INVESTIGATING THE EFFECTS OF DIFFERENT INTENSITIES OF AEROBIC
EXERCISE ON CORTICAL EXCITABILITY IN NON-EXERCISED UPPER LIMB
MUSCLES OF NON-DISABLED YOUNG ADULTS

by

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ABSTRACT

The goal of rehabilitation after stroke is to achieve functional recovery by driving brain recovery. Research has found that highly excitable brain cells can be stimulated easier than less excitable cells. Aerobic exercise (AE) has been shown to increase brain excitability, however the AE level used previously was not feasible for clinical practice. This study sought to test if AE levels lower than those previously established can increase cortical excitability, and to evaluate potential mechanisms underlying this change. Our findings show that exercise at 50 and 40% of heart rate reserve (HRR) for 20 minutes significantly increases cortical excitability and modulates intracortical inhibitory networks. Our findings suggest that AE levels lower than those previously investigated, namely 40% HRR, are effective in increasing cortical excitability, representing a means to prime the brain in advance of rehabilitation. Future work needs to replicate these findings in individuals post-stroke to ensure similar effects.

LIST OF ABBREVIATIONS USED

ADL	Activities of Daily Living
AE	Aerobic Exercise
AMPA	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid
BDNF	Brain-Derived Neurotrophic Factor
bpm	Beat Per Minute
CBF	Cerebral Blood Flow
CIMT	Constraint Induced Movement Therapy
cTBS	Continuous Theta Burst Stimulation
ECR	Extensor Carpi Radialis
EMG	Electromyography
fMRI	Functional Magnetic Resonance Imaging
IPSP	Inhibitory Postsynaptic Potential
GABA	Gamma-aminobutyric Acid
GR	Glucoresponsive
HCAR1	Hydroxycarboxylic Acid Receptor 1
HR	Heart Rate
HRR	Heart Rate Reserve
ICF	Intracortical Facilitation
IHI	Interhemispheric inhibition
IPAQ	International Physical Activity Questionnaire
LICI	Long-Interval Intracortical Inhibition
LTP	Long-Term Potentiation
MCT2	Monocarboxylate Transporter 2
M1	Primary Motor Cortex
MDMA	3,4-Methylenedioxy-Methamphetamine
MEP	Motor Evoked Potential
MET	Metabolic Equivalent of Task
MRI	Magnetic Resonance Imaging
NMDA	N-methyl-D- aspartate

RHR	Resting Heart Rate
RMT	Resting Motor Threshold
rTMS	Repetitive Transcranial Magnetic stimulation
SICI	Short-Interval Intracortical Inhibition
S-R	Stimulus-Response
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation
VO_2	Volume of Oxygen Consumption
WMFT	Wolf Motor Function Test

ACKNOWLEDGEMENTS

CHAPTER 1: INTRODUCTION

Stroke is a brain attack caused by a disruption in blood flow to specific parts of the brain, which results in death of the neural tissue in the affected region. Stroke often occurs in regions of the brain responsible for movement, resulting in the loss of function on the side of the body opposite the side of the damaged brain area (Donnan, Fisher, Macleod, & Davis, 2008). Stroke is the leading cause of long-term disability among adults in the United States and Canada (Evenson & Rosamond, 1999). According to Sawaki et al. (2008), approximately 70% to 88% of people who have had stroke are left with different degrees of motor disability that impact their ability to perform their jobs or even activities of daily living (ADL). As impaired movement is a major consequence of stroke, there is a critical need to rehabilitate those individuals who have experienced a stroke in order to improve their motor performance and restore independence to get better lives.

Research has shown that brain activity patterns, and in turn functional recovery, can be changed positively in response to practice and experience, a process called plasticity. Accordingly, many rehabilitation techniques have been developed to improve motor function in people with stroke. The ultimate goal of these rehabilitation techniques is to stimulate undamaged brain cells to take the actions of the damaged ones, in-turn promoting recovery of lost motor functions. Stimulation of undamaged brain cells can be done through the intensive engagement of the paretic limb(s) in task-oriented training performed in a repetitive manner.

Many studies have shown that highly excitable cells in the area of the brain

responsible for movement (termed the primary motor cortex (M1)) can be stimulated easier and faster than less excitable cells (Rossini and Rossi, 2007). Accordingly, neuronal discharge that occurs as a result of therapy could increase if neurons in M1 were 'ready for stimulation' (i.e., were more excitable). Increased neuronal activity resulting from therapy would in-turn promote greater plasticity and thus better functional recovery. To prepare these neurons for stimulation by the rehabilitative modalities, their resting membrane potential needs to be moved closer to the threshold for depolarization. In other words, neuronal excitability has to be increased before engaging patients in rehabilitation to best facilitate functional recovery. Throughout we refer to this process of altering neuronal excitability prior to rehabilitation as *priming the brain*.

There are numerous modalities that can be used to increase neuronal excitability in M1 (e.g. caffeine, stimulant drugs, and non-invasive brain stimulation), however many have undesired side effects while others are not yet clinically feasible. Thus, there is a need to find a modality that is both safe to use and easy to perform.

Interestingly, previous work has shown that aerobic exercise (AE) potentially modulates M1 excitability within the areas of the cortex that represented the muscles targeted by the exercise. Translating this research into clinical practice for the purpose of stroke rehabilitation is not feasible for people with dense upper extremity hemiparesis however, as using the affected limb to exercise would be difficult especially in the early stages of the stroke. Ideally, priming of the brain would be achieved by exercising non- (or less) affected limbs.

To this end, several studies have been conducted that investigated the effect of lower limb exercise on cortical excitability within the upper limb muscle representation

(McDonnell, Buckley, Opie, Ridding, & Semmler, 2013, Singh, Duncan, Neva, & Staines, 2014 and Smith, Goldsworthy, Garside, Wood, & Ridding, 2014). Results from this work have shown that a single session of exercise does not directly modulate the excitability of cortical neurons in M1, but it may facilitate the induction of experience-dependent plasticity through a change in the amount of inhibition and facilitation in the representation of the non-exercised upper limb muscle.

In the context of using AE as a means to increase cortical excitability and facilitate intracortical changes prior to a bout of rehabilitation therapy, it is critical that patients not be fatigued following the AE, as they need to engage in the task specific therapy component of the intervention immediately thereafter. As such, the exercise intensities and durations used in previous work (20 minutes of AE at 70% of age-predicted maximum HR or ~ 50% heart rate reserve (HRR), and 30 minutes of AE at 40% HRR) may not be feasible for this priming application in clinical practice. This finding leads us to the question posed in the present research, which asks: can lower parameters of AE drive cortical excitability and facilitate intracortical changes in M1?

Accordingly, the goal of the present study was to investigate varying levels of lower limb AE (cycling at different intensities for a fixed duration) to assess if lower intensity AE could still have a positive effect on cortical excitability within the motor representation of a non-exercised upper limb muscle. Our secondary goal was to evaluate potential mechanisms underlying the changes in cortical excitability by assessing the amount of facilitation and inhibition in M1 interneurons after AE at the different levels. The long-term goal of this research is to inform implementation of AE in the clinical setting as a means to prime the brain before neurorehabilitation.

To address these goals, we recruited non-disabled subjects to perform lower extremity AE at three different intensities [30%, 40% and 50% of HRR] for 20 minutes. Using transcranial magnetic stimulation (TMS), stimulus-response curves (SR-curves) were generated before and immediately after each exercise session to evaluate changes in cortical excitability via analysis of motor evoked potential (MEP) amplitude. To evaluate potential mechanisms underlying the changes in cortical excitability, we also assessed the degree of facilitation and inhibition using paired-pulse TMS paradigms including intracortical facilitation (ICF), short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI).

This study found that AE increased cortical excitability, and decreased SICI but these findings were limited to exercise at 40 and 50% of HRR, not 30% HRR. Results also revealed that there was a significant decrease in LICI detected following exercise at 40% HRR. The study found no significant change in ICF after exercise at all the investigated intensities.

The findings of this study suggest that AE parameters lower than those previously investigated, namely 40% HRR for 20 minutes, are effective at increasing neuronal excitability and facilitating network changes and may represent a means to prime the brain in advance of rehabilitation. Future work needs to replicate these findings in individuals post-stroke to ensure similar effects.

CHAPTER 2: BACKGROUND AND RATIONALE

2.1 Stroke and Rehabilitation

Stroke is a leading cause of disability among adults. According to Statistics Canada (2012), stroke affects approximately 50,000 Canadian adults every year. Davis, Taylor, and Tomer (1998) have reported that the number of those who survive their stroke will increase over the next 50 years. Furthermore, the rate of stroke incidence in people aged 20 to 54 years has been significantly increasing in recent years compared with earlier periods (Kissela et al, 2012). Many people who experience a stroke are left with some degree of motor disability. Barreca, Wolf, Fasoli, and Bohannon (2003) reported that in Canada, approximately two-thirds of those individuals who survive their stroke live with neurological deficits that negatively impact on the performance of ADLs. As the number of people living with disability is rising, there is a need for physical rehabilitation to reduce the negative impact of injury on neurological and physical functions and to ensure a better life for survivors of stroke.

Rehabilitation is a major part of comprehensive therapy and care after stroke. Rehabilitation aims to help patients relearn or recover movement patterns that have been lost due to the injury. Older treatment strategies used in clinical rehabilitation had a greater focus on teaching compensatory strategies to patients, with the goal of promoting functional independence more quickly (Kalra, & Lalit, 2010). This compensatory approach would include greater reliance on the less affected limb for ADLs, or promotion of other movement patterns that would be considered atypical. Past decades have seen an increase in the literature addressing rehabilitation strategies that promote recovery of the

affected limb(s) and thus a return to typical functioning. These strategies incorporate patients' more affected limbs into specific activities to promote not only functional recovery but also neural plasticity.

Some studies have found that both structural and functional changes in brain activity correlate with changes in behavioral measures (Askim, Indredavik, Vangberg, & Haberg, 2009 and Dong, Dobkin, Cen, Wu, & Winstein, 2006). For instance, numerous studies have shown that improved performance resulting from motor learning is related to changes in brain activity (Askim et al., 2009). Findings such as these led scientists to focus their attention on studying how experience (such as learning) can drive plasticity in the brain with and without injury. Studying brain plasticity is critical to understanding the mechanisms underlying motor recovery (Kolb, Forgie, Gibb, Gorny, & Rowntree, 1998). Having an understanding of how the brain recovers after neurological insult aids in the development of new rehabilitation strategies to treat the injured brain and promote recovery of lost function.

Research in the last twenty years has generated considerable evidence to support the notion that following injury the nervous system can undergo substantial reorganization, allowing for recovery of lost function. However, it has been shown that plasticity in the brain can in fact be compensatory in nature, and in some instances limit the degree of functional recovery attained (Nudo, 2013). It has been shown that behavioral modification by different rehabilitative interventions plays an important role in shaping the changes in the function and organization of the brain after stroke (Taub, Uswatte & Elbert, 2002). Thus, recent efforts have been made to develop novel rehabilitation strategies to modulate, increase or inhibit plasticity in targeted brain regions

(Dancause, & Nudo, 2011).

Today, many rehabilitation techniques based on functional neurorecovery paradigms have been developed to facilitate the recovery of impaired movement in patients with stroke (Takeuchi & Izumi, 2013). Past research has investigated and provided evidence to support approaches that shape plasticity after brain injury to increase recovery such as non-invasive cortical stimulation and other machine-aided approaches such as robot training (Belda-Lois et al., 2011). Although the majority of these approaches have been shown to be effective in experimental settings, their introduction into routine clinical practice is not likely to occur for some time given the need for advanced training and/or the cost of implementation, both of which are factors that reduce their overall clinical feasibility. This is why we find that therapeutic exercises have become important approaches that form the broad basis of neurorehabilitation of people with stroke.

One of the rehabilitation interventions that has been used for patients post-stroke to promote functional neurorecovery is task-oriented therapy. Task-oriented therapy is a treatment in which the patient engages the affected limb(s) in a series of specific movements and repeats these movements over and over again. A systematic review done by Van Peppen et al. (2004) showed small to large effect sizes for task-oriented therapy; it was particularly effective when applied intensively and early after stroke onset. Furthermore, neurophysiological and neuroimaging studies have been performed to investigate the mechanisms underlying this intervention (Takeuchi and Izumi, 2003). These studies have demonstrated that engaging the paretic limb(s) in repetitive task-oriented training can re-establish neural pathways affected by injury and recruit the brain

areas that have been spared by the injury to assume control of limb function (Candia, Wienbruch, Elbert, Rockstroh, & Ray, 2003 and Nudo, & Milliken, 1996).

One example of task-oriented therapy that has received considerable attention from researchers and clinicians is a technique termed constraint-induced movement therapy (CIMT). CIMT involves constraining movement of the intact or less affected upper limb for most waking hours and engaging the paretic limb in repetitive, meaningful functional activities (Taub, Uswatte, & Pidikiti, 1999). CIMT is based on the concept that behavior modification can lead to changes in the function and organization of the brain which lead to functional recovery (Gauthier et al., 2008). To date, several studies have explored the neural changes in people with stroke who received CIMT. For instance, Pons et al. (1991) reported that sustained use of a body part leads to an increase of cortical representation of that part. Moreover, Gauthier et al. (2008) found that increased use of the paretic limb among stroke patients led to greater improvement of motor function, as well as changes in brain structure that paralleled the functional improvements. As such, task-oriented therapies such as CIMT and other techniques have been the most investigated neurorehabilitation approach commonly prescribed in the clinical setting with regard to the neurophysiological influence on the brain.

Numerous studies conducted in the past decades showed that representational maps in the brain (in somatosensory area) are altered by manipulation of their sensory inputs (Merzenich et al., 1984). Subsequent research has shown that cortical representation in M1 can also be altered by behavioral experience (Nudo, & Milliken, 1996). For instance, Nudo and colleagues worked with intact adult squirrel monkeys to study the effect of upper limb motor skill exercise on the maps of movement

representation in the M1. They found that skilled movement of a certain part of the limb is associated with expansion of the brain region that corresponds to the movement representation of that part. Expanding on this work, Askim et al. (2009) investigated the changes in brain networks in human patients post-stroke treated early by task-oriented therapy, to evaluate the relationship between neuronal activity and functional improvements. They found a significant increase in the activity of several regions in the brain such as contralesional somatosensory cortex (S1) and high lateralization of primary sensorimotor cortex, which both correlated positively with improved hand function. Dong et al. (2006) examined whether motor cortical activation captured during arm-focused therapy can predict paretic hand functional gains. They used serial functional magnetic resonance imaging (fMRI) to assess the brain (bilateral M1 and dorsal premotor (PMd) areas) during performing a pinch task before, midway, and after 2 weeks of CIMT, and used the Wolf Motor Function Test (WMFT) before and after the intervention to evaluate the level of functional recovery. They found a reduction in ipsilateral (contralesional) M1 activation after the first week of training, and this change was correlated with post-therapeutic functional improvements. Overall, research evidence strongly supports the use of a task-oriented approach in rehabilitation, and as such it is recommended (level 1 evidence) as a primary approach to promote functional recovery post-stroke (Lindsay et al., 2010).

2.2 Cortical Excitability

As previously described, neurorehabilitation of patients post stroke depends on the repetitive training of the paretic limb(s) to stimulate neurons in the affected

hemisphere. The stimulation of neuronal pathways in the brain can lead to the

strengthening of existing pathways as well as the 're-wiring' of functional connections,

thus allowing these pathways to inherit functions of the pathways damaged by the neural

insult. Consistent with the principles of long-term plasticity, synapses that are

successfully activated are strengthened, while those that cannot be successfully excited

are weakened (Rossini & Rossi, 2007). Accordingly, the excitability of cortical neurons

(i.e., their resting membrane potential) has an impact on the degree of plasticity that can

occur. For instance, Rossini & Rossi (2007) reported that if neurons were excited, they

could be stimulated faster and easier because highly excitable neurons need to be

stimulated less than depressed neurons in order to elicit muscle activity. Given the link

between neuronal activity, brain plasticity and functional recovery, coupled with the

understanding that neurons whose threshold for depolarization is lower require less

excitatory input to generate an action potential, there is a need to find ways to lower the

threshold of cortical neurons prior to engaging in task-oriented therapies. By increasing

the likelihood of neuronal discharge that occurs as a result of therapy, it stands to reason

that brain plasticity and the resulting functional recovery could be increased.

Indeed, one should understand the mechanisms that control excitability in human

motor cortex after injury. A review by Badawy, Loetscher, Macdonell, & Brodtmann

(2012) points out that excitatory and inhibitory systems in the brain control every aspect

of behavior, from primitive reflexes to abstract thinking and emotions. The level of

excitation or inhibition is determined by the interaction of neurotransmitters and cellular

receptors. This interaction controls the flow of ions through ion channels or through a

complex cascade of intracellular interactions via secondary messengers (Badawy et al.,

2012). Neurological disorders such as stroke are commonly associated with negative changes in the excitability of undamaged areas of the brain. Todd, Butler, Gandevia, & Taylor (2006) and Brasil-Neto et al. (1992) found that reduced afferent input due to transient ischemic block of cutaneous or transient immobilization disrupts the regulation of the M1 excitability. Moreover, brain injury leads to disruption in the inhibitory interactions between the primary motor cortices (Murase, Duque, Mazzocchio , & Cohen, 2004). Specifically, Murase and colleagues reported that movement of the paretic limb is associated with an abnormal level of interhemispheric inhibition (IHI) by the intact (contralesional) hemisphere to the injured one (ipsilesional). This IHI may be the result of a high level of activity of the intact hemisphere during movement of the paretic limb (Liepert, Classen, Cohen, & Hallett, 1998). Also, inhibition in the affected M1 could be the result of abnormal gamma-aminobutyric acid (GABA)-mediated inhibition within the injured hemisphere (Daskalakis, Christensen, Fitzgerald, Roshan, & Chen, 2002). Interestingly, Badawy et al. (2012) suggested that in the early phases following a stroke, increased IHI leads to reduced activity in the unaffected hemisphere, resulting in increased activity of the affected hemisphere, thereby promoting recovery. Furthermore, Gauthier et al. (2007) reported that studies on adult stroke patients have demonstrated positive functional changes in cortical excitability after motor therapy. So, much effort in the past decades has been made to study the pathophysiological basis underlying changes in brain excitability during rehabilitation to better understand the mechanisms underlying recovery and the effect of the various therapeutic approaches used.

The excitability of cortical neurons can be altered by a number of different means, including the use of caffeine (Botella, Bosch, Romero, & Parra, 2001, and Brice, &Smith,

2001), energy drinks (Schwaninger et al., 2002 and Specterman, 2005), drugs (e.g., amphetamines) (Garcia-Munoz, Young, & Groves, 1991), non-invasive brain stimulation such as TMS (Badawy et al., 2012), and AE (Forrester et al., 2006). Caffeine acts as a stimulant by activating the central nervous system. It has been shown to increase motor activity in many behavioral studies in humans (Botella et al., 2001; Brice and Smith, 2001) and animals (Kaplan et al., 1992). Caffeine is a methylxanthine, which antagonizes the depressant effects of adenosine inhibits phos- phodiesterases and 5'-nucleo- tidases, mobilizes calcium, releases catecholamines and ACh, and antagonizes some of the actions of opiates and benzodiazepines, thus enhances the spontaneous firing rate of cortical neurons (Phillis, Edstrom, Kostopoulos, & Kirkpatrick, 1979). Certain concentrations of caffeine are thought to increase synaptic input to the corticospinal neurons, which results in greater corticospinal excitability (Phillis et al., 1979). Wajda and colleagues (1989) studied the changes in the CNS of mice treated with caffeine for three weeks. Their findings showed that caffeine (150-160 mg/kg per day for 3 weeks) led to a significant reduction of GABA in some brain regions including the pons and medulla, which may contribute to the observed increase in excitability (Wajda, Banay-Schwartz, & Lajtha, 1989).

Mobbs, Kow, and Yang (2001) reported that changes in blood glucose might affect neuronal cell function because the brain is metabolically dependent. Accordingly, as brain glucose levels rise, the activity of certain neurons increases such as glucoresponsive (GR) neurons in the hypothalamus. Moreover, Schwaninger et al. (2002) reported that the presence of involuntary muscle contractions in people with diabetes when glucose levels fall very low suggest that there may be a link between blood glucose

levels and the neurophysiology of motor control. These findings led Specterman and colleagues (2005) to examine the effect of glucose- and caffeine-containing energy drinks on corticospinal excitability by measuring the amplitude of MEPs produced before and after the ingestion of these drinks. Their study showed that the combination of caffeine and glucose has no additive effect on cortical excitability than each one alone. However, a study by Orth, Amann, Ratnaraj, Patsalos, & Rothwell (2005) showed that caffeine in a concentration similar to that in a strong cup of coffee does not have a major effect on TMS measures of M1 excitability as they found no significant difference between resting motor threshold (RMT), active motor threshold (AMT), short-interval intracortical inhibition (SICI) or intracortical facilitation (ICF) thresholds when comparing data obtained before and after caffeine ingestion. Moreover, the amount of caffeine needed to increase excitability could produce side effects that impact on behavior as well as physiological responses, and ultimately could negatively impact human health when ingested in high doses. These effects include increased HR, restlessness, irritability, stress, muscle tremors and difficulty sleeping among others (Lieberman, Tharion, Shukitt-Hale, Speckman & Tulley, 2002). Accordingly, caffeine does not seem an appropriate method to increase cortical excitability before neurorehabilitation.

The influence of several drugs on motor excitability has been investigated by using TMS both in healthy subjects and patients (Minelli et al., 2010). Flavel and colleagues (2012) carried out a study to investigate (using TMS) the long-term effect of illicit use of stimulant drugs such as methamphetamine, 'ecstasy' [3,4-methylenedioxy-methamphetamine (MDMA)], and cocaine on human motor cortical excitability. They found significantly larger MEP amplitude in stimulant drug users than in non-drug users

during both relaxation and muscle contraction. Drug users also exhibited significantly greater muscle activity during performance of a given task. These changes may partly underlie the objective reports of movement dysfunction in chronic drug users (Flavel, White, & Todd, 2012). Studies also revealed that drugs like cocaine and amphetamines increased long-interval ICF in motor cortex which in-turn can lead to increased cortical excitability (Sundaresan, Ziemann, Stanley, & Boutros, 2007). The use of these drugs often leads to temporarily increased alertness, mood, and euphoria due to the acute mechanism of action of these drugs on the monoamine neurotransmitters dopamine, norepinephrine, and serotonin (Greco, & Garris, 2003). However, studies in rodents suggest that chronic use of these stimulants (i.e., amphetamines) is associated with long-term changes in monoamine neurotransmission that can cause dopamine deficiency and neurotoxicity due to a combination of mechanisms including mitochondrial dysfunction, oxidative stress, excitotoxicity, and neuroinflammation (Yamamoto, Moszczynska, and Gudelsky, 2010). Although the effect of stimulant drugs on brain excitability have been shown by many studies (Bauernfeind et al., 2003, Sundaresan et al., 2007, and Flavel et al., 2012), the harmful consequences of these drugs make them poor choices for increasing cortical excitability.

