DOES AN AEROBIC EXERCISE INDUCED ALTERATION IN BLOOD LACTATE CONCENTRATION EFFECT BRAIN EXCITABILITY?

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ABSTRACT

Aerobic exercise (AE) is thought to increase corticospinal excitability (CSE), creating an optimal environment for motor learning to occur. During AE, metabolic by-products, including lactate, accumulate in the blood. Increased concentration of lactate during fatiguing exercise has been shown to increase CSE. Our research questions if increased blood lactate concentration mediated by an acute bout of moderate-intensity AE alters CSE. Participants completed two sessions; session 1 consisted of determining peak power output (PPO), and in the second session measures of brain excitability and blood lactate concentration were obtained before, immediately after, and 10-min after a 20-min bout of moderate-intensity AE performed at 60% of PPO. Our results show that no relationship between blood lactate concentration and CSE exists following a bout of moderate intensity AE suggesting that altered lactate concentration is not a factor involved in increasing CSE with AE.

LIST OF ABBREVIATIONS USED

AE – Aerobic Exercise

CSE – Corticospinal excitability

TMS – Transcranial magnetic stimulation

PPO – Peak power output

GXT – Graded exercise test

S-R curve – Stimulus response curve

GABA – Gamma-aminobutyric acid

NMDA – N-Methyl-D-aspartic acid

AMPA - Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

M1 – Primary motor cortex

EMG – Electromyography

MEP – Motor evoked potential

RMT – Resting membrane threshold

AUC – Area under the curve

SICI – Short interval intracortical inhibition

LICI – Long interval intracortical inhibition

ICF – Intracortical facilitation

FDI – First dorsal interosseous

$\dot{V}O_{2max}$ – Maximal oxygen uptake

MET – Metabolic equivalent

RPE – Rating of perceived exertion

LT – Lactate threshold

BDNF – Brain derived neurotrophic factor

IPAQ – International physical activity questionnaire

MCT – Monocarboxylate transporters (MCT2 and MCT4)

MRI – Magnetic resonance imaging

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CHAPTER 1: INTRODUCTION

1.1 Executive Summary

The ability to acquire motor skills is a critical aspect to all human life, particularly in the case of neurological injuries or events that lead to lasting deficits in motor function. In rehabilitative settings the remediation of motor function is important as it leads to improved function and independence (Hubbard et al., 2009). For improvements to occur, the brain undergoes modifications such as the forming and reorganizing of neural connections, this is in response to various stimuli by a process known as neuroplasticity (Khan et al., 2017; Mang et al., 2013; Mateos-Aparicio & Rodríguez-Moreno, 2019). Neuroplasticity is dependent on the excitability of neurons within the cerebral cortex and is often measured through non-invasive means, particularly transcranial magnetic stimulation (TMS) (Rossini et al., 2015).

Research suggests that the excitability of cortical neurons can be altered via interventions such as exercise (El-Sayes, Harasym, et al., 2019); this process is coined exercise-induced neuroplasticity. Exercise is considered to be a mechanism which primes the brain for motor learning, however the mechanisms by which these changes occur are being reviewed on molecular and functional levels (El-Sayes, Harasym, et al., 2019). The current literature shows conflicting findings regarding the mode, intensity, and type of exercise used in these studies. Evidence supports that moderate-intensity aerobic exercise (AE) is successful in increasing corticospinal excitability (CSE) (Baltar et al., 2018; El-Sayes, Turco, et al., 2019; Garnier et al., 2017; Lulic et al., 2017a; MacDonald et al., 2019; Singh et al., 2014; A. E. Smith et al., 2014). However, the findings in the above

studies suggest that there is inter-individual variability associated with the response to exercise which suggests that there may be factors which determine how CSE is mediated by exercise.

It is thought that the accumulation of metabolites during exercise may alter CSE, one of which is lactate. During exercise, blood lactate concentration can increase with increased intensity and duration (Bentley et al., 2001). Individuals who are trained tend to exhibit a higher lactate threshold compared to untrained individuals, meaning that trained individuals are able to exercise for longer before reaching the point where lactate concentration will increase exponentially (Powers & Howley, 2018). These differences in lactate threshold suggests that when an individual exercises at a fixed intensity (e.g., a percentage of peak power output (PPO) or heart rate (HR)) they may be exercising below or above their lactate threshold, ultimately affecting the amount of lactate being produced during exercise. Previous research has indicated a potential positive relationship between blood lactate concentration and CSE during maximal exercise (Coco et al., 2010a), wherein increased blood lactate is associated with increased CSE however, research examining the effects of AE on CSE has yet to monitor the concentration of metabolites during an acute bout of steady-state exercise.

Therefore, the purpose of this study was to determine whether a change in blood lactate concentration resulting from an acute bout of moderate-intensity AE results in altered CSE in healthy adults. Said another way, we were interested in knowing if there was a relationship between increased blood lactate concentration and CSE following a bout of moderate-intensity AE. Secondly, we questioned if an acute bout of moderate-intensity AE altered CSE in healthy adults. It was hypothesized that increased blood

lactate concentration would lead to increased CSE following an acute bout of moderate-intensity AE. The secondary hypothesis is we would observe an increase in CSE following a bout of moderate-intensity AE.

To address the above research questions, our participants completed two sessions. In the first session, participants completed a TMS protocol to ensure they were responsive to TMS and if responsive, participants completed a graded exercise test (GXT) to determine their PPO. The second session occurred within a one-week period and consisted of testing at three time points: prior to exercise (Pre), immediately following exercise (Post), and 10-min following exercise cessation (Post10). The testing protocol consisted of delivery of a stimulus-response (S-R) curve, a measure used to establish changes in CSE, and blood collection to determine lactate concentration. The exercise intervention consisted of 20-min of moderate-intensity cycling at 60% of the participants PPO. Blood lactate measures were also obtained at the 5-min and 15-min mark during the exercise intervention to monitor the participants response to exercise, however these data were not included in the analysis.

Results of a linear regression examining blood lactate concentration and change in CSE was not significant and thus our primary hypothesis was not supported. Further, no change in CSE was determined between timepoints (Pre-Post, Pre-Post10, Post-Post10) following the bout of moderate-intensity AE. Following the AE intervention we observed a range of blood lactate concentration values amongst participants however, majority of participants showed little accumulation of lactate. Only a small number of participants approached or exceeded a value post-exercise that would suggest they were at or near

their lactate threshold. Given the lack of relationship between lactate and CSE, we suspect that lactate is not a factor in driving excitability changes.

CHAPTER 2: BACKGROUND

2.1 Motor Learning

2.1.1 Physiological Components of Learning

The ability to acquire motor skills is critical to many aspects of human life. Acquired or traumatic injuries, including stroke, can lead to long-lasting changes to the central nervous system that result in deficits in function. Deficits in motor function impact an individual's ability to perform activities of daily living and enjoy full participation in other activities including those related to occupation and leisure. Remediation of motor impairment and restoration of function can be achieved through rehabilitation, which facilitates re-learning of motor skills (Krakauer, 2006). The ability for structural and functional changes in the brain to occur is experience-dependent and relies on an underlying mechanism known as neuroplasticity. Neuroplasticity broadly refers to the ability of the nervous system to modify in response to intrinsic and extrinsic stimuli (Khan et al., 2017; Mang et al., 2013; Mateos-Aparicio & Rodríguez-Moreno, 2019). This ability to reorganize and form new connections within the brain is fundamental to learning and is a key component of rehabilitation following neurological injury.

In neurological rehabilitation, task-specific (or task-oriented) training is considered the practice of context specific motor tasks with the provision of feedback related to performance (Hubbard et al., 2009; Teasell et al., 2005). This form of rehabilitation is used to remediate motor impairment and improve performance on functional tasks through practice and repetition, driving neuroplasticity (Hubbard et al., 2009). It is well established that neuroplasticity resulting from engagement in task-

specific therapy is key to recovery of motor function, and as such, task-specific therapy is considered the gold standard in rehabilitation after neurological injury such as stroke, with level I evidence for its effectiveness (*Management of the Upper Extremity Following Stroke*, 2019; Saikaley et al., n.d.).

Within the brain, billions of neural connections exist which allow for the development and execution of behavior. All aspects of behaviour, including memory and learning (inclusive of learning of movement, or motor learning), are dependent on the activation of neurons, which is mediated by inhibitory and excitatory processes in the brain (Badawy et al., 2013). Inhibition is regulated by the neurotransmitter gamma-aminobutyric acid (GABA), which acts on $GABA_A$ and $GABA_B$ receptors (Badawy et al., 2013). Conversely, excitation is regulated by the neurotransmitter glutamate, which acts on N-methyl-d-aspartate (NMDA) and non-NMDA receptors, also known as alpha-amino-3-hydroxy-5-methyl-4-isoaxolepropionic acid or AMPA receptors (Badawy et al., 2013). Briefly, the excitability of a neuron (i.e., its ability to generate a change in resting membrane potential) is determined by the sum of excitatory and inhibitory inputs at any given moment; when excitatory inputs summate to reach the threshold for depolarization, the neuron discharges ('fires'), generating an action potential that traverses its axon to its target. The level of excitation and inhibition of neurons in the brain can be altered in the short- and long-term. Short-term changes are altered by varying the concentration of neurotransmitter or the activity of its receptor (as this affects the neuron's resting membrane potential), as well as the concentration of neurotrophins (i.e., brain derived neurotrophic factor and vascular endothelial growth factor) that may be mediated by aerobic exercise (AE). Short-term changes in excitability resulting from such processes

typically last from seconds to hours. Long-term changes, including long-term potentiation can result in permanent structural changes, including remodelling and growth of the dendrites (Harris et al., 2003). These structural changes are involved in synaptic plasticity that is activity dependent. Long-term potentiation is the process by which neuroplasticity occurs; in its simplest form, long-term potentiation results in an increase in synaptic strength, as the signal between two neurons is strengthened through repeated stimulation (Purves et al., 2004). The idea of long-term potentiation is best captured by the phrase "neurons that fire together, wire together", coined by the famous scientist Donald Hebb.

Activation of the post-synaptic neuron is typically mediated by the binding of glutamate to AMPA receptors, as the NMDA receptors are blocked by a magnesium (Mg^{2+}) ion and requires both the ligand (glutamate) and a voltage to be present to allow the flow of sodium and potassium ions to depolarize the neuron, whereas AMPA receptors require only the ligand (i.e., glutamate) to permit the flow of ions (Purves et al., 2004). With repetitive discharge of the pre-synaptic neuron and the release of glutamate, activation of AMPA receptors provides the voltage necessary to remove the Mg^{2+} ion, opening ion channels in the NMDA receptor permitting the flow of ions. Unlike AMPA receptors, NMDA receptors allow the passage of calcium ions (Ca^{2+}) in addition to others (e.g., sodium, potassium). The passage of Ca^{2+} is critical to long-term potentiation occurring, as Ca^{2+} is a secondary messenger that initiates a cascade of events that results in plasticity. Briefly, once Ca^{2+} enters the post-synaptic neuron, protein kinases are activated, specifically Ca^{2+}/calmodulin-dependent protein kinase and protein kinase C. Activation of these kinases generates new AMPA receptors, which are inserted on the post-synaptic cell membrane. This, in turn increases the neuron's sensitivity to glutamate

(Purves et al., 2004), and the likelihood of activation and facilitating long-term potentiation. Over time, Ca^{2+} influx results in additional changes to the post-synaptic cell (the details of which are beyond the scope of this discussion) that are the basis of memory and motor learning (Badawy et al., 2013; Purves et al., 2004).

Given a precursor for long-term potentiation to occur is activation of the post-synaptic neuron, increasing the excitability of neurons in the brain would create a favourable environment for plasticity to occur, as the resting membrane potential of these neurons would be closer to the threshold for depolarization. Such short-term changes in excitability, mediated by changes in neurotransmitter concentration, receptor activity, and neurotrophins (as indicated above) can be facilitated by various pharmacological agents (including licit and illicit drugs) (McDonnell et al., 2006; Rossini et al., 2015) and AE (El-Sayes, Harasym, et al., 2019; Hariri, 2020; Turco & Nelson, 2021). While various neuroimaging modalities may be used to assess such short-term changes in neuronal excitability (e.g., electroencephalography or magnetoencephalography, among others), non-invasive brain stimulation techniques, most commonly transcranial magnetic stimulation (TMS) are also used to assess short-term neuronal excitability changes (Carmichael, 2012; Rossini et al., 2015).

2.2 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive means of assessing the excitability of the brain. The device works by passing an electrical current through a coil of copper wires, generating a magnetic field, which in turn generates a secondary electrical field that passes through the skull, indirectly stimulating neurons in the region of the brain underlying the coil (see Figure 1) (Griskova et al., n.d.; Rossini et al., 2015).

The excitability of cortical neurons is most often measured through the assessment of corticospinal excitability (CSE), which is performed by stimulating pyramidal neurons within the primary motor cortex (M1). When pyramidal (or upper motor) neurons, located within M1, are activated, the resulting volley of action potentials travels via the corticospinal tract (made up of axons arising from pyramidal neurons) (Badawy et al., 2013; Purves et al., 2004; Rossini et al., 2015), synapsing with lower motor neurons in the spinal cord and ultimately resulting in activation of the contralateral muscle. Activation of the muscle results in a visible 'twitch' (when the intensity of the stimulation is above motor threshold) (Badawy et al., 2013; Rossini et al., 2015). In combination with electromyography (EMG; assessment of the electrical activity related to muscle contraction), the muscle response is known as a motor-evoked potential (MEP), the amplitude of which quantifies the magnitude of the response (Badawy et al., 2013; Rossini et al., 2015). Inclusive of both cortical and spinal level components, the resulting MEP characterizes the CSE. As above, CSE can be obtained using single-pulse TMS (Figure 1).

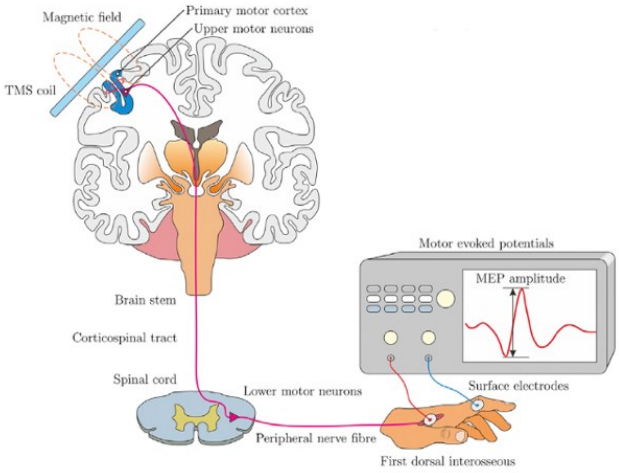

Figure 1. Diagram of the experimental procedure of TMS on the motor cortex. Adapted from Weise et al., (2020).

Single-pulse TMS is a form of TMS in which a single pulse at a fixed intensity is delivered to generate a MEP. When delivered at various percentages of resting motor threshold (RMT, the stimulation intensity required to elicit an MEP with a peak-to-peak amplitude of at least 50μV on 5/10 trials) (Hallett, 2000; Klomjai et al., 2015; Rossini et al., 2015), a stimulus-response (S-R) curve can be generated. Typically, S-R curves require 10 pulses to be delivered at varying intensities (often 100, 110, 120, 130 and 140% of RMT), with a minimum of 3 sec between delivery (Klomjai et al., 2015). Measures associated with the S-R curve (also known as an input-output curve) can be used to assess CSE. The S-R curve provides a sigmoidal shaped plot of the growth in MEP size (indicated by the evoked muscle 'twitch') following the stimulation at the above mentioned intensities (Abbruzzese & Trompetto, 2002; Rossini et al., 2015). A change in the S-R curve can be characterized by the slope of the curve, the area under the curve (AUC) or an upward or downward shift in the curve following some intervention. The shift in the curve may be characterized by the difference between S-R curves taken at two time points (i.e., a change in the AUC or the change in MEP amplitude). Along with characteristics of the S-R curve, alterations in the level of CSE can be identified by a change in RMT or MEP amplitude. For RMT, an increase in the stimulation intensity to achieve RMT indicates a decrease in CSE, while a decrease in the stimulation intensity to achieve RMT indicates an increase in CSE (Livingston & Ingersoll, 2008). For the MEP, an increase or decrease in the (peak-to-peak) amplitude given a consistent intensity of stimulator output would indicate an increase or decrease in CSE, respectively (Cuypers et al., 2014; Kallioniemi et al., 2015; Rossini et al., 2015).

