EXAMINING THE EFFECTS OF HIGH INTENSITY INTERVAL EXERCISE (HIIE) ON CORTICOSPINAL EXCITABILITY AND MOTOR LEARNING

by

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ABSTRACT

High-intensity interval exercise (HIIE) is effective in modulating corticospinal excitability (CSE), and it has been shown to facilitate improvements in motor learning. This study examined the effect of HIIE on motor learning when HIIE was performed prior to multiple sessions of motor task practice. The effect of HIIE on CE was investigated using single and paired-pulse transcranial magnetic stimulation (TMS) before (Pre), directly after (Post 1), and 30 min after (Post 2) HIIE. The effect of HIIE on motor learning was assessed using a complex movement execution (CME) task, during which participants reproduced complex trajectories presented on a touchscreen. Engaging HIIE was shown to significantly increase CE, as evidenced by an increase in CE from Pre to Post 1 ($p=0.048$) and Post 2 ($p=0.003$) time points. However, a significant decrease in intracortical facilitation from Pre to Post 1 ($p=0.031$) and Post 2 ($p=0.002$) time points was also observed. Performing HIIE before engaging in repeated sessions of motor task practice failed to demonstrate a significant effect on motor learning compared to non-exercising controls. Additionally, we were unable to detect a significant correlation between changes in CE and motor learning in participants who underwent TMS and engaged in the CME task after HIIE.

LIST OF ABBREVIATIONS USED

AE	Aerobic exercise
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	Adenosine triphosphate
BB	Biceps brachii muscle
BNDF	Brain-derived neurotrophic factor
bpm	beats per minute
Ca^{2+}	Calcium ion
CaM kinases	Ca^{2+}/calmodulin-dependent protein kinases
CME	Complex movement execution
CP	Creatine phosphate
CS	Conditioning stimulus
CSE	Corticospinal Excitability
EMG	Electromyography
FDI	First dorsal interosseous muscle
GABA	Gamma-aminobutyric acid
Glu	Glutamate
GXT	Graded exercise test
HIIE	High intensity interval exercise
HR	Heart rate
HR_{max}	Heart rate maximum
HRR	Heart rate reserve
ICF	Intracortical faciliation
IHI	Interhemispheric inhibition
IPAQ	International Physical Activity Questionnaire
ISI	Interstimulus interval
LICI	Long-interval intracortical inhibition
LTP	Long-term potentiation
M1	Primary motor cortex
MCP	Metacarpal phalangeal
MEP	Motor-evoked potential
MICE	Moderate-intensity continuous exercise
Mg^{2+}	Magnesium ion
Na^{+}	Sodium ion
NMDA	N-methyl-D-aspartate
PAR-Q	Physical Activity Readiness Questionnaire
PAS	Paired associative stimulation
PO_{max}	Maximal power output
RMT	Resting motor threshold
RPE	Rating of perceived exertion
rpm	revolutions per minute
SAF	Speed accuracy function
SICI	Short-interval intracortical inhibition
S-R	Stimulus-response
S1B1	Learning session 1, block 1

S3B1	Learning session 3, block 1
TMS	Transcranial magnetic stimulation
TS	Test stimulus
VAT	Visuomotor accuracy-tracking task
VO_{2max}	Maximum volume of oxygen consumption rate

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CHAPTER 1: INTRODUCTION

Motor learning is the process of acquiring a novel skill or improving upon an established skill through repetitive task practice and provision of feedback regarding task performance (Newell, 1991). Repetitive motor-task practice drives plasticity in brain areas associated with motor planning and execution, establishing a neural network that underlies successful task performance (Newell, 1991). Repetitive task practice can be made more effective if the brain is more excitable (Ward, 2005). The excitability of cortical neurons is dependent on their resting membrane potentials; excited neurons can propagate neural activity more quickly and more easily than depressed neurons, as they require less additional excitatory input to reach depolarisation (Purves et al., 2012).

Aerobic exercise (AE) has been reported to be an effective mechanism for induction of experience-dependent plasticity, as AE has been shown to result in increased intracortical facilitation and decreased intracortical inhibition (Singh et al., 2014). Therefore, AE is emerging as a potential agent for "priming" the brain prior to engaging in task practice. One form of exercise in particular, high-intensity interval exercise (HIIE), has garnered attention given its use as a training method in many fields, from high performance athletic training to physical rehabilitation (Larsen, 2010; Roig et al., 2012). HIIE involves alternating sets of high- and low-intensity AE.

The first objective of the current study was to investigate whether HIIE results in increased intracortical facilitation and decreased cortical inhibition, relative to levels of corticospinal excitability (CSE) measured prior to exercise. Secondly, the current study examined motor learning outcomes in individuals participating in HIIE, compared to

participants who did not engage in exercise prior to participation in the same motor learning paradigm. The third objective of this study was to examine the relationship between observed HIIE-induced changes in corticospinal excitability and changes in motor learning, assessed via the change in task performance from the beginning of task practice (Session 3) to the retention test (Session 5)

The research objectives were addressed by measuring CSE and motor task performance following HIIE. During session 1, young (19-28 years), healthy participants engaged in a maximal exercise test to determine their maximal power output (PO_{max}). PO_{max} was used in subsequent sessions to define the workload required to achieve the appropriate exercise intensity during HIIE. HIIE was performed on an upright cycle ergometer, involving three 3-min sets of high-intensity exercise (performed at 90% of the participant's maximal power output (PO_{max})), separated by 2-min of low-intensity exercise (50% PO_{max}). During session 2, participants underwent single and paired-pulse transcranial magnetic stimulation (TMS) before, directly after and 30 min after HIIE, to determine HIIE-related changes in CSE. Specifically, changes in CSE were assessed via comparison of stimulus-response curves and intracortical facilitation and inhibition values from pre- to post-HIIE. It was hypothesised that HIIE would result in increased CSE, evidenced by increased intracortical facilitation and decreased intracortical inhibition values, as well as an upward shift in the stimulus-response curve, relative to data collected pre-HIIE.

Sessions 3-5 involved participants engaging in a motor learning paradigm immediately after performing HIIE. The motor learning paradigm used in the present study was a complex movement execution (CME) task; this task was designed and

previously validated in the Laboratory for Brain Recovery and Function (Ingram et al., *Under review*). Briefly, this task asked participants to reproduce complex trajectories presented on a touchscreen, and importantly required upper-limb multi-joint movements to complete. Learning was confirmed via a decrease in performance error from the beginning of task practice (Session 3) to the retention test (Session 5). We previously validated multi-session learning in a large number of young healthy participants using the CME task (Ingram et al., *Under review*), and data collected from these non-exercising participants served as a control group for the present study. Specifically, CME task performance was compared between the group of 15 participants engaging in HIIE prior to task execution and the control group. It was hypothesized that error would be reduced in the participants engaging in HIIE prior to task practice, compared to that of the non-exercising participants.

The third study objective was to determine if there was a relationship between measures of CSE and motor learning task performance following HIIE. This objective was addressed by examining the correlation between the change in motor task performance (from the beginning of CME task practice to the retention test) and change in various measures of CSE from pre- to post-HIIE levels. It was hypothesized that there would a significant, positive correlation between these measures.

The current study sought to extend previous research that has investigated the effects of AE on motor learning (Roig et al., 2012; Skriver et al., 2014). Particularly, the findings will advance our knowledge of the mechanisms through which HIIE facilitates improved motor learning, in turn providing evidence to support its use as a priming agent to be used prior to engaging in task practice. These findings will have important

implications for the recovery of motor skills that have been lost due to neurological injuries such as stroke (Krakauer, 2006).

CHAPTER 2: BACKGROUND AND RATIONALE

2.1 Motor Skill Learning

The acquisition and retention of motor skills play an essential role in daily living. Motor skills such as walking, throwing, or writing are all acquired through repetitive practice. This principle is called motor learning, which can be defined as the process of acquiring a novel skill or improving upon an established skill through repetitive task practice. Motor learning results in a relatively permanent behaviour change, manifested as a change in the ability to execute a motor skill due to practice or experience. In other words, movements can be executed more quickly and accurately with practice (Dayan and Cohen, 2011). Motor skills are typically learned slowly until performance reaches nearly asymptotic levels. Once a skill is acquired, it is generally retained for long periods of time with minimal decay in performance (Luft and Buitrago, 2005). Motor learning can be studied and explained through behavioural and physiological perspectives.

2.1.1 Behavioural view of motor learning

One of the traditional theories of motor skill learning is Fitts and Posner's three stage theory (Fitts and Posner, 1967). As a person learns a movement, they will progress through the three stages of motor skill acquisition: 1) the cognitive stage, 2) the associative stage, and 3) the autonomous stage. During the cognitive stage, the learner must consciously focus on the movement, breaking it down into steps during execution. In this stage, the learner must determine the appropriate sequence of actions to achieve the desired goal (Taylor and Ivry, 2012). In this first stage, there is often a large amount of variance and error in skill performance (Taylor and Ivry, 2012).

Once the basic movement pattern has been determined, the learner enters the associative stage of motor learning. In this stage, attention may be focused on specific details of the motor plan, such as determining appropriate transitions or concentrating on fluency (Taylor and Ivry, 2012). Because this phase is characterised by more subtle adjustments, movements are more consistent from trial to trial and the task outcome is more reliable (Wulf, 2007). At the end of the associative stage the action can be executed in a smooth and coordinated manner (Fitts and Posner, 1967; Taylor and Ivry, 2012).

After extensive practice the learner reaches the final phase of Fitts and Posner's (1967) three stage theory. This stage is termed the autonomous stage, as movement execution requires little to no conscious cognitive effort. In this stage, execution of the learned task is effortless, accurate and consistent (Wulf, 2007).

In the literature surrounding motor skill acquisition, there is an important distinction between motor performance and motor learning (Kantak and Winstein, 2012). The term *motor performance* refers to a transient status of motor behaviour; this can be manifested in the accuracy of task execution at any given point of a practice session (Kantak and Winstein, 2012). In contrast, the term *motor learning* refers to relatively permanent change in the ability to execute a motor skill. Improvements in motor performance within a single practice session are referred to as online gains, and some of the mechanisms that contribute to online gains may not influence learning (Edwards, 2010). Therefore, motor skill learning is often examined using a delayed retention test (Kantak and Winstein, 2012). A retention test is a measurement of skill performance conducted following skill practice but after sufficient time has passed to allow for within-session performance effects to dissipate (Edwards, 2010).

It is also important to note that improvements in motor skill performance can occur after skill acquisition trials and without additional practice. These improvements are termed offline gains (Kantak and Winstein, 2012), and they are reflective of the consolidation of learning.

2.1.2 Physiological view of motor learning - neuroplasticity

The repetitive task practice that underlies successful motor task performance is represented neurologically by structural or functional changes in the brain, or neuroplasticity. Neuroplasticity is defined as structural or functional changes in the nervous system in response to experience, and it is dependent upon the ability of the nervous system to be modified as a result of repetitive activation (Purves et al., 2012). Learning new knowledge and skills, or re-learning skills to support recovery after neurological insult, is dependent on neuroplasticity. Mechanisms of experience-driven neuroplasticity include long-term potentiation, changes in gene expression and protein synthesis.

On a cellular level, plasticity is dependent on synaptic connections between neurons. Synaptic connectivity is constantly changing in response to neural activity. Chemical synapses undergo changes that either strengthen or weaken synaptic transmission. Plasticity at cortical synapses can be short-term or long-term. Short-term changes in synaptic transmission are largely due to changes in the amount of neurotransmitter released from a presynaptic terminal in response to an action potential. These forms of short-term plasticity result in short-lived changes to neurocircuitry, lasting for a few minutes or less. There are also long-lasting forms of synaptic plasticity, such as

long-term potentiation, that result in more permanent changes in brain function (Purves et al., 2012). It is these long-term synaptic changes that are the foundation of learning.

Long-term potentiation (LTP) is characterized by long-lasting increases in synaptic strength, and these changes in synaptic strength are the cellular underpinnings of learning. LTP is a long-lasting enhancement in signal transmission between two neurons that occurs in response to repeated stimulation. In other words, synaptic connections become stronger with frequent, repeated activation.

The key molecules that are involved in LTP are the neurotransmitter glutamate, N-methyl-D-aspartate (NMDA) receptors, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Kandel et al., 2000). NMDA and AMPA receptors are located on the post-synaptic cell and are activated by the binding of glutamate. However, when the post-synaptic cell membrane potential is at resting levels, NMDA receptor channels are blocked by magnesium ions (Mg^{2+}). Therefore, when glutamate is released from the presynaptic neuron during normal, low-frequency synaptic transmission (Figure 1A), it activates only the AMPA receptors. When AMPA receptors are activated sodium ions (Na^{+}) flow through the receptor's ion channel. The Mg^{2+} blockage in the NMDA receptor prevents ions from passing through this receptor. While the influx of sodium ions through the AMPA receptor channel causes a slight depolarization event and is sufficient to elicit a response in the post-synaptic cell, it does not cause LTP (Kandel et al., 2000).

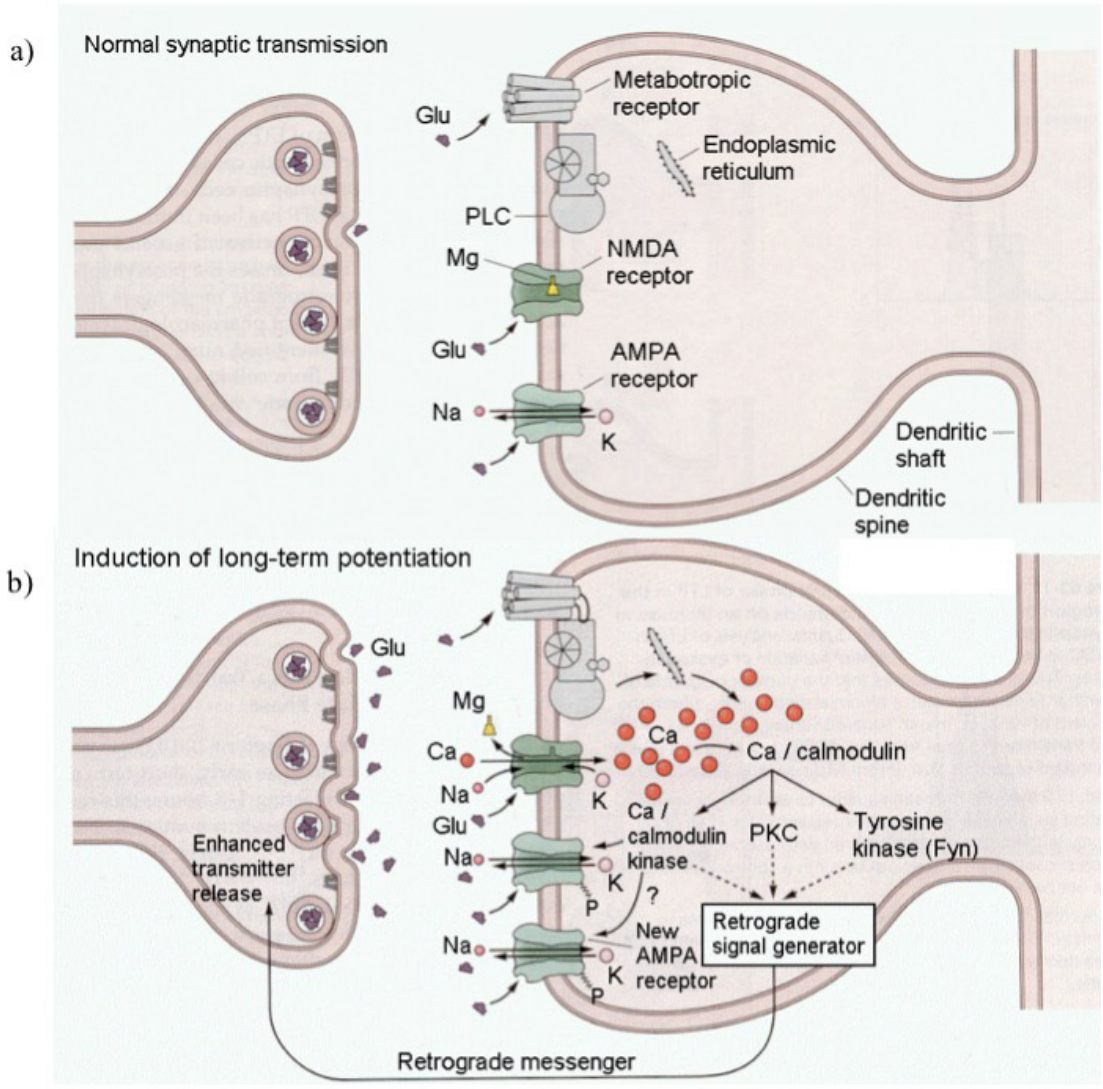


Figure 1. Induction of long term potentiation. (A) During normal synaptic transmission glutamate (Glu) is released from the pre-synaptic terminal and acts on the NMDA and AMPA receptors in the post-synaptic neuron. Sodium (Na) and potassium (K) flow in and out of the AMPA receptor, respectively. The NMDA receptor ion channel is blocked by magnesium (Mg). (B) Long-term potentiation is induced by the influx of calcium (Ca) and a cascade of intracellular signalling. *Retrieved from Kandel et al., 2000.*

PLC: phospholipase C. PKC: protein kinase C. P: phosphate group, signifies phosphorylation.

LTP occurs as a result of high-frequency stimulation (Kandel et al., 2000). Higher frequency action potentials cause greater stimulation of AMPA receptors, allowing more sodium to flow into the post-synaptic cell. The influx of Na^+ causes a large depolarization

event in the post-synaptic cell, triggering the voltage-dependent magnesium blockage of the NMDA receptor to be removed through a process known as electrostatic repulsion. With the Mg^{2+} blockage removed, Na^{+} and calcium ions (Ca^{2+}) are free to flow into the post-synaptic cell through the NMDA receptor (Figure 1B). Calcium acts as an important secondary messenger in the post-synaptic neuron, activating secondary intracellular cascades that underlie long-term potentiation. One mechanism through which calcium induces LTP is by triggering the insertion of new AMPA receptors into the post-synaptic cell membrane at the active synapse. Additionally, the influx of Ca^{2+} causes the activation of Ca^{2+}/calmodulin-dependent protein kinases (CaM kinases), which in turn phosphorylate AMPA receptors and further increase their sensitivity to glutamate. The post-synaptic cell is also thought to release retrograde messengers that act on the pre-synaptic cell to stimulate increased glutamate release (Kandel et al., 2000). Together, these pre- and post-synaptic events strengthen the connections along a specific neural pathway, and this strengthening is the foundation of learning.

2.2 Stroke and Rehabilitation

Motor skill learning is the foundation of motor recovery after stroke. A stroke occurs when blood supply to an area of the brain is interrupted or severely reduced, causing cell death in the affected brain region. When stroke occurs, it often results in motor impairment on the contralateral side of the body (Takeuchi & Izumi, 2013). Stroke is a leading cause of serious, long-term disability that can have lasting physical, social and financial consequences (Clarke & Forster, 2015). A 2015 report compiled for the Heart & Stroke Foundation Canadian Partnership for Stroke Recovery stated that an estimated 405,000 Canadians were experiencing the effects of stroke in 2013 (Krueger et

al., 2015). The prevalence of Canadians living with stroke-induced disability is expected to continue to rise in coming years due to population growth, an increasing incidence of stroke due to the aging Canadian population, and decreasing stroke mortality rates due to improvements in acute stroke care (Statistics Canada, 2014; Krueger et al., 2015). Advances in rehabilitation techniques post-stroke are required to better serve the growing population of Canadians living with the effects of stroke.

The aim of rehabilitation after stroke is to achieve functional recovery by driving brain recovery. For the more than 80% of stroke survivors who experience hemiparesis following stroke (Danells et al., 2004), the goal of rehabilitation is to recover movement patterns that have been lost due to injury. This is often achieved through repetitive task practice, as many repetitions of a task are required for an individual to learn the optimal way to perform a motor task. Skilled motor performance is characterized by the ability to perform a complex motor task, with the flexibility to adapt and refine the skill to meet changing task and environmental demands (Refshauge et al., 2005).

Repetitive task practice can be defined as the repetitive practice of task-specific motor activities. Canadian best practice guidelines for stroke rehabilitation indicate that there is evidence to support the use of repetitive task practice and goal-directed therapies to promote functional recovery after stroke (Hebert et al., 2016). These behavioural interventions capitalize on the process of experience-dependent neural plasticity.

2.3 Neural Repair After Stroke

After stroke, behavioural interventions, such as repetitive task practice, contribute to the adaptation and recovery of neuronal pathways underlying motor skill performance (French et al., 2016). Repetitive task practice strengthens connections within the brain, making it easier for the brain to carry out this task in the future. As indicated above, repetitive task practice is based on the principle of neuroplasticity, or the body's ability to make changes in the organization of the brain's connections as the result of experience.

Neuroplasticity allows for some degree of spontaneous behavioural recovery in the first weeks to months after stroke. The greatest spontaneous gains occur in the first 30 days after stroke, and patients with mild deficits tend to experience a greater degree of spontaneous recovery and recover more quickly than patients with more severe deficits (Duncan et al., 1994; Cramer, 2008).

Stroke has also been shown to cause changes in cortical excitability in the peri-infarct region and beyond. Inhibition is mainly facilitated by the action of gamma-aminobutyric acid (GABA) on GABA receptors, while excitation is mainly facilitated by the action of the neurotransmitter glutamate on NMDA and non-NMDA (such as AMPA) receptors (Kandel et al., 2000). Following a stroke, the peri-infarct region becomes hypoexcitable due to an increase in the tonic GABA current in the affected brain region (Carmichael, 2012). Glutamate, however, has been shown to aid in recovery after stroke; increases in glutamatergic excitability in the peri-infarct region have been shown to parallel functional recovery (Carmichael, 2012).

Additional research has shown that stroke can lead to an imbalance in interhemispheric inhibition (IHI) between the primary motor cortices of the lesioned and the unaffected hemispheres (Murase et al., 2004). Specifically, Murase and colleagues (2004) reported that movement of the paretic limb was associated with an abnormal level of IHI by the intact hemisphere to the injured one. This IHI may be the result of a high level of activity of the intact hemisphere during movement of the contralateral, paretic limb (Liepert et al., 1998).

As described above, brain recovery after neurological injury can be improved if the neurons in the area of the brain responsible for movement (Primary motor cortex; M1) are more excitable (Ward, 2005). This is consistent with the principles of long-term plasticity: synapses that are successfully activated are strengthened, while those that cannot be successfully activated are weakened. As such, the excitability of cortical neurons affects the degree of plasticity and recovery that occurs. Excited neurons (neurons with greater resting membrane potential) can be stimulated more efficiently than less excited neurons (neurons with lower resting membrane potential), as highly excitable neurons need less excitatory input than depressed neurons to generate an action potential and to elicit muscle activity (Purves et al., 2012).

Given the link between neuroplasticity and functional recovery, there is a need to explore methods that can be used to lower the threshold of depolarization of cortical neurons prior to engaging in rehabilitation therapies. Some methods of lowering the threshold for depolarization of cortical neurons and extending increased cortical excitability beyond the spontaneous recovery period are the consumption of caffeine or stimulant drugs, non-invasive brain stimulation, and exercise.

2.4 Transcranial Magnetic Stimulation

One of the objectives of this thesis is to investigate how HIIE modulates CSE. To understand how CSE is measured, it is important to understand the foundations of transcranial magnetic stimulation (TMS).

2.4.1 Principles of Transcranial Magnetic Stimulation

TMS is a non-invasive technique that uses magnetic fields to electrically stimulate neural tissue (Kobayashi and Pascual-Leone, 2003). TMS was introduced into human neurophysiology studies in the 1980s when the tool was first used in humans to study the propagation of nervous signals along the corticospinal tract, spinal roots, and peripheral nerves (Janicak and Dokucu, 2015). A few years later, TMS was proven to be well-suited for exploration of the human cortex as well, and in 1985, Barker et al. showed that the application of a TMS pulse over the motor cortex elicits a motor response in the muscles receiving nervous input from the stimulated cortical region (Barker et al., 1985). Having been shown to be a safe, non-invasive, and non-painful method to activate the human cortex, TMS began to grow in popularity, and the technique is now widely used for research and clinical purposes in neurology, neurophysiology, and psychiatry (Kobayashi and Pascual-Leone, 2003).

A typical transcranial magnetic stimulator consists of a power supply, a large capacitor, a switching mechanism, and an inductor (Papanicolaou, 2017). The inductor in a TMS system is a magnetic coil, which is composed of a tightly wound copper wire encased in plastic. The TMS operator holds the magnetic coil over the subject's scalp to deliver stimulation to the underlying brain region. The power supply serves to charge the

capacitors, which store and then rapidly discharge electricity into the coil. When the coil is discharged, there is a deformation of the copper wire coil that results in an audible click (Papanicolaou, 2017).

TMS is based on Faraday's prinicple of electromagnetic induction. When an electric current is carried through a wire, a magnetic field is formed around it. This magnetic field is then capable, in turn, of inducing a perpendicular electric field in conducting materials (Figure 2). As it applies to TMS, an electric current moving through a TMS coil induces a magnetic field, with lines of magnetic flux forming concentric circles around the wire (Hallet, 2000; Giancoli, 2008). This magnetic field induces an electric field, parallel to the plane of the coil, in proximal conductors such as surface brain tissue (Rotenberg et al., 2014). This magnetic field therefore induces an electric current in the cortex, which can cause the depolarization of cell membranes and the initiation of action potentials (Rotenberg et al., 2014).