TMS is a noninvasive and painless tool for the electrical stimulation of neural tissue, including cerebral cortex, spinal roots, and cranial and peripheral nerves (Kobayashi& Pascual-Leone, 2003). It can be applied over M1 to obtain information about the state of excitability of neuronal circuits *in vivo* in the human brain through producing indirect waves descending along the corticospinal fibers to elicit MEPs (Badawy et al., 2012). In addition to using single pulse TMS to examine cortical

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excitability, repetitive TMS (rTMS) paradigms, along with transcranial direct current

stimulation (tDCS), can be used to alter M1 excitability (Lefaucheur, 2009). As

mentioned in the previous text, stroke can often lead to reduced output from the affected

hemisphere and excessive IHI from the unaffected hemisphere to the affected

hemisphere, which in turn results in motor deficits (Murase, Duque, Mazzocchio, &

Cohen, 2004, Takeuchi, Chuma, Matsuo, Watanabe, & Ikoma, 2005 and Takeuchi &

Izumi, 2012). Techniques such as rTMS or tDCS can enhance recovery of motor function

by reducing IHI by either suppressing the excitability of the unaffected (contralesional)

hemisphere, or increasing the excitability of the affected (ipsilesional) hemisphere

(Takeuchi & Izumi, 2012). It has been argued that the pairing of rehabilitative training

with these neuromodulatory techniques results in more enduring performance

improvements and functional plasticity in the affected hemisphere compared with motor

training or stimulation alone in patients with chronic stroke (Zimerman et al., 2012,

Takeuchi et al., 2005 and Takeuchi et al., 2008). However, although many studies

reported that TMS can be used to excite brain cells prior to rehabilitation, its use as a

therapeutic modality is not widely available due to several reasons. The device is not

commonly available in clinics or rehabilitation centers due to its high cost. The

application of rTMS also requires specialized training, which further increases its cost.

Also, although TMS is considered a safe technique, it has been shown that it can induce

headaches in some individuals (Wassermann, 1998). Wassermann has reported that rTMS

can induce seizures, especially if used in individuals with a history of epilepsy.

Furthermore, there are several contra-indications for using TMS such as: presence of a

pace maker, aneurysm clips heart/vascular clips, prosthetic valves, and intracranial metal

prostheses (Wassermann, 1998). As stroke commonly occurs in an aged population, the possibility of having these contra-indications is high. Consequently, TMS is not potentially the best way to be used as a therapeutic method to prime the brain cells before each rehabilitation session.

2.3 Aerobic Exercise

Aerobic exercise (AE) has been reported to be an effective method for increasing cortical excitability in individuals with traumatic brain injury (Forrester et al., 2006). Forrester and colleagues (2006) conducted a study to compare treadmill-trained and untrained patients with chronic stroke regarding changes in M1 excitability of the quadriceps muscles representation (both paretic and nonparetic). Trained participants performed three treadmill sessions each week for 6 months, at a targeted intensity of 60% HRR for 15 to 20 minutes. After 6 months of training, changes in MEP amplitude resulting from a single bout of treadmill exercise, assessed using single-pulse TMS, were determined. Results showed that treadmill-trained patients had greater MEP amplitude in the M1 representation of the quadriceps muscle for the paretic limb after treadmill exercise relative to the MEP amplitude obtained from the untrained patients. Thus their study indicated that exercise alters M1 excitability in the representation of an exercised lower extremity in people with chronic stroke. Additionally, Balbi and colleagues (2002) reported that muscle contraction potentially modulates M1 excitability within the areas of the cortex that represented the working muscles in healthy individuals. They used single-pulse TMS to measure the amplitude of MEPs from the thenar muscles before and after contractions of different durations (5, 15, and 30 s) and intensities (10%, 25%, and 50%

of maximal voluntary contraction) of the target muscles at several time points and at different intensities and duration of contractions. They found that MEP amplitude increased after the muscle contraction, and that the maximal increase was observed one second after the contraction. As illustrated in Figure 1, MEP amplitude was larger after contractions (filled circles) when compared to both the baseline and control group (open circles) (Balbi et al., 2002).
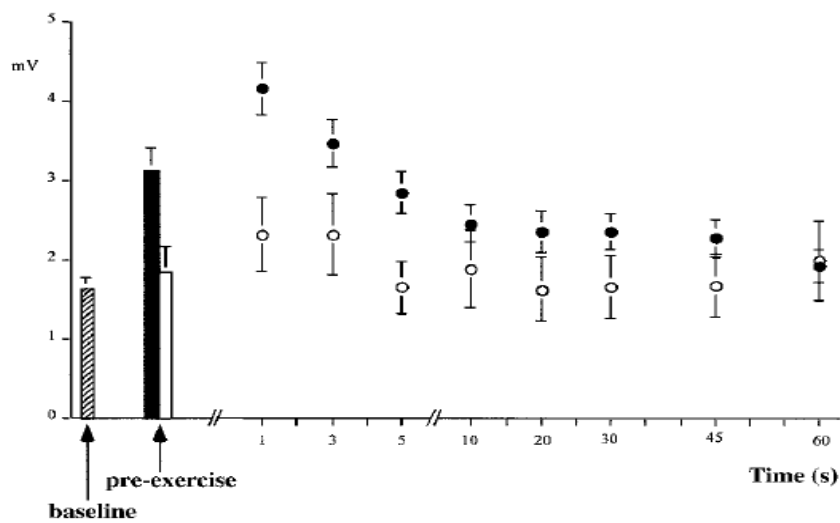
Figure 1. Effect of thenar muscle contraction on MEP amplitude.
Filled circles indicate post-exercise MEP facilitation (each point is the average of the MEP amplitudes following contractions of all intensities and durations for all subjects). Open circles indicate MEP amplitude without previous contraction (each point is the average of MEP amplitudes following no contraction for all subjects). Error bars indicate ±SE. Retrieved from Balbi et al., 2002.

In recent years, research has been done to investigate the effects of AE as a means of enhancing neuroplasticity and functional outcomes after stroke (Kreisel Hennerici, & Bazner, 2007 and Mang, Campbell, Ross, & Boyd, 2013). There is emerging evidence suggesting that AE is neuroprotective, preventing age-related brain atrophy and enhancing performance in both healthy populations and populations with

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neurodegenerative diseases (Cotman & Berchtold, 2002, Kramer, Erickson, & Colcombe, 2006, and Ahlskog, Geda, Graff-Radford, & Petersen, 2011). The effectiveness of exercise with patients post-stroke has been verified by systematic reviews and meta-analyses (Duncan et al., 2011). A recent systematic review done by Austin et al. (2014) showed that thirty-three animal studies have proven that exercise leads to gradual shrinking of the lesion volume after stroke (Austin et al., 2014). Moreover, many studies included in this review report that performing AE after stroke leads to reductions in inflammation and oxidative damage, growth of new blood vessels and an increase of blood flow to the area affected by stroke. Exercise also stimulates recovery mechanisms such as neurogenesis and synaptogenesis, and contributes to synaptic and neuronal plasticity (Austin et al., 2014). Previous studies on animals have shown that physical activity and exposure to enriched environments can elevate the levels of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and reduce GABA-mediated inhibition, which in turn can facilitate neuroplasticity (Smith et al., 2014). Moreover, Cirillo, Lavender, Ridding,& Semmler (2009) suggested that, over the long term, AE can promote increased neural density and survival in the human cortex. Cirillo and colleagues have mentioned that people who regularly take part in large amounts of vigorous physical exercise have a greater neuroplastic response to non-invasive brain stimulation techniques compared to sedentary individuals. The increasing evidence that AE exerts a wide range of benefits among healthy populations led the researchers to assess its effect on individuals with neurodegenerative disorders.

Much research has described the health-promoting and risk-reducing benefits of exercise for patients post stroke (Eich, Mach, Werner, & Hesse, 2004). A meta-analysis

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of the impact of AE post stroke by Pang, Eng, Dawson, & Gylfadottir (2006) included nine studies, of which seven were randomized clinical trials (RCTs), consistently demonstrated that AE improved peak oxygen consumption (VO2), peak workload, walking velocity, and walking endurance, all enabling daily function and independence. Potempa et al. (1996) reported that the average improvement in maximal oxygen consumption ($\dot{V}O_{2max}$) was greater in 13.3% of stroke patients who participated in a 10-week AE training program compared with controls (who did not do the exercise). It has been reported that there is growing evidence that AE improves fatigue, cognition and depressive symptoms in people with neurological conditions (Chaudhuri, & Behan, 2004). Owing to the positive impact of AE, as well as its effect on cardiovascular fitness of neurological patients, AE is recommended by best practice guidelines to be a part of routine neurological rehabilitation and long-term management (Furie, Kasner, Adams, et al., 2011 and Billinger et al., 2014). Furthermore, AE is easy to perform, requires minimal cost relative to other modalities, and is readily accessible in most clinics and rehabilitation centers given the presence of the requisite equipment and expertise. Given all of the benefits associated with AE, in addition to the argument by Balbi et al., (2002) described previously (stating that AE may potentially modulate M1 excitability in working muscles), we can conclude that AE seems to be a good modality to prepare the brain before neurorehabilitation. Questions remain however related to the use of AE as a tool for priming the brain in advance of rehabilitation.

2.4 Effect of a Single Bout of Aerobic Exercise on Cortical Excitability

As outlined previously, one issue related to priming the brain using AE is

deciding which muscle group to target. It is well known that the recovery of upper limb function is a major challenge for stroke survivors (Barreca, 2001). Upper limb recovery is often incomplete and takes a longer time to recover than the lower limb. The longer time for recovery makes it difficult to engage the paretic upper limb in AE to prime the brain before the actual rehabilitation (i.e., engagement in task specific therapy). The good news is that some studies have reported that AE can have a generalized effect on cortical excitability (Takahashi et al., 2011). Researchers have argued that cortical (i.e., M1) excitability can change as a result of contracting remote non-target muscles (Takahashi et al., 2011). In other words, AE could influence M1 excitability even within the areas that control muscles not involved in the exercise. Takahashi and colleagues (2011) mentioned that the spread of cortical excitability from active muscles to non-active muscles in proximal M1 areas might be due to the facilitatory cortical pathways between synergistic arm and leg representations. A study by McDonnell et al. (2013) compared corticospinal excitability and plasticity within the upper limb representation in M1 following a single session of lower limb cycling at either low or moderate intensity. One group in this study was asked to exercise at 57% of age-predicted maximal HR (for 30 min), and the other exercised at 77% of age-predicted maximal HR (for 15 min). Excitability was assessed using TMS to elicit MEPs in the first dorsal interosseus muscle, and M1 plasticity was examined using a continuous theta burst stimulation (cTBS) paradigm. Their findings showed that exercise did not alter cortical excitability; however, they found that low intensity exercise prompted a neuroplastic response to cTBS. Their study results suggested that low intensity (57% of age-predicted maximal HR) exercise has the potential to enhance the effectiveness of motor learning or recovery following brain

damage. Further, Smith et al. (2014) conducted a study to examine whether a single session of 30 minutes of ergometer cycling at two different intensities (40% and 80% of participants age-predicted HRR) was associated with changes in cortical excitability and intracortical inhibition within the hand motor representation. Similar to the previous findings, no changes in cortical excitability were observed, but their findings showed less SICI following exercise at 40% HRR for 30 minutes, and this reduction was sustained for 15 minutes after exercise completion (Figure 2).
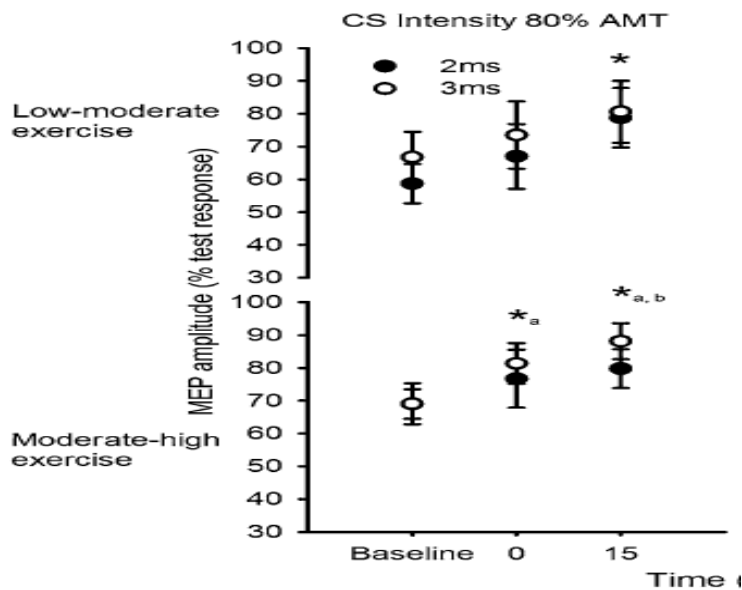
Figure 2. SICI was significantly reduced from baseline following lower limb exercise. This figure shows the amount of SICI recorded from the resting right FDI muscle prior to and at 0 and 15 min following exercise at both the low–moderate (top panel) and moderate–high (bottom panel) exercise intensities. All the circles represent the magnitudes of the conditioned MEPs (after the test stimulus) *reduction from baseline to 15 min, 2 ms ISI; *a reduction from baseline to 0 and 15 min, 2 ms ISI; *b reduction from baseline to 15 min, 3 ms. *$P < 0.05$. Data are shown as group mean ± SEM. Retrieved from Smith et al., 2014.

A more recent study done by Singh et al. (2014) expanded on these results to address the effect of a brief (20 min) session of lower limb AE on the cortical excitability of an upper-limb muscle representation. Singh and colleagues used single-pulse TMS to

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assess input–output curves, and paired-pulse TMS to assess ICF, SICI, and LICI in the extensor carpi radialis muscle in twelve healthy individuals following a single session of moderate intensity stationary biking (70% of their age-predicted maximum HR). Their study results showed that this single bout of AE did not directly modulate the excitability of neurons in M1, as the magnitudes of MEPs did not show significant change following exercise (as shown in Figure 3), but it increased ICF (Figure 4), and decreased SICI (Figure 5), which in turn may facilitate the induction of experience-dependent plasticity.
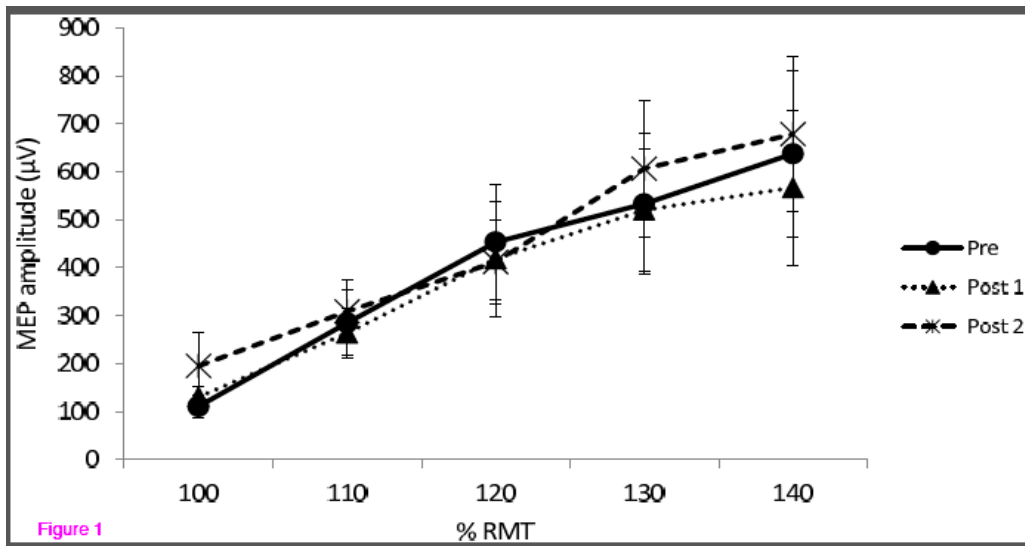
Figure 3. S-R curves pre- and post-exercise in response to stimulation at increasing percentages of resting motor threshold. Post 1: immediately after exercise. Post 2: 30 minutes after exercise (n=12). Bars represent SEM. Retrieved from Singh et al., 2014.
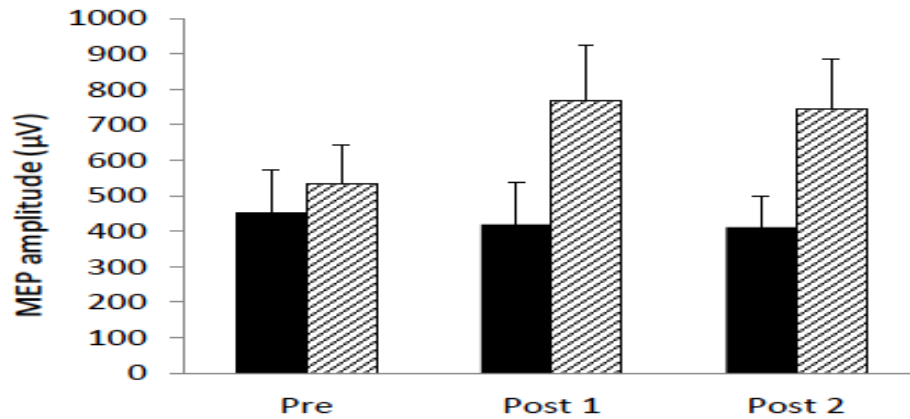
Figure 4. Modulation of ICF following exercise. Induction of ICF across all participants (n=12). Unconditioned single pulse amplitudes at 120% RMT (black bars) are compared to conditioned stimulus amplitudes (striped bars). Bars represent SEM. Retrieved from Singh et al., 2014.
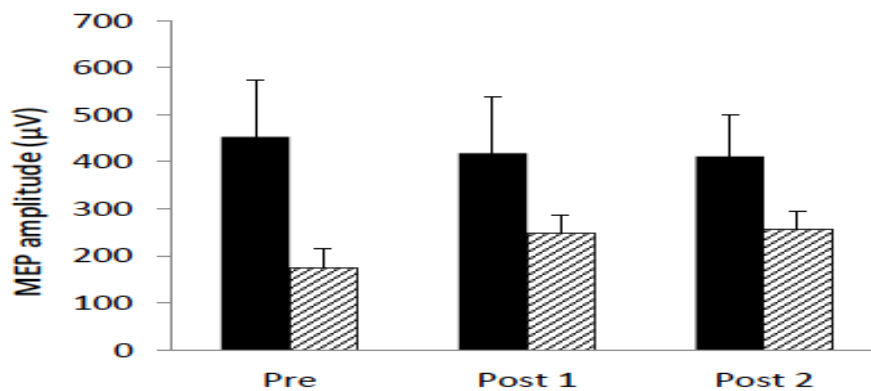
Figure 5. Modulation of SICI following exercise. Retrieved from Singh et al., 2014 Induction of SICI across all participants (n=11). Unconditioned single pulse amplitudes at 120% RMT (black bars) are compared to conditioned stimulus amplitudes (striped bars). Bars represent SEM.

There is strong evidence that AE can modulate the activity and release of neurotransmitters in the brain, such as GABA (γ-aminobutyric acid), which affects the excitability of M1 neurons (Ilic, Korchounov, Ziemann, 2002, and Molina-Luna et al., 2009). GABA is the principal inhibitory neurotransmitter in the CNS and exerts its

effects via multiple receptors, particularly in cortical inhibitory networks. SICI is thought

to be mediated by GABA A receptors (Chen, Corwell, Yaseen, Hallett, & Cohen, 1998),

and LICI is believed to activate GABA B receptors (McDonnell, Orekhov, & Ziemann,

2006). Further, (as described previously) there is evidence in humans showed significant

elevation in serum BDNF level after exercise (Ferris et al., 2007, and McDonnell et al.,

2013). Brunig, Penschuck, Berninger, Benson, & Fritschy (2001) conducted an animal

study to investigate the impact of treatment with BDNF on GABAergic function. They

found that BDNF induced a rapid reduction in postsynaptic $GABA_A$ receptor number that

leads to reduction of GABAergic function. Accordingly, elevation of serum BDNF level

after exercise that has been supported by much human research could contributes to

reduction of inhibition of cortical neurons. Based on these studies, Singh et al. (2014)

suggested that the reduction of SICI and LICI after AE resulted from a reduction in

GABA levels caused by the AE. Moreover, Singh and (2014) reported that it has been

shown that the cortical mechanisms underlying the increase in ICF after AE could be

mediated by glutamatergic interneurons, and possibly N-methyl-D-aspartate (NMDA)

receptors (Liepert, Schwenkreis, Tegenthoff, & Malin, 1997, and Ziemann, Chen, Cohen,

& Hallett, 1998).

   Although AE has been proven to modulate the amount of facilitation and

inhibition in M1, which are both critical to the modulation of cortical output, no

significant differences were observed in MEP amplitudes before and after exercise in any

of the previous studies in this area. Also, research reported that exercise and muscle

contractions led to increase in cortical excitability to exercised and contracted muscle as

evidenced by increase in MEP amplitudes (Balbi et al., 2003, and Forrester et al., 2006).

Taking these findings together with the argument by Takahashi et al. (2011) that cortical excitability can change as a result of contracting remote non-target muscles, significant differences in MEP amplitudes should have been observed after exercise. Examination of these results collectively suggests that the problem in delineating the effects of AE on cortical excitability may be due to the diversity of exercise dosages (intensity and duration) that have been used in the previous studies. On one hand, low dosage exercise might not be enough to alter the neuronal excitability. On the other hand, high intensity or the longer duration exercise often leads to fatigue, which can in turn result in a reduction of excitability in M1 (Zanette et al., 1995 and Bonato et al., 1996). Zanette and Bonato and their colleagues (1996) carried out studies that have supported the argument of Brasil-Neto et al. (1993) that "post-exercise depression of MEPs may be an expression of central nervous system fatigue, probably due to transient depletion of neurotransmitters". Further, based on these findings, it seems that the lack of significant changes in the aforementioned studies in the reduction of MEP amplitude may be the result of fatigue and its effect on neurotransmitter handling. Moreover, Verin and colleagues (2004) showed that MEP amplitudes significantly decreased after exhaustive treadmill exercise. In addition, Sidhu, Cresswell, & Carroll (2012) found that sustained cycling exercise at 75% of maximum HR for 30 minutes does not increase the cortical excitability.

Interestingly, the findings by Smith and Singh and their colleagues (2014), as demonstrated before, showed that the amount of SICI decreased after AE at an intensity of 40% age-predicted HRR for 30 minutes, and 70% of age-predicted maximal HR for 20 minutes respectively. Reduction of inhibition in cortical neurons is critical to prime the

brain of patients with stroke before engaging into rehabilitation session, as previous findings suggest that interventions aimed at decreasing GABA activity might be a useful adjunct to the induction of plasticity in M1. However, in the context of using AE as a means to increase cortical excitability *prior* to a bout of rehabilitation therapy, it is critical that patients not be fatigued following the exercise component of the intervention. We suggested that this level of exercise (i.e., 40% age-predicted HRR for 30 minutes and 70% maximum HR for 20 minutes) may not be feasible for some patients, given the increased frequency of fatigue and thus lower tolerance for AE amongst patients post-stroke (Austin, Ploughman, Glynn, & Corbett, 2014). Thus, the primary issue with the research performed to date is the use AE parameters (intensities and durations) that could not be tolerated as a priming modality before actual therapy. What is needed is the investigation of varying levels of AE to identify the potential parameters to implement in the clinical setting as a means to 'prime' the brain before neurorehabilitation. By investigating multiple exercise levels, it may be possible to identify a potential 'lowest common denominator' for AE needed to result in increased cortical excitability.