Other methods of assessing excitability changes in the brain include paired-pulse TMS. Paired-pulse TMS involves the delivery of two stimuli (pulses) separated by a fixed interstimulus interval (Hallett, 2000; Rossini et al., 2015). The first stimulus is known as the conditioning stimulus and modulates the neuronal response to the second stimulus, which is known as the test stimulus. To assess intracortical phenomena, both stimuli are administered to the same location within a hemisphere, while inhibitory or excitatory influences between brain regions across hemispheres (intercortical) require two coils to deliver stimuli in different locations (Rossini et al., 2015). Paired-pulse techniques can be used to assess short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI) and intracortical facilitation (ICF). Short-interval intracortical inhibition assesses activity of $GABA_A$ receptors and LICI assesses $GABA_B$ receptor activity, providing an indication of the effect of an intervention on inhibitory processes. Using a similar protocol to SICI, ICF provides an indication of the effect of an intervention on facilitatory processes, however the physiological aspects of ICF are poorly understood, but likely have to do with upregulation of glutamate and its receptors (Di Lazzaro et al., 2006). Using methods of single- and paired-pulse TMS, it is possible to examine the impact various interventions have on brain excitability, including AE.

In studies which examine the effects of AE on CSE, a combination of single-pulse and paired-pulse measures were selected. To be discussed in further detail in the subsequent sections, the following studies each indicated a positive change in CSE following a bout of AE (Baltar et al., 2018; El-Sayes, Turco, et al., 2019; Garnier et al., 2017; Lulic et al., 2017a; MacDonald et al., 2019). The above-mentioned studies used TMS to assess excitability within M1. Each study used a S-R curve as an outcome

measure for assessing CSE. Garnier and colleagues (2017) used an S-R curve from 70-130% with 10% intervals to assess the MEP of the abductor pollicis brevis muscle. In the Baltar et al., (2018) study, S-R curves were obtained over the tibialis anterior muscle whereas the Lulic et al., (2017) and the El-Sayes et al., (2019) study protocols focused on obtaining S-R curves form the first dorsal interossei (FDI) muscle from 90-150% RMT and 100-160% RMT. The extensor carpi radialis was used in MacDonald et al., (2019) study to examine the effect of AE on CSE at 100-140% of RMT. Evidence from these studies supports that single-pulse TMS is an appropriate outcome measure to be used to assess CSE in the human motor cortex following AE.

2.3 Aerobic Exercise

It is well known that exercise has many positive benefits on the general health and function of individuals. Most commonly, AE is known for its positive impacts on cardiovascular health, however current evidence highlights that AE has positive impacts on the brain. Participation in AE has shown increased neuromodulatory effects known to mediate neuroplasticity linked to improvements in cognitive functions (El-Sayes, Harasym, et al., 2019; Mang et al., 2013; Müller et al., 2020). Increased neuroplasticity is useful in rehabilitative environments, given that neuroplasticity is a pre-requisite of motor learning. This is important in areas of neurorehabilitation post-stroke, where learning and re-learning motor tasks is of importance. The use of AE as an intervention may generate optimal learning environments for patients in which they can benefit from the full effects of rehabilitation, a process that is often referred to as ‘priming’ (MacDonald et al., 2019). However, the processes by which these environments are generated is still being explored. To understand the underlying mechanisms which AE can be used to improve

neuroplasticity through priming, one must first understand what AE is, and the physiological components of AE.

2.3.1 Variations of Aerobic Exercise

According to the Canadian Society for Exercise Physiology, aerobic physical activities are defined as dynamic activities that involve large muscle groups, which result in substantial increases in heart rate (HR) and energy expenditure (Canadian Society for Exercise Physiology, 2013). Aerobic exercise is defined as an activity that uses large muscle groups, can be maintained continuously and is rhymical in nature (American College of Sports Medicine et al., 2018). The Canadian Society for Exercise Physiology recommends that adults aged 18-64 years accumulate at least 150 minutes of moderate-to-vigorous physical activity per week. Aerobic exercise can be manipulated through adjusting the frequency, intensity, time, and type (i.e., the FITT principle) of exercise prescribed to individuals. Frequency refers to the number of sessions completed within a specific time frame (e.g., 2×/week). Intensity refers to the physical effort required to complete the exercise task. Aerobic exercise intensity can be prescribed through a variety of measures. Measures for quantifying exercise intensity include, energy expended per unit time, power output (PO) (e.g., working at a percentage of the PPO), maximal oxygen uptake ($\dot{V}O_{2max}$), various HR parameters including maximal HR (HR_{max}) or HR reserve (HRR), metabolic equivalents (MET; an objective measure related to the rate the body's oxygen uptake for a given activity as a multiple of resting VO_2) (Sanghvi, 2013), rating of perceived exertion (RPE), and lactate threshold (LT) (McArdle et al., 2015). By modifying the level of any one of these measures, the intensity at which an individual exercises can be altered (e.g., ensuring that the individual is exercising at an intensity

described as low, moderate, or high). The time component refers to the duration that the exercise occurs and can be anywhere from minutes to hours in length (McArdle et al., 2015). With respect to AE in particular, the duration must be greater than two minutes in length. Lastly, the type of exercise refers to the mode used to conduct the activity such as running, walking, cycling or swimming (Canadian Society for Exercise Physiology, 2013; McArdle et al., 2015). Varying exercise based on intensity, time, and duration can modulate the effects of exercise on the individual. In particular, varying intensities, time and type of AE are thought to have effects on brain plasticity and more specifically CSE, however these factors will be discussed in greater detail in the following sections (El-Sayes, Harasym, et al., 2019; Hariri, 2020).

2.3.2 Methods for Prescribing Aerobic Exercise Intensity

In research environments where exercise may be used as a protocol or intervention, it needs to be prescribed relative to a specific measure. For example, relative to an individual's HR_{max}, $\dot{V}O_{2max}$ or PPO. To prescribe exercise relative to individual abilities, physiological testing can, and should be completed. This includes physical exertion or exercise tests, metabolic testing, or a combination of both. A common measure for determining the relationship between exercise intensity and the various integrated body systems is a graded exercise test (GXT) (Beltz et al., 2016). The general protocol for this test is to have participants exercise at an increasing intensity over time until the participant is unable to maintain cadence, or they reach volitional fatigue (Beltz et al., 2016; McArdle et al., 2015). The GXT can be used in a wide variety of populations and allows clinicians to determine data related to metabolic patterns and is considered to be a gold standard in exercise testing (Beltz et al., 2016). From testing, clinicians are able

to determine cardiorespiratory fitness levels through $\dot{V}O_{2max}$ (i.e., the maximum rate of oxygen consumption during incremental exercise), and values such as PPO and LT (Beltz et al., 2016; Jamnick et al., 2018; Pallarés et al., 2016), where PPO is the peak power output achieved during an exercise test and LT refers to the point at which blood lactate concentration begins to increase rapidly (this concept will be discussed in detail in a later section) (McArdle et al., 2015). These measures can be used to prescribe exercise given an individual's fitness level.

Typically prescribed in the low-, moderate-, or high-intensity range, the range of AE intensity varies depending on what physiological metric is being used to characterize it. The American College of Sports Medicine refers to low-intensity exercise as exercise completed in <3.0 METs, moderate-intensity exercise as 3.0-5.9 METs, and high-intensity as ≥ 6.0 METs (American College of Sports Medicine, n.d.). In studies examining AE interventions on CSE (Singh et al., 2016; A. E. Smith et al., 2014), intensity is commonly prescribed relative to an individual's HR_{max}. When prescribed in this manner, low-intensity AE is considered to be between 35-50% of HR_{max}, moderate-intensity between 50-70% HR_{max}, and high-intensity between 70-85% of HR_{max} (Canadian Society for Exercise Physiology, 2013). Another approach to prescribing AE is relative to a participant's HRR, which takes into account the participants resting HR (HR_{rest}) and HR_{max} (McArdle et al., 2015). Low-moderate intensity is considered to be 40% of HHR and moderate-intensity is described as 50-59% HRR and high-intensity is considered to be 60-80% of HRR (Pescatello & American College of Sports Medicine, 2014). The use of HR as a measure for determining exercise intensity is both convenient and well established, as it is predictable when used in conjunction with $\dot{V}O_{2max}$ and vice

versa (McArdle et al., 2015). However, there are caveats associated with the above-mentioned methods of prescribing AE intensity.

2.3.3 Variability in Moderate-Intensity Aerobic Exercise

There are components of exercise intensity which must be considered when prescribing AE. One consideration related to HR is that it is a relative measure and can vary from day-to-day. Particularly, HR is affected by circadian rhythm, physical activity levels, lifestyle factors (smoking and alcohol consumption), and mental stress (Valentini & Parati, 2009) as a result, resting HR may vary. These factors may affect submaximal exercise intensities prescribed regarding HR_{max} and $\dot{V}O_{2max}$ due to varying contributions to the aerobic and anaerobic energy systems (Mann et al., 2013). Exercising at a percentage of $\dot{V}O_{2max}$ or HR_{max} fails to account for metabolic stress differences that occur, such as increases in blood lactate concentration (Mann et al., 2013; D. Meyer et al., 2015). The use of HRR as a function of intensity may be affected by an individual's mental state. Given that HR_{rest} is required for the calculation of HRR, if an individual is predisposed to a stressful circumstance their HR_{rest} will likely be increased, which in turn increases the HR level required for exercise intensity. For exercising at a moderate-intensity, if HR_{rest} is high, it may result in exercise being completed closer to vigorous-intensity given the HR.

In literature examining the effects of AE on CSE, there is variability regarding intensity measures. Rarely, if at all, is exercising at a percentage of PPO used as a measure of intensity in these AE and CSE studies. The use of a continuous ramp style protocol resulted in a similar relationship between PPO and $\dot{V}O_{2max}$ when compared to a step-wise protocol (Lamberts et al., 2012). This relationship between PPO and $\dot{V}O_{2max}$

values highlights that these measures may be used synonymously when prescribing exercise intensity. There is also less variability between sexes when exercising at an intensity relative to PPO compared to more common measures of HR_{max} and $\dot{V}O_{2max}$ (Rascon et al., 2020). For these reasons, a percentage of PPO was selected as the method for prescribing AE, and 60% of PPO specifically as this is considered moderate-intensity AE, which has previously been shown in the literature to elicit an increase in CSE (Hariri, 2020). As above, the use of PPO allows for precise monitoring of load during the session versus an internal measure of intensity such as HR_{max}.

There is variance attributed to exercising at a moderate intensity. By exercising at an intensity relative to PPO, the ability to observe the impacts of moderate-intensity exercise on physiologic functions can be monitored, as the use of PPO in conjunction with RPE and blood lactate samples was found to be more consistent across sexes (Rascon et al., 2020). It is thought that during exercise, some individuals may work at a level higher than their lactate (anaerobic) threshold while others may be working below it, despite exercising at an absolute intensity (T. Meyer et al., 1999). This finding indicates that at the same absolute intensity, individuals may experience varying metabolic responses, for example, whether lactate is being produced and accumulating in the blood, possibly contributing to a change in CSE. Prior to understanding the impacts of lactate concentration on CSE, it is important to understand the current research regarding the effects of AE on CSE and where the current gaps in the literature exist.

2.4 Effects of Aerobic Exercise on the Brain

In addition to the many benefits AE has on general health and fitness, it also plays an interesting role in facilitating and maintaining brain health. Aerobic exercise has

both direct and indirect effects on the brain, where direct refers to increasing concentration and activity of neurotransmitter and neurotrophic factors, and indirect refers to increasing physical fitness capacity (Figure 2) (Mang et al., 2013). However, both effects are linked to positive changes in brain health, including improved cognitive function, mood, and arousal levels. Through previous research, both short- and long-term effects of AE on the brain have been investigated, with a particular focus on CSE.

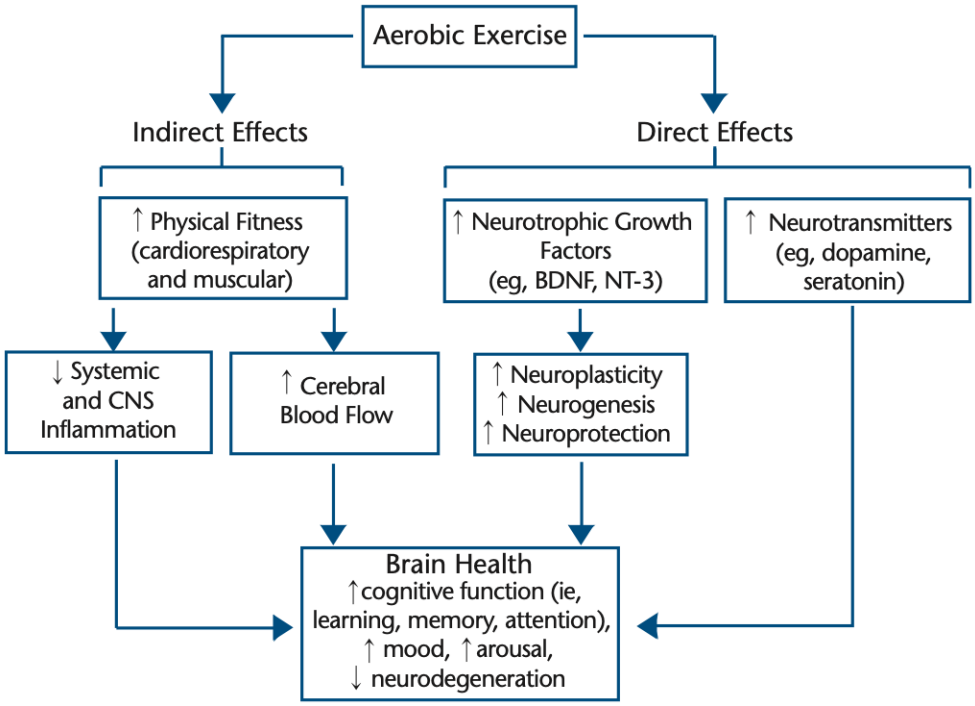

Figure 2. Examples of indirect and direct effects of AE on the brain.
From Mang et al., (2013). Indirect effects refer to the general impacts AE has on health, whereas the direct effects refer to impacts on concentrations of neuromodulatory agents including neurotrophic factors and neurotransmitter concentrations, affecting signaling pathways within the CNS.

The ability for the nervous system to change following a bout of AE is known as exercise-induced neuroplasticity. Work to date has clearly identified that the effect of AE on short-term neuroplasticity occurs on molecular, functional, and behavioural levels, with the details of these effects continuously being explored. These various short-term effects induced by AE are depicted in Figure 3 (El-Sayes, Harasym, et al., 2019).

Changes on a molecular level include increased concentration of neurotrophic factors such as BDNF. As highlighted by El-Sayes et al., (2019) execution of an acute bout of AE may increase neural activity through increased cerebral blood flow. An increase in blood flow within the brain gives opportunity for neurotrophins such as BDNF to cross the blood brain barrier, impacting on neural processes throughout the brain.

On a functional level, changes induced by AE include increased cerebral blood flow, increased glucose and oxygen metabolism, increased concentration of neurotransmitters (specifically, glutamate), increased neural activity and increased receptor activity when assessed via paired-pulse TMS (Maddock et al., 2016). In particular, NMDA receptor activity was altered (Lulic et al., 2017b) and $GABA_A$ and $GABA_B$ receptor activity was reduced (Mooney et al., 2016; Singh et al., 2014). A combination of the molecular and functional effects will eventually lead to behavioural changes related to cognitive and motor function expressed as improvements in memory, retention and reaction time (El-Sayes et al., 2020). The review by El-Sayes and colleagues focused on how altered neurotransmission, neurotrophin concentration, and oxygen metabolism may play a role in driving changes in brain function. However, this review did not include the effects of acute bouts of AE on single pulse TMS measures, which provides information on motor output over a given area of the M1. It also failed to examine the impacts of metabolic by-products that accumulate during AE. Therefore, future studies should include investigations of metabolites, including lactate, on a molecular level and the implications on neuroplasticity.