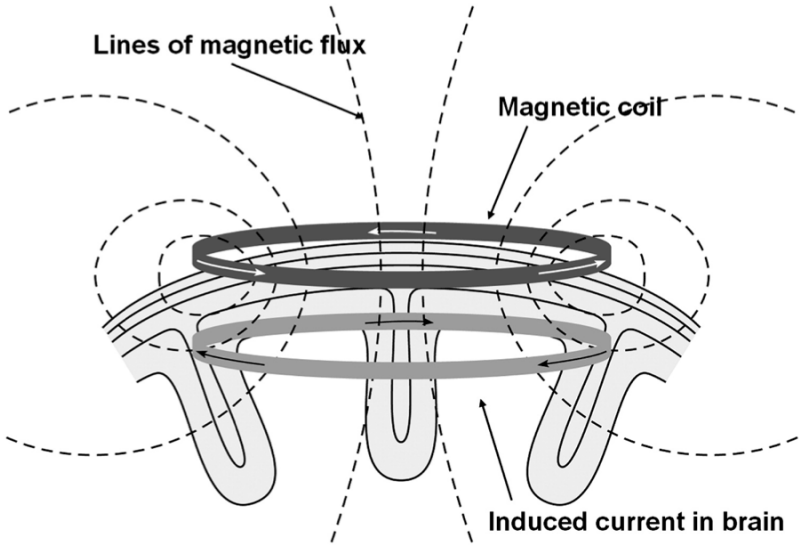


Figure 2. Physics underlying the induction of TMS. Electric current travels through copper wire of the magnetic coil (depicted as a black circle), creating a magnetic field in concentric circles around the wire (the magnetic field is depicted using dashed lines). The magnetic field induces the flow of electric current (depicted as a light grey circle) induced in the underlying brain tissue, parallel to the magnetic coil. *Retrieved from Hallet (2000).*

The magnetic coils used to perform TMS come in many different shapes, as the shape of the coil affects the spatial distribution of the induced electric field. The two most common desings for TMS coils are the round coil, and the figure-eight coil. Rounds coils have an electric field of zero at the centre of the coil and the maximal electric field is induced under the circumference of the loop. It is therefore not possible to target a precise brain region for activation. Figure-eight-shaped coils are more focused, producing maximal current at the intersection of the two round components (Figure 3). This coil design causes the induced electrical current to be two to three times higher under the center of the figure-eight-shaped coil, relative to the current induced near the outer edges of the coil (Groppa et al., 2012). This allows for relatively focused stimulation of a precise cortical region at low to moderate stimulation intensities, making the figure-eight-

shaped coil the more suitable coil design for conditions requiring precise cortical mapping, or applications requiring stimulation of a distinct cortical area.

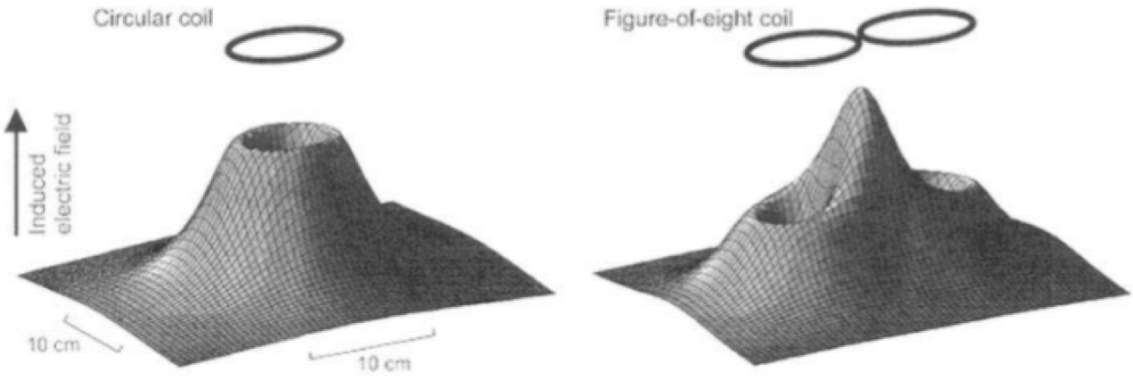


Figure 3. The spatial distribution of the electric field induced below a circular coil (left) and a figure-eight coil (right). *Retrieved from Ilmoniemi et al., 1999.*

2.4.2 Measuring Corticospinal Excitability using TMS

As indicated above, TMS can be used both experimentally, to explore neural excitability or function, and clinically as a diagnostic or therapeutic instrument (Forrester et al., 2006; Bunse et al., 2014; Gorelick et al., 2014; Janicak and Dokucu, 2015). In the present study, single- and paired-pulse TMS will be used for evaluation of exercise-induced changes in CSE.

TMS is used to induce action potentials in motor cortical axons, which then spread to connected cortical regions, along the corticospinal tract and peripheral motor nerves, causing muscle activation. When TMS is applied to the motor cortex, a focal muscle twitch can be produced and electromyography (EMG) can be used to measure the muscle response. The electromyographic muscle response to stimulation, termed a motor-evoked

potential (MEP), is measured using surface electrodes applied to the muscle belly. The peak-to-peak amplitude of collected MEPs can be used to probe the excitability of the corticospinal tract (Klomjai et al., 2015).

Based on the principles outlines above, single-pulse TMS can be used to identify hand muscle representations in the motor cortex, a process referred to as 'hotspotting'. A motor 'hotspot' is the muscle representation within the primary motor cortex (M1) that produces the greatest amplitude MEPs, in the muscle of interest, at a given stimulus intensity. Similarly, single-pulse TMS is used to determine the resting motor threshold (RMT) of the targeted muscles. RMT is generally defined as the lowest stimulation intensity required to elicit MEPs with a peak-to-peak amplitude of 50μV, in the resting muscle, for 5 out of 10 consecutive stimuli (Rossini et al., 1994; Groppa et al., 2012).

Single-pulse TMS can also be used to produce a stimulus-response (S-R) curve. MEPs elicited at various stimulus intensities are used to construct an S-R curve. The slope of, or area under, the S-R curve can be used as an index of excitability (Devanne et al., 1997; Carson et al., 2013; Lulic et al., 2017), and thus can be used as a means to compare CSE under different conditions, such as prior to and after engagement in exercise.

While single-pulse TMS can probe general measures of excitability related to cortical neurons in M1 and the entirety of the corticospinal tract, paired-pulse TMS can be used to gain insight into the excitability of corticocortical connections (Vahabzadeh-Hagh, 2014). Paired-pulse TMS can be used to probe inhibitory or facilitatory systems in the motor cortex (Chen, 2004). Additional information can be gathered based on the

region of stimulation; paired pulses can be applied either within or across hemispheres to measure intra- and inter-hemispheric neuronal connections, respectively. The current study makes use of within hemisphere paired-pulse TMS to examine intra-hemispheric connections.

To deliver paired-pulse TMS, two consecutive pulses are delivered to the same brain region. The first pulse, known as the conditioning stimulus (CS), and the second pulse, known as the test stimulus (TS), are delivered with a set time interval between them, termed the interstimulus interval (ISI). The stimulation intensities of the CS, the TS, as well as the duration of the ISI allow for the investigation of different excitatory and inhibitory corticocortical circuits (Vahabzadeh-Hagh, 2014). Short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI), and intracortical faciliation (ICF) are three different measures of cortical excitability that can be examined through paired-pulse stimulation.

SICI can be probed by delivering a subthreshold (below resting motor threshold) CS followed by a suprathreshold TS at an ISI of 1-5 ms (Figure 4A) (Kujiari et al., 1993). This is termed SICI, as the subthreshold CS inhibits an MEP generated by a test stimulus that follows within this interval of 1-5 ms. SICI probes $GABA_A$-mediated inhibition (Di Lazzaro et al., 2006). LICI is elicited by applying suprathreshold conditioning and test stimuli at an ISI of 50-200 ms (Figure 4B) (Wassermann et al., 1996). Wassermann and colleagues (1996) suggested that LICI is related to a "silent period", or period of suppression of muscle contraction following suprathreshold stimulation. LICI probes $GABA_B$-mediated inhibition (McDonnell et al., 2006). M1 LICI has been shown to be exaggerated in stroke patients (Classen et al., 1997). Paired-pulse TMS can also be used

to examine facilitation. ICF is elicited by a subthreshold CS followed by a suprathreshold TS at ISIs of 8-30 ms (Figure 4C) (Kujari et al., 1993). Physiological processes underlying intracortical facilitation in the human motor cortex are still under investigation, but recent evidence suggests that ICF appears to be mediated by I-wave facilitation (Van den Bos et al., 2018).

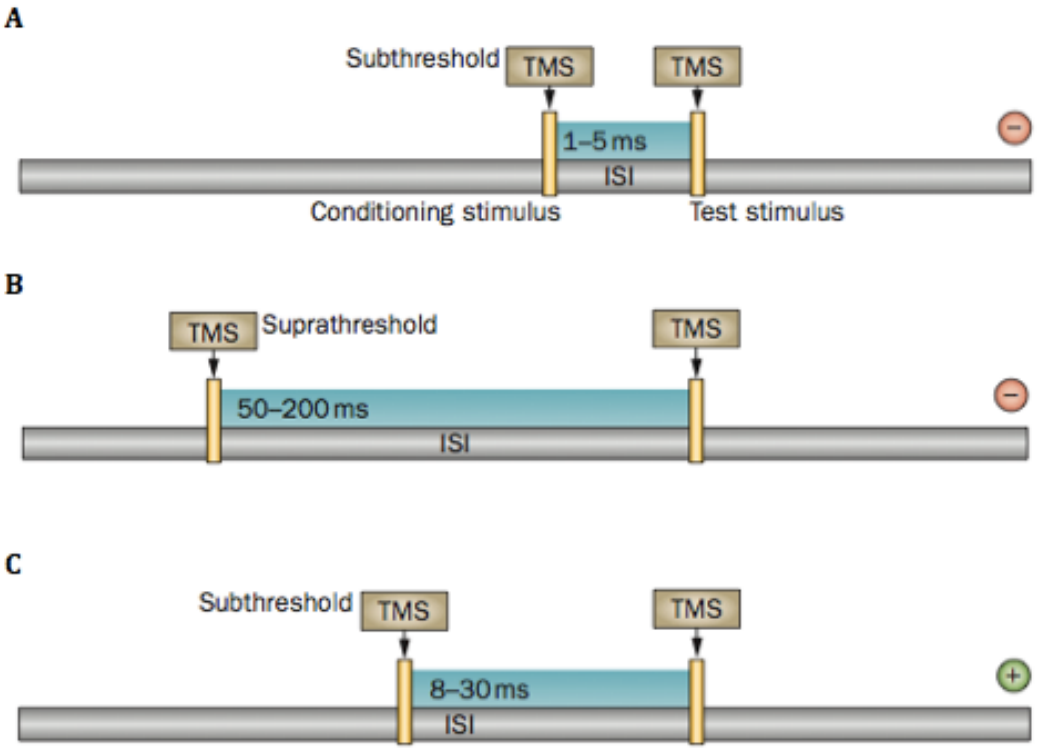


Figure 4. TMS paired-pulse protocols. (A) SICI: a subthreshold CS is followed by a suprathreshold TS after an ISI of 1-5 ms, resulting in inhibition; (B) LICI: a suprathreshold CS is followed by a suprathreshold TS after an ISI of 50-200 ms, resulting in inhibition; (C) ICF: a subthreshold CS is followed by a suprathreshold TS after an ISI of 8-30 ms, resulting in facilitation. *Retrieved from Di Pino et al. 2014*.

Together, the information gathered from single- and paired-pulse TMS will allow for evaluation of changes in cortical excitability resulting from engagement in high-

intensity interval exercise, as well as provide information on the mechanisms underlying the observed changes.

2.5 Aerobic Exercise and the Brain

It is well established that there are many health benefits associated with regular aerobic exercise, such as improving cardiovascular health and reducing the risk of stroke and heart disease (Lucas et al., 2015), diabetes (Charatan, 2001), and neurodegenerative disorders (Ang et al., 2010). In the brain, regular aerobic exercise has been shown to promote angiogenesis (Isaacs et al., 1992), neurogenesis (Saraulli et al., 2017), and synaptic plasticity (Cotman and Berchtold, 2002); processes that are fundamental to learning and rehabilitation after a stroke (Font et al., 2010). The mechanisms that underlie the benefits of aerobic exercise on the brain have been the focus of extensive research over the past few decades, and although significant progress has been made, many of the mechanisms underlying the neurological benefits of exercise remain to be established (Lucas et al., 2015). Consequently, the prescription of AE intensity used to benefit the brain and to facilitate learning is a subject that requires additional focus.

2.5.1 High-Intensity Interval Exercise

High-intensity interval exercise (HIIE) has emerged as an important training method in many fields, from high performance athletic training to rehabilitation (Gibala et al., 2012; Laursen, 2010). This method of exercise involves alternating sets of high- and low-intensity AE. HIIE has been reported to be superior to moderate-intensity continuous exercise (MICE) at improving cardiorespiratory fitness (indicated by comparing VO_{2max} scores measured pre- and post-training) (Hannan et al., 2018).

Importantly, HIIE has also been shown to be suitable for both healthy and 'at-risk' populations, such as patients participating in cardiac (Guiraud et al., 2012; Rognmo et al., 2012; Hannan et al., 2018) or stroke rehabilitation (Carl et al., 2017).

There is considerable variability in the HIIE protocols used in both fitness training and exercise-related research. High-intensity interval exercise often involves repeatedly exercising at a high-intensity for 30 sec to several minutes (referred to as the work interval), separated by low-intensity exercise periods that also range in duration from 30 sec to several minutes (referred to as the recovery interval) (Shiraev and Barclay, 2012). Recovery intervals in HIIE protocols can be inactive, meaning the individual stops exercising for that period, or the recovery period can be active, meaning the individual continues to exercise, but at a lower intensity (Guiraud et al., 2010). The intensity of active recovery intervals varies greatly between HIIE protocols (Guiraud et al., 2010; Schaun and Del Vecchio, 2018). There is also variability in the duration of intervals and the frequency of repetition of intervals used in HIIE protocols in scientific literature.

An additional source of variability in HIIE protocols is the method used to define exercise intensity. Exercise intensity for the work interval and the recovery interval can be defined by any of the following methods: percentage of PO_{max}, percentage of heart rate maximum ($\%HR_{max}$), percentage of heart rate reserve (%HRR), percentage of maximum rate of oxygen consumption ($\%VO_{2max}$), rating of perceived exertion (RPE), or metabolic equivalence (Ross et al., 2016).

HIIE has become an increasingly popular form of exercise due to the large health benefits that have been associated with the training method, and the short time

requirement. For example, research has shown that regular engagement in HIIE is beneficial to cardiorespiratory fitness and vascular health (Warburton et al., 2005; Helgerud et al., 2007; Wisloff et al., 2009). This is due to the interval design of the training method, which allows individuals to work at higher exercise intensities than those that are typically achieved during MICE, making HIIE more challenging for the cardiovascular system (Cassidy et al., 2017). HIIE has also been shown to be important in metabolic disease management, and to be a more effective method to reduce subcutaneous and abdominal body fat than other types of regular aerobic exercise (Tremblay et al., 1994; Trapp et al., 2008; Boutcher, 2011). More recently, research has focused on the effects of HIIE on cerebral health, and the role of HIIE in priming the brain for learning and rehabilitation (Lucas et al., 2015; Haas et al., 2017; Nepveu et al., 2017). The focus of this thesis was to examine the effects of HIIE on CSE and motor skill learning, and to explore the link between HIIE-induced changes in these two variables.

2.5.2 Aerobic exercise and corticospinal excitability

Research has shown that aerobic exercise (AE) is an effective mechanism to increase CSE and to enhance neuroplasticity (McDonnell et al., 2013; Singh and Staines, 2015). For decades, stationary biking has been used in the rehabilitation of locomotor skills after neurological injury, as pedaling has been shown to improve lower extremity motor function, and to have a beneficial role in gait retraining (Raasch and Zajac, 1999; Fujiwara et al., 2005; Yamaguchi et al., 2012). Yamaguchi et al. (2012) examined the effect of pedaling on cortical excitability and found that a short bout of MICE (7 min of pedaling exercise performed at 5Nm and 60rpm) decreased intracortical inhibition (specifically, SICI) in the leg area of the motor cortex.

In addition to the role exercise plays in modulating M1 excitability in working muscle cortical representations, it has been shown that AE can have a generalized effect on CSE, extending to muscles not involved in the exercise (Takahashi et al., 2011; Singh et al., 2014). These finding are particularly advantageous in stroke research, as upper limb recovery after stroke is often incomplete and longer compared to the lower limb. The longer recovery time makes it difficult to engage the paretic upper limb in exercise to prime the brain prior to task specific rehabilitation.

Takahashi and colleagues (2011) examined the effects of strenuous lower limb exercise on CSE in non-exercised upper limb muscles and found large effects on CSE and SICI in the non-exercised muscles. In this study, individuals performed fatiguing intermittent leg press exercise, and paired-pulse TMS was used to examine the effects of the exercise on non-exercised muscles in the arm (first dorsal interosseous muscle: FDI; and biceps brachii muscle: BB). This group found that MEPs were elevated in the non-exercised FDI and BB during short rest periods between the exhaustive leg press exercise, suggesting a spreading of facilitation between cortical areas controlling exercised and non-exercised muscles with proximal M1 representations (Takahashi et al., 2011).

These findings of extension of altered CSE to the non-exercised muscle representation have also been observed in moderate-intensity continuous aerobic exercise (Singh et al., 2014). Research by Singh et al. (2014) found that a single session of moderate-intensity stationary biking can modulate excitability in non-exercised upper limb muscles. Singh and colleagues (2014) used single-pulse TMS to examine changes in stimulus-response curves, and paired-pulse TMS to assess ICF, SICI, and LICI in the extensor carpi radialis muscle following a single 20-min session of MICE (performed at

70% of their age-predicted HR_{max}). Their study results showed that there were no significant differences in MEP amplitudes pre- and post-exercise intervention, indicating that the resting motor threshold of the hand region of M1 was not modulated by cycling exercise (as shown in Figure 5). Singh et al. (2014) did, however, observe immediate and sustained (persisting at 30 min after exercise completion) increases in ICF (Figure 6A), and decreases in SICI (Figure 6B). The group also measured changes in LICI from pre to post exercise, however these changes were not statistically significant (Figure 6C). Singh et al. (2014) proposed that the observed changes in SICI and ICF may facilitate the induction of experience-dependent plasticity.

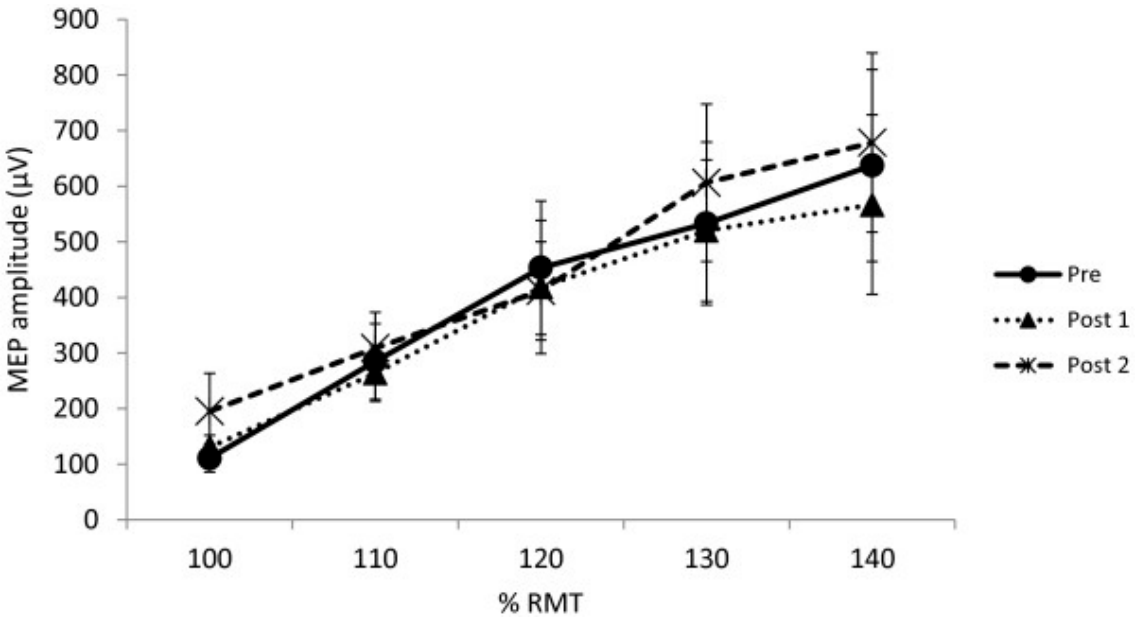


Figure 5. S-R curves pre- and post-exercise in response to stimulation at increasing percentages of RMT. Post 1: immediately after exercise. Post 2: 30 min after exercise (n=12). Bars represent SEM. *Retrieved from Singh et al., 2014.*

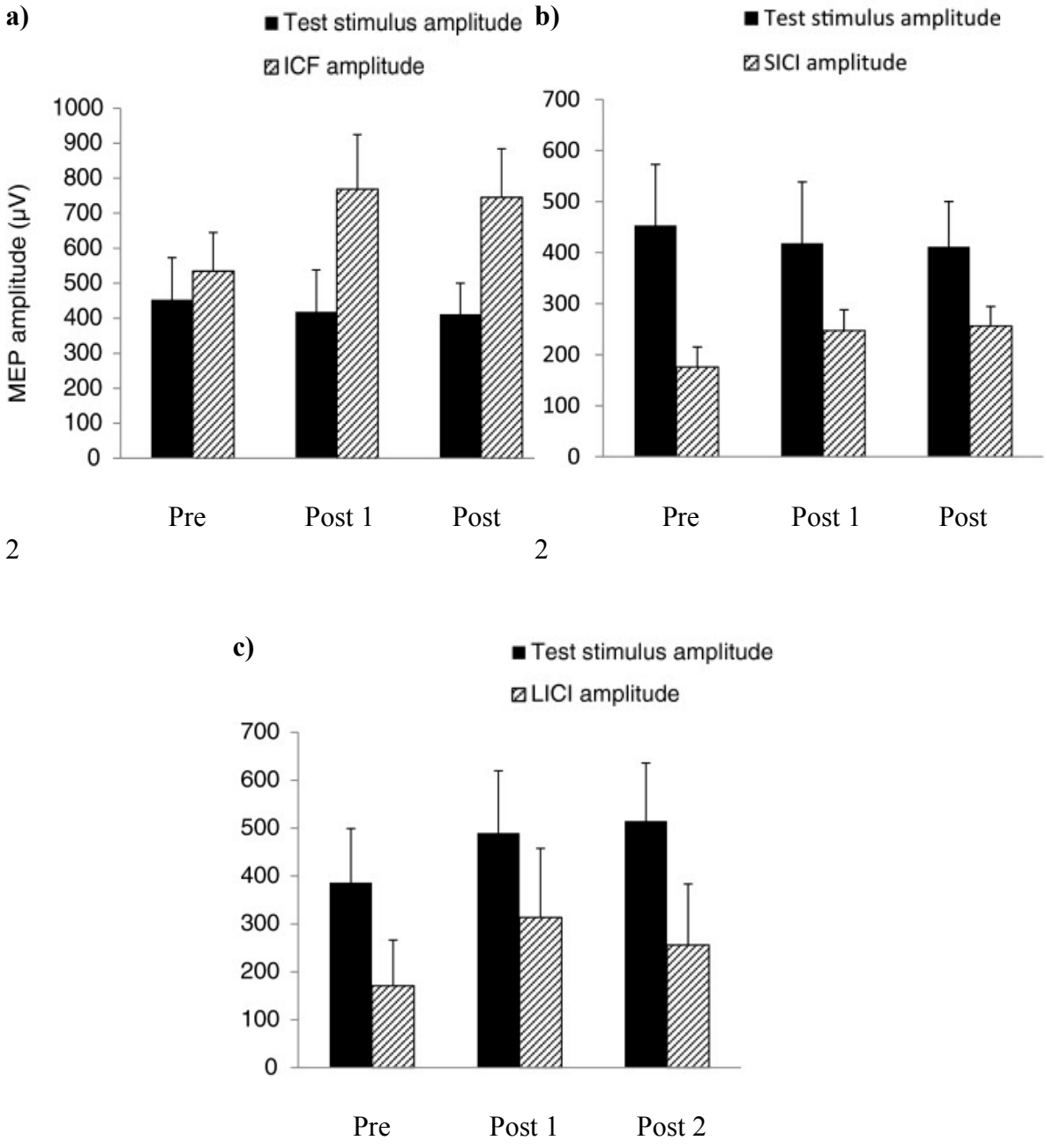


Figure 6. Induction of ICF (A), SICI (B), and LICI (C) across all participants (n = 12, 11, 11, respectively). Unconditioned single pulse amplitudes at 120% RMT (black bars) are compared to conditioned stimulus amplitudes (striped bars). *Modified from Singh et al. 2014.*

2.5.3 Effects of HIIE on Corticospinal Excitability

Recent research has focused on the effects of HIIE on CSE. A study by Mang and colleagues (2014) used single-pulse TMS to test changes in CSE evoked by a paired associative stimulation (PAS) paradigm. PAS is designed to induce LTP-like effects in the primary motor cortex by pairing electrical stimulation of the peripheral nerve associated with a given hand muscle (e.g., ulnar nerve for the first dorsal interosseous) with TMS applied several ms after to the cortical representation of that muscle in M1. In the Mang study, PAS was performed on two separate days in each participant; once after 20-min of HIIE on a stationary bike, and once after a 20-min rest period (Mang et al., 2014). Stimulus-response (S-R) curves were generated using the same stimulation site and intensities immediately pre-PAS (beginning within 5-min after rest/exercise) and post-PAS (beginning within 2-min after PAS). Mang and colleagues found that the slope of the S-R curves showed significantly larger increases when PAS was preceded by aerobic exercise (59.8% increase) compared with rest (14.2% increase, $P = 0.02$) (Figure 7). This finding reveals that a single bout of HIIE increases the CSE of the representational area of a non-exercised upper limb in M1 (Mang et al., 2014). The HIIE protocol used in the study by Mang et al. (2014) consisted of three 3-min blocks of high-intensity cycling performed at 90% of the participants maximal power output, interspersed with 2-min of low-intensity cycling at 50W. Participants were asked to maintain a pedaling cadence greater than 70rpm throughout.

