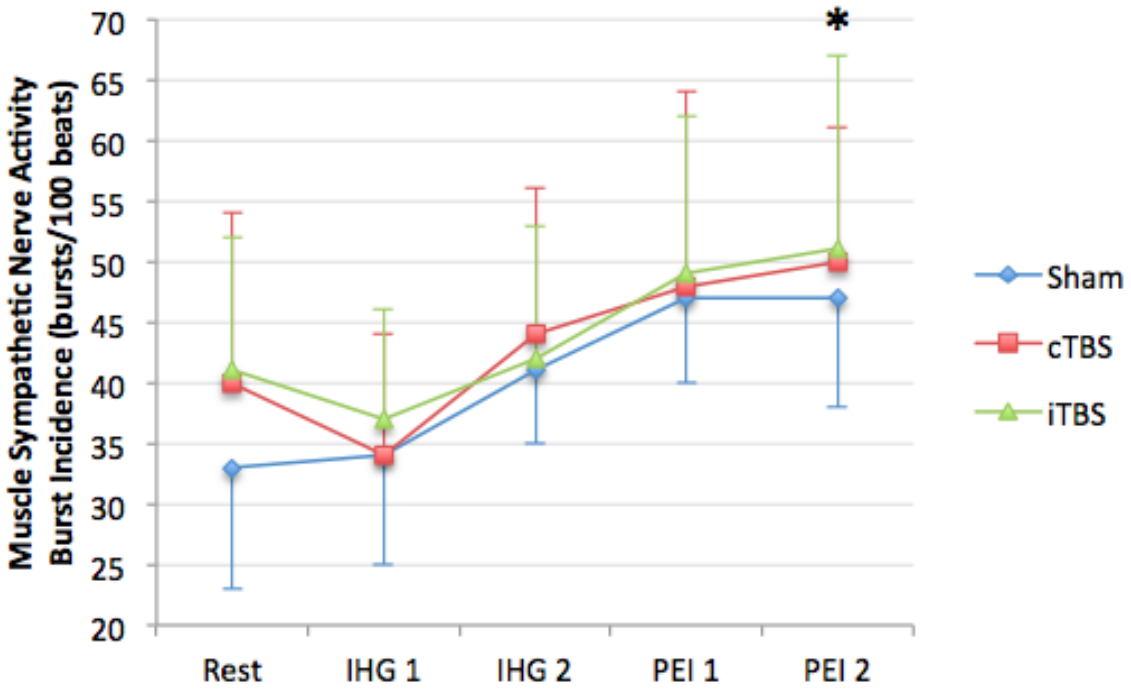

Figure 39. Averaged one-minute muscle sympathetic nerve activity burst incidence recordings post-TBS condition (Sham, cTBS, and iTBS) during IHG and PEI compared to Rest. Data are presented as Means ± Standard Deviations. *, there are significant differences in time with PEI 2 being significantly greater than Rest, IHG 1 and IHG 2. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

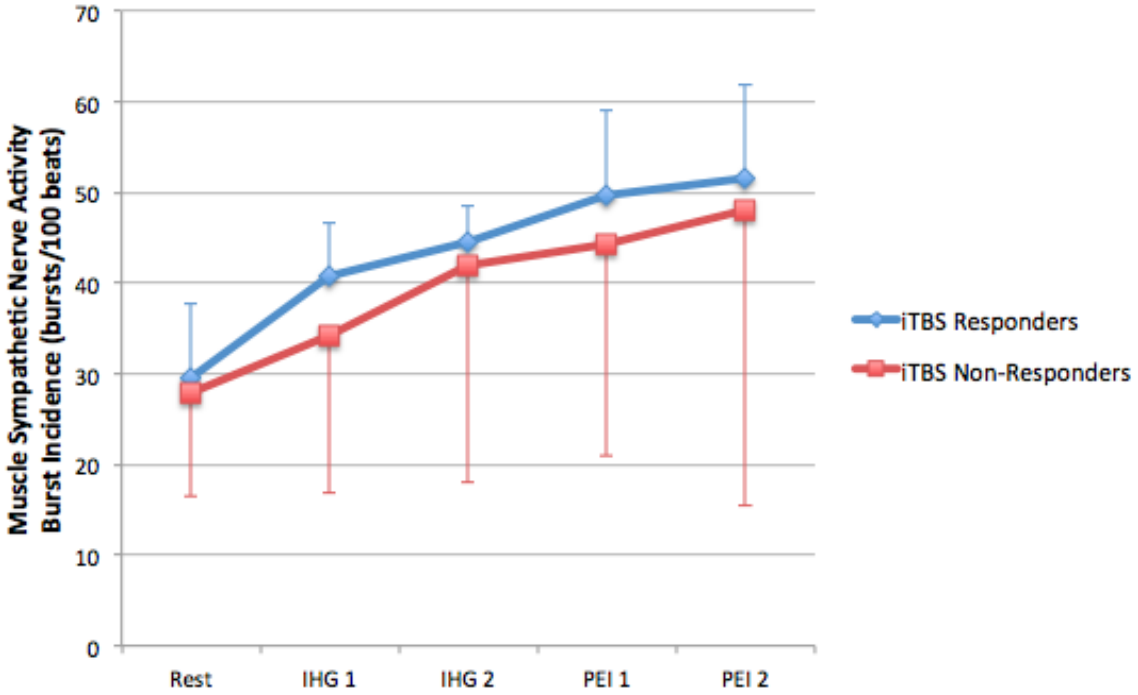

Figure 40. iTBS Responder and Non-responder averaged one-minute muscle sympathetic nerve activity burst incidence recordings post-TBS iTBS during IHG and PEI compared Rest. iTBS, intermittent theta burst stimulation.

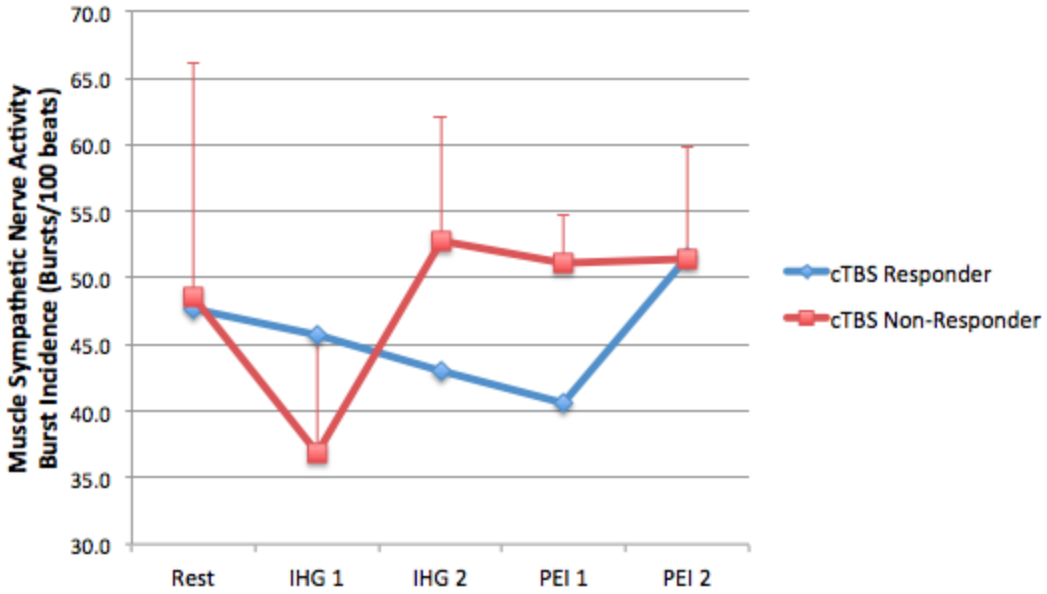

Figure 41. cTBS Responder and Non-responder averaged one-minute muscle sympathetic nerve activity burst incidence recordings post-TBS cTBS during IHG and PEI compared Rest. cTBS, continuous theta burst stimulation

4.4.7 Respiration Rate

There was an interaction effect ($F_{3.1, 34.4}$= 3.5, p = 0.03) for minute-averaged respiration rate collected during IHG and PEI compared to Rest. Specifically, one minute-averaged recordings of respiration rate collected during IHG and PEI compared to Rest significantly differed between iTBS and Sham conditions during the first minute of IHG exercise (p < 0.01). iTBS was also significantly lower than cTBS during the first minute of IHG exercise (p < 0.01). Also, there was a trend toward significance for iTBS to be less than Sham during the second minute of IHG exercise (p = 0.06). There was no main effect of TBS condition on the respiratory rate response to exercise ($F_{2,22}$=1.2, p = 0.33). There also was no main effect of Time ($F_{4, 44}$= 0.45, p < 0.77) (See Figure 42).

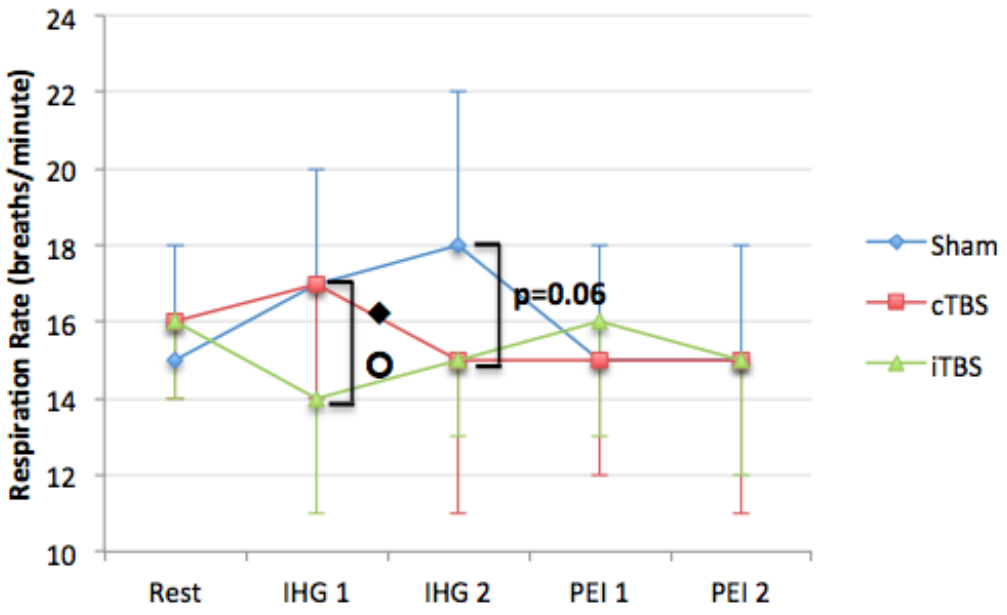

Figure 42. Averaged one-minute respiration rate recordings post-TBS condition (Sham, cTBS, and iTBS) during IHG and PEI compared to Rest. Data are presented as Means ± Standard Deviations. **O** , p < 0.01 between iTBS and Sham. ◆ , p < 0.01 between cTBS and iTBS. There was a trend toward significance between Sham and iTBS at IHG 2, p = 0.06. cTBS, continuous theta burst stimulation; iTBS, intermittent theta burst stimulation.

CHAPTER 5: DISCUSSION

5.1 Overview

The objective of the present study was to determine if using continuous and intermittent transcranial magnetic stimulation protocols to inhibit or facilitate neural activity within the human motor cortex, respectively, alters resting and/or isometric handgrip-mediated cardiovascular responses in a population of young, normotensive men and women. It was hypothesized that at rest, applying iTBS to the flexor digitorum superficialis area of the motor cortex would cause a subsequent increase in HR, MSNA and ABP, while applying cTBS would decrease HR, ABP and MSNA. However, it was hypothesized that iTBS would lessen and cTBS would exaggerate the cardiovascular response to isometric handgrip exercise (compared to Sham TBS) as the magnitude of CC activation would theoretically be lessened and heighted, respectively to maintain the target intensity. Our results showed that MEP amplitudes post-iTBS were significantly higher than baseline (i.e., no TBS) and Sham ($p \leq 0.02$) as expected, but MEPs measured post-cTBS were not inhibited as expected.