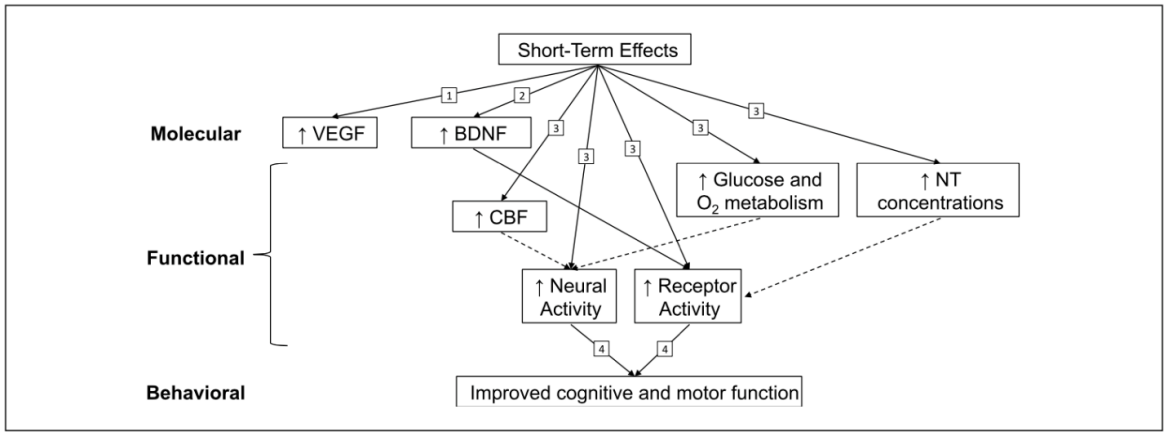

Figure 3. Short-term effects of acute aerobic exercise on neuroplasticity.
From El-Sayes et al., (2019). Acute AE increases peripheral levels of vascular endothelial growth factor (VEGF), and brain-derived neurotrophic factor (BDNF), increases glucose and oxygen metabolism, and neurotransmitters. Increases in neural activity may be mediated by increased cerebral blood flow (CBF) and glucose and oxygen metabolism, whereas increased receptor activity may be mediated by increased concentration of neurotransmitter (NT). These changes may become more substantial with time and repeated exposure, leading to long-term potentiation. Dashed lines indicate speculation of associations. Numbers 1-4 represent references highlighted by the review article.

2.4.1 Effect of Exercise Assessed using TMS

There is evidence which supports the notion that AE can lead to significant (short-term) changes in CSE, however there are conflicting findings, as various intensities and durations of AE have been examined with respect to CSE (Baltar et al., 2018; El-Sayes, Turco, et al., 2019; Garnier et al., 2017; Lulic et al., 2017b; MacDonald et al., 2019; McDonnell et al., 2013; Singh et al., 2016; A. E. Smith et al., 2014). In a recent 2020 scoping review conducted in our laboratory, the effect of AE on CSE was investigated. The review highlighted studies that showed an increase in CSE post-AE in healthy individuals. The common factor associated with studies included in the review is that moderate-intensity AE was the exercise intensity used in interventions where increases in CSE were found (Hariri, 2020). Conversely, studies prescribing low- or high-intensity AE generally did not observe a significant increase in CSE. The AE intensities used in

the studies included in the review were characterized as low-intensity (30, 40-60% HRR, 30% peak VO_2, 57-63% age predicted HR_{max}), moderate-intensity (40-50% of HRR, 60% peak VO_2, 64-76% age predicted HR_{max}), and high-intensity (80% HRR, 77-95% age predicted HR_{max}) (Hariri, 2020). There was variability across the studies with respect to quantifying AE intensities indicating some overlap in the reported intensities. Despite moderate-intensity AE being the common denominator across studies that found increased CSE, the duration which the exercise was prescribed was inconsistent signifying that the optimal AE duration is unknown, though it is suggested that a 20-min bout of AE was adequate to induce neuroplasticity (El-Sayes, Harasym, et al., 2019). A brief review of the current literature was conducted to further examine the inter-study variability across studies examining the effects of AE on CSE.

A study examined the effects of treadmill-based AE at varying intensities and durations on cortical excitability (Baltar et al., 2018). Three exercise intensities and durations ranging from high (77-95% HR_{max}) for 10-min, moderate (64-76% HR_{max}) for 15-min and low (57-63% HR_{max}) for 30-min were prescribed on varying days. Single-pulse TMS was used to assess the motor area of the right tibialis anterior muscle (Baltar et al., 2018). The TMS measures were assessed at multiple time-points including before, immediately after, and at 5-min intervals until the participant was 30-min post intervention. When compared to baseline, there was a significant increase in cortical excitation following moderate-intensity AE, a decrease in cortical excitation following high-intensity AE and no change following low-intensity AE (Baltar et al., 2018). These findings suggest that moderate-intensity AE may increase motor output variables in the lower extremity, which facilitate increases in cortical excitability.

In a 2019 study, the effect of sex and ovarian hormones on short-term AE-induced neuroplasticity was investigated. Males and females were recruited to participate in an acute bout of 20-min moderate-intensity cycling at 65-70% HR_{max} twice ~14 days apart. Single-pulse (MEP, recruitment curves) and paired-pulse (SICI) TMS measures were used to assess neuroplasticity including CSE. This study refuted its hypothesis stating that sex and ovarian hormones do not have an effect of short-term neuroplastic changes induced by acute AE. However, this study revealed that acute AE can cause significant change in neuroplasticity in the M1 and that this finding is similar between males and females (El-Sayes, Turco, et al., 2019), further suggesting that moderate-intensity AE may facilitate increases in CSE.

The effects of two modes of muscle contraction (i.e., eccentric vs. concentric) during treadmill walking was investigated in relationship to changes in CSE. Twelve participants completed two randomized paradigms (the first being exercise alone, and the second being exercise paired with paired associative stimulation), where the participants exercised for 30-min on a treadmill at 60% of HR_{max} with the slope of the treadmill set to +10% and -10% grade. This exercise prescription was not specified to a specific intensity, and respective intensity was not reported. Single-pulse TMS assessed via an input/output curve (also known as a S-R curve) was used to assess the motor output of the abductor pollicis brevis. The results of the study revealed a significant increase in MEP amplitude following both modes of 30-min exercise. MEP amplitude increased 30-min following paired associative stimulation. These findings suggest that sub-maximal exercise on a treadmill can increase CSE within 30-min (Garnier et al., 2017).

As mentioned, an increase in CSE can be characterized through several TMS-based measures, including increased MEP amplitude, decreased RMT, or an upward or downward shift in the S-R curve (Rossini et al., 2015). A 2017 study examined the effect of a 20-min bout of cycling at moderate-intensity (50-70% of age predicted HR_{max} however, participants all maintained approximately 60% of age predicted HR_{max}) (Lulic et al., 2017a). The results showed a significant increase in MEP amplitude at rest and the area under the S-R curve was reported for individuals who identified as highly physically active (Figure 4), but not for those that identified as having a low level of physical activity (Lulic et al., 2017a). The participants' activity levels were identified using the International Physical Activity Questionnaire (IPAQ), a quantifiable measure which looks at the amount of physical activity performed in a 7 day period (Craig et al., 2003). Participants were identified as highly physically active if their MET score exceeded 3000 and were identified as low-moderately active if their MET score was below 3000 (Lulic et al., 2017a). These findings suggest that CSE can be influenced by the physical activity level of the person undergoing the AE. It is possible that other components related to fitness levels may be associated with CSE, including blood lactate concentration (specifically LT), which is discussed below.

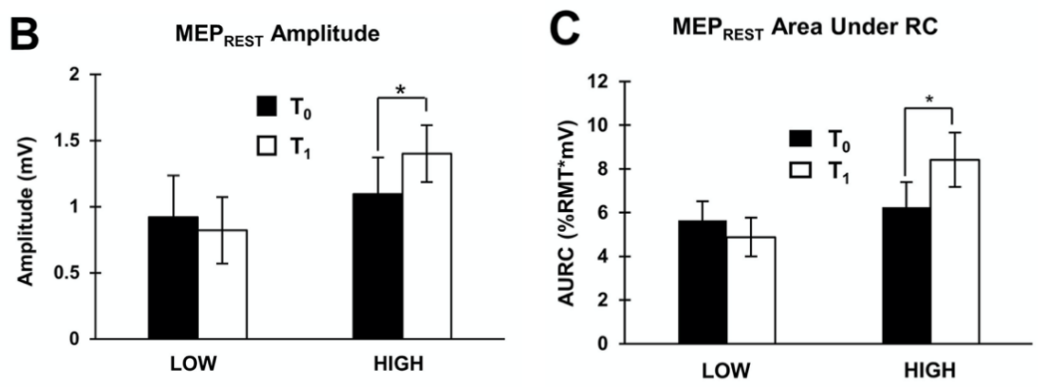

Figure 4. Thresholds and recruitment curves.
From Lulic et al., (2017). B, where T_0 is pre-testing and T_1 is post-testing. MEP amplitudes (mV) were significantly increased in the high physical activity group ('High') compared to the low physical activity group ('Low'). In C, area under recruitment curve (AURC) increased post-AE for the high physical activity group.

A recent study included two experiments to examine the impact of AE intensity and participants' aerobic fitness on CSE. The first experiment prescribed AE as a percentage of HRR with participants cycling for 20-min at three intensities (30, 40, and 50% HRR) in a randomized order. It was found that MEP amplitude increased significantly only after exercising at 40 and 50% of HRR (Figure 5), but not 30% (MacDonald et al., 2019), suggesting there is a lower bound of AE intensity required to drive changes in CSE. In the second experiment, participants aerobic fitness ($\dot{V}O_{2max}$) was first determined via a maximal GXT. The participant's $\dot{V}O_{2max}$ ranged from 22.1-48.2 mL/kg/min. Participants then completed a 20-min bout of cycling-based exercise at 50% of their HRR. Pre-post measures of CSE (via the area under the S-R curve) indicated that while increases in CSE were observed, there was no relationship between participants' aerobic fitness and CSE (Figure 6) (MacDonald et al., 2019).

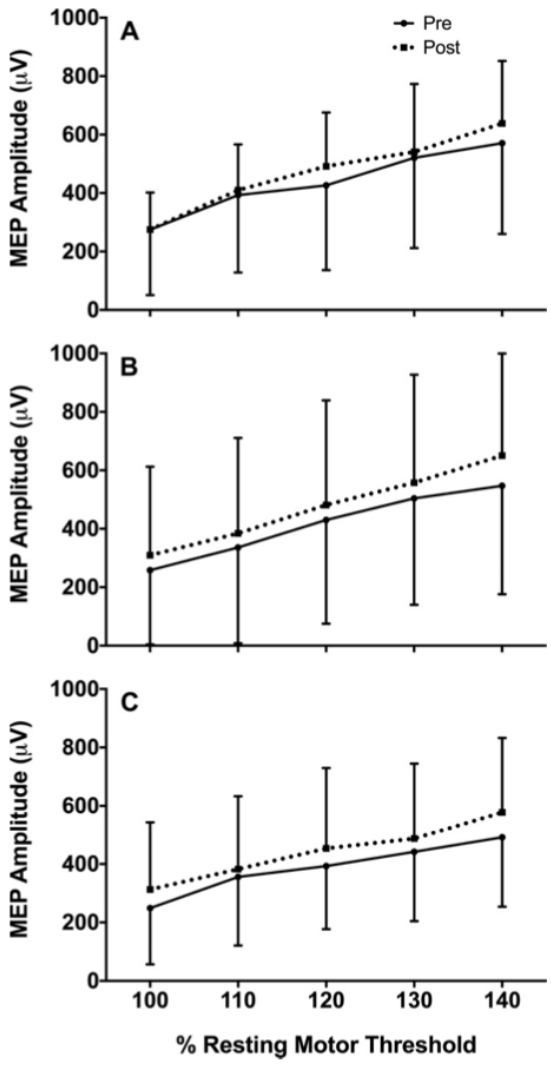

Figure 5. Stimulus response (S-R) curves pre- and post-exercise intervention. From MacDonald et al., (2019). A indicates exercise intensity 30% HRR, B indicates exercise intensity 40% HRR and C indicates exercise intensity 50% HRR. Significant increases in MEP amplitude were observed at 40 and 50% HRR.

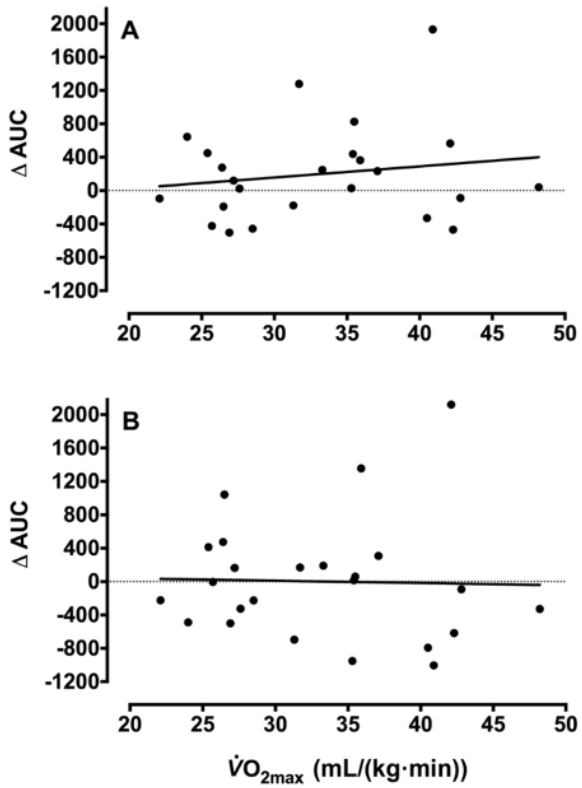

Figure 6. Area under the curve change scores (ΔAUC).
From MacDonald et al., (2019). Area under the curve change scores (ΔAUC) for each participant, plotted as a function of aerobic fitness (measured by maximal oxygen uptake ($\dot{V}O_{2max}$) immediately after exercise (A) and 30 min after exercise (B).

A study by McDonnell et al., (2013) examined the effect of two different cycling-based AE interventions on brain excitability. The first intervention was considered low-intensity (57% of age predicted HR for 30-min) with the second considered moderate-intensity (77% of age predicted HR for 15-min). A no exercise or 'rest' condition was included for comparative purposes as a third condition. Single-pulse TMS was administered pre- and post-exercise to examine MEP amplitude (an average of 15 TMS pulses at each time point). No significant changes in CSE were found following any of the three interventions.

Excitability changes in the upper limb following a single session of lower limb cycling, pre-, immediately post- and post-30 min were examined by Singh et al., (2014).

Several TMS measures were used to assess changes in excitability including, input/output curves, SICI, LICI and ICF of the extensor carpi radialis muscle. The exercise intervention selected was 20-min of cycling at 65-70% of age predicted HR_{max}. Due to its relationship to CSE, BDNF was also monitored in response to exercise during this study. The results of this study indicated significant increases in ICF and decreases in SICI following the exercise intervention. However, no changes were reported for input/output curves and LICI (Singh et al., 2014). Despite the failure to show increased input/output curves, the results indicate that exercise may be favourable for promoting conditions of plasticity in M1.

Other contradictory findings related to the effect of AE on brain excitability are indicated by a 2014 study, which had participants complete a cycling-based AE intervention, in two 15-min bouts, one of which was considered low intensity (40% of their predicted HRR) and the second high intensity (80% of their predicted HRR). In the first experiment, brain excitability was assessed via pre-post changes in active motor threshold, RMT and the S-R curve, while in in the second, SICI was examined. In the first experiment, no change in RMT was reported. However, the expected increase in MEP amplitude with higher stimulus intensities was observed. In the second experiment, a significant reduction in SICI was reported 15-min post-exercise. The findings from experiment two support the notion that AE administered in a single bout reduces $GABA_A$-mediated intracortical inhibition, a hypothesis that was explored regarding exercise-induced neuroplasticity (Singh et al., 2014).

In their 2020 scoping review, Hariri et al. (2020) reported the effects of low-, moderate-, and high-intensity AE on CSE. Table 1 provides a summary of the changes in

CSE from healthy participants (Andrews et al., 2020; Baltar et al., 2018; El-Sayes, Turco, et al., 2019; Garnier et al., 2017; Lulic et al., 2017b; MacDonald et al., 2019; McDonnell et al., 2013; Mooney et al., 2016; Morris et al., 2020; Neva et al., 2017; Singh et al., 2014; A. E. Smith et al., 2014, 2018; Yamazaki et al., 2019). This review further highlights the variability that exists regarding the effect of AE on CSE.