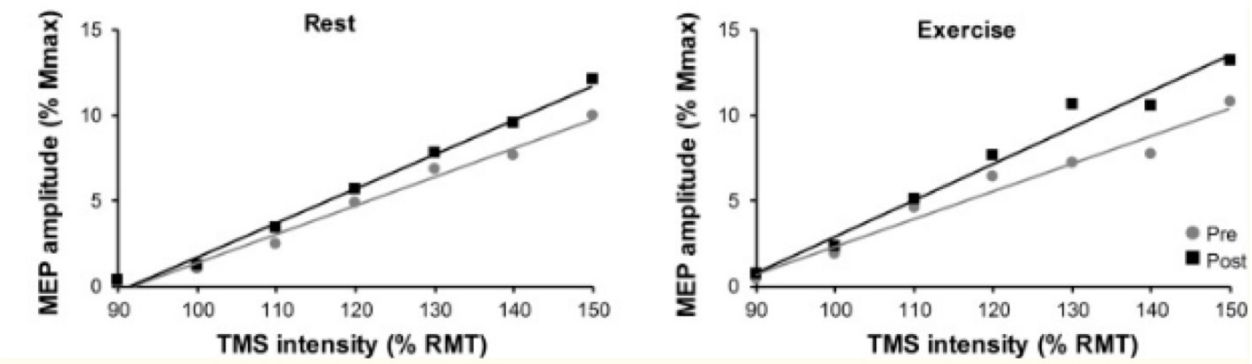


Figure 7. Stimulus-response curves with MEP amplitudes averaged across the group for each TMS intensity pre-PAS and post-PAS, for the rest and exercise conditions. *Modified from Mang et al. 2014.*

2.5.4 Aerobic Exercise and Learning

The benefits of exercise on cognitive and motor skill processes are becoming more widely known (Gomez-Pinilla and Hillman, 2013; Taubert et al., 2015). Despite significant differences in study designs, exercise intervention protocols, and learning tasks used, the literature surrounding exercise as a strategy to facilitate motor learning is beginning to grow. A solid understanding of the effects of exercise on motor skill learning is necessary to inform the development of future exercise interventions for rehabilitation.

Several studies have examined the effects of AE on motor skill performance and learning. It is thought that distinct phases of motor skill learning can be facilitated depending on the timing of a bout of exercise in relation to skill practice. For example, engaging in exercise before task practice is thought to affect skill acquisition and encoding processes, by creating a neural environment conducive to neuroplasticity (Mang et al., 2013), or by simply increasing levels of arousal (Taubert et al., 2015). The mechanisms through which exercise "primes" the brain for neuroplasticity will be discussed below. It is thought that exercising prior to task practice could also affect the

consolidation processes as well, as the neurobiological effects of exercise may endure beyond task practice (McGaugh, 2006; Francisco, 2016). Exercising after task practice can influence only the consolidation phase of learning (Roig et al., 2013).

2.5.5 Effect of HIIE on Motor Learning

Roig and colleagues (2012) explored the effects of HIIE on skill acquisition and motor learning and examined the role of exercise timing. To examine the interaction of exercise timing and motor learning, participants practiced a motor task either before or after a bout of HIIE, or after rest. This study used the same HIIE protocol described above, used in the study by Mang et al. (2014), consisting of three 3-min blocks of high-intensity cycling performed at 90% of the participants maximal power output, interspersed with 2-min of low-intensity cycling at 50W. Motor skill acquisition was assessed during practice and retention was measured 1 hour, 24 hours and 7 days after practice.

Motor skill learning was assessed using a visuomotor accuracy-tracking task (VAT) (Roig et al., 2012). To perform the VAT, subjects were seated in front of a computer monitor (Figure 8A), with their right forearm strapped to an arm support and their hand wrapped firmly around a handle (Figure 8B). The handle was equipped with a potentiometer, used to gauge wrist flexion and extension. Two computer windows were presented to the participant; the first window contained a double sine wave curve that the participant was instructed to track by applying wrist extension and flexion isometric contractions (Figure 8C). The second window provided feedback on performance by

displaying a series of white dots, which represented the distance between the target and the line drawn by the participant (Figure 8D) (Roig et al., 2012).

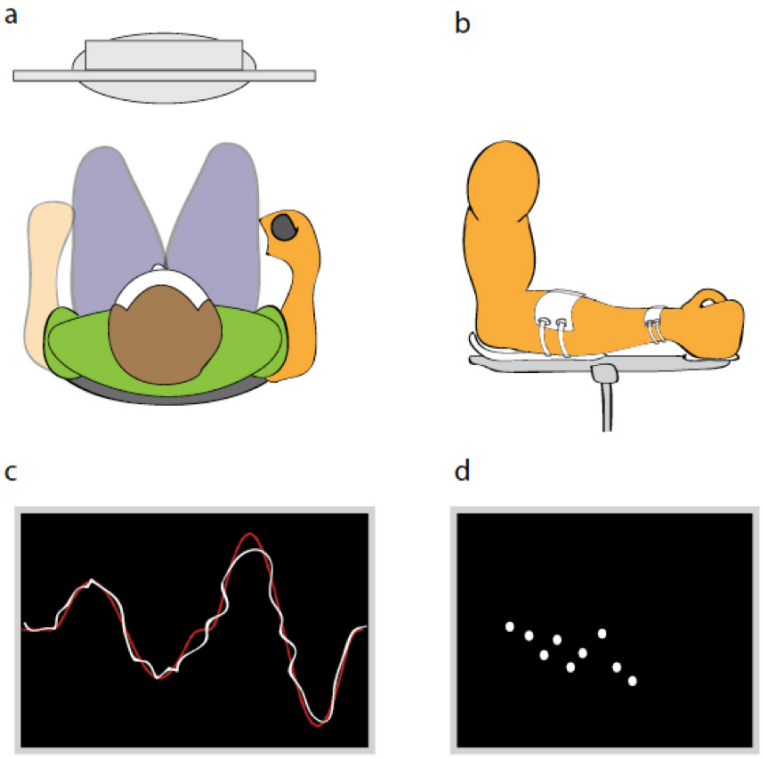


Figure 8. The visuomotor accuracy-tracking task (VAT) used by Roig et al. (2012). (A) Subjects were seated in front of a computer screen with the right forearm placed strapped in an arm support. (B) The forearm was positioned in a neutral semi-prone position, while the hand grasped the handle. (C) The main application window displayed a target consisting of a fixed double sine wave curve (red) that participants had to track with the torque signal (white). (D) The second window provided visual feedback on the performance of the VAT task by displaying a series of white dots on a coordinate axis. *Retrieved from Roig et al., 2012.*

Roig and colleagues (2012) found that there were no significant differences among groups (exercise before VAT, exercise after VAT, and non-exercising control) in the rate of motor skill acquisition, or short-term retention of the motor skill, assessed 1 hour after practice. However, they did observe that both exercise groups showed significantly better retention of the motor skill than the controls at 24 hours and 7 days

after practice (Roig et al., 2012). When comparing retention of the motor skill between exercise groups, Roig and colleagues (2012) found that the participants that exercised after VAT practice showed significantly better retention of the motor skill 7 days after practice. (The current thesis is part of a larger study examining the effects of HIIE on motor skill learning. While the focus of the current thesis was to investigate the effects of HIIE on motor learning when HIIE is performed prior to task practice, a second study group consisting of 15 participants will perform HIIE after task practice. It is the intention of the authors to report the findings from the two exercise groups (i.e. HIIE before task practice, and HIIE after task practice) together, in the future.)

Thomas and colleagues (2016) directly compared the effects of moderate- and high-intensity exercise on motor learning. In this study, exercise was performed after task practice, and motor learning was assessed using the same VAT described above (Roig et al., 2012). The exercise protocols were similar to those used in previous studies by Roig et al. (2012); participants completed a 4-min warm-up, followed by three intervals of 3-min duration on a cycle ergometer separated by a 2-min active recovery interval. The 3-min intervals were performed at 45% PO_{max} for moderate-intensity exercise and 90% PO_{max} for high-intensity exercise. The active recovery intervals were performed at at 25% PO_{max} for moderate-intensity exercise and 60% PO_{max} for high-intensity exercise (Thomas et al., 2016) From baseline (B1) to the end of task acquisition (B5), all groups showed similar skill improvement (Figure 9A; Thomas et al., 2016). In this study, both exercise intensities increased motor memory consolidation compared to rest, however changes were greater after high-intensity exercise than moderate-intensity (Figure 9B; Thomas et al., 2016).

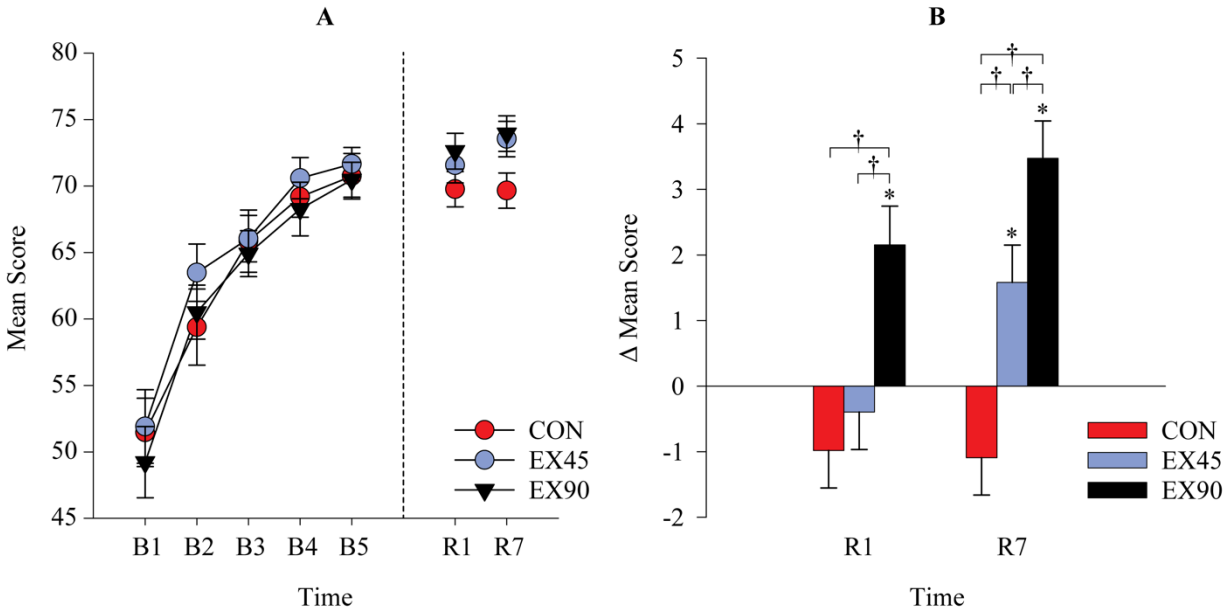


Figure 9. Performance scores on the VAT in the Thomas et al. (2016) study examining the role of exercise intensity (CON = non-exercising control group; EX45 = moderate-intensity exercise group; EX90 = high-intensity exercise group) on motor skill consolidation. (A) Mean scores (± SEM) in the VAT during acquisition blocks 1 to 5 (B1-B5). (B) Changes in means scores (± SEM) in the VAT from the end of skill acquisition (B5) to 1d retention test (R1) and 7d retention test (R7). † Significant between-group difference ($P < 0.05$). *Retrieved from Thomas et al. 2016.*

2.5.6 Neurobiological components of HIIE and learning

Several studies have shown that AE, and HIIE in particular, can be used to improve motor learning (Roig et al., 2012; Statton et al., 2015; Thomas et al., 2016). A study conducted by Skriver and colleagues (2012) examined the potential biochemical mechanisms underlying the observed increases in learning associated with HIIE. While there has been some research into the potential biochemical links between high-intensity exercise and learning in animals (e.g. de Almeida et al., 2013; Morland et al., 2017), the scientific literature surrounding this link in humans has been much less extensive. The focus of this section will be on the work done by Skriver et al. (2012) to examine the

neurobiological components of HIIE and learning, as the HIIE protocol used by this group is very similar to the protocol used in the present project; it can therefore be proposed that similar neurobiological mechanisms are at play.

Skriver et al. (2012) sought to examine the effect of exercise on blood concentrations of brain-derived neurotrophic factor (BDNF) and lactate. Blood samples were drawn when the participant arrived at the laboratory to gather information on baseline concentrations of the above molecules for each participant. The participant then engaged in 10 trials of the VAT used to assess motor skill learning; these trials served as a measure of their baseline performance level. Following the baseline learning task, subjects either engaged in HIIE (test group) or bed rest (control group). Blood samples were then collected again immediately after the 20-min of HIIE or rest. Participants then performed three 5-minute blocks of practice of the learning task. Blood samples were collected after each block of practice, giving the experimenters blood samples from 5-, 10- and 15-min after exercise.

Motor skill acquisition was assessed by retention tests performed 1 h, 24 h, and 7 days after practice. Skriver and colleagues (2012) found that the HIIE group showed better performance than the control group; differences in retention were significant in the tests performed at 24 h, and 7 days after practice. However, differences between groups in test performance 1 h after practice did not reach statistical significance. Effects of exercise on learning are thought to be predominantly regulated by BDNF and lactate (Cotman et al., 2007). Differences between groups in the concentrations of BDNF and lactate were examined at each time point that blood samples were collected. These differences will be discussed below.

2.5.6.1 The role of brain-derived neurotrophic factor on exercise and learning

BDNF is a member of the neurotrophin family of secretory polypeptides that regulate growth and differentiation in the developing nervous system and continue to shape the structure and function of neural circuits throughout life (Park and Poo, 2012). BDNF has been shown to play a critical role in synaptic plasticity and memory-processing in the adult brain (Tyler et al., 2002; Bekinschtein et al., 2007). In particular, BDNF has been shown to induce long-term potentiation (LTP) in the hippocampus, a form of synaptic plasticity thought to underlie learning (Whitlock et al., 2006; Bekinschtein et al., 2008).

Evidence from both animal and human studies supports the idea that BDNF plays an essential role in mediating exercise-induced benefits in learning and plasticity (Cotman et al., 2007). Research by Gomez-Pinilla et al. (2008) examined the influence of exercise on the expression of BDNF in rats using a pharmacological blockade of BDNF expression. This study showed that blocking the action of BDNF in exercising animals abolished exercise-induced enhancement of learning acquisition (evaluated by the Morris water maze test, used to test spatial memory acquisition and retention in animals) (Gomez-Pinilla et al., 2008). Human-based studies have shown that peripheral BDNF concentration was significantly elevated following acute aerobic-exercise (Griffin et al., 2011; Cho et al., 2012; Huang et al., 2014), and research by Skriver et al. also observed an increase in total BDNF concentration after high-intensity exercise, but differences between their exercising participants and non-exercising control group were not significant. However, Skriver and colleagues (2012) did report that higher concentrations of BDNF correlated with better retention 1 h and 7 days (but not 24 h) after practice.

There is debate in the literature as to whether the concentration of BDNF in the blood is reflective of the expression of BDNF in the brain, and even lingering debate as to whether BDNF can cross the blood brain barrier. BDNF appears in higher concentrations in the brain but is also present in the bloodstream; the molecule derives from platelets and circulates in the blood plasma and serum in addition to being produced in the brain tissue (Yamamoto and Gurney, 1990; Rosenfeld et al., 1995). There have been reports that BDNF can cross the blood-brain barrier (Pan et al., 1998), and studies in animals have reported positive correlations between BDNF protein levels in the periphery and the brain (Karege et al., 2002; Klein et al., 2011), suggesting that peripheral concentrations of the molecule may be used to make inferences about levels in the brain (Suliman et al., 2013). However, other studies have challenged the finding that BDNF can cross the blood brain barrier (Pardridge and Sakane, 1998), and others still have not supported the correlation between peripheral and brain BDNF levels (Kyeremanteng et al., 2012).

2.5.6.2 The role of lactate in exercise and learning

Lactate is another metabolite that has been shown to play an important role in neuronal function (Aubert et al., 2005; Costalat et al., 2006). For a long time, cerebral energy metabolism was considered to be an aerobic process, meaning that glucose was thought to be the principle metabolic substrate used to produce energy and drive the cellular processes of the brain (Siesjo, 1978). During this period, elevated levels of cerebral lactate were thought to be associated with damage to the brain, such as stroke (Costalat et al., 2006). However, research has since shown that lactate plays an important role in normal brain function (Prichard et al., 1991; Hu and Wilson, 1997). In the brain, astrocytes can supply neurons with lactate as an energy substrate, through the process of

astrocytic glycogenesis (Brown et al., 2004; Newman et al., 2011). This process is thought to be essential to the maintenance of LTP and memory processing (Newman et al., 2011; Suzuki et al., 2011).

Another very important source of lactate that acts in neuronal processes originates elsewhere in the body, as the by-product of muscle glycolysis (McArdle et al., 2010). In fact, muscles generate the greatest amount of lactate in the body (Andersen et al., 2013; Riske et al., 2016), and it is well known that peripheral blood lactate levels increase significantly with intense exercise (Astrand et al., 2003; McArdle et al., 2010). Peripheral lactate is moved between organs and across the blood-brain barrier with monocarboxylate transporters, where it can be used by the brain as a source of energy. Skriver et al. (2012) showed that higher concentrations of lactate immediately after exercise were associated with better acquisition, and that lactate concentration correlated with better motor skill retention at 1 h, 24 h and 7 days after practice. Other research groups have shown that elevated blood lactate levels are associated with increased excitability in M1 after acute exercise (Ferris et al., 2007; Coco et al., 2010).

As mentioned above, the present study and the research done by Skriver and colleagues used similar HIIE protocols. However, while the two protocols are similar, they are not identical. Primarily, the recovery interval used in research conducted by Skriver and colleagues was performed at 50W, whereas the recovery interval of the protocol used in the present study was performed at 50% of the individual participant's PO_{max}. This recovery period at 50% PO_{max} was selected as this exercise intensity has been shown to facilitate optimal lactate clearance from the working muscles (Riganas et al., 2015). Therefore, while the results found by Skriver et al. (2012) form a useful

foundation, it should not be assumed that identical or equivalent biochemical mechanisms are at play following engagement in the two HIIE protocols.

The reasoning behind this adjustment in protocol was twofold: 1) As stated above, increased intensity of the recovery interval will cause increased blood flow to the working muscles during this period, aiding in lactate clearance. This will allow the participants to recover before engaging in the subsequent high-intensity interval. 2) Additionally, we sought to prescribe the low-intensity recovery interval based each participant's PO_{max}, to ensure that the recovery interval was not easier for some individuals than others, relative to their PO_{max} (e.g. if Participant A achieved a PO_{max} of 150 and Participant B achieved a PO_{max} of 200W, then a recovery interval of 50W would mean Participant A was exercising at 33% of their PO_{max} while Participant B was exercising at 25% of their PO_{max}).

2.5.7 Energy systems involved in HIIE

During exercise, the body depends on three energy systems: the anaerobic alactic system, the anaerobic lactic system, and the aerobic system (Figure 10) (McKee and McKee, 2013). The anaerobic alactic system is also known as the adenosine triphosphate (ATP) – creatine phosphate (CP) system, as this energy system relies on these high energy phosphate molecules. ATP and CP are stored in limited quantities within muscle cells, and can therefore only used to fuel short, powerful bursts of energy. During short, intense physical activity, stored ATP can be used as an immediate source of energy (energy is released during the hydrolysis of ATP into adenosine diphosphate (ADP) and inorganic phosphate). The regeneration of ATP in this energy system is dependent on the

transfer of a phosphate group from stored CP to ADP (McKee and McKee, 2013). The anaerobic alactic system is estimated to provide energy for up to 10 sec of high intensity exercise (Gastin, 2001).

The anaerobic lactic system is also referred to as fast glycolysis. This is the predominant energy system used to fuel high-intensity exercise bouts lasting from approximately 30 sec to 2 min (Gastin, 2001). During glycolysis, glucose is broken down to pyruvate through a series of chemical reactions. For every molecule of glucose converted to pyruvate through fast glycolysis, two molecules of useable ATP are produced (McKee and McKee, 2013). Therefore, although this energy system produces energy relatively quickly, very little energy (ATP) is produced. As indicated in the name, lactate is a by-product of fast glycolysis under anaerobic conditions; through a series of reactions, pyruvate is converted to lactate.

The aerobic energy system is dependent on oxygen and is responsible for most of the cellular energy produced in the body. This system relies on oxidative phosphorylation and produces approximately 18 times more ATP than anaerobic glycolysis (Campbell and Reece, 2005). The aerobic system has an enormous capacity to produce energy, but it is limited by its inability to produce energy quickly (Gastin, 2001). Activities that require a continuous, sustained effort rely on the aerobic system. Research also suggests that during relatively long bouts of high-intensity exercise (e.g. a 3-min interval), both anaerobic and aerobic energy systems are at work. Therefore, there is a considerable contribution of energy by the aerobic system during extended bouts of high intensity exercise.

As the high-intensity intervals performed in the current study are 3-min in duration and are performed at 90% PO_{max}, both anaerobic and aerobic energy systems would be engaged during the HIIE protocol. Therefore, the type of HIIE performed in the present study should be considered intervals of high-intensity aerobic exercise.

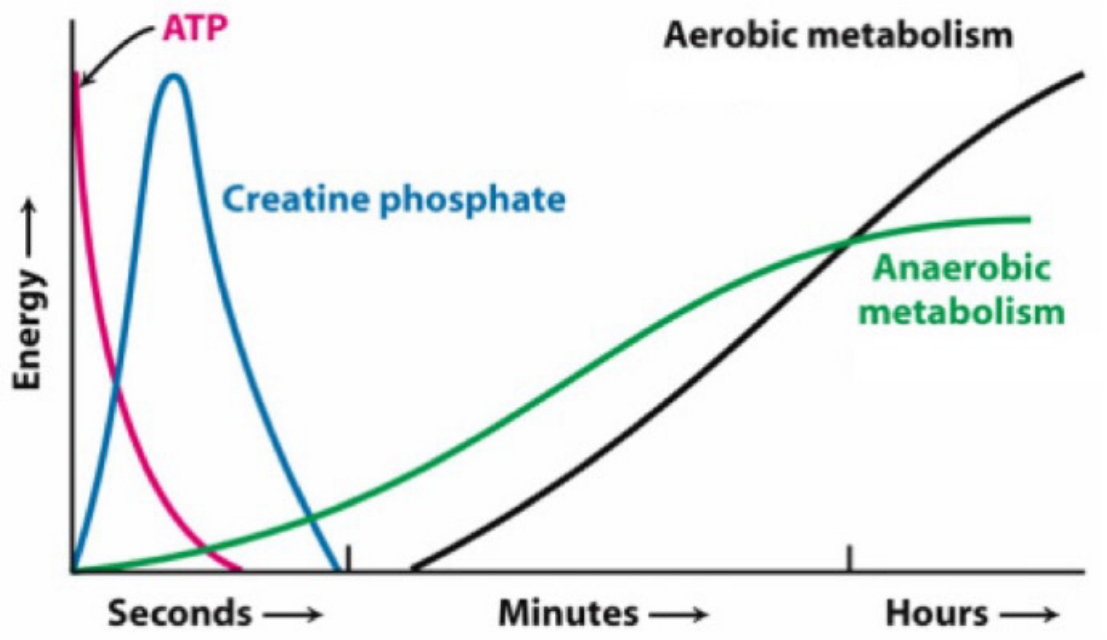


Figure 10. Metabolic energy systems and their contribution to total energy output during all-out exercise of different durations. *Modified from Berg et al. 2012.*

CHAPTER 3: OBJECTIVES AND HYPOTHESES

In the context of using HIIE to increase corticospinal excitability and to enhance motor learning, our objectives and related hypotheses included:

Objective 1: To determine if engaging in HIIE leads to increased CSE after exercise.

Hypothesis 1: Engaging in HIIE will result in an increase in CSE after exercise, as evidenced by:

- An increase in the area under the stimulus-response curve from pre-exercise to post-HIIE.
- Decreased short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI), and increased intracortical facilitation (ICF).

Objective 2: To determine if performing HIIE prior to engaging in an established motor learning paradigm will increase the effectiveness of complex skill acquisition, compared to non-exercising individuals.

Hypothesis 2: Engaging in HIIE prior to the motor learning task will result in optimized motor learning (relative to those who do not perform exercise), as evidenced by:

- A significant main effect of group membership (HIIE vs. non-exercising control) on error in motor skill performance.

- Participants who perform HIIE prior to engaging in the motor learning task will display lower error scores than the control group in both the random and repeated trajectories, when motor skill performance is measured at the retention time point.

Objective 3: To determine if there is a correlation between CSE after HIIE and the extent of motor learning following HIIE.

Hypothesis 3: There will be a significant, positive relationship between motor learning (the change in motor task performance from the beginning of CME task practice to the retention test) and change in various measures of CSE from pre- to post-HIIE levels, as evidenced by:

- A significant positive correlation between changes in motor task performance (operationalized as the change in learning in random and repeated shapes from task familiarization to retention test) and changes in CSE (i.e. changes in AUC, ICF, SICI, and LICI).

CHAPTER 4: METHODS

4.1 Participants

4.1.1 Inclusion and Exclusion of Participants

The HIIE study group included 15 individuals (7 female), aged 19–28 years (average age 22.8 ± 2.8 years), with no self-reported history of neurological disorders. Additional exclusion criteria for study participation included having respiratory disorders, hypertension or other cardiovascular diseases that would preclude participating in exercise, having any contraindications to TMS (discussed below), or smoking.

4.1.2 Participant Recruitment

Prior to recruitment, the Research Ethics Board at Dalhousie University approved the research protocol (REB # 2017-4266). Participants were recruited through word of mouth and via advertisements (see Appendix 1) placed around Dalhousie University. Advertisements included the contact information of the investigators. Participants had the opportunity to respond voluntarily to the study investigator to indicate their interest.