For this purpose, this study aimed to examine the effect of different exercise levels on the excitability of M1 to determine if a level of AE lower than that previously studied can increase cortical excitability. Furthermore, this study aimed to evaluate the mechanisms underlying the changes in cortical excitability by assessing the amount of facilitation and inhibition in M1 after exercising at different levels. Ultimately, this work aims to facilitate the use of exercise as a modality to prime the brain prior to neurorehabilitation.

2.5 Exercise Intensity and Duration

The previous studies in this area have determined the exercise intensities for their participants based on the age-predicted HR formulas. Singh and colleagues (2014) have used the simplest equation (maximum HR = 220 – age) to determine the target exercise HR. Research has reported that this equation gives only a rough estimate of maximum HR and that this number is not the same for every similarly aged person. It has been reported that the validity of this equation has never been established in a sample that included a sufficient number of older adults (Tanaka et al., 2001). This is because older people are more likely to be taking medications that affect their maximum HR (Heyward and Gibson, 2014). Moreover, Smith and colleagues (2014) have used the predicted HRR formula (180– RHR) to determine the target HR in their study, which is may be considered a non-accurate measure of HRR. Ideally, identification of a person's maximum HR is to put the person into a situation where he/she exercised to a maximum degree. The HR value the individual reaches at the completion of the test exercise is considered their maximum HR. Accordingly, the target exercise intensity can then be determined as a fixed percentage of maximum HR or HRR (HRR= maximum HR - RHR).

HRR has been found to be more accurate than maximum HR (Pfitzinger, and Douglas. 1999). Further, it has been shown that the percentage of maximum HR method provides lower value compared to the percentage HRR method, when the same relative intensity is used (Heyward and Gibson, 2014). Thus, in this study we chose to prescribe exercise intensities based on the HRR of the participants.

Our study aimed to compare the effect of three different intensities of AE

(represented by different target HRs for exercise) on M1 excitability and intracortical network changes. We selected our AE intensities such that we could replicate previous work while adding to the body of literature related to our understanding of the effect of AE on cortical excitability. As such, our highest intensity of AE is similar to that investigated by Singh et al. (2014), which was cycling at 70% of age predicted maximal HR for 20 minutes. Our moderate intensity of AE is similar to that used by Smith and colleagues (2014) regarding intensity (40% HRR) but not duration (we use 20 minutes as opposed to 30 minutes; see methods for a description of our exercise duration). We have extended on this previous body of research by including a lower intensity of AE that had yet to be examined, namely 30% HRR.

As described previously, this study prescribed exercise intensities based on the HRR of the participants. In order to replicate the AE intensity that has been used in prior research (i.e., 70% age predicted maximum Maximum HR), the highest intensity that has been investigated in our study had to match this value. Based on the table below, 70% Maximum HR approximately equals 50% HRR.

Table 1. Comparison of methods for prescribing exercise intensity for healthy adults. Retrieved from Heyward and Gibson, 2014.

CR fitness classification	%HRR or %VO₂R	%HRmax	RPE
Poor	30-45	57-67	Light-moderate
Fair	40-55	64-74	Light-moderate
Average	55-70	74-84	Moderate-hard
Good	65-80	80-91	Moderate-hard
Excellent	70-85	84-94	Somewhat hard-hard

HRR = heart rate reserve; RPE = rating of perceived exertion.

The standard parameters of AE (e.g., intensity, and duration) to provide maximal acute neurological benefits have not been reported (Singh et al., 2014). As described previously, selecting the intensities of AE in this study was based on the previous studies in addition to include a lower intensity to test if the effect on M1 is still present at lower level AE. In order to test the impact of intensity, this study aimed to control for the duration of the exercise (i.e., used a fixed duration for all the different intensities). However, previous studies showed diversity in prescribed durations of AE that could influence brain plasticity. Interestingly, research found that 20 minutes of AE elevates the level of dopamine and BDNF in the brain (Hattori, Naoi, & Nishino, 1994, and Schmolesky, Webb, & Hansen, 2013). As demonstrated earlier, the release of facilitatory transmitters, in particular BDNF, reduced the effect of GABA, which in turn leads to reduction in inhibition (Brunig et al., 2001). Additionally, Classen et al. (1998) revealed that a single 20-minute bout of rapid muscle contraction, established a change in the cortical network representing the thumb. Taking these findings together with Singh et al.'s findings, which showed that 20 minute cycling facilitated intracortical changes, supported using 20 minutes as a fixed duration for all three intensities of AE tested in this study.

2.6 Transcranial Magnetic Stimulation (TMS)

Austin and colleagues (2014) argued that in order to advance our understanding of the impact of AE on the brain, researchers must utilize methods to assess neuronal activity in order to monitor exercise effects. There are a number of neuroimaging

modalities that provide information about the activity in the brain (Cramer & Bastings, 2000 and Takeuchi & Izumi, 2013). However, when measuring the excitability of neurons in M1, the most often utilized tool is TMS. As indicated previously, TMS is a non-invasive technique that can be used to obtain the state of brain excitability (Badawy et al., 2012). TMS is applied through a magnetic stimulator, which consists of a set of electrical capacitors that can store and rapidly discharge electricity into a coil. The coil is held in close approximation to the participants scalp. As the electrical current flows through the coil, a magnetic field is generated (perpendicular to the coil) and passes through the scalp and skull, inducing a second electrical flow of current in the brain (perpendicular to the magnetic field; Figure 6) (Hallet, 2000). Different coil types can be used to induce more focal or deeper stimulation. For example, H-coils deliver stimulation at a depth of 6cm, while figure-8 coils produce a more focal pattern of stimulation that penetrates at a depth of 1.5 to 2.5cm.
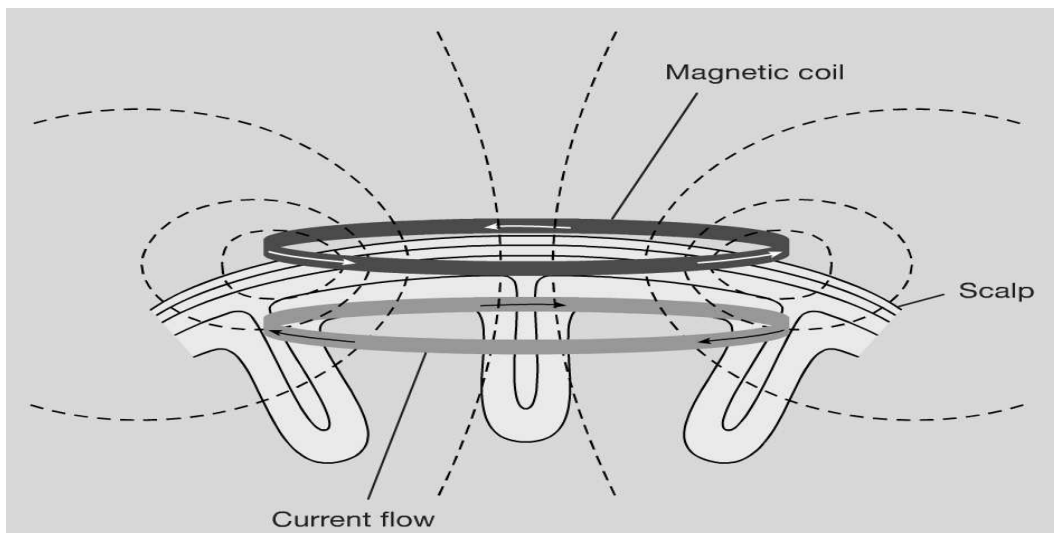
Figure 6. Induction of current in the brain through TMS. Retrieved from Hallet, 2000. The solid black ellipse indicates the magnetic field induced by passing the current through TMS coil (perpendicular to the coil). The dash lines indicate the electrical field induced (perpendicular to the magnetic field). The grey ellipse indicates the current induced into the brain.

The electric current produced in the brain causes a change in the transmembrane current of the neuron, which leads to the depolarization or hyperpolarization of the neuron, and in the case of depolarization, the generation of an action potential. This device can assess cortical excitability of a brain region representing specific muscles through stimulation of the region in M1 corresponding to the muscle. This stimulation produces indirect waves descending along the corticospinal fibers to elicit MEPs in the muscle of interest, detected via electromyography (EMG). Cortical excitability can be assessed based on the amplitude of the resultant MEP (Petersen, Pyndt, & Nielsen, 2003) (Figure 7).
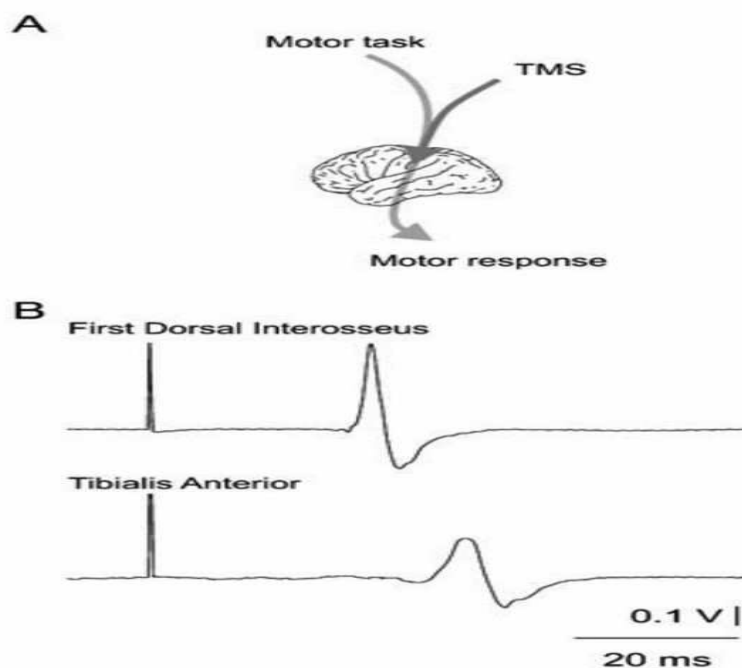
Figure 7. Representation of a motor evoked potential (MEP). Retrieved from Petersen, Pyndt, & Nielsen, 2003

Via TMS, the magnitude of MEPs can be assessed before and after any intervention reported to have an influence on cortical excitability to evaluate the changes,

with increased MEP amplitude representative of increased excitability, and decreased amplitude representative of inhibition. TMS can also be applied to measure the excitatory and inhibitory properties of M1 within and across hemispheres (intra- and inter-hemispheric inhibition respectively).

Interestingly, information obtained experimentally (cellular, synaptic, small local networks) by TMS can be linked with clinical observations (Fatemi-Ardekani, 2008). TMS presents the advantage of precise timing (for single and paired-pulse) and relatively good localization. The main disadvantage is the impossibility to stimulate deep brain structures directly. Most brain imaging techniques allow the investigators to identify brain areas that are active during a given motor, perceptual or cognitive process. However, they cannot tell us whether those areas are necessary for the process. By interfering with the normal functioning of a brain area, as is the case with rTMS, TMS enables inferences about a causal link between this area and behavior. Furthermore, because of the limited duration of the interference it induces, TMS can be used to investigate when a brain area is making its critical contribution to behavior (Cowey, 2005). As a result, TMS has been used heavily in studying the effectiveness of exercise on brain excitability.

2.7 Single and Paired Pulse TMS

TMS has different modes of stimulation, with the effects varying depending on the mode used. Single pulse TMS causes cortical neurons under the site of stimulation to depolarize and discharge an action potential. As indicated above, when stimulation is delivered to M1, it produces muscle activity in the form of a MEP, which can be recorded

using EMG. Another mode of stimulation, rTMS produces longer-lasting effects, which persist past the initial period of stimulation. rTMS can increase or decrease the excitability of the corticospinal tract depending on the intensity of stimulation, coil orientation, and frequency (Fitzgerald, Fountain, & Daskalakis, 2006, and Pascual-Leone, Davey, Rothwell, Wassermann, & Puri, 2002).

As single-pulse TMS has been shown to be a direct way to obtain the state of brain excitability (Badawy et al., 2012), this mode of stimulation will be utilized in the current work to address our primary study objective. In addition, we suggest that it is important to advance our understanding of the mechanisms that control the excitability in M1, and this can be done using paired-pulse TMS. Research has shown that paired-pulse TMS can be used to evaluate excitatory/inhibitory circuits, either within one hemisphere (intracortical circuits) or between the two hemispheres (interhemispheric circuits), to provide information on brain physiology and pathophysiology of various neurological diseases as well as on the mechanisms of brain plasticity (Rossini and Rossi, 2007). Today, the most widely used technique is based on a conditioning test design, which was originally introduced by Kujirai et al. (1993): a conditioning stimulus (CS) is applied prior to a test stimulus (TS) (Moliadze, Giannikopoulos, Eysel, & Funke, 2005). Ilic, Korchounov, & Ziemann (2002) reported that the effect of paired-pulse TMS depends on the intensity of the CS and TS and the interstimulus interval (ISI) between them. Previous studies have demonstrated that the ISI between the CS and TS determines what kind of interaction occurs (Kujirai et al., 1993, Tokimura et al., 1996, and Ziemann et al., 1998). If the CS is below threshold for eliciting an MEP in the target muscle (i.e., sub-threshold), and TS is above the threshold for eliciting a MEP (i.e., supra-threshold), then

the interaction between CS and TS is inhibitory at very short ISIs of 1–5 ms (Kujirai et al., 1993). However, if the CS and TS are close to the MEP threshold (Tokimura et al., 1996), or the CS is clearly above the MEP threshold and TS is below or around the MEP threshold (Ziemann et al. 1998, and Hanajima et al. 2002), then MEP facilitation occurs at discrete ISIs of 1–1.5, 2.5–3.0 and 4.0–4.5 ms. These mechanisms are commonly referred to as short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF), respectively. It is thought that SICI reflects inhibition mediated by $GABA_A$ receptors (Kujirai et al. 1993). Hanajima et al. 2002 reported that the sub-threshold CS produces inhibitory post-synaptic potentials (IPSP) at the corticospinal neurons in M1 that lead to a reduced number of action potentials by the subsequent suprathreshold TS. In contrast, it is thought that ICF reflects direct excitation of the first parts of the axon of excitatory intracortical interneurons by TS, which had been depolarized and therefore made hyperexcitable by the preceding CS (Hanajima et al. 2002). Long interval intracortical inhibition (LICI) is another kind of cortically mediated inhibition, which can be observed with longer ISI between the CS and TS (i.e., 50–200 ms) with both stimuli above the threshold for eliciting an MEP. As mentioned above, SICI reflects inhibition mediated by $GABA_A$ receptors, LICI however, is believed to activate $GABA_B$ receptors (Chen, 2004, and McDonnell, Orekhov, & Ziemann, 2006). Conversely, it has been reported that ICF appears to be mediated by alteration in the activity of glutamatergic interneurons, and possibly NMDA receptors, which can be regulated by several neurotransmitters released by exercise (Liepert, Schwenkreis, Tegenthoff, & Malin, 1997, and Ziemann, Chen, Cohen, & Hallett, 1998). Di Lazzaro et al. (1999) have provided strong evidence, based on cervical epidural recordings of the descending corticospinal

volley that all these interactions occur at the level of the motor cortex; however, the exact mechanisms are not fully understood. Collectively, single and paired-pulse TMS paradigms are ideal methods to examine changes in cortical excitability resulting from single bouts of AE, as well as shedding light on the mechanism(s) underlying the resultant changes.

CHAPTER 3: OBJECTIVES AND HYPOTHESIS

In the context of examining changes in cortical excitability as a result of AE, our objectives and related hypotheses include:

1) To investigate varying lower limb AE intensities to test if lower intensity AE can still modulate the cortical excitability of an upper limb muscle.

   Hypothesis:

   Cortical excitability will increase, as evidenced by increased MEP amplitude, in response to AE at all tested intensities.

2) To evaluate potential mechanisms underlying the changes in cortical excitability by assessing the amount of facilitation and inhibition in M1 after exercising at different intensities.

   Hypotheses:

- The amount of facilitation of cortical neurons in M1 will increase after AE at all tested intensities.

- The amount of short and long inhibition of cortical neurons in M1 will reduce after AE at all tested intensities.

- No significant difference will be found between the amount of reduction in inhibition and the amount of increase in facilitation after AE at all tested intensities.

The long-term goal of this research is to identify the lowest intensity of AE required to increase cortical excitability that could be implemented in the clinical setting as a means to prime the brain before neurorehabilitation.

CHAPTER 4: METHODS

4.1 Participants

4.1.1 Inclusion and Exclusion of Participants

The study included one group of 12 non-disabled individuals, aged 18–40 years (both sexes), with no history of neurological insult, cardiovascular, or pulmonary disorders. Participant safety to perform AE was assessed via the PAR-Q; Appendix 1. Exclusion criteria include having respiratory disorders, hypertension or other cardiovascular disease that would preclude participating in exercise, or having any contraindication to TMS based on the standard TMS screening form (Appendix 2).

4.1.2 Participant Recruitment

Prior to recruitment, the research protocol was approved by the Research Ethics Board of Dalhousie University. Participant recruitment was done through word of mouth and via advertisements (see Appendix 3) placed around Dalhousie University.

4.2 Measures Regarding Participant Characteristics: Screening forms

The following measures were used to screen the study participants regarding their suitability to undertake exercise and confirm the absence of any contraindications to TMS. These screening forms were sent to the participants prior to enrolment such that individuals self-screened to determine eligibility, with the study investigator following up with each participant to confirm eligibility. Additional measures were also used to determine the physical activity level of each participant (described below and included as Appendix 4).

4.2.1 Measures Regarding Suitability to Undertake Exercise

Participants were screened for their suitability to undertake exercise safely using the *PAR-Q* (Canadian Society for Exercise Physiology, Canada) (see Appendix 1). The PAR-Q was developed by the British Colombia Ministry of Health with subsequent revision in 2002 by an expert advisory committee of the Canadian Society for Exercise Physiology. It was designed to identify adults for whom physical activity might be inappropriate or people that should have medical advice concerning the type of activity most suitable for them. The PAR-Q includes 7 questions; if the person answers 'No' to all questions, he/she is deemed safe to partake in any type of exercise. However, if the person answered yes to one or more questions, he/she can participate only in activities that are safe for them after consulting with their doctor. In our study, if the potential participant answered "yes" to any one of the questions, he/she was deemed ineligible.

4.2.2 Measures Regarding Contraindications to TMS

Participants were asked to complete the standard TMS screening form (Rossi, Hallett, Rossini, & Pascual-Leone, 2009) to ensure they do not have any contraindications to TMS including metallic implants, epilepsy or any other neurodegenerative disorders. Participants were excluded from the study if they answered YES to any of the first 10 questions, mentioned experiencing any problems with TMS or MRI in the past, or were taking any medication that can affect brain excitability (Appendix 2).

4.2.3 Measures Regarding Physical Activity Level (secondary measure)

The current physical activity levels of the participants were determined using the self-report short form of the International Physical Activity Questionnaire (IPAQ). The

IPAQ is a form designed to define or describe level of activity of the person in the last 7 days. It was developed in Geneva in 1998, with extensive reliability and validity testing undertaken in 12 countries (14 sites) across 6 continents during 2000 (Ainsworth et al. 2000). The IPAQ queries three specific types of activity undertaken in the three domains including leisure time, domestic and gardening (yard) activities, and work-related and transport-related activity. The IPAQ provides both categorical and continuous indicators of physical activity of the individual. For the present study, categorical scores were used to determine the physical activity level of each subject (Appendix 4). As indicated above, the IPAQ was considered a secondary measure in the present work, to be used only as a means to characterize our population but not for analysis. In other words, we just wanted to know what is the average level of physical activity of our participants when we interpret our findings.

4.3 Experimental Procedures

4.3.1 Study Design

Participants expressing interest in the study were contacted by the study investigator and provided (via email) 1) a brief description of the study and study plan; and 2) the screening forms and IPAQ questionnaire. Following self-screening by the participant, the study investigator contacted the potential participant to confirm study eligibility and schedule the experimental sessions.

Eligible participants were asked to attend 4 experimental sessions in a 7-day period. The 4 sessions included a familiarization session and 3 sessions during which TMS measures (described below) were obtained before and after a bout of AE. In the

familiarization session (considered to be day 1), participants reviewed and signed the informed consent document (Appendix 5), and completed (if they already had not) the screening forms and IPAQ questionnaire, and also completed a maximal exercise test. Participants were instructed to refrain from heavy exercise for at least 48 hours prior to the familiarization session (i.e., the first laboratory visit) as heavy exercise could influence the results of the maximal exercise test. Additionally, participants were asked to refrain from consuming caffeinated beverages and a heavy meal at least 2 hours prior to the session as these could compromise resting and exercise HR and blood pressure responses as well as the ability to complete the maximal assessment due to gastric distress. Lastly, participants were asked to not use the stairs to avoid HR elevation that could affect the reading of resting HR (RHR). During the familiarization session, participants underwent a maximal, graded cycle exercise test to determine their maximal HR (described in detail below). Sessions 2 through 4 involved acquiring the TMS measures and completion of a 20-minute bout of AE (cycling at one of three intensities: 30, 40, or 50% HRR). Before the bout of AE, single-pulse TMS was applied to localize the cortical representation of the target muscle, which is the right extensor carpi radialis (ECR) (termed the hotspot) and to determine the RMT of the participant. Pre- and post-exercise, stimulus–response curves (S-R curve) were obtained to assess changes in corticospinal excitability, and paired-pulse TMS obtained to assess changes in inhibition and facilitation (described in detail below; see Figure 8 for an outline of the experimental timeline). Participants were instructed to refrain from heavy exercise, caffeine and heavy meals for at least 2 hours prior to the experimental sessions. Regarding caffeine, participants were asked to maintain their usual caffeine intake during the day but to

refrain from drinking any caffeinated beverage in the 2 hours before the experimental session. Participants were also asked to maintain a similar diet before each experimental session.
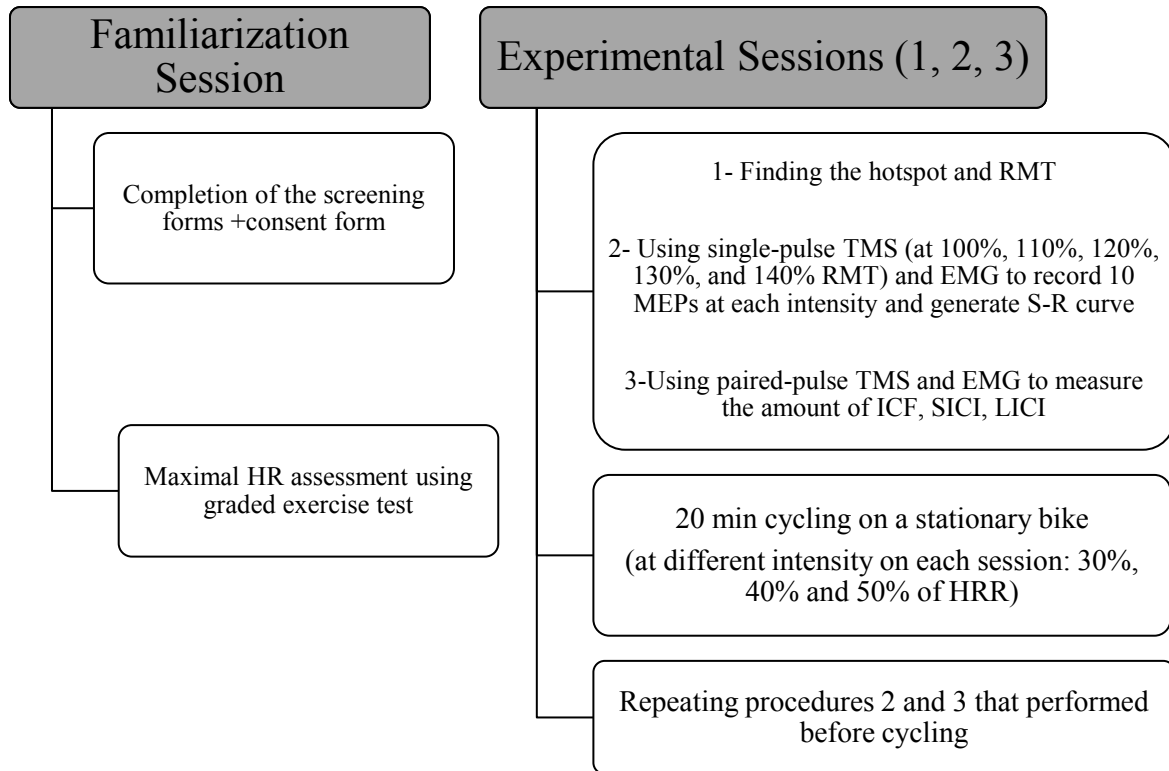
Figure 8. Task familiarization and experimental timeline.

