EXAMINING THE RELATIONSHIP BETWEEN AEROBIC FITNESS AND CORTICAL EXCITABILITY

Monica A. MacDonald

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

at

Dalhousie University
Halifax, Nova Scotia
August 2017

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Because of its ability to enhance cortical excitability, aerobic exercise is receiving interest as a primer for learning and rehabilitation. Previous studies show that physical activity level influences the brain's response to exercise. The purpose of this study was to examine the relationship between aerobic fitness and cortical excitability, as well as exercise-induced changes in excitability. Transcranial magnetic stimulation was used to obtain measures of excitability including the stimulus-response curve and those related to intracortical facilitatory and inhibitory networks before, immediately after, and 30 minutes after a session of aerobic exercise. We hypothesized that 1) increased fitness would be associated with increased cortical excitability at baseline; and 2) increased fitness would be associated with a larger response to the exercise, namely greater changes in cortical excitability relative to baseline. Our results did not support the hypotheses, suggesting that higher aerobic fitness may not result in greater cortical excitability.

LIST OF ABBREVIATIONS AND SYMBOLS USED

Δ – Change in

ACSM – American College of Sports Medicine

AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

APB – Abductor Pollicis Brevis

AUC – Area Under the Curve

BDNF – Brain-derived neurotrophic factor

Ca^{2+} – Calcium ion

CIMT – Constraint-Induced Movement Therapy

CS – Conditioning Stimulus

ECR - Extensor Carpi Radialis

EMG - Electromyography

fMRI – Functional Magnetic Resonance Imaging

GABA – γ-aminobutyric Acid

GXT – Graded Exercise Test

HR – Heart Rate

HRmax – Maximal Heart Rate

ICF – Intracortical Facilitation

IGF-1 – Insulin-like Growth Factor 1

IPAQ – International Physical Activity Questionnaire

ISI – Interstimulus Interval

LICI – Long-Interval Intracortical Inhibition

LTD – Long-Term Depression

LTP – Long-Term Potentiation

M1 – Motor Cortex

MAL – Motor Activity Logs

MEP – Motor Evoked Potential

MRI – Magnetic Resonance Imaging

MT – Motor Threshold

Na^+ – Sodium ion

NMDA – N-methyl-D-aspartate

PAR-Q - Physical Activity Readiness Questionnaire

PAS – Paired Associative Stimulation

RMT – Resting motor threshold

RPE – Rating of Perceived Exertion

S-R – Stimulus-Response

SICI – Short-Interval Intracortical Inhibition

tDCS – Transcranial direct-current stimulation

TMS – Transcranial Magnetic Stimulation

TS – Test Stimulus

VEGF – Vascular endothelial growth factor

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor Dr. Shaun Boe for his tremendous support and guidance throughout my thesis. He has been an exceptional mentor, helping me to become a better researcher, as well as a great confidant, supporting me when I really needed it. He has been everything I could have asked for from a supervisor and I would not have been able to do this without him.

I would also like to thank my committee members, Dr. Derek Kimmerly & Dr. Heather Neyedli, for their input into this project. They helped steer the project in the right direction and provided guidance along the way.

I would like to thank the faculty and staff at Dalhousie's School of Physiotherapy for their support and encouragement throughout my dual program. In particular, I would like to acknowledge Dr. Gail Dechman who has been a great advisor and friend.

I would also like to thank my fellow members of the Laboratory for Brain Recovery and Function for all of their help with the project, particularly Emily, Alex, Sarah and Francesco. The lab is made of an exceptional group of people and I am honoured to be a part of it.

As well, I would like to thank the following funding agencies for their financial support during this project: the Natural Sciences and Engineering Research Council, for the Masters Canada Graduate Scholarship, and the Government of Nova Scotia, for the Nova Scotia Graduate Scholarship.

Finally, I would like to extend my thanks to my family and friends for their unconditional support throughout this program. They have helped many times along the way and I am very grateful. I have some exceptional people in my life.

Many thanks,
Monica

CHAPTER 1: INTRODUCTION

Learning is an integral part of human development. Our capacity to learn comes from the ability of the human brain to change. This ability to change is important not only for learning, but for recovery from brain injury as well. Increases in brain excitability can create an ideal environment for changes associated with learning and recovery (Schabrun & Chipchase, 2012). If motor learning is the aim of therapies used in neurological rehabilitation, it may be beneficial to increase the excitability of the brain prior to such rehabilitation interventions. Priming the brain, or enhancing the sensitivity of the brain prior to motor learning and rehabilitation, may translate into greater clinical outcomes than can be achieved with traditional therapies alone (Stinear, Barber, Coxon, Fleming, & Byblow, 2008).

Priming techniques are gaining interest for use in rehabilitation (Schabrun & Chipchase, 2012). Researchers have begun to investigate the use of aerobic exercise as a means to enhance neuroplasticity and functional outcomes after stroke (Mang, Campbell, Ross, & Boyd, 2013). Regular bouts of aerobic exercise can lead to significant general health benefits; it also has robust effects on brain structure and function (Cotman, Berchtold, & Christie, 2007; Thomas, Dennis, Bandettini, & Johansen-Berg, 2012). Because of the safety and ease of use of aerobic exercise, it has the potential to be a very useful tool for rehabilitation.

Aerobic fitness, one component of physical fitness, is the ability of the body to transport and utilize oxygen in the exercising muscles. Studies have shown that

aerobic fitness can alter brain morphology, whereby individuals with higher aerobic fitness have a decrease in age-related cortical degeneration (Erickson et al., 2011). Aerobic fitness can also influence brain function, as studies have shown improved memory and cognition in individuals with higher aerobic fitness (Colcombe & Kramer, 2003; Colcombe et al., 2004). Although the mechanisms behind these changes remain to be determined, proposed mechanisms include both peripheral and central factors such as reduced inflammatory processes and increased neurotransmitter levels (Cotman et al., 2007).

Transient changes in cortical excitability have been observed after just a single session of exercise (Singh, Duncan, Neva, & Staines, 2014; A. E. Smith, Goldsworthy, Garside, Wood, & Ridding, 2014; Yamaguchi, Fujiwara, Liu, & Liu, 2012); however, the number of studies is still limited**.** Less is known about how fitness level affects these exercise-induced changes. Previous studies have examined levels of physical activity on cortical excitability (Lulic, El-Sayes, Fassett, & Nelson, 2017) and plasticity (Cirillo, Lavender, Ridding, & Semmler, 2009) and found increased excitability in the high activity group. To our knowledge, no studies to date have looked directly at the effects of aerobic fitness on motor cortex excitability.

The purpose of the present study was to examine the relationship between aerobic fitness and cortical excitability at rest, as well as the change in excitability induced by a session of exercise. Measures of cortical excitability were obtained using transcranial magnetic stimulation (TMS). We hypothesized that there would be a positive relationship between fitness and cortical excitability, whereby

individuals with higher aerobic fitness would have increased excitability measures at baseline compared to individuals with lower aerobic fitness. Based on previous studies, we expected that there would be changes in excitability measures in all participants after exercise, characterized by a reduction in inhibition and an increase in facilitation; however, we expected that similar to the relationship observed at baseline, individuals with higher aerobic fitness would have an exaggerated response to the exercise; more specifically, that there would be a relationship between aerobic fitness and the magnitude of response to exercise.

Our results showed no significant relationship between aerobic fitness and cortical excitability at rest, nor following an acute exercise session. Although approaching significance, our results did not show differences in excitability measures in response to exercise. These results suggest that aerobic fitness is not a good predictor of cortical excitability and that, based on previous studies (Cirillo et al., 2009; Lulic et al., 2017) physical activity level may be a better predictor of excitability changes.

CHAPTER 2: BACKGROUND

2.1 Learning

Learning is the foundation of human behaviour. The ability of the brain to change, termed plasticity, is the basis for how learning occurs. Motor-skill learning involves the acquisition of new spatiotemporal muscle activation patterns and the adaptation of these activation patterns (Sanes & Donoghue, 2000). Learning is characterized as a relatively permanent change in motor behavior that is evoked by practice or experience (Ma, Narayana, Robin, Fox, & Xiong, 2011). Brain plasticity and reorganization influence how well motor skills can be learned (Coxon, Peat, & Byblow, 2014). Practicing movements increases cortical excitability, promoting plasticity and resulting in improved performance (Butefisch et al., 2000).

There is heightened plasticity and reorganization that occurs in the brain during motor learning (*see Dayan and Cohen (2011); Doyon and Benali (2005) for review*). Motor learning involves several phases including an early rapid phase, in which improvements can be seen in a single training session of just a few minutes, and a later slow phase in which greater improvements can be seen over longer durations (e.g., several weeks) (Ma et al., 2011). Improvements in behavioural performance are accompanied by changes in brain activity, as shown by a large number of neuroimaging studies (Doyon & Benali, 2005; Floyer-Lea, Wylezinska, Kincses, & Matthews, 2006). Such studies have enabled the identification of specific brain regions and neural networks involved in learning, and have improved our

understanding of the changes that occur during the different phases of motor skill learning.

Boe and colleagues (2012) studied the effect of short-term, single-session motor learning on specific regions of interest using functional magnetic resonance imaging (fMRI). Fourteen participants were trained on a visuomotor task that required the use of bilateral grip force to accurately move a cursor towards a target. Participants were engaged in task performance for less than one hour. Results showed significant training-related improvement in performance, with greater accuracy and a decrease in error magnitude observed following training (Figure 1.A). A significant improvement in performance was also observed across each of the three training blocks, suggesting participants learned progressively over the course of the experiment (Figure 1.B).

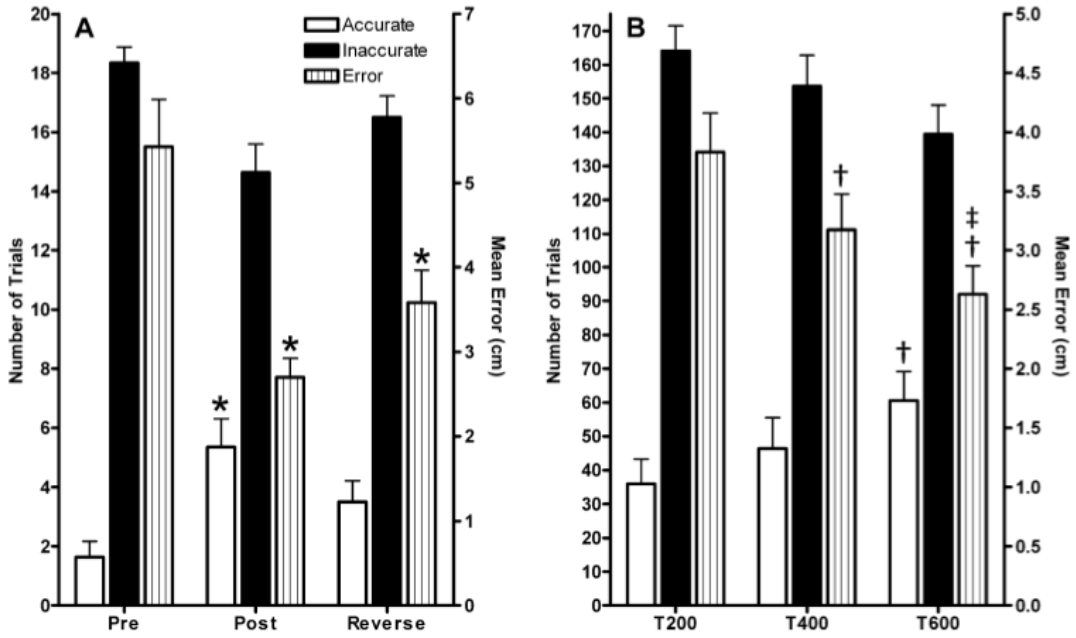

Figure 1: Behavioural improvements associated with short-term learning. ***From Boe et al. (2012).*** **Training was performed in 3 blocks of 200 trials (T200, T400, T600). (A) There was a significant increase in the number of accurate trials (white bars, left y-axis) and a significant decrease in error magnitude (striped bars, right y-axis) in the post- compared to the pre-training condition. (B) Training conditions show a progressive improvement in performance across the three blocks of training. * Signifies statistically significant difference from pre-training; †Significant difference from T200; ‡Significant difference from T400. Bars represent standard error.**

In-line with the behavioural changes (i.e., improved performance), a significant decrease in the extent of spatial activation throughout the whole brain was observed, indicative of a reduced demand on neural resources (Figure 2) (Boe et al., 2012). Examination of the specific regions of interest revealed a similar trend, with significantly reduced numbers of activated voxels in the cerebellum, middle frontal gyrus and primary motor cortex (M1) observed post- relative to pre-training. Although not significantly different from pre-training, activation in the thalamus and supplementary motor area were also reduced post-training. Imaging results support

a training-related attenuation in brain activity linked to increased automaticity in task performance (Boe et al., 2012). Results from Boe and colleagues (2012) were consistent with prior studies of online, short-term motor learning (Doyon & Benali, 2005; Floyer-Lea & Matthews, 2005).

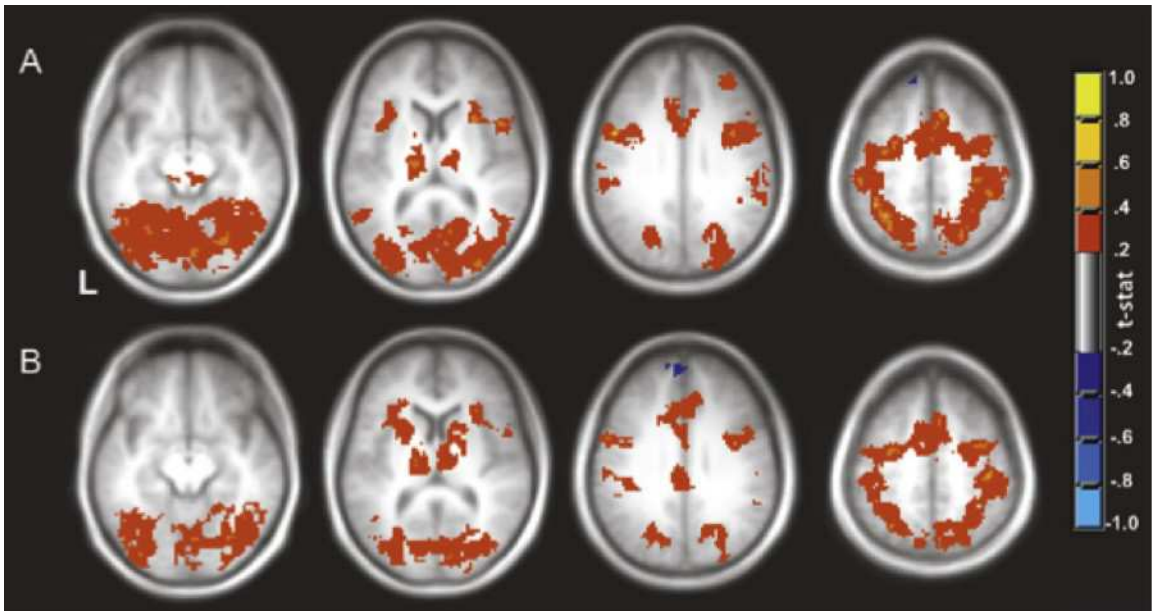

Figure 2: Changes in brain activation associated with short-term learning. ***From Boe et al. (2012).*** **Group statistical parametric maps (t-scored) contrasting conditions relative to baseline activity. Spatial activations decreased from pre- (A) to post- (B). Brain regions shown include the cerebellum (z= −9.5), thalamus (z = 10.5), middle frontal gyrus (z = 30.5), supplementary motor area and M1 (z = 50.5).**

Floyer-Lea and Matthews (2005) examined long-term learning with a visuomotor task in which participants tracked a continuously moving target by varying the grip-force exerted on a pressure sensor. Behavioural performance was characterized by the magnitude of tracking error, and brain activity obtained using fMRI to characterize changes associated with learning. Seven participants took part

in long-term practice, which involved training on the visuomotor tracking task for 3 weeks. Training consisted of 15 min of practice each day, 5 days per week. Training induced a decrease in tracking error, with participants improving from a mean error of 7.0% on the first session to 4.6% after training (Figure 3).

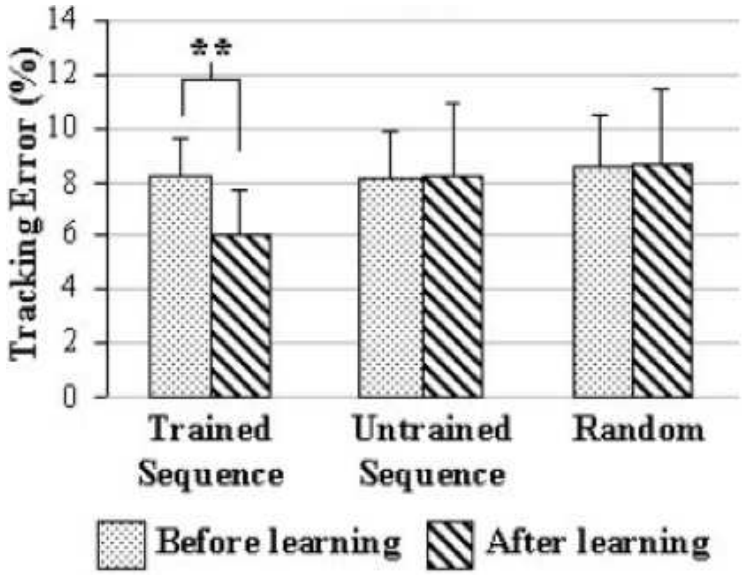

Figure 3: Behavioural results after training, indicative of long-term learning. *From Floyer-Lea and Matthews (2005).* There was a reduction in mean error after training (After Learning) compared to before training (Before Learning). Results were specific to the sequence that was trained. * Signifies statistically significant differences ($P < 0.01$).

In parallel with the training-related changes in performance, increased activity was observed in the left primary somatosensory and motor cortices, as well as in the right putamen (Floyer-Lea & Matthews, 2005). Further analysis of the hand region of M1 showed an increase in the mean percentage signal change and the number of significantly activated voxels during performance of the trained sequence (Figure 4). Overall, results of the study revealed distinct functional changes

associated with long-term learning, including plasticity in the primary somatosensory and motor cortices.

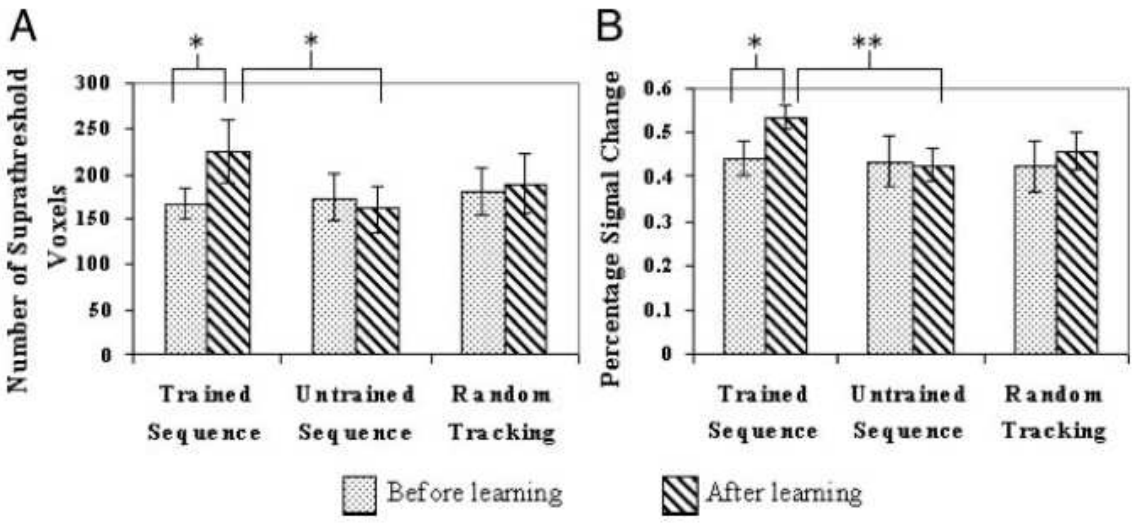

Figure 4: Changes in M1 activation associated with long-term learning. *From Floyer-Lea and Matthews (2005).* (A) Training increased the number of activated voxels ($P < 0.05$) and the extent of activation was larger for the trained sequence than the untrained sequence (after training) ($P < 0.01$). (B) Mean percentage signal change increased ($P < 0.02$) and the trained sequence showed significantly higher activation than the untrained sequence (after training) ($P < 0.05$).

Progression from early to late stages of motor skill learning is characterized by a shift in brain activation from anterior to more posterior regions of the brain (Floyer-Lea & Matthews, 2005). One of the biggest areas of difference is M1: in short-term learning, M1 shows decreased activation as learning progresses; however, long-term learning is associated with increased activation in M1 (Dayan & Cohen, 2011; Floyer-Lea & Matthews, 2005).

Overall, learning, regardless of the stage, is associated with changes in the brain. Specifically, these changes occur through alterations in the strength of the connections between neurons (discussed in detail below). Determining methods to make the brain more susceptible to these changes may assist in learning.

2.2 Neurological Injury and Rehabilitation

Neurological injury, such as that which occurs due to stroke, can cause deficits that result in functional impairments and difficulties with activities of daily living. There are an estimated 60,000 strokes in Canada each year, and more than 400,000 Canadians live with the effects of stroke (Statistics - Heart and Stroke Foundation of Canada. n.d. Retrieved August 13, 2015). These effects include minor to severe impairment, with the latter requiring long-term care. In the days following stroke, spontaneous motor recovery occurs as a result of a heightened state of plasticity in the brain; following this acute stage in which spontaneous motor recovery occurs, further recovery relies on active engagement in rehabilitation using techniques that are based on principles of motor learning.

As indicated above, there is heightened plasticity and subsequent reorganization of the cerebral cortex that occurs shortly after neurological injury. Nudo and Milliken (1996) demonstrated substantial reorganization in M1 after a focal ischemic infarct in adult squirrel monkeys. They used an intracortical microstimulation technique to derive detailed maps of distal forelimb movement representations before and after a surgically induced infarct. Infarcts caused reorganization of movement representations, as shown by the motor maps in Figure

5, whereby changes in location of a colour represent changes in movement representation. The infarct destroyed 21% of digit and 7% of wrist/forearm representation; movement representations, primarily of the digits, underwent reorganization throughout the adjacent, undamaged cortex (Nudo & Milliken, 1996). These changes occurred without intervention and, therefore, were attributed to spontaneous recovery.

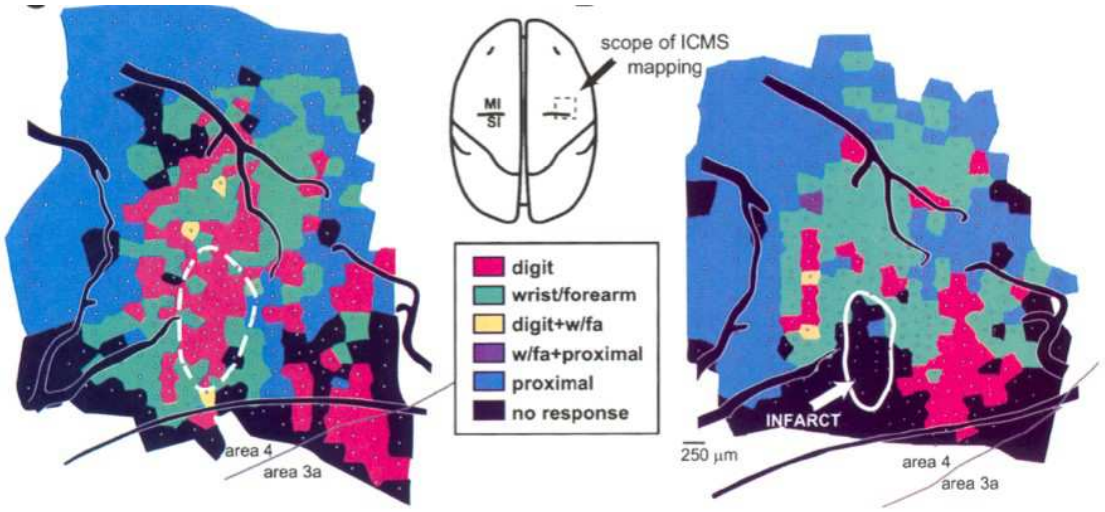

Figure 5: Distal forelimb motor maps before and after infarct. From Nudo and Milliken (1996). Motor maps are derived from intracortical microstimulation. Pre-infarct is represented by the figure on the left and post-infarct is represented by the figure on the right. White dashed lines (left) indicate microelectrode penetrations.

Beyond the spontaneous recovery occurring shortly after a neurological insult, recovery of motor function is facilitated by rehabilitation therapies. Therapies used in rehabilitation are based on the principles of motor learning, as individuals try to relearn motor skills that were lost due to injury (Mang et al., 2013). Similar to the relationship observed between brain activity and behaviour in studies of motor

skill learning (whereby changes in performance are paralleled by changes in brain activation patterns), it follows that the same mechanism plays a role in recovery after neurological damage (Nudo, 2003). Indeed, functional neuroimaging studies indicate that brain plasticity occurs in concert with the functional gains brought about by rehabilitation therapy (*see Schaechter (2004) for review*).

Constraint-induced movement therapy (CIMT) is one such therapy that is used to promote functional recovery post-stroke. Constraint-induced movement therapy, which is used almost exclusively in rehabilitation of upper limb function and use, engages the affected limb in intense, repetitive task practice, while restraining use of the unaffected limb. This therapy has been shown to promote functional recovery throughout all stages of recovery after stroke (Fleet, Page, MacKay-Lyons, & Boe, 2014; Shi, Tian, Yang, & Zhao, 2011), including in patients that had previously reached a plateau (Taub & Morris, 2001). Liepert and colleagues (2000) examined the effects of 12 days of CIMT on functional organization of M1 in relation to clinical recovery. Thirteen patients with chronic stroke (duration of hemiparesis ranging from 0.5 to 17 years) were studied. Clinical recovery was measured using the Motor Activity Log (MAL), which tracked amount and quality of use of the paretic limb in 20 common and important activities of daily living. Transcranial magnetic stimulation was used to assess plasticity in the brain resulting from treatment by mapping the cortical representation of the abductor pollicis brevis (APB), a small intrinsic hand muscle**.** Two baseline measures were collected, one two weeks before the beginning of treatment and one the week before the beginning of treatment, to investigate the stability of pre-treatment measures

(Liepert et al., 2000). Measures were also recorded 1 day, 4 weeks, and 6 months post-treatment to determine short- and long-term effects of the therapy.

Improvements in the amount and quality of use of the limb, assessed using the MAL, is presented in Figure 6. Constraint induced movement therapy had an acute effect, with a significant improvement in amount and quality of use of the affected upper limb observed immediately (1 day) post-treatment (Liepert et al., 2000). Follow-up assessments showed these improvements persevered, with similar MAL scores observed at 4 weeks and 6 months post-treatment.