There were no differences in any of the HRV indices of cardiac autonomic innervation, five-minute-averaged resting measures of HR, MAP, SBP, DBP, MSNA burst frequency, MSNA burst incidence, or respiration rate. However, there was significantly higher SBP measured during the second minute of rest

after iTBS (versus cTBS). In addition, resting MSNA burst frequency was elevated during minutes three, four and five post-cTBS compared to both Sham and iTBS. Resting MSNA burst incidence was also greater post-cTBS (versus Sham and iTBS) during the same resting time points.

There were almost no significant differences between conditions during any point of the IHG exercise or PEI periods except for respiration rate. There were significant differences between the rest and IHG and PEI time points for measured recordings of HR, MAP, SBP, DBP, MSNA, burst frequency, MSNA burst incidence, and respiration rate, as expected, due to the increased metabolic demands of the IHG exercise and entrapment of metabolic byproducts during the PEI period.

5.2 Motor Cortex Excitability: Maximal Evoked Potentials

5.2.1 Sham Condition

The ability of TBS to alter the excitability of the motor cortex was assessed by comparing the changes in MEP amplitude post-Sham, post-iTBS, and post-cTBS to baseline (i.e. no TBS). MEP amplitudes measured post-sham compared to baseline were not significantly different (see Figure 11). This lack of significant difference between sham and baseline was expected; the sham condition consisted of turning the figure-8 coil 90 degrees so that the participant could feel

the weight of the coil on their scalp, and hear the ‘clicking’ noise of stimulation, but no cortical networks were actually being stimulated. With no stimulation to the motor cortex it was expected that the excitability was to remain relatively the same. The lack of difference in excitability between baseline and sham also solidifies that the properties of the sham condition were sufficient for a control condition.

5.2.2 Intermittent Theta Burst Stimulation

Compared to baseline and sham, MEPs measured post-iTBS were significantly facilitated (see Figure 11). This is consistent with past research (49, 52) that investigated TBS effects on motor cortex excitability. There was also a trend towards significance for post-iTBS MEPs measured to be greater than those measured post-cTBS (see Figure 11).

5.2.3 Continuous Theta Burst Stimulation

However, despite consistent results post-iTBS, the lack of depression of motor cortex excitability following cTBS differs from past research $(49, 52)$. This difference in findings may be attributed to various factors influencing the activity of the motor cortex at the time of stimulation. This includes voluntary contraction of the target muscle prior to, during or post stimulation, and the physiological cortical resting state at the time of stimulation, to be discussed in the following

sections. Furthermore, differences in experimental protocol from previous research may be a contributing factor to why we found a lack of change in MEP amplitudes post-iTBS. Huang and colleagues (49) established cTBS and iTBS protocol and the subsequent effects on MEP amplitude. Their protocol for establishing AMT differed from ours in that they defined it as the minimum single pulse stimulation intensity required to produce an MEP of greater than 200 μV in at least five of ten trials while contracting at 20% of their MVC. The current study used a common protocol (TMS Standard Operating Procedure, see Appendix H) of defining AMT as the minimum single pulse stimulation intensity required to produce an MEP of greater than 50 μV in at least five of ten trials while contracting at only 5% of their MVC. While contracting at 20% of MVC would help facilitate greater MEP production, it also causes a greater fatigue that may have affected our IHG exercise. Furthermore, the stronger response (as exhibited by higher MEP amplitudes) observed by Huang and colleagues (49) may explain why they experienced significant results for both iTBS and cTBS. The stronger initial response during the AMT determination is likely an indication that a greater number of their participants than our own could be classified as responders to TMS and thus would have greater MEP responses to the TBS conditions.

5.3 Cardiovascular Response to Exercise

The purpose of the cardiovascular response to exercise is to increase oxygen and nutrient delivery to and the removal of metabolic waste products

from active tissues. The increased MAP that occurs with exercise is essential for maintaining adequate perfusion pressure and blood flow to the contracting muscles. The cardiovascular system is integrated with various other mechanisms to meet the increased metabolic demands of the body at the onset and continually throughout exercise. Isometric exercise such as the one used in the current study is characterized by an increase in systolic, diastolic and mean ABP (37). The neural mechanisms associated with the rise in ABP that occurs during exercise are still not completely understood, but there are two main mechanisms responsible for the initiation and maintenance of the exercise response. The first, “CC” suggests that increases in arterial pressure are due to the direct action of higher brain structures such as the motor cortex. The second is termed the “EPR”, and is responsible for the cardiovascular changes associated with a reflex from active skeletal muscle afferents stimulated by muscle contraction. The EPR is further composed of the Metaboreflex, stimulated by chemical stimuli and the Mechanoreflex, which is stimulated by mechanical (e.g. stretch receptors) stimuli (59).

At the onset of exercise CC and the Mechanoreflex of EPR of the acts to remove parasympathetic tone to achieve the necessary rise in HR to meet the metabolic demands of the exercising muscle. As exercise continues beyond the first minute, a further increase in HR, ABP and MSNA occurs as a result of the accumulation of chemical by-products of skeletal muscle metabolism (such as carbon dioxide, lactic acid, hydrogen and potassium ions) activating the

Metaboreflex (94). The resultant increase in ABP initiated by this reflex is designed to facilitate skeletal blood flow and correct this mismatch. The importance of metabolites compared to mechanical stimuli for the activation of the EPR is especially important during periods of PEI. Occluding blood flow while a limb is contracting traps all the metabolites within the local circulation of the limb. When contraction ceases there is no longer a mechanical stimulus playing a role in activating the EPR, but the chemical by-products resultant from contraction metabolism are trapped within the local circulation and thus still exert their effects to activate the Metaboreflex component of the EPR. Since the participant is no longer contracting, CC signals have ceased by EPR is still activated, thus the PEI acts to separate these two components of cardiovascular control.

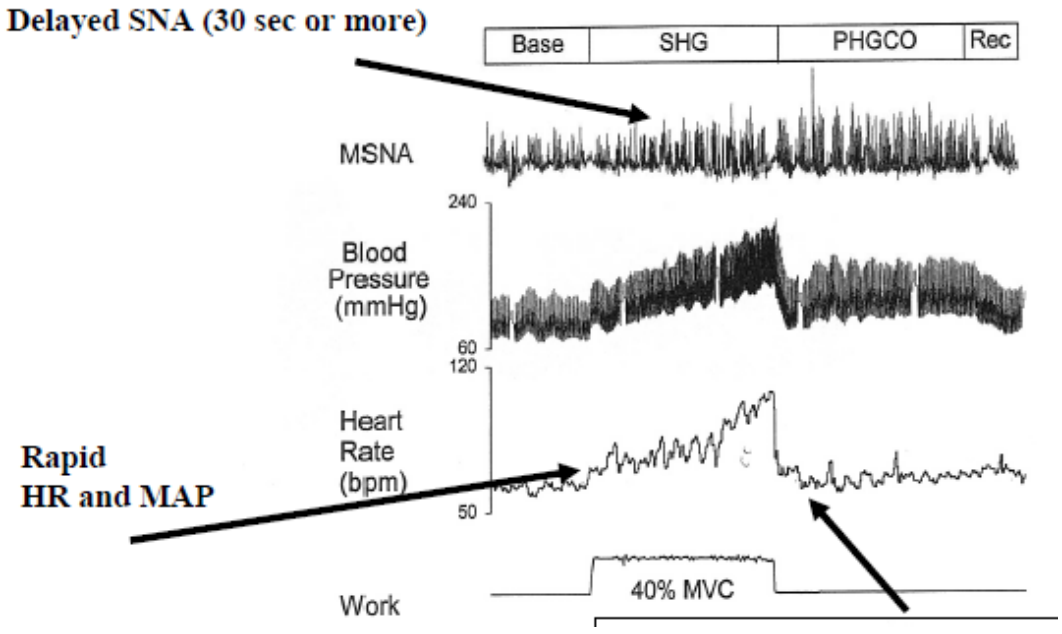

Figure 43. Recordings of MSNA, ABP and HR as they are theoretically supposed to act through periods of baseline (Base), a static handgrip exercise (SHG), post-handgrip circulatory occlusion (PHGCO) and Recovery (Rec).

At the onset of exercise MSNA, blood pressure, and HR increase. The initial increase in HR and ABP during the IHG exercise occurs rapidly due to the activation of CC and the Mechanoreflex. The increase in MSNA is delayed slightly after the onset of exercise with further increases in HR, MSNA and ABP caused by the activation of the Metaboreflex as chemical by-products of exercise metabolism accumulate after a minute of exercise. Immediately after exercise the PEI period begins with blood pressure dropping, but not back to resting levels. Heart rate also drops back to resting levels, while levels of MSNA continue to be elevated. When blood flow to the forearm is restored blood pressure lowers to resting levels and MSNA begins to drop back to resting as the recovery period continues (see Figure 43). Our results show that our IHG and PEI periods followed the expected patterns for HR, MAP, and MSNA (see Figures 32, 33, 38 and 39, respectively) and were similar to other studies with similar experimental protocols (35, 76, 95, 102). Mark et al. (76) had participants perform a 2-minute forearm IHG exercise at 30% of their MVC followed immediately by a 2-minute period of ischemia. They found that during both minutes of the IHG exercise HR and MAP increased, while MSNA only increased significantly during the second minute. During the PEI period HR returned back to resting levels, but MSNA and MAP remained elevated until the recovery period (see Figure 44) (76). Gandevia et al. (35) also had the same pattern of results while utilizing a 2-minute IHG exercise at 33% followed by a 2-minute PEI period. Also, other studies utilizing

just the IHG forearm exercise of 30-35% have also found significant increases in HR, MAP and MSNA (95, 102).

	Control	Handgrip 1st min.	Handgrip 2nd min.	MIR 1st min.	MIR 2nd min.	Recovery
HR (beats/min)	76	88	83	67	63	67
MSA (burst/min X mean burst ampl.)	476	410	1182	1473	1386	603
MAP (mmHg)	100	114	127	122	122	107

Figure 44. Responses of muscle sympathetic activity (MSA), HR and MAP to a 2-minute sustained isometric handgrip exercise, muscle ischemic response (MIR) and recovery found by Mark et al. (76).

Our recordings of HR, MAP, SBP, and MSNA followed the expected patterns that have also been found by other researchers. More specifically, recordings of HR during the first minute of IHG increased significantly above Rest and the second minute of PEI. Furthermore, HR significantly increased further during the second minute of IHG exercise above Rest, the first minute of IHG and both minutes of the PEI period (see Figure 32). Our recordings of MAP increased during the first minute of IHG exercise with further increases during the second minute and dropped, but not back to resting values during the PEI period. Furthermore, Rest was significantly less than both IHG and PEI time points, and the first minute of PEI was significantly less than the second minute of IHG exercise (see Figure 32). Recordings of SBP significantly increased during the second minute of the IHG exercise compared to rest and the first minute of IHG exercise, both minutes of the PEI period were also significantly greater than Rest (see Figure 34). For DBP, both minutes of IHG and PEI were significantly greater than Rest. The

second minute of IHG was also significantly greater than the first minute of both IHG and PEI (see Figure 35). In terms of MSNA, recordings of MSNA Burst Frequency followed the general expected pattern, with IHG minute 2 and PEI minute 2 being significantly greater than the first minute of IHG exercise (see Figure 37). Recordings of MSNA Burst Incidence followed a similar pattern, but only PEI 2 was significantly greater than Rest and both minutes of the IHG exercise (see Figure 39).