4.3.2 Maximal Exercise Test

Before performing the maximal exercise test, participants were asked to sit on the bike quietly for a 5 min period before their resting heart rate (RHR) was obtained using a wrist-mounted monitor (described below). Participants then performed a graded maximal exercise test on a stationary cycle ergometer (Corival 2003, Lode B.V. Medical Technology, Groningen, The Netherlands) using a ramped protocol that was controlled

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externally via Lode Ergometry Manager software (version 10.4.4, Lode B.V., Groningen, The Netherlands). This stationary ergometer modifies resistance to maintain workload (e.g., if we set the power to 50 Watts and the participant was cycling at a lower rate, the ergometer would elevate the resistance to maintain the same workload (i.e., 50 Watts) and vice versa: if the person cycles at a faster rate the resistance was reduced automatically to maintain the same workload). Participants were instructed to start with a 5-min warm-up period of cycling at a workload of 50 Watts. Following this 5-min warm-up, workload was increased by 20 watts/min until cessation of the test. Throughout, HR was measured via the wrist-mounted monitor (Mio global, 2014, Physical enterprises Inc., USA). The Mio monitor permits measurement of HR in real time as well as recording of the HR for offline analysis, with HR data transmitted to an iPhone via Bluetooth using the Mio Global app. Previous work has validated the Mio watch, indicating it provides an accurate measurement of HR during exercise (Stahl, An, Dinkel, Noble, & Lee, 2016), and is a valid HR monitoring instrument for use during graded exercise testing (Olenick, Haile, & Dixon (2015). During the graded maximal exercise test, participants were asked for their Rating of Perceived Exertion (RPE) on a scale of 1-10, where 1 represented "Really Easy" and 10 represented "Maximal: just like my hardest race" (Appendix 6) every 2 minutes for the duration of the test.

Participants were asked to adjust the seat height to a position that they felt comfortable with. Prior to the onset of the graded maximal exercise test, participants were provided instructions related to the test. Briefly, they were told that they had to start with light cycling to warm up before the workload increased after 5 minutes. Also, they were asked to let the observer know if they needed the air conditioner to be turned on or

off during exercise or if they needed a towel or water. Importantly, participants were instructed to give a cue to indicate when they felt they had one minute remaining before they would have to stop the test. Participants were asked not to get off the ergometer when they stopped the graded maximal exercise test, but rather to cycle at a lower workload until their HR returned to approximately their RHR. As indicated above, participants were asked to provide a cue to indicate when they believed they had approximately 1 minute remaining in the test, at which time the final measurement of HR and RPE was made. Again, participants completed the test with a 5-minute cool-down period, during which the workload on the cycle ergometer was reduced to 50 Watts.

To be considered a true maximal exercise test, the following two criteria had to be met:

1. A final RPE on the Borg scale ≥ 7.

2. Maximum HR equal to or within 12 beats of the age-predicted (estimated) Maximum HR calculated by the formula (Tanaka, Monahan & Seals, 2001):

    Estimated Maximum HR = [206.9 – (0.67 × Age)]

As indicated above, RHR was measured via Mio watch at the beginning of the study (after 5 minutes resting with no distractions sitting on the bike). Using RHR and Maximum HR values obtained from the maximal exercise test, HRR was calculated using the formula: **(HRR = Maximum HR – RHR).** Target HR for each of the three different exercise intensities (30, 40 and 50% HRR) were then calculated, based on the required exercise intensity, by the following formula:

Target HR = [% exercise intensity × (HRR)] + RHR

To ensure participants safety, the graded maximal exercise test was terminated if 1) the participant experienced a sudden decrease in HR < 30 beats per minute; or 2) at the participant's own discretion (i.e., if the participant felt that they were not able to continue for any reason).

4.3.3 TMS Protocol

Participants were asked to sit comfortably on a chair in a reclined position, with the right arm placed on a pillow in their lap. Single and paired-pulse TMS was applied through a figure of eight coil (outer diameter of the wing: 70 mm) connected to a MagStim BiStim system, which consists of two MagStim 200^2 magnetic stimulators connected together (The Magstim Company, Whitland, UK). BrainSight neuronavigation (Rogue Research Inc., Montreal, Canada) was used to guide the positioning and orientation of the coil over the target motor region using a template MRI[1]. With the exception of single pulse TMS for hotspot localization and determination of RMT (described below), 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.

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[1] A "template MRI " is an anatomical MRI that is derived from a population of neurologically healthy individuals. Specifically, the anatomical MRI of a sample of these healthy individuals is merged together (called 'warping' in the field of neuroimaging) to create an image of the brain that is representative of the population as a whole.

4.3.3.1 Co-Registration

   To configure our target, each participant's head was co-registered with the template MRI. Co-registration was achieved by aligning three anatomical landmarks on the participant (nasion, right and left pre-auricular points) with the same anatomical landmarks on the digital representation of the participants head obtained via surface reconstruction using the template MRI. Following validation of the co-registration procedure based on the landmarks mentioned above, localization of the hotspot was performed. Using the template MRI, a series of targets arranged in a 5 cm × 5 cm grid, with 7.5-mm spacing between targets, was placed over the cortical surface, centered on the 'hand knob' of the left M1. These targets were used in the localization of the hotspot as described below (See Figure 9).
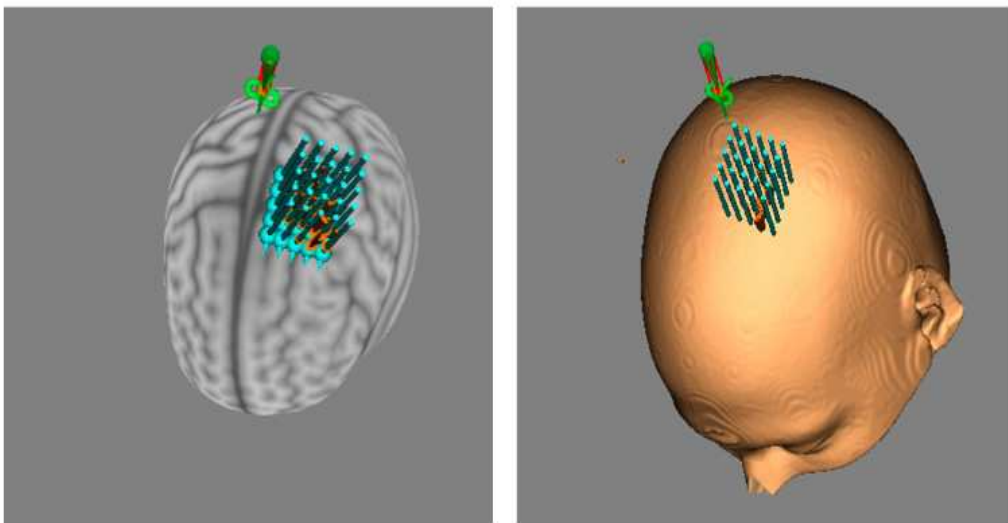
Figure 9. The placement of targets shown in Brainsight. (for the reconstructed cortical surface, left, and head shape, right) including the grid placement over the M1 for hot spot localization.

4.3.3.2 Localization of the Hotspot

As indicated previously, the target muscle was the right ECR, and as such stimulation targeted the left M1. Motor evoked potentials were obtained using self-adhering Ag/AgCl electrodes (1 × 3 cm; Q-Trace Gold; Kendall-LTP, USA) in a bipolar configuration placed on the skin over the muscle belly of the ECR, 5 cm distal to the radiohumeral joint [1 cm interelectrode distance], and the neutral (ground) electrode placed on the olecranon process (Hermens, Merletti and Freriks, 1996). Identification of the ECR muscle was confirmed by asking the participant to extend his/her wrist with radial deviation while palpating the muscle. EMG (that is the MEPs) was obtained using vendor-supplied hardware (Brainsight EMG isolation Unit and Amplifier Pod).

For application of single and paired-pulse TMS, the TMS coil was held in close proximity to the skull with the handle pointing posteriorly and laterally at an angle of approximately 45 degrees to the mid-sagittal line over the left M1 hand area. To identify the motor hotspot of the right ECR, we used the grid over the left M1. Each target on the grid was stimulated to determine the spot (s) that produced the highest amplitude MEPs in the resting muscle for 5 out of 10 stimulation as assessed by MEP amplitude.

4.3.3.3 Determining Resting Motor Threshold

Once the hotspot was located, RMT could be determined. The RMT is defined as the lowest stimulation intensity required to elicit a MEP of minimum amplitude of $50\mu V$ peak-to-peak in the resting target muscle, for 5 out of 10 consecutive stimuli. The RMT was determined for each participant at the beginning of each exercise session, with subsequent stimulation parameters set as a percentage of RMT.

4.3.3.4 Single Pulse stimulation: S-R Curves

After the RMT was determined, the value was recorded on an Excel sheet to calculate the percentages needed for both single and paired-pulse TMS. We then disconnected the Brainsight EMG cables for the electrodes and connected the Signal EMG cables, as EMG was obtained using different hardware during acquisition of the S-R curves and paired-pulse TMS paradigms. EMG during single and paired-pulse TMS was obtained at a sampling rate of 1 kHz, with a bandpass of 25-100 Hz (1902 and Power 1401; Cambridge Electronics Design, UK) using Signal software. In this study, we always performed single-pulse TMS first. Ten single pulses corresponding to each of 5 different stimulus intensities (100%, 110%, 120%, 130%, and 140% of RMT) were delivered over the motor hotspot (total of 50 pulses) to generate a S-R curve. Single pulses were delivered with a fixed interval of 3-seconds between successive stimuli, with the order of delivery randomized. The resulting MEPs were collected and stored for offline analysis to determine effects of AE on corticospinal excitability.

4.3.3.5 Paired Pulse Stimulation

Following completion of the S-R curves, paired-pulse TMS was delivered over the hotspot to measure ICF, SICI and LICI. As described above, ICF consisted of a CS (80% of RMT) and TS (120% of RMT) with an inter-stimulus interval (ISI) of 15 ms; SICI consisted of a CS (80% of RMT) and TS (120% of RMT) with an ISI of 2 ms; LICI consisted of a CS (120% of RMT) and TS (120% of RMT) with an ISI of 100 ms (see Figure 9 for a depiction of ICF, SICI and LICI protocols). In each paired-pulse protocol, ten pairs of stimuli were delivered with a fixed interval of 3 seconds between stimulus pairs. The order in which ICF, SICI and LICI was performed was pseudo randomized

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across participants. Three custom scripts were created, each with a different order of ICF, SICI and LICI (**script A:** ICF, SICI, LICI, **script B:** SICI, ICF, LICI, and **script C:** LICI, ICF. SICI). For each participant, we ran script A, B and C in sessions 1, 2, 3 respectively.
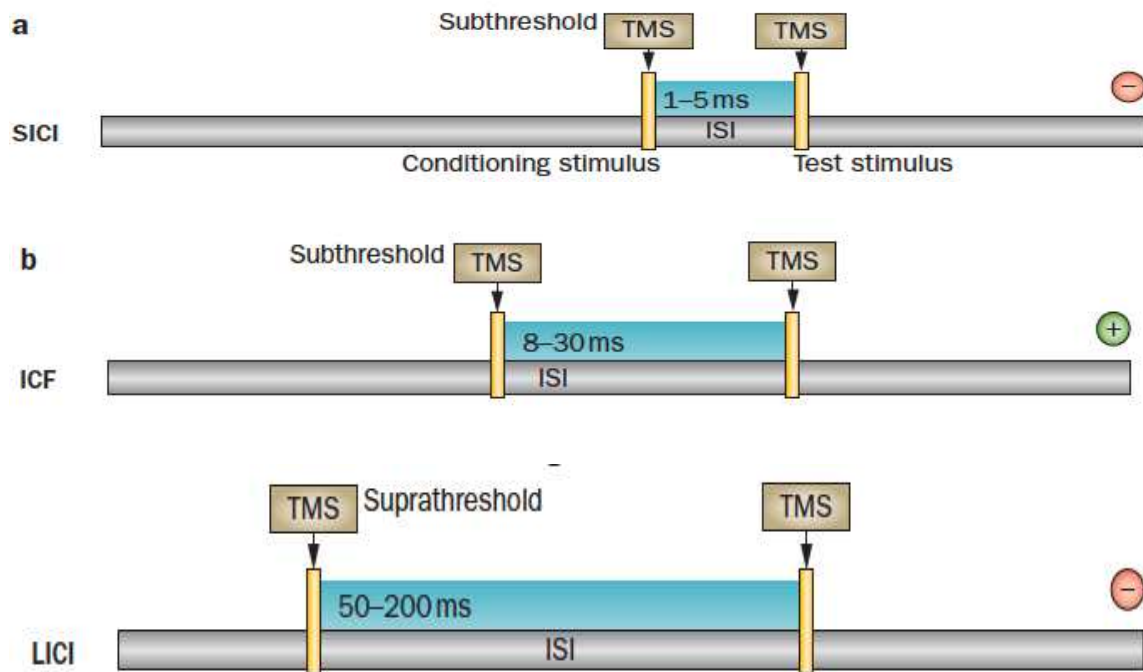
Figure 10. TMS paired-pulse protocols for testing intracortical facilitation and intracortical inhibitory circuitry. In SICI (a), a subthreshold conditioning pulse is followed by a suprathreshold test pulse after a 1–5 ms delay. In ICF (b), a subthreshold conditioning pulse is followed by a suprathreshold pulse after an 8–30 ms delay. In LICI (c), a suprathreshold conditioning pulse precedes a suprathreshold test pulse by 50–200 ms. Retrieved from: Di Pino et al. (2014)

4.3.4 Aerobic Exercise Protocol

In addition to the TMS measures, each of the three experimental sessions involved performing 20 minutes of continuous stationary biking at 30%, 40% or 50% of the participant's HRR as determined via the graded maximal exercise test. To negate the effect of order, the intensity of AE was randomized throughout. Participants were asked

to start with a warm-up consisting of low intensity cycling for between 2-5 minutes, during which the power was increased gradually until they reached the target HR for that session. Participants were asked to maintain a HR within 10 bpm of the target HR throughout the 20-minutes of cycling, with adjustment of workload used to facilitate this. During these 20-minute bouts of AE, participants were instructed to rest their arms comfortably by their sides and not to grip the handlebars to avoid any muscle activity in the upper limbs as our study aimed to test the excitability of a non-exercised upper limb muscle, thus we wanted to make sure that no movement was exerted by our target muscle. Although not obtained during each session, through pilot testing, we confirmed the absence of EMG activity in the ECR that exceeded baseline levels during the 20-minute bouts of AE.

Throughout the 20-minute bouts of AE, HR was measured via the wrist mounted HR monitor (Mio watch) as described previously. Participants had access to their HR in real time via the display on the monitor (as described before, the watch was connected via Bluetooth to an iPhone app (Mio Go)). HR data were sampled once per second. Participants were asked for their RPE score every 2 minutes during the 20-minute cycling.

To reduce experimental bias in the acquisition of the TMS measures, the primary investigator was unware of the exercise intensity assigned for each participant for each of the experimental sessions. Laboratory assistants facilitated completion of the exercise session.

Immediately following the completion of the 20-minute bout of AE, participants moved from the ergometer to the TMS chair for the collection of post-exercise measures.

All TMS measures were repeated post-exercise with the exception of RMT. In general, the pre-exercise assessment was 30 to 45 minutes in duration, while the post-exercise assessment was approximately 10 minutes.

4.4 Data Analysis

4.4.1 Heart Rate During Bouts of Aerobic Exercise

As described earlier, in each experimental session, HR data were recorded during AE via the Mio watch, sampled once per second. Following each experimental session, HR data were exported through the Mio Go app as a text file for offline analysis. Using Microsoft Excel, we averaged every 60 seconds (i.e., 60 samples) to obtain 20 single values, which were then averaged to allow for comparison with the required target HR for that session.

4.4.2 Motor Evoked Potentials (MEPs)

As described above, for single-pulse TMS we performed 10 stimulations at each of the stimulator output intensities (100, 110, 120, 130, and 140% RMT) to generate an S-R curve. Participants in which an *a priori* number of MEPs were not obtained (i.e., less than 6 /10) as a response to stimulation at any intensity were excluded from further analysis (i.e., if stimulation at all the intensities resulted in 6/10 MEPs or more but only stimulation at 120% resulted in 5/10 MEPs or less, that participant was excluded from further analysis of that exercise level). The peak-to-peak amplitude of the MEPs resulting from single and paired-pulse TMS was obtained using custom scripts programmed in Signal. In general, the custom scripts isolated a 50-msec window in which the evoked response occurred, and returned the maximum and minimum value (i.e., the peak-to-peak

amplitude) in the specified window. For single pulse measures, we isolated a window 10 msec following the stimulus (occurring at second 1 in each frame; 1.010 – 1.060), as the typical latency of a MEP to the ECR muscle is between 15 and 20 msec. While localization of the windows was performed in an automated manner, data were reviewed manually to ensure the peak-to-peak amplitude values obtained related to the evoked response (as opposed to artifact for instance). Following analysis of single pulse measures, two Signal data files (pre and post exercise) were saved in each experimental session for each subject. Each file contained 50 frames; 10 frames for each of the different stimulation intensities (i.e., 100, 110, 120, 130, and 140). For analysis, each frame was visually inspected to make sure the timing of the stimuli and the responses were logical (e.g., as the stimulus occurs at 1 second in each frame, the evoked response should appear after a certain latency (15 to 20 msec after the stimulus) depending on the participant's height). Data were saved as a .txt files and exported to Microsoft Excel for further analysis.

For paired-pulse measures, we used a second custom script programmed in Signal to obtain MEP peak-to-peak amplitudes in the manner outlined above. This custom script isolated a window related to the TS; for ICF, SICI and LICI, these windows were 1.020 – 1.070, 1.007 – 1.057, and 1.105 – 1.155 respectively. As in single-pulse analysis, two Signal data files related to paired-pulse TMS (pre and post exercise) were saved in each experimental session for each subject. Each file contained 30 frames; each set of 10 frames represented one of the paired-pulse measures (i.e., ICF, SICI, and LICI). Similar to single pulse TMS, data were saved as a .txt files and exported to Microsoft Excel for further analysis. The average MEP amplitude for each paired-pulse condition for each

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subject was an average of all 10 trials unless there was any evidence of pre-stimulus muscle activity (described below) on the EMG recording or there was some sort of technical failure; in these cases, the trial was removed. Regarding ICF, participants whose average of the 10 trials did not show overall facilitation were excluded from further analysis of ICF data. For SICI and LICI, it is not possible to determine if the absence of an MEP is due to inhibition or technical error. As such, we only removed trials if the TS was preceded by muscle activity (described below). Similar to procedures performed for single pulse TMS data, if less than 6/10 MEPs remained for the paired-pulse TMS measure, that participant was excluded from that part of the analysis.

4.4.3 Pre-Stimulus Muscle Activity

As volitional activity in the target muscle prior to delivery of the TMS pulse will result in increased MEP amplitude, we examined EMG activity in the period immediately preceding the TMS pulse, removing from analysis MEPs where EMG exceeded baseline values. Specifically, our 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). RMS amplitude values were exported along with the MEP amplitude data and included in the Excel spreadsheets. Trials in which the average root mean square amplitude value exceeded the averaged value at baseline plus one standard deviation were removed from subsequent analysis.

For both single- and paired-pulse measures, we examined the data in the Excel spreadsheets, looking at all the frames for both MEP amplitude and RMS amplitude. For both single and paired-pulse measures, we removed MEPs where the stimulus was preceded by a level of EMG activity that was greater than the mean plus one standard

deviation of the baseline value. Similarly, when MEP amplitudes appeared to be low, we reviewed the corresponding trial's Brainsight data (which includes error related to target accuracy), removing from analysis MEPs where the stimulation was too far from the target. As indicated above, if there were less than 6/10 MEPs remaining, data from that participant was removed from further analysis (as described above). For single-pulse measures, each participant's MEPs corresponding to each stimulus intensity and at each exercise level were then averaged to generate an S-R curve. For paired-pulse measures, each participant's MEPs corresponding to each paradigm and at each exercise level were then averaged to produce a single value depicting the degree of ICF, SICI and LICI.

4.4.4 Statistical Analysis

As indicated above, the peak-to-peak amplitude was calculated for each trial, with these values averaged at each of the stimulus intensities, and the average values compared. A one-sample Kolmogorov-Smirnov test (K-S test) was used to assess normality within each individual combination of conditions (e.g., within each of the 30%, 40% and 50% HRR)

To assess changes in cortical excitability within the S-R curves, MEP data were analyzed using three-way repeated-measures ANOVA with TIME (two levels: pre and post), STIMULUS INTENSITY (five levels: 100%, 110%, 120%, 130% and 140% RMT) and EXERCISE INTENSITY (three levels: 30%, 50% and 70% of Maximum HR) as factors.

In all paired-pulse measures, the degree of inhibition or excitation was normalized to the single pulse amplitude at 120% RMT for each time point (i.e., the unconditioned MEP amplitude). For each of SICI and ICF, and LICI, the average amplitude of

conditioned MEPs was expressed as a percentage of the average unconditioned MEP amplitudes at 120% RMT (after single-pulse stimulation). To assess changes in ICF, SICI, and LICI, measures were analyzed using three separate two-way repeated measures ANOVA with TIME (two levels: pre and post) and EXERCISE INTENSITY (three levels: 30%, 40% and 50% of Maximum HR) as factors. Significant main effects in the ANOVA were tested with planned follow-up tests using paired t-tests to detect cortical changes from pre to post for each dependent measure within each group (exercise condition). An a priori alpha of $p < 0.05$ was used to denote significance. All statistical analyses were performed using SPSS v. 23 (IBM Corp., Armonk, NY, USA)

CHAPTER 5: RESULTS

Fifteen participants took part in the study; however, only 12 (8 females, 26.9 ± 3.87 years) completed the whole experiment. One subject was excluded after the first 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). Two subjects didn't complete the sessions due to health conditions unrelated to our study. Physical activity levels of the participants were determined by calculating median MET minutes/week, with each participant categorized as highly active, moderately active or inactive according to guidelines for data processing and analysis of the IPAQ, which refer to weekly amount of physical activity. Scoring showed that 7 participants were considered highly active [4415± 2816 MET-minute/week], with the others found to be moderately active [1464 ± 746 MET-minute/week] (see Table 2).