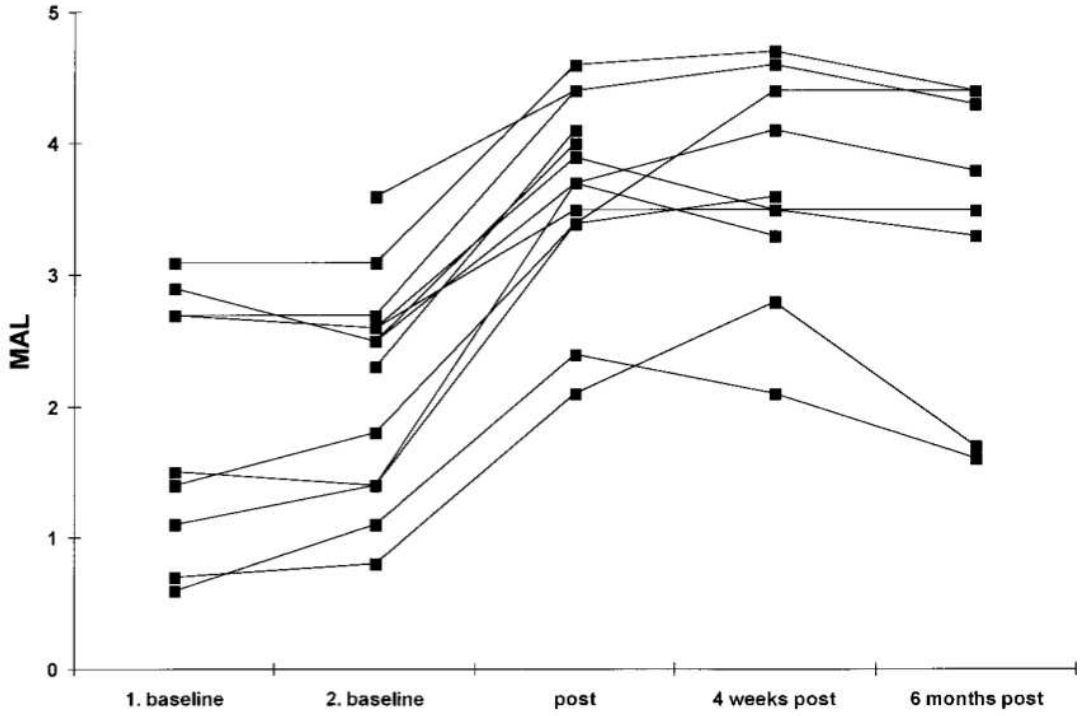

Figure 6: Effect of CIMT on amount and quality of use of the paretic limb. *From Liepert et al. (2000)*. MAL scores were collected at baseline (1. baseline = 2 weeks before treatment; 2. baseline = 1 day before treatment), post (1 day after treatment), 4 weeks post, and 6 months post CIMT. There was no difference in baseline measures, but there was a significant difference between 2. baseline and post, which remained 4 weeks and 6 months post. Note: the maximum score of the MAL is 5.

Using TMS, the size of the cortical motor output map of the APB was measured for both hemispheres at each of the time points (Figure 7). Results showed the number of positions in M1 of the infarcted hemisphere for which activity could be elicited from the APB using TMS at 1 day post-treatment was significantly increased (Liepert et al., 2000). While the number of active positions in the infarcted hemisphere was significantly different than the number in the non-infarcted hemisphere at baseline and at 1 day post-therapy, they became almost identical at 4 weeks and 6 months post-treatment indicating a return of the balance of excitability between the two hemispheres (Liepert et al., 2000).

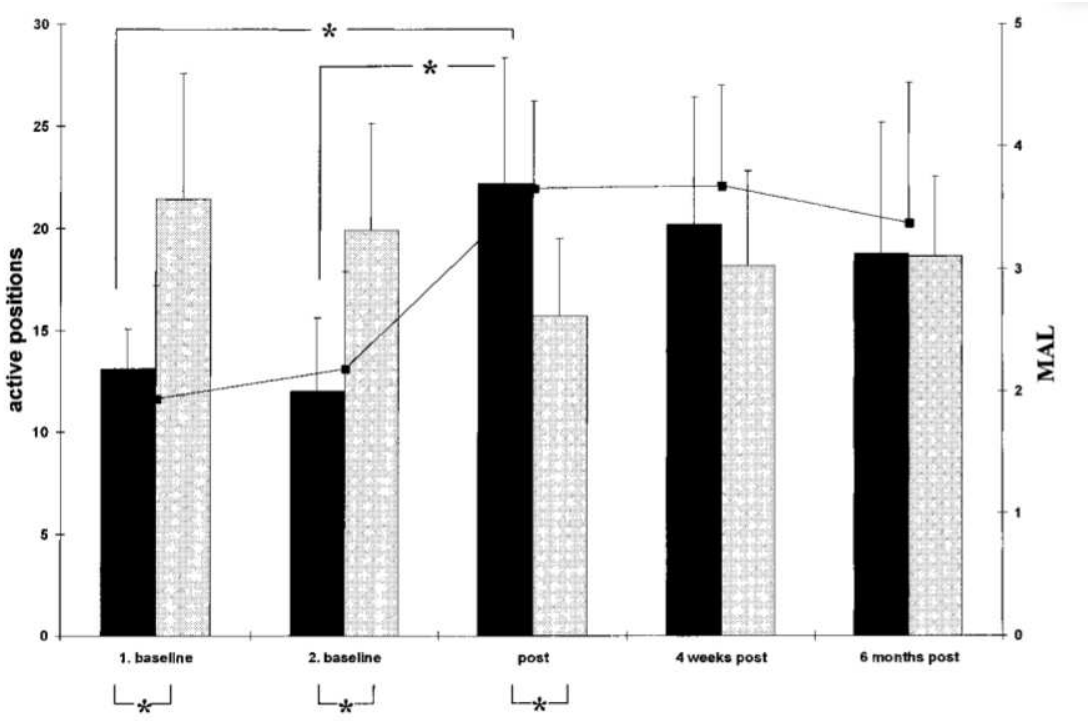

Figure 7: Cortical representation of the APB before and after CIMT. *From Liepert et al. (2000).* Number of active TMS positions in the infarcted (black bars) and noninfarcted (gray bars) hemisphere. Measures were collected at baseline (1. baseline = 2 weeks before treatment; 2. baseline = 1 day before treatment), post (1 day after treatment), 4 weeks post, and 6 months post CI therapy. * Signifies statistically significant differences (P<0.05).

The cortical reorganization found in this study reflects a lasting change in excitability (Liepert et al., 2000). This is likely due to the enhancement of the synaptic strength through the mechanisms of long-term potentiation (LTP). An alternate, and possibly related mechanism, could be a reduction in activity of local inhibitory interneurons, leading to an increase in excitatory connections (Jacobs & Donoghue, 1991).

Given the link between brain plasticity and functional recovery, it is critical to study brain plasticity and ways to promote it. Long-term potentiation is an enhancement of synaptic transmission and efficacy, and is a primary goal of motor learning and rehabilitation following a brain injury (Singh, Neva, & Staines, 2014). Plasticity in M1 is of significant importance, since it is highly involved in motor skill learning (Sanes & Donoghue, 2000). Research has shown that there is considerable plasticity of M1 representations and cell properties following trauma and in relation to everyday experience (Sanes & Donoghue, 2000). Finding ways to promote LTP and motor learning-related neuroplasticity could facilitate functional recovery by boosting the effectiveness of techniques used in rehabilitation.

2.3 Plasticity

The capacity of the brain for continuous alteration in response to experience or injury is known as neuroplasticity. Understanding the mechanisms and circumstances under which plasticity occurs allows one to appreciate the mechanisms underlying learning and rehabilitation, and in-turn to understand how

to facilitate these processes. Repeated firing of the same neural pathways can have an organizational impact on the nervous system (Butefisch et al., 2000). Modifications resulting from this repetitive neural discharge can include alterations in synapse number and strength, as well as changes in the topography of stimulation-evoked movement representations (Adkins, Boychuk, Remple, & Kleim, 2006).

Synapses, the connections between neurons, are the site at which plasticity occurs. Repetitive activation of synapses can result in either strengthening or weakening of the connection between two neurons (Wolters et al., 2003). Briefly, if the presynaptic neuron successfully activates the postsynaptic neuron, the connection between the two will be strengthened; conversely, if the presynaptic neuron is unsuccessful in activating the postsynaptic neuron, the connection between the two will be weakened. Considering this basic principle of synaptic plasticity, the repetition of a given movement reinforces the neural pathways that underlie the movement; these same patterns can be weakened if the movement has not been recently executed (Classen, Liepert, Wise, Hallet, & Cohen, 1998).

Lasting changes related to the strengthening of the connection between neurons, or LTP, is dependent on the activation of the N-methyl-D-aspartate (NMDA) receptors and subsequent changes in cell morphology (Purves, 2012). When a single action potential travels from the pre- to the post-synaptic neuron, glutamate is released from the pre-synaptic cell and binds to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors on the post-synaptic neuron; however, at resting membrane potential, NMDA receptor channels are

blocked by a magnesium ion. The post-synaptic cell must become depolarized, as it does after high-frequency stimulation, for the magnesium ion to be expelled from the NMDA channel. Thus, in the presence of glutamate and a depolarizing current resulting from activation of the AMPA receptors, the magnesium ion is released, allowing the NMDA channels to open and calcium ions (Ca^{2+}) to enter the postsynaptic neuron. The influx of Ca^{2+} activates signal transduction cascades (Lynch, 2004). These cascades lead to a number of processes that result in LTP, including the synthesis and insertion of additional AMPA receptors on the post-synaptic membrane. An increased number of AMPA receptors promote the likelihood that the post-synaptic neuron will become depolarized, providing the necessary current to unblock the NMDA receptors. Increased activation of NMDA receptors results in an influx of Ca^{2+}, which in turn results in gene expression and protein synthesis required for LTP (Purves, 2012).

Synaptic connections cannot be continuously strengthened; consequently, an opposing mechanism to LTP is required. Long-term depression (LTD) is a long-lasting decrease in synaptic strength (Purves, 2012). The Ca^{2+} signal in the postsynaptic cell largely influences whether LTP or LTD occurs: large and fast increases in Ca^{2+} trigger potentiation, whereas small and slow rises in Ca^{2+} lead to depression (Purves, 2012). While high frequency stimulation strengthens the connection between neurons (potentiation), low frequency stimulation has been shown to result in plasticity that ultimately weakens the connection between neurons (depression) (Chen et al., 1997).

Each neuron in the brain receives thousands of inputs, both excitatory and inhibitory, which are capable of modification (Sanes & Donoghue, 2000). It is the summation of these inputs that determine if the neuron will be activated. Increasing the excitability of a given neuron (i.e., reducing the threshold required for a neuron to discharge) increases the likelihood that excitatory signals from presynaptic neurons would be successful in activating the post-synaptic neuron, in-turn, driving LTP and thus increasing the strength of the connection. Increasing the strength of the connections between neurons is important, as this process represents the mechanism that underlies motor skill learning as well as rehabilitation following neurological injury.

2.4 Cortical Excitability: a Primer for Learning

2.4.1 Increasing Excitability and Reducing Inhibition

As mentioned above, it is possible to create an ideal environment in the brain for the induction of LTP by increasing the excitability of neurons and thus reducing the magnitude of the excitatory input required for a neuron to discharge (i.e., bringing the resting membrane potential closer to the threshold for discharge). Indeed, research has shown that highly excitable cells in M1 can be stimulated easier and faster than those that are less excitable (Rossini & Rossi, 2007). Excitability changes may be reflected by an enhancement of corticospinal tract output or by a modulation of intracortical excitability (Singh & Staines, 2015). Increases in cortical excitability following repetitive contractions have been proposed to involve synaptic changes and enhanced short- and long-term potentiation (Griffin & Cafarelli, 2007).

Therefore, increasing cortical excitability can "prime" the brain for lasting changes required for learning and rehabilitation.

As indicated above, a single neuron receives thousands of inhibitory and excitatory inputs; inhibition or excitation brings the neuron further or closer to its threshold for firing, respectively. Cortical activity depends on the balance of these inputs (Chen, 2004). γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system, while glutamate is the primary excitatory neurotransmitter (Purves, 2012). A pharmacological agent that acts as a GABA antagonist reduced local inhibition and led to cortical reorganization within the forelimb area of the rat M1 (Jacobs & Donoghue, 1991). A pharmacological agent that blocks NMDA receptors, a specific subtype of glutamate receptor, led to reduced excitation of the human M1 (Boroojerdi, Battaglia, Muellbacher, & Cohen, 2001). These studies show that increasing facilitatory influences, as well as decreasing inhibitory influences can enhance cortical excitability, leading to plasticity.

Decreases in inhibition may be especially important for the induction of LTP (Coxon et al., 2014). Reduced GABA-mediated inhibition directly modulates the excitability of pyramidal cells (Sanes & Donoghue, 2000) and is associated with motor practice (Butefisch et al., 2000). It seems that a reduction of intracortical inhibition is a necessary precursor to the induction of short-term plasticity (Jacobs & Donoghue, 1991). Furthermore, modulation of GABA is associated with LTP and motor learning (Coxon et al., 2014; Floyer-Lea et al., 2006; Stagg, Bachtiar, & Johansen-Berg, 2011).

2.4.2 Measuring Cortical Excitability using TMS

There are a number of neuroimaging methods that provide information about the activity in the brain; however, when measuring the excitability of neurons in M1, TMS is most frequently used. Transcranial magnetic stimulation is a non-invasive technique that allows for the investigation of the functional state of the brain. A TMS unit consists of a capacitor (or series of capacitors) that store electricity, and a coil consisting of wound copper wire that generates a magnetic field that facilitates delivery of current into the brain. Different coil dimensions lead to different stimulation patterns, however TMS is most commonly delivered using a figure-of-eight coil (Nieminen, Koponen, & Ilmoniemi, 2015). Figure-of-eight coils produce maximal current at the intersection of the two round components, or 'wings', allowing for more focal stimulation (Hallett, 2000). The standard figure-of-eight coil stimulates a surface area ranging from 1 to 2 cm^2, depending on coil dimensions and tissue distribution (Dayan, Censor, Buch, Sandrini, & Cohen, 2013).

Figure 8 shows the induction of an electric current into neural tissue using a TMS coil. An electrical current travelling through the copper wire creates a magnetic field perpendicular to the coil. This magnetic field creates an electrical current in the neural tissue underlying the coil. If placed over M1, a sufficient current will cause the depolarisation of corticospinal neurons and a corresponding muscle response, termed the motor evoked potential (MEP). There are a number of ways to investigate cortical excitability using single- and paired-pulse paradigms, among others.

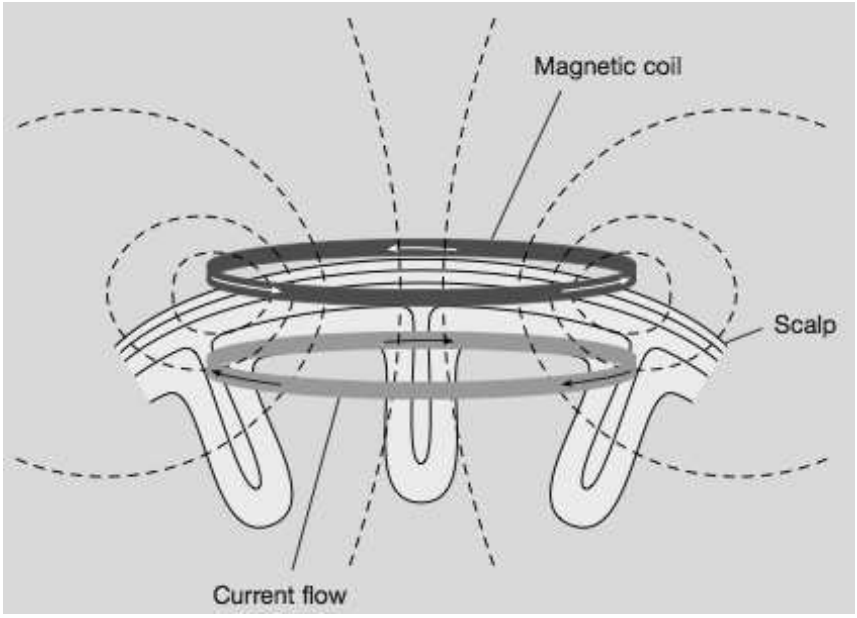

Figure 8: Induction of electrical current using TMS. From Hallett (2000). An electric current travels through the magnetic coil (depicted as a dark circle), creating a magnetic field perpendicular to the coil (dashed lines). An electric field (current flow) is induced in the underlying brain tissue, perpendicular to the magnetic field.

2.4.2.1 Single Pulse Stimulation

As indicated above, the MEP is the muscle response, or motor output, that results when a single pulse of TMS is applied over a given area of M1 (Figure 9). An MEP is obtained via electromyography, in which electrodes are placed over the muscle of interest to record the resultant electrical activity of the muscle. The MEP is an indirect measure of corticospinal tract excitability, reflecting the summation of all inhibitory and excitatory inputs to the descending tract and as such it can be influenced by many factors, both cortical and subcortical (Singh, Duncan, et al., 2014). Several parameters related to the MEP provide information pertaining to motor cortex excitability, including the stimulator output required to elicit an MEP of a given amplitude, or conversely the amplitude or size of the MEP resulting from a

fixed stimulator output. As well, MEPs can be used to 'map' M1, with changes in excitability represented by a change in the size of the cortical area from which potentials can be evoked (Schaechter, 2004).

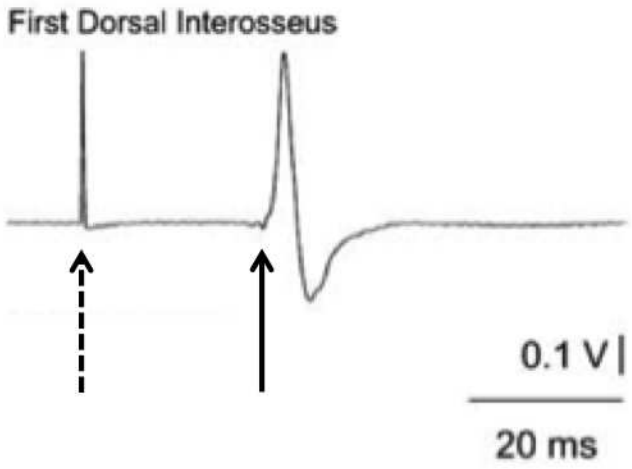

Figure 9: MEP elicited by single-pulse TMS over the hand area of M1 obtained via EMG over the contralateral first dorsal interosseous muscle. The dashed arrow indicates the onset of an artifact resulting from the pulse, with the solid arrow representing the onset of the MEP. ***Modified from Petersen, Pyndt, and Nielsen (2003).***

The stimulus-response (S-R) curve, also referred to as the input-output curve, is the curve resulting when MEPs are elicited at a number of different stimulator intensities (typically between 100 and 140% of resting motor threshold – described below). The slope of the S-R curve is an index of excitability, and provides information about the physiological strength of the corticospinal connections (Devanne, Lavoie, & Capaday, 1997). Numerous studies have demonstrated the utility of the S-R curve for assessing cortical excitability. For instance, Boroojerdi and colleagues (2001) studied the effects of lorazepam, lamotrigine, and d-

amphetamine on cortical excitability using the S-R curve. Lorazepam, which is a positive allosteric modulator of $GABA_A$ receptors, increases GABA potency, resulting in increased inhibition. Similarly, lamotrigine is an inhibitor of voltage-gated sodium ion (Na^+) and Ca^{2+} channels, while d-amphetamine is an indirect agonist of the dopaminergic-adrenergic system, which enhances the release of the neurotransmitters in the central nervous system, and thus should increase excitability. Consistent with the known effects of the drugs, results showed that the S-R curves elicited via TMS were significantly depressed by lorazepam and lamotrigine and were enhanced by d-amphetamine, confirming the utility of the S-R curves for assessing inhibitory and excitatory effects in the brain (Boroojerdi et al., 2001). Similarly, it is possible to assess changes in excitability by examining the area under the curve (AUC), as upward or downward shifts in the S-R curve at each of the stimulator outputs results following interventions, such as aerobic exercise (Lulic et al., 2017).

2.4.2.2 Paired Pulse Measures

Cortical excitability can also be investigated using paired-pulse TMS paradigms, where two single pulses are delivered with a short time interval between them. Pulses can be applied either within or across hemispheres, measuring intra- and inter- hemispheric connections, respectively. Paired-pulse paradigms applied to M1 elicit different measures of excitability than single pulse measures, as they probe inhibitory or facilitatory networks (Chen, 2004). The first pulse, also known as the conditioning stimulus (CS), probes these networks. The second pulse, termed the

test stimulus (TS), follows the CS and produces an MEP. The resulting MEP can be increased (facilitation) or decreased (inhibition) depending on the size of the CS, the TS, and the time interval between them (termed the inter-stimulus interval or ISI).

Kujurai and colleagues (1993) investigated the effects of a subthreshold (i.e., a stimulator output below that required to elicit an MEP) CS on a suprathreshold (i.e., a stimulator output above that required to elicit an MEP) TS and found that the amplitude of the resulting MEP was reduced at ISIs of 1-5 ms. This decrease in MEP amplitude is known as short-interval intracortical inhibition (SICI) (Figure 10 A). SICI is thought to probe the effect of GABA receptor type A ($GABA_A$) modulated inhibitory circuits on corticospinal neurons (Chen, 2004). Because of the difficulties involved in measuring changes in GABA levels in human participants, the assessment of intracortical inhibition is useful as an indirect measure of GABA activity.

Kujurai and colleagues (1993) found that the suppression of the MEP elicited at ISIs of 1-5 ms was followed by an increase in the size of the MEP at ISIs of 10 and 15 ms. Later investigations showed this effect to be intracortical facilitation (ICF), (Figure 10 C) which is present at ISIs of 8-30 ms (Ziemann, Rothwell, & Ridding, 1996). Like SICI, ICF is elicited with a subthreshold CS, followed by a suprathreshold TS. The mechanism behind ICF is not as well understood; however, it is thought to involve activation of glutamatergic interneurons and NMDA receptors (Ziemann, Chen, Cohen, & Hallet, 1998).

Another measure of inhibition, long-interval intracortical inhibition (LICI) (Figure 10 B), is probed when a suprathreshold CS precedes the TS by 50–200 ms

(Valls-Solé, Pascual-Leone, Wassermann, & Hallett, 1992). LICI, unlike SICI, is thought to probe the role of GABA receptor type B ($GABA_B$) inhibitory circuits.

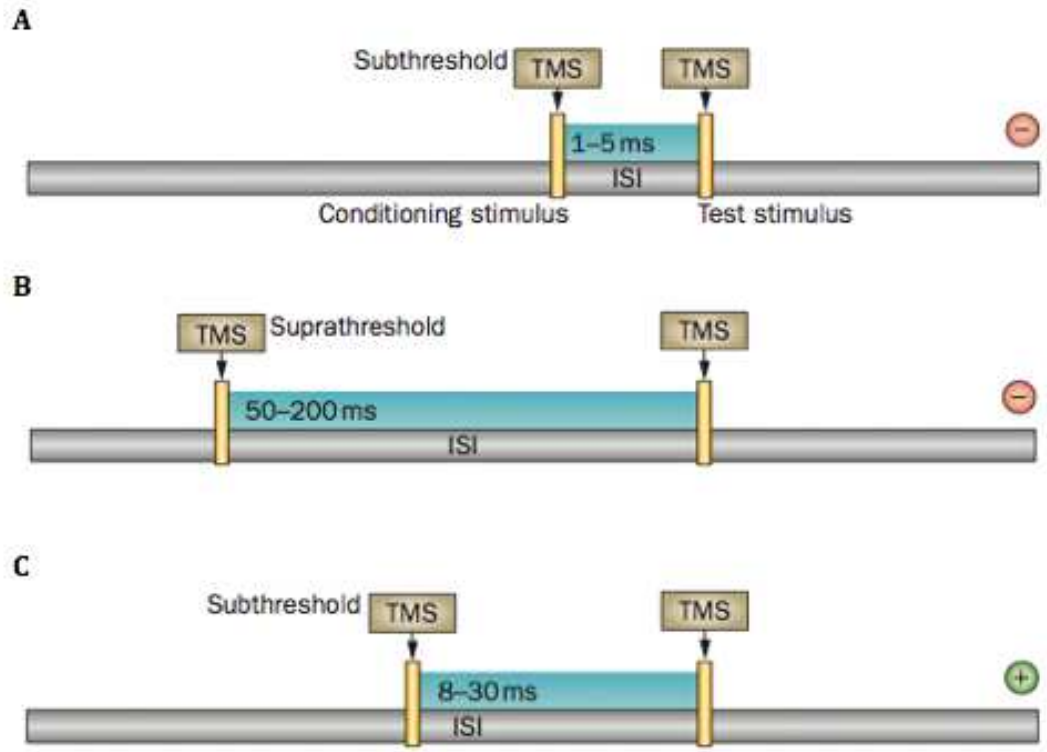

Figure 10: TMS paired-pulse protocols. ***From Di Pino et al. (2014).*** **(A) SICI: a subthreshold CS is followed by a suprathreshold TS after an ISI of 1-5 ms, resulting in inhibition; (B) LICI: a suprathreshold CS is followed by a suprathreshold TS after an ISI of 50-200 ms, resulting in inhibition; (C) ICF: a subthreshold CS is followed by a suprathreshold TS after an ISI of 8-30 ms, resulting in facilitation.**

2.4.3 Methods of Increasing Cortical Excitability

As previously discussed, enhancing M1 excitability can prime the brain for plasticity. Specifically, enhancing excitability by increasing excitatory or reducing inhibitory input increases the probability of discharge of the post-synaptic neuron. Repetitive discharge of the post-synaptic neuron leads to long-term changes (LTP), which is the basis for learning and rehabilitation. There are a number of ways to

alter the excitability of M1, including through the use of caffeine and drugs, changing hormone or blood glucose levels, and using non-invasive brain stimulation such as repetitive TMS (Butefisch et al., 2000; Nicolo, Ptak, & Guggisberg, 2015). Unlike the single or paired pulse techniques described above, repetitive TMS is used to modulate excitability rather than assess it (Di Pino et al., 2014). Depending on the pulse frequency and timing, repetitive TMS can transiently excite or inhibit neural activity (Rossi, Hallett, Rossini, & Pascual-Leone, 2009).

Aside from repetitive TMS, these methods of neuromodulation, including caffeine or drugs, have side effects that in-turn make them less desirable for use as a means of increasing cortical excitability for the purpose of priming the brain prior to learning or rehabilitation. Results from clinical trials on non-invasive brain stimulation techniques, such as repetitive TMS or transcranial direct current stimulation (tDCS), are equivocal, in that the degree of effectiveness in priming the brain appears to be similar; this coupled with the expense and training required to deliver non-invasive brain stimulation reduce its clinical feasibility (Di Pino et al., 2014). One exciting alternative neuromodulatory technique is aerobic exercise. Because aerobic exercise has many general health benefits (Pescatello & American College of Sports, 2014) and is easy and safe to do, it may be a viable option to use as a primer to increase the excitability of the brain before learning or rehabilitation.