4.2 Measures Regarding Participant Characteristics

When an individual expressed interest in participating in the study, they were contacted by the study coordinator and sent 1) an Information Letter (which included a description of the procedures involved in the study, inclusion and exclusion criteria, and possible side effects associated with participation; Appendix 2); 2) a Physical Activity Readiness Questionnaire (PAR-Q; Appendix 3); and 3) a TMS screening form (Appendix

4). Potential participants were asked to complete the screening forms prior to study enrolment in order to self-screen to determine eligibility. The study investigator followed up with each potential participant and contacted those individuals who reported as eligible to schedule the first study session.

4.2.1 Measure Regarding Contraindications to TMS

Participants were screened for contraindications to TMS using a standard TMS screening form (Rossi et al., 2009) (Appendix 4). Participants were excluded from the study if they answered 'yes' to any of the first 8 questions on the screening form, if they indicated that they had any problems with TMS or MRI in the past, if they had metal in their body that made them unsuitable for TMS (specifically metal implanted in their brain or skull, including clips or other brain implants), or if they were taking any medications that could affect brain excitability (e.g. certain medicines used to treat depression, anxiety, and psychotic conditions).

4.2.2 Measure Regarding Handedness

In the laboratory, prior to session 1, the Edinburgh Handedness Inventory was used to assess the dominance of the person's right or left hand in daily activities (Oldfield, 1971) (Appendix 5). The hand deemed the individual's dominant hand was used to perform the CME task.

4.2.3 Measure Regarding Suitability to Engage in Exercise

Participants were screened for their suitability to engage in exercise using the PAR-Q (Appendix 3). The PAR-Q was created by the Canadian Society of Exercise Physiology to determine a person's suitability for exercise. The PAR-Q includes seven questions designed to identify individuals for whom physical activity may be ill advised. If participants answered 'yes' to any of the questions on the PAR-Q he/she was deemed ineligible for the study and advised to consult with a physician to seek approval prior to partaking in physical activity.

4.2.4 Measure Regarding Physical Activity Level (secondary measure)

Physical activity levels of the participants were determined using the International Physical Activity Questionnaire (IPAQ) short form (Appendix 6). The IPAQ is a self-report questionnaire that asks participants to provide information on time spent walking and engaging in vigorous- and moderate-intensity activity and in sedentary activity in the previous seven days. The IPAQ has undergone extensive testing, which has shown it to be a valid and reliable instrument to measure levels of physical activity (Craig et al., 2003).

The IPAQ was used to categorize participants in one of three categories of physical activity: Category 3 – High, Category 2 – Moderate, and Category 1 – Low (The IPAQ Group, 2015; See Appendix 7 for a detailed IPAQ scoring protocol).

As indicated above, the IPAQ was considered a secondary measure in the present study. Information gathered from the administration of the IPAQ was used to characterize the study population but was not used for analysis. The IPAQ was used to determine the

average level of physical activity of study participants, enabling investigators to better interpret the study findings.

4.2.5 Measure Regarding Health History (secondary measure)

Participants were asked to complete a Health History Questionnaire (Appendix 8). On this questionnaire, participants self-reported their height and weight. These measures were then used by the study investigators to calculate body mass index (BMI). Participants were also asked about smoking habits in the previous six months. Participants who indicated that they were a regular smoker at any point in the last six months were excluded from participating in the study, as individuals who smoke are at increased cardiovascular risk (Seron et al., 2014), and high intensity physical activity can increase the risk of adverse cardiac events in susceptible persons (Buttar et al., 2005; Rognmo et al., 2017).

On the Health History Questionnaire, participants were also asked to list the types of exercise in which they partake (e.g. running, swimming, weight lifting, etc.). The information acquired via the Health History Questionnaire was not used for analysis, but rather allowed the study investigators to contextualize their results. This was important, as previous research has shown that there may be differences in exercise-induced changes to CSE in individuals habituated to skill training, endurance training, or strength training (Adkins et al., 2006; Kumpulainen et al., 2015).

4.3 Experimental Procedures

4.3.1 Overview of Testing Sessions

The study consisted of 5 testing sessions, each of which required the participant to engage in HIIE. Sessions 1 and 2 were attempted to be conducted within one week of each other, as were sessions 2 and 3. However, due to participants' schedules this time frame was exceeded in 3 participants (Participant numbers 3, 10 and 15). Sessions 3-5 (motor learning sessions) were required to take place within a period of 5 days, with one day of rest between each session. This 5-day period was required to avoid degradation of learning between sessions.

4.3.1.1 Session 1

During session 1, participants completed an informed consent form (Appendix 9), a Physical Activity Readiness Questionnaire (PAR-Q; Appendix 3), a TMS screening form (Appendix 4) to confirm eligibility for participation, and the Edinburgh Handedness Inventory (Appendix 5) to assess the dominance of their right or left hand in daily activities. Participants were also asked to complete the International Physical Activity Questionnaire (IPAQ; Appendix 6) to categorize their level of physical activity (described above), and to list the types of physical activity in which they partake, as part of the Health History Questionnaire (Appendix 8).

During session 1, participants performed a maximal graded exercise test on a cycle ergometer to determine their PO_{max} (Figure 11). The participant's PO_{max} was then

used to determine the exercise intensity for the subsequent exercise session (described below). This first session took approximately 1 hour to complete.

4.3.1.2 Session 2

The second session examined the effects of HIIE on CSE (Figure 11). In this second session, TMS was performed at three time points to obtain measures of CSE: TMS was performed 1) before HIIE to collect information on the participant's baseline levels of CSE; 2) directly after exercise to examine the immediate effects of HIIE on CSE; and 3) 30 min after engaging in HIIE, to examine any lasting changes in excitability. An outline of the HIIE protocol is described below. This session took approximately 2 hours to complete.

4.3.1.3 Sessions 3, 4 and 5

The third, fourth and fifth sessions were required to take place within a period of 5 days, with one day of rest between each session. Sessions 3 - 5 were identical. During these sessions, participants performed HIIE, directly followed by engagement in the motor learning task (Figure 11). An outline of the HIIE protocol and the motor learning task are described below.

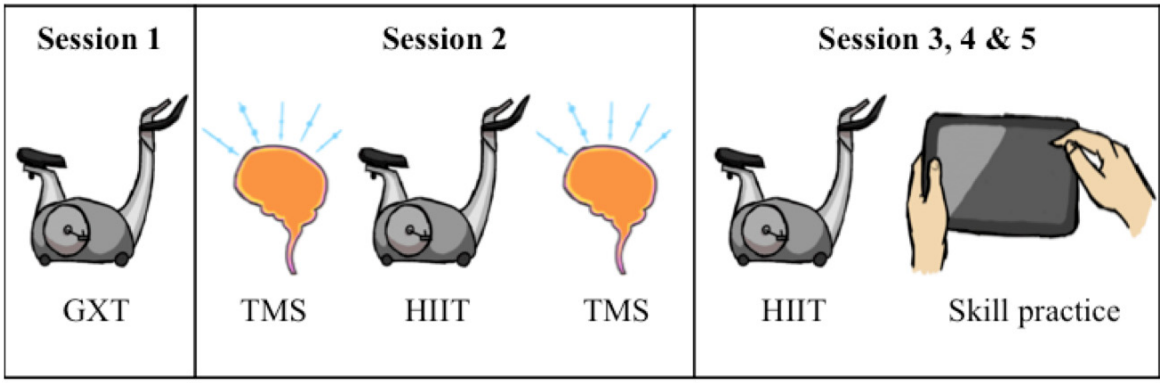


Figure 11. Overview of experimental procedures during study sessions.

4.3.2 Participant Instructions

As participants would be exercising during each study session, they were asked to wear or bring comfortable clothing (shorts and t-shirt or other appropriate exercise apparel). Participants were able to use the locker rooms in the Laboratory for Brain Recovery and Function to change their clothing before and/or after the study sessions. Participants were also asked to refrain from consuming caffeine, heavy meals, and alcohol for at least 2 hours before testing, to avoid significant exertion or exercise on the day of testing, and to get adequate sleep (6-8 hours) the night before the test to ensure they were well rested.

4.3.3 Maximal Exercise Test

During Session 1, the participant performed a graded maximal exercise test (GXT) to determine his/her maximal power output. The GXT was completed on an upright stationary cycle ergometer (Lode Corival Ergometer, Lode B.V., the Netherlands). This ergometer was able to electronically modify resistance to maintain workload; to do this, an electromagnetic braking force adjusted the resistance if the participant increased or

decreased pedaling rate, in order to keep the power output constant. The ergometer was electronically controlled using Lode Ergometry Manager 10 (Lode B.V., the Netherlands), which adjusted the power output automatically.

The participant was asked to wear a wrist-mounted Mio heart rate monitor (Mio Global, Physical enterprises Inc., USA) during each study session. The Mio heart rate monitor measured and displayed heart rate (HR) in real time, and it was also synced with Wahoo Fitness iPad app (Wahoo Fitness L.C.C., USA) so that HR data could be recorded for subsequent analysis.

Throughout the GXT, participants were instructed to aim to maintain a pedaling cadence of approximately 75 revolutions per minute (rpm). Specifically, participants were instructed to stay within 5rpm of the targeted cadence, resulting in a target cadence range of 70-80 rpm. The target pedaling cadence for the GXT was set as 70-80rpm, as this was the target cadence selected for the HIIE protocol used in following test sessions. Failure to maintain a cadence of at least 70 rpm for a period greater than 10 s resulted in termination of the GXT. This requirement of a minimum pedalling cadence throughout the GXT and the HIIE protocol was line with previous studies that use PO_{max} to define HIIE protocol intensities (Roig et al., 2012; Ostadan et al., 2016).

It is important to maintain a constant cycling cadence during maximal exercise testing and subsequent exercise prescription, as variable pedaling cadences affect oxygen uptake, and alter the expected relationship between oxygen uptake and work rate (Cooper and Storer, 2001). Heart rate, stroke volume, cardiac output and blood pressure have been shown to increase with increased cadence, despite constant workload (Gotshall et al.,

1996). It was therefore important to set a fixed cycling cadence throughout the study to control for the hemodynamic changes associated with varying pedal cadence at a constant workload.

Throughout the GXT, participants were also instructed not to grip the handlebars. As an alternative, they were told to rest their arms comfortably by their sides or to rest their forearms against the handlebars. This was also done in subsequent study sessions, to avoid prolonged activation of the hand muscles that would be probed during TMS investigation. HIIE was prescribed based on each participant's PO_{max}. Therefore, it was important that the participant was able to achieve their PO_{max} at the same cadence at which they would be expected to pedal during subsequent test sessions, and that this PO_{max} could be achieved while the participant was assuming the same posture that would be required in subsequent test sessions (i.e., arms by their sides or forearms resting against the handlebars).

The GXT began with a 5-min warm-up period, during which participants cycled at a workload of 40 Watts (W). Following this 5-min warm-up, workload was increased by 20 W every minute until exhaustion, which was determined by the participant's inability to maintain a minimum pedaling cadence of 70-80 rpm, despite verbal encouragement. This GXT is designed to be short in duration; the test has been used previously (Lanzi et al., 2014; Lanzi et al., 2015), and typically ranges between 8 and 12 min in length.

The outlined maximal GXT protocol is also consistent with the recommendation made by Buchfuhrer et al. (1983) that work rate increments should be selected appropriately in order to attain maximal effort in approximately 10-min (±2 min). This

recommendation was made on the basis that longer tests waste time and supply no additional information. Additionally, longer maximal effort tests contribute to a participant's inability to achieve indicators of maximal effort (see below) due to decreased motivation, increased discomfort (e.g. seat discomfort on the cycle ergometer), increased body temperature, greater dehydration, or respiratory muscle fatigue (Buchfuhrer et al., 1983).

Participants were asked for their rating of perceived exertion (RPE) based on the Borg scale (Borg, 1982; Appendix 10) at the end of the warm up period, and with 10 s remaining in each block of increased workload during the test. The GXT was terminated if the participant experienced any of the indications listed in Table 1.

Table 1. General indications for stopping an exercise test. *From Pescatello et al. (2014)*.

General Indications for Stopping an Exercise Test in Low-Risk Adults
• Onset of angina or angina-like symptoms
• Shortness of breath, wheezing, leg cramps, or claudication
• Signs of poor perfusion: light-headedness, confusion, ataxia, pallor, cyanosis, nausea, or cold and clammy skin
• Failure of HR to increase with increased exercise intensity
• Participant requests to stop
• Physical or verbal manifestations of severe fatigue
• Failure of the testing equipment

Participants were asked to notify investigators when they believed they have approximately 1 min remaining in the test. A final measurement of RPE was made at this time. When the participant reached exhaustion, determined by the participant expressing that he/she was exhausted, or by his/her inability to maintain a minimum-pedaling cadence of 70 rpm, workload was reduced to 40W for a 5-minute cool down period. This cool-down period was included to bring the participant's HR back down to approximately resting level.

Maximal power output (PO_{max}) was defined as the workload (W) of the last full, one-minute block the participant was able to complete while maintaining a cadence of 70-80 rpm. PO_{max} was used to define the participant's prescribed workload for the HIIE protocol used in subsequent study sessions.

4.3.3.1 Qualifying Maximal Effort during the Graded Exercise Test

The GXT was terminated when the participant reached his or her symptom-limited maximal power output, or when the participant was no longer able to maintain a pedaling cadence of 70 rpm. Participants were asked to indicate which of the following symptoms lead them to end the test:

- Breathlessness
- Leg fatigue
- Breathlessness and leg fatigue
- General Fatigue
- Physical Discomfort

- Chest Pain
- Palpitations
- Dizziness
- Dry Mouth
- Other

The requirements for attaining maximal effort during graded exercise testing are variable in the literature. Heart rate (HR) is often used to qualify maximal effort; it is, however, used variously as a peak exercise HR > 85% (Borg, 1982) or > 95% (Katzel et al., 2001) of age-predicted maximum, or a HR within 5 beats per minute (bpm) (Paterson et al., 1999) or 10 bpm of age-predicted maximum (Howley et al., 1995).

Age-predicted maximum HR is based on the following equation described by Tanaka et al. (2001):

$$\text{Age-predicted } HR_{max} = 208 - (0.7 \times \text{age})$$

In addition to the various uses of HR to qualify maximal effort, it has been suggested that achievement of some percentage of HR_{max} is a problematic criterion in and of itself (Howley et al., 1995; Kolata 2001). There are substantial interindividual differences in HR_{max} among individuals of the same age (Tanaka et al., 2001). Both a meta-analysis and a laboratory-based study by Tanaka et al. (2001), carried out to determine a generalized equation for predicting HR_{max} in adults, found substantial variation across the entire examined age range, with standard deviations ranging from 7 to 11 bpm. Achievement of some percentage of HR_{max} is therefore not an ideal requirement

for maximal exertion, as individuals falling in the lower half of this distribution would not achieve their age-predicted HR_{max} even when working maximally, while those at the upper end of the distribution would achieve the same estimate while working sub-maximally.

Due to the variability in the literature and limitations mentioned, HR was not used to qualify maximal effort during the GXT. HR data will nonetheless be collected for each participant throughout the GXT, and it will be used to inform the investigator on the subject's ability to tolerate the maximal exercise test.

Borg's RPE (Appendix 10) is also frequently used as a marker of maximal effort. An RPE of at least 18 on the Borg scale at the final stage of exercise is often used as a criterion for maximal effort (Tanaka et al., 1997; Cress and Meyer, 2003). This criterion will also be used in the present study to qualify maximal effort.

4.3.4 High Intensity Interval Exercise Protocol

The following HIIE protocol was used during study sessions 2-5. HIIE was performed on the same cycle ergometer used for the GXT. Maximal power output (PO_{max}) determined from the GXT was used to inform prescription of cycling intensity for the participant's individualized HIIE protocol.

The HIIE protocol involved three, 3-min sets of high-intensity cycling, separated by 2-min of low intensity cycling. Participants performed a 5-min warm-up at 50W at the beginning of the session to elevate their HR. The high-intensity intervals required participants to cycle at 90% of their PO_{max} for 3-min. The low-intensity intervals

consisted of pedaling at 50% PO_{max} for 2 minutes. Upon completion of the third high-intensity interval, participants entered a 5-min cool down period, also at 50W. In total, this HIIE protocol required participants to exercise for 23 min. Participants were instructed to maintain a cadence between 70 and 80 rpm throughout the exercise session.

The outlined HIIE protocol was modified from research conducted by Mang et al. (2014, 2016). The HIIE protocol used by Mang et al. (2014) also included a 5-min warm-up at 50W, three 3-min sets of high intensity cycling, separated by 2-min of low-intensity cycling, and a 5-min cool down. Mang and colleagues defined high-intensity intervals as 90% of participant's maximal workload, and low intensity intervals as a standardized 50W. We chose to modify this protocol so that cycling intensity of the low intensity exercise interval was also based on the participant's PO_{max}. This was done to ensure that participants were all engaged in equivalent workloads relative to their maximal PO. The low-intensity exercise interval was set at 50% PO_{max} after exploration of the literature surrounding the influence of recovery exercise intensity on lactate clearance in the working muscles (Riganas et al., 2015).

As indicated above, throughout the HIIE sessions, participants were instructed not to grip the handlebars. As with the GXT, participants were told to rest their arms comfortably by their sides or to rest their forearms against the handlebars. This was done so that changes in CE within the hand muscle M1 representation could be attributed to the widespread effects of the HIIE protocol on motor cortex excitability, rather than prolonged activation of the studied muscle.

HR data and RPE on the Borg scale were collected during each bout of HIIE (sessions 2-5). This information was used to characterize performance and to inform the investigator on the participant's ability to tolerate the HIIE protocol. Participants were asked for their RPE (Borg, 1982; Appendix 10) at the end of the warm up period, and with 20-30 sec remaining in each interval.

4.3.5 Transcranial Magnetic Stimulation Protocol

TMS was performed during session 2 to assess CSE prior to engagement in HIIE, and again directly after and 30-min after the completion of the HIIE protocol.

To perform TMS, participants were asked to sit on a reclined chair, with their head resting comfortably in a headrest, and their right arm placed in a relaxed position on a pillow in their lap. TMS was delivered through a figure of eight coil (Magstim Double 70mm Alpha Coil) connected to a Magstim BiStim^2 system (The Magstim Company Ltd, UK). The Magstim BiStim^2 system consisted of two Magstim 200^2 units (The Magstim Company Ltd, UK) joined through a connecting module, allowing for paired-pulse TMS to be delivered though a single coil.

BrainSight neuronavigation system (Rogue Research Inc., Canada) was used in combination with a template MRI to position the TMS coil over the target motor region. The template MRI (MNI-152) is an averaged anatomical MRI that was derived from a sample of 152 neurologically healthy individuals from the Montreal Neurological Institute (MNI), in order to create an image of a brain that is meant to be representative of the population as a whole.

4.3.5.1 Co-registration

To configure the target position for stimulation, the participant's head was co-registered to the template MRI using the BrainSight neuronavigation software and a Polaris optional position sensor (Northern Digital Inc., Canada). The Polaris sensor contains two infrared cameras, emitters and associated electronics, and is connected to the BrainSight computer. The Polaris optical position sensor monitors the space in front of its cameras for trackers. Trackers associated with BrainSight are tools with three affixed retro-reflective markers (Figure 12). The retro-reflective markers are spheres that reflect infrared light emitted by the Polaris sensor emitters. The Polaris optical position sensor calculated the position and orientation of the tracker tool based on the information the position sensor receives from those markers. To perform the TMS protocol outlined below three trackers were used:

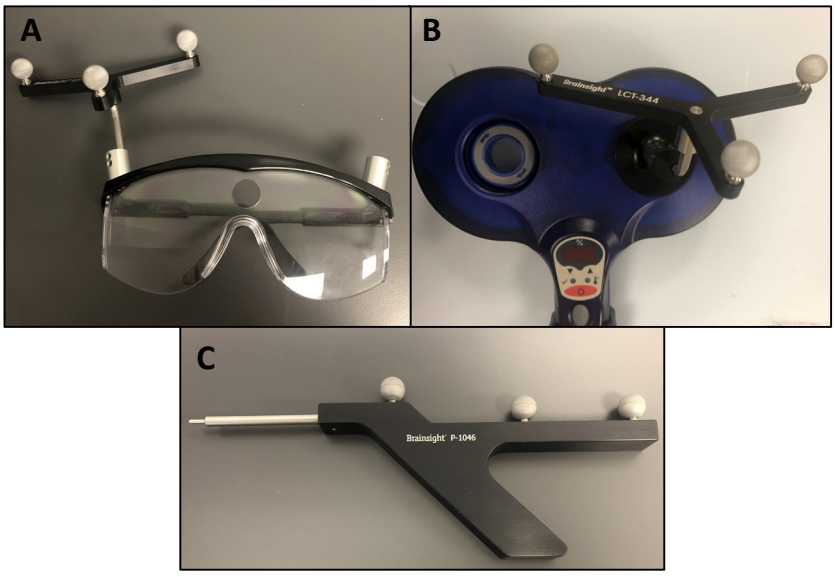


Figure 12. BrainSight trackers with retro-reflective markers in a pattern recognizable by the Polaris sensor. (A) Subject tracer. (B) Coil tracker. (C) Pointer tool.

To begin the TMS session, the participant was asked to wear glasses with an attached tracker, called the subject tracker. The subject tracker monitors the position of the subject's head. The participant's head was then co-registered to the template MRI by aligning three anatomical landmarks on the participant's head (nasion, left and right pre-auricular points) with the same anatomical landmarks on the template brain (obtained via surface reconstruction using the template MRI).

4.3.5.2 Localization of the Motor Hotspot

The target muscle for analysis was the right FDI and, therefore, stimulation targeted the left M1. The hand motor hotspot is commonly used in TMS practice. Anatomical and imaging studies have placed the hand representation within M1 in a region of the central sulcus called the "hand knob". (Yousry et al., 1997; Boroojerdi et al., 1999). The FDI was targeted as this muscle is heavily recruited during the motor task used in the current experiment (described below).

Prior to TMS mapping of M1, electrodes were placed on the FDI muscle. By measuring MEPs in response to stimulation at various grid points, the TMS operator was able to localize the hand motor hotspot, specifically the right FDI muscle representation in the left M1. Muscle activity of the right FDI muscle was collected using EMG. The EMG signal was acquired using self-adhering electrodes (1 x 3 cm; Q-Trace Gold; Kendall-LTP, USA) in a mono-polar configuration; one electrode was placed over the muscle belly of the FDI (approximately 1 finger breadth proximal to the 2nd metacarpal phalangeal (MCP) joint), and a second electrode was placed on the first phalanx of second digit. Identification of the FDI muscle was confirmed by asking the participant to

abduct his/her second digit while the experimenter resisted the movement and palpated the muscle. The EMG signal was sampled at 1000Hz with a bandpass of 25-100 Hz (1902 and Power 1401; Cambridge Electronics Design, UK) and stored for offline analysis.

To begin localization of the motor hotspot, the TMS coil was held over the left M1, in close proximity to the skull. The TMS coil was positioned with the coil handle pointed posteriorly, at an angle of approximately 45 degrees to the mid-sagittal plane. A series of 25 targets, arranged 5 x5 grid with 7.5mm spacing between targets, was then overlaid on the template brain, with the midpoint (location 2, 2) centered on the estimated location of the FDI muscle representation on the left M1 (Figure 13). To identify the motor hotspot of the right FDI, each target on the grid was stimulated to determine the spot that produced the highest amplitude MEPs for 5 out of 10 stimulations.

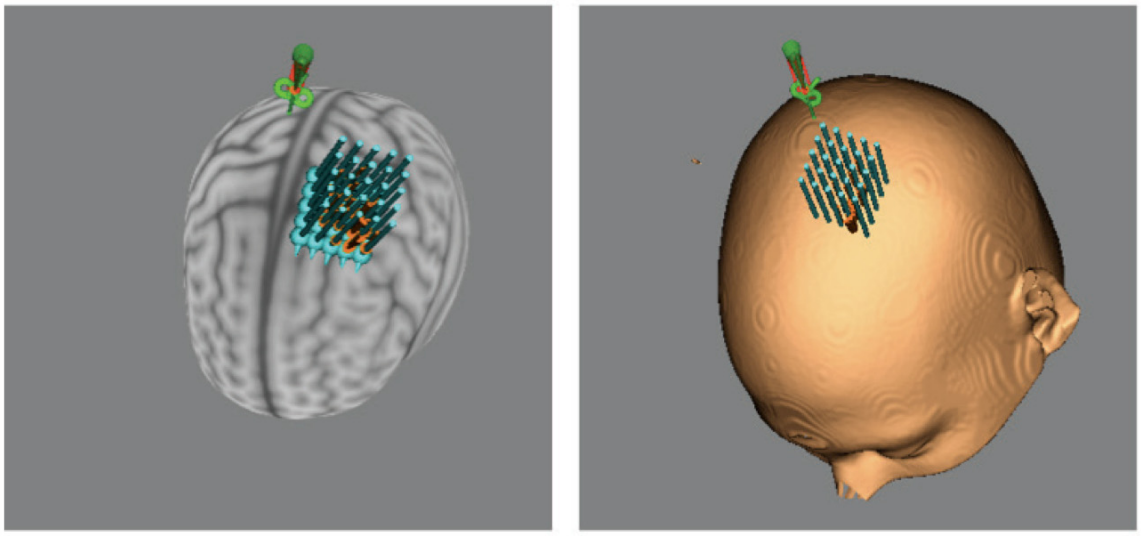


Figure 13. Stimulation target grid placement over the hand-knob of the left M1, shown in BrainSight. (Left, reconstructed cortical surface; right, head shape).

4.3.5.3 Determining Resting Motor Threshold

Once the motor hotspot was located, the resting motor threshold (RMT) was determined. RMT was defined as the lowest stimulation intensity required to elicit a MEP with a peak-to-peak amplitude of 50μV, in the resting FDI muscle, for 5 out of 10 consecutive stimuli. The RMT was measured for each participant prior to engagement in HIIE. Subsequent stimulation parameters were then set as a percentage of RMT.