Table 2. Participant characteristics

Subject Code	Sex	Age	MET-Minute/week according to IPAQ	Physical Activity Level according to IPAQ
1	Male	30	2160	Highly active
2	Female	25	10026	Highly active
3	Female	32	1059	Moderately active
4	Female	25	5880	Highly active
5	Male	25	4584	Highly active
6	Female	28	990	Moderately active
8	Male	21	3120	Highly active
9	Female	25	2772	Moderately active
10	Female	28	1113	Moderately active
11	Female	25	2160	Highly active
12	Male	24	2972	Highly active
15	Female	35	1386	Moderately active

5.1 Exercise Results

All participants were able to complete the graded maximal exercise test as per our criteria, with HR values within 12 beats of their respective estimated Maximum HR based on the equation described earlier (Estimated maximum HR = [206.9 – (0.67X Age)]) (as shown in Table 3).

Table 3. Graded maximal exercise test results compared to estimated age-predicted maximum heart rate (beats per minute; bpm)

Subject code	Estimated Age-predicted Maximum HR	Actual Maximum HR Attained on Maximal Exercise Test
1	186.8	187
2	190.15	185
3	185.46	178
4	190.15	182
5	190.15	186
6	188.14	179
8	192.83	190
9	190.15	183
10	188.14	177
11	190.15	181
12	190.82	187
15	183.45	172

For the exercise component of the experimental sessions, our findings show that all the participants were able to maintain their HR within range of the required target HR when comparing the average HR achieved in each session to the target HR that was required at that session (as defined by Table 4). The average HR maintained by the participants was 107 ± 7.15, 116 ± 7.17, and 126 ± 6.55, while RPE score ranged from 1-2, 2 -3, and 3 - 4 during cycling at 30%, 40%, and 50% HRR respectively (see Appendix 6)

Table 4. Required target HR (bpm) for each session compared to the averaged HR has been maintained during cycling (Mean ± SD).

Subject Code	30% HRR		40% HRR		50% HRR	
	Required Target HR	Average HR Maintained	Required Target HR	Average HR Maintained	Required Target HR	Average HR Maintained
1	113	115 ± 2.6	124	124 ± 2.5	135	134 ± 3.0
2	102	103 ± 3.0	113	113 ± 2.7	124	124 ± 2.6
3	113	111 ± 5.3	121	122 ± 1.8	131	131 ± 2.7
4	99	98 ± 5.2	111	109 ± 5.6	122	122 ± 1.8
5	102	103 ± 5.3	113	110 ± 6.6	124	120 ± 4.0
6	102	103 ± 2.0	112	111 ± 3.8	123	123 ± 1.8
8	97	98 ± 1.9	106	105 ± 2.0	116	115 ± 1.6
9	116	115 ± 1.4	125	125 ± 1.4	133	133 ± 1.5
10	108	108 ± 3.4	118	117 ± 2.0	127	126 ± 1.8
11	109	108 ± 2.5	120	120 ± 1.9	131	131 ± 1.7
12	119	119 ± 1.7	127	127 ± 1.5	136	136 ± 1.7
15	100	99 ± 2.6	110	113 ± 2.7	120	121 ± 4.9

5.2 TMS Results

5.2.1 Single Pulse TMS

Using the criteria outlined in the data analysis section, one participant was removed from analysis of two exercise-levels (30 and 50% HRR) because they did not meet the minimum criteria for number of MEPs. Thus we included 11, 12 and 11 participants for the 30, 40 and 50% HRR analysis, respectively. For all measures, pre-exercise responses were considered as baseline values. Kolmogorov-Smirnov test (K-S test) indicated that none of the cases showed a significant deviation from normality (p < .05).

Our results show an increase in MEP amplitude after exercise at 50 and 40% HRR as evidenced by the post- compared to pre-exercise S-R curves (Figures 11 and 12), with

the average MEP amplitude at each stimulation intensity larger compared to those obtained before exercise. For instance, pre-exercise MEP amplitude was 491 µV at 140 % RMT, with a post-exercise value of 587 µV after exercise at 50% HRR. At 40% HRR, an average MEP amplitude of 558 µV resulted by stimulation at 130% RMT after exercise, compared to a pre-exercise value of 504 µV when stimulating at the same intensity. Figures 11 through 13 display the S-R curves with the average MEP amplitude evoked in response to varying stimulus intensities at each time point for each exercise condition. Statistical analysis supported these findings: the three-way repeated measure ANOVA showed a significant main effect of stimulation intensity ($F_{4, 40}$ = 61.038, p = .000, η^2=. 859). The effect size is a partial eta squared which shows that the effect of stimulation intensity is large. In addition, there was a significant main effect of time point (pre-post) relative to exercise ($F_{1, 10}$=11.939, p=. 006, η^2=. 544). However, no main effect of exercise level was observed ($F_{2, 20}$ = .194, p = .825, η^2=. 859).

Planned follow-up tests for the observed main effects (time point) performed to assess the difference after exercise at each level revealed that MEP amplitude increased significantly after exercising at 50% ($F_{1, 10}$=21.052, p<0.001, η^2=. 678) and 40% of HRR.
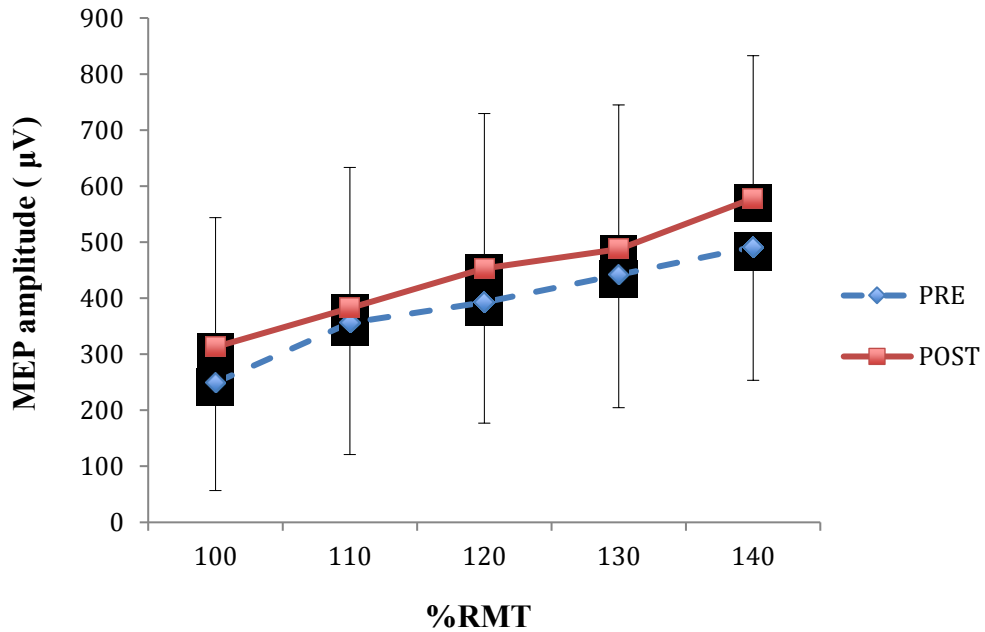
Figure 11. S-R curves before and after exercise at 50% HRR. S-R curves pre- and post-exercise in response to stimulation at increasing percentages of RMT (n=11). Bars represent SD.
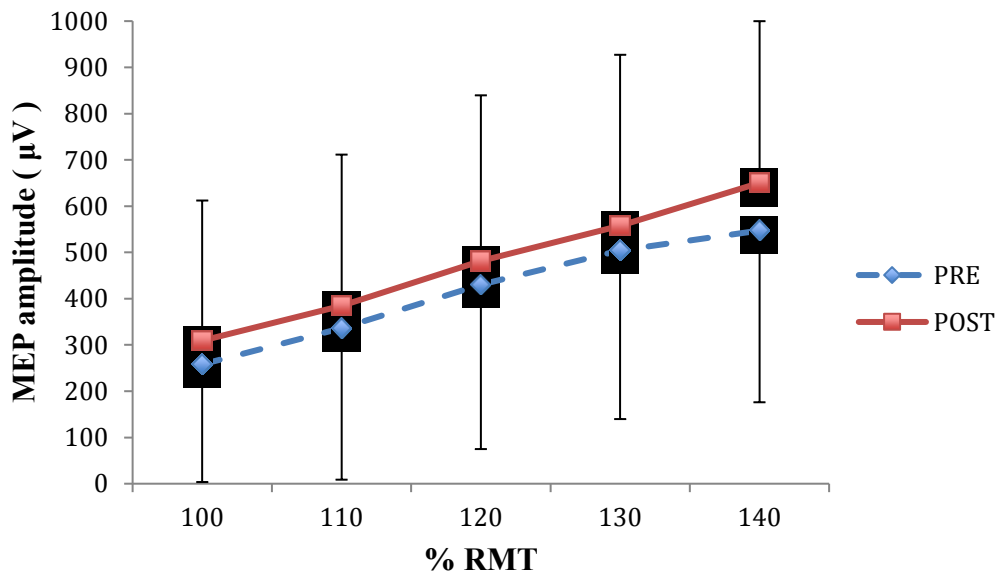
Figure 12. S-R curves before and after exercise at 40% HRR. S-R curves pre- and post-exercise in response to stimulation at increasing percentages of RMT (n=11). Bars represent SD.
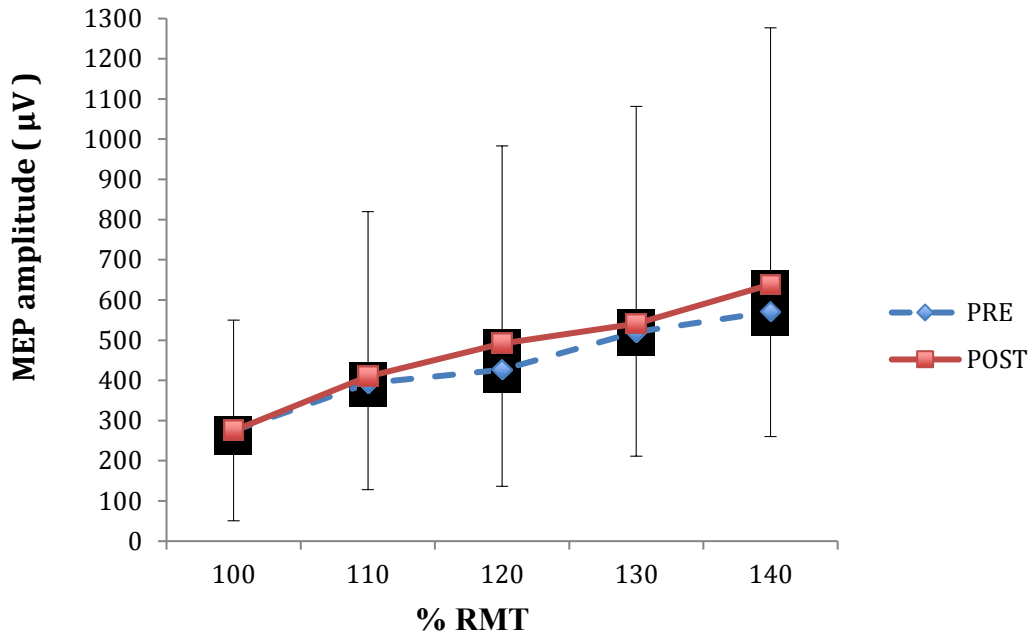
Figure 13. S-R curves before and after exercise at 30%HRR. S-R curves pre- and post-exercise in response to stimulation at increasing percentages of RMT (n=11). Bars represent SD.

5.2.2 Paired Pulse TMS

Average paired-pulse responses across all subjects in each exercise condition are shown in Figures 14 through 22. The above-noted exclusion criteria resulted in one participant being removed from the paired-pulse analysis (ICF, SICI and LICI analysis) after exercising at both 30 and 50% HRR. The averaged unconditioned and conditioned MEPs for each paired-pulse measure before and after AE at each level are shown in Table 5.

Table 5. Average MEPs ± SD for paired-pulse TMS measures

	ICF				SICI				LICI			
	Pre		Post		Pre		Post		Pre		Post	
	UC	C	UC	C	UC	C	UC	C	UC	C	UC	C
50%	393 ± 216	418 ± 223	453 ± 276	511 ± 295	393 ± 216	108 ± 80	453 ± 276	163 ± 102	393 ± 216	93 ± 56	453 ± 276	109 ± 56
40%	408 ± 348	446 ± 367	481 ± 341	554 ± 370	408 ± 348	144 ± 157	481 ± 341	255 ± 260	408 ± 348	75 ± 72	481 ± 341	133 ± 122
30%	408 ± 277	453 ± 284	492 ± 184	546 ± 188	408 ± 277	103 ± 44	492 ± 184	198 ± 98	408 ± 277	92 ± 52	492 ± 184	154 ± 84

UC: unconditioned MEP amplitude, C: conditioned MEP amplitude

Two-way repeated measures ANOVA of ICF showed no significant main effect for either time point ($F_{1, 10}$=2.004, p=0.187) or exercise level ($F_{2, 20}$=1.254, p=0.307) on amount of facilitation, and there was no interaction between intensity and time point ($F_{2, 20}$ =3.091 p=0.068). Interestingly, time point related to exercise had a significant main effect on amount of SICI ($F_{1, 10}$ =33.845 p=0.000) and LICI ($F_{1, 10}$ =5.294 p=0.044). In other words, SICI and LICI decreased significantly pre- to post-exercise at all intensities. However, no main effect of exercise level was observed for SICI and LICI.

5.2.2.1 Intracortical Facilitation (ICF)

Our data demonstrated that MEP amplitude did not increase significantly after exercise (changes were not significant), as the two-way ANOVA revealed no main effect for ICF related to time point. Accordingly, no follow-up was performed to test the effect of ICF on each exercise level separately. At 50% HRR, pre-exercise values were 107.1 ± 4.82 % of unconditioned stimulus amplitude (i.e., a 7.1% facilitation), whereas post-exercise values increased to 114.6 ± 10.58 % of unconditioned stimulus amplitude (i.e., a

14.6% facilitation; see Figure 14). At 40% HRR, pre-exercise values were 112.8 ± 10.87 % of unconditioned stimulus amplitude (i.e., a 12.8% facilitation), whereas post-exercise values increased to 122.1 ± 18.57 % of unconditioned stimulus amplitude (i.e., a 22.1% facilitation; see Figure 15). At 30% HRR, pre-exercise values were 113.3 ± 21.78 % of unconditioned stimulus amplitude (i.e., a 13.3% facilitation), whereas post-exercise values were found to be 112.1 ± 15.18 % of unconditioned stimulus amplitude (i.e., a 12.1% facilitation; see Figure 16).
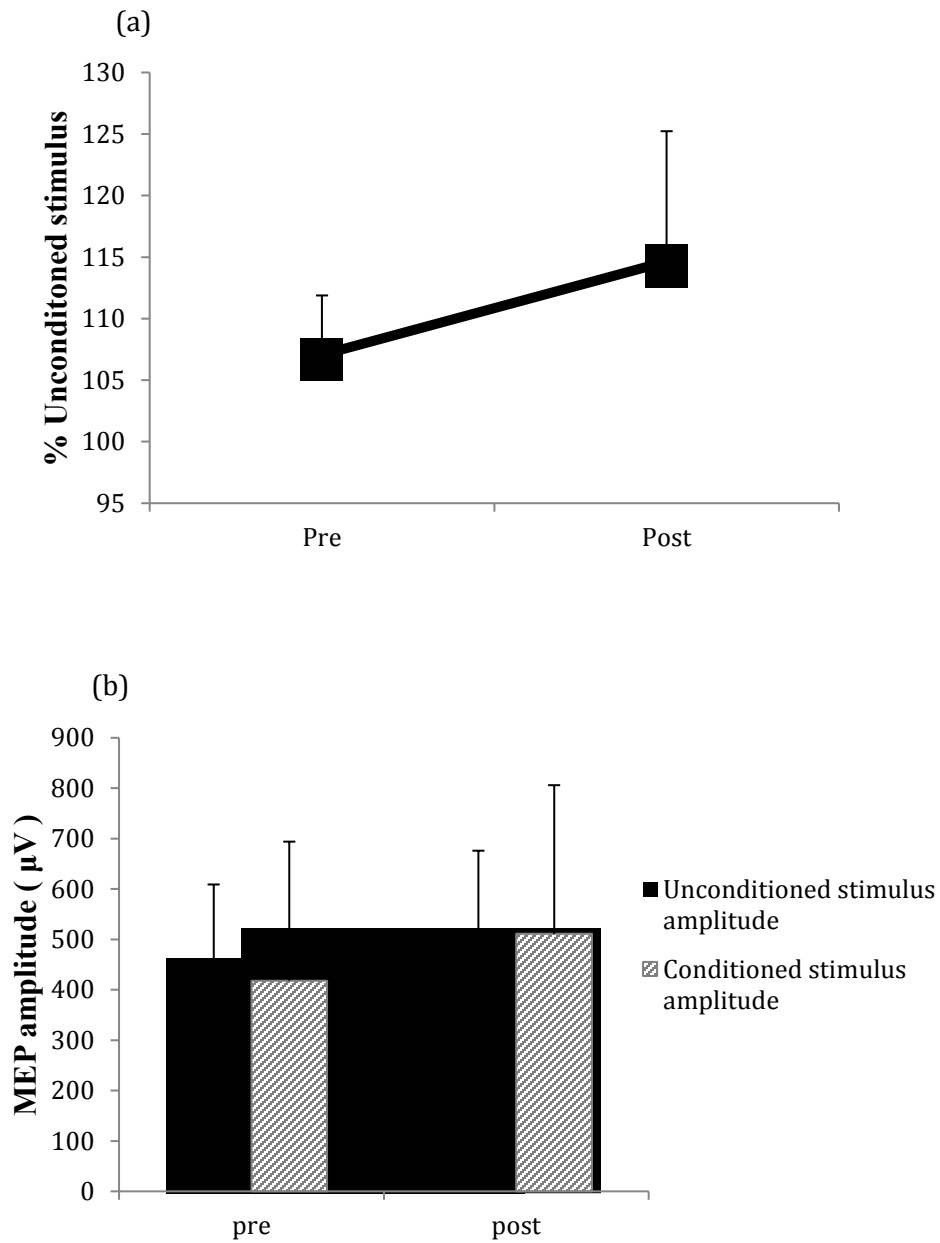
Figure 14. Modulation of ICF following exercise at 50% HRR. (a) Induction of ICF across all participants (n=11) at each time point. (b) Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SD.
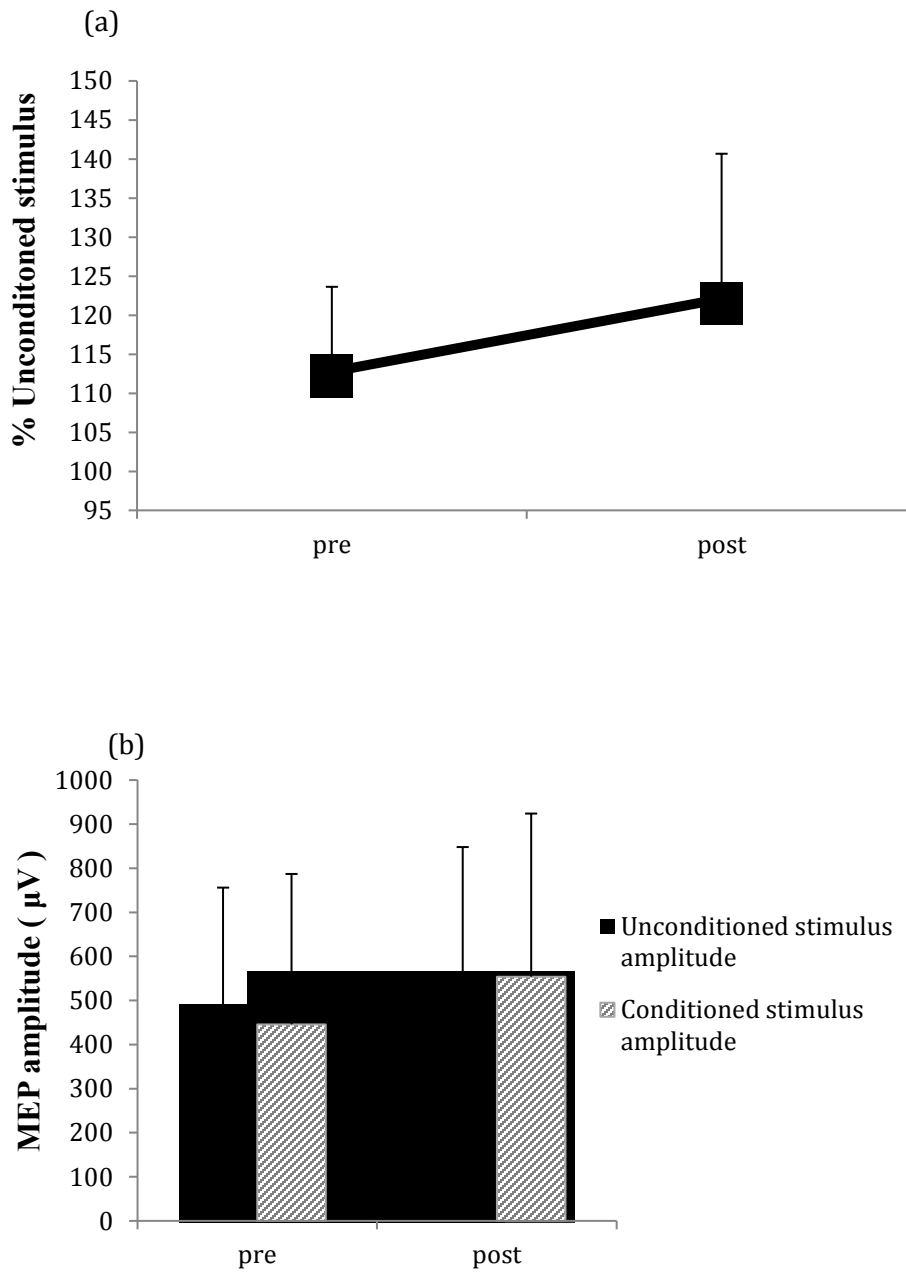
Figure 15. Modulation of ICF following exercise at 40% HRR. (a) Induction of ICF across all participants (n=12) at each time point (b) Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SD.

Figure 16. Modulation of ICF following exercise at 30% HRR. (a) Induction of ICF across all participants (n=11) at each time point (b) Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SD.