2.5 Aerobic Exercise

2.5.1 Physical Activity and Aerobic Fitness

Physical activity is defined as "any bodily movement produced by the contraction of skeletal muscles that results in a substantial increase in caloric requirements over resting energy expenditure" (Pescatello & American College of Sports, 2014). It is well known that physical activity is recommended for general health benefits: the American College of Sports Medicine (ACSM) recommends that adults get at least 150 minutes of moderate intensity exercise per week (Pescatello & American College of Sports, 2014).

There are a number of characteristics that relate to the ability of individuals to perform physical activity. One of these health-related, physical fitness components, is cardiorespiratory endurance, also known as aerobic fitness. Aerobic fitness is the ability to perform large muscle, dynamic, moderate-to-vigorous intensity exercise for prolonged periods of time (Pescatello & American College of Sports, 2014). It is dependent on the ability of the circulatory and respiratory system to supply oxygen to the contracting muscles. Maximum oxygen consumption (VO_2max) is the maximum rate of oxygen utilization of the muscles during aerobic exercise and is the criterion measure of cardiorespiratory (aerobic) fitness (Heyward, 2010). Physical activity level has been shown to be positively related to aerobic fitness (Hagströmer, Oja, & Sjöström, 2007).

2.5.2 General Health Benefits of Aerobic Exercise

Regular exercise has a number of general health benefits including improvement in cardiovascular and respiratory function, reduction in cardiovascular disease risk factors, and overall decreased morbidity and mortality (Pescatello & American College of Sports, 2014). Accompanying these more general health effects are specific neurophysiological benefits, including decreased anxiety and depression (Pescatello & American College of Sports, 2014) and reduced risk for Alzheimer's disease and dementia (Kramer & Erickson, 2007). Aerobic exercise benefits memory and cognition, and the associated cortical changes have been studied in areas such as the hippocampus and pre-frontal cortex (discussed below). Fewer studies have examined the effects of aerobic exercise on M1, however results suggest that acute (McDonnell, Buckley, Opie, Ridding, & Semmler, 2013) as well as chronic (Cirillo et al., 2009) exercise can promote plasticity. As well, increases in cortical excitability have been observed after just a single session of aerobic exercise (Singh & Staines, 2015). Because improvements in motor function are a primary goal of training in motor tasks and rehabilitation, understanding how exercise can affect M1 is important.

2.5.3 Effects of Aerobic Exercise and Fitness on the Brain

Aerobic exercise has robust effects on both the structure and function of the brain (*see Cotman et al. (2007); Thomas et al. (2012) for review*). Aerobic fitness enhances cognitive function and memory (Colcombe & Kramer, 2003; Colcombe et al., 2004), enhances resting state functional efficiency in cognitive networks (Voss et

al., 2010), and protects against age-related degeneration (Erickson et al., 2011). These global effects are due to the ability of exercise to trigger critical molecular and cellular processes that support brain plasticity (Knaepen, Goekint, Heyman, & Meeusen, 2010). Therefore, exercise is an exciting tool to be used to not only improve general health but also promote brain function.

Colcombe and Kramer (2003) performed a meta-analysis on 18 longitudinal studies that examined the effects of cardiovascular fitness training on cognitive function. In the examined studies, participants (aged 55 to 80) had to have been randomly assigned to a control group or an exercise group, which was required to have an aerobic fitness component. The results of the meta-analysis showed that cardiovascular fitness training can reduce age-related declines in cognition. Specifically, results showed that regardless of the cognitive task, aerobically trained participants showed significantly greater improvements than control participants who did not participate in a similar exercise regimen (Colcombe & Kramer, 2003). Overall the fitness training was found to have robust benefits for cognition, with the largest benefits occurring for executive-control processes (Colcombe & Kramer, 2003).

To add to the previous study, Colcombe and colleagues (2004) examined the relationship of aerobic fitness and cognition in cross-sectional and longitudinal studies of older adults. The two studies reported on in this particular publication (detailed below) examined cognition, as measured by performance on a flanker task, and task-related brain activity, as measured by fMRI. Results showed increased fitness was associated with better performance on a test of executive functioning in

aging humans (Colcombe et al., 2004). Imaging results showed increased activation in frontal and parietal regions important in attentional networks.

In the first experiment (cross-sectional design of 41 older adults), individuals were classified as having high or low fitness based on a VO_2max test. Behavioural results from the Flanker task showed that the participants with higher fitness levels (i.e., higher VO_2max) were more efficient in dealing with the conflicting cues compared to those with lower fitness levels (i.e., lower VO_2max). Accompanying the behavioural results, fMRI showed that participants with high fitness levels demonstrated significantly greater activation in cortical regions associated with attentional control compared to their lower fitness counterparts (Figure 11) (Colcombe et al., 2004).

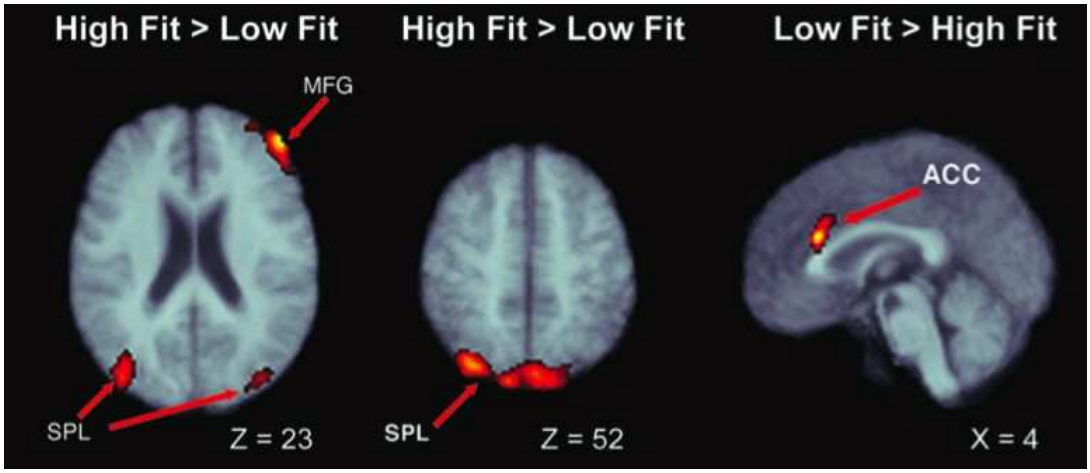

Figure 11: Differences in cortical activation as a function of aerobic fitness. *From Colcombe et al. (2004)*. Superior parietal lobule (SPL) and right middle frontal gyrus (MFG) had increased activation in high fit individuals, while the anterior cingulate cortex (ACC) had increased activation in low fit individuals. Z and X scores report level of cross-section.

The second experiment (longitudinal design of 29 older adults) was an extension of the first in which participants trained 3 times per week for 6 months and were tested on the same behavioural and neuroimaging measures as the first experiment (Colcombe et al., 2004). One group engaged in aerobic training, while the other (control group) participated in stretching and toning exercises. Cognitive testing and neuroimaging were performed 1 week before the beginning and 1 week after the completion of the intervention. The aerobically trained group had improved behavioural results that coincided with changes in brain activation. Overall, differences observed between the aerobically trained individuals and the controls for behavioural and neuroimaging results were comparable to those found in the first experiment between the high and low fitness groups (Colcombe et al., 2004).

In addition to improvements in cognitive function and related changes in functional brain activity, aerobic exercise has also been shown to have structural effects on the brain. Erickson and colleagues (2011) performed a randomized control trial examining the effect of aerobic exercise on age-related hippocampal degeneration. In the study, 120 adults (aged 58-80 years) were divided into either an aerobic exercise group, or a group who performed stretching and toning exercises (control). Individuals participated in 3 sessions per week over a one-year period. MRI results showed that the aerobic exercise group had an increase in volume of the left and right hippocampus by 2.12 and 1.97%, respectively, while the control group displayed a 1.40 and 1.43% decline in volume (Erickson et al., 2011). Similar to the control group data, previous work has shown that hippocampal

volume decreases 1 to 2% annually (Raz et al., 2005); the increase in hippocampal volume in the aerobic exercise group indicates that aerobic exercise training is effective in reversing the age-related loss in hippocampal volume.

Although the majority of studies have examined older adults, the benefits of increased aerobic fitness on brain volume have been demonstrated in younger populations as well (Chaddock et al., 2010). In a cross-sectional study of 21 high fitness and 28 low fitness pre-adolescent children, those with higher aerobic fitness levels had larger hippocampal volumes. Furthermore, larger hippocampal volumes were associated with superior memory performance (Chaddock et al., 2010). Given the evidence, it appears that aerobic exercise has beneficial effects on brain function and structure, regardless of the population.

2.5.4 Potential Mechanisms Underlying the Effect of Aerobic Exercise on the Brain

It is apparent that structural and functional changes occur in the brain due to exercise (as discussed above). Animal studies show that exercise leads to increased angiogenesis (Black, Isaacs, Anderson, Alcantara, & Greenough, 1990; Swain et al., 2003) and neurogenesis (van Praag, Shubert, Zhao, & Gage, 2005). It is reasonable to speculate that these same processes (i.e., angiogenesis and neurogenesis) underlie the large-scale changes seen in human studies. As indicated previously, aerobic exercise is associated with enhanced task-induced and resting-state brain activity (Colcombe et al., 2004; Voss et al., 2010), as well as increased cerebral blood flow (Pereira et al., 2007). Aerobic exercise also indirectly affects cognitive decline by reducing risk factors such as diabetes, hypertension and cardiovascular disease

(Cotman et al., 2007). A common mechanism may explain the benefits of aerobic exercise on the brain: indeed, it has been proposed that the induction of central and peripheral growth factors leads to the structural and functional changes observed as a result of aerobic exercise training (Cotman et al., 2007).

Growth factors are associated with neuroprotection and synaptic plasticity (Cotman & Berchtold, 2002). Brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1) may regulate the effects of exercise on learning, whereas exercise-dependent stimulation of angiogenesis and hippocampal neurogenesis seem to be regulated by IGF-1 and vascular endothelial growth factor (VEGF) (Cotman et al., 2007). BDNF, IGF-1, and VEGF have all been shown to be up-regulated with exercise (Ding, Vaynman, Akhavan, Ying, & Gomez-Pinilla, 2006; Knaepen et al., 2010; Kramer & Erickson, 2007).

BDNF is critical for the growth and survival of neurons and plays a key role in the development of LTP (Cotman & Berchtold, 2002). Furthermore, it has been found to facilitate learning (Tokuyama, Okuno, Hashimoto, Li, & Miyashita, 2000) and may promote the induction of additional AMPA receptors at neural synapses (Bolton, Pittman, & Lo, 2000). Exercise increases levels of BDNF, as demonstrated in animal models (Neeper, Gomez-Pinilla, Choi, & Cotman, 1996), as well as in humans (*see Knaepen et al., 2010 for review*). In animal studies, exercise increases BDNF in several brain regions, with the most robust responses found in the hippocampus (Cotman & Berchtold, 2002). In humans, systemic levels of BDNF are increased for 10 to 60 minutes following a single bout of aerobic exercise (Knaepen et al., 2010).

Systemic, as well as hippocampal, levels of IGF-1 are increased after exercise (Ding et al., 2006; Schwarz, Brasel, Hintz, Mohan, & Cooper, 1996). This increase is important for neurogenesis and improved memory (Trejo, Carro, & Torres-Aleman, 2001). IGF-1 and BDNF work together on a common pathway and IGF-1 can mediate the effects of BDNF (Ding et al., 2006).

IGF-1 interacts with VEGF to influence exercise-induced neurogenesis and angiogenesis (Cotman et al., 2007). VEGF is triggered by oxygen deprivation and in-turn stimulates the formation of new blood vessels (Thomas et al., 2012). BDNF and VEGF play different roles in neurogenesis; while VEGF promotes the proliferation of new neural cells, BDNF promotes their survival and incorporation into neural networks (Thomas et al., 2012).

In addition to central mechanisms, exercise indirectly reduces several peripheral risk factors for cognitive decline (Cotman et al., 2007). Inflammation interferes with growth factor signaling in the periphery and in the brain. Inflammation can result from health modifiers, such as metabolic syndrome and hypertension. Exercise can reduce pro-inflammatory conditions, and thereby indirectly promote growth factor cascades. Overall, exercise directly and indirectly promotes growth factors that drive exercise-mediated brain responses (Figure 12).

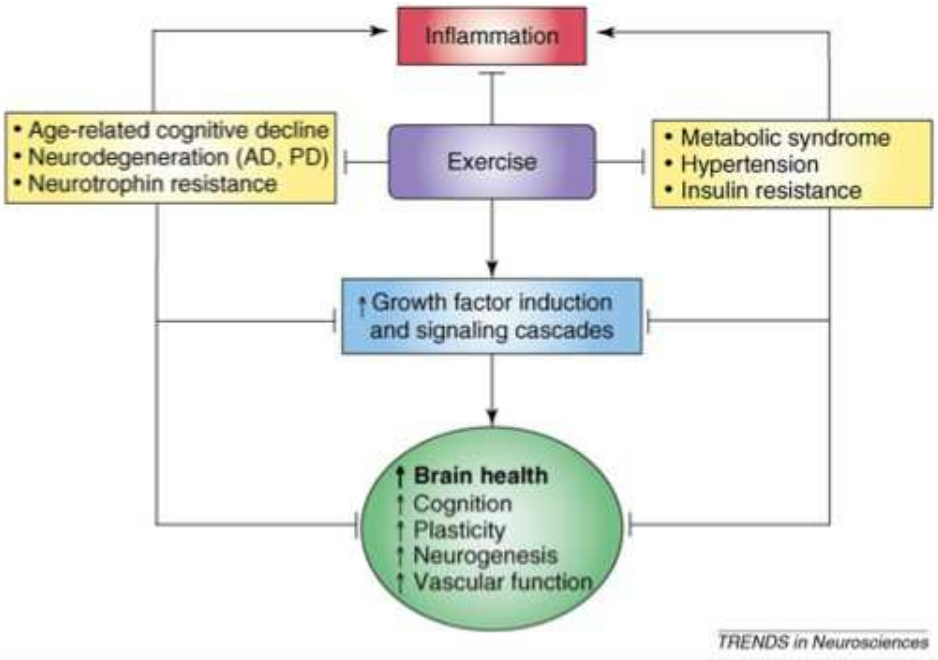

Figure 12: Exercise effects: central and peripheral factors on brain health. From Cotman et al. (2007). Exercise promotes growth factor cascades, a direct means of mediating brain function, and reduces peripheral risk factors for cognitive decline, indirectly affecting brain health.

2.5.5 Effects of Aerobic Exercise on M1

While aerobic exercise-induced changes in hippocampal volume demonstrate a positive impact of exercise on brain structure and function, we are particularly interested in the modulatory effects of exercise on M1 given its role in motor skill learning and rehabilitation. Cirillo and colleagues (2009) examined the effect of physical activity level on paired associative stimulation (PAS), a non-invasive brain stimulation protocol that can induce neuroplastic change within M1 through LTP-like mechanisms (Stefan, Kunesch, Benecke, Cohen, & Classen, 2002; Stefan, Kunesch, Cohen, Benecke, & Classen, 2000). Specifically, plasticity in M1 was probed in 14 highly active and 14 sedentary individuals, grouped based on a self-reported physical activity questionnaire. Highly active participants performed >150 min per

day of moderate-to-vigorous aerobic activity at least 5 days per week, whereas the sedentary group performed <20 min per day of physical activity no more than 3 days per week. Results showed that compared with sedentary individuals, highly active individuals had an increased neuroplastic response to PAS (Cirillo et al. 2009), indicating that high levels of physical activity can increase the capacity for cortical plasticity. The input-output curve (i.e., the S-R curve) was 35% steeper in active individuals when combined before and after PAS, suggesting an increased strength of corticospinal connections that are activated at higher TMS intensities in active individuals; however, no differences were observed in measures of inhibition between physically active and sedentary participants, suggesting that GABAergic, specifically $GABA_A$, mechanisms in M1 are not influenced by regular physical activity. (Cirillo et al., 2009).

Lulic and associates (2017) examined how physical activity level influences M1 excitability. Participants were divided into two groups based on the International Physical Activity Questionnaire (IPAQ), a self-report measure of physical activity. Individuals with low-to-moderate activity scores were combined into one group and individuals with high activity scores into another. This study used TMS to assess M1 excitability via MEPs obtained from the first dorsal interosseous muscle. Corticospinal excitability was examined by measuring MEP amplitude at rest and during tonic contraction. Recruitment (S-R) curves were obtained and slope as well as area under the curve (AUC) was calculated. Intracortical networks were examined via SICI, ICF and short-interval intracortical facilitation (SICF). Measures were acquired before and following a 20-minute bout of

cycling at 50-70% of age predicted maximal heart rate (HRmax) using the formula 220 – age. While SICI and ICF were reduced following exercise for both groups, MEP recruitment curve amplitudes at rest and AUC values were increased following exercise in the high activity group only (Lulic et al., 2017). These findings indicated that exercise altered corticospinal excitability depending on the level of physical activity. The authors propose that this may be due to an increased uptake of BDNF within the CNS in the high activity group after exercise or other possible physiological adaptations, such as increased stroke volume, increased brain perfusion, and muscle adaptations that may reduce fatigue (Lulic et al., 2017).

2.5.6 Single Session Effects of Aerobic Exercise on the Brain

Research suggests that even a single session of aerobic exercise can create an ideal environment for the early induction of plasticity (*see Singh and Staines (2015) for review*). Several studies have demonstrated changes in cortical excitability after just a single session of exercise, with these effects observed in both the exercised (Yamaguchi et al., 2012), and perhaps more interestingly, a non-exercised muscle (Singh, Duncan, et al., 2014; A. E. Smith et al., 2014). The fact that increased cortical excitability can be induced in a non-exercised muscle has important implications for rehabilitation; up to 75% of those individuals that survive their stroke experience upper limb impairments that persist into the chronic stage (Mang et al., 2013). Having an intervention that can have a 'priming effect' (i.e., increase excitability) of the cortical representation of the upper extremities without causing fatigue is very relevant as increasing cortical excitability, specifically lowering inhibition, has been

shown to increase the potential for LTP to occur (Jacobs & Donoghue, 1991). These transient changes found after a single session of exercise have the potential to prime the brain for more lasting changes necessary for learning and rehabilitation to occur (Singh & Staines, 2015).

2.5.6.1 Excitability of Exercised Muscle Representations after a Single Exercise Session

Yamaguchi and colleagues (2012) examined cortical excitability in the tibialis anterior and soleus muscles, two lower-limb muscles, after a 7-minute session of cycling at 60 rpm at a resistance of 5 Nm. Although they found no change in the amplitude of the single-pulse MEPs obtained, they found that active pedaling led to a decrease in SICI. Specifically, applying a sub-threshold CS followed by a supra-threshold TS at ISIs of 2 and 3 ms resulted in a smaller MEP than a TS given alone (Yamaguchi et al., 2012). Figure 13 shows the SICI ratio of the soleus and tibialis anterior muscles before, as well as immediately, 15 and 30 min following the cycling session. The SICI ratio is the resulting paired pulse MEP amplitude, shown as a percentage of the TS alone. The ratio increased immediately after exercise, meaning a reduction in inhibition.

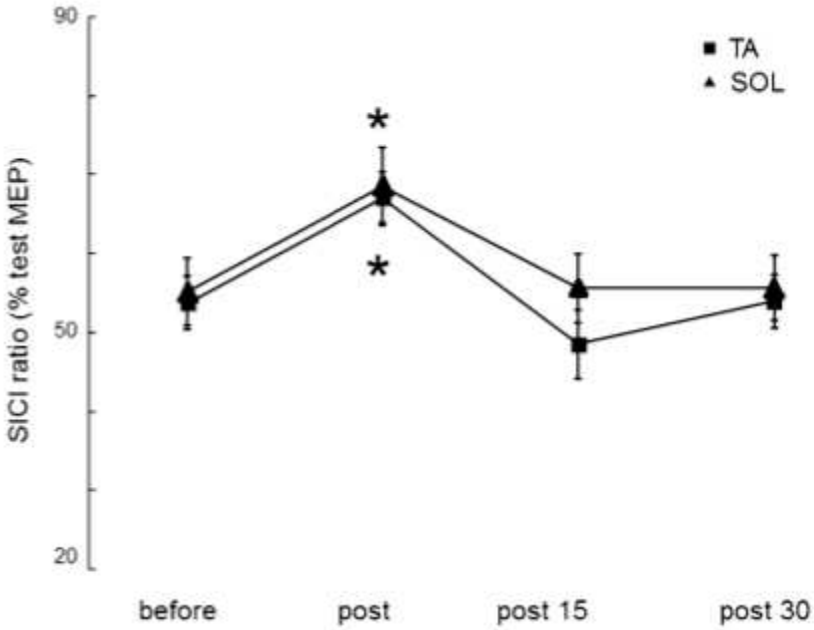

Figure 13: Active pedaling reduces inhibition (SICI) in tibialis anterior (TA) and soleus (SOL) muscles. ***From Yamaguchi et al. (2012).*** **Measures are reported before, immediately after (post), 15 min after (post 15), and 30 min after (post 30) 7 minutes of active pedaling. Data for the TA muscle represent an average of 10 participants, while SOL muscle data represent an average of 6 participants. Error bars are standard errors. * Signifies statistically significant differences from baseline.**

2.5.6.2 Excitability of Non-Exercised Muscle Representations after a Single Exercise Session

As outlined above, a single session of aerobic exercise has also been shown to induce excitability changes in the cortical representation of non-exercised muscles (Singh, Duncan, et al., 2014; A. E. Smith et al., 2014). Singh and colleagues (2014) examined the effects of a 20-minute cycling session on upper limb cortical excitability. S-R curves, SICI, LICI and ICF in the extensor carpi radialis (ECR), a wrist extensor muscle, were measured prior to (Pre), immediately following (Post 1) and 30 minutes following (Post 2) a cycling session at 65-70% age-predicted HRmax. Results showed no changes over time in corticospinal excitability, as measured by

the S-R curve. However, there were differences found in inhibitory and facilitatory networks. Specifically, a significant suppression in SICI was found at 30 minutes post-exercise (Figure 14); while not reaching significance, LICI displayed a similar trend to SICI (Figure 15). In addition to the suppression of inhibition, there was a significant increase in ICF immediately following exercise (Figure 16).

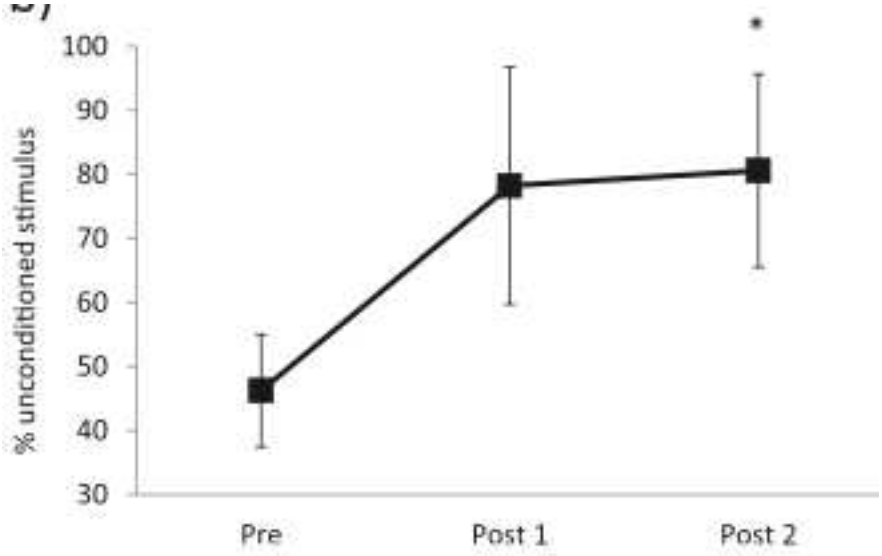

Figure 14: SICI of the ECR muscle following exercise. From Singh, Duncan, et al. (2014). The size of the TS is reported as % unconditioned stimulus at 120% RMT. An increase in the % unconditioned stimulus signifies a reduction in inhibition. Bars represent standard error of the mean. * Signifies a statistically significant difference from pre-exercise ($p < 0.05$).

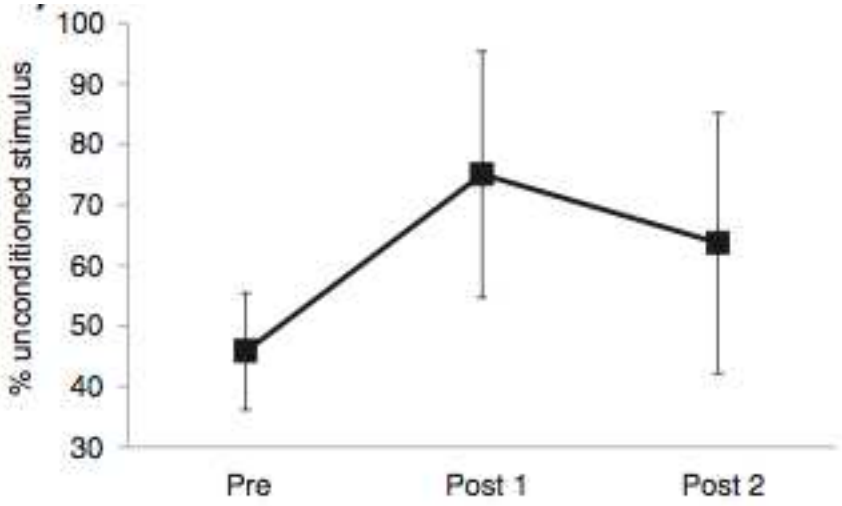

Figure 15: LICI of the ECR muscle following exercise. From Singh, Duncan, et al. (2014). The size of the TS is reported as % unconditioned stimulus at 120% RMT. An increase in the % unconditioned stimulus signifies a reduction in inhibition. Bars represent standard error of the mean.

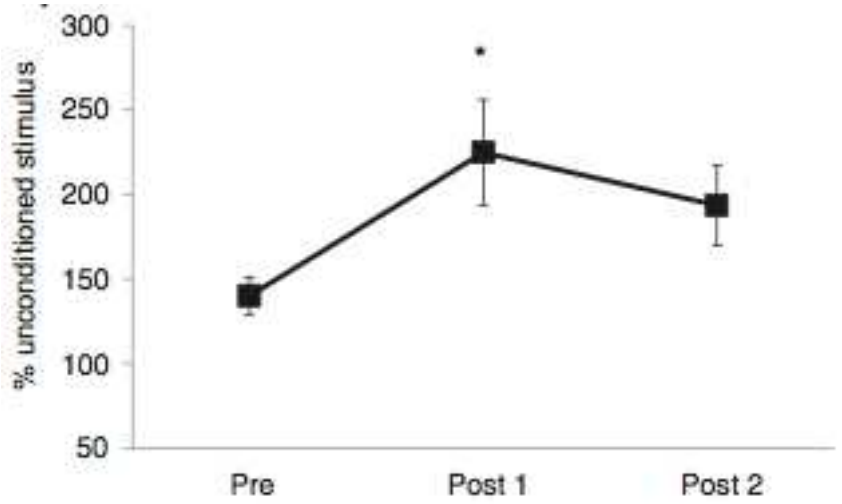

Figure 16: ICF of the ECR muscle following exercise. From Singh, Duncan, et al. (2014). The size of the TS is reported as % unconditioned stimulus at 120% RMT. An increase in the % unconditioned stimulus signifies an increase in facilitation. Bars represent standard error of the mean. * Signifies a statistically significant difference from pre-exercise ($p < 0.05$).