Table 1. Changes in CSE following AE interventions. Adapted from Hariri, 2020.

Study Name	Changes in Corticospinal Excitability		
	↑	↓	—
Singh (2014)			—
McDonnell (2013)			—
McDonnell (2013)			—
Mooney (2016)			—
Garnier (2017)	↑		
Garnier (2017)	↑		
El-Sayes (2019)	↑		
Smith (2014)			—
Smith (2014)			—
Lulic (2017)	↑		
Morris (2020)			—
Smith (2018)			—
MacDonald (2019)			—
MacDonald (2019)	↑		
MacDonald (2019)	↑		
Baltar (2018)			—
Baltar (2018)	↑		
Baltar (2018)		↓	
Andrews (2020)			—
Neva (2017)			—
Yamazaki (2019)			—
Yamazaki (2019)			—

Note. An increase in CSE is symbolized by a green 'up' arrow, a decrease in CSE is symbolized by a red 'down' arrow, and no change in CSE is symbolized by a blue 'dash.' Details related to the protocols used in each study is available here.

As highlighted in the previous sections, there is discrepancy in the literature regarding the impact of AE on CSE, with some studies showing a positive effect on CSE (i.e., increasing CSE) with others showing no effect. Most of the studies listed above focus on increased CSE following a bout of moderate-intensity AE prescribed at a percentage of HR_{max} or HRR. Given that there may be variability associated with prescribing exercise through relative intensities, it may be of benefit to prescribe the AE intensity relative to PPO, as PPO is an absolute value. When exercise is prescribed in relation to threshold/absolute measurements (i.e., PPO) compared to relative measurements (i.e., HR_{max}, HRR, $\dot{V}O_{2max}$) it is possible that absolute intensities may limit variation across metabolic and respiratory response to exercise (Mann et al., 2013), making absolute intensities optimal for prescribing exercise.

The current literature involving AE and the brain indicates positive effects related to optimal environments for neuroplasticity, however the exact dose of exercise which is recommended to induce these changes is not yet known and is still being explored (Nicolini et al., 2020). Depending on the exercise intensity (i.e., low, moderate, or high) various physiological changes occur. These physiological changes may be due to exercise induced increases in the concentration of metabolites such as BDNF, glucose, and lactate, all of which are used by the central nervous system on a molecular or functional level (Coco et al., 2010b, 2014; El-Sayes, Harasym, et al., 2019; Mang et al., 2013; Müller et al., 2020; Perciavalle et al., 2010a; Yang et al., 2014a). These metabolic changes may drive short-term changes in CSE, facilitating altered receptor activity, and in particular NMDA receptor activity within neurons in the brain as reported by Yang et al., (2014a).

Altered receptor activity within the brain may ultimately lead to increased CSE, however the exact mechanisms related to increased CSE continue to be explored.

2.5 Lactate

2.5.1 The Production of Lactate

Lactate is a by-product, which accumulates in the blood during glucose metabolism or Glycolysis, a process which occurs anaerobically (i.e., without the presence of oxygen) (Powers & Howley, 2018). Glycolysis refers to the breakdown of glucose or glycogen to form two molecules of pyruvate, which can later be converted to lactate. The process of Glycolysis is broken down into two phases, where phase one is known as the energy investment phase and phase two being the energy generation phase (Powers & Howley, 2018). When glucose is broken down during the energy investment phase, two stored adenosine triphosphates (ATP) are required to form phosphates. However, if glycogen is broken down, only one ATP molecule is required (Powers & Howley, 2018). In the energy generation phase, high energy electron carrier molecules nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD) allow for hydrogen (H^+) to be transported for later metabolic processes. When NAD^+ is reduced, it forms NADH (Powers & Howley, 2018). Under the presence of oxygen, the H^+ ions are shuttled to be used in the mitochondria during aerobic metabolism but when oxygen is not present, pyruvate accepts the H^+ ions and forms lactate (Powers & Howley, 2018), allowing Glycolysis to continue. The process of pyruvate converting to lactate is catalyzed via the enzyme lactate dehydrogenase (LDH) (Powers & Howley, 2018).

Aerobic metabolism occurs via two related pathways: 1) the citric acid cycle (CAC) and 2) the electron transport chain (ETC). The CAC allows for complete

oxidation of macronutrients (carbohydrates, fats, and proteins) via FAD and NAD^+. The CAC begins when pyruvate forms acetyl-CoA to enter the CAC for oxidation and produces 3 NADH and $FADH_2$ for the ETC (Powers & Howley, 2018). As a result, electrons are released, which travel to the ETC promoting the formation of ATP via oxidative phosphorylation. The ETC allows for aerobically produced ATP via $FADH_2$, NADH and water (Powers & Howley, 2018). The production and removal of lactate can occur within the skeletal muscle (Cruz et al., 2012) and the brain (Magistretti & Allaman, 2018). During light- to moderate-intensity exercise, metabolic processes occur via aerobic metabolism, meaning that oxygen is required for the production of ATP (Powers & Howley, 2018). With increasing exercise intensity and duration, blood lactate concentration will increase, specifically as individuals' approach/surpass their lactate threshold (LT).

Lactate threshold is a parameter of aerobic function, which is commonly used in exercise physiology and exercise prescription settings where it is used to assess physical working capacity (Belman & Gaesser, 1991; Powers & Howley, 2018). Lactate threshold is defined as the point where blood lactate tends to increase exponentially during graded exercise testing, commonly at 4 mmol/L (Goodwin et al., 2007; McArdle et al., 2015). There are four potential mechanisms for explanation of LT during incremental exercise, being: 1) lack of available oxygen for the muscle, 2) increased muscle glycogen breakdown due to heightened epinephrine, 3) recruitment of fast-twitch muscle fibers and 4) declined rate of lactate removal (Powers & Howley, 2018). A concept known as maximal lactate steady state is used to establish the peak aerobic power achieved without the accumulation of lactate for metabolic energy (Beneke et al., 2011). This concept

suggests that lactate concentration will level out during longer duration AE. The average value of maximal lactate steady state in individuals is approximately 4.0 mmol/L, ranging from 1.5 mmol/L to 7.0 mmol/L (Goodwin et al., 2007). With exercise, lactate is released into the blood stream following production from type II muscle fibers, where it can be used as an energy source by other cells (including, type 1 muscle fibers, cardiac fibers and the liver) (Cruz et al., 2012; Donovan & Pagliassotti, 2000). Secondary, an increase in blood lactate concentration results in build-up of lactate in the working muscle, that is then shuttled through the blood stream where it may be used by other organs (Coco et al., 2010a; Magistretti & Allaman, 2018).

2.5.2 Response of Lactate During Exercise

Following an exercise-induced increase in lactate, the body may respond by allowing lactate to act as a direct fuel source for the heart, skeletal muscle (Powers & Howley, 2018) and brain (Barros, 2013; Magistretti & Allaman, 2018), as well as being used as a substrate in the liver for the synthesis of glucose (Powers & Howley, 2018). During exercise, lactate will accumulate with increased intensity. An acute bout of AE will cause an increase in substrates important for metabolic functioning such as glucose, oxygen and lactate (Moscatelli, Valenzano, Petito, Triggiani, Cilibert, et al., 2016). The lactate shuttle is a concept which allows for the transportation of lactate around the body to be used as a fuel source (Powers & Howley, 2018). There are two methods to support the notion of the lactate shuttle hypothesis, including the intracellular lactate shuttle and the intercellular lactate shuttle (Brooks, 1986b; Todd, 2014). The intracellular lactate shuttle suggests that monocarboxylate transporters (MCT) allow for lactate molecules to be transported across the mitochondrial intermembrane space where lactate is upregulated

into the skeletal muscles (Todd, 2014). The intercellular lactate shuttle occurs when there is an excess of lactate generated via fast-twitch muscle fibers, and the lactate is transported to areas able to metabolize lactate such as slow-twitch muscle fibers (Todd, 2014). Lactate is known to attach to red blood cells altering the pH of blood, however this uptake is only proportional during AE, as with maximal exercise the rate at which lactate is produced exceeds the rate at which it can be metabolized (Gladden, 2004; Todd, 2014).

During AE, blood lactate concentrations may vary depending on the exercise intensity. Indeed, there is individual variability in response to exercise and training due to physiological and genetic factors (Mann et al., 2013). It is suggested that LT can be used as a parameter for establishing exercise intensity, as blood lactate concentration provides an indication of aerobic status and endurance performance (Powers & Howley, 2018). It is known that individuals who are considered trained exhibit increased LT in comparison to untrained individuals. This is evident in the relationship between LT and $\dot{V}O_{2max}$, where trained individuals reach their LT between 65-80% of their $\dot{V}O_{2max}$ and untrained individuals reach their LT at 50-60% of their $\dot{V}O_{2max}$ (Powers & Howley, 2018). With aerobic training, LT has been shown to increase (Goodwin et al., 2007). Since blood lactate concentration is a commonly used measure in exercise physiology (Goodwin et al., 2007; Mann et al., 2013; McArdle et al., 2015), aerobic training in conjunction with LT leads to improved endurance performance in runners and cyclists, suggesting that the same may occur for healthy individuals. Knowing this, we can expect that untrained and trained individuals may experience varied responses to blood lactate concentration depending on the exercise intensity. Understanding that LT values may vary based on

training status suggests that blood lactate concentration may be a factor involved in AE-induced alterations in CSE.

When individuals engage in AE that is prescribed at a fixed intensity relative to their own maximum (e.g., age predicted HR_{max}, HRR or PPO), some may be working below LT while others could be working above LT owing to their aerobic fitness. Typically, during exhaustive exercise interventions (i.e., maximum GXTs, as used in Coco et al., 2010), blood lactate concentration will increase exponentially, as participants reach their maximum work capacity. During steady-state AE however, it is likely that some individuals may experience an accumulation of blood lactate, while others may not, with the variability attributable to differences in aerobic fitness (and thus LT) across participants. As neither blood lactate concentration nor participants aerobic fitness are routinely assessed in studies examining the effect of AE on CSE, there is a considerable gap in knowledge. These changes in blood lactate concentration may be a factor driving the variability observed in CSE changes following AE interventions.

Within the brain, lactate is a valued contributor to normal function and is used by neurons and muscles during exercise (Schurr, 2008; Todd, 2014). Glucose is the preferred substrate for cerebral metabolism, however lactate acts as a substrate during periods of stress (e.g., exercise) (Barros, 2013; Magistretti & Allaman, 2018; Riske et al., 2017). When participating in high-intensity exercise, lactate concentration in arterial blood increases, which contributes to the consumption of lactate by the brain when the amount of glucose is decreased (Barros, 2013; Dalsgaard et al., 2004; Quistorff et al., 2008). The switch of fuel for metabolism from glucose to lactate during exercise allows for maintenance of optimal function of the brain in low glucose conditions (Barros, 2013;

Wyss et al., 2011). It is suspected that increased blood lactate concentration is associated with increased CSE, when assessed using TMS. Previous work has investigated these exercise-induced increases in blood lactate concentration with respect to brain excitability, which is discussed in the following section (Coco et al., 2010a; Moscatelli, Valenzano, Petito, Triggiani, Cilibert, et al., 2016; Perciavalle et al., 2010b).

2.5.3 How the Brain Responds to Lactate

The ability of the brain to produce and use lactate continues to be investigated. Lactate is thought to act as an important energy substrate for neural function (Todd, 2014). Lactate is a metabolite, which is used by neurons during exercise (Overgaard et al., 2012). Astrocytes metabolize glucose during exercise forming lactate as a by-product. Neurons not only receive lactate produced at the level of astrocytes but also from the blood stream (Brooks, 1986a; Magistretti & Allaman, 2018). This finding suggests that lactate can be shuttled between astrocytes and neurons, a concept coined the astrocyte-neuron-lactate shuttle hypothesis (Magistretti & Allaman, 2018; Overgaard et al., 2012). The communication between astrocytes and neurons is said to be crucial for memory formation (Müller et al., 2020; Suzuki et al., 2011). When exercise is involved, this hypothesis is said to contribute up to 33% of total energy substrate consumed, which ultimately supports the notion that lactate produced during exercise is metabolized by both astrocytes and neurons within the brain due to its ability to cross the blood brain barrier (Proia et al., 2016; Riske et al., 2017). Therefore, lactate, whether accumulated via Glycolysis in the periphery or through its production in the brain, is involved in homeostatic functions (Magistretti & Allaman, 2018).

Secondly, lactate may modulate cortical excitability based on preliminary findings where increased excitability in pyramidal cells under the presence of lactate in the hippocampal region of the brain was documented (Sada et al., 2015). Other evidence has been reported supporting the notion that lactate may play an important role in modulating metabolic functions, however the response of lactate may be dependent on factors related to signal pathways, transporters and the target neurons (Magistretti & Allaman, 2018). van Hall et al., (2009) showed that 30-min of cycling at 75% of $\dot{V}O_{2max}$ increased the uptake of lactate in the brain from rest conditions. The authors proposed that lactate concentration may be a confounding factor associated with exercise-induced plasticity due to its interaction with BDNF. It was hypothesized that lactate is associated with increased NMDA receptor activity within neurons (Müller et al., 2020; Yang et al., 2014a). Specifically, it is thought that an increase in blood lactate concentration is associated with increased NADH and Ca^{2+}, upregulating glutamate receptor activity (NMDA) (Yang et al., 2014a) and thus synaptic connectivity, a form of short-term neuroplasticity. Whether this effect of increased lactate concentration from AE manifests as increased corticospinal excitability is not entirely known.

2.6 Blood Lactate and Corticospinal Excitability

The impacts of neuromodulatory agents on brain excitability have been investigated in previous literature, particularly when induced by AE (Coco et al., 2014, 2014; Mang et al., 2013; Moscatelli, Valenzano, Petito, Triggiani, Ciliberti, et al., 2016; Perciavalle et al., 2010a). As mentioned, when one exercises, various metabolic reactions occur contributing to physiological changes. Of specific interest, blood lactate has been explored as an agent that can influence CSE. A series of studies have examined the effect

of blood lactate concentration on brain excitability following various exercise interventions. However, there are conflicting results as to whether blood lactate is associated with changes in brain excitability. Table 2 contains a summary of the following studies, including the participant demographics, methods, intervention and results (Coco et al., 2010b, 2014; Moscatelli, Valenzano, Petito, Triggiani, Ciliberti, et al., 2016; Perciavalle et al., 2010a).

Table 2. Summary table of the impact of blood lactate on CSE.

Author	Participants	Methods	Intervention	Results
Coco et al., (2014)	8 healthy males Mean age: (28.0±6.3 years) All participants were right-handed.	Hand-grip strain gauge dynamometer to produce tonic contraction. Cortical excitability assessed using 1mV resting motor threshold. Looking to find a compound MEP value. Capillary blood lactate measured end, post 5-min and post 10-min intervention.	Participants replicated 30% of MVG for 1 min and relaxed for 15 seconds. This was repeated until volitational fatigue.	Blood lactate increased at end of exercise, returning to pre-test levels by 10-min. MEP decreased at end of exercise and improved after 5-min before returning to pre-test levels. At end to post 5-min cortical excitability decreased and lactate levels increased, suggesting an increase in blood lactate is associated with decreased cortical excitability.

Coco et al., (2010a)	17 adult men Mean age: A) 20.8±2.2 years B) 22.5±2.2 years	Single pulse TMS at 120% of threshold intensity. Capillary blood lactate levels were measured, and MT were measured. All 17 participants completed an exhaustive exercise intervention (A). Only 6 of the 17 participants received an intravenous lactate infusion (B). Testing occurred pre, post, post 5-min and post 10-min.	Exhaustive exercise: Maximal multistage dis-continuous incremental cycling test on a mechanically braked cycle ergometer. Participants cycled at a constant RPM of 60, load increased by 30 watts ever 3 min until volitational fatigue or the subject was unable to maintain cadence. Intravenous lactate: a 2mEq/mL lactate solution was delivered intravenously	Exhaustive exercise: Increases in blood lactate and decreased in MT were observed. Intravenous lactate: increases in blood lactate and decreased in MT were noted.