4.3.5.4 Stimulus-response curve measures

After localization of the hotspot, and determination of the RMT, a pre-HIIE stimulus-response (S-R) curve was measured. An S-R curve is a plot of MEP amplitude over increasing TMS intensity. The S-R curve was created by delivering 50 single pulses of different stimulus intensity over the motor hotspot; 10 single pulses were delivered at each of the following stimulus intensities: of 100%, 110%, 120%, 130%, and 140% of RMT. The order of the intensity of these 50 pulses was randomized using the same Signal software (Signal v 6.0, Cambridge Electronics Design, UK) used to collect and analyze the corresponding EMG data. These single pulses were delivered with a fixed 3-second interval between successive stimuli, and order of stimulus intensity was randomized. The peak-to-peak MEP amplitude for each stimulus was measured, and the average amplitude evoked by the 10 pulses at each stimulus intensity was calculated. The averaged MEP amplitudes were then used to generate an S-R curve. S-R curves were generated prior to, directly following and 30 min following HIIE.

4.3.5.5 Paired Pulse Measures

Intracortical networks were investigated using paired-pulse TMS. Intracortical facilitation (ICF), short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI) will be assessed before, directly after, and 30 min after HIIE. To perform paired pulse TMS, the coil was placed over the motor hotspot, and two successive stimuli (a condition stimulus (CS) and test stimulus (TS)) were delivered at varying percentages of RMT and interstimulus interval (ISI) based on the paradigm being performed. ICF was assessed using a CS of 80% of RMT, a TS of 120% of RMT, with an ISI of 15 ms; SICI was measured using a CS of 80% of RMT, a TS of 120% of RMT, with an ISI of 2 ms; and LICI was assessed using a CS of 120% of RMT, a TS of 120% of RMT, with an ISI of 100 ms. In the paired-pulse protocol, thirty pairs of stimuli were delivered with a fixed interval of 3 sec between stimulus pairs. The order in which ICF, SICI and LICI were measured was pseudo randomized across participants. Three paired pulse paradigms were created, each with a different order of ICF, SICI and LICI (script A: ICF, SICI, LICI, script B: SICI, ICF, LICI, and script C: LICI, ICF. SICI). For each participant, the same paired-pulse script was run prior to, directly after, and 30 min after HIIE.

The MEPs measured during each paradigm (ICF, SICI, LICI) were compared to an unconditioned MEP amplitude evoked at 120% RMT, obtained from the S-R curve at the corresponding time point. S-R curves and paired-pulse paradigms were software controlled (Signal v 6.0, Cambridge Electronics Design, UK) to ensure consistency across participants.

4.3.6 Motor Learning Task

Participants engaged in the motor learning task during sessions 3, 4 and 5, immediately after performing the HIIE protocol outlined above. Study sessions 3 and 4, and 4 and 5 were separated by one rest day, to allow the participant to recover from the HIIE. The motor learning task used in the current study was a complex movement execution (CME) task. This task was developed in our lab – the Laboratory for Brain Recovery and Function – and a previous study has demonstrated that participants were able to learn novel, complex movements in three days (Ingram et al., *Under review*) (Figure 14). The motor learning task requires reproduction of a complex movement trajectory that involves multi-joint upper limb movements.

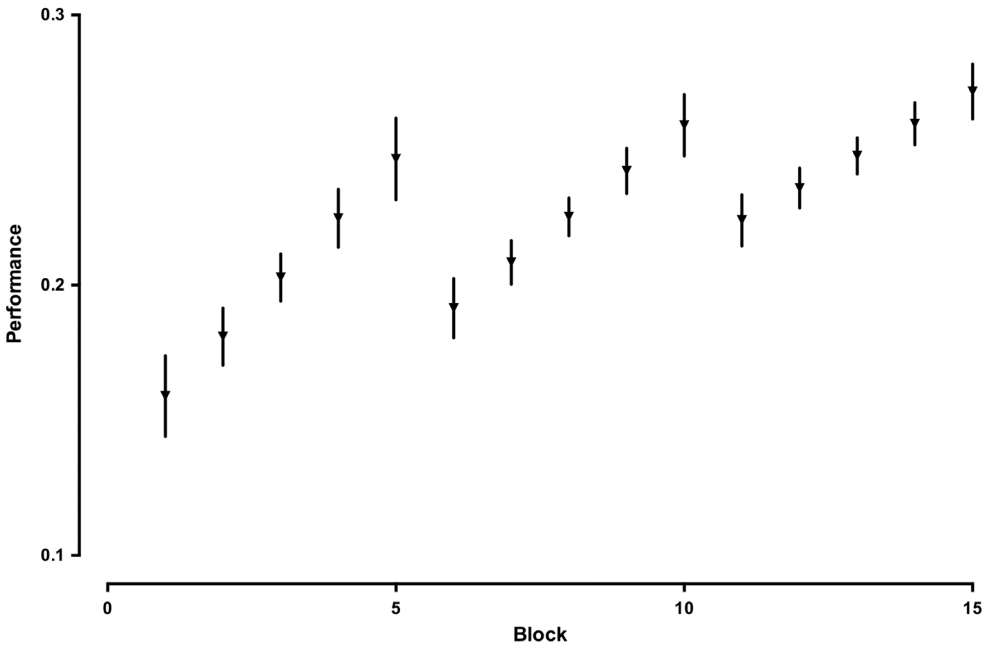


Figure 14. Learning across experimental blocks for control group (no exercise group). Performance is operationalized as the difference between repeated and random speed accuracy function (SAF) shifts, and is presented as mean ± SD of the posterior distribution of the shift. The term 'performance' is used here (as opposed to error) owing to the analysis approach used in this particular study (i.e., Ingram et al). Taken from Ingram et al., *Under Review*.

After completing HIIE, participants were seated at a desk, with a touchscreen, located inside of a testing box, in front of them (Figure 15). The testing box was intended to help reduce visual distractions while the participant was completing the task. Participants were asked to wear headphones, both to listen to the tutorial at the beginning of the task, and to block noise for the remainder of the task. The touchscreen was connected to a control computer, which produced the complex trajectories, and recorded and stored the data.

Figure 15. The experimental set up and equipment used for the CME task: (1) 'control' computer running the TraceLab program, (2) testing box, used to reduce distractions and (3) touchscreen, where the participant observed and reproduced complex trajectories.

Participants were asked to learn a complex trajectory on a touchscreen using custom programmed software we refer to as TraceLab. TraceLab is used to create "traceability experiments" where both simple and complex trajectories can be created using reusable components (Alhindawi et al., 2013; Ingram et al., *Under review*). For this particular experiment, TraceLab was used to create five repeated trajectories (Figure 16) as well as random trajectories of varying complexity.

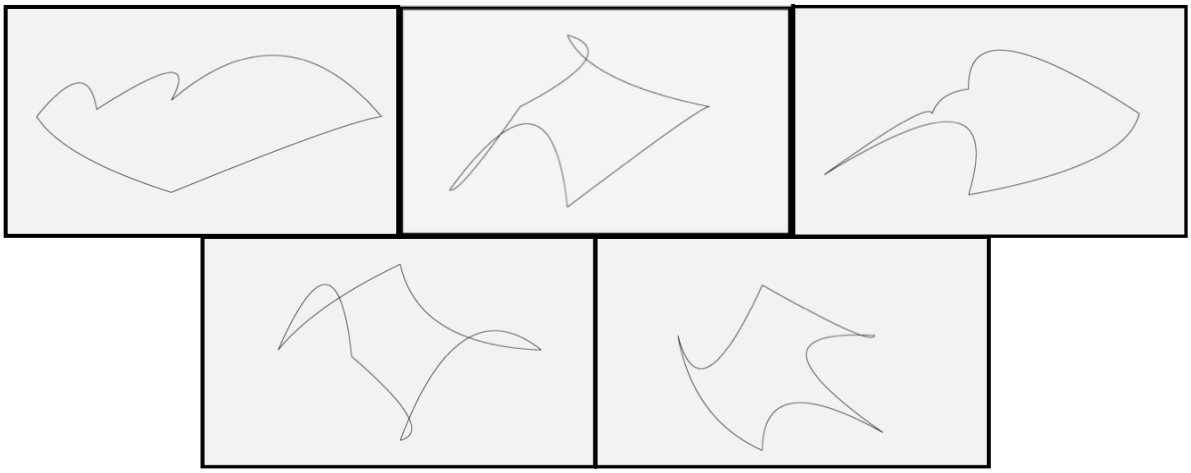


Figure 16. Each participant trained with one of these five complex trajectories for half (n=50) of their trials, with the other half (n=50) being randomly generated.

The repeated trajectories were chosen by generating 10,000 random trajectories (on TraceLab), which were then analyzed to determine their complexity. Complexity was as measured by: (1) total absolute curvature (a measure of how much the trajectory curves), and (2) approximate entropy (a measure of the irregularity or how unpredictable the trajectory is) (Brook, Bruckstein & Kimmel, 2005; Pincus, 1991). The five repeated trajectories used in the CME task were selected from the 10,000 random trajectories generated by TraceLab by first reducing the number of possibilities to those within 0.25 standard deviations of the mean complexity (using the measures listed above).

Subsequently, each trajectory was visually inspected and excluded if it did not meet the following criteria, designed to optimize their use with the touchscreen.

The trajectory must:

1. Be reasonably far from the edge of the touchscreen, to allow for effective tracing,
2. Move close to the origin of trajectory prior to the end of figure,
3. Be centered roughly in the middle of the touchscreen,
4. Move through all four quadrants of the touchscreen.

Finally, when the trajectories were narrowed down to less than ten based on the factors listed above, the final five were randomly selected. The random trajectories were then selected to incorporate similar characteristics as the repeated trajectories.

As stated previously, participants were asked to perform complex trajectories on a touchscreen. Each session included performance of 50 trials of one repeated trajectory, and 50 trials of random trajectories, for a total of 100 trials per session. Each of the 100 trials were performed at one of five different, randomly selected animation speeds (500ms, 1000ms, 1500ms, 2000ms or 2500ms), where the participant attempted to match the speed at which they traced the trajectory to the speed at which the trajectory had just previously been produced on the screen.

Each trial began with a white dot moving across the touchscreen, animating the trajectory to be reproduced. Each trial (trajectory) started and finished at the same, predetermined location. The start and end point of the trajectory was also the point from

which participants were asked to reproduce the trajectory. When the animation of the trajectory was complete, a red dot appeared at this point.

When the animation of the trajectory was complete, participants started the task by placing the index finger of their dominant hand on the red dot. Once the participant placed their finger on the red dot, the colour changed to green, indicating that the screen has registered their touch and prompting them to recreate the trajectory (Figure 17). When the participant had completed the trial, their finger returned to the dot, the dot changed colour from green to red, indicating the completion of the movement and the end of the trial. Upon completion of each trial, participants received a visual display of their performance; the trajectory the participant produced was overlaid onto the animated trajectory.

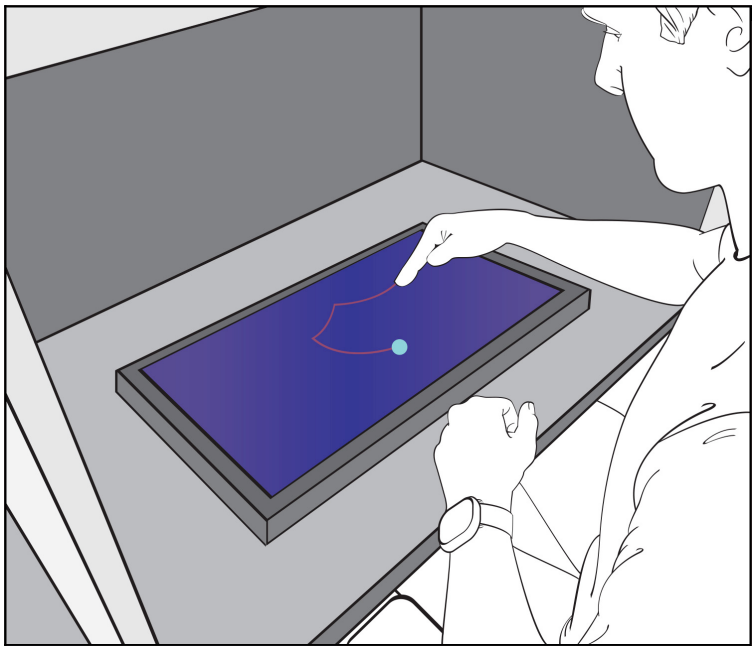


Figure 17. Example trial depicting a typical trajectory. Note that the traced lines shown above are for descriptive purposes and no such feedback was provided to participants during task execution. Participants received feedback of their performance in comparison to the animated trajectory at the end of each trial.

As previously stated, participants engaged in the motor learning task during sessions 3, 4 and 5. These sessions were scheduled approximately 48 hours apart to enhance recovery from HIIE during the previous session. The investigators anticipated participants would demonstrate a within session performance improvement as well as improvement between sessions. Within session performance improvement was characterized by temporary changes in motor behaviour during a single tracing session. If changes in performance last longer than the training session, and improvements in task execution are retained and demonstrated during the subsequent tracing session, this will be indicative of motor learning (Shumway-Cook & Woollacott, 2001).

4.4 Data Analysis

4.4.1 Analysis of EMG Data

Analysis of MEP data was performed in line with previous work in our laboratory. During TMS, Signal software (Signal v 6.0, Cambridge Electronics Design, UK) was used to externally control the stimulator by setting stimulus intensity and timing. Through the use of Signal, stimulus intensity, type (i.e. single pulse, ICF, SICI, LICI) were recorded along with the EMG signal, facilitating offline analysis of MEPs. The peak-to-peak amplitude of MEPs was determined using custom scripts programmed for Signal. In general, the custom scripts isolated a 50ms period in which the MEP should have occurred, and then returned the peak-to-peak amplitude (i.e. the difference between the maximum and minimum values) that occurred in that specified time period.

To examine single pulse measures used to construct the S-R curve, a 50 ms analysis period began 10ms after the stimulus was delivered (which occurred 1 s into each Signal frame). The window of analysis began 10ms after stimulus was delivered, as the typical latency of a MEP in the FDI after TMS is between 15 and 25 ms. Therefore, the interval of interest for single pulse measures was from 1.010-1.060 s.

To examine paired pulse measures, a 50 ms analysis period was also used. This period was temporally linked to the occurrence of the MEP evoked by the TS. As the TS for ICF, SICI, and LICI occurred at 1.015 s, 1.002 s, and 1.100 s respectively, the analysis periods for these paradigms were 1.020 – 1.070 s, 1.007 – 1.057 s, and 1.105 – 1.155 s.

For both single and paired-pulse measures, the Signal script was run and returned the peak-to-peak amplitude within the set analysis window (outlined above). The EMG data was also manually reviewed to ensure that the peak-to-peak amplitude values obtained were logical and related to the evoked response (as opposed to artefact). Data files were saved as .txt files and then exported to Microsoft Excel for further analysis.

4.4.1.1 EMG Data Reduction: Number of MEPs

As described above, for single pulse TMS, 10 pulses were delivered at each of the selected stimulator intensities (100, 110, 120, 130, and 140% RMT) to generate an S-R curve. If a minimum of 4/10 MEPs were not obtained as a response to stimulation at any intensity for a given time point, the participant was excluded from further analysis at that time point (e.g., if only 3/10 MEPs were obtained for the 110% RMT intensity for the second post-HIIE TMS collection, the single pulse data for that participant was removed from the analysis at that time point).

Data reduction methods vary between the paired-pulse measures examining intracortical facilitation, and those examining intracortical inhibition. When analyzing ICF data, participants who did not display overall facilitation at the pre-exercise timepoint were excluded from further analysis of ICF data (i.e, if the average of the 10 ICF trials was not greater than the average unconditioned MEP amplitude at 120% RMT for the same time point, the participant was removed from subsequent analysis). Additionally, if a minimum of 4/10 MEPs were not obtained as a response to the ICF TS, the participant was excluded from further analysis for that paradigm. When examining SICI and LICI data, it is not possible to determine if the absence of a MEP is due to inhibition or technical error, therefore there was no minimum number of MEPs required for a participant to be included in SICI or LICI data analysis. However, participants in which intracortical inhibition could not be induced pre-exercise were excluded from the corresponding analysis (i.e. if the average of the 10 SICI or LICI trials was greater than the average unconditioned MEP amplitude at 120% RMT from the Pre-HIIE time point, the participant was removed from subsequent analysis for that paradigm).

4.4.1.2 EMG Data Reduction: Pre-Stimulus Muscle Activity

As voluntary activity in the target muscle prior to stimulation will result in increased MEP amplitude, we examined EMG activity in the period immediately before delivery of the TMS pulse. If the EMG activity prior to stimulation exceeded baseline values, the subsequent MEP was removed from analysis. Specifically, the Signal scripts calculated the average root mean square (RMS) amplitude in a 70 ms window (0.025 to 0.095) before the TMS pulse (or before the CS for the paired-pulse paradigms). RMS amplitude values were exported along with the MEP amplitude data and included in the

Excel spreadsheets. The baseline EMG activity was determined by calculating the average RMS value of all of the trials for each stimulation intensity (single pulse: 100%, 110%, 120%, 130%, 140% RMT) or paradigm (ICF, SICI and LICI). For each trial, if the RMS amplitude of the EMG activity before the stimulus was greater than baseline plus one standard deviation, the frame was flagged for manual inspection. If upon visual inspection the increased RMS amplitude was determined to be from movement prior to the TMS pulse (as opposed to electrical noise), the corresponding MEP was removed from subsequent analysis.

4.4.2 Statistical Analysis: TMS Data

Statistical analysis of the TMS data were performed using GraphPad Prism (version 7.00, GraphPad Software, La Jolla California USA, www.graphpad.com).

4.4.2.1 Stimulus-Response Curve

S-R curves were constructed for each participant at each time-point: Pre-HIIE (before engaging in HIIE), Post 1 (directly after HIIE), and Post 2 (30 min after HIIE). The average peak-to-peak MEP amplitude was calculated for each trial and the 10 MEPs obtained at each stimulus intensity were averaged (or fewer than 10 if trials were removed; see data reduction methodology above). These values were then plotted to produce an S-R curve. The area under the curve (AUC) was calculated as the integral under the function (S-R curve); this measure provided a global estimate of CSE (Peri et al., 2017). A one-way repeated measures ANOVA was used to examine the effect of time (three levels: Pre, Post 1 and Post 2) on the area under the S-R curve.

4.4.2.2 Paired-pulse measures

Paired-pulse measures were analyzed to assess changes in intracortical inhibition and facilitation. For each paired-pulse paradigm (SICI, ICF, and LICI), the average amplitude of conditioned MEPs were expressed as a percentage of the average unconditioned MEP amplitude at 120% RMT (after single-pulse stimulation). To assess changes in ICF, SICI, and LICI, measures were analyzed using three separate one-way ANOVAs with time (three levels: Pre, Post 1 and Post 2) as a factor.

4.4.3 Analysis of CME Data

Motor learning was examined using the CME task. Error was quantified as the mean point-by-point difference (in millimeters) between the stimulus trajectory (what the participant observed) and the response trajectory (what the participant executed). The primary outcome measure determining performance on the motor learning task was the difference in task performance from the first block of task practice (learning session 1, block 1*; S1B1) to the retention test (learning session 3, block 1*; S3B1). We used learning session 3, block 1 as a retention test, to measure CME skill performance following 2 days of skill practice. We did not use the final block of task performance (i.e. learning session 3, block 5) to measure learning, as performance in this block would have been influenced by within-session performance effects in addition to learning. Random trajectories were used in order to allow differentiation between general learning of the task versus actual learning of the repeated complex trajectory. Learning was

* Note: learning session 1 was actually the third study session, and learning session 3 was actually the fifth and final study session.

operationalized as the difference in error between trial types (i.e. repeated and random trajectories) and a reduction in shape error (in millimeters) from S1B1 to S3B1 will indicate that learning has occurred.

One of the main objectives of this thesis (Objective 2) was to determine if performing HIIE prior to engaging in an established motor learning paradigm will increase the effectiveness of complex skill acquisition, compared to non-exercising individuals. To meet this objective, learning was compared between two groups of participants: the HIIE group (15 participants who performed HIIE prior to engaging in the CME task), and the control group. The control group data were collected during a previous study in our laboratory and came from 15 individuals who performed only the CME task (i.e. they did not engage in the prescribed HIIE protocol directly prior to CME task performance).

4.4.3.1 CME Task Data Reduction

Prior to statistical analysis, the CME task data were reduced to only include meaningful trials. Participants performed 100 trials of the CME task during each of the three motor learning sessions, for a total of 300 trials per participant. Therefore, data were collected from 4500 trials performed by the HIIE group, and 4500 trials performed by the control group. In total, data were collected from 9000 trials. The first step in data reduction was to remove outlier trials based on movement time (i.e., the time required to complete tracing of the trajectory). The CME task was equipped with a pre-programmed method to remove outliers based on movement time. Briefly, this method removed trials if the movement time to animation time ratio was less than 0.5 or greater than 2.5. This

was programmed into the task analysis to remove trials in which the participant made an error on the touch screen which resulted in the trial being cut short (i.e., the participant touched the final point of the trajectory while attempting to execute the shape, unintentionally ending the trial) or erroneously extended the trial duration (i.e., the participant missed the red button marking the final point of the trajectory, extending the duration of the trial).

Outliers in error score were calculated independently for each participant and animation time (500ms, 1000ms, 1500ms, 2000ms, 25000ms). Z-scores (standardized residuals) were calculated for performance error, and trials in which error was greater than 2 standard deviations from the mean were removed.

4.4.4 Statistical Analysis: CME Data

To examine learning, we were interested in changes in error from the first block of task performance (S1B1) and the first block of performance on the last day of the study (S3B1). We were also interested in the difference in error between the repeated and random trajectories at each of these time points. Statistical analysis of the CME task data was performed using a 3-way repeated-measures ANCOVA with factors of group (HIIE group vs. non-exercising control group), trajectory type (repeated and random), and time (learning session 1, block 1 vs. session 3, block 1), and movement time as a covariate. Statistical analyses of the CME task data were performed using 'SPSS'.

4.4.5 Exploring the Link between CSE and Learning

The third objective of this thesis was to examine the relationship between HIIE-induced changes in CSE and motor learning task performance after HIIE. This

relationship was evaluated by examining the correlation between various TMS parameter change scores and a 'learning score'. The learning score used to examine this correlation was reflective of each participant's performance on random and repeated shapes during both the first block of task practice (S1B1) and at the retention timepoint (S3B1). The learning score was calculated using the following equation:

$$Learning\ score =$$

$$(S1B1Error_{random} - S1B1Error_{repeated}) - (S3B1Error_{random} - S3B1Error_{repeated})$$

For single pulse TMS, an AUC change score was calculated by taking the difference of AUC values from the pre- and post-HIIE conditions (i.e. for each participant, their Pre-HIIE AUC value was subtracted from their Post 1 AUC value and Post 2 AUC value, respectively). For paired-pulse TMS measures, change scores were calculated for ICF, SICI and LICI. As outlined above, the amplitude of MEPs resulting from the paired-pulse TMS were normalized to unconditioned MEP amplitude evoked at 120% RMT, (obtained during single pulse stimulation at the corresponding time point). The change score for paired-pulse measures was therefore calculated from the difference in the average normalized MEP from Pre to Post 1, and Pre to Post 2 (e.g. each participant's Pre-HIIE normalized SICI value was subtracted from their Post 1 normalized SICI value). This was done for both post-HIIE timepoints (Post 1 and Post 2) for each paired-pulse measure.

4.4.5.1 Statistical Analysis: Correlation between CSE and Learning

After change scores were calculated, statistical analysis was performed to determine if there was a relationship between learning score and change score for any of the TMS parameters. Kendall's Rank Correlation coefficient (Tau) was used, as it is recommended with small sample sizes. Kendall's Tau is a measure of the relationship between columns of ranked data. Therefore, the learning scores and the various TMS change scores were ranked, and the correlational analysis was performed. This statistical analysis was performed using "R: A language and environment for statistical computing" (R Foundation for Statistical Computing, Vienna, Austria).

CHAPTER 5: RESULTS

Twenty-one participants were recruited for the present study, of which six participants were unable to complete the study; one participant was excluded as they were unable to complete the GXT, another participant was excluded as they were unable to complete the HIIE protocol, and three additional participants were excluded as we were unable to elicit consistent MEPs in response to TMS. A total of 15 participants (7 females, 14 right-handed, 22.8 ± 2.8 years) completed all five study sessions (See Table 2 for additional participant characteristics).

Table 2. Participant characteristics.

Participant Number	Age	Sex	Maximum Power Output from GXT (W)	Height (cm)	Weight (kg)	BMI	IPAQ Continuous Score (MET-min/week)	IPAQ Categorical Score
1	20	F	140	170.2	61.2	21.1	3266	High
2	19	M	140	183.0	70.0	20.9	4039	High
3	22	M	280	178.0	110.0	34.7	2655	High
4	21	F	140	162.6	54.4	20.6	1746	Moderate
5	26	M	240	185.4	81.7	23.8	3084	High
6	20	M	240	182.9	84.8	25.3	4346	High
7	21	F	160	167.6	61.2	21.8	2628	Moderate
8	24	M	180	170.2	59.0	20.4	1425	Moderate
9	25	M	260	188.0	93.0	26.3	2973	Moderate
10	20	M	220	180.0	75.0	23.1	5493	High
11	22	F	180	161.1	59.0	22.8	4692	High
12	28	F	160	149.9	52.2	23.2	984	Moderate
13	25	F	160	140.0	64.0	24.8	1464	Moderate
14	22	F	140	165.1	72.6	26.6	1554	Moderate
15	27	M	280	175.3	88.4	28.8	3693	High

MET: Metabolic equivalent. 1 MET = 1 kcal/kg/hour

Motor learning data were compared to a control group collected in our laboratory as part of a previous study. The non-exercising control group included 15 individuals (9

female), aged 18-30 years (average age 23.5 ± 4.3 years). The control group was collected as part of a previous study in our laboratory. These participants did not complete the IPAQ (Appendix 6) or a Health History Questionnaire (Appendix 8) as part of the screening procedures for the previous study. Therefore, we do not have data on participant characteristics such as height, weight and BMI, or information regarding physical activity leading up to study participation for this group.