In another study examining a non-exercised muscle, Smith and colleagues (2014) investigated the influence of aerobic exercise on corticospinal excitability, as measured by S-R curves and SICI. In this study, two different exercise intensities

were used. In both experiments, participants completed a 5-min warm-up period followed by two 15 min blocks of cycling either at 40 % or at 80 % of their predicted HR reserve (calculated by (180 − rest HR) × X% + rest HR). MEPs were obtained from the first dorsal interosseous, an intrinsic hand muscle, before, immediately after, and 15 minutes after exercise. Similar to Singh and colleagues (2014), results showed no changes in corticospinal excitability (as measured by the S-R curves) but there was a significant reduction in inhibition (SICI) following exercise (A. E. Smith et al., 2014). There was no significant effect of exercise intensity. Interestingly, this study included participants based on self-reported physical activity level and omitted individuals who were highly active. In their limitations, they suggested that the reduction in SICI may have been due to fitness level as opposed to physical activity (A. E. Smith et al., 2014).

2.6 Summary of the Current Literature

Exercise has robust effects on the body, as well as the brain. Cortical changes have been observed after chronic exercise, as demonstrated in cardiovascular fitness training studies (Colcombe & Kramer, 2003; Colcombe et al., 2004; Erickson et al., 2011), as well as after single sessions of aerobic exercise (McDonnell et al., 2013; Singh & Staines, 2015). The majority of the literature has examined the effects of aerobic fitness on cognition and memory, and the underlying cortical structures and networks (i.e., the hippocampus and pre-frontal cortex); however, less is known about the effects of exercise on M1. Increased levels of physical activity seem to promote heightened excitability (Lulic et al., 2017) and plasticity in M1 (Cirillo et al.,

2009), and single sessions of aerobic exercise lead to reduced inhibition (Singh, Duncan, et al., 2014) and increased excitability (Lulic et al., 2017) in M1. The level of aerobic fitness may have an effect on cortical excitability, yet to date no study has examined the effect of a single session of aerobic exercise on cortical excitability while considering pre-existing fitness level. The present study sought to expand on the previous literature by studying the effect of pre-existing fitness level on cortical excitability before and after an exercise session.

Given the link between brain plasticity and learning, it is critical to study ways to promote the mechanisms through which plasticity occurs (Sanes & Donoghue, 2000). Plasticity in M1 is of significant importance, as M1 has been shown to be involved in motor-skill learning (Sanes & Donoghue, 2000). Significant research has shown that aerobic exercise enhances plasticity and the factors that underlie it (Cotman & Berchtold, 2002). Understanding how fitness level influences the effect of a single session of aerobic exercise on the promotion of cortical excitability can inform practices related to the administration of exercise programs to facilitate learning and rehabilitation.

2.7 Purpose of the Present Study

To this end, this study examined the relationship between aerobic fitness and cortical excitability, as well as the relationship between aerobic fitness and the excitability changes induced by an exercise session. Transcranial magnetic stimulation was used to obtain measures of excitability before and after a single session of aerobic exercise.

2.7.1 Study Objectives

1. To determine if there is a relationship between aerobic fitness and cortical excitability
2. To determine if aerobic fitness influences cortical excitability changes following a single session of exercise.

2.7.2 Hypotheses

1. There will be a positive relationship between aerobic fitness and cortical excitability, where increases in fitness will be associated with increases in excitability, as evidenced by:
    a. Greater AUC values with increasing fitness
    b. Lower levels of SICI and LICI and greater ICF with increasing fitness
2. There will be a positive relationship between aerobic fitness and the magnitude of change in cortical excitability measures from baseline, where increases in fitness will be associated with greater change, as evidenced by:
    a. Greater increase in AUC values of the S-R curve relative to baseline measures
    b. Greater reductions in SICI and LICI, and greater increases in ICF in individuals with high aerobic fitness relative to baseline levels

Chapter 3: METHODS

3.1 Participants

Thirty-five participants were recruited for the present study (described below). The number of participants was determined to be adequate given the proposed analysis approach. A previous study by List and associates (2013) used multiple regression to examine the correlation between a TMS-based measure of brain excitability (resting motor threshold) and a number of predictors including age, sex, years of education, and MRI-derived structural parameters (cortical thickness and coil-to-cortex distance). Using a sample size of 30 participants, the study found that two of the variables (cortical thickness and coil to cortex distance) best predicted cortical excitability ($F = 9.51$, $p=0.001$, $R^2=0.40$). Based on the findings of this previous work, as well as the fact that we were using simple linear regression, we believed our sample size would provide adequate power for the proposed analysis.

Individuals aged 18-40 years, with no history of neurological insult, cardiovascular, or pulmonary disorders were recruited for the present study. Our recruitment methods allowed us to recruit individuals of varying fitness level to achieve representation across the continuum of aerobic fitness. Participants' suitability to take part in the study was determined by the Physical Activity Readiness Questionnaire (PAR-Q) and a TMS screening form (described below). Fitness level was determined via an exercise test to obtain each participant's

maximal oxygen consumption (VO_2max), and the International Physical Activity Questionnaire (IPAQ) was used to determine each participant's typical activity level. As well, participants were asked what types of activities they were involved in. Single and paired-pulse TMS paradigms were used to assess cortical excitability before, immediately after and 30 minutes after a single session of aerobic activity. The study procedures are described in detail below.

3.1.1 Physical Activity Readiness Questionnaire (PAR-Q)

Participants were screened for their suitability to undertake exercise using the PAR-Q (see Appendix 1). The PAR-Q was created by the Canadian Society of Exercise Physiology to determine a person's suitability for exercise and the need for a physician's approval prior to engaging in physical activity. If participants answered "yes" to any of the questions they were deemed ineligible for the study.

3.1.2 TMS Screening Form

Participants were screened for contraindications to TMS using a standard TMS screening form (Rossi et al., 2009) (see Appendix 2). Participants were excluded from the study if they answered "yes" to any of the first 10 questions, if they indicated that they had any problems with TMS or MRI in the past, or if they were taking any medication that can affect brain excitability.

3.1.3 International Physical Activity Questionnaire (IPAQ)

Participants' activity levels were determined based on the IPAQ (see Appendix 3). The IPAQ is a self-report questionnaire that can be used to calculate an activity score to determine if participants engage in low, moderate or high levels of physical activity. The IPAQ has been shown to be a reliable, valid instrument to measure activity levels and is related to assessment by accelerometer (Craig et al., 2003). There is a short- and long-form of the IPAQ available, assessing physical activity undertaken in four domains including leisure time, domestic and gardening activities, work-related and transport-related physical activity. While comparable data is found between both forms, the long form provides detailed information on domains of physical activity (Craig et al., 2003). For the present study, researchers were interested in total physical activity level, as opposed to time spent in each activity domain, and therefore the short form of the IPAQ was used.

The IPAQ gives both categorical and continuous indicators of physical activity for each participant. Based on IPAQ scoring criteria, an individual is categorized as "highly active" if they engage in vigorous-intensity activity at least 3 days per week or a combination of walking, moderate-intensity or vigorous-intensity activities 7 or more times per week. "Moderately active" individuals are those who participate in vigorous-intensity activity for at least 20 minutes per day, 3 or more days per week, moderate-intensity activity and/or walking of at least 30 minutes per day 5 or more days per week, or 5 or more days per week of any combination of walking, moderate-intensity or vigorous intensity activities. Individuals who do not meet the

criteria for moderate or high levels of activity are classed as being in the "low activity" category.

In addition to the IPAQ, participants were asked to list the types of physical activity they are involved in. While this data, as well as data collected from the IPAQ, was not included in the formal data analysis, it provided additional information regarding participants' typical physical activity. This information is important, as there may be differences in excitability measures between skill-trained and endurance-trained athletes (Adkins et al., 2006; Kumpulainen et al., 2014).

3.1.4 Screening

The following inclusion and exclusion criteria were used to determine if individuals were suitable to take part in the present study. These were based on our population of interest and excluded populations that have increased health risks with exercise.

3.1.4.1 Inclusion criteria include

1. Individuals ages 18-40
2. Suitability to perform exercise safely (as assessed by the PAR-Q; Appendix 1)

3.1.4.2 Exclusion criteria include

1. Having respiratory disorders, hypertension or other cardiovascular diseases that would preclude participating in exercise
2. Having a Body Mass Index ≥ 30kg/m^2 and a waist circumference >102 cm for men and >88 cm for women

3. Individuals who smoke
4. Having any contraindications to TMS (as assessed by the TMS screening form; Appendix 2)

3.1.4.3 Eligibility Justification:

1. The age range (18-40) was set to avoid the effect of aging on the brain as well as to exclude older adults who are at higher cardiovascular risk
2. Having any contraindication to TMS increases the risk of TMS-related adverse events
3. Cardiovascular or respiratory diseases are contraindications for the maximal exercise test
4. Individuals who have high BMI, high waist circumference and who smoke have increased risk of cardiovascular disease (Pescatello & American College of Sports, 2014)

3.2 Data Collection Protocol

3.2.1 Overview of Testing Sessions

When an individual presented an interest in participating in the study, they were sent the PAR-Q and TMS screening forms. Potential participants were asked to review the screening forms, and told that if they answered 'YES' to any of the questions they would be ineligible to participate. At this point the participant was asked to inform the research team that they are not eligible, without disclosing the reason for their ineligibility. Having the participants self-screen in this manner allows us to screen out participants prior to obtaining informed consent and

scheduling a study visit. The research team contacted those participants who were deemed eligible, and a first study visit was scheduled.

In the first session, participants completed informed consent, the PAR-Q and TMS screening forms, so that the researcher could verify participant's eligibility. Participants also completed the IPAQ to allow for their level of physical activity to be determined. During this first session, participants performed a maximal graded exercise test (GXT) on a cycle ergometer to determine their aerobic fitness and HRmax. Participant's HRmax was used to determine the exercise intensity for the subsequent exercise session (described below).

In the second session, participants engaged in a bout of aerobic exercise, with TMS measures of cortical excitability obtained before, immediately after and 30 minutes after the exercise. Sessions were attempted to be scheduled within one month of each other. Participants were asked during the second session if they significantly differed their training from before the first session. An overview of the testing sessions is presented in Figure 17. Figure 18 is a visual representation of the testing sessions, indicating which measures were used to answer each hypothesis.

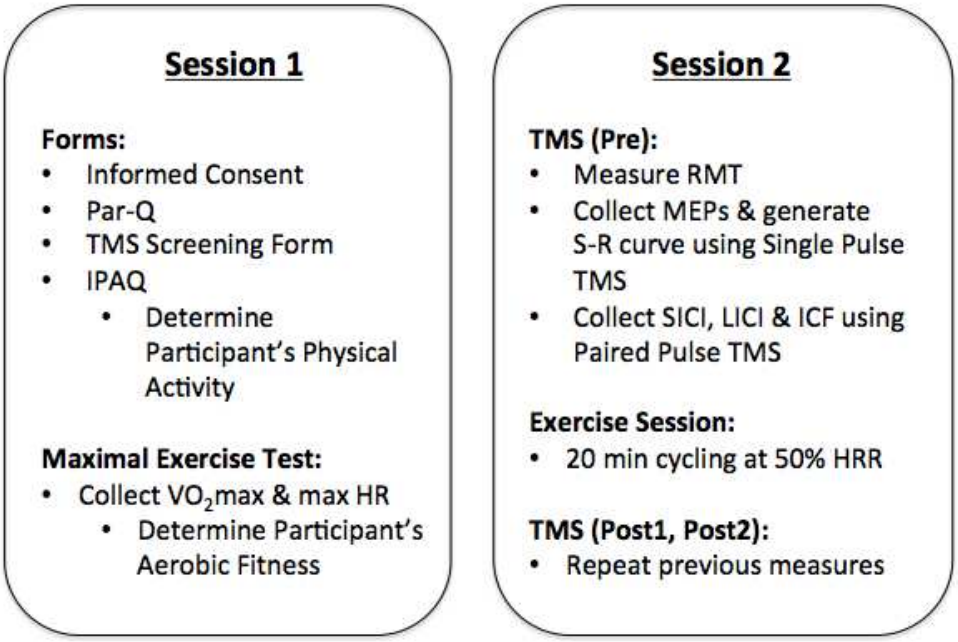

Figure 17: Experimental testing sessions. In session 1 participants completed the following forms: Informed Consent, Physical Activity Readiness Questionnaire (Par-Q), Transcranial magnetic stimulation (TMS) Screening form, and the International Physical Activity Questionnaire (IPAQ). Also during session 1, participants completed a maximal exercise test to determine aerobic fitness as measured by maximal oxygen consumption (VO_2max) and maximal heart rate (HR). In session 2, TMS measures were collected prior to (Pre), immediately after (Post1) and 30 min after (Post2) a 20 min cycling session at 50% heart rate reserve (HRR). At the Pre time-point, resting motor threshold (RMT) was determined. At all time-points, single pulse TMS at varying percentages of RMT was used to generate motor evoked potentials (MEPs) and stimulus-response curves. Also at all time-points, paired pulse TMS was used to determine short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI) and intracortical facilitation (ICF).

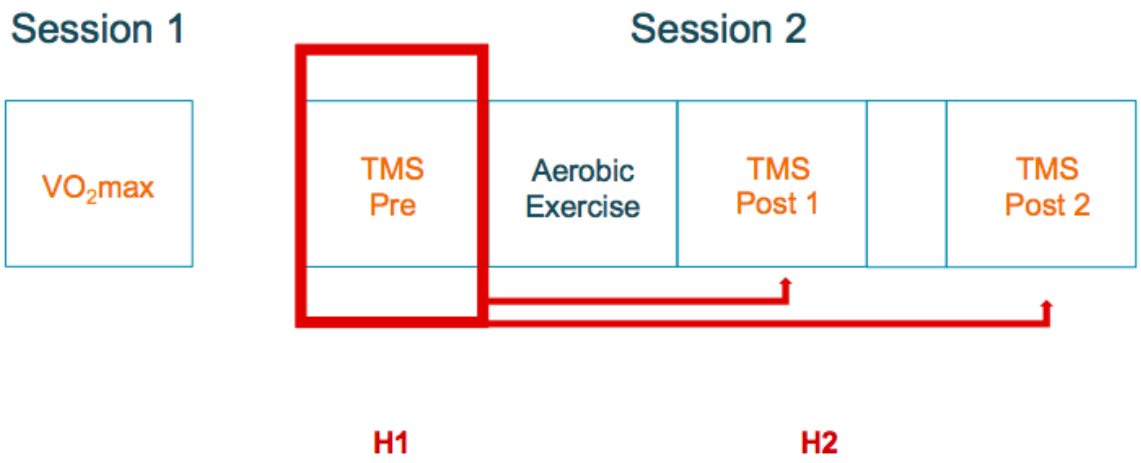

Figure 18: Depiction of experimental testing sessions. A graded maximal exercise test was completed during session 1 to determine participant's aerobic fitness, as measured by maximal oxygen consumption (VO_2max). During the second session, cortical excitability measures using transcranial magnetic stimulation (TMS) were collected before (Pre), immediately after (Post 1) and 30 minutes after (Post 2) an aerobic exercise session. TMS measures collected before exercise were used to address hypothesis 1 (H1) and the change scores between Pre and Post 1 and Pre and Post 2 were used to address hypothesis 2 (H2).

3.2.2 Participant instructions

Participant instructions were given before exercise testing, based on the ACSM recommendations, as explicit instructions increase test validity and data accuracy (Pescatello & American College of Sports, 2014). Participants were asked to wear comfortable clothing (shorts and t-shirt or other appropriate exercise apparel) and refrain from ingesting food, alcohol and caffeine within 3 hours of testing. Participants were asked to avoid significant exertion or exercise on the day of testing and get adequate sleep (6-8 hours) the night before the test to ensure that they were well rested.

3.2.3 Maximal Exercise Test

As outlined above, a maximal GXT was completed in the first session to determine each participant's level of aerobic fitness as measured by his or her VO_2max. The GXT was conducted in the Dalhousie School of Physiotherapy's Exercise Physiology Laboratory. The GXT was completed on an upright stationary cycle ergometer (Ergoselect 200P, Ergoline, Bitz, Germany). This ergometer is electronically braked, which means an electromagnetic braking force adjusts the resistance for slower or faster pedaling rates, keeping the power output constant. The ergometer was controlled by proprietary software (JLab LABManager 5.3.04, Cardinal Health, Germany) so that the power output was adjusted automatically. Ergoline only guarantees the accuracy of power output at pedalling cadence of 40 rpm and higher. As such, failure to maintain a cadence of at least 40 rpm resulted in GXT termination.

The session began with a 3 minute warm up period, followed by an incremental ramp GXT where power output increased every minute. The workload, measured in Watts (W), was determined by the participant's IPAQ score, as work rate increases should be based on amount and intensity of regular physical activity (Wasserman, 2012). As such, participants' average amount of time spent doing vigorous activity was also taken into consideration when determining workload to be used in the test protocol. The goal was for participants to reach exhaustion within 8-12 minutes. Participants with low physical activity levels began with a warm up at 25 W, followed by the test where the power increased by 15 W every minute. For participants with high physical activity levels, warm up began at 50 W and then

during the test the power increased 20 W every minute. An additional protocol was created for participants who had high levels of vigorous activity, as the workload did not increase to a sufficient extent for some participants to reach maximum in the ideal 8-12 minute range. This protocol consisted of a warm up at 75 W while the test began at 100 W and increased by 25 W every minute.

The volume and content of expired oxygen and carbon dioxide was analyzed using the Oxycon Mobile® portable metabolic unit. The Oxycon Mobile® measures ventilation and gas exchange breath-by-breath and is attached to the body using a vest system. Participants wore a facemask that contained an airflow sensor (Hans Rudolf, Inc., Kansas City, MO) to measure ventilation as well as a gas sampling port. The facemask and sensor were sanitized after each use. A sampling line was used to direct expired air to a sensor unit that measures O_2 and CO_2 concentrations using a microfuel cell and thermal conductivity, respectively. Ventilation and gas concentration data were stored in the transmitting unit and then relayed to the receiving unit attached to a computer. The Oxycon Mobile has high validity and reliability (Rosdahl, Gullstrand, Salier-Eriksson, Johansson, & Schantz, 2010). Data were collected on a breath-by-breath basis and VO_2 data were averaged every 20 seconds for further analysis. Other physiological parameters (HR, respiratory rate, gas exchange, and oxygen saturation were measured by the Oxycon Mobile® continuously throughout the test. A secondary HR measure was recorded using a wrist-mounted monitor (Mio Alpha Watch, Mio Global, Physical Enterprises Inc.). Heart rate was collected every second, with every 20 seconds of data (i.e., 20 samples) averaged to create values used in the subsequent analysis.

Participants were asked for their rating of perceived exertion (RPE) based on the 6-20 Borg scale (Borg, 1982) at the end of warm up and every 2 minutes during the test. Participants were asked to provide a cue when they believed they had approximately one minute remaining in the test. A final measurement of RPE was made at the termination of the test. The GXT was also terminated if the participant experienced any of the indications listed in Table 1. Participants ended with a minimum 3-minute "cool down" period at the same power as the warm-up to decrease their HR to near resting levels.

Table 1: General Indications for stopping an exercise test. From Pescatello and American College of Sports (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 • Noticeable change in heart rhythm • Participant requests to stop • Physical or verbal manifestations of severe fatigue • Failure of the testing equipment

Many individuals do not attain a VO_2 plateau and so other criteria may be used to support the judgement of achieving a VO_2max (Heyward, 2010). For the present study, a plateau in VO_2 was defined as a difference <2.1ml/kg/min in the last minute of the test. If a plateau in VO_2 was not observed, 2 of the following 3 criteria must have been achieved: 1) reaching a peak HR that was at least 95% of age predicted max (based on Tanaka et al. 2001); 2) a final RPE on the Borg scale ≥17; or 3) a resting exchange ratio (VCO_2/VO_2) ≥ 1.15.

Maximum HR was defined as the peak HR of the averaged data. Resting HR was collected during the beginning of the study while the participant was resting on the ergometer. Resting HR was measured again during the second session in case participants had elevated HR due to anticipation of the exercise session. Resting HR was averaged over the entire rest period. Heart rate reserve (HRR) was calculated based on maximal HR and resting HR to determine target HR for each participant for the subsequent exercise session. Target HR for the exercise session was determined using Equation 1.

Eq. 1 Target HR = [50% x (HRmax – HR rest)] + HR rest

3.2.4 Aerobic Exercise Session

The second test session was performed in the Laboratory for Brain Recovery and Function (School of Physiotherapy, Dalhousie University) to allow access to TMS equipment. For the aerobic exercise session, an upright stationary cycle ergometer was used (Lode Corival V3, Lode BV, Groningen, The Netherlands) under manual control. Participants cycled for 20 minutes at 50% of HRR, which is equivalent to approximately 70% of HRmax. The session duration was chosen based on the AEROBICS Canadian Stroke Best Practice Recommendations (MacKay-Lyons, 2012), which suggest that aerobic exercise sessions for patients post-stroke should be greater or equal to 20 minutes. As well, the duration and intensity of exercise mimic a previous study (Singh, Duncan, et al., 2014) which allowed for comparison of results. As in session one, HR was monitored continuously throughout the exercise using the wrist-mounted HR monitor (Mio Alpha Watch, Mio Global, Physical

Enterprises Inc.). Estimated workload was determined based on the maximal exercise test in session 1, where the workload corresponding to the target HR was used. Participants were instructed to perform a graded 5-minute warm-up starting at 50% of the estimated workload at the beginning of each session to elevate HR into the target zone, and a 5-minute cool-down period at 50% of the estimated workload at the end of the session to bring HR back down to resting rate. If the participant's HR deviated from the target HR by more than 10 beats, the power was adjusted to bring HR back to the desired level. If the participant's HR was too low, the power was increased and if the participant's HR was too high, the power was decreased. Ratings of perceived exertion were collected throughout using the Borg Scale, as per the previous session (explained above). Throughout the exercise session, participants were instructed to rest their arms comfortably by their sides and not to grip the handlebars. Participants were instructed not to grip the handlebars in order to avoid contraction of the target upper limb muscle (discussed below). Although not obtained during each session, electromyography (EMG) was collected during pilot testing to confirm the absence of muscle activity from the target muscle during exercise.

3.2.5 Muscle Activity

Evoked muscle activity (i.e., the MEP) of the right extensor carpi radialis muscle (ECR) was obtained using EMG during the TMS paradigms. An upper limb muscle was chosen owing to the interest in the generalized effects of aerobic exercise on cortical excitability. Recent literature (Singh, Duncan, et al., 2014) as

well as current work in the Laboratory for Brain Recovery and Function at Dalhousie University, utilizes the ECR for TMS measures. Obtaining these measures from the same muscle across studies facilitates comparison of results. Motor evoked potentials were acquired using self-adhering electrodes (1 x 3 cm; Q-Trace Gold; Kendall-LTP, USA) in a bipolar configuration with a 1 cm inter-electrode distance. Electrode placement was based on Seniam guidelines (European Commission, 1999); briefly, electrodes were placed 5 cm distal to the radiohumeral joint, and the neutral (ground) electrode placed on the olecranon process. Identification of the ECR muscle was facilitated by asking the participant to extend his/her wrist with radial deviation while the experimenter palpated the muscle. Motor evoked potentials (e.g., the EMG signal) were obtained using vendor supplied hardware (Brainsight EMG isolation Unit and Amplifier Pod) during identification of resting motor threshold (see below for details), while MEPs during all subsequent TMS procedures were acquired using Signal software, sampled at 1000Hz with a bandpass of 25-100 Hz (1902 and Power 1401; Cambridge Electronics Design, UK) and stored for offline analysis.

3.2.6 TMS

Transcranial magnetic stimulation was delivered before, immediately after, and 30 minutes after the exercise session on the second day of testing (refer to Figure 18). Resting motor threshold, S-R curves, and paired-pulse measures were collected (discussed below) for the right ECR at each time-point. During stimulation, participants were seated comfortably on a chair in a reclined position, with the right

arm placed on a pillow in their lap. Transcranial magnetic stimulation was applied through a 70 mm figure-of-eight coil connected to a Magstim BiStim2 magnetic stimulator (Magstim, Whitland, UK). Brainsight 2™ (Rogue Research Inc., Montreal, Canada) neuronavigation was used to guide the positioning and orientation of the coil over the target motor region using a template MRI. A template MRI is an anatomical MRI that is derived from a population of neurologically intact individuals, meant to be representative of the population as a whole.

3.2.6.1 Co-registration and Localization of the Motor Hotspot

To configure the target position, the participant's head was co-registered to the template MRI using the neuronavigation software. Prior to the TMS session, co-registration was achieved by aligning three anatomical landmarks on the participant (nasion, right and left pre-auricular points) with the corresponding anatomical landmarks on the template brain (MNI152_T1_1mm). A 5 x 5 grid with 7.5mm spacing was overlaid on the template brain with the mid-point (location 2, 2) centered on the estimated location of the ECR muscle representation of the left M1 (Kleim, Kleim, & Cramer, 2007). Stimulator output was set to 55% and points on the grid were stimulated starting from 2,2 with the coil positioned tangentially to the scalp with the handle at a 45^0 angle to the posterior. To identify the motor hotspot of the right ECR we worked outwards from the centre in a counter-clockwise manner to determine the location(s) that produced the highest amplitude MEPs for 5 out of 10 stimulations, termed the 'motor hotspot'.

3.2.6.2 Resting Motor Threshold (RMT)

Once the motor hotspot was located, the resting motor threshold (RMT) was determined. The RMT is defined as the lowest stimulation intensity required to elicit a minimum MEP peak-to peak amplitude of 50μV in the resting ECR muscle, for 5 out of 10 consecutive stimuli. The RMT was measured for each participant prior to the exercise session, and used to determine stimulator output in the subsequent TMS measures.

3.2.6.3 S-R Curve

After localization of the hotspot, and determination of the RMT, 10 single pulses were delivered at stimulus intensities of 100%, 110%, 120%, 130%, and 140% of RMT, with a fixed interval of 3-seconds between successive stimuli. The order of stimulus intensity was randomized. The peak-to-peak MEP amplitudes were calculated for each stimulus and the average of the 10 pulses was calculated for each stimulus intensity. The average MEP amplitudes were then used to generate an S-R curve. As indicated previously, single-pulse measures were collected prior to, immediately after, and 30 minutes following the exercise session.