Moscatelli, Valenzano, Petito, Triggiani, Ciliberti et al., (2016)	24 participants Mean age Athletes: 26.5±5 years Mean age Non-athletes: 25.5±4.8 years	TMS: MEPs were measured and averaged over 5 consecutive responses and the RMT and MEP amplitude were determined in each subject before fatiguing hand-grip exercises and at the end of the exercise. RMT was expressed as a percentage of maximum output power. To determine MVG: Right hand in hand dynamometer, grip to produce an isometric contraction. Average of highest 3 values of maximal isometric force generated by dominant hand. Capillary blood lactate was measured pre, post, 3-min post and 10-min post.	Fatiguing exercise: After subsequent rest, subjects were asked to reproduce 30% of the MVG for 4 seconds, relaxed for 2 seconds and then repeated for 10 min	Total Population (athletes and non-athletes): An increase in blood lactate concentration was associated with increased RMT and decreased MEP amplitudes. Suggesting that, an increase in blood lactate concentration was associated with decreased brain excitability.

Perciavalle et al., (2010b)	41 participants 20 Females: Mean age 21.0±1.6 years 21 Males: Mean age 21.7±1.8 years	Capillary blood lactate levels, blood glucose and MT were measured, post, post 5-min and post 10-min following the intervention. TMS stimulus intensity was set to 120% of RMT value. TMS was delivered in single pulse.	Subjects performed a maximal multistage discontinuous incremental cycling test on a mechanically braked cycle ergometer at 60 rpm increased by 30 watts every 3 min until volitational fatigue or they could not maintain cadence.	Female: Significant differences were found as an increase in blood lactate levels lead to decreased MT. Male: Significant increases in blood lactate levels and decreases in MT were reported. Following post 5-min, a significant difference between MT for males and females were found, in which females saw a greater increase in motor cortex excitability due to an increase in blood lactate in comparison to men. Suggesting the females are more excitable than males.

Note. MVG, maximal voluntary grip; mV, millivolts; MEP, motor evoked potential; TMS, transcranial magnetic stimulation; MT, motor threshold; RPM, revolutions per minute; RMT, resting motor threshold

Several studies have used interventions that result in fatigue to induce changes in blood lactate concentration. For instance, Coco and colleagues (2014) examined blood lactate concentration and brain excitability before and after a fatiguing intervention. Participants were required to replicate 30% of their pre-determined maximum voluntary grip using a hand-held dynamometer. The participants were asked to maintain a contraction at 30% of their maximum voluntary grip for 1 min, repeating the contraction with 15-second breaks between repetitions until failure or volitational fatigue (Coco et al., 2014). Cortical excitability was evaluated using TMS via the right flexor digitorum superficialis muscle of the contralateral hand of the hand involved in the fatiguing protocol. Briefly, before completion of the fatiguing protocol, the intensity of TMS required to elicit a MEP 1mV in amplitude in 5/10 trials was identified (Coco et al., 2014). Upon completion of the fatiguing protocol TMS was delivered at the same intensity as prior to the intervention. Blood lactate concentration and MEP amplitude were measured at pre-, immediately post-, post- 5-min and post- 10-minu of the fatigue intervention. The results of the study demonstrated that at the end of the exhaustive exercise and at the post- 5-min timepoint cortical excitability was decreased, and blood lactate concentrations were increased compared to baseline (Figure 7). As blood lactate concentration increased immediately post-exercise, MEP amplitude decreased, with a return to pre-exercise MEP amplitude as blood lactate concentration returned to pre-exercise levels. This finding suggests that an increase in blood lactate concentration results in decreased CSE.

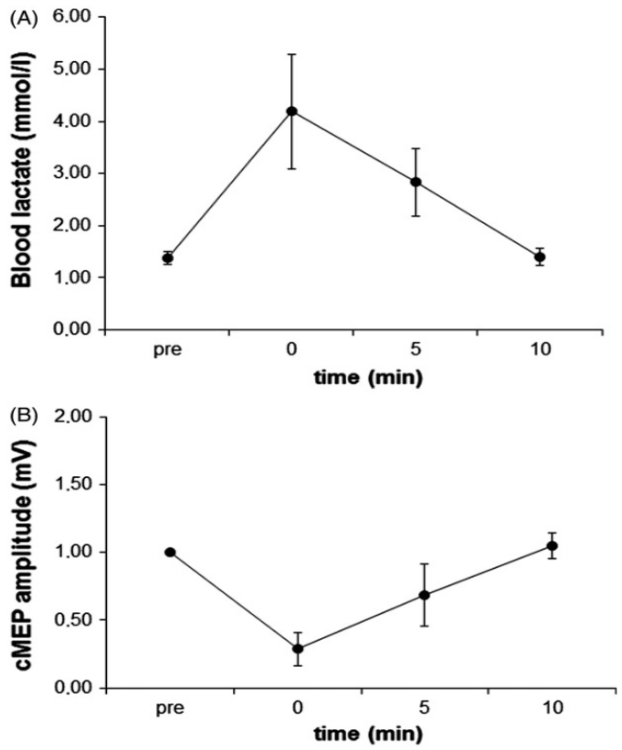

Figure 7. Blood lactate levels (mmol/L) and contralateral compound MEP (cMEP) amplitudes (mV) pre, post (0), post 5-min (5) and post 10-min (10) intervention. From Coco et al., (2014). Blood lactate levels increased significantly pre to 0 (1.3 mmol/L to 4.2 mmol/L) before beginning to decrease at 5 to 2.8 mmol/L and then recovering to pre-exercise levels of 1.4 mmol/L. cMEP amplitudes decreased significantly from pre to 0 (1 mV to 0.3 mV), before increasing to 0.7 mV at 5, and 1.1 mV at 10.

A more recent study used a similar approach to examine cortical excitability and lactate concentration in Taekwondo athletes and non-athletes (Moscatelli, Valenzano, Petito, Triggiani, Ciliberti, et al., 2016). Participants completed a hand-grip to fatigue intervention where participants produced 30% of their pre-determined maximum voluntary grip for 4 sec on and 2 sec off for a duration of 10-min. The flexor digitorum superficialis muscle was selected to assess changes in RMT (RMT%), MEP amplitude (mV) and blood lactate levels (mmol/L) from pre- to post- (end), post- 3-min (3 min) and post- 10-min (10 min) for athletes and non-athletes (Moscatelli, Valenzano, Petito, Triggiani, Ciliberti, et al., 2016). The relevant finding associated with this study was that

an increase in blood lactate was associated with a significant increase in RMT (Figure 8) and lower amplitude MEPs, with the latter finding depicted in Figure 9, showing that as blood lactate concentration decreased, MEP amplitude increased. This finding of decreased brain excitability with increased blood lactate concentration was observed in both athletes and non-athletes.

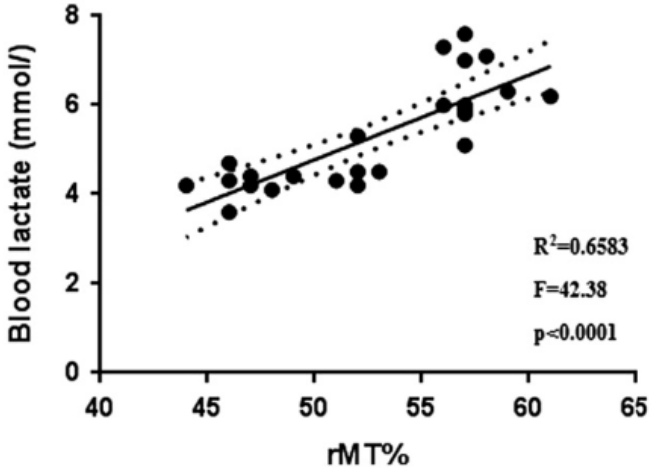

Figure 8. Correlation between blood lactate concentration and resting motor threshold, post fatigue intervention for total population.
From Moscatelli et al., (2016). A linear regression analysis revealed a significant, positive correlation between blood lactate and RMT (p<0.0001). Meaning that, as blood lactate concentration increased, a higher percentage of stimulator output was required to elicit RMT. The finding suggests that increased blood lactate concentration is associated with increased RMT values, signifying decreased brain excitability.

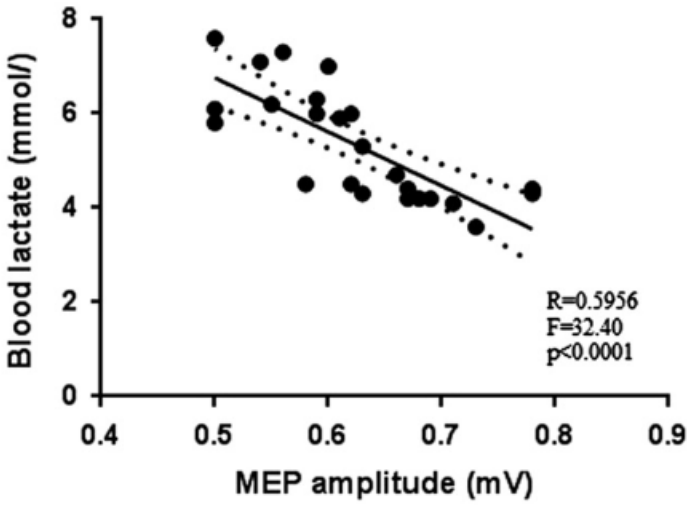

Figure 9. Correlation between blood lactate concentration and MEP amplitude (mV). From Moscatelli et al 2016. Linear regression revealed a significant negative correlation between blood lactate concentration and MEP amplitude (mV) (p<0.0001) post fatiguing intervention. This finding suggests that increased blood lactate concentration was associated with smaller MEP amplitudes, signifying decreased CSE.

In relation to exercise interventions, maximal exercise tests appear to be the common method of choice when examining the relationship between blood lactate concentration and brain excitability (Coco et al., 2010b; Perciavalle et al., 2010b). In one study, both male and females were examined in order to determine if there were sex differences related to blood lactate concentration and motor cortex excitability (Perciavalle et al., 2010b). The study examined capillary blood lactate concentrations and motor threshold (MT) values of the FDI pre-, post-, post- 2-min and post- 10-min following a maximal multistage discontinuous incremental cycling test. TMS was delivered in single pulses at 120% of the predetermined RMT value. An increase in blood lactate concentration was related to an increase in motor cortex excitability, with this increase observed in both males and females, however females showed a heightened response.

Coco et al, (2010) reported similar findings to that of Perciavalle et. al. (2010b). In this study participants blood lactate concentration and motor threshold values (obtained via the FDI) were assessed pre-, post-, post- 5-min and post- 10-min following an exhaustive exercise intervention. The study also included an intravenous lactate infusion of six participants. The lactate infusion was implemented to determine if the increase in lactate alone is capable of influencing motor cortex excitability. As displayed in Figure 10, in both conditions (i.e., exhaustive exercise and lactate infusion) there was a significant negative correlation between increased blood lactate concentration (mmol/L) and MT (%) at pre-, post-, post 5-min and post 10-min (Coco et al., 2010a). Therefore, the main finding from Coco et al., 2010, is that following a bout of exhaustive exercise, blood lactate concentration increased and was associated with a significant decrease in MT. This finding suggests that an increase in blood lactate concentration was associated with increased excitation of the motor cortex (Figure 10).

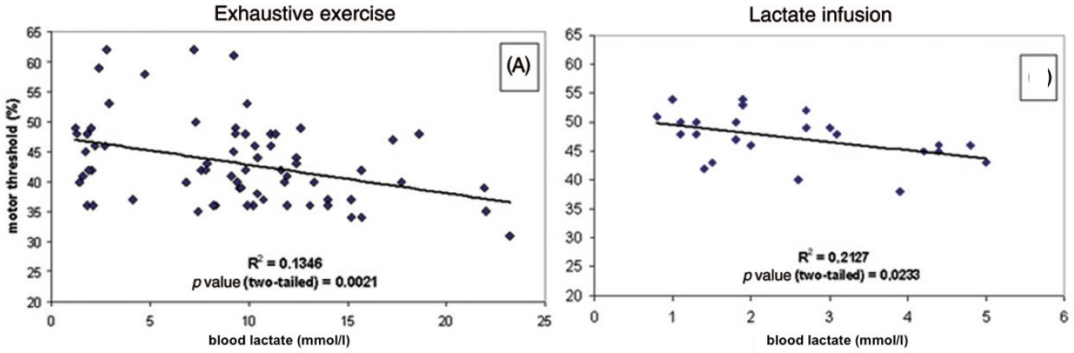

Figure 10. Relationship between blood lactate and motor threshold values for motor cortex excitation for total subject population.
From Coco et al., (2010). Where the figure on the left highlights the relationship between motor threshold (%) values and blood lactate (mmol/L) following a bout of exhaustive exercise and the figure on the right highlights the relationship between motor threshold (%) and blood lactate (mmol/L) following an intravenous lactate infusion.

Collectively, the findings from these studies indicates there is variability in the impact of increased concentration of lactate on brain excitability. In some studies there was an increase in CSE with increased blood lactate concentration (Coco et al., 2010a; Perciavalle et al., 2010b), whereas other studies found no relationship between increased blood lactate concentration and CSE or highlighted a decrease in CSE following an increase in lactate concentration (Coco et al., 2014; Moscatelli, Valenzano, Petito, Triggiani, Ciliberti, et al., 2016).

Across the aforementioned studies different exercise interventions were used, including those that involved exercise to fatiguing hand-grip exercise (Coco et al., 2014; Moscatelli, Valenzano, Petito, Triggiani, Ciliberti, et al., 2016) and exhaustive exercise (Coco et al., 2010a; Perciavalle et al., 2010b). To our knowledge, the effect of altered blood lactate concentration during steady state AE has not been investigated. The literature examined in the previous sections regarding the effect of AE on CSE showed moderate-intensity AE to be optimal in facilitating significant alterations in CSE (El-Sayes, Harasym, et al., 2019; Hariri, 2020). However, there is variability in the response both across and within studies (i.e., some participants show increased CSE while other do not). As none of these studies examined the effects of metabolic by-products, which may accumulate during AE, it is possible that the variability in the brains response to AE is attributable to a change in lactate concentration in the blood and should be considered in such work.

2.7 SUMMARY

Review of prior literature highlights the relationship between AE and CSE, and the potential role that a change in blood lactate concentration may play in mediating CSE.

Through previous evidence, we've learned that moderate-intensity AE drives increases in CSE when assessed via TMS. A gap in the literature linking the effect of an acute bout of moderate-intensity AE on CSE and the inter-individual variability of metabolites, which accumulate during physical activity and exercise exists. It is important to understand whether these metabolites impact CSE. Further, there is variability in past work regarding the methods for prescribing AE in that approaches have included working at percentages of HR_{max} or $\dot{V}O_{2max}$. These methods are subject to variability across participants, and therefore, an absolute measure of intensity, such as working at a percentage of PPO, would provide a better approach as the work performed by a given participant would be less variable compared day-to-day. Understanding that at a moderate-intensity of exercise, some individuals may be working above their lactate threshold and others may be below, which may explain the varying results (i.e., increased, decreased or no change in CSE) across AE studies where CSE was examined. This poses the question, is there a confounding variable associated with alterations in CSE?

There is variability that exists in these studies, as in some instances, changes in CSE are observed, while in other instances it is not, despite moderate-intensity AE interventions being used. Metabolic by-products, such as lactate, accumulate in the blood during intense physical activity and exercise. However, this accumulation varies between individuals. During moderate-intensity exercise, some participants may exceed their lactate threshold (i.e., transition from aerobic to anaerobic systems) while others remain below their lactate threshold. This variability in lactate production and accumulation may explain the variability observed in studies examining AE and CSE yet changes in lactate concentration have yet to be considered in this body of work.

CHAPTER 3: STUDY OBJECTIVE AND RESEARCH QUESTIONS

To our knowledge, no studies have examined the effect of altered blood lactate concentration on CSE following an acute bout of moderate-intensity AE. Understanding blood lactate concentrations during steady-state moderate-intensity AE will aid in the understanding regarding the potential relationship between lactate and brain excitation as assessed by CSE; specifically, whether changes in CSE are in response to a change in blood lactate concentration. Hence, the purpose of this study is to determine whether a change in blood lactate concentration induced by an acute bout of moderate-intensity AE will alter CSE. The results from this study will indicate whether blood lactate is a mediating factor involved in increased CSE induced by moderate-intensity AE.

Our research seeks to answer two questions: Firstly, does a change in blood lactate concentration induced by an acute bout of moderate-intensity AE alter CSE in healthy adults? Given the findings of previous studies showing group-level effects of AE on CSE, we also sought to determine if an acute bout of moderate-intensity AE will increase CSE. We hypothesized that in healthy participants there would be a positive relationship between blood lactate concentration and CSE in that increased blood lactate concentration would result in increased CSE. Regarding our secondary question, we anticipated an overall increase in CSE following a bout of moderate-intensity AE when comparing pre and post measures.