One participant was eliminated from the TMS portion of the analysis due to excessive artefact in the EMG signal that interfered with the ability to distinguish MEPs. Of the 14 remaining participants, 12 had sufficient data to be included in the S-R curve analysis, and 14, 13, and 7 participants had sufficient data to be included in ICF, SICI, and LICI paired pulse analyses, respectively (Table 3). All 15 participants were included in the CME portion of the analysis.

Table 3. Number of participants included in each part of the data analysis.

Analysis	S-R	ICF	SICI	LICI	CME
Number of participants included	12	14	13	7	15

5.1 Exercise Results

One participant was unable to complete the GXT and was eliminated from the study. Another participant was unable to complete the HIIE protocol during the second study session, and therefore we did not perform post-HIIE TMS on the participant, nor did the participant engage in subsequent motor learning sessions. Of the 15 participants who completed the 5 study sessions, 4 participants were unable to complete the high

intensity intervals of the HIIE protocol at 90% of their PO_{max} (determined from the GXT performed in session 1) (Table 4). During the participant's first time performing their personalized HIIE protocol, if he/she was unable to maintain the required pedalling cadence (i.e. a minimum of 70rpm), the workload of the high intensity interval was reduced to 85% or 80% of the participant's PO_{max}. This was done so that the participant was able to complete the exercise session, as well as continue to participate in the study. The participant's personalized HIIE protocol was then adjusted so that all future high intensity intervals would be performed at that same intensity (i.e. either 85% or 80% of PO_{max}, depending on which intensity the participant had demonstrated that they could complete).

Table 4. Participants' POmax and individualized HIIE protocols. Shading is used to indicate the percentage of POmax at which participants exercised.

Participant Number	Sex	Maximum Power Output from GXT (W)	Maximum RPE			High-intensity interval intensity (W)			Low-intensity interval intensity (W)
			GXT	HIIE		90% PO_{max}	85% PO_{max}	80% PO_{max}	50% PO_{max}
				S2	S3-S5				
1	F	140	19	15	15	126	119	112	70
2	M	140	18	16	15-17	126	119	112	70
3	M	280	20	17	17-18	252	238	224	140
4	F	140	18	19	19-20	126	119	112	70
5	M	240	20	19	19-20	216	204	192	120
6	M	240	20	19	17-19	216	204	192	120
7	F	160	19	20	17-20	144	136	128	80
8	M	180	18	19	18-19	162	153	144	90
9	M	260	19	18	18	234	221	208	130
10	M	220	20	19	19	198	187	176	110
11	F	180	18	18	17-18	162	153	144	90
12	F	160	18	18	17-18	144	136	128	80
13	F	160	18	16	15-17	144	136	128	80
14	F	140	19	19	17-19	126	119	112	70
15	M	280	19	18	17-18	252	238	224	140

S2: Session 2 (TMS session). S3-S5: Session 3-Session 5 (CME task sessions).

5.2 TMS Results

5.2.1 Single Pulse TMS

S-R curves were constructed using the average MEP amplitude evoked by varying percentages of RMT at each time point (Figure 18). Overall, MEP amplitude increased as a function of stimulator output within each time-point.

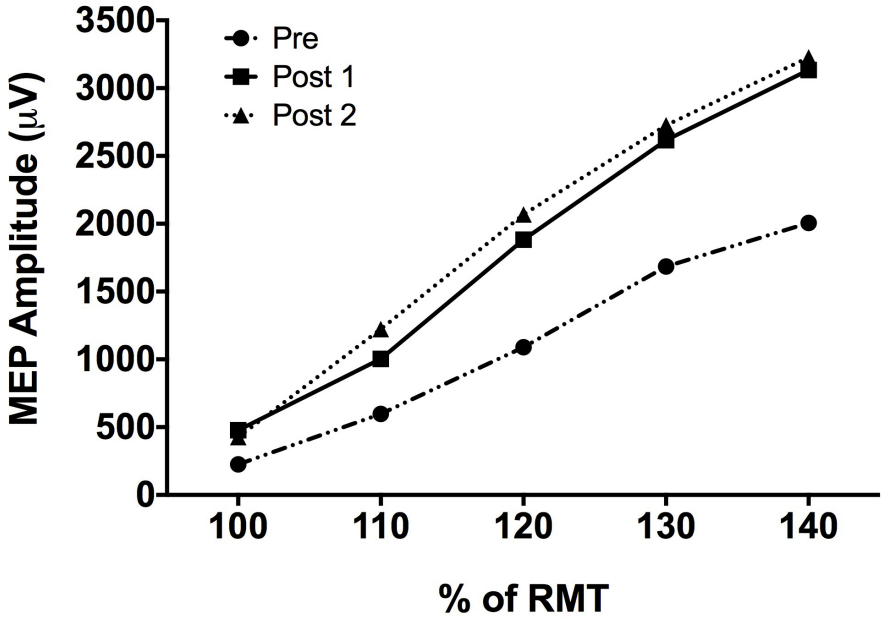


Figure 18. Stimulus-response curves before and after HIIE. S-R curves pre- and post-HIIE in response to stimulation at increasing percentages of RMT (n=12).

We observed an increase in the AUC values in many of the participants from the pre-HIIE time point to post 1 (n=8), and from pre-HIIE to Post 2 (n=10). D'Agostino and Pearson normality test indicated that AUC values from Pre-HIIE and Post 1 were not normally distributed ($p < 0.05$). Therefore, a square root transformation was performed on all of the AUC values, and subsequent normality tests indicated that the transformed data were normally distributed ($p > 0.05$). A one-way repeated measures ANOVA showed a significant main effect of time point (Pre, Post 1, Post 2) on AUC ($F_{2, 22} = 7.34$, $p =$

0.0048). Post hoc comparisons (Tukey's multiple comparisons test) revealed that there was a difference between Pre and Post 1 AUC ($p = 0.048$), and Pre and Post 2 AUC ($p = 0.003$) values. No difference between Post 1 and Post 2 AUC values were observed ($p = 0.795$). The average Pre-HIIE AUC value was 4489.1 ± 2078.8, compared to 7309.8 ± 4889.6 at Post 1 and 7845.2 ± 4530.2 at Post 2. Overall, our data demonstrate that AUC was significantly increased immediately after HIIE (Post 1; $p = 0.048$) and 30 min after HIIE (Post 2; $p = 0.003$) compared to before engaging in exercise (Pre).

5.2.2 Paired Pulse TMS

5.2.2.1 Intracortical Facilitation (ICF)

D'Agostino and Pearson normality test indicated that the ICF data from Pre-HIIE were not normally distributed ($p < 0.05$). Therefore, a square root transformation was performed. Subsequent normality tests indicated that the transformed data passed normality tests ($p > 0.05$). A one-way repeated measures ANOVA showed a significant main effect of time point (Pre, Post 1, Post 2) on average MEP amplitude ($F_{2, 26} = 6.10$, $p = 0.013$) for ICF paired-pulse measures. Post hoc tests (Tukey's multiple comparisons test) revealed that there was a significant difference between average MEP amplitude evoked by ICF from Pre to Post 1 ($p = 0.031$), and from Pre to Post 2 ($p = 0.002$). However, there was no significant difference in MEP amplitude evoked by ICF between Post 1 and Post 2 ($p = 0.987$). Average pre-HIIE MEP amplitude during ICF was 171.4 ± 58.5% of unconditioned stimulus amplitude (i.e. 71.4% facilitation). Average Post 1 and Post 2 MEP amplitudes were 125.9 ± 62.3%, and 119.2± 41.5% of the unconditioned stimulus amplitude from their respective timepoints (Figure 19). Our ICF data

demonstrate that MEP amplitude was significantly decreased immediately after HIIE (Post 1) and 30 min after HIIE (Post) compared to before exercise (Pre).

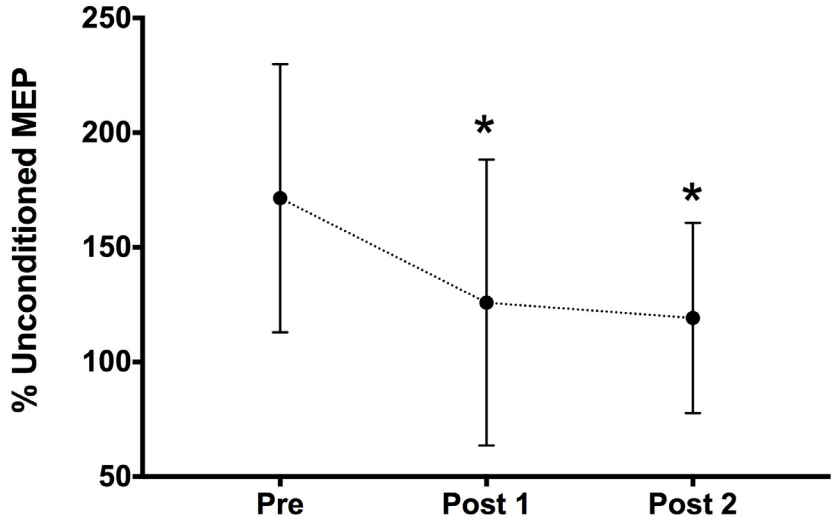


Figure 19. Induction of ICF across all participants (n=14) at each timepoint as a percentage of unconditioned MEP amplitude from the corresponding time point. Bars represent SD. Asterisks indicated values that are significantly different from pre-HIIE values ($p < 0.05$).

5.2.2.2 Short-Interval Intracortical Inhibition (SICI)

D'Agostino and Pearson normality test indicated that the SICI data from Post 1 and Post 2 were not normally distributed ($p < 0.05$). Following the same data processing steps used above, a square root transformation was performed. However, follow-up normality tests (D'Agostino and Pearson) revealed that the SICI data from Post 1 and Post 2 were still not normally distributed ($p < 0.05$). The original SICI data were then transformed again, this time using a logarithmic transformation. Normality testing of the logarithmically transformed data revealed that SICI data from Pre and Post 1 were normally distributed. Normality could not be assessed for the Post 2 dataset, as the sample

size (n=7) was too small. (There were originally 13 participants included in the SICI analysis, but 6 of these participants did not experience MEPs during the Post 2 time point. As the logarithm of zero is not defined, these participants were removed from subsequent analyses using data from the Post 2 time point. One of the 13 participants was removed from the Pre time point for the same reason.)

A one way repeated-measures ANOVA was performed using the six participants who had data for each time point following the logarithmic transformation. The ANOVA revealed that there was no significant main effect of time point (Pre, Post 1, Post 2) on average SICI MEP amplitude ($F_{2, 10} = 1.06$, $p = 0.3603$. A paired t-test was also used to compare the means of SICI MEP amplitude from Pre and Post 1, as this analysis could include 12 participants who still had data for each of these time points following the logarithmic transformation. The paired t-test, with the additional participants included, also confirmed that there was no significant difference between the mean MEP amplitude evoked by SICI at the Pre and Post 1 timepoints. Average pre-HIIE MEP amplitude during SICI was 37.1 ± 20.6% of unconditioned stimulus amplitude. Average Post 1 and Post 2 values were 49.3 ± 55.0%, and 36.5± 28.0%, respectively (Figure 20). Overall, when probing SICI, our data show that MEP amplitude did not change immediately (Post 1) or 30 min after (Post 2) engaging in HIIE ($p > 0.05$).

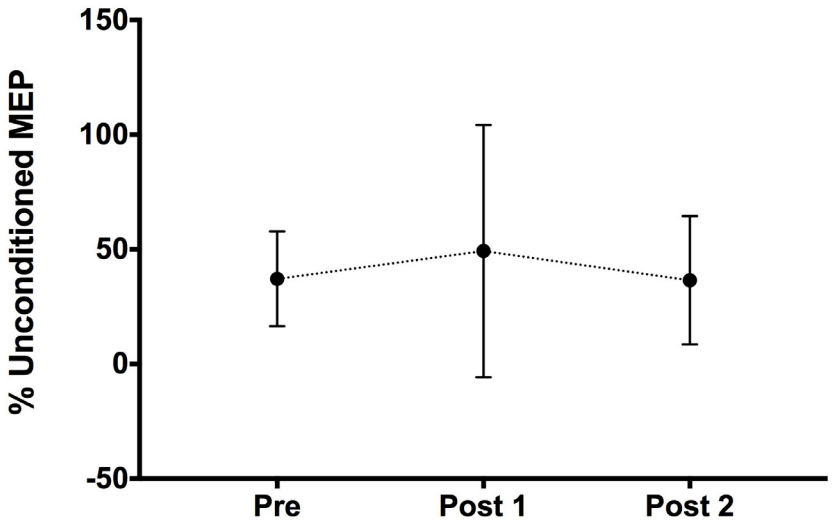


Figure 20. Induction of SICI across all participants (n=13) at each timepoint as a percentage of unconditioned MEP amplitude from the corresponding time point. Bars represent SD.

5.2.2.3 Long-Interval Intracortical Inhibition (LICI)

Normality tests were performed as described above, however the sample size (n=7) was too small to run a D'Agostino and Pearson normality test. Instead, Shapiro-Wilk normality tests were used, and they indicated that although the Post 2 data were normally distributed, the datasets from Pre and Post 1timepoints were not. A square root transformation was performed, and subsequent Shapiro-Wilk normality tests indicated that the transformed Pre dataset was now normally distributed, as well as the Post 2 dataset. However, the Post 1 dataset was still not normally distributed after the square root transformation. It was not possible to perform logarithmic or reciprocal transformations (the two other transformation methods typically used to reduce the positive skew displayed by our data), as these transformations cannot be done if a data point is zero. Removing participants who did not experience MEPs (i.e. average amplitude was zero) in response to LICI at one or more time points from the analysis

would have reduced the sample size for the LICI portion of the analysis from seven to four. Therefore, a paired t-test was performed to compare the two data sets that were normally distributed following the square root transformation. A paired t-test revealed that there was no significant difference between mean MEP amplitude evoked following LICI at the Pre and Post 2 time points ($p = 0.900$). When probing LICI, average pre-HIIE MEP amplitude was 16.2 ± 20.9% of unconditioned stimulus amplitude. Average Post 1 and Post 2 values were 24.6 ± 35.5%, and 11.3± 11.6% of unconditioned MEP amplitude, respectively (Figure 21). Our LICI data demonstrate that MEP amplitude did not change immediately (Post 1) or 30 min after (Post 2) performing HIIE ($p > 0.05$).

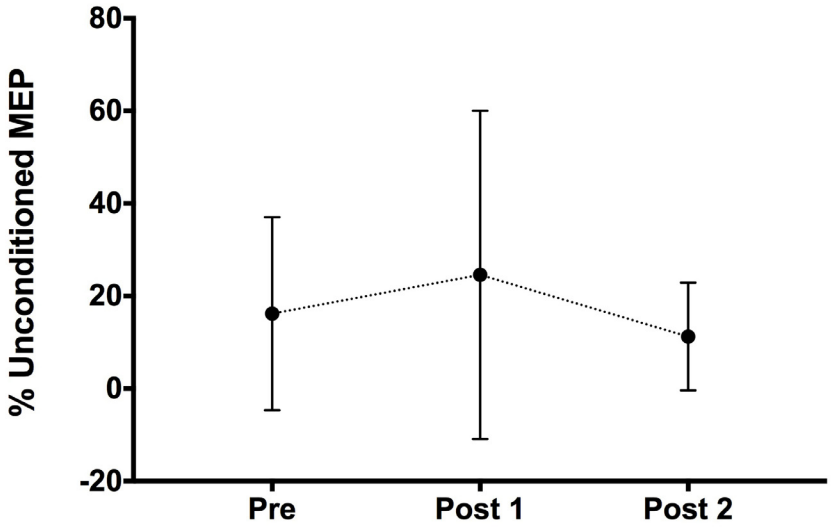
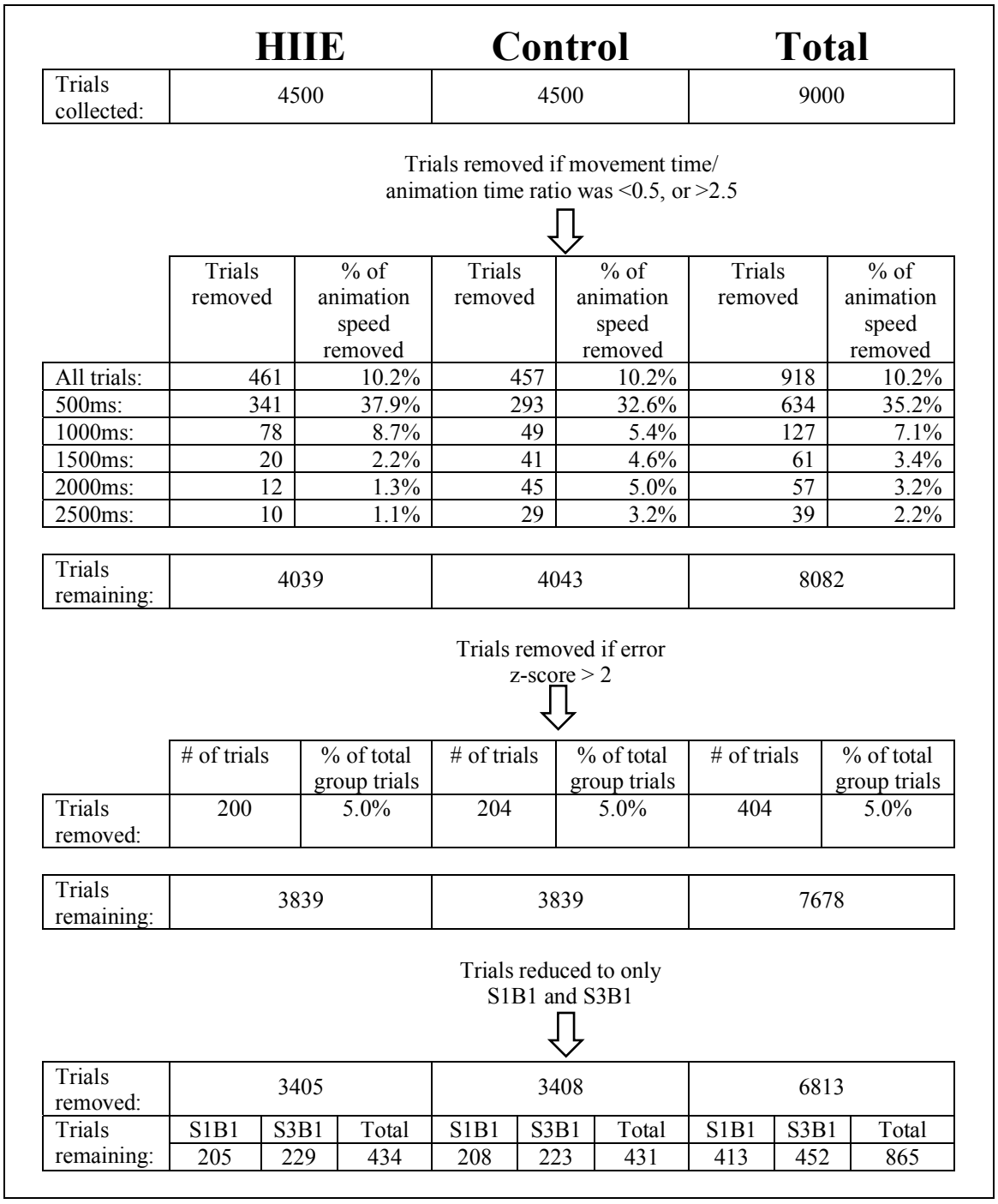


Figure 21. Induction of LICI across all participants (n=7) at each timepoint as a percentage of unconditioned MEP amplitude from the corresponding time point. Bars represent SD.

5.3 Complex Movement Execution (CME) Task Results

Prior to performing data analysis, the data were reduced to only include meaningful trials. (*See above: Section 4.5.3.1 CME Task Data Reduction*) The first step in data reduction was to remove trials based on movement time or error. This step caused 461 (of 4500) trials to be removed from the HIIE group, and 457 (again, of 4500) trials to be removed from the control group (see Figure 22 for an overview of the number of trials eliminated in each stage of data reduction).



	HIIE	Control	Total
Trials collected:	4500	4500	9000

Trials removed if movement time/ animation time ratio was <0.5, or >2.5

	Trials removed	% of animation speed removed	Trials removed	% of animation speed removed	Trials removed	% of animation speed removed
All trials:	461	10.2%	457	10.2%	918	10.2%
500ms:	341	37.9%	293	32.6%	634	35.2%
1000ms:	78	8.7%	49	5.4%	127	7.1%
1500ms:	20	2.2%	41	4.6%	61	3.4%
2000ms:	12	1.3%	45	5.0%	57	3.2%
2500ms:	10	1.1%	29	3.2%	39	2.2%

Trials remaining:	4039	4043	8082

Trials removed if error z-score > 2

	# of trials	% of total group trials	# of trials	% of total group trials	# of trials	% of total group trials
Trials removed:	200	5.0%	204	5.0%	404	5.0%

Trials remaining:	3839	3839	7678

Trials reduced to only S1B1 and S3B1

Trials removed:	3405			3408			6813		
Trials remaining:	S1B1	S3B1	Total	S1B1	S3B1	Total	S1B1	S3B1	Total
	205	229	434	208	223	431	413	452	865

Figure 22. Overview of methods used to reduce CME task data, and the number of trials removed at each stage.

Upon further analysis, it was determined that this method caused 37.9% and 32.6% of the 500ms trials to be removed from the HIIE group and the control group, respectively (in comparison only 1.1-8.7% of trials were removed from any of the other time points for either group). Therefore, the 500ms timepoint was excluded from subsequent analysis. This caused an additional 559 and 607 trials to be removed from the HIIE and control groups, respectively.

Outliers in error score were calculated independently for each participant and animation time (1000, 1500, 2000 and 2500 ms). Z-scores (standardized residuals) were calculated for performance error, and trials in which error was greater than 2 standard deviations from the mean were removed. This caused 200 trials and 204 trials to be removed from the HIIE dataset and the control dataset, respectively.

The average error per block (20 trials) was plotted to visually examine effects of figure type (random vs. repeated) and group membership (HIIE group vs control) on task performance (Figure 23).

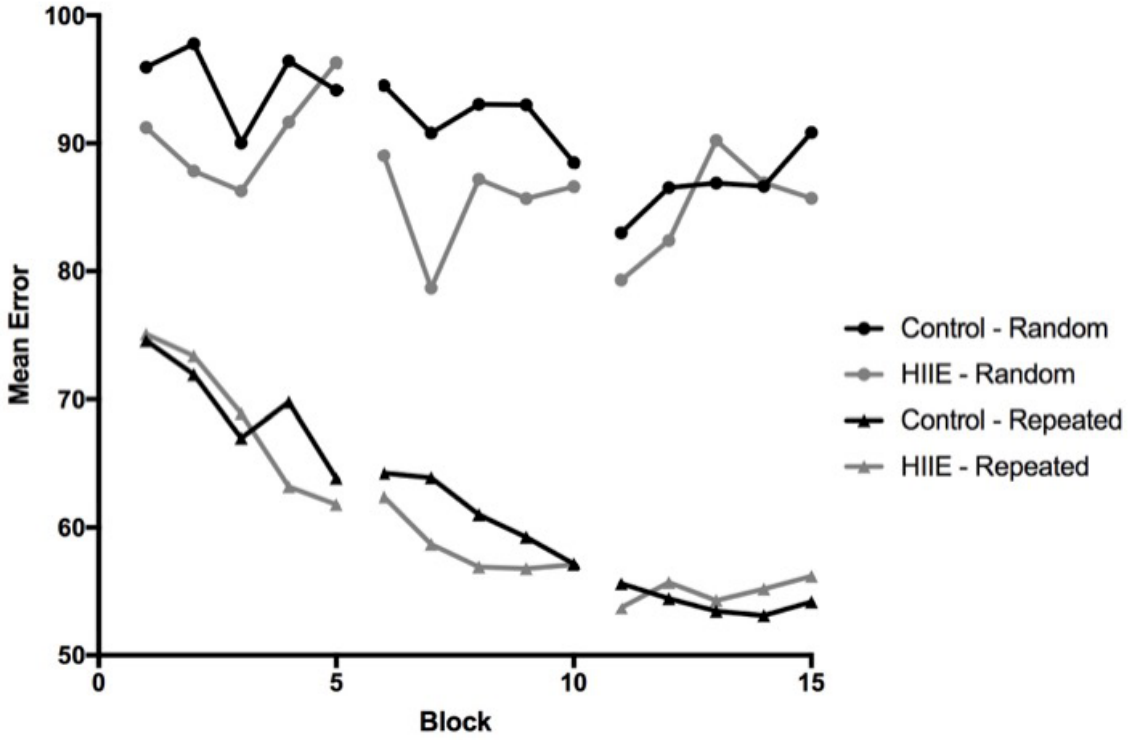


Figure 23. Mean error (in mm) per block, plotted for each combination of group and figure type. Blocks 5, 10 and 15 mark the end of learning sessions 1, 2, and 3, respectively.

To examine learning, we were interested in changes in error from the first block of task performance (S1B1) to the first block of performance on the last day of the study (S3B1). We were also interested in differences in error between random and repeated trajectories; random trajectories were used to differentiate between general learning of the task versus actual learning of the repeated complex trajectory. Statistical analysis included a 3-way repeated-measures ANCOVA with factors of group (HIIE group vs. non-exercising control group), trajectory type (repeated and random), and time (learning session 1, block 1 vs. session 3, block 1), and movement time as a covariate.