5.4 Factors Influencing TBS Aftereffects

5.4.1 Voluntary Contractions

Previously, it has been found that voluntary contractions, such as those used to assess AMT and MVC, can facilitate MEPs for several minutes (97). This may bias the results of the subsequent measurements of the experiment. In the current experiment, AMT assessment was performed prior to the measurement of MVC and then a ten-minute rest period before TBS stimulation. The motor cortex activation caused by AMT determination and MVC measurement likely did not affect any post-TBS results because of the long period of time separating the voluntary contraction and stimulation. This period of time was used for the determination of the MSNA recordings, during which the participants were instructed to remain as still and relaxed as possible. Although, since a neuronavigation system was not used, a pre-TBS check was done to ensure the

coil was placed on the optimal stimulation location. This involved the participant doing a slight contraction of about 5% while single TMS pulses were delivered approximately every five seconds while monitoring for large peak-to-peak MEPs. This slight contraction immediately prior to the experimental TBS protocols could potentially have had an effect on the measurements obtained after stimulation.

Huang et al. (47) showed that a weak voluntary contraction (~10% MVC) during TBS abolished the typical aftereffects observed, as well, a contraction lasting one minute after TBS was applied reversed the usual facilitation and inhibition observed (47). To avoid the possible contribution of voluntary muscle contractions on TBS effects,

Martin et al. (77) instructed participants to remain completely relaxed throughout the experiment and discarded any data that showed an increase in EMG activity indicative of a muscle contraction. They also instructed all participants to not perform any strong contractions in the hours prior to the experiment and underwent a 30-minute rest period prior to any data collection and TBS application. However, they still experienced a large amount of inter-individual variability in MEP amplitude post-cTBS of the first dorsal interosseous muscle and no significant changes in MEP size in the biceps muscle. While they concluded that inadvertent muscle contractions could be to blame for their results, they did speculate that differences in the functional state of the motor

cortex, induced by longer-term neuronal activity prior to the experimental session may influence the magnitude and direction of changes caused by TBS (77).

Several other studies have also investigated the possibility of the reversal of expected effects of cTBS (inhibition) and iTBS (facilitation) because of movement prior to, during or after stimulation. Stockel and colleagues (106) had participants perform 150 right finger abductions (motor training) followed by iTBS at 80% AMT, or Sham, with MEP tests performed at baseline, post motor training and post-iTBS. They tested motor performance by measuring peak speed during ten of the previously practiced finger movements. Motor performance decreased 63% following iTBS stimulation, but not sham. MEP amplitudes increased 38% and 37% for iTBS stimulation and sham groups, respectively, after motor training. However, MEP amplitudes decreased 22% below baseline after iTBS stimulation, while they increased by 51% above baseline for the sham group. Furthermore, there was a great degree of inter-individual variation within the stimulation group, with 8 of 12 participants experiencing the decrease in MEP amplitudes post-iTBS, while the majority of the sham group had increased or unchanged MEP amplitudes post-sham condition (106). Participants in the current study were asked to gently squeeze the handgrip dynamometer, while single TMS pulses were delivered prior to cTBS and iTBS application, to ensure the coil was in the optimal stimulation site. This muscular contraction is comparable to the movements performed by Stockel et al. (106), and may have influenced the direction of change in MEP amplitude elicited by the TBS protocols. However, the

iTBS condition still resulted in facilitated MEPs as expected, while the cTBS remained unchanged compared to baseline and sham, instead of being inhibited. Other factors may have contributed to cTBS not resulting in inhibition, but further research is needed to investigate the susceptibility of the excitability outcome of different TBS protocols.

Iezzi et al. (51) discovered that repeated phasic movements of the target muscle preceding TBS reversed the effects of iTBS and cTBS, which resulted in iTBS producing inhibition and cTBS producing facilitation (51). This is similar to the results of Huang et al. (47) who investigated the aftereffects of TBS if the target muscle was tonically contracted immediately after TBS application. They discovered that this tonic voluntary contraction increased the facilitative effects of iTBS, but reversed the inhibitory effects of cTBS to facilitation (49, 51, 77).

While there are differences between many of these studies investigating the effect that muscle contractions performed prior, during or post stimulation, it highlights that the activity of the motor cortex as determined by muscle activity is an important factor in the determination of aftereffects of TBS protocols. The differences in MEP amplitude results can be attributed to many differences in experimental protocols between research groups including the duration and type of contraction, as well as the timing in reference to the application of stimulation. Regardless of including a rest period, instructing participants to not voluntarily contract muscle and discarding data with unintended contractions, it is difficult to

determine whether or not cortical excitability is in a “neutral” state for each individual participant. Furthermore, many studies that are similar in protocol to the current study, in that they all involve pre-stimulation contractions for AMT and/or MVC determination, yet still result in MEPs being both facilitated and inhibited. This then leads to the issue of the physiologic resting state of the brain and motor cortex being influenced by factors other than the contraction of the target muscle.

5.4.2 Mental Resting State

Several studies have suggested that external factors such as emotion and attention can affect the resting state of the cortex at the time of stimulation, which may influence the aftereffects of TBS protocols. Koganemaru et al. (60) tested whether long-term potentiation (LTP)-like plasticity induced by iTBS in the human primary motor cortex was modulated by the emotional state of the participant at the time if stimulation. Participants underwent three sessions of iTBS while viewing a series of positive, neutral or negative images during stimulation application (60). MEPs were measured pre-stimulation and starting immediately after stimulation up to 30 minutes post-stimulation. It was determined that emotion does have an effect on LTP-like plasticity as MEP amplitudes were facilitated compared to pre-stimulation and neutral and positive images, for 30 minutes, when negative images were viewed during iTBS (60). This may have implications to the current study as some individuals reported higher feelings of

discomfort than others. Some participants' experimental sessions were longer than others due to difficulty with MSNA data collection and/or optimal stimulation site and AMT determination. In some participants the process of finding the optimal nerve-recording site for MSNA recordings was much longer and/or painful than others. Some participants experienced more severe leg cramps, 'pins and needles' or involuntary muscle twitches which could contribute to a more negative emotional state. Conversely, there were some participants who reported the experimental procedures were enjoyable thus we can assume they had more of a neutral or positive emotional state. To further complicate the issue of emotional state, some participants appeared and/or outwardly expressed more discomfort and negative emotions during some portions of the experiment than others. This was especially true for the last TBS condition to be applied as participants had been sitting as still as possible for a period of about two hours before the last TBS condition was completed. While no current studies to our knowledge, have examined if discomfort modulates the aftereffects of TBS, discomfort can likely be associated with a negative emotional state. This wide and varied range of emotions experienced by participants may have had a modulatory effect on the aftereffects of the TBS conditions in our study.

Attention has also been proposed as a possible modulator of the direction of the aftereffects of different repetitive TMS protocols (19, 105). Conte et al. (19) aimed to determine if attentional processes in humans influence the change in MEP amplitude size elicited by repetitive TMS. They tested a facilitative repetitive

TMS protocol of 5 Hz in ten trains of ten stimuli during three different mental attentional conditions; full muscular and mental relaxation with eyes closed ("relaxed" condition), attention directed to the target hand of the rTMS ("target hand" condition), and attention directed to the non-target or contralateral hand ("non-target hand" condition). They found that these different mental attention states highly influenced the magnitude and length of MEP facilitation. The condition in which participants directed their mental attention to the target hand of repetitive TMS had far greater and longer-lasting MEP facilitation than the conditions in which the participants were relaxed or directed attention to the opposite hand (19). Conte et al. (19) suggested that the motor preparation (involuntary or imaging the movement repetitive TMS produced) may contribute to the enhanced facilitation. This increase in primary motor cortex excitability during motor preparation is consistent with past studies that investigated motor preparation with TMS that show increased MEP amplitudes prior to the onset of a voluntary movement (19, 36, 88, 104). MEP amplitude results (see Figure 11) from the current may have been influenced by the participant's attention towards the motor task that occurred after stimulation. The significant facilitation seen post-iTBS (see Figure 11) may have been due to participant's mental attention towards the IHG exercise that was to occur after the rest period following stimulation. Furthermore, while Conte et al. (19) did not investigate attention effects while using an inhibitory TMS pattern, MEPs measured post-cTBS in our study may have also been inadvertently facilitated by mental attention.

5.4.3 Conditioning Pulse/ Priming

Condition pulse or priming with multiple pulses in various patterns of frequencies and lengths prior to the TMS condition has been shown to change the aftereffects of TBS and TMS conditions applied (52). Iezzi, et al. (52) used 5 Hz repetitive TMS as a primer to induce short-term potentiation (STP) in efforts to modulate subsequent TBS-induced LTP and long-term depression (LTD)-like plasticity in the human motor cortex. They found that when TBS was primed with 10 suprathreshold 5 Hz rTMS trains delivered within at least 60 seconds of TBS, the rTMS induced STP abolishes the subsequent LTP or LTD-like plasticity of TBS (52). While the current study didn't intend to use a priming pulse sequence, immediately before TBS application, single pulse TMS was used o ensure the coil was placed over the optimal stimulation site. These single pulses could have potentially acted as an unintentional priming stimulus in some participants whose optimal stimulation site took more time and pulses to confirm. It is thus possible that the lack of facilitated MEPs post-iTBS in some participants, and the lack of inhibited MEPs post-cTBS at the group level could be attributed to abolished LTP and LTD-like plasticity from the single pulses potentially acting as a primer.

It is important to note that while the group results show no overall significant decrease in MEP amplitudes post-cTBS, some participants did experience a depression in MEP amplitude. This inter-individual variability may also contribute

to the lack of significant results and will be discussed later as a limitation to the current study.

5.5 Theta Burst Stimulation Effects: Motor Cortex Plasticity Mechanisms

Plasticity within the nervous system is the ability of the neural tissue to undergo changes that strengthen or weaken synaptic transmission based on the activity of the neurons. Homeostatic metaplasticity is the theory that a neural system strives to maintain equilibrium within a particular physiological range. The threshold for bidirectional changes in plasticity through the induction of LTP or LTD varies according to the recent history of synaptic transmission. These homeostatic adjustments don't allow for the excessive expression of either LTP or LTD, thus keeping neural activity within a range that is considered usefully dynamic (106).

5.5.1 Long-Term Potentiation versus Long-Term Depression

Huang and colleagues measured cortical excitability before and after the application of different TBS protocols over the motor cortex (49). They found that MEPs were suppressed for up to 60 minutes after cTBS and was facilitated for up to 15 minutes after iTBS. The opposing effects on cortical excitability from differing TBS protocols are not surprising given that previous work on animals has used intermittent TBS protocols (similar to iTBS protocol) to consistently facilitate synaptic connection (11, 42, 49). Similarly, longer trains of stimulation,

similar to the cTBS protocol has been used to produce suppression (42, 107). Takita et al. (107) speculate that cTBS may reduce the efficacy of transmission through the synaptic connections that are recruited when evoking an MEP, while iTBS increases transmission via synaptic connections of neurons.

5.6 Potential Cortical Pathways Involved with Autonomic Cardiovascular Control Affected by TBS

While it has been well documented that TBS affects the excitability of the motor cortex, there are not many studies conducted that have demonstrated whether TBS alters autonomic outflow through the motor cortex (49, 52). When Krogh and Lindhard first termed CC as the higher brain control of autonomic function that is responsible for the rapid increase in HR observed at the onset of exercise, there was no way of determining where exactly CC was located in the brain (63). Since then many researchers have used various techniques to help elucidate where CC originates, ultimately exposing the motor cortex as an area of importance (72, 100). Our study found that iTBS has the ability to change the excitability of the motor cortex (see Figure 11). Furthermore, we found evidence that iTBS as well as cTBS may potentially affect autonomic outflow via the human motor cortex

Past research has shown that TMS can alter HRV due to connections between the cortex and autonomic centers located within the brainstem (6, 75). Clinical manifestations in patients suggest there is a link between cortical structures and

autonomic centers; for example, stroke often results in ectopic beats and atrial fibrillation (a cardiac arrhythmia characterized by an irregular and rapid heart beat) (83). Cabrerizo demonstrated that rTMS can induce changes in heart rhythm (10). They found excitatory rTMS (trains of stimuli of 1-second at 10Hz for a total of 50 pulses as the pulse train was repeated 5 times at intervals of 1 minute) reduced the cardiac interval (i.e., increased HR) in 7 of 10 participants, although, the other 3 participants had increased time between successive heart beats (RR interval). While this demonstrates a great deal of inter-individual variability, it still provides evidence that TMS can induce changes on autonomic centers via the cortex (10).