CHAPTER 4: METHODOLOGY

4.1 Participants

Sample size was estimated to be 29 participants using a moderate effect size (f = 0.5), assuming a one-tailed, $\alpha = 0.05$ and $\beta = 95\%$ power for a linear regression model (one predictor value) (G*Power, v3.1) where the effect of blood lactate concentration on CSE was observed. Individuals identifying as male and female between the ages of 18-40 years of age were eligible to participate, with the goal of obtaining near equal distribution of males and females. Participants had no self-reported history of neurological, cardiovascular (including hypertension), pulmonary disorders, and were deemed safe for exercise. Participants were required to complete screening protocols for non-invasive brain stimulation (TMS) and for blood collection to ensure eligibility for participation. The study was approved by the Dalhousie University Health Sciences Research Ethics Board and each participant provided written, informed consent. Participants were included in the study based on the following inclusion and exclusion criteria.

Inclusion criteria included:

1. Individuals aged 18-40 years of age.
2. Suitability to perform exercise safely (as assessed by the PAR-Q+).
3. Suitability to engage in non-invasive brain stimulation (TMS).
4. No self-reported history of neurological, cardiovascular, or pulmonary disorders.

Exclusion criteria include:

1. Having respiratory disorders, hypertension or other cardiovascular diseases that would preclude participating in exercise.

2. Having any contraindications or non-responsive to TMS (as assessed by the TMS screening form).
3. Having a Body Mass Index ≥ 30kg/m^2
4. Is a regular smoker.

4.2 Study Outline

Participants completed two sessions. The first session took approximately 90-min and consisted of 'hotspotting' (explained in 'Transcranial Magnetic Stimulation' below) via TMS to ensure responsiveness to the stimulation. Participants who were responsive to TMS were administered a GXT to determine their PPO. The second session took approximately 120-min to complete and involved a 20-min bout of moderate-intensity AE bookended by an S-R curve and measurements of blood lactate concentration. These measures were completed before (Pre), immediately after (Post) and 10-min post (Post10) AE. Blood lactate measures were collected at the 5-min and 15-min mark following AE to monitor the participant's blood lactate response to exercise. Figure 11 demonstrates the procedures of the study.

Session 1 Overview

Session 2 Overview

Figure 11. Visual representation of components involved in session 1 and 2.

4.3 Procedures

4.3.1 Session One

Participants were asked to refrain from caffeine, heavy meals, and alcohol for at least 2 hours prior to the study. As well, participants were encouraged to avoid significant exertion or exercise prior to the study, get 6-8 hours of sleep, and to stay well hydrated prior to completing session 1 and 2. Prior to the start of session 1, all participant completed the relevant documentation to ensure eligibility for participation (Consent Form – Appendix A, Physical Activity and Readiness Questionnaire – Appendix B, and TMS screening form – Appendix C). A series of questionnaires including a health history questionnaire (Appendix D) and the International Physical Activity Questionnaire (IPAQ) (Appendix E) were also administered at this time. Finally, participants were provided with a brief verbal overview of the study and the participants' height (cm) and weight (kg) was recorded to the nearest tenth.

Maximal Graded Exercise Test

A GXT was administered to determine each participant's PPO, which was then used in the subsequent session to determine the intensity of the AE intervention. The GXT was administered on a stationary up-right cycle ergometer (Lode Corival Cpet, Lode BV, Gronigen, Netherlands). The ergometer was electronically braked with a modifiable load (resistance) in watts (W) and was adjusted via external software provided by the manufacturer to ensure the workload was maintained adequately during the duration of the test. Each participant was equipped with an arm mounted HR monitor (Polar Electro Inc., 2022, Lake Success, NY, USA) to monitor HR during the duration of the test. Polar arm-worn optical HR monitors are valid for obtaining HR measures during

moderate and high-intensity exercise (Hettiarachchi et al., 2019). The device sampled HR every second and HR measures were recorded every 2-min manually during the GXT to monitor the participants response to exercise.

The GXT protocol mirrored that of prior work in the laboratory (Lanzi et al., 2014, 2015). Participants were seated in a chair for 5-min prior to the start of the test to determine their resting HR. Resting HR was used during the cool-down portion of the test to ensure that the participants HR returned to resting levels. Participants began the test with a short warm-up where they cycled for 5-min at an intensity of 40 W. This warm-up period allowed for familiarization with the bike and the ability to prepare themselves for strenuous exercise. Following the warm-up, the workload increased by 20 W every minute following a ramp protocol until the participant was unable to complete the test further (i.e., exhaustion), the participant asked to stop, their cadence dropped below 60 RPM or for other reasons (summarized in Table 3). Self-reported rating of perceived exertion (RPE) on a scale of 6-20 was monitored for the duration of the test using the Borg Scale (Borg, 1982) (Appendix F). Participant RPE was recorded immediately following the warm-up and every 2-min during the GXT. The investigator held the Borg Scale in front of the participant and asked for a verbal rating. Given that the GXT was designed to be short in duration the length typically ranged between 8 and 12-min. Participants were instructed to provide a cue when they believed they had approximately 1-min remaining in the test. A final measurement of RPE and HR was performed at that time. Each participant completed a 5-min cool down period at an intensity of 50 W to help lower their HR to at, or near resting levels. Participants were instructed to avoid holding or gripping the handlebars of the ergometer while doing the GXT and while

completing the AE intervention during session 2 to avoid potential activation of the hand and forearm muscles being investigated through TMS.

To be considered a true maximal GXT, the following two criteria must be met:

1. A final RPE on the Borg scale ≥ 17.
2. Peak HR equal to or within 12 beats of the participant's age predicted HR_{max}, determined by the formula (Tanaka et al., 1997): Age predicted HR_{max} = [206.9 – (0.67 × Age)].

Table 3. General indications to stop an exercise test.
Adapted from, (American College of Sports Medicine et al., 2018).

General Indications of Stopping an Exercise Test in Adults
- Onset of angina or angina like symptoms - Drop in (systolic blood pressure) SBP >/= 10 mmHg with an increase in work rate of if SBP decreases below the value obtained in the same position prior to testing - Excessive rise in blood pressure (BP): SBP >/= 250 mmHg and/or diastolic pressure >115 mmHg - Shortness of breath, wheezing, leg cramps and claudication - Signs of poor perfusion: light-headedness, confusion, ataxia, pallor, cyanosis, nausea or cold and clammy skin - Failure of HR to increase with increased intensity - Noticeable change in heart rhythm by palpation or auscultation

Peak power output (PPO) was defined as the maximum wattage (recorded via the ergometer) achieved during the GXT. Moderate-intensity (i.e., 60%) was determined by multiplying the PPO by 0.6. If participants were unable to maintain 60% of PPO during the moderate-intensity AE session the wattage was decreased by 10 W for a maximum of two decrements.

Transcranial Magnetic Stimulation

Transcranial magnetic stimulation was delivered during both sessions at the specified testing points, however measures of CSE were only recorded during the second session. During the first session, RMT was determined to ensure the participant was

responsive to TMS via hotspotting. For session two, RMT was determined via hotspotting and then used to determine the stimulator intensity for the S-R curves and measurement of CSE. Stimulation was applied through a 70 mm figure-of-eight coil (Magstim Double 70mm Alpha Coil) connected to a Magstim BiStim^2 magnetic stimulator (Magstim, Whitland, UK). During stimulation, participants were seated comfortably on a chair in a slightly reclined position, with the right arm placed on an armrest for comfort. Brainsight 2^{TM} (Rogue Research Inc., Montreal, Canada) neuronavigation was used to guide the position and orientation of the coil over the target motor region using a template MRI. A template MRI is an anatomical MRI that is derived from a population of 152 neurologically healthy, meant to be a non-biased representation of the population.

Muscle Activity

Electromyography (EMG) was acquired from the first dorsal interosseous (FDI) muscle during TMS. An upper limb muscle was chosen owing to the interest in the generalized effects of AE on cortical excitability (i.e., exercise performed with the lower limb impacting excitability of the representation of an upper limb muscle). Moreover, recent literature (Lulic et al., 2017b; A. E. Smith et al., 2014) used the FDI in determination of CSE, thus obtaining these measures from the same muscle across studies facilitates comparison of results. The EMG signal was acquired using self-adhering electrodes (1 × 3 cm; Q-Trace Gold; Kendall-LTP, USA) in a bipolar configuration with a 1 cm inter-electrode distance, sampled at 1000Hz with a bandpass of 1-500 Hz (1902 and Power 1401; Cambridge Electronics Design, UK) and stored for offline analysis. Electrodes were placed on the FDI muscle belly, approximately one

centimeter proximal (active electrode) and distal (reference electrode) to the metacarpophalangeal joint. To confirm the accuracy of the electrode placement, the participant was asked to abduct their second digit, while the researcher palpated the muscle belly.

Co-registration and Localization of the Motor Hotspot

To configure the target position (in this study the representation of the FDI muscle in M1), the participant's head was co-registered to the template MRI using the neuronavigation software and a Polaris optimal position sensor (Northern Digital Inc., Canada). Within the Polaris sensor are two infrared cameras, emitters, and associated electrics, which communicate with the BrainSight computer. There are retro-reflective markers that capture movement via the Polaris optical sensor because they reflect infrared light emitted by the sensor. These trackers are affixed to the subject tracker, coil tracker and pointer tool, highlighted in Figure 12. These tools are of importance because they calculate the position and orientation of the devices based on the information provided by them in space. Prior to the TMS session, the participant was asked to wear the glasses with the attached tracker, so their head position could be monitored. Co-registration was achieved by aligning five anatomical landmarks on the participant (glabella, nasion, right and left pre-auricular points and tip of nose) with the corresponding anatomical landmarks on the template MRI (MNI152_T1_1mm).

Figure 12. TMS glasses and pointer for co-registration

The muscle of interest targeted in the study was the FDI, where the left M1 was targeted. The FDI was selected because of its role in studies utilizing TMS as the hand representation within M1, known as the "hand knob" (Yousry et al., 1997). Secondly, given that participants were instructed to execute all exercise interventions (GXT and AE intervention) with their hands relaxed at their sides, there is limited involvement and pre-activation of the muscle prior to the stimulation. In previous exercise studies where inconsistencies regarding cortical responses were seen, the FDI was the selected muscle highlighting potential variability across studies (El-Sayes, Turco, et al., 2019; Lulic et al., 2017a; McDonnell et al., 2013; A. E. Smith et al., 2014).

A 5 × 5 grid with 7.5mm spacing was overlaid on the template brain with the mid-point (location 2, 2) centered on the estimated location of the FDI muscle representation of the left primary motor cortex (M1) (Kleim et al., 2007). Stimulator output was set to 40% and points on the grid stimulated starting from 2,2 (Figure 13) with the coil positioned tangentially to the scalp with the handle at a 45° angle to the posterior. To

identify the motor hotspot of the right FDI muscle, we worked outwards from the centre point of the grid (2,2) in a counter-clockwise manner to determine the location(s) that produce the highest amplitude MEPs for 5 out of 10 stimulations.

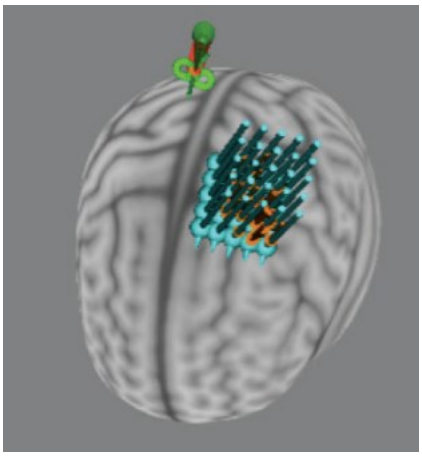

Figure 13. Target grid for hand-knob of FDI over M1.

Determining Resting Motor Threshold

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

4.3.2 Session Two

Exercise Intervention and Testing/Aerobic Exercise Protocol

The AE intervention was performed on the same cycle ergometer and consisted of a 20-min bout at 60% of the participant's PPO as determined in session one. This exercise intensity and duration were consistent with moderate-intensity AE prescribed relative to PPO utilized in previous studies where moderate-intensity exercise was

prescribed in relation to PPO (Mekari et al., 2015; Pinot & Grappe, 2014). As in session one, HR was monitored throughout the intervention using the arm mounted Polar HR monitor.

Participants performed a 5-min warm-up at the beginning of the session to prepare for exercise. The warm-up involved cycling at a 50 W workload for 2-min; for the remaining 3-min the workload increased gradually to the participants predetermined exercise intensity (e.g., if 60% of a participants PPO equates to 150 W, following a 2-min warm-up at 50 W the participant will gradually increase from 50 W to 150 W over the course of 3-min). Participants were allotted a 5-min cool-down period at the end of the session to return HR back to near resting levels. Throughout the exercise session, participants were instructed to rest their arms comfortably by their sides and not to grip the handlebars. During the AE intervention, participants maintained 60% of their PPO. Participants were instructed that if they were unable to maintain the 60% intensity, the intensity could be lowered by 10 W decrements up to two times during the session. The workload was maintained electronically using the ergometer's software. As above, participants maintained 60% PPO for the duration of the test (unless requested to decrease as mentioned above), and therefore the resistance (wattage) was adjusted electronically to maintain their PPO. Participants were instructed to maintain a cadence of 60-80 RPM throughout the AE intervention to maintain an optimal RPM for cycling (Coast et al., 1986).

Transcranial Magnetic Stimulation

Transcranial magnetic stimulation in session two was delivered in a similar manner as session one, however during the second session a custom-programmed

paradigm via Signal (Signal v 6.0, Cambridge Electronics Design, UK) software was used. Single-pulse TMS measures were delivered through this custom-programmed paradigm, which controlled the stimulator externally by setting the stimulator intensity and subsequent timing of the TMS coil at the aforementioned timepoints (Pre, Post, and Post10).

Stimulus-Response Curve

After localization of the hotspot, and determination of the RMT, a pre-AE stimulus-response (S-R) curve was obtained. An S-R curve is a plot of MEP amplitude over increasing TMS intensity. The S-R curve was created through the delivery of 50 single pulses of varying intensity over the motor hotspot; 10 single pulses were delivered at each of the following stimulus intensities: of 100%, 110%, 120%, 130%, and 140% of RMT. These single pulses were delivered with a variable interval of 3 or 4-seconds between successive stimuli. The pulse intensity was randomized via Signal software (Signal v 6.0, Cambridge Electronics Design, UK) which was also used to collect and analyze the corresponding EMG data. The delivery of pulses was randomized to avoid an anticipatory effect of participants knowing when a pulse would be delivered to avoid preferential activation of the FDI muscle. The peak-to-peak MEP amplitude for each stimulus was measured, and the average amplitude evoked by the 10 pulses at each stimulus intensity was calculated. The averaged MEP amplitudes were then used to generate an S-R curve. S-R curves were generated at the following timepoints: Pre, Post and Post10 minutes following the bout of AE.

The use of single-pulse measures only was established as the purpose of this study was to examine the relationship between lactate concentration and CSE. The simplest

approach to this measure was to see if there is a single change (i.e., increase, decrease or no change) following a bout of AE. Given the variability in previously conducted literature involving the effects of AE on CSE, we decided to use only single-pulse measures to assess if there is a change occurring in the brain following an increase in blood lactate concentration. Secondly, given the time between our post-exercise sessions (10-min), we were limited in time between testing points on including both a S-R curve and paired-pulse measures. Therefore, we made the decision to include only single-pulse measures in conjunction with blood lactate measures.

Blood Lactate Concentration Measurements

A 20 uL sample of blood (like that required for regular blood-glucose testing using a glucometer) was collected via finger-tip and analyzed using a Biosen R-Line Lactate and Glucose Analyzer (EFK Diagnostics, Barleben, Germany) to determine the concentration of lactate ions in the blood. Blood samples were collected at various timepoints during the second session of the study including, before (Pre; resting value), during AE at min 5 and min 15, end (Post), and 10-min after (Post10) completion of the 20-min bout of AE. At each time point, 3 blood samples were obtained to allow for an average measure of blood lactate concentration to be determined at each time-point to ensure accuracy. As a result, a total of 15 samples were obtained (i.e., 3 samples at 5 timepoints).