3.2.6.4 Paired Pulse Measures

Intracortical networks were investigated using paired-pulse TMS to probe SICI, LICI and ICF. As with the S-R curve, the TMS coil was placed over the motor hot spot, and two successive stimuli (CS and TS) delivered at varying percentage of

stimulator output and ISI based on the paradigm being performed. SICI was measured using a CS of 80% of RMT, a TS of 120% of RMT, and an ISI of 2 ms. Identical magnitudes of CS and TS were used for measuring ICF, but with an ISI of 15 ms. To assess LICI, a CS of 120% of RMT was followed by a TS of 120% of RMT with an ISI of 100 ms (refer to Figure 10 for a depiction of the paired-pulse paradigms). Ten pairs of stimuli were delivered for each measure, with a fixed interval of 3 seconds between stimulus pairs. The order of SICI, LICI, and ICF was randomized; specifically, three custom scripts were created, each with a different order of ICF, SICI and LICI. The script used for each participant remained constant throughout session 2, such that pre- and post-exercise TMS procedures used the same script (i.e., the order of paired-pulse measures was the same for each participant, but differed across participants). Paired-pulse measures were collected prior to, immediately after, and 30 minutes following the exercise session. The resulting MEPs were compared to the single-pulse measure at 120% RMT at the corresponding time-point.

With the exception of single pulse TMS for hotspot localization and determination of RMT, during which the stimulator was under manual control, delivery of stimuli (i.e., control of the stimulator) was based on custom scripts programmed using Signal software (Signal 6.03c x86 Unicode, Cambridge Electronic Design Ltd., UK). Briefly, Signal enables external control of the stimulator via a hardware interface, including setting stimulus intensity and timing. Through the use of Signal, details related to the nature of the stimuli (i.e., intensity and type) are recorded along with the MEP, facilitating offline analysis.

3.3 Data Analysis

3.3.1 IPAQ Scoring

Data processing and analysis followed the "Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ)", as found on the IPAQ website (International Physical Activity Questionnaire. (n.d.). Retrieved August 11, 2015). Calculating total activity score for the short form requires summation of the duration (in minutes) and frequency (in days) of walking, moderate-intensity and vigorous-intensity activities. Continuous physical activity scores can be calculated in Metabolic Equivalent of Task (METs). Physical activity can also be categorized into low, moderate, or high levels. A sample calculation can be observed in Appendix 5.

3.3.2 Heart Rate During Aerobic Exercise

As described earlier, HR was recorded during the bout of moderate-intensity exercise in session 2 via the Mio watch, sampled once per second. Data were exported through the Wahoo fitness app as a text file for offline analysis. Using Microsoft Excel, we averaged HR data from minute 5 to minute 25 to allow for comparison with the required target HR for that session. Standard deviation was also calculated for each participant.

3.3.3 Motor Evoked Potentials (MEPs)

Analysis of MEP data was performed in line with past work in the Laboratory for Brain Recovery and Function at Dalhousie University. For single and paired-pulse measures, peak-to-peak amplitude of the resultant MEPs was obtained using scripts custom programmed in Signal. The custom scripts isolated a 50 msec window in which the evoked response occurred, and returned the peak-to-peak amplitude (i.e., the minimum and maximum values) in the specified window. For single pulse measures, the 50 msec window began 10 msec after the stimulus (occurring at second 1 in each frame) as the typical latency of a MEP to the ECR muscle is between 15 and 20 msec. Thus the window of interest for single pulse measures was from 1.010 – 1.060 sec. The custom script used for the paired pulse analysis also isolated a 50 msec window, in this case temporally linked to the occurrence of the TS; for ICF, SICI and LICI, these windows were 1.020 – 1.070, 1.007 – 1.057, and 1.105 – 1.155 respectively. For both single and paired-pulse measures, after the automated script was run the resultant data was visually inspected (i.e., each frame that contained a single trial and thus one MEP) to ensure that 1) the timing of the stimuli and the responses were logical (e.g., the evoked response should appear after a certain latency (15 to 20 msec after the stimulus) depending on the participant's height); and 2) the peak-to-peak amplitude values obtained related to the evoked response (as opposed to artefact). Data files were then saved as .txt files: three files per participant (1 pre- and 2 post-exercise) for each of the single and paired-pulse measures, with the paired-pulse files including SICI, LICI and ICF data. Lastly, data files were exported to Microsoft Excel for further analysis.

In addition to extracting peak-to-peak amplitude values, the EMG data files were examined to detect pre-stimulus muscle activity, as the presence of volitional activity in the target muscle immediately preceding the delivery of the TMS pulse will result in increased MEP peak-to-amplitude, and thus artificially large MEPs. Specifically, trials in which excessive muscle activity was observed prior to the TMS pulse were removed from further analysis. To facilitate this analysis, the custom scripts calculated the average root mean square amplitude in a 70 msec window (0.025 to 0.095) before the TMS pulse (or CS in the case of paired-pulse paradigms). The root mean square amplitude values were exported along with the MEP peak-to-peak amplitude values and included in the Excel spreadsheets. For removal of individual trials, we defined excessive EMG activity as trials in which the average root mean square amplitude value exceeded the average value at baseline plus one standard deviation. Individual trials that met the criteria for excessive EMG activity were flagged in the Excel spreadsheet using the 'IF' function and removed from subsequent analysis (i.e., the MEP peak-to-peak amplitude value was not included in the participant's average as described below).

As outlined above, 10 stimulations were performed at each of the stimulator output intensities (100, 110, 120, 130 and 140% RMT) to generate an S-R curve. Participants in which more than 6/10 MEPs were rejected due to not meeting our inclusion criteria at any intensity were excluded from further analysis (e.g., if only 3/10 MEPs were obtained for the 120% RMT intensity for the second post-exercise TMS collection, the S-R curve data for that participant would be removed from that portion of the analysis). The S-R curve for each participant and time-point (pre-

exercise, immediately post-exercise and 30 min post-exercise) was then generated by averaging the MEP peak-to-peak amplitude at each of the stimulus intensities and across time-points (pre, post1 and post2). Similar to the single pulse measures, if less than 4/10 MEPs remained following the analysis outlined above, the participant's data was excluded from that part of the analysis. The amount of facilitation or inhibition was determined by averaging the remaining MEP peak-to-peak amplitude for each participant, paradigm (SICI, LICI, ICF) and time-point, and then expressing this average peak-to-peak amplitude as a percentage of the unconditioned stimulus obtained at the same stimulator output (120% RMT) and time-point during the single pulse data collection. For ICF specifically, participants whose average MEP peak-to-peak amplitude did not show overall facilitation (i.e., exceeding 100% of the unconditioned peak-to-peak amplitude obtained at 120% RMT) were excluded from further analysis of the ICF data. Given SICI and LICI are expected to produce inhibition, it is not possible to determine if the absence of a MEP is due to inhibition or some other technical error. As such, trials obtained during SICI and LICI were only removed from analysis if the stimulus was preceded by excessive muscle activity (as described above).

3.3.4 Statistical Analysis

Stimulus response curves were plotted for each participant at each time-point, and AUC determined for each. Briefly, AUC analysis involves calculating the area under the curve, where the curve is represented by a series of connected XY points in which the area is computed for a given baseline (in this instance Y = 0).

Subsequent analyses of S-R curve data utilized the AUC value or the AUC change score, which was calculated by subtracting the AUC value for the pre-exercise condition from either of the post-exercise conditions (i.e., post1 and post2). As indicated above, for paired-pulse measures, the degree of facilitation (ICF) and inhibition (SICI and LICI), was expressed as a percentage of the unconditioned MEP amplitude at 120% RMT for the corresponding time-point. To facilitate our analysis, we also calculated change scores for each of ICF, SICI and LICI, in which the change score was calculated by subtracting the percentage value pre-exercise from either of the post-exercise conditions. For all measures, normality of the data was examined using the D'Agostino & Pearson omnibus K2 test (Stephens, 1986). In all instances of a non-Gaussian distribution, outliers were removed to ensure normality of the residuals, as the analyses used (analysis of variance (ANOVA) and regression) are susceptible to error when the residual values are not normally distributed.

To aid in comparison to prior work looking at aerobic exercise and cortical excitability, we examined the general effect of the single exercise session on excitability measures by comparing pre- and post-exercise measures using one-way repeated measures ANOVA. Specifically, for the S-R data we looked at change in AUC value as a function of time (pre, post1 and post2), while for paired-pulse data we looked at the change in percentage of the unconditioned MEP as a function of time (pre, post1 and post2). To address our specific hypotheses related to the influence of aerobic fitness (i.e., VO_2max) on the excitability variables (S-R curve, SICI, LICI and ICF) we employed linear regression. Specifically, we regressed VO_2max values against AUC values (S-R curve) and percentage of unconditioned MEP (ICF, SICI and

LICI) for the 'pre' time-point, and VO_2max values against the AUC change scores (S-R curve) and percentage of unconditioned MEP (ICF, SICI and LICI) for the post1 and post2 time-points. Where applicable, an *a priori* alpha of $p < 0.05$ denoted statistical significance. All statistical analyses were performed using Prism 6 (v. 6.0a, GraphPad Software Inc, CA, USA).

CHAPTER 4: RESULTS

Of the 35 participants recruited for the study, data from 29 (14 females, 1 left-handed, 25.9 ± 2.8 years) were included in the analysis. Two participants were excluded at the beginning of the first testing session, as they did not meet the inclusion criteria. An additional two participants were excluded during the second experimental session, as we were unable to localize a hotspot for the ECR muscle (i.e., no response was elicited as a result of stimulation). Finally, two participants were eliminated during pre-processing owing to an excessively noisy baseline in the EMG data that precluded the ability to distinguish MEPs. Of the 29 remaining participants, some data had to be removed from various aspects of the analysis owing to the criteria described above (e.g., not a sufficient number of MEPs). Refer to Table 2 for the number of participants included in data analysis for each stimulation paradigm and associated analysis. Participant demographics are provided in Table 3, arranged according to their VO_2max values (ascending smallest to largest).

Table 2: Number of participants included in data analysis for each stimulation paradigm.

Participants			
Total for Analysis:		Initial	After removing outliers
S-R	Pre	29	26
	Post 1	27	24
	Post 2	27	24
SICI	Pre	28	28
	Post 1	26	26
	Post 2	26	26
LICI	Pre	26	21
	Post 1	24	24
	Post 2	23	20
ICF	Pre	0	0
	Post 1	0	0
	Post 2	0	0

Table 3: Participant demographics. Participants are ranked in order of maximal oxygen consumption (VO_2max; ml/kg/min) score. Age, sex, body mass index (BMI; kg/m^2), International Physical Activity Questionnaire (IPAQ) Score (in Met-minutes per week) and Category are also included for each participant.

Participant #	VO_2max	Sex	Age	BMI	IPAQ Score	IPAQ Category
1	22.1	M	24	28.5	320	Low
2	24.0	F	25	28.3	2364	Moderate
3	25.4	F	23	28.2	1182	Moderate
4	25.7	F	26	28.8	7848	Moderate
5	26.4	M	24	36.9	1902	High
6	26.5	M	28	34.2	1893	Moderate
7	26.9	F	24	22.8	12288	High
8	27.2	F	27	23.8	2946	Moderate
9	27.6	F	26	24.7	3972	High
10	28.5	F	25	20.0	1542	Moderate
11	31.0	M	29	24.7	2448	High
12	31.3	F	23	20.2	1417	Moderate
13	31.7	M	26	25.2	3252	High
14	33.3	F	36	22.6	4938	High
15	34.1	M	25	29.6	12342	High
16	35.3	F	25	22.2	4386	High
17	35.4	F	26	22.0	2887.5	High
18	35.5	F	23	19.4	2142	Moderate
19	35.9	M	23	22.9	4830	High
20	37.1	F	27	20.5	7224	High
21	37.2	F	28	20.2	3154	High
22	37.7	M	25	26.4	9306	High
23	40.5	M	25	24.2	11196	High
24	40.9	M	29	23.8	5079	High
25	42.1	M	24	21.2	1782	Moderate
26	42.3	M	28	20.2	3873	High
27	42.8	M	25	25.0	3666	High
28	46.1	M	22	21.3	2337	Moderate
29	48.2	M	29	22.3	3158	High

4.1 Maximal Exercise Test Results

All participants were able to complete the GXT as per our criteria. Although not all participants were able to achieve HR values within 95% of their respective estimated HRmax, based on the equation described earlier (Estimated HRmax = [206.9 – (0.67 x Age)]), all participants achieved a max RPE ≥17 and a RER ≥ 1.15 (Table 4). 24 participants saw a measurable plateau in VO_2max values (1.34 ± 1.07). As observed in Figure 19, participants achieved a range of VO_2max values (22.1 to 48.2), with an average value of 33.7 ± 7.0 mL/kg/min observed across the group.

Table 4: Results from the Maximal Exercise Test, including maximal oxygen consumption (VO_2max; ml/kg/min), maximal work (watts), maximal respiratory exchange ratio (RER), maximal heart rate (HRmax; beats per minute), and the corresponding percentage of age-predicted HRmax. Maximal rating of perceived exertion (RPE) was based on the 6-20 Borg scale. A plateau was achieved if a difference of VO_2 less than 2.1ml/kg/min in the last minute of the test.

Participant #	VO_2max	Max Work	HRmax	% Age Predicted HRmax	Max RPE	Max RER	Plateau achieved?
1	22.1	157	162	84.9	20	1.31	Yes
2	24.0	205	174	91.5	19	1.21	Yes
3	25.4	187	203	106.0	17	1.31	Yes
4	25.7	172	187	98.7	20	1.31	Yes
5	26.4	229	174	91.2	19	1.24	Yes
6	26.5	250	178	94.6	19	1.22	No
7	26.9	163	186	97.5	20	1.28	Yes
8	27.2	178	174	92.2	19	1.38	Yes
9	27.6	199	188	99.2	19	1.32	Yes
10	28.5	166	180	94.7	19	1.34	Yes
11	31.0	214	174	92.8	20	1.36	Yes
12	31.3	208	193	100.8	20	1.37	Yes
13	31.7	214	191	100.8	19	1.34	Yes
14	33.3	194	187	102.3	20	1.42	Yes
15	34.1	282	194	102.0	20	1.39	No
16	35.3	218	163	85.7	19	1.24	Yes
17	35.4	210	195	102.9	20	1.24	Yes
18	35.5	202	167	87.2	19	1.2	Yes
19	35.9	254	177	92.4	18	1.43	Yes
20	37.1	214	175	92.7	19	1.36	Yes
21	37.2	199	188	99.9	20	1.28	Yes
22	37.7	318	181	95.2	19	1.25	No
23	40.5	258	169	88.9	18	1.31	No
24	40.9	314	156	83.2	17	1.27	Yes
25	42.1	262	194	101.7	20	1.36	Yes
26	42.3	345	166	88.2	19	1.37	Yes
27	42.8	318	192	101.0	18	1.29	No
28	46.1	286	190	98.9	20	1.46	Yes
29	48.2	300	203	108.3	19	1.23	Yes

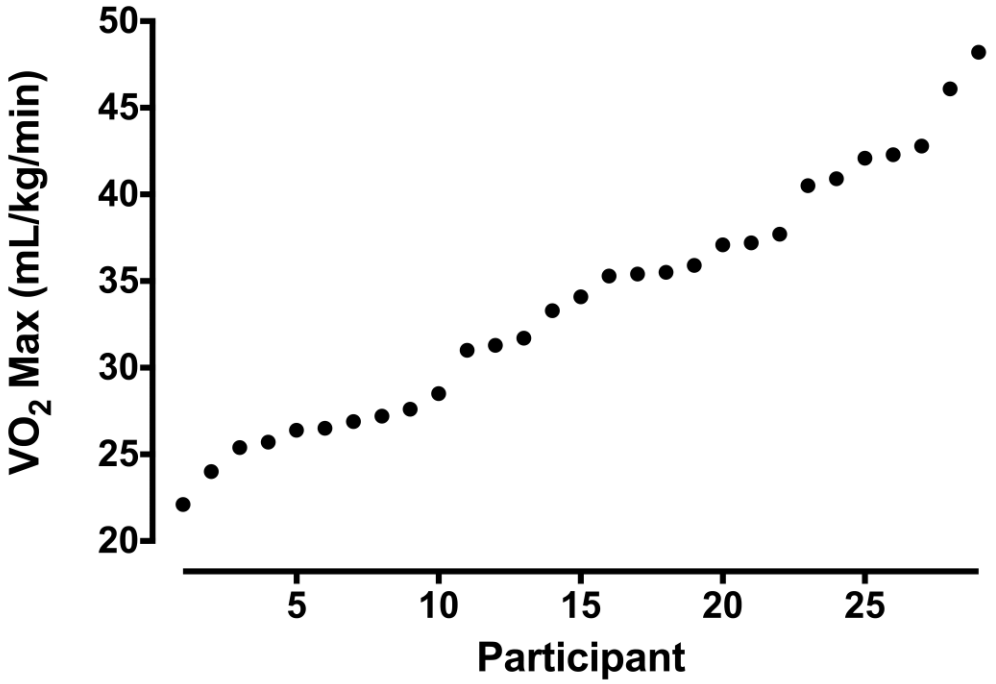

Figure 19: Aerobic fitness level, measured by maximal oxygen consumption (VO_2max), by participant.

4.2 Moderate-Intensity Exercise Results

On average, 19.6 ± 14.5 days separated the first and second experimental sessions (Table 5). As outlined in the methods, participants were asked at the beginning of the second session if they had significantly changed their training since the first session, to which none replied that they had. Also displayed in Table 5 is each participant's target HR (126 ± 9.1) for session 2, which was 50% HRR based on the results of GXT. The average HR achieved during the exercise session (128 ± 9.8) as well as the range of RPE for each participant during the 20-minute exercise session are also shown (Table 5). In general, participants were able to maintain their HR within 10 beats of their target. Participants 2 and 29 were greater than 10 beats under their target HR, but had high RPE's (11-16 and 13-16, respectively).

Participant 19 had an average HR in excess of 10 beats greater than their target but had a lower RPE (9-12).

Table 5: Moderate-intensity exercise data. Participant's maximal oxygen consumption (VO_2max) was determined in session 1. Number of days session 2 occurred after session 1 is included. Target heart rate (HR; beats per minute) was calculated based off the HR data from session 1. Average HR is the data from session 2 (beats per minute ± standard deviation). The range of rating of perceived exertion (RPE) values, based on the 6-20 Borg scale, is included.

Participant #	VO_2max	Days post session 1	Target HR	Average HR	RPE
1	22.1	15	116	117 ± 4.0	12-16
2	24.0	6	126	111 ± 17.9	11-16
3	25.4	2	147	150 ± 4.1	9-13
4	25.7	1	127	128 ± 6.7	11-14
5	26.4	7	129	133 ± 3.1	10-15
6	26.5	28	120	124 ± 4.6	8-13
7	26.9	13	134	137 ± 5.7	10
8	27.2	10	120	126 ± 5.0	10-12
9	27.6	41	138	138 ± 4.6	11-16
10	28.5	2	130	134 ± 4.7	9-15
11	31.0	23	122	127 ± 2.7	15-18
12	31.3	7	133	133 ± 6.3	12-13
13	31.7	19	125	126 ± 7.5	11-14
14	33.3	21	133	137 ± 5.0	11-13
15	34.1	11	134	136 ± 5.9	12-13
16	35.3	24	114	120 ± 3.3	12-13
17	35.4	22	131	138 ± 5.4	8-13
18	35.5	37	118	114 ± 5.7	11-17
19	35.9	9	119	132 ± 5.0	9-12
20	37.1	16	125	127 ± 3.8	12-15
21	37.2	17	122	125 ± 3.7	12-13
22	37.7	28	124	123 ± 7.1	11-16
23	40.5	61	112	117 ± 6.5	11-15
24	40.9	12	107	109 ± 3.8	10-16
25	42.1	5	133	139 ± 3.3	11-16
26	42.3	46	114	115 ± 4.6	11-13
27	42.8	38	133	141 ± 3.4	12-13
28	46.1	27	123	126 ± 8.6	9-12
29	48.2	19	139	125 ± 6.4	13-16

4.3 TMS Results

4.3.1 Single Pulse TMS Results

The average peak-to-peak amplitude for the resultant MEPs for each participant, stimulation intensity and time-point are shown in Table 6. Overall, MEP amplitude increased as a function of stimulator output within a time-point. While only 4/10 MEPs at any one stimulator output was required for inclusion in the data analysis, on average the number of MEPs included for each participant was higher (see Table 7).

Table 6: Average MEP peak-to-peak amplitudes for each participant for each stimulation intensity and time-point. Numbers in parentheses are standard deviations. 'X' indicates data that was not used for analysis.

		100%			110%			120%			130%			140%		
P#	**VO_2**	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2
1	22.1	262 (69)	240 (106)	172 (59)	257 (81)	306 (110)	229 (98)	334 (147)	350 (95)	256 (81)	446 (140)	327 (76)	376 (75)	501 (80)	440 (116)	494 (157)
2	24.0	385 (98)	498 (190)	193 (74)	557 (191)	796 (139)	552 (407)	673 (186)	892 (146)	476 (145)	913 (139)	941 (162)	775 (212)	817 (190)	1024 (94)	712 (309)
3	25.4	119 (95)	252 (251)	205 (117)	502 (312)	631 (302)	438 (104)	512 (148)	594 (173)	923 (326)	760 (207)	895 (107)	770 (171)	811 (121)	886 (208)	836 (150)
4	25.7	104 (89)	74 (34)	165 (73)	217 (139)	114 (42)	205 (99)	280 (49)	262 (120)	308 (169)	506 (138)	283 (120)	478 (168)	474 (99)	346 (182)	430 (160)
5	26.4	240 (168)	92 (44)	86 (40)	542 (159)	419 (289)	402 (121)	880 (482)	1066 (246)	1057 (386)	1038 (317)	1214 (226)	1450 (422)	1147 (376)	1371 (232)	1351 (335)
6	26.5	200 (62)	179 (90)	510 (222)	648 (328)	388 (180)	739 (358)	558 (236)	578 (253)	1020 (165)	766 (179)	784 (235)	968 (317)	738 (155)	818 (172)	1003 (134)
7	26.9	493 (169)	315 (119)	365 (65)	547 (181)	381 (106)	489 (178)	535 (94)	443 (218)	515 (134)	655 (212)	623 (203)	426 (139)	874 (279)	625 (117)	613 (231)
8	27.2	278 (116)	405 (142)	322 (139)	501 (61)	526 (142)	429 (221)	507 (160)	640 (158)	642 (81)	704 (139)	678 (137)	791 (104)	840 (222)	688 (162)	824 (142)
9	27.6	100 (29)	177 (47)	147 (77)	281 (99)	198 (89)	167 (62)	325 (170)	508 (175)	398 (91)	673 (151)	587 (125)	441 (73)	713 (211)	659 (118)	561 (76)
10	28.5	387 (142)	264 (55)	441 (120)	427 (132)	344 (130)	480 (119)	646 (112)	502 (336)	576 (151)	567 (186)	456 (154)	449 (145)	621 (122)	506 (129)	385 (147)
11	31.0	327 (533)	X	X	281 (174)	X	X	613 (713)	X	X	853 (552)	X	X	1235 (490)	X	X
12	31.3	406 (205)	350 (71)	286 (103)	468 (68)	691 (285)	498 (211)	838 (146)	709 (129)	540 (160)	809 (173)	638 (160)	517 (82)	705 (122)	561 (143)	551 (144)
13	31.7	191 (116)	254 (165)	197 (142)	353 (289)	651 (285)	223 (101)	418 (138)	927 (516)	522 (390)	347 (155)	696 (226)	591 (143)	450 (163)	633 (319)	347 (134)
14	33.3	133 (67)	208 (82)	235 (136)	348 (184)	288 (88)	283 (98)	371 (115)	453 (96)	344 (45)	333 (92)	448 (180)	491 (188)	354 (136)	500 (151)	500 (81)
15	34.1	453 (67)	X	X	185 (112)	X	X	299 (165)	X	X	412 (170)	X	X	443 (233)	X	X

		100%			110%			120%			130%			140%		
P#	**VO_2**	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2
16	35.3	95 (39)	239 (91)	153 (152)	542 (250)	414 (196)	321 (179)	739 (237)	836 (204)	477 (210)	911 (164)	879 (205)	504 (190)	703 (262)	742 (219)	522 (257)
17	35.4	114 (74)	590 (516)	120 (52)	149 (133)	222 (109)	158 (164)	294 (136)	351 (256)	287 (292)	402 (246)	399 (174)	344 (168)	263 (76)	411 (72)	397 (188)
18	35.5	458 (113)	507 (141)	333 (57)	437 (240)	559 (103)	428 (157)	422 (189)	576 (60)	431 (217)	459 (98)	840 (299)	582 (134)	358 (128)	648 (176)	355 (134)
19	35.9	137 (40)	268 (104)	354 (151)	183 (32)	258 (120)	599 (191)	370 (58)	337 (168)	781 (466)	448 (164)	631 (265)	740 (254)	441 (199)	588 (125)	701 (173)
20	37.1	125 (124)	250 (157)	265 (159)	235 (226)	317 (162)	331 (118)	212 (98)	325 (114)	234 (71)	241 (93)	221 (81)	299 (109)	307 (125)	301 (150)	428 (106)
21	37.2	688 (187)	743 (436)	767 (492)	1707 (516)	1621 (497)	1601 (516)	2293 (402)	2422 (405)	2655 (558)	2610 (327)	2710 (290)	2719 (456)	2727 (384)	2872 (260)	2934 (458)
22	37.7	155 (53)	1088 (411)	440 (102)	310 (175)	2026 (944)	801 (221)	360 (131)	3436 (414)	1323 (494)	504 (145)	3938 (205)	1479 (289)	471 (106)	3645 (514)	1394 (461)
23	40.5	125 (131)	111 (135)	176 (266)	215 (209)	257 (261)	201 (291)	347 (78)	326 (99)	180 (75)	794 (410)	611 (227)	333 (141)	948 (521)	623 (258)	596 (313)
24	40.9	363 (169)	827 (336)	325 (135)	695 (217)	1063 (338)	422 (210)	928 (141)	1522 (293)	606 (119)	911 (127)	1470 (282)	647 (159)	928 (232)	1282 (219)	678 (242)
25	42.1	272 (128)	166 (72)	255 (72)	383 (106)	269 (87)	754 (412)	628 (200)	882 (217)	1434 (530)	625 (245)	959 (212)	1319 (220)	826 (296)	1115 (410)	1345 (391)
26	42.3	381 (291)	202 (70)	181 (54)	303 (163)	239 (62)	205 (28)	309 (132)	262 (83)	214 (50)	513 (486)	292 (90)	260 (83)	420 (193)	322 (113)	272 (115)
27	42.8	151 (44)	136 (75)	215 (127)	684 (157)	550 (130)	603 (180)	777 (189)	809 (132)	746 (245)	716 (197)	776 (200)	771 (126)	874 (183)	794 (69)	739 (139)
28	46.1	356 (292)	138 (121)	383 (310)	1324 (922)	606 (149)	1108 (1363)	1287 (317)	625 (141)	1223 (1137)	1823 (1012)	571 (156)	975 (486)	1606 (899)	813 (460)	782 (191)
29	48.2	92 (34)	193 (109)	142 (62)	368 (104)	327 (48)	241 (109)	340 (102)	395 (85)	264 (85)	418 (152)	416 (129)	340 (116)	443 (129)	400 (98)	295 (107)

Table 7: Number of MEPs averaged for each participant for each stimulation intensity and time-point. 'X' indicates data that was not used for analysis.