5.2.2.2 Short-Interval Intracortical Inhibition (SICI)

The amount of SICI was significantly decreased after exercising at 50% HRR (p =0.01). Prior to exercise, an average of 27.6 ± 9.83 % of unconditioned stimulus amplitude was elicited after the TS, while an average of 38.7 ± 15.12 % of unconditioned stimulus amplitude was observed following exercise (Figure 17). Similarly, SICI was decreased significantly after exercising at 40% HRR (p =. 002). An average of 34.18 ± 20.57 % of unconditioned stimulus amplitude was elicited after the TS before exercise, with an average of 51.29 ± 21.33 % of unconditioned stimulus amplitude observed following exercise (Figure 18). SICI did not show significant change after exercise at 30% HRR (p=. 075), with 31.47 ± 14.3 % unconditioned stimulus amplitude compared to 46.09 ± 24.9 % observed for pre- to post-exercise respectively (Figure 19).

(a)

(b)

Figure 17. Modulation of SICI following exercise at 50% HRR. (a) Induction of SICI across all participants (n = 11) at each time point. (b) Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SD. Asterisks indicate values significantly different from pre-exercise (p <0.05).

(a)

(b)

Figure 18. Modulation of SICI following exercise at 40% HRR. (a) Induction of SICI across all participants (n = 12) at each time point. (b) Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SD. Asterisks indicate values significantly different from pre-exercise (p <0.05).

(a)

(b)
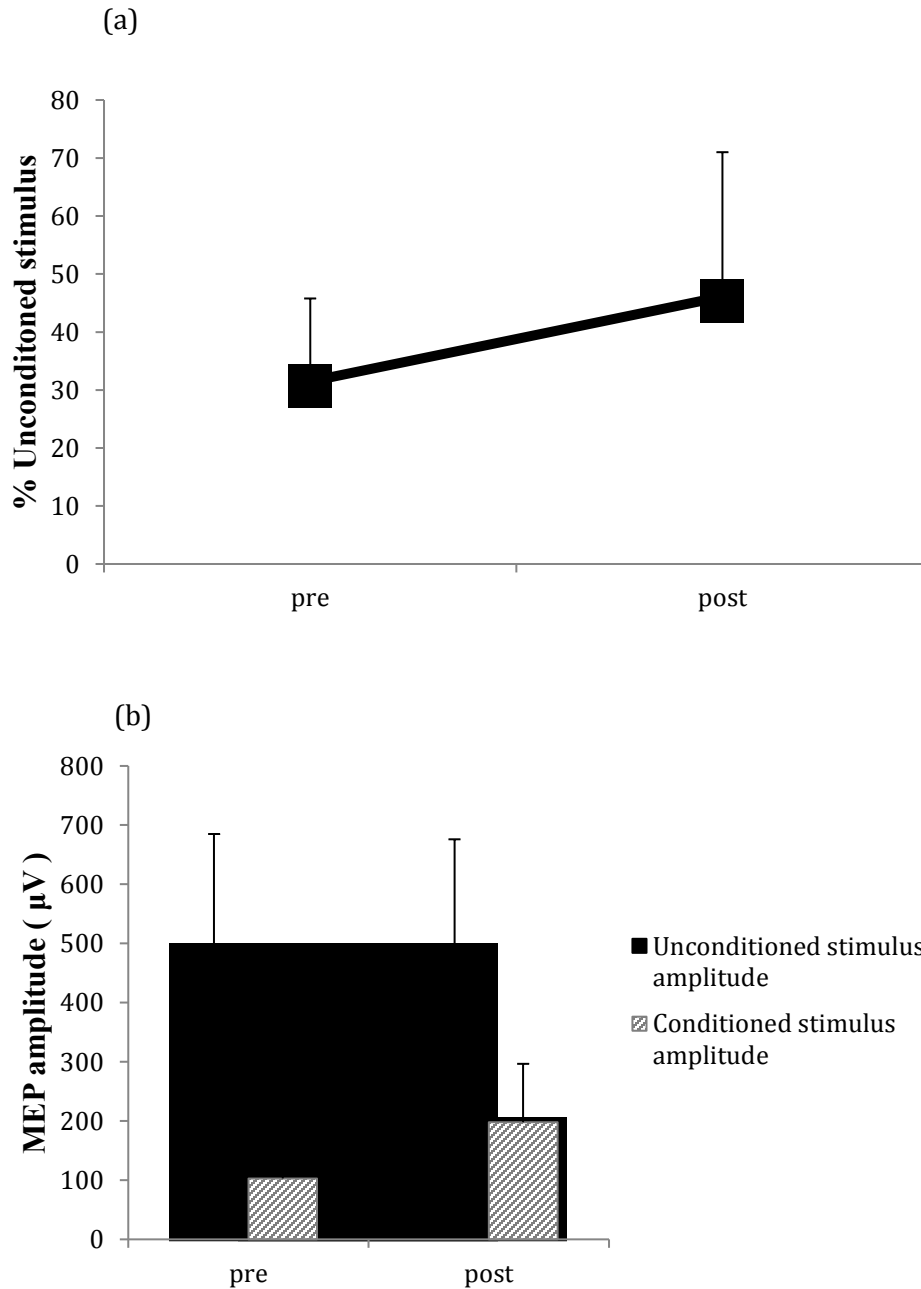
Figure 19. Modulation of SICI following exercise at 30% HRR. (a) Induction of SICI across all participants (n = 11) at each time point. (b) Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SD.

5.2.2.3 Long-Interval Intracortical Inhibition (LICI)

Results from the LICI analysis demonstrated a similar trend as SICI. Pre-exercise inhibition showed an average of $29.31 \pm 19.37\%$ of unconditioned stimulus amplitudes while inhibition after exercise at 50% HRR decreased the test stimulus amplitude to $28.42 \pm 17.6\%$ of the unconditioned stimulus amplitude. Interestingly, these values were not significantly different (p=. 883, Figure 20). Results did however show that the amount of LICI decreased significantly after exercise at 40%HRR (p=0.022). Prior to exercise, an average of $19.92 \pm 12.84\%$ of unconditioned stimulus amplitude was elicited after the TS; following exercise, an average of $29.31 \pm 14.74\%$ of unconditioned stimulus amplitude was observed (Figure 21). Exercising at 30% HRR did not show significant change (p=0.255). An average of $26.58 \pm 18.47\%$ of unconditioned stimulus amplitude was obtained before exercise, compared to $33.77 \pm 19.5\%$ of unconditioned stimulus amplitude after exercise (Figure 22).
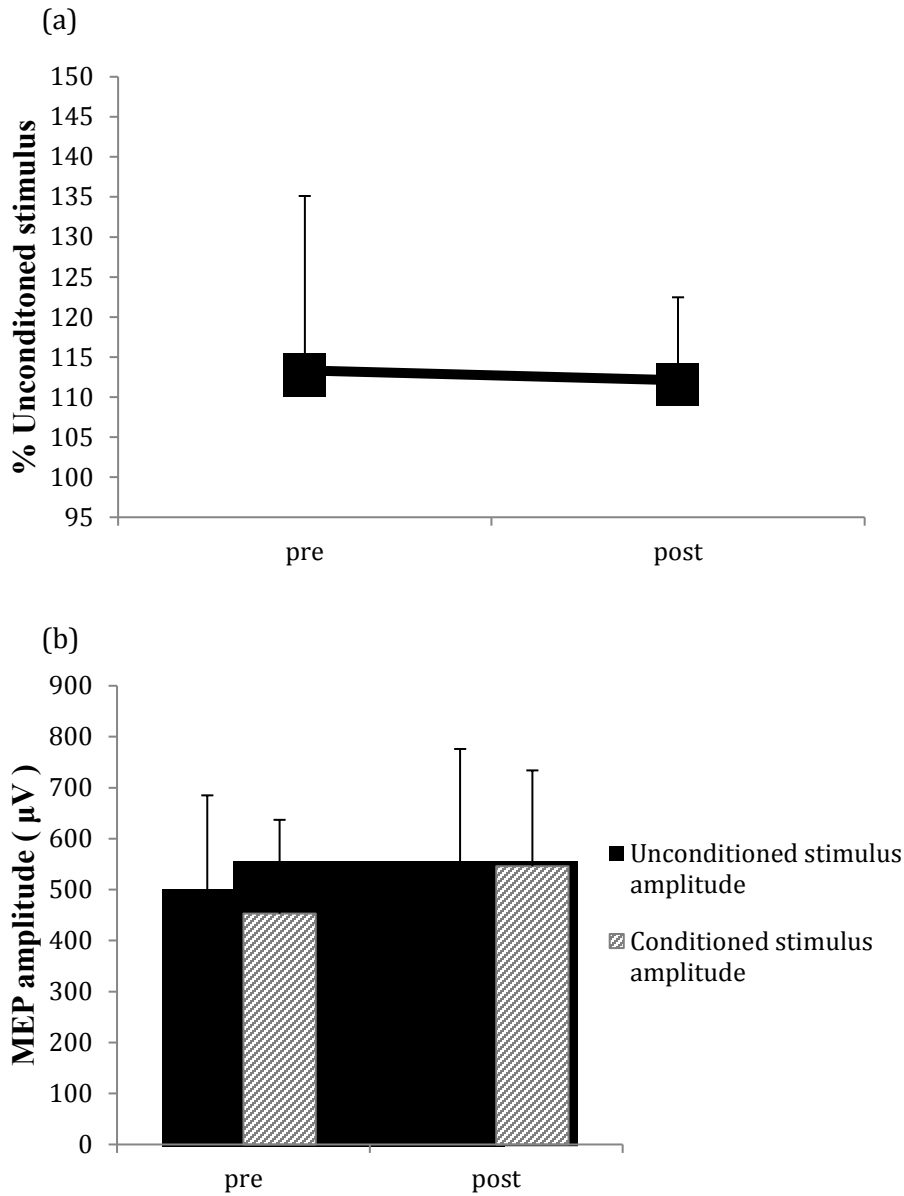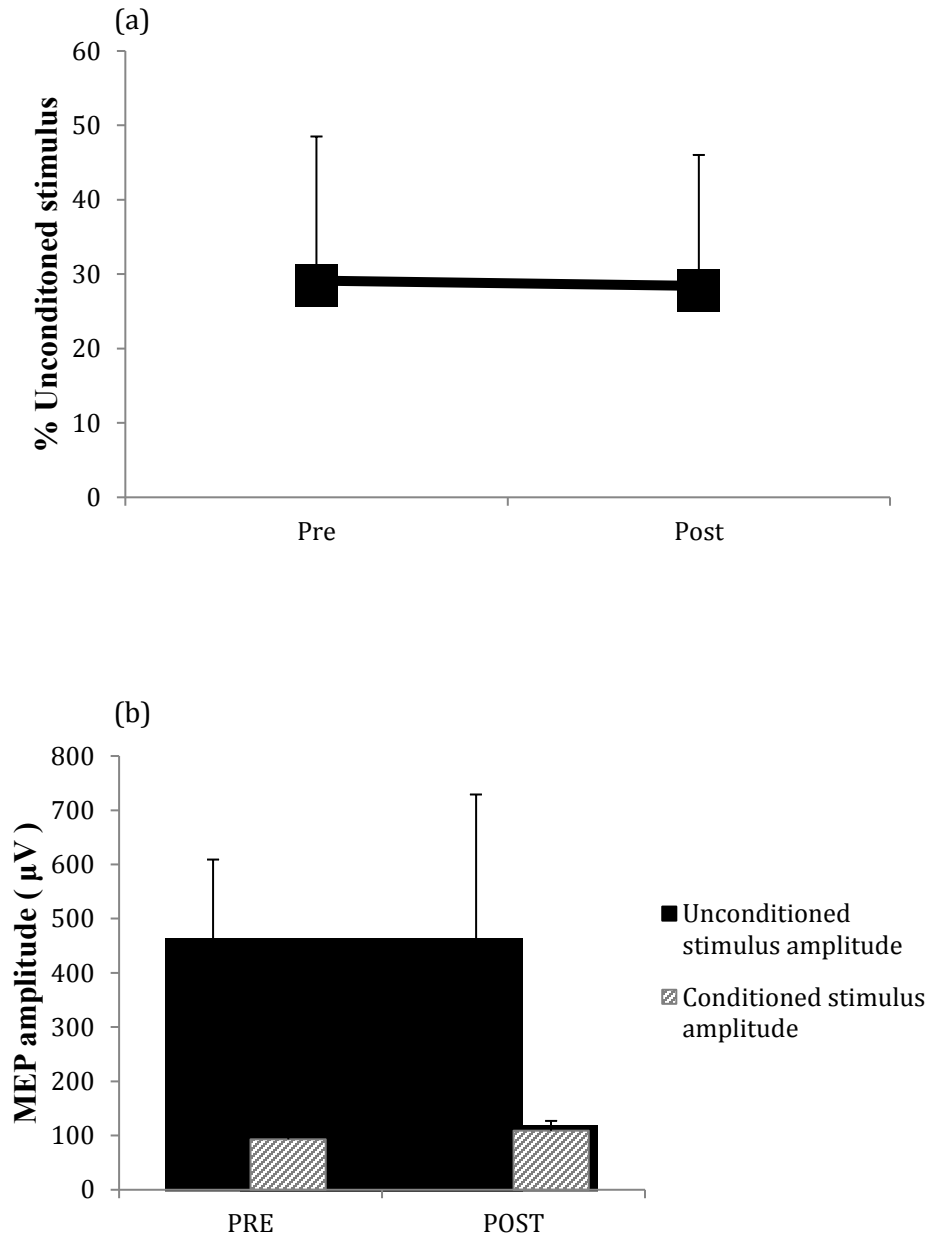
Figure 20. Modulation of LICI following exercise at 50% HRR. (a) Induction of LICI across all participants (n = 11) at each time point. (b) Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SD.
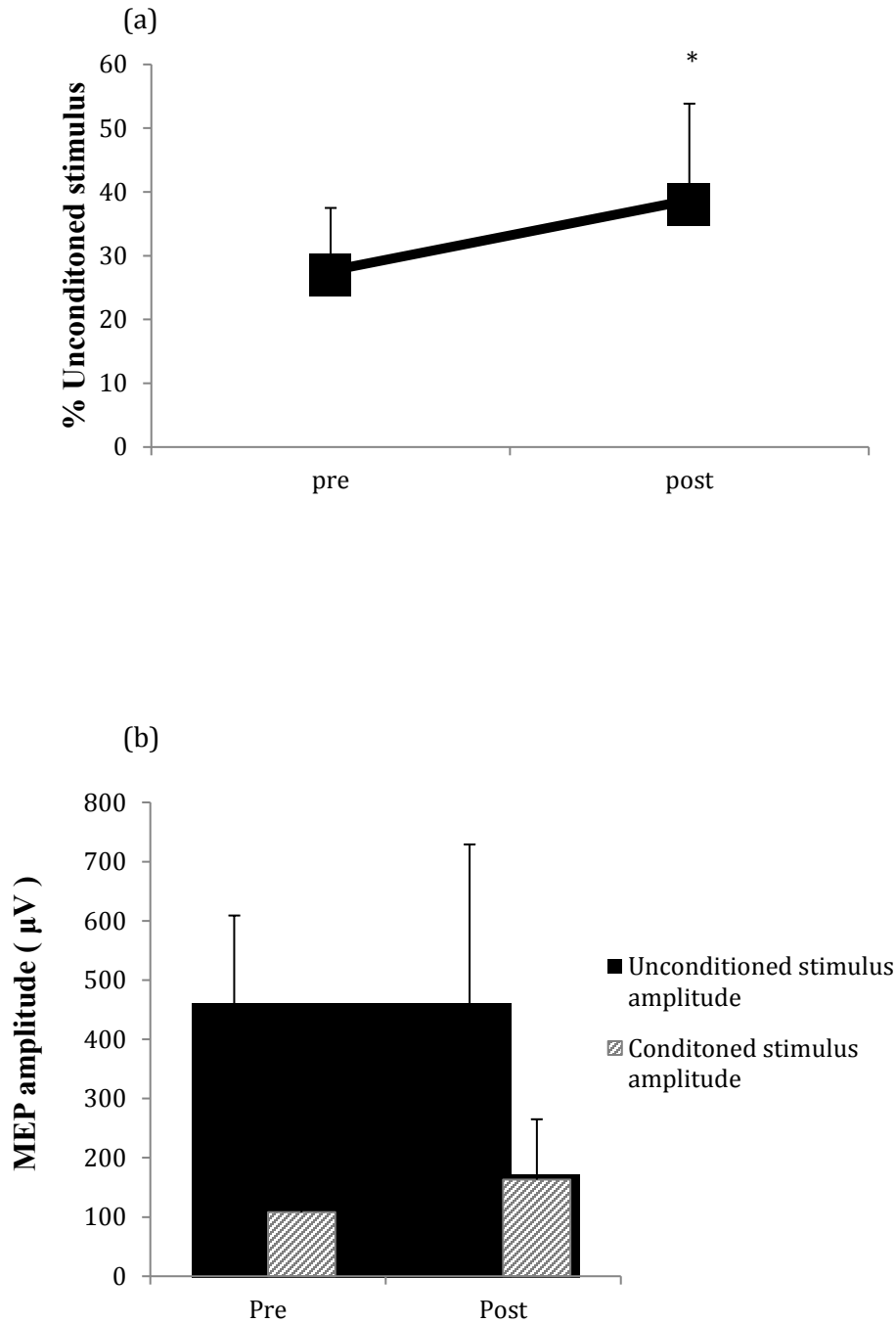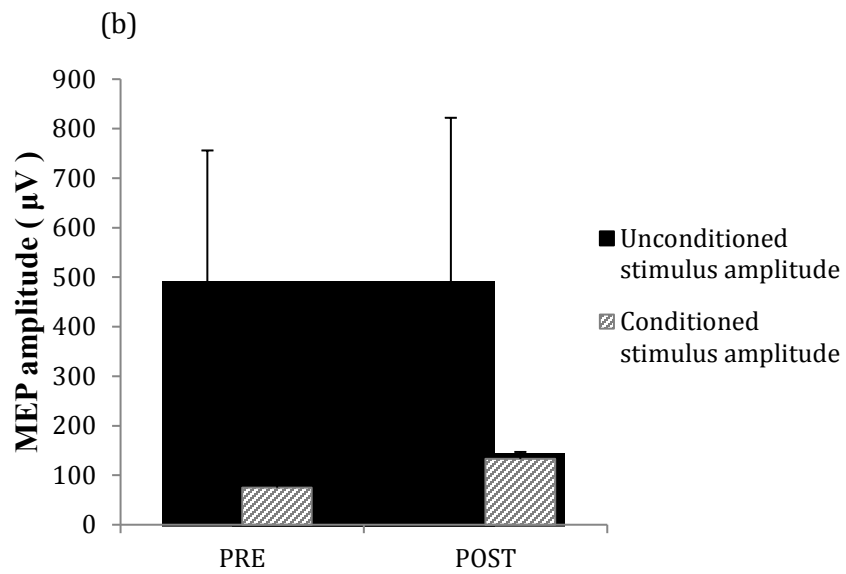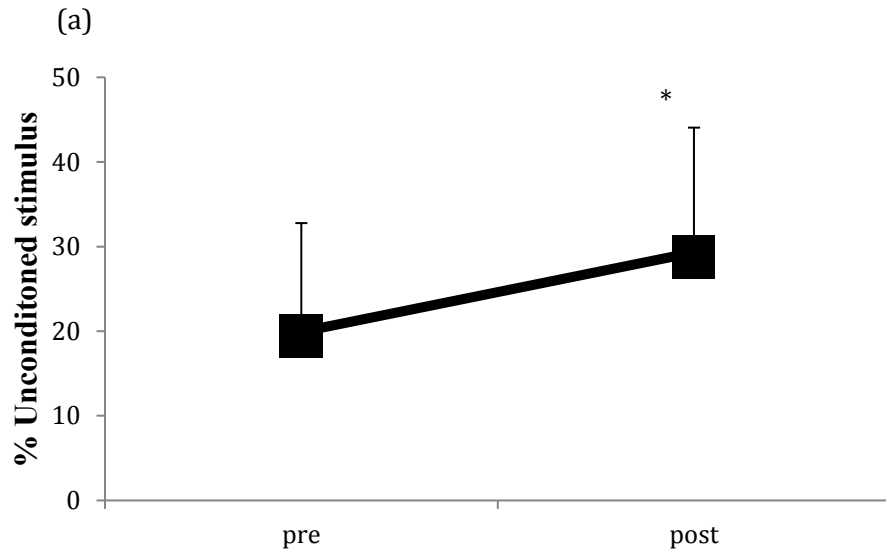
Figure 21. Modulation of LICI following exercise at 40% HRR. (a) Induction of LICI across all participants (n = 12) at each time point. (b) Unconditioned single pulse amplitudes at 120% RMT are co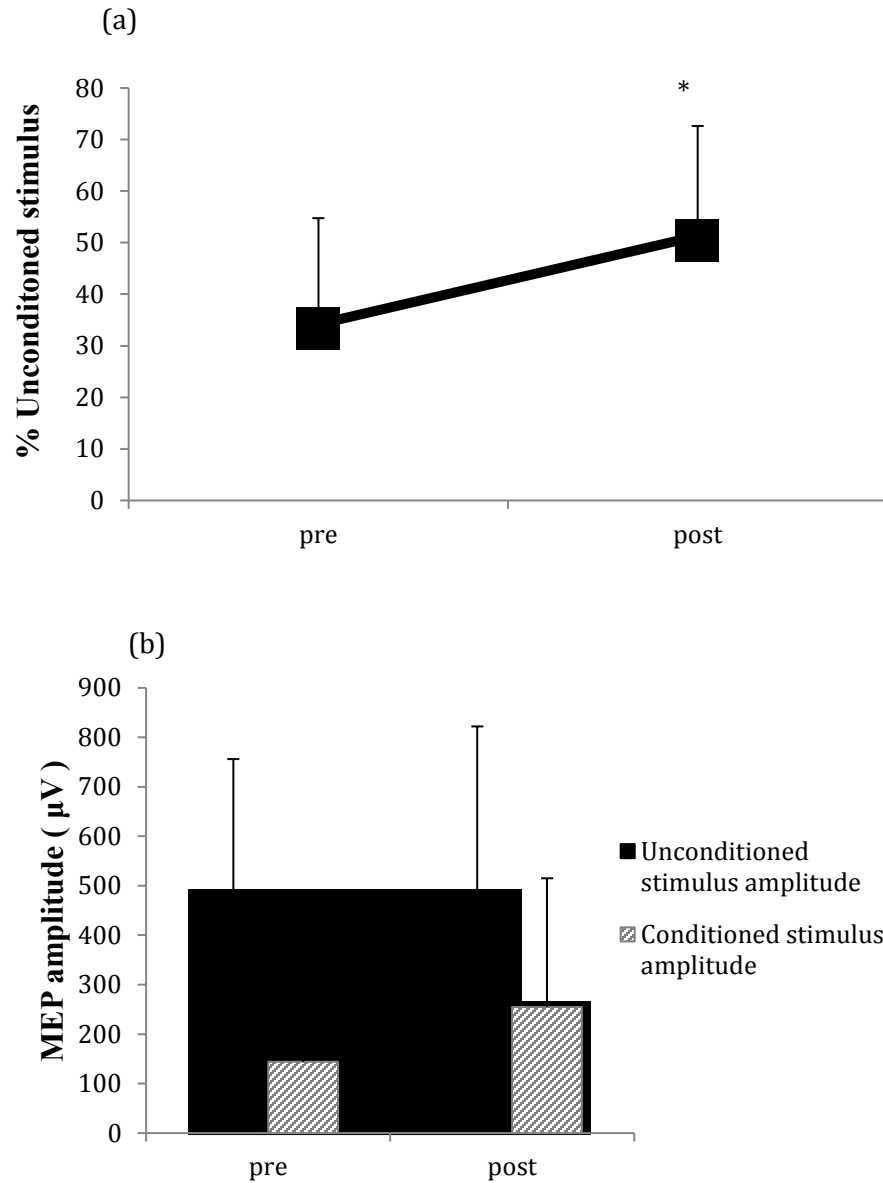mpared to conditioned stimulus amplitudes. Bars represent SD. Asterisks indicate values significantly different from pre-exercise (p <0.05).