Our data demonstrate that there was a significant effect of movement time ($F_{1, 856}$ = 22.052, p < .000, ηp^2 = 0.025), figure type ($F_{1, 856}$ = 159.779, p < .000, ηp^2 = .157), and time ($F_{1, 856}$ = 84.846, p < .000, ηp^2 = .090) on error during CME task execution (Table 5). There was also a significant interaction effect of figure type and time ($F_{1, 856}$ = 4.273, p = .039, ηp^2 = .005) on CME task error. This interaction effect shows that learning occurred, as there was a significant difference in error between in repeated shapes and the random shapes at baseline and retention. However, the effect of group membership (i.e. HIIE group membership, vs. non-exercising control) was not significant ($F_{1, 856}$ =.268, p = .605, ηp^2 < .000). Additionally, the interaction effect of group, figure type and time ($F_{1, 856}$ =.209, p = .648, ηp^2 < .000) on CME task error (mm) was insignificant, signifying that engaging in HIIE prior to task practice did not significantly affect CME task learning.

Table 5. Tests of effects of independent variables and covariate on error during CME task.

Source	df	F	Significance (p)	Partial Eta Squared (ηp^2)
Movement time	1	22.052	.000	.025
Group	1	.268	.605	.000
Figure type	1	159.779	.000	.157
Time	1	84.846	.000	.090
Group * Figure type	1	1.291	.256	.002
Group * Time	1	.325	.568	.000
Figure type * Time	1	4.273	.039	.005
Group * Figure type * Time	1	.209	.648	.000
Error	856			

Although exercise was not shown to significantly influence motor task performance, learning was observed in both groups. Learning was evidenced by 1) a smaller error value for the repeated shapes compared to random shapes, and 2) by a decrease in error from learning S1B1 to learning S3B1 (Table 6). In the HIIE group, average error in random shapes was 84.89 ± 30.16mm, compared to 63.90 ± 21.82mm for the repeated shapes. In the control group, average error for random and repeated shapes were 89.38 ± 32.52mm and 64.59 ± 23.34mm, respectively.

In the HIIE group, there was a decrease in overall error (calculated from the average error of both random and repeated shapes) from S1B1 (82.82 ± 27.81mm) to S3B1 (66.12 ± 26.14mm). The same trend was observed in the control group, where CME task error was also observed to decrease from S1B1 (85.28 ± 31.57mm) to S3B1 (68.75 ± 27.82mm).

Table 6. Mean error observed in each group for figure type and timepoint. Units of error are mm from the animated figure trajectory.

Group	Figure Type		Time	
	Random	**Repeated**	**S1, B1**	**S3, B1**
HIIE	84.89 ±30.16	63.90 ±21.82	82.82 ±27.81	66.12 ±26.14
Control	89.38 ±32.52	64.59 ±23.34	85.28 ±31.57	68.75 ±27.82

S1, B1 denotes learning session 1, block 1; S3, B1 denotes learning session 3, block 1

These findings do not support our second hypothesis that engaging in HIIE prior to the motor learning task will result in optimized motor learning relative to those who did not perform exercise.

5.4 Relationship between CSE and learning

Next, we examined the relationship between HIIE-induced changes in CSE and CME task performance. Specifically, we examined the correlation between a participant's 'learning score' and their various change scores for each TMS paradigm and timepoint. As explained above, the learning score used to examine this correlation was reflective of the participant's performance on random and repeated shapes during both the first block of task practice (S1B1) and at the retention timepoint (S3B1) (See *Section 4.5.5 Exploring the Link between Corticospinal Excitability and Learning* for a more detailed description of how the learning score was calculated). Learning scores and the various TMS change scores were calculated for each participant (Table 7).

Table 7. Data used to correlate learning change score and TMS parameter change score.

Participant	Learning change score	Post 1 – Pre AUC change score	Post 2 – Pre AUC change score	Post 1 – Pre ICF change score	Post 2 – Pre ICF change score	Post 1 – Pre SICI change score	Post 2 – Pre SICI change score
1	12.45	-1280	883	-128.77	19.64	N/A	N/A
2	5.09	3956	3035	-88.32	-91.54	-30.21	-32.38
3	1.53	2607	1182	-70.93	-53.20	19.76	-35.58
4	-1.72	-216	7244	31.65	-77.27	2.75	-15.56
5	-3.62	12012	2273	-57.48	-4.88	-9.56	22.53
6	12.16	4742	6297	-33.27	-81.32	0.14	-3.32
7	3.45	-1166	-306	31.14	-20.51	-7.18	-23.014
8	3.35	-795	-408	5.60	-41.47	28.59	-3.89
9	-14.85	N/A	N/A	N/A	N/A	N/A	N/A
10	5.99	2493	1658	-76.19	-27.24	-9.20	14.40
11	-0.22	N/A	N/A	-192.18	-180.12	157.78	49.98
12	3.37	5416	4505	-28.29	-23.13	-36.03	-28.85
13	3.33	3752	8818	17.05	-81.80	19.03	-21.14
14	4.12	2328	5092	-9.59	-11.94	2.87	17.53
15	31.28	N/A	N/A	-37.76	-56.36	18.88	51.10

After change scores were calculated, statistical analyses were performed to determine if there was a correlation between learning and any of the TMS parameters. Due to the small sample of participants who displayed LICI (n = 7 for Pre to Post 1 comparison; n = 6 for Pre to Post 2 comparison), change in learning values were not correlated with change in LICI values. Our data show that there was no significant correlation between learning and any of the measures of CSE examined (Table 8).

Table 8. Correlation of learning change score with TMS parameter change score.

Learning correlated with:	Kendall's Tau	Significance (p)
Post 1 to Pre AUC-change score	-.15	.55
Post 2 to Pre AUC-change score	-.09	.74
Post 1 to Pre ICF-change score	-.21	.33
Post 2 to Pre ICF-change score	.14	.52
Post 1 to Pre SICI-change score	-.13	.59
Post 2 to Pre SICI-change score	.07	.77

CHAPTER 6: DISCUSSION

The first objective of this thesis was to investigate the effects of HIIE on CSE. The second objective was to examine the effects of performing HIIE prior to motor task practice on motor learning. In collecting the data necessary to address these first two objectives, we also had the data required to address a third objective: examining the relationship between HIIE-induced changes in corticospinal excitability and motor learning performance when HIIE is performed prior to task practice. Overall, the objective of this thesis was to advance our knowledge of the mechanisms through which HIIE may facilitate motor learning.

In designing our study, we sought to contribute to the literature surrounding the effects of HIIE on motor learning. Aerobic exercise, and more specifically HIIE, has been reported to be an effective mechanism to induce experience-dependent plasticity (Roig et al., 2012). Specifically, engaging in AE creates a neural environment conducive to neuroplasticity by increasing CSE. Work by Singh et al. (2014) showed an increase in ICF and a reduction in SICI after moderate-intensity exercise, and studies by Mang et al. (2014) and Ostadan et al. (2016) used single-pulse TMS to demonstrate that a single bout of HIIE increases general CSE. We sought to extend on these findings and probe the intracortical networks (i.e. ICF, SICI and LICI) that could be responsible for the observed changes in CSE following HIIE. Given the link between increased CSE, neuroplasticity and learning, we were also interested in the effects on performing HIIE prior to engaging in a motor learning task on task performance and learning. Previous research by Roig et al. (2012) has shown increased skill retention when HIIE is performed prior to engaging in a motor learning task. Our study was designed so that participants would partake in

both TMS and the motor learning task after HIIE (over multiple study sessions), allowing us to examine the link between CSE and motor skill performance and learning.

To address our objectives, we recruited 15 (7 female) young (19-28 years), healthy participants to participate in our study. We obtained measures of CSE before, directly after, and 30 min after a bout of HIIE. Specifically, we used single-pulse TMS to assess general changes in CSE from one timepoint to the next, and we used paired-pulse TMS to probe intracortical facilitatory and inhibitory networks. In three subsequent study sessions, participants engaged in a complex movement execution task designed to measure changes in motor task performance. Data on CME task performance were then compared to data previously collected from non-exercising participants. We then sought to explore the link between CSE and motor learning performance.

Our single-pulse TMS results showed a general increase in CSE, evidence by a significant increase in area under the S-R curve from pre-exercise levels to levels measured directly after and 30 min after HIIE. Our paired-pulse TMS results revealed that, surprisingly, intracortical facilitation was significantly decreased immediately after HIIE (Post 1) and 30 min after HIIE (Post) compared to before exercise (Pre). Our results also revealed that there was no significant effect of HIIE on SICI or LICI, when comparing levels of inhibition observed before (Pre) and after (Post 1 and Post 2) HIIE. Our CME task results show that learning occurred in both the HIIE and control groups. However, engaging in HIIE prior to task practice was not shown to significantly effect CME task performance. Additionally, no correlation was observed between changes in motor task performance (operationalized as the change in learning in random and repeated shapes from S1B1 to S3B1, see *Section 4.4.5*) and changes in CSE (i.e. changes

in AUC, ICF, or SICI). Limitations of the present study and possible reasoning for our findings are discussed below. As the observed changes in CSE will be discussed in the context of our motor learning results, our second objective related to motor learning will be addressed first.

6.1 Main Findings: Motor Learning

The second objective of this thesis was to examine the effects of HIIE on learning a novel motor skill over consecutive practice sessions. Specifically, this objective was addressed by having participants perform HIIE directly prior to engaging in motor task practice. It was hypothesized that engaging in HIIE prior to the motor learning task would result in optimized motor learning (relative to those who do not perform exercise). Our results did not support this hypothesis, as the effect of group membership (i.e. HIIE group vs. control group) was insignificant. Additionally, the interaction effect of group, figure type and time was insignificant, signifying that engaging in HIIE prior to task practice did not significantly affect CME task learning. However, our results did show a significant interaction effect of figure type (random vs. repeated) and time (S1B1 vs S3B1) on error, which shows greater learning for repeating versus random shapes over time. This finding indicated that learning did occur in both the HIIE and control groups. Learning was demonstrated by 1) a smaller error value for the repeated shapes compared to random shapes, and 2) by a decrease in error from learning S1B1 to learning S3B1 (Table 5).

The null impact of HIIE on motor learning is inconsistent with previous literature. Research by Roig et al. (2012) showed enhanced motor learning in individuals who performed HIIE prior to task practice.

Our study methods deviate from those used by Roig et al. (2012) in several important ways, which could explain why our results differ. One of the key differences between our study and the work done by Roig and colleagues (2012) was the HIIE protocol. Both protocols included three 3-min bouts of high-intensity exercise, separated by 2 min of low-intensity exercise. In both studies, the high-intensity interval was performed at 80-90% of the participants PO_{max}. However, the low-intensity interval was performed at 50W in the study performed by Roig et al. (2012), and performed at 50% PO_{max} in the current study. The reasoning for this change in HIIE protocol is explained in detail above (see Section *2.5.6.2 The role of lactate in exercise and learning*). Consequently, our participants performed their low-intensity recovery intervals at 70-140W. It is possible that the increased intensity of the low-intensity recovery interval used in our study made the protocol too hard, and that increased exertion interfered with HIIE-induced facilitation of motor learning that has been reported in previous studies (Roig et al., 2012).

This reasoning is further supported by the difference in the level of exertion experienced by the participants in the current study and those in the study by Roig et al. (2012). In both studies, the Borg scale was used to record the subjective level of perceived exertion experienced by participants at the end of the last high-intensity interval. While average RPE following HIIE reported by participants in the study by Roig and colleagues (2012) was 11.69 ± 0.67, average RPE in the current study was 18.00 ± 1.41 (Average Session 2 RPE; Session 2 was used so the RPE values being compared were collected after the first time participants engaged in the HIIE protocol in the respective studies). For the sake of a rough comparison, the average level of perceived

exertion reported by participants in the study by Roig and colleagues (2012) would have been between "light" (11) and "somewhat hard" (13), while the average RPE reported by our participants was between "very hard" (17) and "extremely hard" (19). This difference shows that not only were the participants in the present study performing more work relative to their PO_{max} due to the increased low-intensity interval, but they also perceived that they were exerting more effort. RPE is a recognized integrated marker of homeostatic disturbance during exercise (Eston, 2012; Mann et al., 2017). The discrepancy in the RPE values reported after HIIE in the present study and the study by Roig and colleagues (2012) is noteworthy, as the homeostatic stress associated with an exercise bout has important implications on extent to which the responses of individuals performing an "equivalent" exercise bout can be compared (Mann et al., 2017).

The reasoning behind the discrepancy in RPE values reported in the two studies is unclear. The present study and the study by Roig and colleagues (2012) used different criteria to classify participant fitness. IPAQ data from the present study revealed that all participants were moderately to highly physically active (Table 2), and VO_2 peak scores from the study by Roig et al. (2012) revealed that their participants had average to excellent aerobic fitness (44.6 - 64.1ml/kg/min). Interestingly, research by Mang and colleagues (2014) using the same HIIE protocol as Roig et al. (2012) to examine the effects of HIIE on motor learning, reported average RPE values more similar to those observed in the present work (16.45 ± 2.22; Mang et al., 2014). Despite the higher RPE values reported, Mang and colleagues observed an effect of HIIE on plasticity (as assessed via PAS) and learning of an implicit motor task (Mang et al., 2014). The discrepancy in RPE values across the three studies (the current study, Roig et al. 2012,

and Mang et al., 2014) suggest that differences in participant characteristics may influence the response to AE (more specifically HIIE), and perhaps more importantly that there may be an upper limit to the benefits of exertion, beyond which positive effects of HIIE on cortical excitability and learning are no longer observed. As outlined above, RPE is marker of disturbance to the system, and as such higher RPE values may be associated with induction of an environment in the brain that is not conducive to plasticity.

As physical activity behaviour and aerobic fitness are two fundamentally different measures, it is difficult to compare the two groups (i.e. the HIIE group in the present study, and participants in the study by Roig et al., 2012). Previous work suggests cortical response to aerobic exercise may be better predicted by physical activity than aerobic fitness (Lulic et al., 2017; MacDonald, 2017). Research by Lulic and colleagues (2017) has recently shown that exercise-induced changes in CSE are related to physical activity, such that individuals with higher activity levels had a greater response to exercise. Additionally, work by MacDonald (2017) demonstrated that there was no significant relationship between aerobic fitness (measured using VO_2max) and cortical excitability.

Another important difference between the present study and previous work done by Roig and colleagues (2012) is the tasks used to measure motor task performance and learning; we used the CME task to measure motor learning, while they used the VAT. The lack of significant differences between the HIIE group and the control group could be due to intrinsic characteristics of the CME task. The CME task introduces a brand-new motor skill: the production of the repeated trajectory. Therefore, engaging in the CME task leads to the acquisition of a novel motor skill and the consolidation of a novel motor plan. The VAT, used in previous studies, required participants to match the movement of

a sinusoidal curve across a computer screen by using wrist movements to control an on-screen cursor; wrist extension moved the cursor upward and flexion moved the cursor downward. Modulating wrist flexion and extension is required to execute various common tasks (such as using cutlery or writing). Therefore, the improvements in VAT performance can be attributed to improvements in a pre-existing motor plan. It could therefore be suggested that the link between HIIE and motor 'learning' demonstrated in these previous studies is only applicable to the improvement of existing motor skills, and that these findings do not extend to the acquisition and consolidation of a *novel* motor plan, such as is presented in the CME.

The third key difference between our study and the work done by Roig and colleagues (2012) was our experimental design; specifically, the studies were different in terms of the frequency of both task practice and exercise. In our study, participants engaged in the CME task in three sequential study sessions (separated by one day of rest). Data from the third day of CME task engagement was used as a retention test to examine learning (specifically, the first block of practice from the third motor learning session (S3B1) was used as a measure of skill retention.) In the study conducted by Roig and colleagues (2012), motor skill acquisition was assessed following one practice session, using retention tests at 1 hr, 24 hr, and 7 days after task practice. Importantly, participants in the study by Roig et al. (2012) only engaged in HIIE prior to the task practice. They did not perform HIIE prior to skill retention testing. In their study, they observed that the exercise group showed significantly better skill retention 24h and 7 days after acquisition (but not at 1hr). It is possible that had we included additional tests of skill retention, we may have observed a significant change in motor learning over this extended time period.

However, this was not possible to do in the present study, as our control group data had already been collected and testing had not been structured in such a way for that group. Additionally, learning had never been validated over this extended time period using the CME task.

Another important difference in our experimental design was having the participant engage in HIIE prior to each time they performed the CME task. This was done so that our study design would be more applicable to the rehabilitation of motor skills (such as following a stroke). Roig and colleagues (2012) have demonstrated that exercising before motor skill practice increases learning. However, for this research to be applicable to clinical practice, it is important to understand the combined effect of several sessions of concurrent exercise and learning. Our study sought to examine if those improvements in performance were still significant the next session, when the person has just performed exercise again. It is possible that our lack of significant findings was due to the introduction of HIIE prior to each time motor performance was measured. Specifically, it is possible that engaging in HIIE prior to skill practice did result in increased consolidation of the motor skill; however, by performing exercise again before the subsequent motor skill practice session, it was not possible to distinguish these effects. It can be argued that this reasoning is further supported by extrapolating findings from Roig et al. (2012). While Roig and colleagues (2012) demonstrate that the exercise group showed significantly better retention of the motor skill at 24 hr and 7 days after practice, there was no significant difference in skill retention between exercise and control groups at 1 hr after exercise. Roig and colleagues (2012) also found that there was no significant difference in skill acquisition between participants who performed HIIE

before task practice and the control group. It could therefore be suggested that while engaging in HIIE facilitates increased motor learning, this effect is not evident directly after HIIE has been performed.

6.2 Main Findings: Corticospinal Excitability

6.2.1 Stimulus-Response Curves

As indicated above, we observed that engaging in HIIE increased CSE relative to pre-exercise levels. Our results show that AUC was significantly increased immediately after HIIE (Post 1) and 30 min after HIIE (Post 2) compared to levels measured before engaging in exercise (Pre). These findings support study Hypothesis 1 that engaging in HIIE would result in an increase in CSE. Specifically, these findings support the first part of Hypothesis 1, that an increase in the area under the stimulus-response curve would be observed from pre- to post-HIIE. These findings are in line with a previous study from our laboratory examining the effects of moderate-intensity exercise on CSE (Khan, 2016). Findings from Khan's thesis (2016) demonstrated that there was a significant upward shift in the S-R curves generated after exercise compared to baseline, when MICE was performed at 40% and 50% of HRR.

Our findings are also in line with previous research focusing on the effects of HIIE on CSE. A study by Ostadan et al., (2016) examined the effects of HIIE on CSE. This study by Ostadan and colleagues used an HIIE protocol similar to the one used in the current study; the exercise bout started with 2 min of warm-up at 50 W followed by three 3-min blocks of high-intensity exercise, separated by 2-min blocks of moderate-intensity exercise. The high-intensity intervals were performed at 85–90% of the participant's

VO_2peak attained during a previous GXT, and the low-intensity intervals were performed at 25% of the maximum workload achieved during the GXT. CE was assessed from MEP amplitude evoked in the FDI through single-pulse TMS applied to M1. Ostadan and colleagues reported that CSE was elevated for 2 hours after exercise, compared to non-exercising participants (2016). The potential mechanisms underlying the observed increase in CSE following HIIE will be discussed below.

6.2.2 Paired-Pulse Measures

We used three paired-pulse measures to assess changes in cortical excitability. These measures (ICF, SICI, and LICI) were used to probe various intracortical networks that contribute to cortical excitbaility. Paired-pulse TMS was used to probe ICF, which is an intracortical facilitatory circuit. Recent evidence suggests that ICF is mediated by I-wave facilitation (Van den Bos et al., 2018). Intracortical inhibition was assessed by studying SICI and LICI. These inhibitory networks are thought to be mediated by $GABA_A$ and $GABA_B$, respectively.

6.2.2.1 Intracortical Facilitation

As indicated above, we hypothesized that intracortical facilitation would be increased by engaging in HIIE. However, our results indicated that ICF was significantly decreased following HIIE. A one-way repeated measures ANOVA showed a significant main effect of time point (Pre, Post 1, Post 2) on average MEP amplitude for ICF paired pulse measures. Follow-up tests revealed that MEP amplitude evoked by ICF was significantly decreased from Pre to Post 1 timepoints, and from Pre to Post 2 timepoints. The difference in MEP amplitude evoked by ICF from Post 1 to Post 2 was not

significant. These findings are in opposition to our hypothesis. To our knowledge, our study was the first to use paired-pulse TMS to probe changes in intracortical networks following HIIE. Our hypothesis was based on findings of increased ICF following engagement in moderate intensity exercise (e.g. Singh et al., 2014). However, it is possible that such increases in this facilitatory network do not extend to HIIE. The potential mechanisms underlying the observed decrease in ICF following HIIE will be discussed below.

6.2.2.2 Intracortical Inhibition

We also used paired-pulse TMS to examine SICI and LICI. As stated previously, we hypothesized that intracortical inhibition (both SICI and LICI) would be decreased following engagement in HIIE. Overall, for both SICI and LICI paired-pulse measures, our data show that MEP amplitude did not increase significantly directly after or 30 min after engaging in HIIE. As mentioned above, we are not aware of any previous studies examining changes in SICI or LICI in response to HIIE. However, previous studies of moderate-intensity AE have shown decreases in inhibition following MICE. Specifically, research by Singh et al. (2014) showed that SICI was significantly decreased from Pre to Post 2 (30 min after exercise). They also observed a decrease in LICI from Pre to Post 1 (immediately after exercise) and Post 2, however, these differences were not statistically significant (Singh et al., 2014).

6.2.3 Potential mechanisms underlying observed changes in CSE

The current study used four mechanisms to measure HIIE-induced changes in cortical excitability: 1) single-pulse TMS to examine general CSE, and paired-pulse TMS

to examine 2) ICF, 3) SICI and 4) LICI. The four TMS methods used probe fundamentally different aspects of motor CSE (Ilic et al., 2002). Our results demonstrated an increase in general CSE assessed using single-pulse TMS, and a decrease in ICF assessed using paired-pulse TMS. Previous studies have reported a similar disconnect between single and paired-pulse measures of CE (Ilic et al., 2002; Singh et al., 2014). In the exercise and TMS literature, Singh and colleagues (2014) reported an increase in ICF and a decrease in SICI following a 20 min bout of MICE. Both of these reported changes are reflective of increased cortical excitability. However, Singh et al. (2014) also reported that no differences were observed in single-pulse S-R curves following exercise. TMS is often also used to examine the mechanisms through which psychiatric medications modulate cortical excitatory and inhibitory effects. A study by Ilic and colleagues (2002) investigated the effects of the selective serotonin re-uptake inhibitor sertraline on human motor cortex excitability. This study used TMS to demonstrate that sertraline resulted in a steeper S-R curve and depressed paired-pulse facilitation.

These findings of increased general CSE assessed using single-pulse TMS and decreased ICF using paired-pulse TMS reported by Ilic et al. (2002) are in line with the findings from the current study. Although the observed increase in the area under the S-R curve (increased excitability) and decrease in ICF (decreased excitability) may appear incongruous, these findings can be explained by the fundamentally different aspects of CSE probed by the two measures. Single-pulse TMS assesses the overall excitability of the corticospinal tract, which is reflective of all inhibitory and excitatory inputs, both cortical and subcortical, to the descending motor neuron (Singh et al., 2014). ICF is

reflective of the activity of a particular pool of cortical interneurons, which is one of many inputs to the descending neuron (Singh et al., 2014).

6.2.4 Further examination of CSE following HIIE

The current thesis is part of a larger two group study in which 15 participants performed HIIE before CME task practice, and 15 participants performed HIIE after CME task practice. TMS data were collected for all 30 participants before, directly after and 30 min after performing HIIE. It is possible that examining data from all 30 participants may allow us to observe significant changes in intracortical inhibition.

6.3 Main Findings: Link between CSE and Motor Learning

The third objective of this study was to determine if there was a relationship between increased CSE after HIIE and motor learning, when motor skill practice is preceded by HIIE. We hypothesized that there would be a significant, positive relationship between the change in motor task performance (from the beginning of CME task practice to the retention test) and change in the various measures of corticospinal excitability from pre- to post-HIIE levels. However, the correlations between change in motor task performance and each of the measures of excitability examined (AUC, ICF and SICI) were far from significant (i.e. $p = .33$ to $p = .77$).

The learning score used to examine this correlation was reflective of each participant's performance on random and repeated shapes during both the first block of task practice (S1B1) and at the retention timepoint (S3B1). Specifically, for each participant, mean error in execution of the repeated shapes was subtracted from mean

error in the random shapes for S1B1 and S3B1. The difference in error from S1B1 to S3B1 was then calculated.

As we did not observe a significant difference in CME task execution between participants who performed HIIE prior to task practice and our non-exercising controls, it is not surprising that no significant correlations between learning and CSE were detected. Nonetheless, future studies examining this link using the CME should use an altered measure of learning. Our learning score did not take into account the various speeds at which the task was performed. Bayesian multi-level modelling could be used to model the data, creating one metric that could take both figure type (random vs. repeated) and speed into account at each time point.

6.4 Limitations

There are a number of limitations to the current study. The first of which is that our study did not take into account the effects of regular physical activity on HIIE-induced changes in CSE. Recent research from Lulic and colleagues (2017) demonstrated that physical activity level influences motor cortex excitability. This finding was not controlled for in the present study. However, we did administer the IPAQ to collect information on participants' physical activity leading up to study participation, and this was used to categorize participants' level of physical activity as high, moderate or low. This data will be included in the analysis examining the TMS data from both groups in the larger two group study outlined above (i.e. 15 participants who performed HIIE before CME task practice, and 15 participants who performed HIIE after CME task practice). As the effect of regular physical activity is a limitation to a number of studies

using exercise to modulate CSE, an upcoming study in our laboratory will attempt to further explore the link between regular physical activity and changes in CE in response to three different intensities of exercise.