Each blood sample was obtained via the lateral aspect of the finger pads of the distal digits on the left hand. The area was cleaned and sterilized using an alcohol swab. The skin was punctured with a small disposable lancet (ACCU-Chek Safe-T Pro Plus Blood Lancet, 23-gauge, 1.8 mm depth) to obtain a blood sample. A gentle squeeze was

applied around the puncture site to ensure that the drop of blood was large enough to fill the capillary. The capillary tube was held at a slight angle (approximately 45 deg) using a capillary holder, to ensure that the tube was filled with no air bubbles. Once the capillary was full, it was placed into a pre-filled micro test tube. The lid of the test tube was closed and then the inverted 10 times to ensure that the solution is homogenously mixed. Participants were provided with bandages as required. For each sample, a new disposable lancet was used and was disposed of in the appropriate biohazardous waste container.

4.4 Data Analysis

4.4.1 Participant Demographics

Participant demographics were calculated via a Microsoft Excel document (Microsoft Corporation. (2018). *Microsoft Excel*. Retrieved from https://office.microsoft.com/excel). Descriptive statistics were completed to determine the mean, standard deviation (SD), and range of values for all participants.

4.4.2 IPAQ Scores

The participants IPAQ scores were calculated using the appropriate IPAQ scoring guidelines (see Appendix G) via a Microsoft Excel document. The scores were determined by calculating the frequency and duration of physical activity for vigorous-intensity, moderate-intensity, and walking activities for each participant. The scores were calculated using an equation regrading METs associated with the intensity of activities mentioned (described as MET-minutes/week). Each participant was then scored as high (>1500 METs), moderate (>600 METs) or low (<300 METs) regarding their physical activity.

4.4.3 Lactate Data

The device required a calibration using standard solutions and quality control materials to ensure that the device was reading accurately. Once calibrated, the homogenously mixed test tubes were placed in their intended position for the commencement of the measurement. When all 15 samples were inputted into the device the analysis process began, and results were displayed on the device and recorded by hand. Lactate data was reviewed manually, and values which were greater or less than 2 mmol/L when compared to the remaining values at a specific timepoint were removed from the subsequent analysis.

4.4.4 EMG Data

Analysis of our MEP data was conducted in the same manner as work previously conducted in the laboratory. A CFS file containing the EMG data from all timepoints and obtained using Signal was imported into RStudio (RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/). Analysis of the EMG data (which contained the MEPs) occurred in four automated steps. First, each trial was trimmed to 1200ms and included a 1000ms pre-pulse window. Second, trials were baseline corrected by subtracting the median value obtained from samples within that timepoint. Third, 60Hz line noise was removed using a Discrete Fourier Transform filter. Fourth, the peak-to-peak amplitude of the MEP was determined by selecting the region of the signal containing the MEP (10-50ms post-pulse) and then finding the minimum and maximum peaks within that window. The difference between the minimum and maximum peaks was then determined. As voluntarily contraction in the target muscle prior to the delivery of the

TMS pulse can alter MEP amplitude the pre-TMS pulse EMG activity was examined to ensure the absence of muscle activity during the pre-pulse period. Briefly, we determined the root mean square amplitude of the EMG signal in the 500ms period prior to the TMS pulse and compared it to the amplitude in the first 500ms. Trials in which the pre-pulse EMG amplitude exceeded 2 standard deviations of the first 500ms were removed from analysis.

MEPs with low signal-to-noise ratio were also removed from analysis. Trials where the amplitude of the MEP was smaller than the amplitude range (max - min) of the 1000 ms pre-TMS pulse EMG window were flagged and removed automatically. Similarly, MEPs with amplitudes <25 mV and with peak onsets that were less than a plausible latency (< 15 ms post-pulse) were also excluded. The MEP data were reviewed manually to ensure the MEP peak-to-peak amplitudes were logical and a represented a true MEP response.

The script determined the number of MEPs obtained at each stimulator intensity. A minimum of 4/10 MEPs were required at each intensity and time point for it to be included in further analysis (e.g., if only 2/10 MEPs were obtained at 100% RMT intensity at the post timepoint, the participants data at that timepoint was removed). In instances where data for specific intensities was removed due to having less than 4/10 MEPs, the data were extrapolated (Prism 9.5.1) to fill the missing data points to create a complete S-R curve at each timepoint. Extrapolation occurs by extending the x values beyond the range of data (typically equal to half the difference between Xmin and Xmax). These values were then divided into 1000 line segments, and the software aims to

extrapolate within the range of the x value data points, and the extrapolation is determined through the line segment to be as accurate as possible.

4.5 Statistical Analysis

Statistical analysis was completed in SPSS (IBM Corp. Released 2021. IBM SPSS Statistics for Macintosh, Version 28.0. Armonk, NY: IBM Corp). A series of one-way repeated measures ANOVAs were conducted to assess changes in blood lactate concentration and CSE as a factor of time (Pre, Post, Post10). Change scores of blood lactate and CSE data between timepoints were calculated and used in a linear regression model to assess the relationship between blood lactate and CSE. Outliers were removed based on the boxplots of CSE data at Pre, Post and Post10 timepoints (see Appendix J).

4.5.1 CSE Data

Three S-R curves were generated for each participant (one for each timepoint). Peak-to-peak amplitude of the MEPs included in the analysis were averaged for each intensity (i.e., > 4/10 trials at 100% RMT were averaged to a single data point). The average MEP amplitude was then plotted to create the S-R curve. Area under the curve (AUC) was calculated (Prism 9.5.1), as AUC provides a global estimate of CSE (Peri et al., 2017). A change score between the AUC values from Pre-Post, Pre-Post10, and Post-Post10 were calculated for each participant to be used in the statistical analysis. To ensure that the data were normally distributed, a Shapiro-Wilks test was conducted ($p>0.05$). To assess if CSE was altered as a result of the AE intervention, the AUC change scores were analyzed via one-way repeated measures ANOVA with the factor of time (three levels: Pre, Post, Post10). Mauchly's test for sphericity was completed and if violated, a Greenhouse Geisser correction was applied.

4.5.2 Lactate Data

A change score for blood lactate concentration was first calculated between Pre-Post, Pre-Post10, and Post-Post10 for each participant. To assess the change in blood lactate values following moderate-intensity AE, a one-way repeated measures ANOVA was completed with the factor of time (three levels: Pre, Post, Post10). Bonferroni adjustments were completed for post hoc comparisons where appropriate. To ensure that the data was normally distributed, a Shapiro-Wilks test was conducted ($p>0.05$).

4.5.3 Relationship Between CSE and Lactate

A linear regression was used to determine the relationship between blood lactate concentration and CSE at 3 timepoints (Pre-Post, Pre-Post10, and Post-Post10). Change scores from excitability measures (AUC values) and blood lactate concentrations were used. Significance was denoted throughout by an alpha value of $p<0.05$.

CHAPTER 5: RESULTS

5.1 Participant Demographics

A total of 41 participants were recruited to participate in this study, however eight were deemed unresponsive to TMS during the first session and therefore were ineligible to participate in the subsequent sessions, two participants requested to stop data collection during the second session, three participant's data were incomplete due to a technical issue with the equipment, one participant experienced an adverse event during the second session, and three participants were removed during the statistical analysis due to being outliers. As a result, a total of 24 participants (13 female) were included in the analysis. The average age of these participants was 23.7 ± 2.7 years with an average height and weight of 169.9 ± 11.0 cm and 71.6 ± 15.9 kg. All participants had a body mass index (BMI) of <30 (average 24.5 ± 3.3). On average, participants completed the 2 sessions within 4 ± 2 days. Each participant was scored based on their IPAQ results as being either highly (MET value > 3000 METs) or moderately (MET value < 3000 METs) physically active, where 7 participants were categorized as moderately physically active. Demographic data for each participant is provided in Table 4.

Table 4. Participant demographics

Participant #	Sex	Age	Height (cm)	Weight (kg)	BMI	IPAQ Score	IPAQ Category
P001	F	21	155.0	56.8	23.6	15648	High
P002	F	25	152.0	65.0	28.1	2772	Moderate
P003	M	21	182.0	87.0	26.3	7890	High
P005	F	29	170.0	74.4	25.8	1716	Moderate
P006	M	23	183.0	89.0	26.6	7278	High
P007	F	25	163.0	61.5	23.1	8625	High
P008	M	22	162.0	66.4	25.3	4293	High
P009	F	24	166.0	54.0	19.6	4548	High
P010	F	28	158.0	68.0	27.2	7236	High
P011	M	26	172.0	81.3	27.5	5706	High
P012	F	23	167.0	79.1	28.4	4266	High
P013	M	21	171.5	67.0	22.8	9570	High
P016	F	20	160.0	63.2	24.7	4434	High
P017	M	24	187.0	101.5	29.0	1520	Moderate
P019	M	24	184.0	87.5	25.8	3200	High
P021	F	23	168.0	58.9	20.9	5370	High
P022	F	24	174.0	68.9	22.8	2214	Moderate
P023	F	23	170.0	66.4	23.0	2373	Moderate
P024	F	19	152.0	37.7	16.3	928	Moderate
P026	M	25	165.0	65.5	23.0	5859	High
P027	M	24	182.0	72.8	22.0	3199	High
P029	M	21	187.5	100.0	28.4	3297	High
P030	F	23	164.0	53.4	19.9	3426	High
P034	M	30	183.0	96.4	28.7	1386	Moderate

5.2 Maximal Exercise Test

Of the 24 participants, seven did not reach 95% of age-predicted HR and one did not reach the required RPE (i.e., between 16-20). The remaining 17 participants met the criteria for a GXT. A range of PPO values were obtained (128 to 360 W) with the average PPO being 236.2 ± 52.8 W. Details pertaining to the GXT for individual participants are shown in Table 5.

Table 5. Summary of individual GXT results.

ID	PPO (W)	Time of Test (min:sec)	HRmax (bpm)	95% of age predicted max	Max RPE	True GXT? Y/N
P001	198	8:58	203	183	19	Y
P002	210	9:00	196	180	19	Y
P003	238	9:37	193	183	20	Y
P005	206	8:20	197	178	18	Y
P006	337	12:49	176	182	19	N
P007	226	8:20	190	181	20	Y
P008	211	9:02	203	183	20	Y
P009	201	8:05	175	181	18	N
P010	236	9:43	175	179	16	N
P011	360	17:00	183	180	20	Y
P012	221	8:55	167	182	18	N
P013	230	10:08	194	183	19	Y
P016	208	8:25	201	182	20	Y
P017	257	11:00	183	182	18	Y
P019	306	13:25	174	182	19	N
P021	215	8:43	181	182	19	Y
P022	237	9:46	181	181	17	Y
P023	203	8:10	178	182	20	N
P024	128	4:25	187	185	19	Y
P026	238	9:54	163	181	19	N
P027	318	13:42	185	181	19	Y
P029	293	12:40	189	183	19	Y
P030	216	8:48	189	182	20	Y
P034	176	7:45	177	177	20	Y

Note: Age-predicted HR was determined using the following equation 206.9 – (0.67 x age) by Tanaka et al., 2001.

5.3 Moderate-Intensity Exercise

Intensity of the AE session ranged from 77 W to 216 W with the average PPO for the moderate-intensity AE being 141.8 ± 31.7 W. Participant RPE scores ranged from 10-19 during the session. During AE, three participants were unable to maintain the prescribed AE intensity and requested that the intensity be decreased. The average HR for

participants during moderate-intensity AE was 160 bpm and ranged from 138 to 179 bpm. Table 6 reports individual participant data related to the moderate-intensity AE intervention.

Table 6. Summary of moderate-intensity AE results for each participant.

Participant #	60% PPO (W)	Days since session 1	Average HR (bpm)	RPE range
P001	119	3	172	13-15
P002	126	2	174	13-17
P003	143	2	158	13-16
P005	124	2	161	13-14
P006	202	8	158	13-17
P007*	136	6	167	13-17
P008	127	1	172	12-19
P009	121	2	153	12-14
P010	142	6	153	11-13
P011	216	5	163	11-13
P012	133	3	138	11-14
P013	138	2	177	13-15
P016*	125	6	164	14-16
P017	154	2	179	14-16
P019	184	2	147	12-17
P021	129	6	157	12-15
P022	142	6	157	11-18
P023	122	7	156	12-15
P024*	77	3	170	15-17
P026	143	2	148	13-19
P027	191	6	156	13-17
P029	176	7	166	11-17
P030	130	6	160	10-18
P034	104	5	146	14-18

Note: * indicates participants who requested a decrease in intensity.

5.4 Lactate

5.4.1 Descriptive Lactate Statistics

The average blood lactate concentration ranged from 0.76-3.27 mmol/L, 0.92-6.06 mmol/L and 0.89-3.47 mmol/L for the Pre, Post, and Post10 timepoints, respectively. The average blood lactate concentration prior to exercise was 1.5 ± 0.7

mmol/L (Pre), 5-min into the exercise it was 3.9 ±1.1 mmol/L, and 15-min into exercise it was 4.1 ± 1.3 mmol/L. Post-exercise values were 3.0 ± 1.2 mmol/L (Post) and 2.0 ± 0.9 mmol/L (Post-10). Individual participant data related to blood lactate concentration is reported in Table 7.

Table 7. Average blood lactate concentration at each timepoint for each participant.

ID	Pre	During AE		Post	Post-10
		5-min	15-min		
P001	0.97	5.32	5.71	5.12	2.90
P002	1.37	2.41	2.98	4.56	3.30
P003	1.00	3.42	3.99	2.41	1.48
P005	1.32	3.13	2.89	1.26	0.91
P006	1.79	5.74	5.22	3.33	2.47
P007	2.18	3.76	4.30	3.74	2.24
P008	0.82	3.78	4.23	2.51	1.52
P009	2.80	2.16	2.12	3.37	1.13
P010	2.79	3.55	4.67	2.55	1.46
P011	1.00	3.42	3.99	2.41	1.48
P012	0.99	3.67	3.78	2.56	1.45
P013	1.83	4.01	2.67	0.92	0.93
P016	2.43	4.33	5.92	1.52	0.93
P017	0.95	2.81	2.73	1.02	0.89
P019	1.44	5.26	8.45	6.06	3.47
P021	0.76	4.42	4.74	2.88	2.73
P022	1.28	3.94	4.25	3.84	2.07
P023	1.24	4.92	5.23	3.68	1.84
P024	1.11	3.82	2.89	2.06	2.48
P026	0.82	3.22	3.48	2.36	1.64
P027	1.51	4.75	4.01	3.33	2.84
P029	3.27	6.84	4.84	3.27	3.08
P030	1.28	3.00	4.27	2.77	3.23
P034	1.33	3.44	4.02	3.93	1.34

5.4.2 Change in Lactate Across Time

Figure 14 provides the average blood lactate concentration from all participants at each time point (N=24). Following analysis via a Shapiro Wilk's test, blood lactate

concentration values were determined not to be normally distributed (Pre [p=0.00525], Post [p =0.571], and Post10 [p =0.0577]). A log transformation was subsequently applied, and re-analysis determined the data were normally distributed (Pre [p =0.0618], Post [p =0.109], and Post10 [p =0.0867]). Mauchly's test for sphericity indicated that the assumption was violated (χ^2 (2) = 11.536, $p < 0.05$) and a Greenhouse-Geisser correction applied estimates of sphericity ($\varepsilon = 0.710$). Results of the one-way repeated measures ANOVA showed the main effect of time (Pre-Post, Pre-Post10, and Post-Post10) was significant (F(1.420, 32.669) = 19.750, $p < 0.001$, $\eta^2 = 0.462$). Post-hoc comparison (Bonferroni adjusted) indicated that the mean score for blood lactate concentration at Pre values (M = 1.478, SD = 0.681) was significantly different than Post (Post (M= 2.959, SD = 1.213). However, blood lactate values from Post10 (M=1.993 SD = 0.850) did not significantly differ from the Pre values.