(a)

(b)
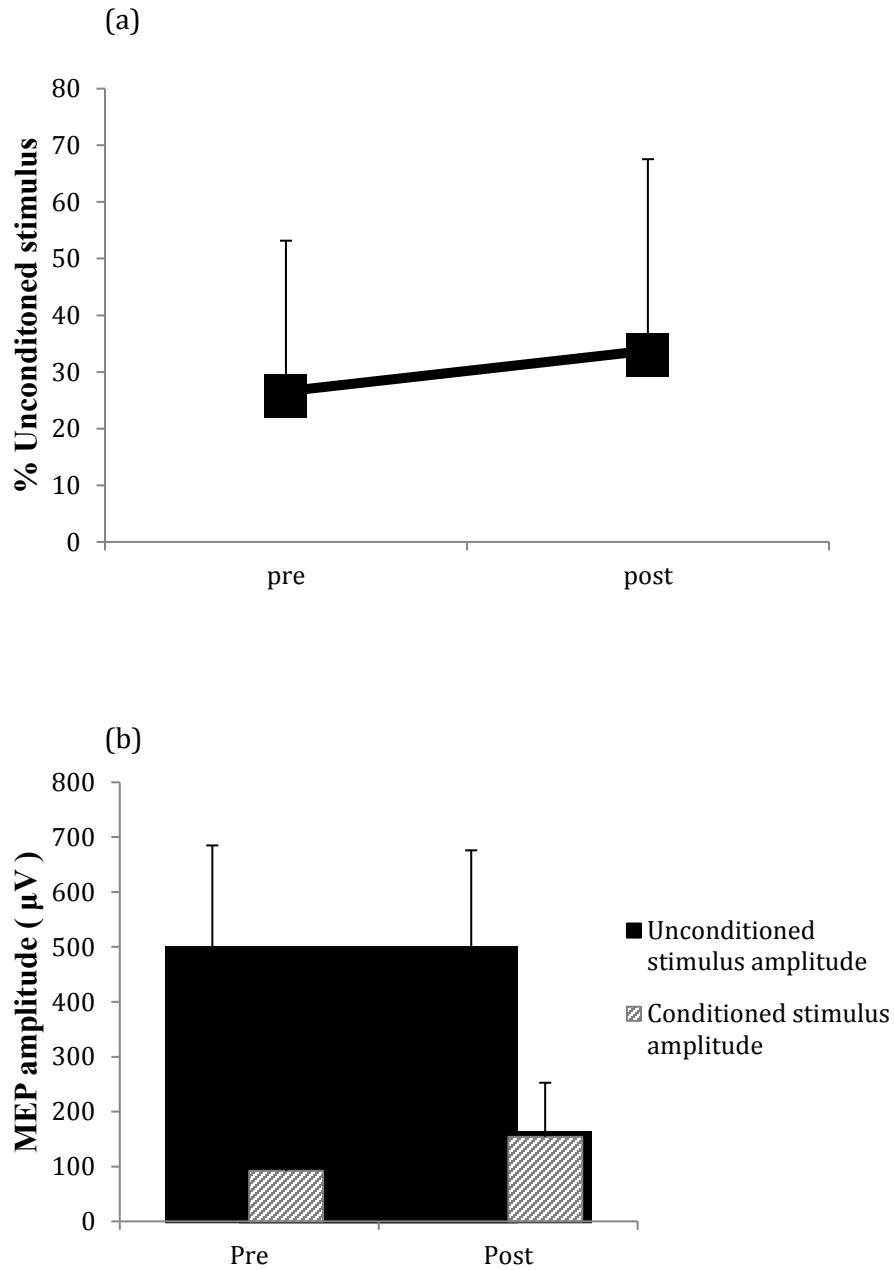
Figure 22. Modulation of LICI following exercise at 30% HRR. (a) Induction of LICI across all participants (n = 11) at each time point. (b) Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SD.

CHAPTER 6: DISCUSSION

The primary objective of this study was to attempt to find a lower AE level that can still drive excitability by examining the effect of AE involving the lower limbs at various intensities on upper limb motor excitability. A secondary objective was to investigate the potential mechanisms underlying the changes in cortical excitability by assessing the amount of facilitation and inhibition in M1 after exercising at different intensities. Our long-term goal is to inform implementation of AE in the clinical setting as a means to prime the brain before neurorehabilitation.

 In selecting our exercise levels, we sought to replicate previous findings showing increased cortical excitability resulting from AE at 70% age-predicted HRmax (50% HRR), and also assessing two lower levels: 40% HRR that has been investigated previously but with different duration, and 30% HRR. The three exercise intensities were performed for a fixed duration (20 minutes) in order to address our primary objective. We hypothesized that cortical excitability and the amount of facilitation of cortical neurons would increase in response to AE at all levels. We also hypothesized that the amount of inhibition of neurons in M1 would be reduced after all AE levels. It was anticipated that no significant difference would be found between the amount of reduction in inhibition and the amount of increase in facilitation after all AE levels.

To address our objectives, we recruited twelve non-disabled young adults, and obtain measures related to cortical excitability before and after 20-minute bouts of AE at 30, 40 and 50% of HRR based on a graded maximal exercise test. Measures of cortical

excitability and the degree of facilitation/inhibition were obtained using single- and paired-pulse TMS applied to the ECR muscle representation in M1.

In general, our results showed that participants were able to maintain their heart in proximity of the required target HR for all the exercise levels (see Table 4). Statistical analysis revealed that AE modulated cortical excitability, however, these findings were limited to 40 and 50% of HRR only, as evidenced by the significant increase in MEP amplitude post-exercise. Our results also revealed that there was no main effect of AE at all three exercise levels on ICF. Results showed that the amount of SICI decreased following exercise at all intensities but that the decrease was significant only after 40 and 50% HRR, not 30% HRR. Surprisingly, LICI decreased significantly only after exercise at 40% HRR. By conducting this study, we identify that lower intensity AE can still modulate cortical excitability and lead to intracortical changes.

6.1 Cortical Excitability

The main concept that led to the development of this research question and interest in examining changes in excitability is the concept of "*priming the brain prior to rehabilitation*". Rehabilitation of people with neurological disorders aims at inducing brain plasticity to achieve functional recovery. Rehabilitative modalities induce plasticity by 'rewiring' connections in the brain, including intact or surviving neurons taking on the action of damaged cells (see Chapter 2 for a review of this literature). Briefly, previous work has found that lowering the resting membrane potential of these neurons to a level closer to the threshold for depolarization makes it easier for re-wiring of neural connections to occur (Rossini & Rossi, 2007). Thus, the brain can be stimulated faster to

best facilitate functional recovery. Accordingly, it is critical to prime the brain before engaging individuals into clinical rehabilitation sessions by altering neuronal excitability.

The excitability of cortical neurons can be altered by a number of different means, such as caffeine, drugs (e.g. amphetamines), energy drinks and non–invasive brain stimulation (Badawy et al., 2012, Botella et al., 2001, Brice & Smith, 2001, Garcia-Munoz et al., 1991, Schwaninger et al., 2002, and Specterman, 2005). However, introduction of these methods to prime the brain before rehabilitation is not recommended, as most of these methods have side effects on the human body and some are not yet clinically feasible. As noted previously, AE has been reported to be an effective method for increasing cortical excitability of individuals with traumatic brain injury (Balbi et al., 2002, and Forrester et al., 2006).

6.2 Benefits of Exercise

As outlined in the preceding chapters, emerging evidence has described that AE exerts a wide range of benefits among healthy populations and individuals with neurodegenerative disorders (Zigmond & Smeyne, 2010). Green, Maiorana, & Cable (2008) reported that exercise involving the lower limbs affects vascular functioning in upper limb muscles. Research has also demonstrated that AE is neuroprotective, and can prevent age-related brain atrophy (Ahlskog et al., 2011, Cotman & Berchtold, 2002, and Kramer et al., 2006). Smith and colleagues (2010) have reported that a single session of moderate intensity cycling can induce a 20% increase in global cerebral blood flow (CBF). It has also been shown that 20 minutes of moderate to high-intensity AE elevates the level of dopamine and BDNF in the brain (Hattori, Naoi, & Nishino, 1994, and

Schmolesky, Webb, & Hansen, 2013), which in turn has an effect on the induction of

LTP and related postsynaptic modification (Gold et al., 2003, and Rojas et al., 2006). As

well, research has shown that AE increases the activity and release of some

neurotransmitters, including serotonin and norepinephrine (Goekint et al., 2012, Gomez-

Merino, Béquet, Berthelot, Chennaoui, & Guezennec, 2001, Kitaoka et al., 2010,

Meeusen et al., 1997, and Zouhal, Jacob, Delamarche, & Gratas-Delamarche, 2008).

Overall, the tremendous benefits of exercise on the brain in addition to its effect on the

whole body has been proven by research (see Chapter 2). In relation to exercise and

excitability, research has reported that AE leads to generalized increase in intracortical

excitability (Hillman, Erickson, & Kramer, 2008, Naugle, Fillingim, & Riley, 2012, and

Smith, Paulson, Cook, Verber, & Tian, 2010). The mechanisms underlying the change in

cortical excitability in response to AE are not entirely clear; however, Singh and

colleagues (2014) reported that there is strong evidence that exercise can modulate

activity and release of neurotransmitters in the brain, which in turn may contribute to the

altered excitability of neurons.

As indicated above, previous research has shown that exercise has an influence on

cortical excitability of exercising muscle (Yamaguchi, Fujiwara, Liu, & Liu, 2012).

Additionally, Takahashi and colleagues (2001) reported that lower limb resistance

exercise affects cortical excitability in non-exercised hand muscles. They suggested that

the potential mechanism for their findings might be due to the presence of facilitatory

cortical pathways between synergistic arm and leg representations, thus the spread of

excitability from active muscles to non-active muscles in proximal M1 areas.

Furthermore, Smith et al. (2014) and Singh et al. (2014) have investigated whether the

effect of exercise on cortical excitability of a region in M1 representing a specific muscle is dependent on the involvement of that muscle in the exercise itself. Specifically, they assessed the change in the upper limb motor excitability in response to single bout of lower limb cycling at 40% HRR for 30 minutes and 70% of age predicted maximal HR for 20 minutes respectively, with restriction of any movements of the upper limbs muscles. Surprisingly, their results showed that the single-pulse TMS measures did not correlate with the paired-pulse TMS data. Although they found that MEP amplitude of inactive hand muscles were unchanged by exercise, their findings revealed that lower limb exercise caused an immediate and sustained reduction of SICI and increase in ICF (found by Singh et al.) of an upper limb muscle, which is considered an indirect way of altering brain excitability. In this study, we aimed at replicating the findings of Singh et al. (2014) in addition to examining the intensity used in Smith et al. (2014; albeit with a shorter duration) and one additional lower intensity of AE (30% HRR). Our study revealed similar results as previous work regarding an alteration in intracortical inhibition after exercising at both the replicated intensity (i.e., 50% of HRR used in this study, corresponding approximately to the 70% age-predicted HRmax used previously) and one of the lower intensities tested here (40% HRR or ~54-66% HRmax). Additionally, our results showed a change in long interval intracortical inhibition (LICI), but only at 40% of HRR. Interestingly, and unlike the findings of Singh and Smith and their colleagues, we observed a change in cortical excitability, evidenced by altered S-R curves post-compared to pre-exercise. As we used a similar duration of AE, outcome measures (S-R curve, ICF, SICI, and LICI), and similar target muscle to those used in previous work by Singh et al. (2014), our findings are discussed relative to their work, in particular, and in

relation to the underlying mechanisms below.

Understanding the changes that occur in the brain in response to a single session of AE can aid in the interpretation of the TMS-related findings of the present work. It should be noted that while the changes occurring in the cortex as a result of AE are likely the cause of our observed changes, there are other factors, namely sub-cortical changes at the level of the spinal cord not examined here that could influence our results. These cortical changes are described below.

Much research has previously reported that interneurons in M1 release either excitatory or inhibitory neurotransmitters (namely glutamate and GABA, respectively) onto pyramidal neurons; these neurotransmitters in turn control and regulate the gain of synaptic inputs. Given excitation of these pyramidal neurons through the excitatory neurotransmitter (glutamate), action potentials are generated and the corresponding motor command is sent to the lower motor neurons via the corticospinal tract to be executed. However, the majority of neurotransmitter release mediated by interneurons in M1 is inhibitory (i.e., they utilize GABA), which weakens synaptic activity through an overall inhibitory effect. Previous findings suggest that interventions aimed at decreasing GABA activity might be a useful adjunct to the induction of plasticity in M1. As indicated previously, AE results in an alteration in the release and activity of neurotransmitters in the brain. Unfortunately, the measurement of GABA levels in human participants is difficult, and thus there is only limited evidence available on GABA levels immediately following exercise. Previous work in animals has shown that 60 minutes of treadmill running caused up to a 76% decrease in striatal GABA levels, although it should be noted that these data did not reach statistical significance (Meeusen et al., 1997). Further, it is

thought that hypotension reported after exercise could be the result of the down-regulation of GABA signaling on baroreceptor neurons (Chen, Bechtold, Tabor, & Bonham, 2009). Taking these findings together, it is suggested that a single bout of AE increases GABA levels and its related activity in M1, thus decreasing the amount of inhibition that could affect cortical output, and in-turn cortical plasticity and reorganization (Chen et al., 1998). Correspondingly, intracortical inhibition can be taken as an indirect measure of GABA activity. As GABA exerts its effects via multiple receptors in cortical inhibitory networks (namely $GABA_A$ and $GABA_B$ receptors as indicated previously), different measures can be taken, including SICI and LICI, to test if GABA activity changes after exercise (Chen, 2004, Ilic, Korchounov, & Ziemann, 2002, McDonnell, Orekhov, & Ziemann, 2006, and Molina-Luna et al., 2009). Further research; however, is needed regarding measuring changes in neurochemical factors in humans' brain that affect brain excitability.

Previous research has also found that rapid excitability changes can also be mediated by alterations in receptor activity, and in particular NMDA receptors. As indicated, the release of several neurotransmitters and neuromodulators including dopamine, serotonin, norepinephrine and BDNF have been found to increase after a single bout of AE. These neurotransmitters and neuromodulators could play a role in the modulation of M1 excitability via regulating the activity of α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid (AMPA) and NMDA receptors (Liepert, Schwenkreis, Tegenthoff, & Malin, 1997, and Ziemann, Chen, Cohen, & Hallett, 1998). Raising the level of these chemicals could potentiate the activity of AMPA receptors, increasing current flow and, in-turn, activation of NMDA receptors resulting in an influx of Ca^{2+}, a

secondary messenger critical to the induction of LTP. Given increased current flow

through both AMPA and NMDA receptors coupled with the influx of Ca^{2+}, greater

depolarization of the neuron is achieved (i.e., increasing cortical excitability), decreasing

the threshold for action potential generation and ultimately creating an enhanced

environment for the induction of synaptic plasticity (Schiffer et a., 2011, and Yang,

2014).

In addition to the reduction of GABA levels and increase in the release of

excitatory neurotransmitter(s), previous studies have found that there is a corresponding

increase in brain lactate uptake as a response to AE (Ide, Schmalbruch, Quistorff, Horn,

and Secher, 2000). Indeed, research has reported that the lactate receptor HCAR1

(hydroxycarboxylic acid receptor 1) is co-localized with MCT2 (monocarboxylate

transporters) which in turn increases the activity of the membrane passage (i.e., the flow

of ions through the cell membrane), that could be linked to rapid excitatory

neurotransmission (Bergersen, 2015). To this end, it has also been shown that increased

lactate levels correspond to increased M1 excitability following a bout of AE (Coco et al.,

2010), as evidenced by a decreased motor threshold, which corresponds to an increase in

cortical excitability.

Lastly, previous studies have reported that increased cerebral blood flow and the

related activation of the stress response caused by exercise could also contribute to

changes in both the level and activity of neurotransmitters, thus providing a means for

modulating cortical networks (i.e., inhibition and facilitation) and cortical excitability.

Although much of the work regarding neurochemical changes in response to exercise

remains hypothetical, it is the foundation of a model describing how M1, and more generally the brain as a whole, may benefit from a single bout of AE.

6.3 Main Findings and Underlying Potential Mechanisms

6.3.1 S-R Curves

As indicated, this study found that AE involving the lower limbs increased the corticospinal excitability of the non-exercised upper limb muscle as evidenced by the increase in MEP amplitude post-exercise. Our results show that the S-R curves generated were shifted upward (i.e., increased in amplitude) after exercise at each level. However, these increases were significant only after exercising at 40 and 50%, but not 30% HRR. This result did not confirm the first hypothesis of the study, namely that cortical excitability would increase in response to all AE levels. Also, the shift in the S-R curves reveal that the MEP amplitudes were increasing as a response to increasing the stimulator output intensity (e.g., the MEP amplitudes resulting as a response to stimulation at 130% RMT were larger than those resulting from stimulation at 110 % RMT). This trend was expected as previous TMS studies showed that MEP amplitudes increased as a response to increasing the stimulator input. Interestingly, the replicated exercise intensity (70% Maximum HR) did not show the same upward shift in S-R curves in Singh and colleagues' study (2014). They explained their findings in relation to the fact that the final corticospinal output is influenced by many cortical and sub-cortical (spinal level) factors. It is critical to understand the mechanisms that may underlie the changes in excitability observed in our study. As demonstrated earlier, there is strong evidence that exercise can modulate both the level and activity of neurotransmitters in the brain. All of

these changes could contribute to the modulation of cortical excitability via activating

AMPA and NMDA receptors, which in-turn increase current flow and Ca^{2+} influx leading

to increased synaptic activity. Increasing synaptic activity makes it easier to depolarize

M1 to produce MEPs (i.e., neurons in M1 do not need a large stimulus to depolarize as

the threshold for depolarization is lowered in response to the preceding bout of AE).

Regarding the disparity in findings between the current and previous work, we suggest

that one reason that our study reports an increase in excitability after a single bout of AE

while the previous studies have not may be that we used a more precise measure of

maximum HR for each individual instead of using the age-predicted formulas as they did

(Singh et al., 2014, and Smith et al., 2014). Use of a more accurate process for identifying

maximum HR (and subsequently target HR) may have facilitated our AE sessions,

ensuring an adequate intensity of AE was achieved. This explanation however does not

explain our finding of increased cortical excitability at 40% HRR (an intensity lower than

their 70% of age-predicted maximum HR). An alternative explanation could be that their

participants 'overshot' their target HRs and in fact exercised at a level greater than 70%,

resulting in fatigue and in-turn a depletion of neurotransmitter levels in the brain that

accompanies high intensity AE. It should be noted that while Singh and colleagues did

not observe statistical significance in their pre-post S-R curve analysis, they did report an

upward shift in the post-exercise results, indicating that cortical excitability was

modulated in response to AE, just not at a level great enough to overcome variability in

their data and achieve significance. Lastly, it is also possible that a difference in study

design could have contributed to the disparity in findings. Specifically, Singh and

colleagues did not measure S-R curves first (i.e., before the paired-pulse measures) as we

did, as their four measures were randomized across the participants (S-R, ICF, SICI and LICI). It is possible that preceding the S-R curve measurement by paired-pulse measures, which include activating facilitatory and inhibitory networks in MI, may have affected the overall excitability of M1.

6.3.2 Intracortical Facilitation (ICF)

As described earlier, the study also hypothesized that the amount of facilitation of cortical neurons in M1would increase after all AE levels. Our results did not confirm this hypothesis as analyzing our data using two-way ANOVA found that exercise had no main effect on the amount of ICF. Further, it was obvious that the amount of facilitation in the conditioned MEPs after exercise was not large enough to be significantly different (see Table 4). Also, inspection of the data reveals a high degree of variability between the participants, which also impacts on the ability to show significant changes pre- to post-exercise (Table 4). However, we cannot report here that AE does not modulate ICF, as previous studies have indeed showed facilitation following exercise at 70% of age-predicted Maximum HR (Singh et al., 2014). Our inability to replicate these findings at the corresponding level of AE (i.e., 50% HRR) could be due to the high degree of variability observed between participants. The high degree of variability is a common issue in TMS studies, as people respond differently to TMS. The cortical mechanism(s) underlying the increase in ICF after exercise is not clearly delineated; however, as described earlier, it has been reported that facilitation could be mediated by glutamatergic interneurons, and possibly NMDA receptors that are activated by the presence of the excitatory neurotransmitter (glutamate among others) that are released in response to AE (see related discussion in section 6.2). It was surprising that although we observed a

change in corticospinal excitability (evidenced by the upward shift in the S-R curve), there was no significant change in ICF. As demonstrated before, the cortical networks underlying facilitation and inhibition influence corticospinal excitability. However, previous studies have shown the same disconnect between these single and paired-pulse measures (Sanger, Garg, & Chen, 2001, Ilic, Korchounov, & Ziemann, 2002, and Roy, 2009). Further, Singh and colleagues (2014) indicated that the final corticospinal output is influenced by many other cortical and sub-cortical (spinal level) factors. Thus, the increase in corticospinal excitability in our study could be affected by factors other than the intracortical changes (represented by increased facilitation). It is likely however that our lack of ability to show changes in facilitation (i.e., ICF) is due to the high degree of variability in our data at 40 and 50% of HRR (Table 4).

6.3.3 Short Interval Intracortical Inhibition (SICI)

Our results revealed that the amount of SICI was reduced following exercise, as evidenced by increased amplitude of the conditioned MEPs after exercise (see Table 4); however the reduction was significant after AE at only 40 and 50% HRR. This finding did not confirm our hypothesis that the amount of SICI will be reduced after all AE intensities. Interestingly, our results replicate previous findings by Smith et al., (2014) and Singh et al., (2014). As explained earlier, the majority of interneurons in M1 release the inhibitory neurotransmitter GABA. Previous work examining AE and its effects on the brain show that AE leads to a reduction in the amount of GABA release. Our findings related to SICI support this notion, in that a single session of AE decreased intracortical inhibition (measured via SICI), a finding attributed to the reduction of GABA release and in-turn reduced activity on $GABA_A$ receptors. Although exercising at 30% HRR did not

show a significant reduction in the amount of SICI, the averaged conditioned MEP amplitude was larger post exercise. The lack of significance may be due to the large variability between participants, as well as the fact that this intensity of exercise may not have been adequate to induce a similar brain response as higher intensity exercise (see 6.4).