The importance of the sympathetic nervous system is demonstrated when there is an interruption in ongoing sympathetic activity, such as by autonomic failure or a transection of the spinal cord. This disruption in sympathetic activity causes a wide variety of problems, especially when focusing on the cardiovascular system, including hypotension and bradycardia. Currently, most studies that have investigated the importance of which, and how cortical structures regulate sympathetic activity used experimental protocols that only focus on short-lasting sympathetic activation (22, 23). Schlindwein and colleagues (100) aimed to identify the brain regions associated with sympathetic activity generation at rest using FDG-PET while measuring autonomic function parameters including blood pressure, HR, HRV and plasma catecholamines (100). Unlike the current study, Schlindwein and colleagues (100) controlled for attention by having all their participants focus on an emotionally neutral visual presentation, dots, that moved

either clockwise or randomly at a slow rate. They found that the autonomic nervous system at rest seems to be at least partially connected to the activity of motor regions, specifically the caudate nuclei and the primary motor cortex. Their results showed that sympathetic activity, measured by plasma norepinephrine and heart rate, is positively correlated to primary motor cortex activity such that when activity in the motor cortex increases it predicts high sympathetic activity at rest (100). While we found no significant differences between conditions at rest for HR (and did not measure plasma norepinephrine), there was a trend towards HR post-cTBS being significantly less than post-iTBS. This supports our hypothesis that at rest, applying iTBS to the motor cortex would cause a subsequent increase in cardiovascular measures, while applying cTBS would cause a decrease by increasing and decreasing, respectively, the sympathetic activity directed toward the cardiovascular system.

Furthermore, SBP was elevated post-iTBS during Rest minute 2 (see Figure 18) and MAP during the full Rest period was elevated post-iTBS compared to post-cTBS (see Figure 33). The amplitudes of MEPs measured post-iTBS demonstrated it was successful in causing motor cortex facilitation, which was then followed by the increase in SBP and MAP. This may indicate that facilitating the motor cortex caused a subsequent increase in sympathetic activity, which ultimately resulted in a significant rise in MAP and SBP. This rise in MAP and SBP may be evidence that stimulation to the motor cortex may modulate cardiac autonomic balance and affect the actions of the baroreflex. The baroreflex acts to

maintain a homeostatic blood pressure, with an increase in sympathetic activity causing an increase in blood pressure; when facilitative stimulation to the motor cortex was applied, the elevation in MAP and the momentary increase in SBP is an indication that an increase in sympathetic activity was likely affecting this mechanism.

Macefield et al. (72) used single-pulse transcranial magnetic stimulation to determine whether a time-locked cortical stimulus could interfere with the sympathetic vasomotor drive to human skeletal muscle (72). They applied excitatory single-pulse TMS to the motor cortex and vertex at 0, 200, 400 and 600 ms after the R-wave of the ECG while measuring HR, blood pressure, pulsatile blood flow and MSNA from the peroneal nerve. It was found that excitatory cortical stimulation over the vertex caused a transient inhibition of the sympathetic discharges and decreased skin blood flow, when the stimulus was delivered 200-400 ms after the R-wave of the electrocardiogram, which was followed by an increase in sympathetic activity. Cortical stimulation over the hand area of the primary motor cortex also caused inhibition of sympathetic bursts and decreased skin blood flow, but to a smaller degree (72). This demonstrates a dependence of the stimulus timing on the cardiac cycle to modulate MSNA by the arterial baroreceptors. More importantly, the second sympathetic burst following the R-wave is the one that is temporarily inhibited due to conduction delays. Macefield et al. (72) states that the inhibition in sympathetic burst observed was caused when cortical excitation summed with the baroreceptor input to increase

inhibitory drive on the muscle vasoconstrictor outflow. The timing is important because when a stimulus is applied with no delay in accordance with the R-wave the baroreceptor afferent volley has yet to arrive at the brainstem, and there needs to be at least a 150 ms delay to allow this to happen. Since the stimulus is delivered at 200 ms post R-wave, it interferes with the central processing within the baroreceptor. Furthermore, a delay any more than 200 ms, such as those delivered at a 400 and 600 ms delay, occur too late to have any effect on baroreceptor activity associated with the second burst of sympathetic activity, and perhaps too early to affect the third burst (72). This inhibition in sympathetic activity found by Macefield et al. (72) is similar to our findings regarding MSNA. In the current study resting MSNA Burst Frequency was elevated during minutes three, four and five post-cTBS compared to both Sham and iTBS. MSNA Burst Frequency post-cTBS was also greater than iTBS during the Rest period (see Figure 21). Resting MSNA Burst Incidence was also greater post-cTBS compared to Sham and iTBS, during Rest minutes 3, 4 and 5 (see Figure 26). This may represent the effects of iTBS and possibly cTBS, despite cTBS failing to cause a change in MEP amplitude immediately post-stimulation, lasting into the rest period after testing MEP amplitudes with single pulse stimulation. While there was no difference found post-cTBS in motor cortex excitability that does not exclude cTBS from affecting cardiac autonomic function via the motor cortex. The inhibitory effects of cTBS may have peaked later than the time at which we measured the MEP amplitudes. This is consistent with a theory presented by Huang et al. (49). They speculate that single trains of TBS, regardless of the

protocol effects, produce a mixture of facilitation and inhibition on synaptic transmission. However, the effect of facilitation accumulates faster than those of inhibition. If it is assumed that facilitation and inhibition effects saturate at some level then eventually inhibition overcomes facilitation. Therefore, a short intermittent protocol such as iTBS would cause the rapid accumulation of facilitation but cTBS would initially produce facilitation but that would saturate and inhibition would ultimately dominate. This view by Huang et al. (49) is consistent with the findings of animal studies in which opposing effects of LTP and LTD have been induced by the similar stimulation protocols (11, 42, 43, 107). Furthermore, this pattern of initial transient facilitation followed by inhibition, is consistent with the pattern of ABP fluctuations experienced by many stroke patients. Approximately two-thirds of stroke patients experience an acute period of hypertension followed by more long-term hypotension (92). While our study found consistent results in terms of the aftereffects of iTBS, our cTBS MEP amplitudes are not consistent with previous results. While this could be due to other factors explained previously, the timing of inhibition saturation for cTBS could have been slower than anticipated hence the lack of low MEPs measured immediately after the application of the cTBS condition. As time progressed through the rest period inhibition may have eventually overcome the initial facilitation, which would explain why cTBS had an effect on MSNA burst frequency and incidence compared to Sham and iTBS in the final three minutes of rest (See Figures 29 and 31).

Our recordings of Respiration rate is further evidence supporting our hypothesis that iTBS would lessen the cardiovascular response to isometric handgrip exercise (compared to Sham TBS) as the magnitude of CC activation would be lessened to maintain the target intensity. Respiration rate measured post-iTBS was significantly less than Sham and cTBS during the first minute of IHG exercise (see Figure 42). This could be attributed to the effect of iTBS on cortical excitability because iTBS was the only condition to have an effect on cortical excitability, as demonstrated by the increased MEP amplitude found post-iTBS compared to the Sham condition (see Figure 10). This significant difference, along with a trend towards significance of iTBS being less than sham during the second minute of IHG exercise (see Figure 42) is consistent with our hypothesis and follows the patterns of inhibition of our MSNA recordings.

Previous work has shown that human stroke patients have disturbed regulation of blood pressure, HR and MSNA during rest, exercise and PEI periods (61, 81). Stroke patients also have low HRV, an indication of deficient autonomic adaptation, baroreflex insensitivity and a predictor of a poor prognosis post-stroke (61). Stroke patients often have motor function deficits, especially those who have had a stroke in the motor cortex area (15, 16, 67, 68, 111). Stroke patients with a motor cortex lesion is comparable to applying cTBS to the motor cortex as cTBS temporarily causes inhibition thus acting as a lesion in the cortical tissue of M1 (49). Our elevated resting recordings of MSNA burst frequency and burst incidence mimic that of stroke patients (see Figures 22 and

26). Furthermore, in a study of stroke patients compared to age-matched, healthy controls, the stroke patients experienced a significant increase in blood pressure, HR and MSNA during a 2-minute 35% IHG exercise, just like that used in the current study. However, when compared to the control group the magnitude of these cardiovascular responses was attenuated (81). These results suggest that isometric exercises performed by those who have suffered a stroke may successfully activate the EPR, but the attenuated response may be related to brain lesions affecting the activation of CC. While we had no evidence of an attenuated cardiovascular response following cTBS, our results still provide evidence that cTBS did have an effect and the motor cortex may be implicated in CC control.

5.7 Limitations

It is well known that the modulatory effects of rTMS are dependent on the stimulation parameters including intensity, frequency, train length, inter-train interval, the total number of pulses delivered per session, coil type, the direction of the current and the placement of the coil on the scalp in respect to the cortex, specifically the area being stimulated. While many researchers have found relatively consistent effects of cTBS and iTBS paradigms over the motor cortex in humans, a great number of other studies have found differing results similar to those obtained in the current study in which iTBS caused facilitation of MEPs but cTBS had not effect (see Figure 11). These opposing and mixed results may be

due to various factors and limitations associated with TMS that will be explained in the following sections.

5.7.1 Sham Condition

Since there were no differences found in any experimental variables between the sham condition and baseline one can assume the sham condition works as true control condition despite some experimental shortcomings. All participants underwent single pulse TMS during the first visit to ensure the researcher could find their optimal stimulation site and their AMT was below the threshold needed to perform rTMS within the power confines of the device. Since all participants underwent the optimal stimulation site and AMT procedures during the first visit and before any data collection on the second visit, all were aware of the feeling that TMS delivers on the scalp and surrounding areas despite never undergoing rTMS before. This posed an issue for the sham because participants could feel the weight of the coil on their head and hear the auditory noise produced by TMS but the feeling on the scalp was absent. Although the sham condition was always performed first before the two TBS conditions many participants were aware during the sham condition, or deduced after the first TBS condition was applied that the first condition was the control. While there is currently no way to perform a control that elicits the same feeling on the scalp as TBS conditions without actually stimulating the cortex, the lack of significant differences between baseline and sham leads to the notion that it is a sufficient control. Despite our

sham condition being a sufficient control, there are several modifications that could be made in the future to strengthen our reliability. Since participants would be able to tell the difference between actual stimulation and sham quite easily, the sham condition had to be performed prior to both the iTBS and cTBS conditions, thus the conditions couldn't be truly randomized. In the future using surface electrodes for skin stimulation while using a shielded TMS coil would enhance the sham condition as it would provide a similar feeling on the scalp and the coil could be oriented the correct way on the scalp. This is considered the gold standard of TMS sham conditions and would allow the sham to be included in the randomization of the conditions (32). Furthermore, it should be stated that the differences observed after stimulation of iTBS and cTBS cannot be attributed to auditory arousal due to the loud clicking noise of the TMS device as no changes were witnessed during the sham condition.