		100%			110%			120%			130%			140%		
P#	**VO_2**	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2
1	22.1	9	9	7	6	9	7	8	8	8	8	9	8	9	8	8
2	24.0	8	8	9	8	8	8	8	8	9	9	9	9	8	9	8
3	25.4	7	5	7	8	8	7	8	8	9	8	9	9	8	9	9
4	25.7	8	4	9	8	8	9	7	9	9	8	7	9	6	8	9
5	26.4	8	7	6	7	9	7	8	7	8	9	8	9	8	9	8
6	26.5	8	8	7	8	8	8	8	9	8	9	9	8	9	8	5
7	26.9	7	9	7	8	9	9	9	8	8	9	8	8	9	8	9
8	27.2	8	9	8	7	9	9	7	9	8	9	9	8	8	9	8
9	27.6	6	7	4	7	8	8	8	8	9	9	9	8	8	9	9
10	28.5	8	7	6	7	9	9	8	7	8	9	8	8	9	9	8
11	31.0	4	X	X	7	X	X	7	X	X	8	X	X	9	X	X
12	31.3	8	7	8	7	9	9	8	7	9	8	8	9	8	8	8
13	31.7	7	7	8	8	9	6	8	9	7	8	9	8	7	9	8
14	33.3	8	7	8	9	8	9	9	7	6	8	8	8	8	9	7
15	34.1	7	X	X	8	X	X	8	X	X	8	X	X	9	X	X
16	35.3	6	7	7	8	9	9	9	9	9	8	8	9	8	9	8
17	35.4	6	8	5	8	8	6	8	8	7	8	9	9	7	7	8
18	35.5	8	9	6	8	7	7	9	7	8	9	9	7	6	9	8
19	35.9	6	8	9	8	8	8	7	9	8	7	9	9	8	8	7
20	37.1	7	7	7	7	9	8	9	9	6	8	8	8	8	9	8
21	37.2	6	5	8	9	8	9	8	8	8	9	8	8	8	8	9
22	37.7	8	9	8	7	8	8	8	8	9	9	8	7	8	9	10
23	40.5	7	8	6	8	7	7	8	8	8	9	7	9	9	8	9
24	40.9	7	9	8	8	8	7	8	8	8	8	7	8	8	9	8
25	42.1	7	9	7	7	7	9	8	9	9	8	8	8	8	8	8

		100%			110%			120%			130%			140%		
P#	**VO_2**	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2	Pre	Post1	Post2
26	42.3	8	7	9	7	9	8	9	9	9	9	7	9	8	9	7
27	42.8	5	6	6	9	8	8	9	9	9	8	8	7	9	7	9
28	46.1	7	8	7	9	8	8	7	9	9	8	8	6	8	8	8
29	48.2	8	7	8	8	8	8	9	7	9	7	9	8	9	8	9
Average		7.1	7.4	7.2	7.7	8.3	8.0	8.1	8.2	8.2	8.3	8.3	8.2	8.1	8.4	8.1
SD		1.1	1.3	1.3	0.8	0.7	0.9	0.7	0.8	0.9	0.6	0.7	0.8	0.8	0.6	0.9
Min		4	4	4	6	7	6	7	7	6	7	7	6	6	7	5

Pre- and post-exercise (post1 and post2) values were analyzed independently. For all measures, pre-exercise responses were considered as baseline values and used to address our first hypothesis. Post-exercise responses (post1 and post2) were considered the exercise induced change from baseline and were used to address our second hypothesis. For S-R data, 29 and 27 participants were analyzed for the pre- and post-exercise time-points, respectively. As outlined in the methods, a change score was calculated for post1 and post2 values, with these change scores used in the analysis. Analysis indicated that the residuals for the AUC data were not normally distributed ($p<0.05$), and thus 3 participants (21, 22 and 28) for the pre-exercise time-point and 5 participants (11, 15, 21, 22 and 28) for the post-exercise time-points were identified as outliers and subsequently removed from the analysis. As a result, data from 26 and 24 participants remained in the analysis of the S-R curve data for our first and second hypotheses respectively (see Table 6).

While we observed an increase in AUC values in many of the participants in post1 relative to pre (n=15) and post2 relative to pre (n=11), we did not observe a main effect of time on AUC values ($F(2,23) = 1.120$, $p = 0.3268$). To address our first hypothesis, linear regression was performed using the VO_2max and AUC values. Results of this analysis revealed a non-significant relationship in relation to the slope of the line differing from zero ($F(1, 24) =0.6110$, $p=0.4420$) and an R^2 value of 0.02483. The relationship between VO_2max and AUC values for the pre-exercise time-point is shown in Figure 20 (panel A). As indicated by the line of best fit, a negative non-significant relationship exists between VO_2max and AUC, suggesting

that higher aerobic fitness does not equate to increased cortical excitability in general. While not formally a part of the analysis, Figure 20 also depicts the relationship between VO_2max and AUC values for the post1 (panel B) and post2 (C) time-points. Similar to that observed for the pre-exercise time-point, these results suggest that higher aerobic fitness does not equate to a heightened response to a single session of aerobic exercise. Linear regression was performed on the VO_2max values and AUC change scores for the post1 (Figure 21, panel A) and post2 (Figure 21, panel B) time-points. For Post 1, results revealed a non-significant relationship in relation to the slope of the line differing from zero ($F(1, 22) = 0.6210$, $p=0.4391$) and an R^2 value of 0.02745. Similar results were found for the post2 time-point ($F(1, 22) = 0.01663$, $p=0.8986$; $R^2 = 0.0007551$). Again, these findings indicate that higher aerobic fitness does not equate to a heightened response to a single session of exercise.

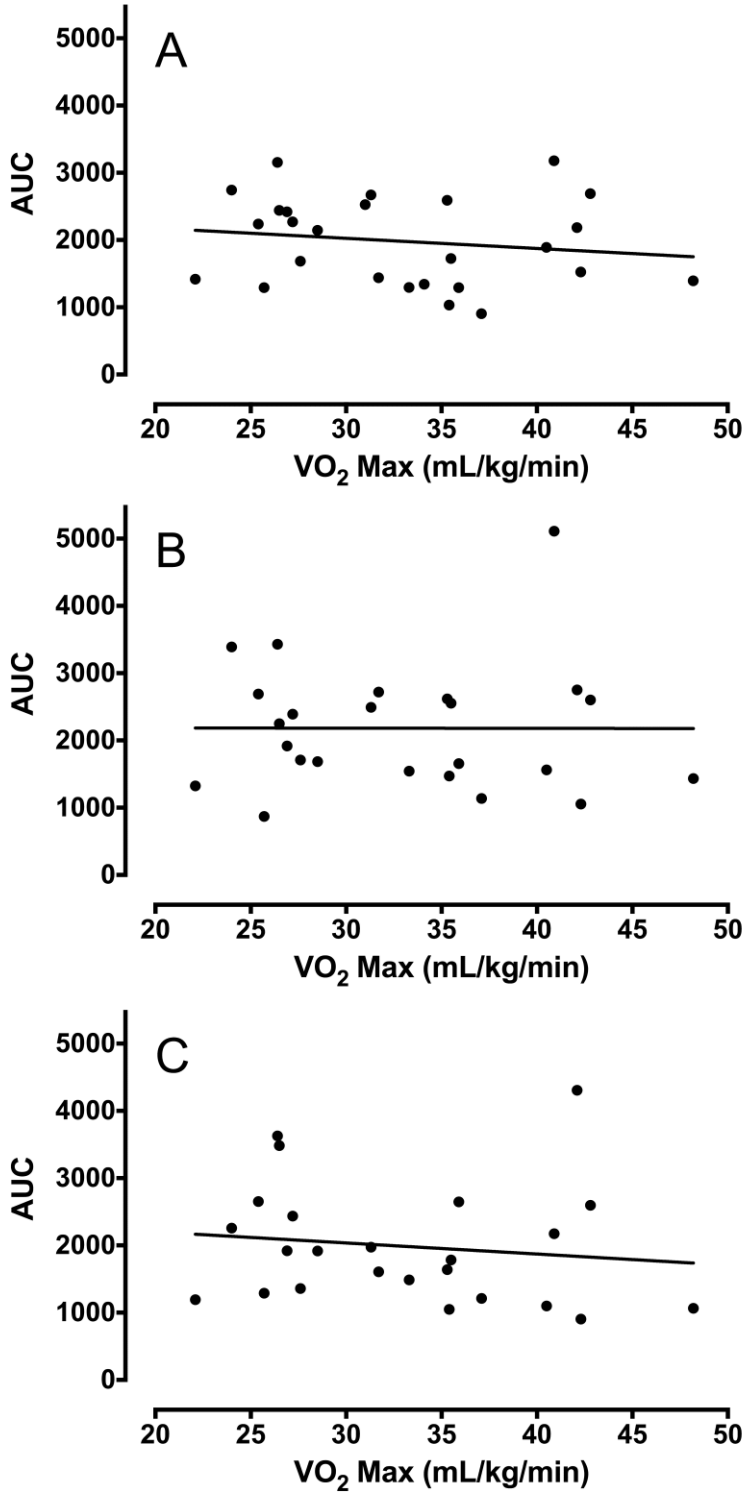

Figure 20: Area under the stimulus-response curve (AUC) values for each participant, plotted as a function of aerobic fitness (VO_2max) prior to exercise ('pre'; A); immediately after exercise ('post1'; B); and 30 min after exercise ('post2'; C). Solid lines represent the line of best fit.

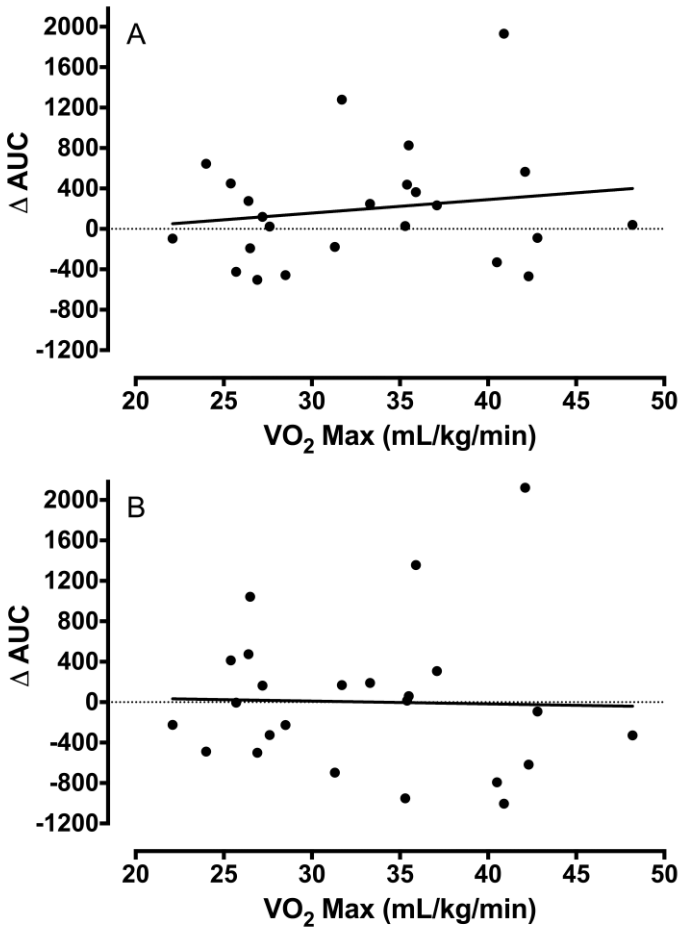

Figure 21: Area under the stimulus-response curve change scores (ΔAUC) for each participant, plotted as a function of aerobic fitness (VO_2max) immediately after exercise ('post1'; A); and 30 min after exercise ('post2'; B). Change score is calculated from baseline, or pre exercise. Solid lines represent the line of best fit.

4.3.2 Paired-Pulse TMS Results

Consistent with the approach used for the S-R analysis (above), pre- and post-exercise (post1 and post2) values were analyzed independently. For all measures, pre-exercise responses were considered as baseline values and used to address our first hypothesis. Post-exercise responses (post1 and post2) were considered the exercise induced change from baseline and were used to address our

second hypothesis. As outlined in the methods, a change score was calculated for post1 and post2 values, with these change scores used in the analysis.

4.3.2.1 SICI Results

For the SICI component of the analysis, 28 and 26 participants were analyzed for the pre- and post-exercise time-points, respectively. Analysis of the residuals revealed a normal distribution, and as such no outliers were removed from the analysis, and all participants (28 and 26 for hypotheses 1 and 2 respectively) were carried forward in the analysis. Values for SICI are shown in Table 8, reported as the percentage of the unconditioned MEP (i.e., 120% RMT) for the corresponding time-point. Participants had 7/10 MEPs or more for each time-point (Table 9).

Table 8: SICI values for pre- and post-exercise. Values represent the % of the unconditioned MEP obtained at 120% of RMT for the corresponding time-point.

Participant #	VO_2max (ml/kg/min)	% of unconditioned MEP		
		Pre	Post1	Post2
1	22.1	42	40.0	73.4
2	24.0	X	X	X
3	25.4	11.8	8.5	10.7
4	25.7	10.8	20.0	16.4
5	26.4	5.8	3.7	3.6
6	26.5	2.7	18.2	12.8
7	26.9	22.9	48.9	40.4
8	27.2	19.0	24.7	16.4
9	27.6	1.7	1.4	6.1
10	28.5	45.9	14.5	30.4
11	31.0	6.8	X	X
12	31.3	27.5	22.6	51.8
13	31.7	16.0	4.9	17.4
14	33.3	13.8	24.5	16.6
15	34.1	14.0	X	X
16	35.3	5.4	7.7	6.2
17	35.4	20.8	75.6	38.7
18	35.5	50.5	73.6	44.0
19	35.9	8.1	9.3	9.6
20	37.1	40.9	46.0	57.4
21	37.2	2.4	8.5	8.1
22	37.7	34.2	18.3	28.1
23	40.5	7.3	17.3	10.2
24	40.9	26.0	9.5	73.3
25	42.1	14.5	10.3	11.1
26	42.3	27.4	46.6	32.5
27	42.8	20.8	6.6	4.5
28	46.1	7.5	5.1	13.2
29	48.2	4.8	7.7	18.1

Table 9: Number of MEPs averaged for each participant for each stimulation intensity and time-point. 'X' indicates data that was not used for analysis.

Participant #	VO_2max (ml/kg/min)	Pre	Post1	Post2
1	22.1	8	9	9
2	24.0	X	X	X
3	25.4	8	8	8
4	25.7	7	9	9
5	26.4	8	9	9
6	26.5	9	8	8
7	26.9	8	9	8
8	27.2	9	8	8
9	27.6	8	8	8
10	28.5	8	7	9
11	31.0	9	8	7
12	31.3	8	8	9
13	31.7	8	8	9
14	33.3	8	8	8
15	34.1	9	9	8
16	35.3	9	8	9
17	35.4	9	8	9
18	35.5	8	9	9
19	35.9	7	8	8
20	37.1	9	9	9
21	37.2	9	9	9
22	37.7	9	8	8
23	40.5	9	8	9
24	40.9	9	8	8
25	42.1	7	8	9
26	42.3	10	7	8
27	42.8	9	9	8
28	46.1	9	8	8
29	48.2	8	8	8
Average		8.4	8.3	8.4
SD		0.7	0.6	0.6
Min		7	7	7

Repeated measures ANOVA was performed to determine if there was a change in SICI as a result of the exercise across all participants, which revealed no main effect of time-point (pre, post1 and post2; $F(2,23) = 2.810$, $p = 0.0779$). While trending towards significance (refer to Figure 22, panel A), this result suggests that inhibition specific to SICI did not change as a result of the single session of exercise.

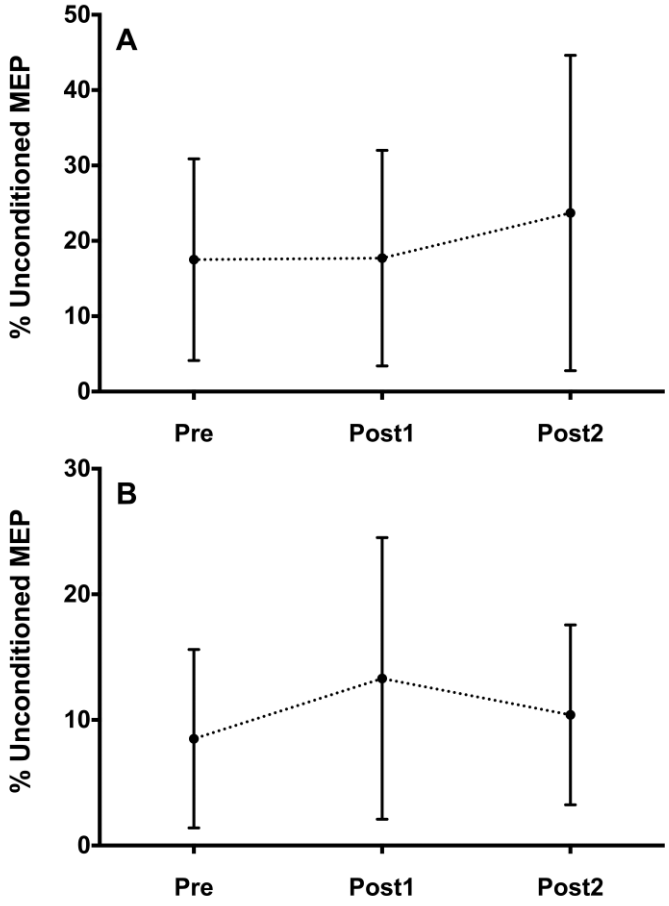

Figure 22: Averaged short-interval intracortical inhibition (panel A) and long-interval intracortical inhibition (panel B) values for Pre, Post1 and Post2 time-points. Bars represent standard deviation.

To address our first hypothesis, linear regression was performed using the VO_2max and SICI values from the pre-exercise time-point (Table 8). Results of this analysis revealed a non-significant relationship in terms of the slope of the line differing from zero ($F(1, 26) = 0.1612$, $p=0.6913$) and an R^2 value of 0.006162. The relationship between VO_2max and SICI values for each participant at the 'pre' time-point is illustrated in Figure 23 (panel A). This finding for VO_2max and SICI values indicates that higher aerobic fitness does not result in a reduction in SICI. For illustrative purposes, the relationship between VO_2max and SICI values for the post1 and post2 time-points are also depicted in Figure 23 (panels B and C respectively). A similar relationship was observed, suggesting in response to a single bout of aerobic exercise, SICI is not reduced relative to aerobic fitness (as measured by VO_2max). To formalize this finding (and address hypothesis #2), we again performed linear regression on the VO_2max values, this time with the post-exercise (post1 and post2) SICI change scores. This analysis revealed a non-significant finding ($F(1, 24) = 0.02513$, $p=0.8754$) for the post1 time-point, with an R^2 value of 0.001046 (Figure 24, panel A). A similar finding was observed for the post2 time-point ($F(1, 24) = 0.02288$, $p=0.8810$; $R^2 = 0.0009523$; Figure 24, panel B).

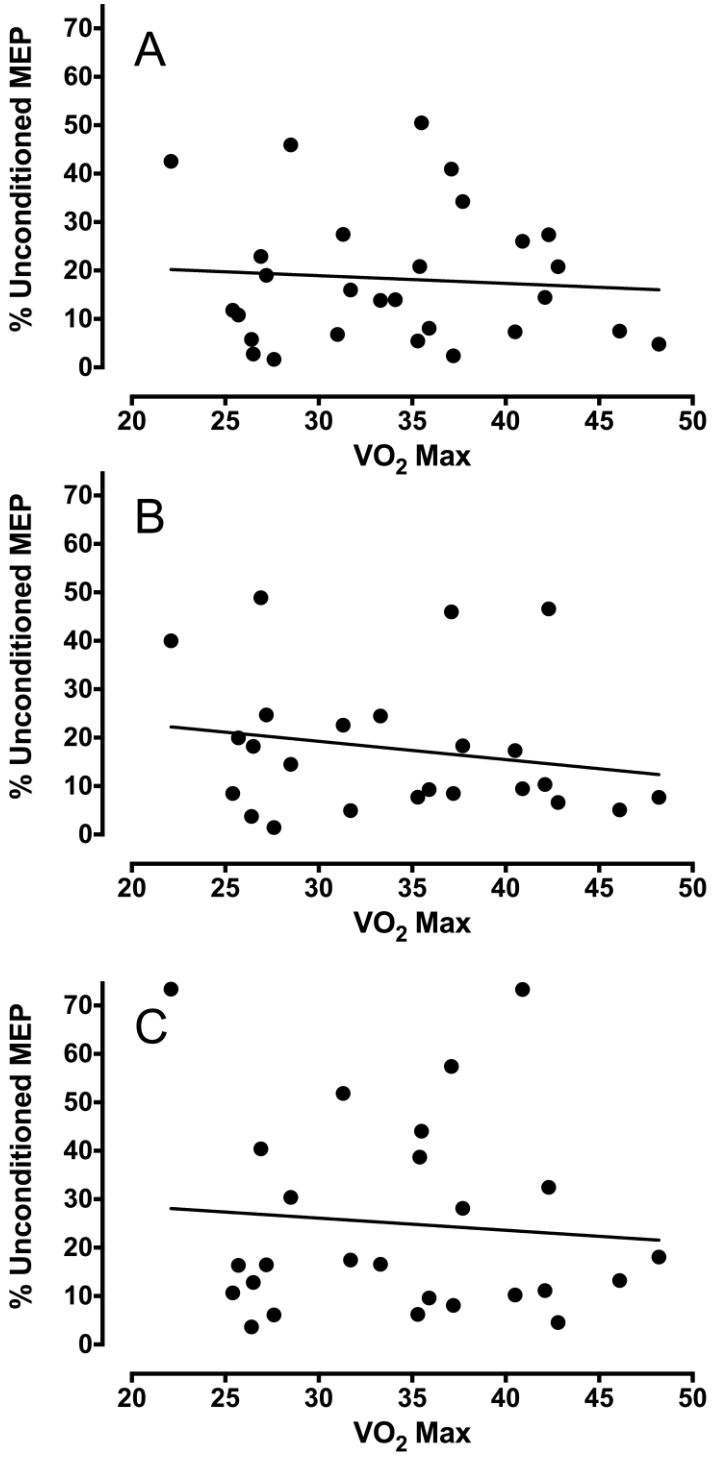

Figure 23: Short interval intracortical inhibition (SICI) for each participant (reported as % of the unconditioned MEP), is plotted as a function of aerobic fitness (VO_2max) prior to exercise ('pre'; A); immediately after exercise ('post1'; B); and 30 min after exercise ('post2'; C). Solid lines represent the line of best fit.

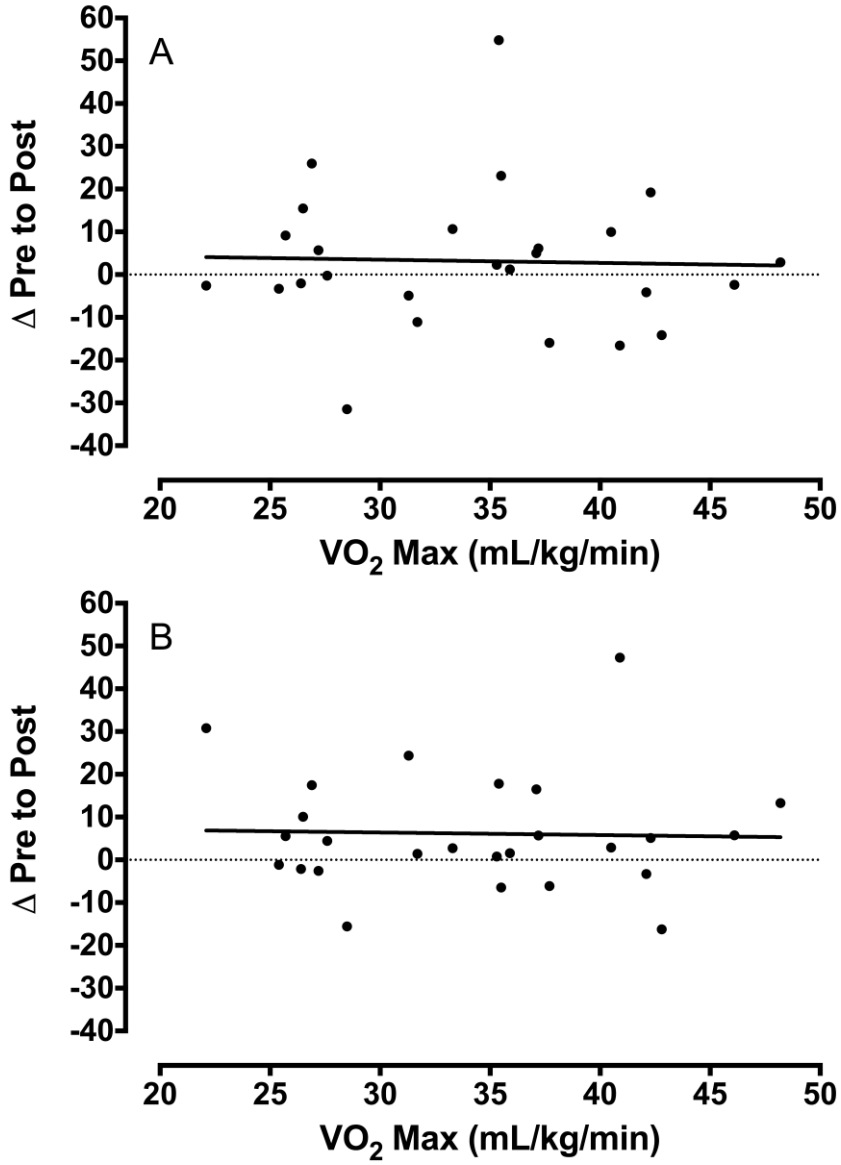

Figure 24: Change in short interval intracortical inhibition (SICI) for each participant (reported as change from pre-), is plotted as a function of aerobic fitness (VO_2max) immediately after exercise ('post1'; A); and 30 min after exercise ('post2'; B). Solid lines represent the line of best fit.