6.3.4 Long Interval Intracortical Inhibition (LICI)

The amplitude of the conditioned MEPs increased significantly only after exercise at 40% HRR, which indicates reduction in LICI after exercise at this intensity. This finding did not support our hypothesis that the amount of LICI will reduce significantly as a response to AE at all intensities. The reason that the decrease in inhibition was not statistically significant after 50% HRR exercise is most likely due to a technical failure during stimulation or slight variations in the stimulation site due to differences in brain morphology among individuals. As mentioned earlier in the data analysis section, we included all the conditioned MEPs in the LICI and SICI paradigms even if there were no MEPs present. The reason for this is that we are not able to differentiate if these low amplitudes (or even an absent MEP) were due to the induction of inhibition (in this case LICI) or due to technical errors in the delivery of the TMS pulses. This could be one reason for the lack of significance, namely we were unable to elicit the MEP due to technical error, and thus did not obtain MEPs post-exercise that could well have demonstrated a decrease in LICI (i.e., had increased amplitude relative to the unconditioned stimulus). Interestingly, our study found that LICI was reduced significantly after exercise at 40% HRR. It stands to reason that given the mechanism underlying LICI (described below), and in light of reduced LICI at 40% HRR, we should

have observed LICI reduction following AE at 50% HRR as well. Again, it is most likely that this inability to demonstrate reduced LICI at 50% HRR is due to technical error in obtaining our sample of MEPs. Given our finding of reduced LICI at 40% HRR, we can report that AE modulates the amount of LICI even though other studies did not detect significant changes in LICI. Our result here could support the idea described above that a single session of AE can lead to decrease in both the level and activity of GABA in the brain. Reduction in the amount of LICI resulted in this study supported that the release of GABA was reduced, and in-turn this mediated the activity of $GABA_B$ receptors. As with SICI, the potential factors relating to the lack of significance of AE at 30% HRR related to a reduction in amount of LICI is described below (6.4)

6.3.5 The Difference Between AE Levels

Further, our results revealed no main effect of exercise level, which confirmed our hypothesis that we expected no significant difference between the amount of reduction in inhibition and the amount of increase in facilitation after all, tested levels of AE. Accordingly, if both AE at 40 and 50%HRR for 20 minutes lead to modulation of cortical excitability and lead to changes in the cortical network by decreasing the amount of short and long interval intracortical inhibition, it is likely feasible to choose a lower intensity of AE that would potentially result in less fatigue.

6.4 Exercise Intensities

One of the aims of this study was to replicate findings related to the exercise intensity and duration used in previous work (i.e., 70% age predicted Maximum HR or ~ 50% HRR for 20 minutes) in addition to testing two lower AE levels: (40 % HRR that

investigated previously but with shorter duration (20 instead of 30 minuets), and AE at 30% HRR for 20 minutes, to investigate the effect of each of these AE intensities on cortical excitability of a non-exercised upper limb muscle. However, our significant findings, in terms of cortical excitability and inhibitory/facilitatory networks, were observed after AE at 50 and 40% HRR but not 30% HRR. The primary explanation behind these findings is most likely related to the fact that exercise at this intensity (30% HRR) is considered 'very light exercise' for the healthy young individuals who participated in this study. Evidence to support this statement comes from the related RPE scores (reported every two minutes during exercise), which were between 1 and 2, representing 'really easy' and 'easy', respectively. As RPE is correlated with the feeling of fatigue, it seems that AE at 30% HRR was not accompanied with a sense of fatigue (at least for our population). Research has reported that the sensation of fatigue is associated with a modulation of activity in multiple brain centers with increases in prefrontal cortical activity, which in turn could contribute to increased cortical excitability (González-Alonso et al., 2004, Hilty, Langer, Pascual-Marqui, Boutellier, & Lutz, 2011, and Nybo & Nielsen, 2001). Further, as discussed earlier, it has been shown that increased lactate levels resulting from more intense AE correspond to increased M1 excitability. Accordingly, the lack of a sense of fatigue could be a reason that AE at 30% HRR did not show significant changes. Researcher has shown that higher exercise intensities are associated with a greater release of neurotransmitters in brain (Kashihara, & Nakahara, 2005). So, it is suggested that lower exercise intensity lead to a reduced effect in terms of changing the level and activity of neurotransmitters in M1, thus resulting in a lower degree of modulation for both cortical networks and overall cortical

excitability. Accordingly, perhaps the changes associated with alteration in activity at GABA$_A$ and GABA$_B$ receptors do not occur at 30% (i.e., it is not intense enough to cause the cascade of cellular and other related events that happen with AE at 40 and 50% HRR). This could be a reason that the amount of short and long interval intracortical inhibition did not change significantly after AE at 30% HRR. As a result, exercising at 30% HRR does not seem to be effective for priming the brain in advance of neurorehabilitation. However, given our study design, we cannot indicate that 40% HRR for 20 minutes is the 'lowest common denominator' for the modulation of cortical excitability, as we did not test other AE intensities between 30 and 40% HRR. Thus, further research could be performed to increase the resolution of the data related to the threshold of AE that could still modulate cortical excitability in M1.

6.5 Clinical Use of Exercise

Best practice guidelines have recommended including AE to be a part of routine neurological rehabilitation and long-term management (Furie et al., 2011 and Billinger et al., 2014). Despite the fact the recovery of upper limb function is a major challenge for survivors of stroke (Barreca, 2001), the majority of these clinical AE interventions performed in rehabilitation clinics before the actual session involve lower limb exercises, such as walking, pedaling and running, owing to the benefits of these types of exercise on cardiovascular fitness of people with neurological disorders. When previous studies found that effects of AE are not limited to the heart and lungs but also has a generalized effect on the CNS, researchers intended to test the effect of single session lower limb exercise on M1 excitability. Many positive effects have been found as demonstrated

earlier and surprisingly, it has been shown that lower limb exercise modulates cortical excitability in non-exercised upper limb muscles due to cortical pathways between synergistic arm and leg representations (Takahashi et al., 2001). Further studies have been conducted to support this finding. These studies collectively suggested that pre-rehabilitation moderate-to-high-intensity AE is sufficient to prime M1 to undergo experience-dependent plasticity. However, in the context of using AE as a means to increase cortical excitability prior to a bout of rehabilitation therapy, it is critical that patients not be fatigued following the AE, as they need to engage in the task specific therapy component of the intervention immediately thereafter. Accordingly, AE intensities and durations previously investigated may not be feasible for some patients especially in the early stage of rehabilitation or if they have general body weakness. For this purpose, we sought to examine if AE at levels lower than those previously examined would increase cortical excitability. We also intended to replicate the same AE intensities used before but with more accurate measures (will be described in the following section) to confirm previous findings.

6.6 Contribution of This Research

Most studies to date that have tested changes in cortical excitability as a response to a single session of AE have used an age-predicted estimation to determine the maximum HR or even HRR of their participants. In the present work, we wanted to use a more accurate method to determine the maximum HR, and thus utilized a graded maximal exercise test. Subsequently, to determine target HRs for our participants' exercise prescription, we used the percent HRR. We prefer this method as percentage of

HRR more closely approximates the percent values as VO_2 reserve method (the difference between the maximal aerobic capacity and resting oxygen consumption) (Lounana, Campion, Noakes, & Medelli, 2007, and Swain & Leutholtz, 1997,). Adding to the previous findings, our results revealed significant changes in M1 excitability, as evidenced by increased MEP amplitudes after exercise. Further, a significant change in LICI has been found after exercise, which has not been shown before. This study also confirms that SICI reduces after AE, which may facilitate the induction of plasticity. In general, observations in this study taken together with previously findings may have important implications for the use of AE to prime the brain prior to the rehabilitation session in treating upper limb motor deficits

6.7 Limitations

There are several limitations associated with this research that need to be considered when interpreting the results.

First, the aim of the study was to test the effect of exercising the lower limbs on the cortical excitability of the non-exercised upper limb motor representation in M1. Thus, it is critical to check that the tested upper limb muscle exerted no activity during the exercise session and this can be done using EMG. In this experiment, subjects were instructed to keep their arms at their sides, and to not move their hands nor grip the handlebars during the 20-minute bouts of AE. During the AE sessions, we continuously watched for upper limb movements; however, we did not monitor for muscle activity via EMG. As such, it is possible that the non-exercised upper limb muscle (ECR muscle) under investigation was active during the exercise session. As we outlined previously,

pilot work in our laboratory examining muscle activity via EMG revealed no activity in the ECR during cycling. Similarly, previous work examining the same muscle (Singh and colleagues' study) reported no detectable muscle activity the ECR, flexor carpi radialis (FCR), or first dorsal interosseous (FDI) during cycling.

Another possible limitation of our study was that we tested the changes in cortical excitability resulting from AE immediately after the exercise, but not at later time points. Specifically, we did not undertake a second round of TMS measures to examine if the effect of AE on brain excitability, facilitation, short and long intracortical inhibition persisted over time. Knowing the longer-term effect of AE would better inform the clinical implementation of exercise as a priming agent.

Also, although participants were asked to complete the IPAQ to determine their level of physical activity, we did not formally include the differences in physical activity between our participants in the analysis. These differences in activity level could have influenced the results obtained. As indicated previously, the IPAQ was used in this study only to characterize the participants regarding their level of physical activity. We suggest that measuring fitness level directly (i.e., through assessment of VO_2 max) would be a more accurate method in order to differentiate subjects regarding physical activity level. Given that our participant group was fairly homogenous in relation to IPAQ scores, it is unlikely that inclusion of physical activity level in the analysis would have altered our findings. Going forward however, it would be important to study participants of varying fitness level to determine the effect of prior fitness level on the brains response to AE.

As indicated above, another limitation in our study was that we only tested three exercise intensities, and that relatively large gaps exist between each (i.e., 10% HRR

between each level). As such, we are unable to conclude if other intensities of AE would be effective in modulating cortical excitability in a manner similar to those observed here. Specifically, we are unable to conclude that 40% HRR is the lowest intensity of AE effective at modulating cortical excitability, as it may be possible that an intensity between 30 and 40% HRR would have a similar effect. Thus, we suggest further research to test the effect of these exercise intensities on M1 excitability.

Lastly, we did not control for sex differences in this study, which may have increased the variability of the findings and influenced the outcomes of this study. As demonstrated, our study included a larger number of females than males. Research has suggested that the phase of the menstrual cycle in females may influence cortical excitability, as excitability can be increased as a response to high circulating estradiol levels (Smith, Adams, Schmidt, Rubinow, & Wassermann, 2002).). In addition, Kuo, Paulus, & Nitsche (2006) reported that sex affects modulation of cortical plasticity. In this work they used cathodal tDCS to diminish cortical excitability, and a short duration tDCS (4 s tDCS, which produces no after-effects) to induce inhibition. Their study showed that female group displays a prolonged response to cathodal tDCS and more inhibition than male group. Accordingly, differences in hormonal levels are possibly the reason that participants respond differently to the TMS, which could affect our results. However, Smith and colleagues (2014) revealed that sex has no influence of on amount of SICI. Thus, the effect of sex on both cortical excitability and intracortical changes may be not entirely clear yet.

CHAPTER 7: CONCLUSION

The primary objective of this study was to examine if AE at intensities lower than those previously examined would increase cortical excitability, ultimately resulting in the identification of a potential 'lowest common denominator' for AE and cortical excitability that could be implemented in the clinical setting as a means to prime the brain before neurorehabilitation.

The results of the study indicated that AE at an intensity lower than that previously examined (70% of age-predicted HRmax) can still modulate the excitability of M1. Moreover, our findings demonstrated intracortical network changes after exercise at this intensity (40% HRR), evidenced by a decrease in intracortical inhibition. These network changes are critical to facilitate the induction of experience-dependent plasticity, indicating that AE creates favorable conditions under which more permanent changes may occur.

However, these findings were not observed after exercise at the lowest intensity examined in this study, namely 30% HRR; thus we could not identify what is the potential lowest intensity that can still modulate cortical excitability and result in cortical network changes. Further research is needed to investigate the effect of intensities lower than AE at 40% HRR on excitability changes.

In regard to priming the brain before neurorehabilitation, we can conclude that AE at intensities lower than those previously investigated, namely 40% HRR, are effective in increasing cortical excitability and facilitating intracortical changes in a population of aerobically fit young adults. Future work needs to replicate these findings in individuals post-stroke to ensure similar effects.

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Physical Activity Readiness Questionnaire for Everyone (PAR-Q, from the Canadian Society of Exercise Physiologists)

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 <u>and</u> 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 <u>any other reason</u> 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 <u>all</u> 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.

<u>Informed Use of the PAR-Q</u>: 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 _____ WITNESS _____
or GUARDIAN (for participants under the age of majority)

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.

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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):

1

113

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.

2

If you answer, "yes" to questions #1 through 8 below, you are ineligible for this study. Please contact the researcher to let them know that you are not eligible; you do not have to tell why you are not eligible.

APPENDIX 3

Recruitment Poster

Volunteers Needed…

We are recruiting for a study using brain stimulation to look at how aerobic exercise alters brain function

You will visit the Laboratory for Brain Recovery and Function at Dalhousie University for 4 visits; each one will take about 2 hours.

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

You will be compensated for any costs incurred from parking (up to $10 / visit). To be eligible to volunteer, you must be in good health and be between 18 and 40 years of age

Who to Contact: Hawazin Khan, Graduate Student / **Supervisor:** Dr. Shaun Boe

Email: Hawazin.Khan@dal.ca **Phone:** (902) 210-7755

Where: School of Physiotherapy, Dalhousie University

Study Title: Investigating the effects of different intensities of aerobic exercise on cortical excitability in non-exercised upper limb muscles of non-disabled young adults

AE on brain study Hawazin.Khan@dal.Ca (902) 210-7755	AE on brain study Hawazin.Khan@dal.Ca (902) 210-7755	AE on brain study Hawazin.Khan@dal.Ca (902) 210-7755	AE on brain study Hawazin.Khan@dal.Ca (902) 210-7755	AE on brain study Hawazin.Khan@dal.Ca (902) 210-7755	AE on brain study Hawazin.Khan@dal.Ca (902) 210-7755	AE on brain study Hawazin.Khan@dal.Ca (902) 210-7755	AE on brain study Hawazin.Khan@dal.Ca (902) 210-7755	AE on brain study Hawazin.Khan@dal.Ca (902) 210-7755	AE on brain study Hawazin.Khan@dal.Ca (902) 210-7755

International Physical Activity Questionnaires (IPAQ)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRES

IPAQ: SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS

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 in 12 countries (14 sites) across 6 continents during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages. IPAQ is suitable for use in regional, national and international monitoring and surveillance systems and for use in research projects and public health program planning and evaluation. International collaboration on IPAQ is on-going and an international prevalence study is under development.

Using IPAQ

Worldwide use of the IPAQ instruments for monitoring and research purposes is encouraged.

It is strongly recommended, to ensure data quality and comparability and to facilitate the development of an international database on health-related physical activity, that

- no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments,
- if additional questions on physical activity are needed they should follow the IPAQ items,
- translations are undertaken using the prescribed back translation methods (see website)
- new translated versions of IPAQ be made available to others via the web site to avoid duplication of effort and different versions in the same language,
- a copy of IPAQ data from representative samples at national, state or regional level be provided to the IPAQ data storage center for future collaborative use (with permission) by those who contribute.

More Information

Two scientific publications presenting the methods and the pooled results from the IPAQ reliability and validity study are due out in 2002.

More detailed information on the IPAQ process, the research methods used in the development of the IPAQ instruments, the use of IPAQ, the published papers and abstracts and the on-going international collaboration is available on the IPAQ web-site.

www.ipaq.ki.se

This is the final SHORT LAST 7 DAYS SELF-ADMINISTERED version of IPAQ from the 2000/01 Reliability and Validity Study. Completed May 2001.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

In answering the following questions,

◆ **vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder that normal.

◆ **moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder that normal.

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

Think about *only* those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**   ⇨   1b.   How much time in total did you usually spend on one of those days doing vigorous physical activities*?*

or   _____ **hours** _____ **minutes**

   none

2a.   Again, think *only* about those physical activities that you did for at least 10 minutes at a time. 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**   ⇨   2b.   How much time in total did you usually spend on one of those days doing moderate physical activities*?*

or   _____ **hours** _____ **minutes**

   none

3a.   During the last 7 days, on how many days did you **walk** for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

_____ **days per week**   ⇨   3b.   How much time in total did you usually spend walking on one of those days*?*

or   _____ **hours** _____ **minutes**

   none

The last question is about the time you spent <u>sitting</u> on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.

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

____ **hours** _____ **minutes**

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

APPENDIX 5

CONSENT FORM

Project Title: "Investigating the effects of different intensities of aerobic exercise on cortical excitability in non-exercised upper limb muscles of non-disabled young adults"

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Introduction

We invite you to take part in a research study being conducted by Hawazin Khan who is a student at Dalhousie University as part of her MScPT (Rehabilitation Research) degree. The supervisor for this study is Dr. Shaun Boe (MPT, PhD), Assistant Professor in School of Physiotherapy at Dalhousie University. Participation in the research is up to you and you can leave the study at any time. There will be no negative impact on your studies if you decide not to participate in the research. The information below tells you about what you will be asked to do and about any benefits, risks, or discomforts that you might experience. You should discuss any questions you have about this study with Hawazin Khan.

Purpose and Outline of the Research Study

This study wants to test if low intensity aerobic exercise can affect your brain cells. This will be done by measuring changes in your brain just before, and after cycling exercise at three different intensities, and comparing the results.

This study will be conducted in Dr. Shaun Boe's Laboratory for Brain Recovery and Function, located at School of Physiotherapy in in the Forrest Building at Dalhousie University.

Who Can Participate in the Research Study?

You may participate in this study if you are between 18 and 40 years old with no known medical illnesses.

You will not be eligible for this study if you:
- Are under the age of 18 or older than 40 years
- Have told by your doctor that you are not allowed to perform exercise

- Have been diagnosed with medical condition that affects the nervous or cardiorespiratory systems
- Have been prescribed high blood pressure or any other heart medications
- Have a history of epilepsy, concussions, prior brain infarction, implanted metal in your brain (such as aneurysm clips) or regular migraines
- Are pregnant.

How many people are taking part in the study?

The study will include one group of 12 individuals.

What You Will Be Asked to Do?

To help us achieve our goal, we will ask you to come in for 4 sessions *over a period of 7 days* (1 familiarization and 3 experimental sessions) with a total time commitment of ~8 hours.

During the familiarization session (day 1):

You will be asked first to complete the screening forms (PAR-Q and TMS screening form), and the IPAQ questionnaire that have been emailed to you and to sign this informed consent form.

You will then be shown the equipment we will use in the study and you will have a chance to ask any questions. After this, we will direct you to a private change room if you need to change into comfortable clothing for the duration of the test. You will begin the session by sitting on a stationary bike quietly for 5 minutes to measure your RHR. Then, you will start cycling on the stationary bike and your heart rate will be monitored to determine your maximal heart rate for aerobic exercise. The cycling part of this session

takes a different amount of time for each person, but should last between 15 and 20 minutes. In total, this first session will last approximately 2 hours.

<u>During each of the 3 experimental sessions</u>

Each of the participants will perform the following testing procedures:

Transcranial Magnetic Stimulation (TMS)

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

Muscle activity

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

Cycling exercise

For this study we will be asking you to perform some cycling exercise on a stationary bicycle. When performing the cycling exercise you will be sitting upright with your hands resting comfortably in your lap.

Watch to monitor your heart rate ('Mio watch')
The 'Mio watch' is simply a watch that acts as a heart rate monitoring device that allows

one to measure one's heart rate in real time or record the heart rate for later analysis.

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

After you finish the TMS session, you will cycle on a stationary bike for 20 minutes (plus 5 min warming-up and 5 min cooling-down). Throughout we will monitor your heart rate using the Mio watch (outlined above). As you finish cycling, you are going to transfer back to the TMS chair to let us take the brain measurements again. After this is done, the testing is completed. The same protocol will be repeated in all three experimental sessions, but the exercise intensity will be different each day. Each experimental session will last approximately 2 hours.

Possible Benefits, Risks, and Discomforts

BENEFITS:

We cannot guarantee or promise that you will receive any benefits from this research; however this study has the potential to benefit society through the generation of knowledge regarding the effect of aerobic exercise on the brain. In the longer-term, we anticipate these results will impact on the implementation of aerobic exercise in the clinical rehabilitation of people with motor deficits resulting from brain injuries like stroke.

RISKS:

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

Potential risks during Maximal Exercise Testing:

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

Potential risks of using TMS:

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

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

Common risks: 1-10% people have experienced headaches, which are caused by muscle tension. In the case of a headache, you will be advised to take whatever pain medication you usually take for mild headaches, which in most cases promptly resolves the discomfort.

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

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

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

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

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

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

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

What you will receive for taking part:

There is no compensation for being part of this research study. However, information regarding your general cardiovascular health including resting and maximal heart rate will be provided to you upon request. Juice and snacks will be provided after you complete the session. If you are bringing your own vehicle to the study, parking will be covered up to $10 per session if you provide a receipt.

How your information will be protected:

The research team will keep any personal health information about you in a secure and confidential location (namely the Laboratory for Brain Recovery and Function at

Dalhousie University) for 7 years and then destroy it. You should know that the results of this study will be made available to the scientific community. This will happen through publication in a scientific journal. Neither your name nor any reference to you will be used in creating or publishing these results. This means that *you will not be identified in any way in our reports*. The people who work with your information have special training and have an obligation to keep all research information private. When this data becomes available in a publication you may access the results if you are interested. To ensure confidentiality of participant information each participant will be assigned an identification code. Each code and its files will be labeled and stored in a secured file folder. The folder will be on a computer in Dr. Shaun Boe's office in Forrest building.

If You Decide to Stop Participating

You are free to leave the study at any time. If you decide to stop participating at any point in the study, you can also decide whether you want any of the information that you have contributed up to that point to be removed or if you will allow us to use that information. You can decide to withdraw your study data within a one-month period after completion of your final testing session. After that time, it will become impossible for us to remove it because it will already be analyzed.

How to Obtain Results

You can obtain the group results of the study once they are published by including your contact information at the end of the signature page.

Questions

We are happy to talk with you about any questions or concerns you may have about your participation in this research study. Please contact Hawazin Khan at Hawazin.Khan@dal.Ca or (902) 210-7755 or Dr. Shaun Boe at s.boe@dal.Ca or (902) 494-6360 at any time with questions, comments, or concerns about the research study.

We will also tell you if any new information comes up that could affect your decision to participate.

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

Signature Page

Project Title:

"Investigating the effects of different intensities of aerobic exercise on cortical excitability in non-exercised upper limb muscles of non-disabled young adults"

Lead Researcher:

Hawazin Khan, MScPT (Rehabilitation Research) Candidate,

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

_____   _____

Participant's Signature   DATE

_____   _____

Print Name of Participant   DATE

_____   _____

Signature of Witness   DATE

I would like to receive a copy of group results:

_____   _____

Participant's Signature   DATE

Please contact me at (your e-mail address):

_____

APPENDIX 6

Borg Rating of Perceived Exertion Scale

1 - 10 Borg Rating of Perceived Exertion Scale	
0	Rest
1	Really Easy
2	Easy
3	Moderate
4	Sort of Hard
5	Hard
6	
7	Really Hard
8	
9	Really, Really, Hard
10	Maximal. Just like my hardest race