5.7.2 Coil Position

The determination of the optimal stimulation site was done manually as described in the methods section. A more precise and reliable method of determining the optimal stimulation site of individual participants involves the use of an anatomical magnetic resonance imaging (MRI) and a TMS neuronavigation system. All participants undergo a brain MRI to generate a high-resolution anatomical brain image that the neuronavigation system uses to determine and mark the optimal stimulation location within the motor cortex to stimulate the

muscle of interest. This allows the TMS coil to be placed systematically in the same location with the same, correct orientation and tilt every time TBS is applied throughout the session. This eliminates the need to go through the tedious process of attempting to find the site in the initial visit and subsequent visit. It also makes re-checking for the optimal stimulation location using single-pulse TMS prior to each TBS application unnecessary thus saving time and some discomfort for the participant. It may also be of interest to note that many participants found the scalp cap that marked the general location of this stimulation site uncomfortable as time passed throughout the experiment and the use of neuronavigation system would have eliminated this discomfort. The neuronavigation system may have also reduced the dropout rate as it may have been able to find the optimal stimulation site faster and more accurately than our manual method on the individual whose site was difficult or impossible to find

TMS acts to excite the axons of cortical neurons rather the cell bodies as demonstrated from studies involving measurement of the strength-duration (S-D) time constant, a measure of how stimulation threshold varies in connection with the duration of the stimulation pulse. Estimate measures of the S-D time constant for TMS application on the brain resulted in similar values of those when TMS was applied over large diameter peripheral nerve axons (4, 26, 90). The direction of the stimulating coil and thus the current is imperative as cortical neuron axons are activated by the difference in their potential along their length. If the current is applied perpendicular to an axon’s length the stimulation is far less effective than

if applied longitudinally. It has been found that points at which an axon bends out of a locally uniform electric field are most easily stimulated because this is the location where the length differential of the field is maximal (3). Therefore, depending on the orientation of the coil and thus the direction of the current, the coil can stimulate different neuron axons (96). One consequence of this is that that stimulation threshold depends on the coil orientation; the threshold of the hand area of the motor cortex is usually lowest when the coil is approximately pointed in the posterior to anterior direction, with the coil handle pointed 45 degrees to the tangential. The figure-8 coil during our study may not have been placed and held within the confines of the correct orientation to optimally stimulate the nerve axons of the forearm region of the motor cortex. The individual differences in brain morphology, specifically the location and orientation of the axons of neurons in the primary motor cortex, may play a role in the level of stimulation that actually occurs. This potentially led to some degree of error when stimulation was applied and may have had some effect on our measured outcomes such as MEP amplitude.

5.7.3 Non-Focal Property of TMS

Non-invasive brain stimulation such as that of TMS is a complex process that is not completely understood. Direct stimulation of a motor nerve is far more simplistic; a single electrical stimulus causes the production of an action potential that travels away from the site of stimulation to the target muscles to produce a

muscle twitch. A single electrical stimulus to the motor cortex of the brain will cause action potentials in adjacent neuron axons, but also activates other neurons in other locations due to complex synaptic networks.

Originally, TMS coils were relatively non-focal due to their large single circular shape. Many modifications have been made to enhance the focal abilities of the device to more specifically target the cortical region of interest. The coil size has been reduced and the figure-8 shape design was introduced to cause an overlap of magnetic fields that create one strong point of stimulation at the center of the coil. Many studies have been designed for the sole purpose of detecting potential changes in MEP amplitudes that outlast the application of TBS. Many stimulation studies use the motor cortex as the site of application because it is possible to measure the size of the EMG response using a single TMS pulse as an objective measure of cortical excitability. However, these results are often weak and highly variable between individuals (73). This means that in some cases of TMS the stimulation will target a mixture of different neural networks (both the targeted location and other areas not intended to be stimulated) that could potentially lead to interacting effects that interfere with the final physiological outcome. Siebner (103) used PET to show TMS to the premotor cortex decreases regional cerebral blood flow in the premotor and motor cortex bilaterally, but also found other remote areas such as the putamen and cerebellum were affected (103). This is an example that while one may intend to stimulate a single area when using TMS, various unintended areas may be affected and thus be considered when

interpreting results. In the current study the best efforts were made to ensure the hand area, specifically the FDS, was consistently being stimulated, there is no way to ensure this was the only neural network being activated

5.7.4 Stimulation Intensity

The stimulation intensity of the experimental TBS conditions was determined as 80% of the AMT. The stimulation output maximum during AMT was set as 70% because of the limitations of the Magstim. The TBS protocols heat up the coil at a faster rate, and requires a greater amount of power, than the single pulse protocol used to find AMT. This means the Magstim can only be set at 56% of stimulator output during TBS before it shuts down prior to completing the full TBS protocol. Since we determined 80% of AMT to be the optimal stimulation intensity for the TBS protocols, any participant with an AMT of greater than 70% had to be excluded because the TBS protocols could not be successfully completed.

5.7.5 Inter-individual Variability

The majority of studies using TMS on the motor cortex have focused on the differential effects on neurological patients versus their healthy counterparts. However, the effects of TMS on the healthy subjects alone greatly differ from person to person, yet this variability is often not reported (69). The results exhibited by iTBS and cTBS protocols have been shown by several and varied

studies to be weak and highly variable from one individual to the next (73, 77, 117). Despite Huang and colleagues (47, 49) finding significant results showing that iTBS induced facilitative effects on MEP amplitude and cTBS had the opposite effect, they state that intra- and inter-individual variability of cortical excitability may influence the response of TBS. Specifically, it is highly likely that the same TBS paradigm can trigger different effects in different individuals and effects may depend on the resting state of cortical neurons at the time of stimulation (47, 49).

Martin et al. (77) investigated the effects of cTBS on the cortical region projecting to the biceps and the region projecting to the first dorsal interosseus (FDI) muscle. MEPs measured post stimulation from the biceps showed no significant inhibition and actually were slightly facilitated for the first ten minutes post-stimulation. They also noted inter-individual variability was high, with 5 of 8 subjects exhibiting a small decrease in MEP size (that was highly variable and short-lived), and the other three subjects exhibited overall facilitation. MEPs recorded from the FDI were significantly suppressed 5 minutes post-cTBS and remained suppressed for the full 35-minute recording period. Although the inter-individual variability was smaller for the FDI than the biceps, three subjects still showed either no change in MEP size or a facilitation of MEPs following cTBS (77). This type of inter-individual variability was also observed in the current study with 8 of 12 participants demonstrating facilitated MEPs post-iTBS and the remaining 4 demonstrating lower MEP amplitudes compared to baseline.

Furthermore, only 2 of our 12 participants recorded a decrease in MEP amplitude following cTBS stimulation and the remaining 10 participants actually recording higher MEPs compared to baseline. Several participants experienced facilitation after both TBS protocols and actually had MEPs that were highest in peak-to peak amplitude post-cTBS than iTBS.

5.7.6 Intra-individual Variability

Researchers have also investigated intra-subject variability by repeating the iTBS and cTBS protocols on three subjects leading to mixed results (77). Some participants who previously showed facilitation in the FDI or biceps muscles showed either no change or a reversal of that facilitation during the second bout of the protocol, while some participants had similar results during both visits (77).

Vernet and colleagues (115) found results consistent with Huang et al. (49) and our hypothesized results in that cTBS caused MEP suppression. However, they did note that while an obvious MEP suppression was observed in most of their ten participants during both of two visits, two participants during one visit, and one participant during both visits showed the opposite effect (48, 49, 115). While this type of inter- and intra-individual variability seems to be highly common it is not always reported thus causing a general assumption that TBS protocols may result in more consistent patterns of facilitation and inhibition than actually occurs.

5.7.7 Determination of the Effects of Theta Burst Stimulation

In the current study, after the condition stimulation (Sham, inhibitory or facilitative stimulation) was administered to the participant at 80% of the AMT, the effectiveness of the stimulation was confirmed by using single pulse TMS while the muscles were weakly contracted (~5%). The MEPs' amplitude post-stimulation were recorded and used to determine the stimulation effects on cortical excitability. After the sham stimulation it was expected that there would be no changes in the maximum peak-to-peak amplitude of the MEP because no stimulation was applied. It was anticipated that the inhibitory TBS condition (cTBS) would decrease the MEP (compared to baseline) after a single pulse of stimulation, while following the facilitative stimulation (iTBS), a single pulse would result in an increased MEP amplitude. While we did find that MEPs measured post-iTBS were facilitated, those measured post-cTBS did not have the anticipated resulted of being inhibited.

There are other potential methods for determining the effect of the TBS protocols based on how the different protocols affect the nervous system physiology. As previously discussed, stimulation of the motor cortex produces a muscle twitch on the contralateral side, which is measured using EMG and called an MEP. This measurement however is an indirect measure of the output of the motor cortex since it has been filtered by spinal cord synaptic activity. The most direct way to measure the stimulation effects is to record activity in the corticospinal output

neurons by implanting electrodes in the epidural space on the surface of the spinal cord at the high cervical level (usually C2). This type of recording is usually reserved for animal models but a few opportunities have occurred in humans for this type of recording. Early studies were performed on patients who were anaesthetized during surgery (7-9, 28, 109) and showed TMS caused a series of volleys or waves down the corticospinal tract even thought the anaesthesia caused a depression in the physiological response. Years later two separate groups (28, 58) used epidural electrodes implanted in the spinal cord of individuals to treat chronic pain to record descending volleys induced by TMS. Done while the individuals were conscious, this was the first time this was done and triggered a series of studies done by Di Lazzaro and collegues using the same method (27). They found that the composition of the descending waves depended on stimulation intensity, the nature of the stimulus and the direction that the current is applied.

TMS causes the modulation of indirect waves (I-waves) (29, 30). In terms of the motor cortex, the cascade of activity from the brain to the target muscle can be measured by recording the activity of the corticospinal tract. This is a central recording of the descending pathways while a single stimulation pulse is applied over M1. It results in a series of electrical discharges at about 600 Hz (Volz, 2014). The earliest wave is believed to originate directly from the activation of the axons of corticospinal neurons (27) and is thus called the direct or D wave. The later waves originate from the indirect, mono and poly-synaptic activation of

corticospinal neurons and thus are called “I” waves and named in order of their appearance (I1, I2, and I3). These waves reflect the activity generated within the neural network of the motor cortex. cTBS decreases the amplitude of the earliest I-wave, I1. Oppositely, iTBS increased the amplitude of the later I-waves (29, 30). Therefore TBS differentially affects cortical circuits based on what protocol is being used. Furthermore, this early inhibition by cTBS and later facilitation by iTBS is thought to originate from separate pools of interneurons (29, 30). Therefore, we can use this information to measure the effects of both iTBS and cTBS by measuring the amplitudes of these I-waves. Another way we could have confirmed the effects of iTBS and cTBS was by measuring latency, the speed of conduction of the TBS effects. This method is more simplistic than measuring than I-wave amplitudes and measures the time between when the stimulation occurs to the onset of the MEP. Furthermore, this information can be used to determine if certain individuals are more responsive to either iTBS or cTBS by seeing which individuals have a greater recruitment of early or late I-waves. In addition, early I-waves, such as those affected by cTBS, are believed to originate from monosynaptic excitatory connections to pyramidal cells. In comparison, late I-waves, those affected by iTBS, are generated by more complex oligosynaptic circuits (29, 30). This difference in the way different TBS conditions effects the brain could provide insight into why effects differ from person to person.

Paired-pulse stimulation could have also been used to examine the effects of TBS, however, the current study lacked the equipment necessary to perform this

type of TMS. Paired pulse can be used to examine TBS effects by administering an initial conditioning pulse which is strong enough to activate the cortical neurons, but still weak enough as to not have any effect on descending pathways (no generation of a MEP). Then a second stimulus is applied after a short interval of time at a suprathreshold level to determine the effect by measuring the precise latency of the onset of the MEP.

5.8 Advantages of TBS

The most obvious advantages of TBS protocols used in this study versus other rTMS protocols are the shorter stimulus application time and the lower stimulation intensity necessary to elicit an effect. Both of these factors contribute to more comfortable stimulation condition especially when an individual is undergoing various or repeated protocols. Despite this, some participants still noted a discomfort during the TBS protocols, mainly related to muscle twitching of the face or scalp caused by current. It is interesting to note that individuals with higher AMT were not always the individuals who complained of this discomfort leading to the notion that some individuals may be more susceptible to the effects of discomfort of TBS.