Another limitation of the current study is the control group that was used. As mentioned previously, the control group consisted of 15 non-exercising participants who engaged in the same motor learning task; the CME task. However, as the control data were collected for a different study, data collection was not structured to make it the ideal control group for the current study. Ideally, participants in the control group would have been asked to rest upon arriving at the laboratory each day; this rest period would have been 23 minutes, to match the duration of the HIIE protocol. Following the rest period, participants could then complete the CME task. Participants would also have been given clear instructions to refrain from vigorous exercise throughout the day on which CME task practice occurred, to eliminate any confounding effects of other bouts of exercise on motor skill acquisition and consolidation. Another issue with the control group is that data collection was not structured to occur every other day. As participants in the original CME task study did not perform intense exercise, they were able to complete task practice on consecutive days. CME task practice in the present study occurred every other day, to allow for sufficient time for participants to recover from the HIIE that preceded the motor learning task.

There are a few independent variables that influence CSE that were not tightly controlled for in the present study. The first is time of day at which the exercise sessions were performed. Although we did attempt to control for this, not all experimental sessions were completed at the same time of day due to participants' schedules. Another variable

known to influence CSE is caffeine. Participants were asked to refrain from consuming caffeine for at least 2 hours before the test, but adherence to this request was not confirmed.

Another limitation of the present study was that we did not account for timing of the menstrual cycle in female participants, which is known to affect cortical excitability (Smith et al., 1999). Progesterone metabolites are known to enhance the action of GABA (Smith et al., 1999), thus increasing cortical inhibition, and estradiol is known to enhance excitatory neurotransmission (Smith et al., 2002). Smith and colleagues used paired-pulse TMS to probe intracortical networks in females during various stages of the menstrual cycle (Smith et al., 1999; Smith et al., 2002). Cortical excitability was shown to increase from the early follicular (low estradiol, low progesterone) phase to the late follicular (high estradiol, low progesterone) phase of the menstrual cycle (Smith et al., 2002). Cortical excitability was also shown to decrease from the late follicular phase to the luteal (high estradiol, high progesterone) phase (Smith et al., 1999; Smith et al., 2002). Because timing of the menstrual cycle was not controlled for in female participants in the current study, additional variability was introduced into the data.

It should also be noted that numerous previous studies that have demonstrated that HIIE can be used to facilitate improved motor learning used only male participants (Roig et al., 2012; Skriver et al., 2014; Thomas et al., 2016). It can therefore be argued whether the significance of their findings can be extrapolated to females, and to females at all stages of their menstrual cycle. While it is important that this research continues to be conducted in both male and female participants, future studies should consider analysing data from the two sexes separately.

A final limitation of the present study was screening for CME task participation, and follow-up questionnaires regarding attentiveness during task execution. Other studies examining motor learning use the Rapid Visual Processing test to measure sustained attention, and also employ tests of spatial working memory. It has also been suggested that due to the complexity of the CME task it would be beneficial to add a questionnaire about learning disabilities such as dyslexia to our pre-study questionnaires. Certain participants expressed that they found the task difficult more strongly than others. While anecdotal, this could perhaps show that the task was in fact more difficult for some individuals than others.

6.5 Conclusion

Performing HIIE was shown to significantly increase CSE, as evidenced by an increase in AUC from Pre to Post 1 and Post 2 timepoints. We also observed a significant decrease in ICF from Pre to Post 1 and Post 2 timepoints. No significant changes in SICI or LICI were detected. Performing HIIE before engaging in repeated sessions of motor task practice failed to demonstrate a significant effect of HIIE on motor learning compared to non-exercising controls. Additionally, we were unable to detect a significant correlation between changes in CSE and motor learning in participants who underwent TMS and engaged in the CME task after HIIE.

Future research is needed to further examine the observed decrease in ICF, as this study was the first to examine HIIE-induced changes in intracortical networks. Additional studies investigating HIIE-induced changes in overall CSE and ICF (and other measures of intracortical network excitability) should be performed with other motor tasks that

have been shown to be responsive to the effects of HIIE on motor learning (e.g. the VAT, Roig et al., 2012). These same measures of CSE should also be examined following additional HIIE protocols, to determine if the observed changes in CSE vary between protocols and the level of exertion experienced by the participants.

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APPENDIX 1: Recruitment Form

How does exercise affect learning?

Volunteers Needed!

We are recruiting for a study using brain stimulation to look at how exercise (specifically, High Intensity Interval Training) affects motor learning.

You will be asked to attend 5 sessions, on separate days.
One of these sessions will determine your maximum heart rate. This will require you to do a maximal exercise test.

The study will also involve transcranial magnetic stimulation (TMS). TMS allows us to look at brain function to measure the properties of the brain, like how easy it is to turn on a particular brain region.

You will be asked to participate in 5 sessions, which will take about 6.5 hours in total. This study will be performed in the Laboratory for Brain Recovery and Function in the School of Physiotherapy.

You will receive $10 / visit. To be eligible to volunteer, you must be between 18 and 40 years of age and not have any brain, lung or heart conditions. You must also have normal or corrected-to-normal vision.

Who to Contact: Emily Rogers, Graduate Student
Email: emily.rogers@dal.ca

Study Title: Understanding the effects of high intensity interval training (HIIT) on cortical excitability and motor learning

Exercise & Learning emily.rogers@dal.ca	Exercise & Learning emily.rogers@dal.ca	Exercise & Learning emily.rogers@dal.ca	Exercise & Learning emily.rogers@dal.ca	Exercise & Learning emily.rogers@dal.ca	Exercise & Learning emily.rogers@dal.ca	Exercise & Learning emily.rogers@dal.ca

APPENDIX 2: Information Letter

INFORMATION LETTER- **Examining the Effect of High Intensity Interval Training on Motor Learning**

Dear participant,

Thank you for your interest in "Examining the Effect of High Intensity Interval Training on Motor Learning", a study being conducted by Emily Rogers, a Masters of Science (Rehabilitation Research) candidate; Allison Keller, a Bachelor of Kinesiology with Honours candidate; and Dr. Shaun Boe from School of Physiotherapy at Dalhousie University. We are investigating how performing High Intensity Interval Training (HIIT) before learning a new skill affects your ability to learn that skill. In order to participate you must:

- Be between 18 and 40 years of age
- Have normal or corrected-to-normal vision
- Be a non-smoker
- Never have been diagnosed with a cardiovascular, respiratory, or neurological disease
- Have never been told that you are not allowed to perform aerobic exercise by your doctor.
- Be eligible for non-invasive brain stimulation as per a screening form.

There are 4 files attached in this email:

- Two screening forms, called the PAR-Q and the TMS Screening Form. We ask you to complete these screening forms; if you answer, "yes" to any item on the PAR-Q, or any of the first 9 questions on the TMS screening form, you are ineligible to take part of this study. In this case, email the researcher that you are not eligible (you do not have to tell what is the reason for ineligibility).
- An informed consent form: please read this form as it describes what you will be doing as part of the study, as well as outlines any risks and benefits.
- A questionnaire called the IPAQ that will help us determine your physical activity level (You can have a look at it, but don't worry about filling it out at this time).

If you are eligible to participate in the study, we will ask you to attend 5 laboratory sessions within a three-week period (a total time commitment of ~6.5 hours). Participants will be compensated $10 per visit, whether they complete the session or not. Participation is voluntary and you are free to withdraw from the study at any time, without consequences. You are not obliged to answer any questions or participate in any activities that you find objectionable or which make you feel uncomfortable. All information we collect is confidential.

On testing days, we will ask you to please bring clothing you are comfortable in (there will be somewhere private where you may change), to perform cycling exercise.
In preparation for each of the next testing session, we ask that you follow these guidelines:

- Maintain the **similar diet:** eat same amount and same type of food (at the same timing) at least 2 hours before you come to the test.

(e.g. if you're scheduled to start the session at 4 p.m., and you had a light sandwich and a piece of fruit during the time between 2 to 4 p.m., we ask that you have a similar meal and similar portion during the same time, before the next sessions)

- Please refrain from **caffeine, heavy meals, and alcohol** for at least 2 hours before the test as these subtances are known to have effects on the things we will be measuring.

If you are interested in this study or have any questions, please reply to this email and we will arrange a time that is convenient for you. Please feel free to contact emily.rogers@dal.ca or (902) 521-3045 if you have any questions.

Thank you for your interest,
Emily Rogers

APPENDIX 3: Physical Activity Readiness Questionnaire (PAR-Q)

Participant ID: _____

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.

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.

CSEP | SCPE

APPENDIX 4: TMS Screening form

LABORATORY FOR
BRAIN RECOVERY AND FUNCTION
SCHOOL OF PHYSIOTHERAPY
DALHOUSIE UNIVERSITY
Inspiring Minds

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.

Participant Study ID: _____

PLEASE COMPLETE THE QUESTIONS BELOW

Questions	**Yes**	**No**
1. Do you have epilepsy or have you ever had a convulsion or a seizure?	☐	☐
2. Have you ever had a head trauma that was diagnosed as a concussion or was associated with a loss of consciousness?	☐	☐
3. Do you have any hearing problems or ringing in your ears?	☐	☐
4. Do you have cochlear implants?	☐	☐
5. Are you pregnant or is there any chance that you might be?	☐	☐
6. Do you have an implanted neurostimulator (e.g., DBS, epidural/subdural, VNS)?	☐	☐
7. Do you have a cardiac pacemaker or intracardiac lines?	☐	☐
8. Do you have a medication infusion device?	☐	☐

9. Have you ever had a fainting spell or syncope (loss of consciousness)? ☐ ☐
If yes, please describe on which occasion:

10. Are you taking any medications? ☐ ☐

(Taking medications that alter brain excitability, such as medicines used to treat depression, anxiety, and psychotic conditions, would make you ineligible for study participation.)

If yes, please list:

11. Do you have metal in the brain, skull or elsewhere in your body (e.g., splinters, fragments, clips, etc.)? If so, please specify: ☐ ☐

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: ☐ ☐

If you answer, "yes" to any of the first 8 questions you are ineligible for this study. If you answered yes to questions 9 -13, researchers will discuss with you how, if at all, this affects your participation in the study.

* 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 5: Edinburgh Handedness Inventory

Participant ID: _____

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	☐☐	☐☐

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 6: International Physical Activity Questionnaire (IPAQ)

Participant ID: _____

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
(August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week**

☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

APPENDIX 7: Calculating Continuous and Categorical IPAQ Scores

At A Glance
IPAQ Scoring Protocol (Short Forms)

Continuous Score

Expressed as MET-min per week: MET level x minutes of activity/day x days per week

	Sample Calculation
MET levels	**MET-minutes/week for 30 min/day, 5 days**
Walking = 3.3 METs	3.3*30*5 = 495 MET-minutes/week
Moderate Intensity = 4.0 METs	4.0*30*5 = 600 MET-minutes/week
Vigorous Intensity = 8.0 METs	8.0*30*5 = 1,200 MET-minutes/week
	TOTAL = 2,295 MET-minutes/week

Total MET-minutes/week = Walk (METs*min*days) + Mod (METs*min*days) + Vig (METs*min*days)

Categorical Score- three levels of physical activity are proposed

1. **Low**

   - No activity is reported **OR**
   - Some activity is reported but not enough to meet Categories 2 or 3.

2. **Moderate**

   Either of the following 3 criteria
   - 3 or more days of vigorous activity of at least 20 minutes per day **OR**
   - 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day **OR**
   - 5 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum of at least 600 MET-minutes/week.

3. **High**

   Any one of the following 2 criteria
   - Vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week **OR**
   - 7 or more days of any combination of walking, moderate- or vigorous-intensity activities accumulating at least 3000 MET-minutes/week

From: The IPAQ Group. (2015). *Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire*.

APPENDIX 8: Health History Questionnaire

Participant ID: _____

1. Age: _____

2. Sex
    a. Male
    b. Female

3. What is your approximate weight (kilograms)? _____
   To convert from pounds to kilograms, multiply by 0.454

4. What is your approximate height (meters)? _____
   To convert from inches to meters, multiply by 0.0254

5. Please calculate your approximate BMI (*you will be provided with a calculator*):
   BMI = weight/ height^2 = kg/m^2 = _____

6. At any point in the last 6 months were you a regular smoker?
    a. Yes
    b. No

7. If you do any exercise, what types of exercise do you do? Please be specific (i.e.running, swimming, soccer, basketball, etc.)

   _____

   _____

   _____

APPENDIX 9: Informed Consent

DALHOUSIE UNIVERSITY

LABORATORY FOR BRAIN RECOVERY AND FUNCTION
SCHOOL OF PHYSIOTHERAPY

CONSENT FORM

Project Title: Examining the Effect of High Intensity Interval Training on Motor Learning

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Introduction

You have been invited to take part in a research study. A research study is a way of gathering information on a treatment, procedure or medical device or to answer a question about something that is not well understood. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study.

Please read this carefully. Take as much time as you like. Mark anything you don't understand, or want explained better. After you have read it, please ask questions about anything that is not clear.

The researchers will:

- Discuss the study with you
- Answer your questions
- Keep confidential any information which could identify you personally
- Be available during the study to deal with problems and answer questions

You are being asked to take part in this study because you replied to our advertisement, you meet the study requirements, and you are free of any brain injury or disease and meet the inclusion criteria for the study.

Purpose and Outline of the Research Study

We learn new skills by practicing them over and over again. This repetitive practice causes connections in our brain to strengthen, which is the basis for learning. Exercise has been shown to make strengthening brain connections easier by increasing how excitable the brain is. This study will examine how one specific kind of exercise, called High Intensity Interval Training (HIIT) changes brain excitability as well as how this kind of exercise helps with learning a new skill. The information gathered in this study will tell us a lot about how exercise affects the brain, and how exercise can be used to make learning new skills better.

Who Can Participate in the Research Study?

You may participate in this study if you are between 18 and 40 years old, and have no self-reported history of neurological (brain), cardiovascular (heart), or pulmonary (lung) disorders. You must also have normal or corrected-to-normal (that is you wear glasses or contact lenses) vision. Additionally we will ensure you can undergo all of the study procedures by screening for specific conditions (we describe this below).

You will not be eligible for this study if you:
- Are a smoker
- Have ever been told by your doctor that you are not allowed to perform exercise
- Have any conditions precluding participation in non-invasive brain stimulation, as determined by screening

How many people are taking part in the study?

30 individuals will be participating in this study.

What You Will Be Asked to Do?

Screening
You be asked to complete some questionnaires to see if you can take part. This is called screening. It is possible that the screening results will show that you can't be in the study. The research team will discuss these with you.
We will do two screening tests. The first is a questionnaire to determine if you can participate in transcranial magnetic stimulation (TMS; described in the next section). We will be using this technique to assess brain excitability. This set of questions will take about 5 minutes to complete. The answers to the questions will determine whether or not you have any conditions that could possibly cause you harm if you were to have brain stimulation (TMS). The second is a questionnaire to determine if it is safe for you to exercise. This questionnaire, called the PAR-Q, will take about 3 minutes to complete.

Following screening, if you are eligible to participate, you will be asked to attend 5 sessions *over a period of 3 weeks* for a total time commitment of ~6.5 hours.
All session must occur within a 3-week time period. Sessions 3-5 (the learning sessions) must be completed within 3 days. All sessions will take place in the Laboratory for Brain Recovery and Function (Dalhousie University).

During the maximal exercise test session (session 1, 1.5 hours):

You will be asked first to complete the screening forms (PAR-Q and TMS screening form), a Health History Questionnaire and the International Physical Activity Questionnaire (IPAQ) that have been emailed to you and to sign this informed consent form. The Health History Questionnaire will ask questions about your age, sex, height, weight, and the types of exercise you do, if any. The IPAQ will ask you about the time you spent being physically active in the **last 7 days**.

You will then be shown the equipment we will use in the study and you will have a chance to ask any questions. After this, we will direct you to a private change room if you need to change into comfortable clothing for the duration of the test. You will begin the session by sitting on a stationary bike quietly for 5 minutes to measure your resting heart rate. Then, you will start cycling on the stationary bike and your heart rate will be monitored to determine your maximal heart rate for aerobic exercise. The cycling part of this session takes a different amount of time for each person, but should last between 15 and 20 minutes. In total, this first session will last approximately 1.5 hours.

During the brain activity assessment session (session 2, 2 hours):
Each of the participants will perform the following testing procedures:

Transcranial Magnetic Stimulation (TMS)
TMS is a machine that uses electricity to create a magnetic field. TMS involves delivering brief magnetic pulses over different locations on your head. Basically a TMS machine stores electricity, and then uses this electricity to make a magnetic field in a small coil that is held over your head. The magnetic field creates a flow of electrical current in your head. We can use TMS to measure the properties of the brain like how easy it is to turn on a particular brain region.

Muscle activity
Activity in your muscles will be measured using electromyography (EMG). EMG involves attaching two electrodes (like stickers) to the skin over the muscles of the forearm. Because of the location of these electrodes, it would be best to wear a short-sleeved shirt for the study

High Intensity Interval Training (HIIT) on a stationary bike
HIIT will be performed on a stationary bicycle, and will involve three 3-minute sets of high intensity exercise, separated by 2 minutes of low intensity exercise.

Watch to monitor your heart rate ('Mio watch')
The 'Mio watch' is simply a watch that acts as a heart rate monitoring device that allows us to measure your heart rate in real time or record the heart rate for later analysis.

Overview of session 2
As you arrive, you will be asked to sit in a reclined position on a chair and the TMS coil will be positioned on or near your head. You will be asked to keep your head as still as possible. This procedure is not painful. You will hear a clicking noise as the current flows through the coil. When determining the position of the TMS coil, the pulses may cause your finger to move. You may also feel some tingling sensations on the head where the TMS coil is located. During this part of the study, we will record muscle activity from your hand as we have described above. Following this, you will experience magnetic pulses for approximately 5 minutes. We will ask you to wear disposable earplugs (which we will provide) while you receive the magnetic stimulation to protect your hearing from the clicking noises.

After you finish the TMS session, you will complete HIIT (plus 5 min warming-up and 5 min cooling-down). Throughout we will monitor your heart rate using the Mio watch (outlined above). As you finish cycling, you are going to transfer back to the TMS chair to let us take the brain measurements again. This time we will take brain measurements twice, immediately after you finish HIIT, and again 30 minutes after exercise. After this is done, the testing is completed. In total, this second session will last approximately 2 hours.

During the learning assessment sessions (sessions 3-5, 1 hour each):

We will be using a training task to teach you a new skill. At the beginning of the study, you will be assigned to one of two study groups. Group 1 will perform the previously described HIIT protocol (three 3-minute sets of high intensity exercise, separated by 2 minutes of low intensity exercise) immediately before doing the training task. Group 2 will perform HIIT immediately after completing the training task.

To perform the training task, you will be seated comfortably in a chair facing a table with the task set up in front of you. The task involves using a touchscreen monitor on which you will perform the task. The training task involves watching a white circle move on the screen in different patterns, always beginning and ending at the same location. After the white circle disappears, a red circle will prompt you to begin. You will recreate the pattern by tracing the pattern on the touchscreen using your fingertip. Once you return to the red circle the trial is over. You will be provided with breaks to make sure you do not tire. During the study we will not be able to talk to you about your performance of the learning sessions, but we can discuss these results with you after the study session.

Possible Benefits, Risks, and Discomforts
BENEFITS:

This study has the potential to benefit society through the generation of knowledge regarding the effect of aerobic fitness and exercise on the brain, as well as the effect of exercise on learning a new skill.

RISKS:

Presented here are the potential risks and discomforts that may arise throughout the duration of the study.

Potential risks during Maximal Exercise Testing:
Nearing the end of the exercise test, you will experience shortness of breath, muscular fatigue, and an increased heart rate, while dizziness, nausea, muscular pain and profuse sweating may occur. These symptoms should subside as soon as the test is over, or shortly thereafter. If these symptoms persist or worsen, investigators qualified in first aid response will monitor the participants' condition and call for medical assistance if required. Some solutions to help reduce symptoms include slowly walking around, small sips of water or lying down with the legs elevated above the heart. An active cool down period is prescribed to alleviate any symptoms arising from the maximal exercise. The cool down period will be considered complete when the heart rate of the participant falls below 50% of their age-predicted maximum heart rate. Studies have shown that only an average of 2.4 in 10000 participants will experience any adverse outcomes from this protocol that will require immediate medical treatment and this represented a population of variable health.

Potential risks of using TMS:

TMS has been approved in Canada for both therapeutic and research use, and has been used in various studies worldwide since 1985. TMS has been shown to be extremely safe as long as proper safety precautions are taken. In general, the TMS procedure produces no pain and causes no known short-term or long-term damage of any kind. We will contact you if any new risks are discovered during the time of this study. Please contact us if you experience any effects that you feel may be a result of your participation in the study.

TMS is painless, although some forms of TMS can cause tingling or twitching of muscles in the face, which may lead to soreness. This is not likely to occur in this study, as we are not using that form of TMS.

Common risks: 1-10% people have experienced headaches, which are caused by 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.

Rare risks: .01-.1% people have experienced the following:

- In rare cases, seizures have been known to occur after TMS. However, the risk of seizure is *very low* except in people with epilepsy or people taking certain medications. You will be asked to complete a TMS screening form, and precautions will be taken to ensure your safety. Despite these precautions, TMS can induce a convulsion even in people who do not have brain lesions, epilepsy or other risk factors for seizures. However, only 16 cases of convulsions induced by TMS in participants without risk factors for epilepsy have been reported despite the fact that many thousands of subjects have been studied world-wide. The overall risk for seizures during TMS is thought to be less than 1 in 1,000 patients. As with seizures in general, the seizures induced by TMS are usually brief and without serious physical consequences. In total, only 2 instances of seizure have been reported in participants undergoing the forms of magnetic stimulation that will be used during this study. In both of these cases, the participants were diagnosed with a neurological disorder and each were taking medications that alter brain excitability.

As indicated above, TMS produces a loud clicking noise when the current passes through the coil. This loud click can result in tinnitus and transient decreased hearing if no ear protection is used. To prevent this adverse effect both the TMS operator and participants wear earplugs during the application of TMS. Studies have shown that earplugs can effectively prevent the risk of hearing disturbances.

TMS is generally safe unless you have metal or magnetized objects in your body. Examples of these metal objects are cardiac pacemakers, surgical clips (e.g., aneurysm clips in your head), artificial heart valves, cochlear implants, metal fragments in your eyes, electronic stimulators, and implanted pumps. If you have any of these, you will not be able to participate in this study.

<u>Potential risks of recording muscle activity (EMG)</u>

There is minimal risk related to the use of this technique. The electrodes lie on top of the skin (like a sticker on your skin) and a conductive gel provides the contact between the skin and the electrodes. In uncommon instances (.01- .1%) it is possible that your skin may be sensitive to the conductive gel, alcohol or adhesive used in the application of the electrodes. In such cases a rash or reddening of the skin is possible. This usually goes away in less than 24 hours.

To minimize the risks associated with this study researchers are trained in Emergency first aid with CPR "C"/AED.

If for any reason we find information that may show a possible health risk, we will explain the issue to you and strongly recommend that you visit your family doctor. You will no longer be eligible to participate in the study.

What you will receive for taking part:

There is $10 per session given to participants taking part in this research study. This money is meant to cover the cost related to travel to the lab sessions and will be provided regardless of whether you complete the session or not.

Juice and snacks will be provided after you complete the session.

How your information will be protected:
Privacy: Protecting your privacy is an important part of this study. Every effort to protect your privacy will be made. No identifying information (such as your name) will be sent outside of Dalhousie University. If the results of this study are presented to the public, nobody will be able to tell that you were in the study.
If you decide to participate in this study, the research team will look at your personal information and collect only the information they need for this study, such as your;

- Name
- Age
- Information from the study questionnaires

Confidentiality: In order to protect your privacy and keep your participation in the study confidential, you will be de-identified using a study code. For the purpose of data analyses, all participants will only be identified by their study code (e.g. s001). All hard copy data associated with the study (including this consent form) will be stored in a locked cabinet in a secured laboratory that is accessible only to lab personnel via personalized pin codes and who are trained in confidentiality. All data collected will be stored on a secure, password-protected server in the Laboratory for Brain Recovery and Function. No documentation will exist (hard copy or electronic) that links your name with your study code.

Data retention: Information that you provide to us will be kept private. Only the research team at Dalhousie University will have access to this information. We will describe and share our findings in theses, presentations, public media, journal articles, etc. We will be very careful to only talk about group results so that no one will be identified. This means that ***you will not be identified in any way in our reports***. The people who work with us have an obligation to keep all research information private. Also, we will use a participant number (not your name) in our written and computer records so that the information we have about you contains no names. All your identifying information will be securely stored. All electronic records will be kept secure, password protected server in the Laboratory for Brain Recovery and Function.

If You Decide to Stop Participating
You may choose not to continue your participation in the study at any time. If you decide not to take part in the study or if you leave any session early, your data will be automatically withdrawn from the study. If you complete your session, your data may be withdrawn up until the point of data analysis.

How to Obtain Results

If you would like a description of the results at the end of the study, you can obtain a short description of these results by visiting boelab.com in approximately 12 months. You may request data related to your maximal exercise testing from the investigator. Otherwise no individual results will be provided.

Questions

We are happy to talk with you about any questions or concerns you may have about your participation in this research study. Please contact Emily Rogers at Emily.Rogers@dal.ca or (902) 521-3045 or Dr. Shaun Boe at s.boe@dal.ca or (902) 494-6360 at any time with questions, comments, or concerns about the research study. We will also tell you if any new information comes up that could affect your decision to participate.

If you have any ethical concerns about your participation in this research, you may also contact Catherine Connors, Director, Research Ethics, Dalhousie University at (902) 494-1462, or email: ethics@dal.ca

Signature Page

Project Title:
Examining the Effect of High Intensity Interval Training on Motor Learning

Lead Researcher:
Shaun Boe, MPT, PhD

I have read the explanation about this study. I have been given the opportunity to discuss it and my questions have been answered to my satisfaction. I agree to take part in this study. However I realize that my participation is voluntary and that I am free to withdraw from the study at any time.

Participant's Signature	DATE
Print Name of Participant	DATE
Signature of Witness	DATE

APPENDIX 10: Borg Rating of Perceived Exertion Scale

Rating	Perceived Exertion
6	No exertion
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

From: G.A.U. Borg., "Psychological Bases of Physical Exertion" in Medicine and Science in Sports and Exercise, 14: 377-81, 1982.