4.3.2.2 LICI Results

For the LICI component of the analysis, 26, 24 and 23 participants were analyzed for the Pre, Post 1 and Post 2 time points, respectively. Analysis of the residuals revealed a non-Gaussian distribution for the pre- and post2 time-points, and as such 5 (7, 10, 12, 14, 20) and 3 (9, 10, 14) participants identified as outliers and removed from subsequent analysis. Thus analysis of the LICI data included 21, 24 and 20 participants for the pre-exercise (hypothesis #1) and post-exercise (post 1 and 2 respectively; hypothesis #2). Values for LICI are shown in Table 10, reported as the percentage of the unconditioned MEP (i.e., 120% RMT) for the corresponding time-point. On average participants had considerably greater than 4/10 MEPs for each time-point, although a few data points were an average of only 1-3 MEPs. Average number of MEPs included for each participant for each time-point is presented in Table 11.

Table 10: LICI values for pre- and post-exercise. Values represent the % of the unconditioned MEP obtained at 120% of RMT for the corresponding time-point.

Participant #	VO_2max (ml/kg/min)	% of unconditioned MEP		
		Pre	Post1	Post2
1	22.1	12.0	21.2	17.5
2	24.0	X	X	X
3	25.4	2.2	5.2	1.3
4	25.7	X	X	X
5	26.4	10.2	6.2	10.4
6	26.5	1.4	11.1	0.8
7	26.9	54.2	63.6	47.1
8	27.2	6.0	3.3	4.6
9	27.6	15.2	12.0	45.3
10	28.5	59.1	30.4	8.7
11	31.0	1.9	X	X
12	31.3	33.9	25.5	37.6
13	31.7	8.1	21.6	23.6
14	33.3	56.3	95.1	101.9
15	34.1	5.5	X	X
16	35.3	3.6	7.4	10.4
17	35.4	4.1	37.6	6.5
18	35.5	10.2	5.7	8.0
19	35.9	11.3	2.7	2.2
20	37.1	31.4	17.7	11.1
21	37.2	17.5	6.3	15.3
22	37.7	2.1	1.0	2.3
23	40.5	6.9	34.9	22.4
24	40.9	6.5	1.8	7.6
25	42.1	9.2	12.3	10.4
26	42.3	2.1	25.7	11.2
27	42.8	12.5	6.3	X
28	46.1	X	X	X
29	48.2	8.6	16.7	20.9

Table 11: Number of MEPs averaged for each participant for each stimulation intensity and time-point. 'X' indicates data that was not used for analysis.

Participant #	VO_2max (ml/kg/min)	Pre	Post1	Post2
1	22.1	8	9	9
2	24.0	X	X	X
3	25.4	2	5	4
4	25.7	X	X	X
5	26.4	8	9	9
6	26.5	5	7	7
7	26.9	8	8	9
8	27.2	9	7	8
9	27.6	6	8	1
10	28.5	9	8	8
11	31.0	4	X	X
12	31.3	9	9	9
13	31.7	6	6	5
14	33.3	9	9	8
15	34.1	8	X	X
16	35.3	5	3	5
17	35.4	9	7	8
18	35.5	8	9	8
19	35.9	4	6	6
20	37.1	7	8	8
21	37.2	2	8	9
22	37.7	8	5	8
23	40.5	4	8	7
24	40.9	8	8	8
25	42.1	8	9	9
26	42.3	8	8	8
27	42.8	5	8	X
28	46.1	X	X	X
29	48.2	9	9	8
Average		6.8	7.5	7.0
SD		2.2	1.5	2.4
Min		2	3	1

As outlined in the methods, a repeated measures ANOVA was performed to determine if there was a change in LICI due to the single exercise session. Results of

this analysis indicated no main effect of time-point (pre, post1, post2; $F(2,16) = 2.831$, $p = 0.0963$), indicating that a significant change in inhibition specific to LICI was not observed. The relationship can be seen in Figure 22(panel B).

To address our first hypothesis, linear regression was performed using the VO_2max and LICI values from the pre-exercise time-point (Table 10). Results of this analysis revealed a non-significant relationship in terms of the slope of the line differing from zero ($F(1, 19) =0.03043$, $p=0.8634$), with an R^2 of 0.001599. The relationship between VO_2max and LICI values for each participant at the 'pre' time-point is illustrated in Figure 25 (panel A). This finding for VO_2max and LICI values indicates that higher aerobic fitness does not result in a reduction in LICI, however unlike SICI we did observe a positive slope (i.e., as VO_2max increases, the degree of inhibition decreases). Similar to SICI, the relationship between VO_2max and LICI values for the post1 and post2 time-points are also depicted in Figure 25 (panels B and C respectively). A similar relationship was observed, suggesting in response to a single bout of aerobic exercise, LICI does not have a significant reduction relative to aerobic fitness (as measured by VO_2max), however, again a positive slope is observed. To formally address hypothesis #2, linear regression was performed on the VO_2max values and post-exercise (post1 and post2) LICI change scores. This analysis revealed a non-significant finding ($F(1, 22) =0.3097$, $p=0.5835$) for the post1 time-point, with an R^2 of 0.01388 (Figure 26, panel A). A similar finding was observed for the post2 time-point ($F(1, 18) =0.9118$, $p=0.3523$; $R^2 = 0.04822$; Figure 26, panel B).

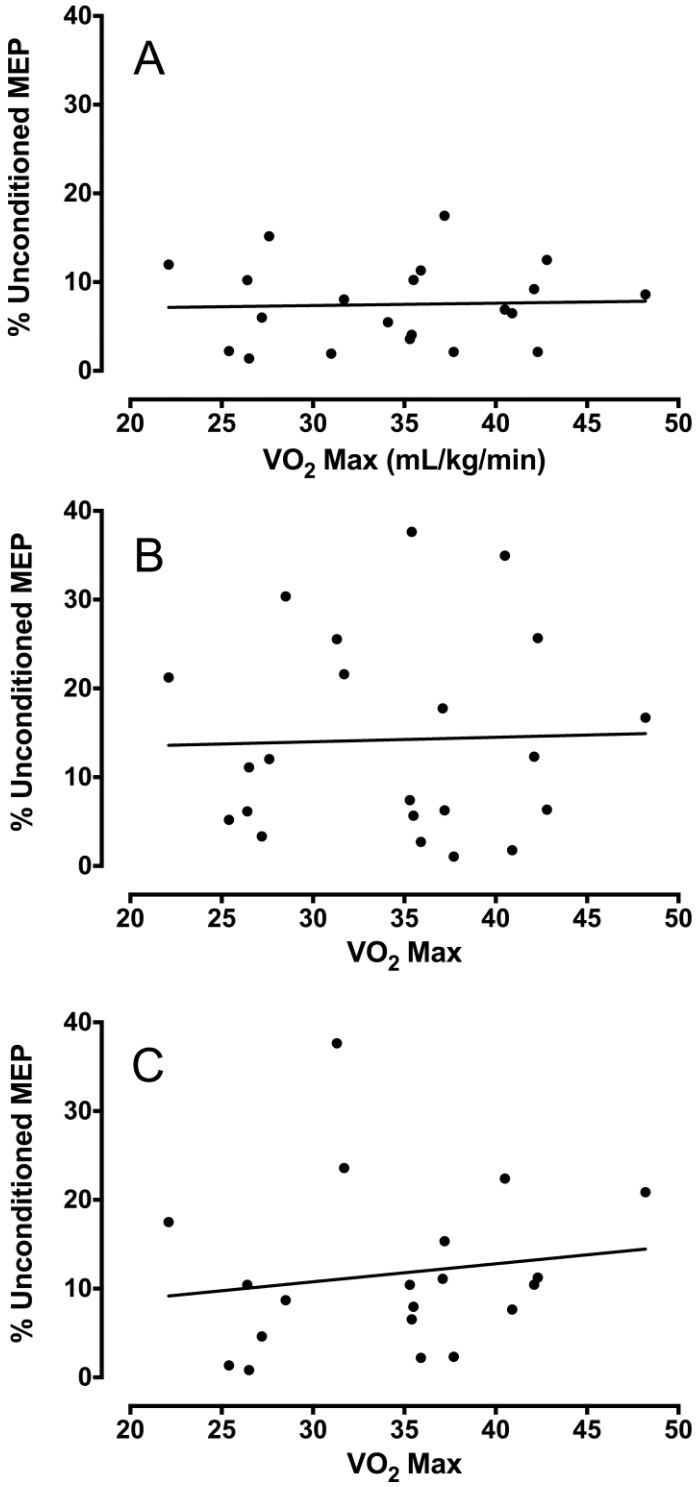

Figure 25: Long interval intracortical inhibition (LICI) for each participant (reported as % of the unconditioned MEP), is plotted as a function of aerobic fitness (VO_2max) prior to exercise ('pre'; A); immediately after exercise ('post1'; B); and 30 min after exercise ('post2'; C). Solid lines represent the line of best fit.

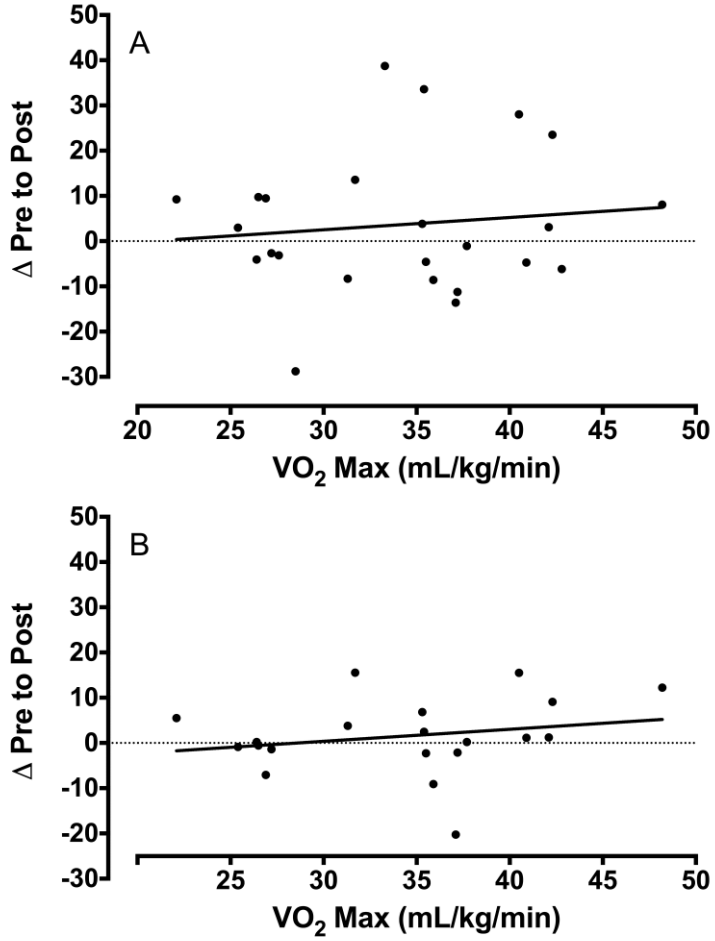

Figure 26: Change in long interval intracortical inhibition (LICI) for each participant (reported as change from pre-), is plotted as a function of aerobic fitness (VO_2max) immediately after exercise ('post1'; A); and 30 min after exercise ('post2'; B). Solid lines represent the line of best fit.

4.3.2.3 ICF Results

As per the criteria set out in the methods, only 3 of 26 participants remaining after preliminary analysis showed facilitation (i.e., ICF) at the pre-exercise time-point (i.e. % of the unconditioned MEP > 100). As such, no analysis was performed for ICF.

CHAPTER 5: DISCUSSION

Aerobic exercise has received increased interest owing to its potential for use as an agent for priming the brain for learning and rehabilitation. Improved performance on cognitive tasks (i.e., improved learning) has been shown after single sessions of aerobic activity (Chang, Labban, Gapin, & Etnier, 2012) and in physically active individuals (Colcombe & Kramer, 2003). Priming the brain for long lasting changes (i.e., LTP) is thought to be associated with an increase in cortical excitability (Griffin & Cafarelli, 2007) and changes in intracortical networks (i.e., decreases in intracortical inhibition) (Butefisch et al., 2000). Research has shown that a single session of aerobic exercise is associated with changes in cortical excitability (McDonnell et al., 2013; Singh, Duncan, et al., 2014; A. E. Smith et al., 2014). A recent study found that these exercise-induced changes were related to physical activity level (Lulic et al., 2017), such that individuals with higher activity levels had a greater response to exercise. What has not been previously studied is the relationship between aerobic fitness and cortical excitability, and how aerobic fitness affects the cortical response to exercise. Thus, the purpose of the present study was twofold: 1) to determine if there was a relationship between aerobic fitness and cortical excitability and 2) to determine if aerobic fitness influenced cortical excitability changes following a single session of exercise. Our hypotheses were that 1) there would be a positive relationship between aerobic fitness and cortical excitability, where increases in fitness would be associated with increases in

excitability, as evidenced by greater AUC and ICF, and lower levels of SICI and LICI; and 2) there would be a positive relationship between aerobic fitness and the magnitude of change in cortical excitability measures from baseline, where increases in fitness would be associated with greater change, as evidenced by greater increases in AUC values and ICF, and greater reductions in SICI and LICI relative to baseline measures. While our results displayed a similar trend to previous studies in response to exercise (Singh et al., 2014), the findings were not significant. Contrary to our hypotheses, there was little to no relationship between fitness and excitability measures or between fitness and the excitability response to exercise. Possible reasoning for our findings are discussed below.

5.1 Effects of Aerobic Fitness, Acute and Regular Aerobic Exercise on the Brain

Studies have shown that there are numerous neurophysiological responses that occur with acute and regular exercise. A number of these responses that occur during and after a single session of exercise are thought to influence changes in cortical excitability. Singh and Staines (2015) report extensively on these changes, which include changes in neurotransmitter amount and activity, altered cerebral blood flow and cortisol levels, and increases in BDNF. In short, the changes that occur with regard to these neuromodulatory agents result in a change in the overall excitability of the brain. Acute aerobic exercise affects cerebral blood flow and impacts the use of resources required for cerebral function, including oxygen, glucose and lactate (Coco et al., 2010; Gonzalez-Alonso et al., 2004), while regular exercise is associated with persistent changes in the cerebral vasculature. Animal

studies have found that regular exercise is associated with increased metabolic capacity and increased angiogenesis in M1 (Kleim, Cooper, & VandenBerg, 2002; McCloskey, Adamo, & Anderson, 2001). High levels of aerobic fitness are associated with increased cerebral blood flow (Pereira et al., 2007). As the availability of substrates (i.e. oxygen and lactate) is critical for brain function, cerebral blood flow is no doubt a factor contributing to changes in cortical excitability. Indeed, increases in blood lactate are associated with increases in M1 excitability (Coco et al., 2010).

Levels of BDNF are variable between participants, and therefore studies have demonstrated mixed findings. Overall the majority of studies suggest an increase in BDNF levels in response to acute exercise *(see Knaepen et al., 2010 for review)*. Although a number of aerobic training studies have been shown to result in increased resting levels of BDNF, the majority of studies have not reported such an increase (Knaepen et al., 2010; Schmolesky, Webb, & Hansen, 2013). There is growing evidence showing that there is an inverse relationship between aerobic fitness and BDNF levels, but that higher fitness is associated with an increase in the response (or change) in BDNF to acute aerobic exercise (Cho et al., 2012).

Acute exercise has been shown to have an intensity dependent relationship with cortisol, where cortisol levels decrease after low and moderate intensity exercise and increase after high intensity, exhaustive exercise (McDonnell et al., 2013). The decrease in cortisol elicited by aerobic activity facilitates the induction of plasticity (McDonnell et al., 2013). On the other hand, high levels of cortisol strongly inhibit the induction of plasticity in M1 (Sale, Ridding, & Nordstrom, 2008). Regular exercise is associated with inhibition of the hypothalamic-pituitary-adrenal axis, the

pathway that produces cortisol (Droste, Chandramohan, Hill, Linthorst, & Reul, 2007), which is why we expected to see increased excitability in individuals of increased fitness; however our results did not align with our hypotheses.

Overall, regular physical activity and level of aerobic fitness induce changes in the brain at rest and after a session of exercise. While the evidence suggests that level of aerobic fitness influences cortical excitability at rest and in response to exercise, the evidence is somewhat conflicting. The results of the present study do not support a relationship between aerobic fitness and cortical excitability.

5.2 Cortical Excitability

5.2.1 Area under the curve (AUC)

In their recent study examining the influence of activity level (assessed using the IPAQ) and cortical excitability, Lulic and associates (2017) found an increase in AUC values from pre- to post-exercise, but only in individuals with higher activity levels. In the current study, there was no association between AUC and time (i.e., pre- to post-exercise), suggesting that the AUC remained the same from pre- to post-exercise. This finding could be due to the fact that we grouped all participants together for this analysis (i.e., the repeated measures ANOVA), as opposed to separating them into two groups, as Lulic and associates (2017) did. It is possible that if we had separated our participants into two groups we could have seen differences in the higher fitness individual's pre to post1, as the change scores for post1 demonstrated a positive, although non-significant, relationship. This 'grouped' approach to analysis was not appropriate of course, given we recruited individuals

with a range of VO_2max values as opposed to two dichotomous groups (i.e., one with low and one with high VO_2max values). In regards to the relationship between fitness and AUC values, our results demonstrated a slightly negative, non-significant relationship at baseline ('pre'). This relationship approached zero (i.e., a flat line) immediately after exercise. Cho and associates (2012) found that basal BDNF levels are inversely correlated with aerobic fitness levels, where higher levels of fitness are associated with lower resting BDNF levels, but that the correlations become positive immediately after high intensity exercise. Because BDNF levels are related to increased cortical excitability, this is a possible explanation for our AUC results. Specifically, the lower BDNF levels in higher fitness individuals at baseline would result in less excitability, whereas the larger change scores observed in our higher VO_2max participants (post1 specifically, Figure 21, panel A) could be indicative of a small (albeit non-significant) change in excitability. The present results however do not support this notion that BDNF levels increase immediately post-exercise. As stated previously however, changes in excitability attributed to an increased uptake of BDNF have been found in individuals with high physical activity (Cho et al., 2012), which does not necessarily equate to a high VO_2max value. As such, the absence of a relationship between cortical excitability measures and aerobic fitness may be an indication that such a relationship does not exist.

5.2.2 Intracortical networks

There are a variety of paradigms that are thought to elicit different networks through the use of different stimulation parameters (i.e., different ISI, CS, and TS).

The parameters used in this study for inhibition (SICI and LICI) have been shown to illicit a decreased in the size of the MEP in response to the TS in comparison to the unconditioned stimulus (Kujurai et al., 1993; Valls-Solé et al., 1992), and did so in the current study for all participants for which a measurable MEP was obtained. However, although the parameters meant to induce facilitation (ICF) used in the present study have been used in previous studies (Kujurai et al., 1993), we were only able to elicit facilitation in a small number of participants and therefore did not pursue formal analysis of this data.

Although our results did not reach significance, there was a trend towards decreased SICI ($p = 0.0779$; see Figure 22 panel A) and LICI ($p = 0.0963$; see Figure 22 panel B) pre- to post-exercise. Previous studies have demonstrated similar trends in changes in inhibition in response to an acute bout of aerobic exercise. Singh and associates (2014) displayed similar trends in SICI and LICI due to a single session of exercise (refer to Figures 14 & 15), although their results for SICI demonstrated a significant difference pre to post2. Although they displayed a similar trend, our values for percent of the unconditioned MEP for both SICI and LICI were much smaller than the ones found in the study by Singh and associates (2014). Specifically, prior work reported relatively high percentages of the unconditioned MEP for both SICI and LICI, even at the pre-exercise time-points (Singh, Duncan, et al., 2014), while even our post-exercise time-points were well below the 100% level. This finding may be explained by technical reasons, namely error in the localization of the TMS coil, however this is an unlikely explanation, as the error would have had to

be systematic (i.e., across all participants) given the similarly low values observed for all participants.

Short- and long- interval intracortical inhibition provide information about different pathways: SICI is thought to probe GABA receptor type A (Chen, 2004), while LICI is thought to probe GABA receptor type B mediated-inhibition (Valls-Solé et al., 1992). Brain derived neurotrophic factor is thought to mediate intracortical circuitry by influencing $GABA_A$ receptor activity. Increased BDNF reduces $GABA_A$ receptor activity (Jovanovic, Thomas, Kittler, Smart, & Moss, 2004), and so we would expect a decrease in SICI with increased BDNF. Baseline SICI measurements (i.e., the % of unconditioned stimulus) show a slight negative relationship to aerobic fitness (i.e., increased inhibition with increased fitness). This is consistent with Cho and associates (2012) who found BDNF is negatively correlated with fitness. Of note, Cirillo and associates (2009) and Lulic and associates (2017) observed no differences in SICI between physically active and sedentary participants, suggesting that GABAergic, specifically $GABA_A$, mechanisms in M1 are also not influenced by regular physical activity.

The mechanism behind ICF is less understood but is thought to involve activation of glutamatergic interneurons and NMDA receptors (Ziemann, Chen, Cohen & Hallett, 1998). We are unsure why such a small number of participants in the current study showed ICF. We hypothesized that there would have been a positive relationship between fitness and the ICF change scores, as exercise stimulates BDNF to a greater extent in fit individuals (Cho et al., 2012) and BDNF stimulates glutamate release (Jovanovic, Czernik, Fienberg, Greengard, & Sihra,

2000). Our lack of understanding of the mechanism(s) underlying ICF, coupled with findings that ICF values have been found to be quite variable in past research (Lulic et al., 2017; Singh, Duncan, et al., 2014), suggest that it may not be the best means of assessing excitability in response to exercise.

Of note, there was significant variability in our data, which is evident in the size of the standard deviations (see Fig. 22 for instance). However, this variability is comparable to other studies in this area (Singh, Duncan, et al., 2014). For instance, Figures 14-16 (from Singh and colleagues 2014) also have a high degree of variability as evidence by the size of the standard error.

5.3 Aerobic fitness vs. Physical Activity

The criterion measure for aerobic fitness is VO_2max. Maximal oxygen consumption is a measure of the functional capacity of the cardiovascular system. It is the limit of the body to respond to aerobic exercise and is often used to measure changes after aerobic training. The Fick equation describes VO_2max as a product of both central and peripheral factors, specifically, cardiac output (i.e., the amount of blood pumped per minute) and tissue oxygen uptake (i.e. the amount of oxygen taken from the blood to be used by the muscles). There are many factors that predict VO_2max, including sex, age, genetics and aerobic training (Heyward, 2010).

Although aerobic fitness is an objective measure of aerobic training, it is not the ideal measure for measuring regular aerobic activity, as there are a multitude of factors that influence it. Tools that measure activity level include accelerometers and subjective assessment tools, such as the IPAQ. The IPAQ has a fair to moderate

relationship (*N* 781, 0.30, 95% CI 0.23–0.36) with accelerometer measures (Craig et al., 2003). Lulic and associates (2017) and Cirillo and associates (2009) used IPAQ to separate participants based on activity levels and found increased excitability in response to exercise and PAS, respectively, in the high activity groups. In the present study, although our independent variable of interest was fitness level, we collected both VO_2max through the GXT as well as Met-minutes per week of physical activity through the IPAQ. The relationship between participants' VO_2max and their IPAQ score for the present study can be observed in Figure 27. There was only a small correlation between VO_2max and IPAQ score and some individuals with low VO_2max values were quite active. Previous studies have also found a weak positive correlation ($p = 0.21$, $P < 0.05$) between aerobic fitness and IPAQ (Hagstromer et al., 2006). It appears from the present study that VO_2max might not reflect activity, and perhaps increased physical activity level might impact on the factors that drive cortical excitability more so than just having a high VO_2max value.

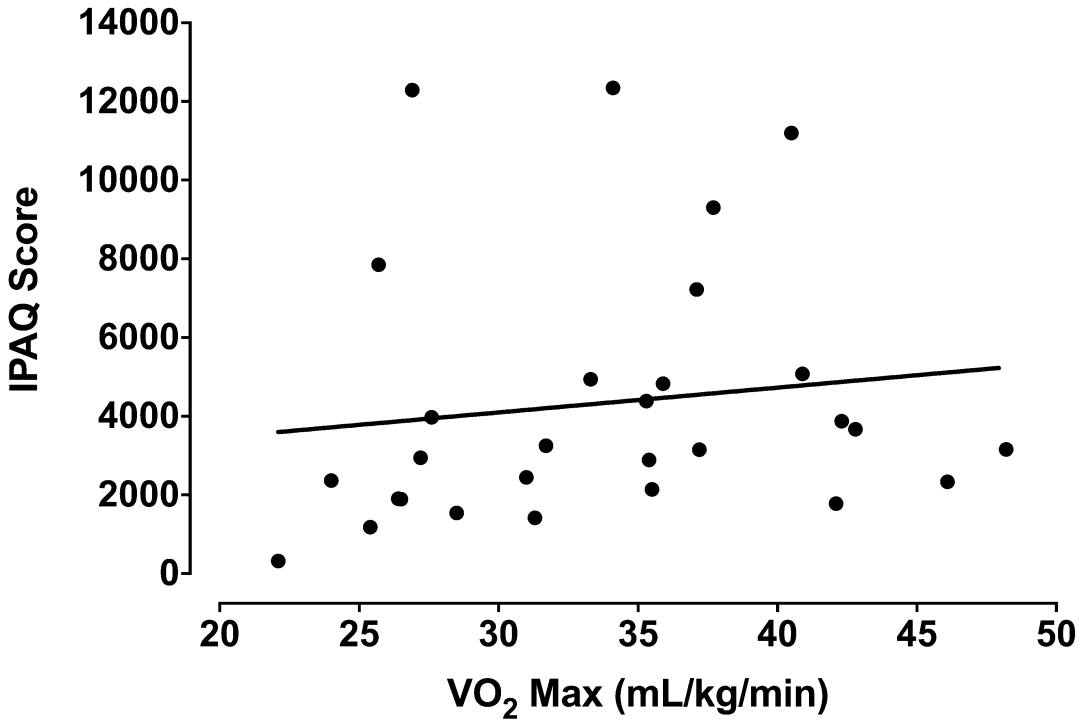

Figure 27: The relationship between self-reported physical activity (IPAQ Score) and aerobic fitness (VO_2max). The solid black line represents the line of best fit.

5.4 Limitations

There are a number of limitations in the current study. While we controlled for HR during the second exercise session, we did not control for RPE, resulting in a large range of RPEs. Participant's physical or emotional strain can influence the release of certain neurotransmitters (McMorris et al., 2009), which would then influence cortical excitability. Future studies should closely control for both HR and RPE.

There were a few independent variables that we did not control for that we are aware influence cortical excitability. Cortisol fluctuates throughout the day,

affecting cortical excitability (Kanaley, Weltman, Pieper, Weltman, & Hartman, 2001). Although we did attempt to control for this, due to logistics not all experimental sessions were completed at the same time of day. We completed the majority of the sessions in the afternoon and early evening, with 14 of the 29 sessions beginning between 12:30pm and 3:30pm, and 11 beginning between 4:00pm and 5:45pm. Timing of the menstrual cycle is another known factor that affects cortical excitability (M. J. Smith et al., 1999) which we did not control for in the present study.