5.9 Future Directions

Future studies investigating the connection between the motor cortex and the control of the autonomic nervous system, particularly cardiovascular autonomic control should focus on controlling inter-individual variability. This variability can highly affect TBS aftereffects and therefore skew results to oppose the majority of previous TBS work. This inter-individual variability could be controlled by having more strict guidelines for accepting participants as responders to TMS. Only individuals who have a substantially strong reaction (as demonstrated by high MEP amplitudes) to TMS should be included in research studies. Perhaps the TMS Standard Operating Guidelines (Appendix H) should be revised to the standards of Huang et al. (49), where AMT is defined as the minimum stimulus intensity necessary to elicit an MEP of greater than 200 μV in at least 5 of ten trials. While this may be more difficult to obtain in participants and will likely lead to a greater participant drop out rate (due to some participants' MEPs not reaching 200 μV before reaching the maximum stimulator intensity), the participants who are able to reach this standard are more likely to have a stronger MEP response to TBS. Huang and colleagues (49) seem to have great success using this AMT protocol as they found significant results for facilitated MEPs post-iTBS and inhibited MEPs post-cTBS with only 9 subjects.

TMS has already been used as a novel treatment for those suffering from depression, epilepsy, Parkinson' disease, among others. The next step is to find

a novel treatment for cardiovascular diseases that affect such a large population of individuals. Since TBS may induce changes in synaptic transmission in the motor cortex through LTP and LTD-like effects, it holds potential value as a therapeutic aid for various neurologic disorders such hypertension and other cardiovascular diseases. Furthermore, since TBS has been shown to have longer lasting effects with less stimulation time than traditional TMS protocols, it provides patients with a relatively tolerable treatment option. Perhaps the manipulation of stimulation intensity and frequency, to create new TBS patterns and protocols will be beneficial to explore, and could be personalized to the individual to maximize efficacy of the treatment. Currently more research is needed in this area to ensure TMS application to those with treatment-resistant cardiovascular disease is safe and effective.

Further studies are needed to better understand the mechanisms involved in TBS changes in cortical excitability, and the safety aspect associated with different neurological disorders. TBS protocols combined with techniques such as fMRI, and EEG may provide more clarification on how it affects the healthy human brain, and the brain of those suffering from neurological disorders and injuries such as stroke. Cardiovascular diseases that are resistant to traditional treatments could potentially be helped through the modulation of cortical networks through the application of TBS. Furthermore, different populations of people may be affected by TBS differently, therefore future work should include performing TBS on select populations of people including older adults, athletes

and over-trained individuals with marked indicators of over active sympathetic drive. Future studies utilizing TBS to examine the location of CC could also broaden the scope of the dependent variables measured to include things such as PET cerebral blood flow. The blood flow response found using PET cerebral blood flow to TBS examined in conjunction with PEI protocol could help elucidate the location of CC, potentially helping solidifying the role of the motor cortex.

Future studies should focus more on stroke patients, as they are a population of individuals that could potentially benefit the most from an established TBS therapy. Past studies focusing on animals have shown that stimulation combined with motor training tasks encourages recovery after bilateral ablation of the forelimb area of the motor cortex (12, 13, 84). Studies using TMS have shown that the excitability of the motor cortex is reduced shortly after stroke, and the cortical representation of the affected muscles (motor map) is decreased (15, 16, 111). Stroke rehabilitation programs have been shown to increase the motor map of the injured hemisphere relative to that measured immediately post-injury (111). Studies utilizing constraint-induced movement therapy, in which the uninjured limb is restricted for a period of time, have shown mixed results in terms of enlarging the motor map of the affected limb. Some have shown that this treatment aids in increasing the size of the motor map of the affected limb by focusing on goal-directed movements of the affected limb (67, 68, 118). However, other researchers have found that extreme use of the affected limb (consistent with constraining the unaffected limb, so that the affected limb is

forced into performing movement tasks) can result in lesion enlargement and cause greater motor impairment (50, 62). The results of these studies suggest that there may be periods during stroke recovery that are particularly vulnerable to either further injury or enhanced recovery. These time periods should be considered when applying TMS as a potential therapeutic aid in stroke patient recovery. In particular, future research conducted on stroke patients could focus on individuals who have suffered a stroke within a 6-month time frame as recovery does not usually continue after this point. If possible, TMS interventions should be applied within the first few weeks post-stroke as this is the time period when the greatest recovery occurs and this could maximize the therapeutic potential of TMS (84). Further research on stroke patients beyond this 6-month recovery plateau should be done, as TMS could be the novel treatment that may help induce cortical changes that aid in recovery.

5.10 Conclusion

The objective of the present study was to determine if using transcranial magnetic stimulation to inhibit or facilitate, using cTBS and iTBS, neural activity within the human motor cortex would elicit significant changes in HR, MSNA and ABP during rest and isometric handgrip exercise in a population of healthy, young men and women. Results from measuring MEP amplitudes post-TBS determined that iTBS did facilitate the excitability of the motor cortex, but cTBS failed to produce inhibition of MEPs as demonstrated by previous researchers

(49, 52). Despite this, there were still some significant changes in cardiovascular measures post-stimulation. There was significantly higher SBP measured during the second minute of rest and higher MAP during the entire five minute Rest period after iTBS compared to cTBS. There was also a significant difference in MSNA burst frequency between cTBS and Sham, with cTBS being significantly higher at rest minutes one, three, four and five. Furthermore, cTBS was also significantly greater than iTBS during rest minutes three, four and five. There was a significant difference in MSNA burst incidence between iTBS and cTBS, with cTBS being significantly higher at rest minutes three, four and five. Furthermore for MSNA burst incidence, cTBS was also significantly greater than Sham in the group data during rest minutes three four and five. These cardiovascular changes suggest that modulation of the excitability of the human primary motor cortex may have an influence on cardiovascular control. While we did observe the expected cardiovascular response to the IHG exercise and PEI period, there were few differences between conditions. Our lack of inhibition following cTBS may have been due to influencing factors such as pre-stimulation voluntary contraction and. the high degree of inter-individual variability present in our study. Although this type of variability in studies utilizing TMS has recently been highlighted as a common occurrence by many researchers. Despite these issues, the results provide further evidence that there is a potential link between the human motor cortex and the control of cardiovascular function.

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APPENDIX A:
GRAPHICAL DEPICTION OF HYPOTHESIS

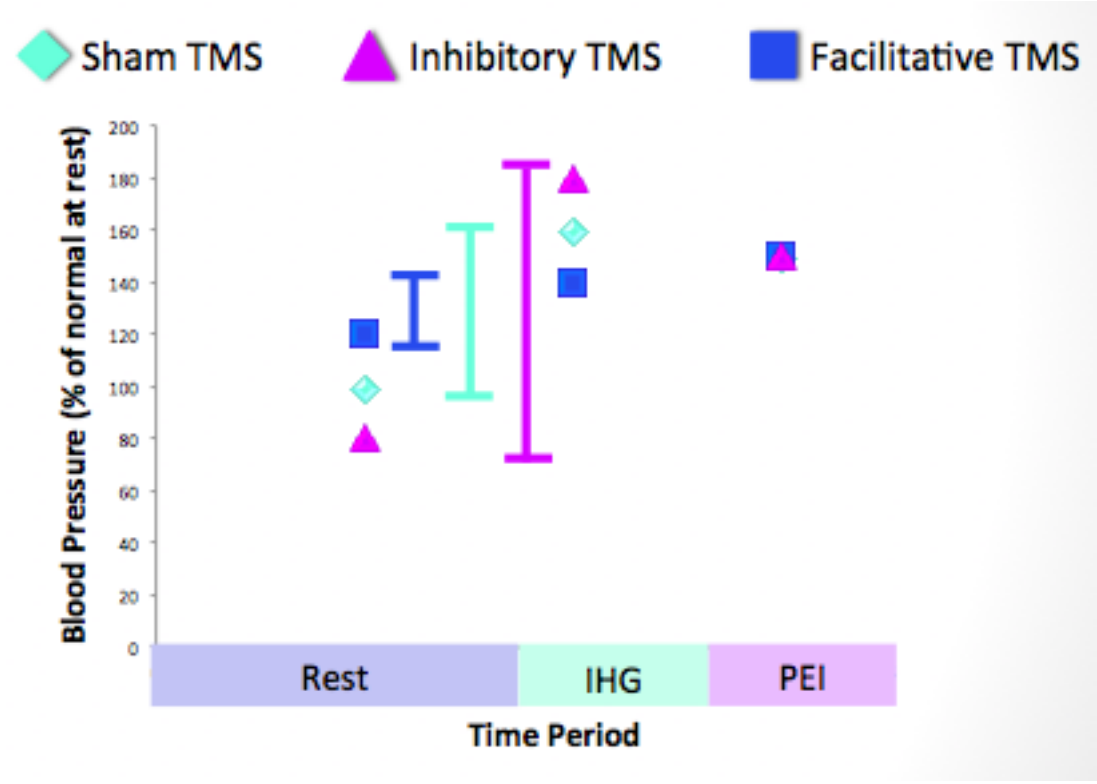
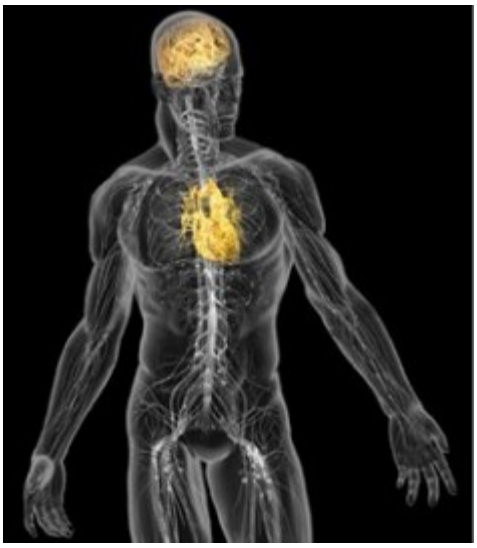


Figure 45. It was hypothesized that facilitating the hand area of the motor cortex would cause a subsequent increase in HR, ABP and MSNA, while inhibiting the motor cortex would cause a decrease in HR, ABP and MSNA at rest. However, during exercise, compared to Sham, the excitatory effects of iTBS would lessen the cardiovascular response to isometric handgrip exercise as the magnitude of CC activation would be lessened compared to the target exercise intensity. Similarly, during exercise, compared to Sham, the inhibitory effects of cTBS would exaggerate the cardiovascular response to the isometric handgrip exercise as the level of CC activation would be heightened in effort to maintain the exercise intensity. The Sham condition was hypothesized to produce no effect on HR, ABP or MSNA due to the lack of actual stimulation being applied to the scalp.

APPENDIX B:
RECRUITMENT POSTER

Capital Health

DALHOUSIE UNIVERSITY
Inspiring Minds



ARE YOU INTERESTED IN YOUR BRAIN, HEART HEALTH AND EXERCISE?

Researchers within the Division of Kinesiology invite healthy men and women to participate in a study looking at the effect of a safe and non-invasive brain stimulation machine on the blood pressure response to handgrip exercise.

You may be eligible to participate if you:

- Are between 19-64 years of age
- Have no history of seizures
- Do not suffer from any medical conditions
- Are not pregnant

You will need to attend 1-2 sessions (~3 hours each) at the Human Cardiovascular and Exercise Physiology Laboratory located at the Dalplex (2360 South Street).

For more information about or to volunteer for this study, please contact:

Julie Weir (B.Sc.) at julie.weir@dal.ca or

Derek Kimmerly (Ph.D.) at 494-2570 or dskimmerly@dal.ca

CDHA-RS/2014-112 Version #2, September 16th, 2013

APPENDIX C:
HEALTH HISTORY QUESTIONNAIRE

NAME:

ADDRESS:

CITY:

POSTAL CODE:

PHONE: (home) **(work)**

DATE OF BIRTH:

E-mail address:

Name:

1.	Do you consider yourself to be in good health?	Yes	No
2.	Do you exercise on a regular basis?	Yes	No
	If yes, what type of exercise:		
	How often:		
3.	Have you ever had high blood pressure?	Yes	No
4.	Have you ever had migraines?	Yes	No
5.	Have you ever had chest pain, heart disease or a heart murmur?	Yes	No
6.	Are you taking any medications?	Yes	No
7.	Do you drink alcohol more than twice a week?	Yes	No
8.	Do you take coffee or any other stimulants?	Yes	No
	If yes, approximately how many cups/day _____ ?		
9.	Do you smoke?	Yes	No
10.	Have you taken any medication that might stimulate or depress your nervous system?	Yes	No
11.	Have you ever fainted?	Yes	No
12.	Is there any possibility that you may be pregnant?	Yes	No
13.	Do you suffer from epilepsy or ever had a seizure?	Yes	No

APPENDIX D:
PRE-STUDY INSTRUCTIONS AND CONTACT INFORMATION FOR PARTICIPANTS

Study Title: Uncovering the role of the human primary motor cortex in blood pressure regulation during exercise using transcranial magnetic stimulation

Investigators: Derek S. Kimmerly, *PhD* , Julie Weir, BSc.

Location: Exercise and Cardiovascular Physiology Laboratory, Dalplex, 6260 South Street, Halifax, NS, B3H 1T8

Contact Email:
Dr. Derek Kimmerly: dskimmerly@dal.ca
Miss Julie Weir julie.weir@dal.ca

Pre-study Reminders:

1. Please avoid the following for 24 hours before the study session:
    - Intense physical activity (running, bicycling, weight training, etc.)
    - Alcoholic beverages
    - Caffeinated products (coffee, tea, chocolate, etc.)
    - Nicotine containing products (cigarettes, Nicorette gums, etc.)
2. Eat a light meal ~3 hours before each study and write down the contents of this meal.
3. Drink plenty the night before (~5 hours before bed) and during the morning (~1 cup per hour)

APPENDIX E:
PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada Santé Canada

continued on other side...

APPENDIX F:
EDINBURGH HANDEDNESS INVENTORY

Please indicate your preferences in the use of hands in the following activities *by putting a check in the appropriate column*. Where the preference is so strong that you would never try to use the other hand, unless absolutely forced to, *put 2 checks*. If in any case you are really indifferent, *put a check in both columns*.

Some of the activities listed below require the use of both hands. In these cases, the part of the task, or object, for which hand preference is wanted is indicated in parentheses.

Please try and answer all of the questions, and only leave a blank if you have no experience at all with the object or task.

	Left	Right
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking Match (match)		
10. Opening box (lid)		
TOTAL (count checks in both columns)		

Difference	Cumulative TOTAL	Result

Scoring:

Add up the number of checks in the “Left” and “Right” columns and enter in the “TOTAL” row for each column. Add the left total and the right total and enter in the “Cumulative TOTAL” cell. Subtract the left total from the right total and enter in the “Difference” cell. Divide the “Difference” cell by the “Cumulative TOTAL” cell (round to 2 digits if necessary) and multiply by 100; enter the result in the “Result” cell.

Interpretation (based on Result):

- below -40 = left-handed
- between -40 and +40 = ambidextrous
- above +40 = right-handed

APPENDIX G:
TRANSCRANIAL MAGNETIC STIMULATION (TMS) SCREENING FORM

Below is a questionnaire used to determine whether potential participants are suitable for research studies using transcranial magnetic stimulation (TMS). Please complete the questions honestly and to the best of your knowledge. This information, as well as your identity, will be kept completely confidential.

Participants Study ID: _____

Participants Age: _____

PLEASE COMPLETE THE QUESTIONS BELOW

	YES	NO
1. Do you have epilepsy or have you ever had a convulsion or a seizure?	□	□
2. Have you ever had a fainting spell or syncope (loss of consciousness)? If yes, please describe on which occasion:	□	□
3. Have you ever had a head trauma that was diagnosed as a concussion or was associated with a loss of consciousness?	□	□
4. Do you have any hearing problems or ringing in your ears?	□	□
5. Do you have cochlear implants?	□	□
6. Are you pregnant or is there any chance that you might be?	□	□
7. Do you have metal in the brain, skull or elsewhere in your body (e.g., splinters, fragments, clips, etc.)? If so, please specify:	□	□
8. Do you have an implanted neurostimulator (e.g., DBS, epidural/subdural, VNS)?	□	□
9. Do you have a cardiac pacemaker or intracardiac lines?	□	□
10. Do you have a medication infusion device?	□	□
11. Are you taking any medications? (please list):	□	□
12. Did you ever undergo TMS in the past? If yes, were there any problems:	□	□
13. Did you ever undergo MRI in the past? If yes, were there any problems:	□	□

* TMS screening form is from the International Consensus Guidelines:

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

APPENDIX H:
TMS STANDARD OPERATING PROCEDURE

Standard Operating Procedure: Transcranial Magnetic Stimulation (TMS)

Purpose: This standard operating procedure describes the procedures for the use of transcranial magnetic stimulation (TMS) equipment.

Responsibility: Faculty researchers or supervisors, undergraduate and graduate students

1.0 Background

Transcranial magnetic stimulation (TMS) has been used in a growing number of laboratories worldwide since 1985. TMS is a widely used technique that excites cortical tissue in humans for both experimental and clinical purposes. TMS involves using a series of brief magnetic pulses applied on the outside of the head over cortical regions of the brain. Briefly, TMS relies on the properties of electromagnetic induction; a rapidly changing magnetic field is generated when a high-voltage current is passed through a coil. When this coil is held in close proximity to any electrically conducting medium, such as the brain, this time-varying magnetic field induces current in a direction opposite to the original current in the coil (**Figure 1**).[1,2] TMS can be applied one stimulus at a time, single-pulse TMS, in pairs of stimuli separated by a variable interval, paired-pulse TMS, or in trains, repetitive TMS (rTMS). Single-pulse TMS can be used, for example, for mapping motor cortical outputs, studying central motor conduction time, and studying causal chronometry in brain-behavior relations. In paired pulse techniques TMS stimulation can be delivered to a single cortical target using the same coil or to two different brain regions using two different coils. Paired pulse techniques can provide measures of intracortical facilitation and inhibition, as well as study cortico–cortical interactions.[3]
Below is a point-by-point description of the procedure.

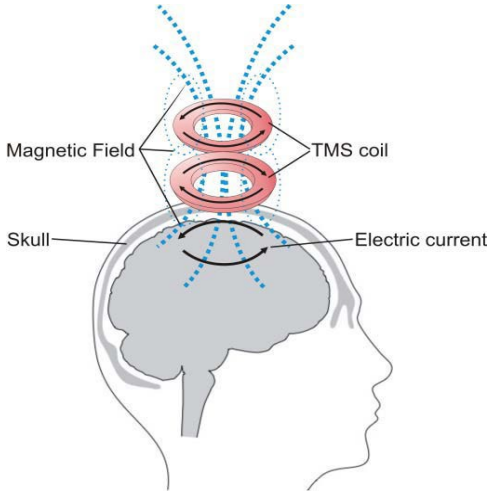


Figure 1. Schematic of current flow direction in a magnetic coil and the induced current in the brain

2.0 Determining the Resting Motor Threshold using Single Pulse Transcranial Magnetic Stimulation

2.1 Using the neuronavigation* system pre-loaded with the participants anatomical MRI, position the TMS stimulation coil over the 'hand knob' region of the primary motor cortex of the hemisphere desired for stimulation [this location approximates the C3 position on the international 10-20 system for EEG (i.e., 50% of the measured distance between the vertex of the head and the left preauricular point)].

2.2 Starting at 30% of the stimulator output, deliver single TMS pulses while monitoring for (a) participant sensation of the stimulus in a target muscle and (b) the occurrence of a motor evoked potential (MEP) in a target muscle of the right hand or forearm.

2.3 If the single TMS pulse does not elicit a MEP/sensation then reposition the stimulation coil 0.5 cm anterior; if the MEP/sensation is still absent, move the coil 0.5 cm to the left or right as required. The effect of stimulation will be re-checked at each new position using the same stimulus intensity. If no suitable location elicits a response then the process will be repeated using greater stimulus intensity.

2.4 Increase the stimulus intensities in steps of 5% until a MEP can be seen. The objective here is to locate the motor 'hotspot' for the target muscle (the predominant motor cortical representation controlling the target muscle). This motor 'hotspot' is the location that yields the largest MEP amplitude using the minimal necessary stimulator intensity.

2.5 Determine the resting motor threshold (RMT) at the motor 'hotspot'. Increase or decrease the stimulus intensity until a MEP (of at least 50 μV) can be seen on 5 of 10 trials of stimulation on the motor 'hotspot'.

2.6 For muscles of the hand and forearm, MT is usually in the range of 45-60% of the maximum stimulator output with a 70 mm typical figure-of-eight coil; if MT is not evident before increasing the stimulus intensity to 70% of the maximum stimulator output, stop the experiment.

3.0 Performance of Repetitive Transcranial Magnetic Stimulation (rTMS)

3.1 The RMT determined in section 2 above will be used to guide the stimulus intensity for 1 Hz
rTMS.

3.2 Localization of target sites will be determined based on the objectives of the study. In most cases, the location of target sites will be determined using a participant's anatomical or functional MRI co-registered with the neuronavigation system. Use of the neuronavigation system to guide the position of the stimulator coils provides the ability to focally stimulate (on the order of millimeters) a given region of the brain.

3.3 A computer program will control the timing of the rTMS application such that stimuli will be applied using a research ethics board (REB) approved stimulation paradigm that meets consensus guidelines for the safe application of TMS.[3] A typical stimulation paradigm used in rTMS studies includes stimulation at 1 Hz or lower with the intensity of stimulation not to exceed 140 % of an individual's RMT (see figure 2 below).

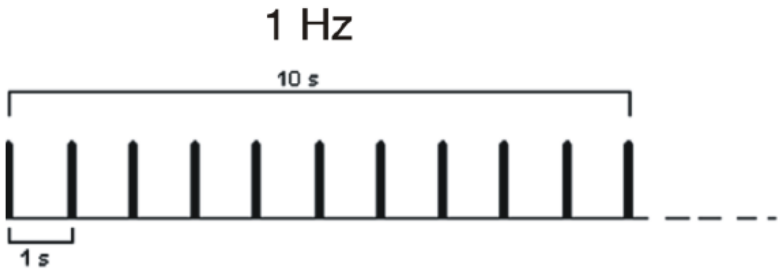


Figure 2. Example of 10 s of rTMS at 1 Hz.

4.0 Description of rTMS Procedure for Research Participants

The following information will be provided to research participants in the informed consent letter:
"As a participant in this study, rTMS will be used to stimulate different regions of your brain. rTMS involves delivering brief magnetic pulses over different locations on your head. You will be asked to keep your head as still as possible. rTMS uses a magnetic stimulator which is basically a set of electrical capacitors that can store and rapidly discharge electricity into a coil of electrical wires that are encased in plastic. The plastic case rests against your head. As electrical current flows through the coil, a magnetic field is generated that passes through the skull and induces a second electrical flow of current in the brain that persists for a very brief time (< 1 second).
This procedure is not painful. You will hear a clicking noise as the current flows through the coil and may experience involuntary activation of different muscle groups depending on the position of the coil over the head. You may also feel some tingling sensations on the head where the TMS coil is located. Ear plugs will be provided during stimulation for added comfort. We will first determine the location within the motor cortex that evokes a muscle response. We will then determine the minimum amount of brain stimulation necessary to evoke that response."