Also due to logistics, the time between exercise sessions was not well controlled. While participants denied changing their physical activity level between sessions, this cannot be confirmed beyond the self-report. Although it is improbable that participants would have changed their aerobic training to a sufficient extent to increase their VO_2max, we cannot count this out.

Although we recruited participants with a range of VO_2max values, perhaps this range was insufficient to observe the full trend. Our participants, as a group, seemed to have low VO_2max values, with all participants scoring between poor and good, and none scoring excellent or superior according to Heyward (2010; refer to Table 12).

Table 12: Aerobic Fitness Classifications based on age and sex. ***From Heyward (2010).***

Cardiorespiratory Fitness Classifications: VO2max (ml x kg^{-1} x min^{-1})					
Age (yr)	**Poor**	**Fair**	**Good**	**Excellent**	**Superior**
			WOMEN		
20-29	≤35	36-39	40-43	44-49	50+
30-39	≤33	34-36	37-40	41-45	46+
40-49	≤31	32-34	35-38	39-44	45+
			MEN		
20-29	≤41	42-45	46-50	51-55	56+
30-39	≤40	41-43	44-47	48-53	54+
40-49	≤37	38-41	42-45	46-52	53+

Finally, while we opted to perform an analysis on each of the independent variables, an analysis of covariance may have been better approach, as one model could have addressed our hypotheses. However, given our findings and the lack of a relationship between any of our measures and the independent variable (VO_2max), it is unlikely that an alternate approach to the analysis would have modified the results.

5.5 Future Directions

Future studies may consider looking further into the mechanisms behind the changes in cortical excitability due to exercise; for example, measuring BDNF and corticospinal excitability, simultaneously. Future studies should also attempt to control for both HR and RPE during the exercise session, time of day and menstrual cycle.

Creating a study where aerobic fitness and excitability were measured before and after a training period would be very informative. Assessing changes between a control, a strength-trained, and an aerobically-trained group would add to the current research. As well, comparing cortical excitability to physical activity as measured by an accelerometer has not been done. Finally, data from the present study could be examined to determine if the IPAQ or VO_2 is a better predictor for cortical excitability.

5.6 Conclusions

It is clear that there are many possible mechanisms that influence the cortical excitability response to acute and regular exercise. Aerobic fitness has unique influences on the body and the brain that we had hypothesized would influence excitability measures at baseline and in response to an exercise session. Although our results were non-significant, this study adds to the research in this area, as it is the first study to examine the relationship between aerobic fitness and cortical excitability. Our results suggest that aerobic fitness is not a predictor of cortical excitability at rest and in response to exercise. It seems based on previous studies that physical activity level may be a better predictor of cortical excitability. It is important to understand the determinants of cortical excitability, as increasing excitability creates an environment for longer lasting changes in the brain that relate to learning. Understanding factors that underlie brain plasticity can better inform the application of priming techniques for learning and rehabilitation.

REFERENCES

Adkins, D.L., Boychuk, J., Remple, M.S., & Kleim, J.A. (2006). Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord. *J Appl Physiol, 101*, 1776-1782.

Black, J. E., Isaacs, K. R., Anderson, B. J., Alcantara, A. A., & Greenough, W. T. (1990). Learning causes synaptogenesis, whereas motor activity causes angiogenesis in cerebellar cortex of adult rats. *Proc Natl Acad Sci U S A, 87*(14), 5568-5572.

Boe, S. G., Cassidy, R. J., McIlroy, W. E., & Graham, S. J. (2012). Single session motor learning demonstrated using a visuomotor task: Evidence from fMRI and behavioural analysis. *J Neurosci Methods, 209*(2), 308-319. doi: 10.1016/j.jneumeth.2012.06.016

Bolton, M. M., Pittman, A. J., & Lo, D. C. (2000). Brain-Derived Neurotrophic Factor Differentially Regulates Excitatory and Inhibitory Synaptic Transmission in Hippocampal Cultures. *J Neurosci, 20*(9), 3221-3232.

Borg, G.A.V. (1982). Psychophysical bases of perceived exertion. *Med Sci Sports Exerc, 14*(5), 377-381.

Boroojerdi, B., Battaglia, F., Muellbacher, M., & Cohen, L. G. (2001). Mechanisms influencing stimulus-response properties of the human corticospinal system. *Clin Neurophysiol, 112*, 931-937.

Butefisch, C. M., Davis, B. C., Wise, S. P., Sawaki, L., Kopylev, L., Classen, J., & Cohen, L. G. (2000). Mechanisms of use-dependent plasticity in the human motor cortex. *Proc Natl Acad Sci U S A, 97*(7), 3661-3665. doi: 10.1073/pnas.050350297

Chaddock, L., Erickson, K. I., Prakash, R. S., Kim, J. S., Voss, M. W., Vanpatter, M., . . . Kramer, A. F. (2010). A neuroimaging investigation of the association between aerobic fitness, hippocampal volume, and memory performance in preadolescent children. *Brain Res, 1358*, 172-183. doi: 10.1016/j.brainres.2010.08.049

Chang, Y. K., Labban, J. D., Gapin, J. I., & Etnier, J. L. (2012). The effects of acute exercise on cognitive performance: a meta-analysis. *Brain Res, 1453*, 87-101. doi: 10.1016/j.brainres.2012.02.068

Chen, R. (2004). Interactions between inhibitory and excitatory circuits in the human motor cortex. *Exp Brain Res, 154*(1), 1-10.

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

Cho, H. C., Kim, J., Kim, S., Son, Y. H., Lee, N., & Jung, S. H. (2012). The concentrations of serum, plasma and platelet BDNF are all increased by treadmill VO(2)max performance in healthy college men. *Neurosci Lett, 519*(1), 78-83. doi: 10.1016/j.neulet.2012.05.025

Cirillo, J., Lavender, A. P., Ridding, M. C., & Semmler, J. G. (2009). Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals. *J Physiol, 587*(Pt 24), 5831-5842. doi: 10.1113/jphysiol.2009.181834

Classen, J., Liepert, J., Wise, S. P., Hallet, M., & Cohen, L. G. (1998). Rapid plasticity of human cortical movement representation induced by practice. *J Neurophysiol, 79*, 1117-1123.

Coco, M., Alagona, G., Rapisarda, G., Costanzo, E., Calogero, R. A., Perciavalle, V., & Perciavalle, V. (2010). Elevated blood lactate is associated with increased motor cortex excitability. *Somatosens Mot Res, 27*(1), 1-8. doi: 10.3109/08990220903471765

Colcombe, S. J., & Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: A meta-anayltic study. *Psychol Sci, 14*(2), 125-130. doi: 10.1073/pnas.0400266101

Colcombe, S. J., Kramer, A. F., Erickson, K. I., Scalf, P., McAuley, E., Cohen, N. J., . . . Elavsky, S. (2004). Cardiovascular fitness, cortical plasticity, and aging. *Proc Natl Acad Sci U S A, 101*(9), 3316-3321.

Cotman, C. W., Berchtold, N. C., & Christie, L. A. (2007). Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci, 30*(9), 464-472.

Cotman, C. W., & Berchtold, N.C. (2002). Exercise: a behavioral intervention to enhance brain health and plasticity *Trends Neurosci, 25*(6), 295-301.

Coxon, J. P., Peat, N. M., & Byblow, W. D. (2014). Primary motor cortex disinhibition during motor skill learning. *J Neurophysiol, 112*(1), 156-164. doi: 10.1152/jn.00893.2013

Craig, C. L., Marshall, A. L., Sjostrom, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., . . . Oja, P. (2003). International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc, 35*(8), 1381-1395.

Dayan, E., Censor, N., Buch, E. R., Sandrini, M., & Cohen, L. G. (2013). Noninvasive brain stimulation: from physiology to network dynamics and back. *Nat Neurosci, 16*(7), 838-844. doi: 10.1038/nn.3422

Dayan, E., & Cohen, L. G. (2011). Neuroplasticity subserving motor skill learning. *Neuron, 72*(3), 443-454. doi: 10.1016/j.neuron.2011.10.008

Devanne, H., Lavoie, B.A., & Capaday, C. (1997). Input-output properties and gain changes in the human corticospinal pathway. *Exp Brain Res, 114*, 329-338.

Di Pino, G., Pellegrino, G., Assenza, G., Capone, F., Ferreri, F., Formica, D., . . . Di Lazzaro, V. (2014). Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat Rev Neurol, 10*(10), 597-608. doi: 10.1038/nrneurol.2014.162

Ding, Q., Vaynman, S., Akhavan, M., Ying, Z., & Gomez-Pinilla, F. (2006). Insulin-like growth factor I interfaces with brain-derived neurotrophic factor-mediated synaptic plasticity to modulate aspects of exercise-induced cognitive function. *Neuroscience, 140*(3), 823-833. doi: 10.1016/j.neuroscience.2006.02.084

Doyon, J., & Benali, H. (2005). Reorganization and plasticity in the adult brain during learning of motor skills. *Current Opinion in Neurobiology, 15*, 161-167. doi: 10.1016/j.conb.2005.03.004

Droste, S. K., Chandramohan, Y., Hill, L. E., Linthorst, A. C., & Reul, J. M. (2007). Voluntary exercise impacts on the rat hypothalamic-pituitary-adrenocortical axis mainly at the adrenal level. *Neuroendocrinology, 86*(1), 26-37. doi: 10.1159/000104770

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., . . . Kramer, A. F. (2011). Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S A, 108*(7), 3017-3022. doi: 10.1073/pnas.1015950108

European Commission, Directorate General for Research, Commission of the European Communities, & Biomedical and Health Research Programme. (1999). *SENIAM : European recommendations for surface electromyography*. Netherlands: Roessingh Research and Development.

Fleet, A., Page, S. J., MacKay-Lyons, M., & Boe, S. G. (2014). Modified constraint-induced movement therapy for upper extremity recovery post stroke: what is the evidence? *Top Stroke Rehabil, 21*(4), 319-331. doi: 10.1310/tsr2104-319

Floyer-Lea, A., & Matthews, P. M. (2005). Distinguishable brain activation networks for short- and long-term motor skill learning. *J Neurophysiol, 94*(1), 512-518. doi: 10.1152/jn.00717.2004

Floyer-Lea, A., Wylezinska, M., Kincses, T., & Matthews, P. M. (2006). Rapid modulation of GABA concentration in human sensorimotor cortex during motor learning. *J Neurophysiol, 95*(3), 1639-1644. doi: 10.1152/jn.00346.2005

Gonzalez-Alonso, J., Dalsgaard, M. K., Osada, T., Volianitis, S., Dawson, E. A., Yoshiga, C. C., & Secher, N. H. (2004). Brain and central haemodynamics and oxygenation during maximal exercise in humans. *J Physiol, 557*(Pt 1), 331-342. doi: 10.1113/jphysiol.2004.060574

Griffin, L., & Cafarelli, E. (2007). Transcranial magnetic stimulation during resistance training of the tibialis anterior muscle. *J Electromyogr Kinesiol, 17*(4), 446-452. doi: 10.1016/j.jelekin.2006.05.001

Hagströmer, Maria, Oja, Pekka, & Sjöström, Michael. (2007). The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutrition, 9*(06). doi: 10.1079/phn2005898

Hallett, M. (2000). Transcranial magnetic stimulation and the human brain. *Nature, 406*, 147-150.

Heyward, Vivian H. (2010). *Advanced fitness assessment and exercise prescription*. Champaign, IL: Human Kinetics.

Jacobs, K. M., & Donoghue, J. P. (1991). Reshaping the cortical motor map by unmasking latent intracortical connections. *Science, 251*(4996), 944-947.

Jovanovic, J. N., Czernik, A. J., Fienberg, A. A., Greengard, P., & Sihra, T. S. (2000). Synapsins as mediators of BDNF- enhanced neurotransmitter release. *Nature, 3*(4), 323-329.

Jovanovic, J. N., Thomas, P., Kittler, J. T., Smart, T. G., & Moss, S. J. (2004). Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating GABA(A) receptor phosphorylation, activity, and cell-surface stability. *J Neurosci, 24*(2), 522-530. doi: 10.1523/JNEUROSCI.3606-03.2004

Kanaley, J. A., Weltman, J. Y., Pieper, K. S., Weltman, A., & Hartman, M. L. (2001). Cortisol and Growth Hormone Responses to Exercise at Different Times of Day. *The Journal of Clinical Endocrinology & Metabolism, 86*(6), 2881-2889.

Kleim, J. A., Cooper, N. R., & VandenBerg, P. M. (2002). Exercise induces angiogenesis but does not alter movement representations within rat motor cortex. *Brain Res, 934*, 1-6.

Kleim, J. A., Kleim, E. D., & Cramer, S. C. (2007). Systematic assessment of training-induced changes in corticospinal output to hand using frameless stereotaxic transcranial magnetic stimulation. *Nat Protoc, 2*(7), 1675-1684. doi: 10.1038/nprot.2007.206

Knaepen, K., Goekint, M., Heyman, E.M., & Meeusen, R. (2010). Neuroplasticity - exercise-induced response of peripheral brain-derived neurotrophic factor. *Sports Med, 40*(9), 765-801.

Kramer, A. F., & Erickson, K. I. (2007). Capitalizing on cortical plasticity: influence of physical activity on cognition and brain function. *Trends Cogn Sci, 11*(8), 342-348. doi: 10.1016/j.tics.2007.06.009

Kujurai, T. , Caramia, M.D., Rothwell, J. C., Day, B.L., Thompson, P.D., Ferbert, A., . . . Marsden, C.D. (1993). Corticocortical inhibition in human motor cortex. *Journal of Physiology, 471*, 501-519.

Kumpulainen, S., Avela, J., Gruber, M., Bergmann, J., Voigt, M., Linnamo, V., & Mrachacz-Kersting, N. (2014). Differential modulation of motor cortex plasticity in skill- and endurance-trained athletes. *Eur J Appl Physiol*. doi: 10.1007/s00421-014-3092-6

Liepert, J., Bauder, H., Wolfgang, H. R., Miltner, W. H., Taub, E., & Weiller, C. (2000). Treatment-induced cortical reorganization after stroke in humans. *Stroke, 31*(6), 1210-1216.

List, L., Kubke, J.C., Lindenberg, R., Kulzow, N., Kerti, L., Witte, V., & Floel, A. (2013). Relationship between excitabiity, plasticity and thickness of the motor cortex in older adults. *Neuroimage, 83*, 809-816. doi: 10.1016/j.neuroimage.2013.07.033

Lulic, T., El-Sayes, J., Fassett, H. J., & Nelson, A. J. (2017). Physical activity levels determine exercise-induced changes in brain excitability. *PLoS One, 12*(3), e0173672. doi: 10.1371/journal.pone.0173672

Lynch, M.A. (2004). Long-term potentiation and memory. *Physiological Reviews, 84*(1), 87-136. doi: 10.1152/physrev.00014.2003

Ma, L., Narayana, S., Robin, D.A., Fox, P.T., & Xiong, J. (2011). Changes occur in resting state network of motor system during 4 weeks of motor skill learning. *Neuroimage, 58*(1), 226-233. doi: 10.1016/j.neuroimage.2011.06.014

MacKay-Lyons, M., Macko, R., Eng, J., Billinger, S. Hafer-Macko, C., Suskin, N., Tang, A., Giacomantonio, N., Prior, P., Che, M., Dromerick, A., Thornton, M., Reid, R., Unsworth, K, LaGrand, C., Firth, W., & Che, M. (2012). AEROBICS: Aerobic exercise recommendations to optimize best practices in care after stroke. doi: http://strokebestpractices.ca/wp-content/ uploads/2013/07/AEROBICS-FINAL-July-2013.pdf.

Mang, C. S., Campbell, K. L., Ross, C. J. D., & Boyd, L. A. (2013). Promoting neuroplasticity for motor rehabilitation after stroke: Considering the effects of aerobic exercise and genetic variation on brain-derived-neurotrophic factor. *Physical Therapy, 93*(12), 1707-1716. doi: http://dx.doi.org.ezproxy.library.dal.ca/10.2522/ptj.20130053

McCloskey, D. P., Adamo, D. S., & Anderson, B. J. (2001). Exercise increases metabolic capacity in the motor cortex and striatum, but not in the hippocampus. *Brain Res, 891*, 168-175.

McDonnell, M. N., Buckley, J. D., Opie, G. M., Ridding, M. C., & Semmler, J. G. (2013). A single bout of aerobic exercise promotes motor cortical neuroplasticity. *J Appl Physiol, 114*, 1174-1182. doi: 10.1152/japplphysiol.01378.2012.-Regular

McMorris, T., Davranche, K., Jones, G., Hall, B., Corbett, J., & Minter, C. (2009). Acute incremental exercise, performance of a central executive task, and sympathoadrenal system and hypothalamic-pituitary-adrenal axis activity. *Int J Psychophysiol, 73*(3), 334-340. doi: 10.1016/j.ijpsycho.2009.05.004

Neeper, S. A. , Gomez-Pinilla, F., Choi, J., & Cotman, C. W. (1996). Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res, 726*, 49-56.

Nicolo, P., Ptak, R., & Guggisberg, A. G. (2015). Variability of behavioural responses to transcranial magnetic stimulation: Origins and predictors. *Neuropsychologia*. doi: 10.1016/j.neuropsychologia.2015.01.033i

Nieminen, J. O., Koponen, L. M., & Ilmoniemi, R. J. (2015). Experimental Characterization of the Electric Field Distribution Induced by TMS Devices. *Brain Stimul, 8*(3), 582-589. doi: 10.1016/j.brs.2015.01.004

Nudo, R. J. (2003). Adaptive plasticity in motor cortex: implications for rehabilitation after brain injury. *Journal of Rehabilitation Medicine, 35*(0), 7-10. doi: 10.1080/16501960310010070

Nudo, R. J., & Milliken, G. W. (1996). Reorganization of movement representations in primary motor cortex following focal ischemic infarcts in adult squirrel monkeys. *J Neurophysiol, 75*(5), 2144-2149.

Pereira, A. C., Huddleston, D. E., Brickman, A. M., Sosunov, A. A., Hen, R., McKhann, G. M., . . . Small, S. A. (2007). An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad Sci U S A, 104*(13), 5638-5643. doi: 10.1073/pnas.0611721104

Pescatello, Linda S., & American College of Sports, Medicine. (2014). *ACSM's guidelines for exercise testing and prescription*. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins Health.

Petersen, N. T., Pyndt, H. S., & Nielsen, J. B. (2003). Investigating human motor control by transcranial magnetic stimulation. *Exp Brain Res, 152*(1), 1-16. doi: 10.1007/s00221-003-1537-y

Purves, Dale. (2012). *Neuroscience*. Sunderland, Mass.: Sinauer Associates.

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., . . . Acker, J. D. (2005). Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb Cortex, 15*(11), 1676-1689. doi: 10.1093/cercor/bhi044

Rosdahl, H., Gullstrand, L., Salier-Eriksson, J., Johansson, P., & Schantz, P. (2010). Evaluation of the Oxycon Mobile metabolic system against the Douglas bag method. *Eur J Appl Physiol, 109*(2), 159-171. doi: 10.1007/s00421-009-1326-9

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol, 120*(12), 2008-2039. doi: 10.1016/j.clinph.2009.08.016

Rossini, P. M., & Rossi, S. (2007). Transcranial magnetic stimulation: Diagnostic, therapeutic, and research potential. *Neurology, 68*, 484-488.

Sale, M. V., Ridding, M. C., & Nordstrom, M. A. (2008). Cortisol inhibits neuroplasticity induction in human motor cortex. *J Neurosci, 28*(33), 8285-8293. doi: 10.1523/JNEUROSCI.1963-08.2008

Sanes, J.N., & Donoghue, J. P. (2000). Plasticity and primary motor cortex. *Annu Rev Neurosci, 23*, 393-415.

Schabrun, S. M., & Chipchase, L. S. (2012). Priming the brain to learn: the future of therapy? *Man Ther, 17*(2), 184-186. doi: 10.1016/j.math.2011.12.001

Schaechter, J. D. (2004). Motor rehabilitation and brain plasticity after hemiparetic stroke. *Prog Neurobiol, 73*(1), 61-72. doi: 10.1016/j.pneurobio.2004.04.001

Schmolesky, M. T., Webb, D. L., & Hansen, R. A. (2013). The Effects of Aerobic Exercise Intensity and Duration on Levels of Brain- Derived Neurotrophic Factor in Healthy Men. *Journal of Sports Science and Medicine, 12*, 502-511.

Schwarz, A. J., Brasel, J. A., Hintz, R. L., Mohan, S., & Cooper, D. M. (1996). Acute effect of brief low- and high-intensity exercise on circulating insulin-like growth factor (IGF) I, II, and IGF-binding protein-3 and its proteolysis in young healthy men. *The Journal of Clinical Endocrinology & Metabolism, 81*(10), 3492-3497. doi: 10.1210/jcem.81.10.8855791

Shi, Y. X., Tian, J. H., Yang, K. H., & Zhao, Y. (2011). Modified constraint-induced movement therapy versus traditional rehabilitation in patients with upper-extremity dysfunction after stroke: a systematic review and meta-analysis. *Arch Phys Med Rehabil, 92*(6), 972-982. doi: 10.1016/j.apmr.2010.12.036

Singh, A. M., Duncan, R. E., Neva, J. L., & Staines, W. R. (2014). Aerobic exercise modulates intracortical inhibition and facilitation in a nonexercised upper limb muscle. *BMC Sports Science, Medicine & Rehabilitation, 6*(23).

Singh, A. M., Neva, J. L., & Staines, W. R. (2014). Acute exercise enhances the response to paired associative stimulation-induced plasticity in the primary motor cortex. *Exp Brain Res, 232*(11), 3675-3685. doi: 10.1007/s00221-014-4049-z

Singh, A. M., & Staines, W. R. (2015). The effects of acute aerobic exercise on the primary motor cortex. *J Mot Behav, 47*(4), 328-339. doi: 10.1080/00222895.2014.983450

Smith, A. E., Goldsworthy, M. R., Garside, T., Wood, F. M., & Ridding, M. C. (2014). The influence of a single bout of aerobic exercise on short-interval intracortical excitability. *Exp Brain Res, 232*(6), 1875-1882. doi: 10.1007/s00221-014-3879-z

Smith, M. J., Keel, J. C., Greenberg, B. D., Adams, L. F., Schmidt, P. J., Rubinow, D. A., & Wassermann, E. M. (1999). Menstrual cycle effects on cortical excitability. *American Academy of Neurology, 53*(9), 2069-2072.

Stagg, C. J., Bachtiar, V., & Johansen-Berg, H. (2011). The role of GABA in human motor learning. *Curr Biol, 21*(6), 480-484. doi: 10.1016/j.cub.2011.01.069

Stefan, K., Kunesch, E., Benecke, R., Cohen, L. G., & Classen, J. (2002). Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. *The Journal of Physiology, 543*(2), 699-708. doi: 10.1113/jphysiol.2002.023317

Stefan, K., Kunesch, E., Cohen, L. G., Benecke, R., & Classen, J. (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain, 123*, 572-584.

Stephens, Michael A. (1986). Tests based on EDF statistics. *Goodness-of-fit Techniques, 68*, 97-193.

Stinear, C. M., Barber, P. A., Coxon, J. P., Fleming, M. K., & Byblow, W. D. (2008). Priming the motor system enhances the effects of upper limb therapy in chronic stroke. *Brain, 131*(Pt 5), 1381-1390. doi: 10.1093/brain/awn051

Swain, R. A., Harris, A. B., Wiener, E. C., Dutka, M. V., Morris, H. D., Theien, B. E., . . . Greenough, W. T. (2003). Prolonged exercise induces angiogenesis and increases cerebral blood volume in primary motor cortex of the rat. *Neuroscience, 117*(4), 1037-1046. doi: 10.1016/s0306-4522(02)00664-4

Taub, E., & Morris, D.M. (2001). Constraint-induced movement therapy to ehance recovery after stroke. *Current Atherosclerosis Reports, 3*(4), 279-286.

Thomas, A. G., Dennis, A., Bandettini, P. A., & Johansen-Berg, H. (2012). The effects of aerobic activity on brain structure. *Front Psychol, 3*, 86. doi: 10.3389/fpsyg.2012.00086

Tokuyama, W., Okuno, H., Hashimoto, T., Li, Y. X., & Miyashita, Y. (2000). BDNF upregulation during declarative memory formation in monkey inferior temporal cortex. *Nat Neurosci, 3*(11), 1134-1142.

Trejo, J. L., Carro, E., & Torres-Aleman, I. (2001). Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. *J Neurosci, 21*(5), 1628.

Valls-Solé, Josep, Pascual-Leone, Alvaro, Wassermann, Eric M., & Hallett, Mark. (1992). Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section, 85*(6), 355-364. doi: http://dx.doi.org/10.1016/0168-5597(92)90048-G

van Praag, H., Shubert, T., Zhao, C., & Gage, F. H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *J Neurosci, 25*(38), 8680-8685. doi: 10.1523/JNEUROSCI.1731-05.2005

Voss, M. W., Prakash, R. S., Erickson, K. I., Basak, C., Chaddock, L., Kim, J. S., . . . Kramer, A. F. (2010). Plasticity of brain networks in a randomized intervention trial of exercise training in older adults. *Front Aging Neurosci, 2*. doi: 10.3389/fnagi.2010.00032

Wasserman, Karlman. (2012). *Principles of exercise testing and interpretation including pathophysiology and clinical applications* (5th ed.. ed.). Philadelphia: Philadelphia : Wolters Kluwer Health/Lippincott Williams & Wilkins.

Wolters, A., Sandbrink, F., Schlottmann, A., Kunesch, E., Stefan, K. , Cohen, L. G., . . . Classen, J. (2003). A temporally asymmetric hebbian rule governing plasticity in the human motor cortex. *J Neurophysiol, 89*, 2239-2345.

Yamaguchi, T., Fujiwara, T., Liu, W., & Liu, M. (2012). Effects of pedaling exercise on the intracortical inhibition of cortical leg area. *Exp Brain Res, 218*(3), 401-406. doi: 10.1007/s00221-012-3026-7

Ziemann, U., Chen, R., Cohen, L. G., & Hallet, M. (1998). Dextromethorphan decreases the excitability of the human motor cortex. *Neurology, 51*(5). doi: 10.1212/WNL.51.5.1320

Appendix 1 – Physical Activity Readiness Questionnaire (PAR-Q)

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

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

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

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

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

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

If you answered

YES to one or more questions

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

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

NO to all questions

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

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

→ **DELAY BECOMING MUCH MORE ACTIVE:**

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

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

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

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

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

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

NAME _____

SIGNATURE _____ DATE _____

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

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

CSEP | SCPE

Appendix 2 – 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.

Participants Study ID: _____

Participants Age: _____

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

12. Did you ever undergo TMS in the past? If yes, were there any problems:

13. Did you ever undergo MRI in the past? If yes, were there any problems:

* TMS screening form is from the International Consensus Guidelines:

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

Appendix 3 – International Physical Activity Questionnaire (IPAQ)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

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

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

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 4 – Borg Rating of Perceived Exertion (RPE) 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.

Appendix 5 - Calculating Continuous and Categorical PA Score (Using IPAQ)

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