5.0 Description of Risks Associated with Use of rTMS for Research Participants

The following information will be provided to research participants in the informed consent letter:
A series of adverse effects that can be induced by TMS have been identified. However, there is no evidence that the procedure is harmful if appropriate guidelines are followed.[3,9,10]

*The following are **risks and discomforts** that are possible when undergoing rTMS and are communicated in writing to participants in the informed consent document:*
a) The procedure is painless, although it can cause muscles to contract immediately after stimuation, which may lead to residual soreness caused by muscle fatigue over the duration of the experiment.
b) Approximately 1 in every 10 research participants undergoing TMS experience headaches or dizziness, which are believed to be due to excessive muscle tension. In the case of a headache, you will be advised to take whatever pain medication you usually take for mild headaches, which in most cases promptly resolves the discomfort (for example acetaminophen promptly resolves the discomfort in most cases).
c) Approximately 1 in every 100 research participants undergoing TMS experiences

neck stiffness and pain. This is believed to be due to the straight posture of the head and neck during the application of TMS. In the case of a headache, you will be advised to take whatever pain medication you usually take for mild headaches, which in most cases promptly resolves the discomfort (for example acetaminophen promptly resolves the discomfort in most cases). Participants are asked to advise the researcher at the first opportunity if they experience any neck stiffness or soreness. In this situation, the participant may opt to withdraw from the study or to rest and change posture for several minutes before the procedures are resumed.
d) TMS produces a loud clicking noise when the current passes through the coil. This loud click can result in tinnitus (i.e., “ringing” in the ears) and temporary decreased hearing if no ear protection is used. To prevent this adverse effect all research participants receiving TMS and those researchers delivering TMS will be expected to wear earplugs.
e) The use of single, paired pulse, or low frequency (repetitive) TMS has never induced a seizure in a healthy participant. However, there is the possibility that TMS can induce a convulsion even in the absence of brain lesions, epilepsy or other risk factors for seizures. Only 7 cases of convulsions have been reported using single pulse TMS in patients with pre-existing brain damage despite extensive use in both the healthy and patient population. In the case of high frequency repetitive TMS the risk of seizure is reported at less than 1% in healthy young adults and only one seizure has ever been reported in a normal participant following this higher frequency stimulation.[3]
f) The overall risk for seizures during TMS is thought to be in the order of 1 in 1000 studies. In the event a participant does experience a seizure, emergency services via 911 will be contacted. One member of the research team will stay with the participant at all times. A second member of the research team is responsible for calling emergency services via 911 and then Dalhousie Security (ext 4109) to inform campus police of the location of the incident and facilitate the arrival of emergency personnel.

Pregnancy
Women who are or could be pregnant may not participate in TMS studies because the potential effects of magnetic fields on the fetus are unknown. Study personnel will follow an established protocol in screening women who may be of childbearing age. Briefly, if the potential participant is a female in reproductive years, she must either (a) confirm that she practices an appropriate method of contraception, or (b) have a negative urine pregnancy test to proceed with the MRI and/or TMS session. Appropriate methods of contraception include abstinence, birth control pills or implanted hormonal contraceptives, contraceptive barriers such as a diaphragm or condom, intra-uterine device, and having only partner(s) who have a history of vasectomy.

***** We will not be using a neuronavigation system. To determine the optimal stimulus location, we will use single-pulse TMS to systematically cover the primary motor cortex until the great motor evoked potential of the hand/wrist is found (as described in the Methods section).

Adapted from the University of Waterloo Human Research Ethics Committee UWSOP # 214: Protocol for Repetitive Transcranial Magnetic Stimulation (TMS) of the Brain With permission from Dr. M Vesia

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APPENDIX I:
TMS LAB SAFETY DOCUMENT

Purpose: The purpose of this document is to outline the policies in place to ensure the safe operation of TMS by lab personnel. Additionally, a standard operating procedure is described in the rare instance that an emergency situation occurs.

Responsibility: Faculty researchers or supervisors, undergraduate and graduate students

1.0 General

Transcranial magnetic stimulation (TMS) may only be performed by lab personnel or associated investigators who have the following:

I. First responder certification
   a. This includes a current certificate in first aid and CPR from a recognized institution (e.g., Heart and Stroke, St. John's Ambulance)

II. Completion of a TMS training module, which includes:
   a. Familiarity with the safe operation of the TMS hardware and software
      i. Computers
      ii. Trigger cables
      iii. TMS unit front panel, amplifiers, coils and here applicable the neuronavigation unit (Brainsight 2)
   b. Have read and reviewed pertinent literature related to the safe operation and application of TMS (e.g., International Consensus Guidelines first published by Wasserman 1998 and updated by Rossi et al., 2009). This literature is available electronically (PDF) and in hard copy in the Laboratory
   c. Have read and reviewed the Laboratory standard operating procedure for TMS

III. Have a study protocol with current approval from an affiliated research ethics board (which includes Capital District Health Authority REB, IWK Health Centre REB or the Dalhousie University REB)

2.0 TMS Procedures

I. TMS Screening Form

For all participants, the TMS screening form must be completed and signed prior to participation in any protocol using TMS. As per international consensus guidelines, affirmative response ('yes') to one or more of the questions does not represent an absolute contraindication to TMS, however the risk/benefit ratio to participation should be considered by the Principal Investigator. Note that an affirmative response to question #6 (regarding pregnancy) requires investigators to obtain further information (see SOP for TMS section 5.0). If a participant is engaged in subsequent studies, a new form must be completed.

II. Earplugs

All participants (study participant and members of the research team) must

wear protective earplugs throughout the duration of the TMS procedures

III. Study personnel present during TMS

There must be a minimum of two lab personnel or associated investigators (who meet the criteria listed above) present during any TMS procedure.

IV. Response to an emergency situation

While a seizure has never been reported with single pulse or repetitive TMS (at low frequency) using healthy participants, the lab personnel and/or associated investigator must be familiar with the Lab Policy for a seizure, which is outlined below

i. One of the two investigators will remain with the study participant at all times while the other 1) calls 911 for emergency assistance; and 2) contacts Dalhousie security services at extension 4109 Dalhousie Security (ext 4109) to inform campus police of the location of the incident to facilitate the arrival of emergency personnel.

APPENDIX J:
DATA COLLECTION FORM

TMS and Handgrip Exercise Study

Date: _____ Time of Day:_____
Name: _____ Study ID#: _____
Date of Birth: _____ Height (cm): _____ Weight (kg):____
Resting Blood Pressure: _____ BMI (kg/m^2): _____
Time of last Meal: _____Room Temperature (ºC): _____

Main Testing Day: 3 Conditions (~3 hours)

- ❑ Meet participant and explain procedures and equipment
- ❑ Have participant thoroughly read through and complete medical history, handedness and TMS Safety questionnaires (if not done previously)
- ❑ Measure participant's height and weight. Calculate body mass index (BMI).
- ❑ Have participant sign consent form if they meet all inclusion criteria and remind of ability to withdraw at any time.
- ❑ Have participant mould mouth guard (if not previously done)
- ❑ Measure participant's forearm and mark electrode placement.
- ❑ Equip participant with electrocardiogram (ECG) and electromyography (EMG) electrodes and ground bracelet (Non-dominant arm)
- ❑ Equip participant with Portapres® cuffs (Dominant arm)
- ❑ Equip participant with Pneumotrace II® respiration band
- ❑ Measure and equip participant with scalp cap.
- ❑ Equip participant with neck brace.
- ❑ Collect 5-10 minutes of resting data in Lab Chart.
- ❑ Perform a MVC and record size in Newtons on Lab Chart comments and turn off Portapres.
- ❑ Test Magstim away from participant
- ❑ Set Magstim to single pulse mode.
- ❑ Determine optimal location on scalp of hand area of the motor cortex (add pulse number to Lab Chart)
- ❑ Mark stimulation location on the participant's individual scalp cap
- ❑ Determine AMT of participant and add as a comment in Lab Chart. Also note the average amplitude size and pulse number and mark as comment in LabChart.
- ❑ Perform two, 5-second maximal voluntary contractions of non-dominant hand and record in Newtons.

- ❑ Calibrate hand dynamometer to percentage of maximal voluntary contraction force.
- ❑ Have participant practice the 120-second 35% exercise protocol including the 120-second post-exercise circulatory occlusion protocol.
- ❑ Map fibular nerve using mild external stimulation
- ❑ Locate best recording site (strongest and cleanest signal)
- ❑ Turn on Portapres, insert square wave into and calibrate LabChart channel (0mmHg and 200mmHg)
- ❑ Turn off Physiocal after 3 cycles
- ❑ Measure blood pressure with Carescape and comment in LabChart.
- ❑ Continuously record ECG, EMG, Portapres® blood pressure, respiration and MSNA data for 5 minutes while participant sits quietly.
- ❑ Perform single pulse TMS to confirm stimulation site location
- ❑ Turn off Portapres
- ❑ Turn Magstim on repetitive mode (Sham parameters)
- ❑ Perform Sham TMS
- ❑ Perform a single pulse test and compare AMT to pre-TMS. Note the amplitude as a comment on LabChart.
- ❑ 10 minutes of seated rest while continuously record ECG, EMG, Portapres® blood pressure, respiration and MSNA data
- ❑ Turn off Physiocal after 3 cycles
- ❑ Perform isometric handgrip exercise for 2 minutes
- ❑ Inflate arm cuff for 2-minute post-exercise ischemia period 5 seconds before PEI period.
- ❑ Deflate arm cuff.
- ❑ Continuously record ECG, EMG, Portapres® blood pressure, respiration and MSNA data for 5 to 10 minutes while participant sits quietly for the Recovery period.
- ❑ Turn off Portapres
- ❑ After 5 minutes perform a single pulse to test effects of TMS condition. If AMT amplitude is similar to initial test continue protocol. If it is significantly higher or lower wait 5 minutes and perform the single test again.
- ❑ Once AMT is back to resting level continue protocol
- ❑ Perform single pulse TMS to confirm stimulation site location
- ❑ Perform TMS (facilitative or inhibitory)
- ❑ Perform a single pulse test and compare AMT to pre-TMS. Note the amplitude as a comment on LabChart.
- ❑ 10 minutes of seated rest while continuously record ECG, EMG, Portapres® blood pressure, respiration and MSNA data

- ❑ Turn off Physiocal after 3 cycles
- ❑ Perform isometric handgrip exercise for 2 minutes
- ❑ Inflate arm cuff for 2 minute post-exercise ischemia period 5 seconds before PEI period.
- ❑ Deflate arm cuff.
- ❑ Continuously record ECG, EMG, Portapres® blood pressure, respiration and MSNA data for 5 to 10 minutes while participant sits quietly for the Recovery period.
- ❑ Turn off Portapres
- ❑ After 5 minutes perform a single pulse to test effects of TMS condition. If AMT amplitude is similar to initial test continue protocol. If it is significantly higher or lower wait 5 minutes and perform the single test again.
- ❑ Once AMT is back to resting level continue protocol
- ❑ Perform single pulse TMS to confirm stimulation site location
- ❑ Perform TMS (facilitative or inhibitory)
- ❑ Perform a single pulse test and compare AMT to pre-TMS. Note the amplitude as a comment on LabChart.
- ❑ 10 minutes of seated rest while continuously record ECG, EMG, Portapres® blood pressure, respiration and MSNA data
- ❑ Turn off Physiocal after 3 cycles
- ❑ Perform isometric handgrip exercise for 2 minutes
- ❑ Inflate arm cuff for 2 minute post-exercise ischemia period 5 seconds before PEI period.
- ❑ Deflate arm cuff.
- ❑ Continuously record ECG, EMG, Portapres® blood pressure, respiration and MSNA data for 5 to 10 minutes while participant sits quietly for the Recovery period.