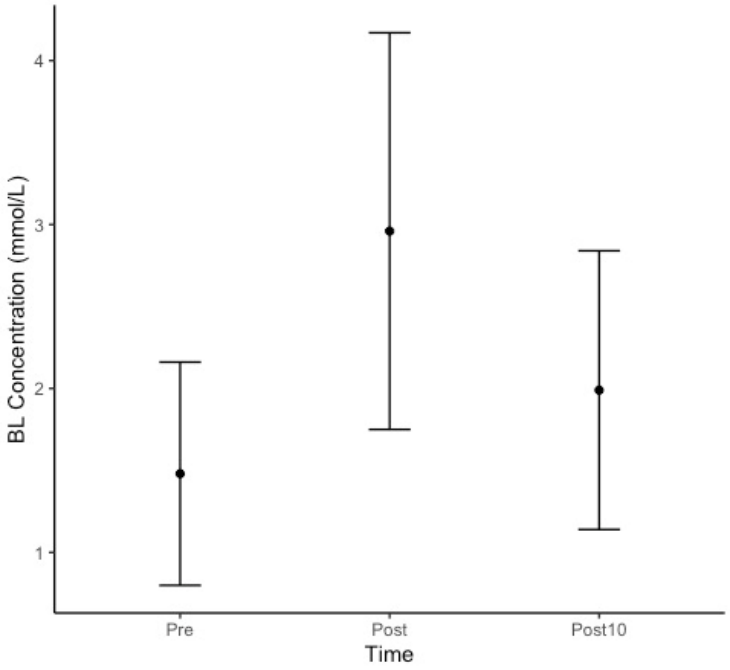

Figure 14. Average blood lactate concentration at Pre, Post and Post10.
Error bars denote SD.

The histogram in Figure 15 highlights the range of blood lactate concentration observed across participants, and more specifically the frequency with which certain blood lactate concentration values were observed within the sample. At Pre, 16 participants had a blood lactate concentration between 0.5 and 1.5 mmol/L. At Post, blood lactate concentration ranged from 2.0 to 4.0 mmol/L. Only 3 participants had a blood lactate concentration greater than 4.0 mmol/L following AE. At Post10, blood lactate concentration values ranged from 0.5 to 3.5 mmol/L.

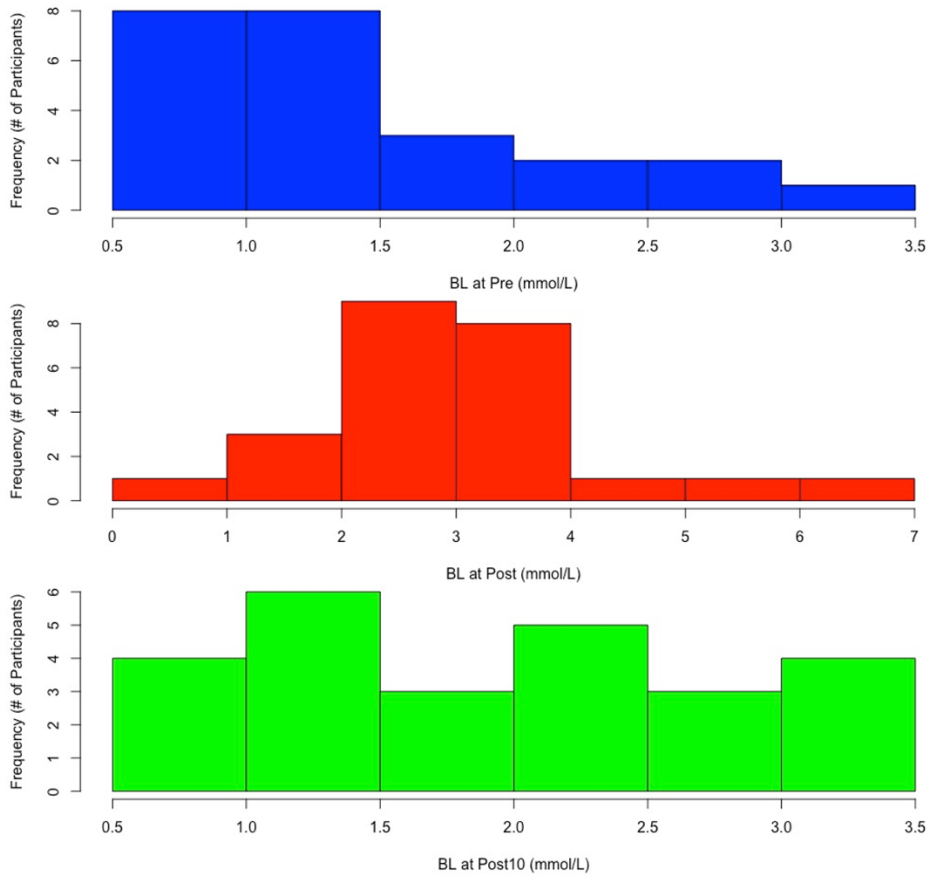

Figure 15. Frequency of blood lactate concentration at Pre, Post, and Post10
Note: Where Pre (blue), Post (red), Post10 (green). BL: blood lactate. The x-axis denotes the range of blood lactate values (BL) and the y-axis is the number of participants within each range.

5.5 CSE Analysis

5.5.1 Descriptive CSE Statistics

The AUC data were not normally distributed as per the Shapiro Wilk's test (Pre [p=0.0156], Post [p =0.00296], and Post10 [p =0.00179]). Therefore, a log transformation was applied to normalize the data, and follow-up testing confirmed a normal distribution (Pre [p=0.914], Post [p =0.199], and Post10 [p =0.127]). Detailed AUC data for each participant are shown in Table 8, while Table 9 reports participant's AUC values for each timepoint.

Table 8. Average of MEP amplitude at each stimulator intensity for each participant.

	100			110			120			130			140		
	Pre	**Post**	**Post10**	**Pre**	**Post**	**Post10**	**Pre**	**Post**	**Post10**	**Pre**	**Post**	**Post10**	**Pre**	**Post**	**Post10**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
P001	252.4 (63.1)	156.6 (78.5)	197.4 (100.8)	449.7 (181.5)	265.5 (37.4)	362.9 (189.8)	1047.4 (256.6)	595.3 (225.7)	601.3 (200.4)	1492.4 (270.9)	1581.4 (327.5)	1403.0 (326.3)	2158.2 (263.9)	1904.1 (181.1)	1801.5 (196.3)
P002	/	380.2 (379.5)	544.1 (355.2)	121.0 (131.9)	606.9 (665.0)	834.1 (366.1)	567.9 (274.4)	2652.2 (1207.2)	2798.0 (12.9)	919.8 (324.5)	3557.4 (937.1)	3827.6 (1180.2)	1748.3 (460.8)	4293.0 (745.7)	4039.1 (875.2)
P003	246.3 (193.2)	1495.7 (2883.7)	/	1030.4 (531.0)	1010.7 (1096.4)	1214.9 (617.1)	2514.9 (794.6)	1631.9 (781.2)	2351.2 (757.3)	2960.6 (678.6)	2569.4 (478.8)	2862.6 (860.1)	3265.6 (764.5)	3357.0 (1533.5)	4003.3 (967.2)
P005	/	/	/	1204.6 (620.4)	526.0 (333.5)	716.4 (394.8)	1479.2 (1010.3)	1239.7 (960.5)	1995.0 (1397.2)	2726.0 (999.5)	2759.8 (1254.2)	3124.2 (731.5)	4592.8 (2153.7)	3274.9 (1557.4)	4396.4 (1007.2)
P006	/	/	/	459.1 (279.6)	388.2 (354.1)	888.1 (392.8)	1387.4 (819.4)	1280.1 (725.1)	1729.6 (704.5)	1675.2 (760.9)	2713.2 (1106.5)	2750.6 (1176.5)	2908.0 (1402.9)	2885.1 (1498.4)	576.5 (999.9)
P007	468.2 (403.8)	399.6 (280.1)	236.2 (118.2)	1151.0 (1302.5)	914.0 (443.0)	812.9 (679.5)	1945.6 (985.9)	2242.7 (797.2)	2101.0 (1167.0)	3257.4 (1203.6)	3286.9 (1032.1)	2803.8 (1059.0)	3741.3 (1350.1)	3602.4 (1055.1)	2622.9 (724.2)

P008	394.1 (233.6)	282.3 (140.9)	264.1 (131.9)	1164.8 (623.8)	851.3 (697.6)	510.8 (336.7)	1221.7 (581.4)	1114.6 (747.6)	1363.6 (260.3)	1915.4 (993.0)	1245.8 (616.6)	1401.9 (346.1)	2093.3 (679.1)	1419.4 (396.0)	1314.6 (406.8)
P009	565.5 (310.7)	464.7 (544.2)	449.8 (329.8)	1877.9 (944.6)	685.5 (241.7)	1074.0 (590.7)	2951.8 (1227.3)	1923.7 (717.4)	2123.9 (1215.9)	3778.7 (955.0)	2913.6 (1070.2)	3136.4 (1277.2)	4740.7 (580.8)	3451.6 (574.7)	4436.0 (619.3)
P010	731.6 (1112.3)	581.3 (567.4)	/	2104.8 (997.8)	948.1 (633.7)	1604.2 (993.3)	3108.9 (1150)	2263.1 (1270.8)	2482.8 (1157.3)	4594.8 (905.7)	3335.9 (929.5)	4016.4 (1100.6)	5643.5 (871.9)	3050.6 (474.1)	3673.1 (1203.4)
P011	822.1 (450.8)	341.4 (133.2)	655.9 (325.4)	2992.2 (852.6)	1052.2 (292.6)	1980.3 (739.6)	3366.9 (708.6)	1716.3 (666.8)	2716.0 (506.2)	3154.1 (682.7)	2383.3 (779.0)	3177.1 (564.6)	3737.9 (817.9)	3288.4 (1002.3)	4120.6 (868.9)
P012	/	/	/	510.3 (316.2)	637.1 (330.3)	747.9 (345.1)	908.7 (410.8)	1296.9 (324.6)	949.7 (349.9)	1104.9 (624.2)	1399.4 (502.7)	865.3 (527.3)	1089.3 (480.3)	1586.8 (807.2)	1188.9 (381.1)
P013	638.6 (525.8)	239.8 (178.7)	881.5 (587.1)	1248.4 (586.8)	1267.8 (527.5)	1482.5 (783.2)	3373.3 (1325.7)	2238.5 (896.8)	3002.6 (554.8)	5534.7 (860.8)	3592.1 (1328.7)	3587.8 (734.5)	6570.1 (954.0)	5077.4 (893.6)	4182.8 (753.9)
P016	1781.4 (1831.0)	2561.3 (1676.7)	3703.0 (1364.5)	5993.9 (1211.2)	4260.3 (943.2)	5089.7 (1592.7)	6962.0 (1599.8)	5660.8 (844.1)	6786.4 (1237.8)	8165.8 (921.3)	5824.2 (854.3)	7719.3 (1057.4)	8399.9 (765.1)	6891.7 (1574.3)	7752.8 (674.7)

P017	/	596.2 (388.5)	627.4 (285.5)	902.4 (631.8)	2245.0 (850.2)	2324.0 (777.3)	1810.1 (604.9)	4173.6 (1687.7)	3222.0 (664.5)	2254.5 (718.9)	3728.1 (1421.6)	4390.5 (1164.4)	2796.8 (827.2)	4421.53 (1265.5)	4336.4 (762.1)
P019	2328.5 (1991.4)	3656.4 (1573.7)	4527.9 (1411.2)	6072.4 (1363.9)	5633.1 (1880.1)	6614.4 (2144.9)	7067.9 (1043.2)	6509.0 (1200.9)	6977.2 (897.4)	7663.8 (951.7)	7384.2 (1178.0)	7861.7 (299.1)	8066.1 (467.8)	7573.4 (768.9)	7999.7 (7383.0)
P021	976.1 (618.5)	1037.5 (556.4)	2001.2 (763.3)	3125.3 (1731.1)	2851.9 (1927.0)	4687.8 (1308.2)	4421.9 (2036.4)	5041.2 (1581.3)	6581.0 (1285.6)	6886.2 (1172.4)	6147.7 (1352.5)	7432.02 (1441.7)	6320.9 (2621.1)	6803.0 (1105.9)	7672.2 (793.9)
P022	346.9 (361.7)	245.5 (331.7)	607.4 (631.7)	1185.5 (701.5)	562.4 (413.7)	1108.6 (594.5)	1643.0 (660.2)	756.3 (343.2)	1699.8 (619.2)	2695.4 (697.1)	1581.8 (781.4)	2140.8 (919.4)	2182.8 (562.6)	1912.1 (1133.2)	2483.2 (785.2)
P023	217.1 (195.4)	186.0 (256.2)	517.5 (847.5)	641.1 (333.3)	446.0 (250.6)	908.5 (456.5)	1003.4 (654.8)	1294.5 (757.6)	1539.8 (1031.2)	2689.9 (830.8)	1950.3 (1361.5)	2174.0 (929.4)	3006.1 (848.2)	2137.2 (1083.7)	3061.0 (33.5)
P024	736.0 (774.7)	1061.2 (910.7)	1038.4 (649.6)	1718.4 (702.2)	2332.8 (1258.5)	2467.5 (1108.4)	4219.7 (1406.6)	6358.2 (2187.8)	5973.6 (2319.7)	6117.3 (2084.9)	7813.5 (2089.1)	7777.6 (1879.2)	8594.2 (816.7)	9466.3 (379.6)	9287.1 (701.3)
P026	176.6 (138.9)	358.7 (377.2)	630.0 (629.2)	1833.7 (1400.1)	2390.4 (1167.9)	1782.9 (480.4)	2597.5 (891.6)	4046.4 (1411.7)	3651.2 (1486.6)	3070.2 (847.1)	5193.6 (1209.6)	5006.7 (1513.9)	4693.6 (1159.4)	5445.6 (1520.5)	7007.69 (941.08)

P027	79.3 (76.5)	/	/	547.0 (411.5)	224.5 (104.0)	615.4 (656.0)	1713.9 (1127.7)	812.6 (410.3)	1068.8 (859.0)	3362.1 (2105.6)	1473.5 (484.7)	2304.9 (936.1)	5485.5 (1376.7)	1943.1 (493.2)	2788.8 (864.0)
P029	273.8 (299.5)	447.4 (404.8)	658.1 (632.5)	1206.4 (1003.7)	832.1 (505.6)	1880.3 (1084.0)	2992.1 (1207.2)	2374.5 (1107.8)	3714.5 (1072.3)	5210.0 (44.1)	3297.4 (494.0)	3369.3 (596.1)	5431.7 (1256.7)	3850.5 (983.2)	4472.4 (1852.1)
P030	/	136.5 (97.0)	896.8 (1395.9)	1081.8 (1207.5)	1682.9 (1617.0)	2100.5 (1469.5)	5996.9 (1859.5)	6458.1 (1561.7)	6779.2 (628.1)	6694.4 (627.1)	6305.8 (939.2)	6576.4 (711.8)	7005.2 (555.0)	6315.5 (1036.6)	6727.0 (808.3)
P034	853.6 (429.4)	976.9 (798.8)	680.8 (576.9)	1107.4 (456.0)	841.5 (5.6)	1177.6 (729.4)	1353.2 (431.6)	953.9 (385.6)	767.5 (484.7)	1224.(303.3)	1499.5 (752.3)	1122.7 (449.1)	1271.0 (267.7)	1599.4 (1033.8)	972.0 (523.5)

Note: / indicates data that were omitted due to not meeting the MEP requirements (<5/10 MEP amplitude greater than 50 μV), data were then extrapolated using statistical software during the equation of the AUC using the S-R curve data.

Table 9. Average AUC values at Pre, Post and Post10 for each participant.

ID	Pre	Post	Post10
P001	4195	3472	3590
P002	2422	9153	9462
P003	8262	7638	6487
P005	7104	5900	5995
P006	4746	5630	5880
P007	8459	8445	8262
P008	5546	4062	3713
P009	11262	7481	7862
P010	12996	8363	7926
P011	11793	6967	8052
P012	2813	3808	3864
P013	13761	9757	10293
P016	26212	20472	21872
P017	5914	12656	12750
P019	26001	25141	26558
P021	18082	17961	20279
P022	6789	3979	4706
P023	5946	4852	5481
P024	16721	21768	21891
P026	9936	14533	14061
P027	8404	3370	3938
P029	12261	8653	9807
P030	16735	17673	18470
P034	4747	4583	3894

5.5.2 Change in CSE Across Time

Mauchly's test revealed the assumption of sphericity was violated (χ^2 (2) = 49.302, $p < 0.001$) and a Greenhouse-Geisser correction applied estimates of sphericity ($\varepsilon = 0.528$). The repeated measures one-way ANOVA revealed the effect of time (Pre-Post, Pre-Post10, and Post-Post10) was not significant ($F(1.056, 24.292) = 0.361$, $p = 0.565$, $\eta^2 = 0.015$). Figure 16 shows the S-R curve obtained at each timepoint.

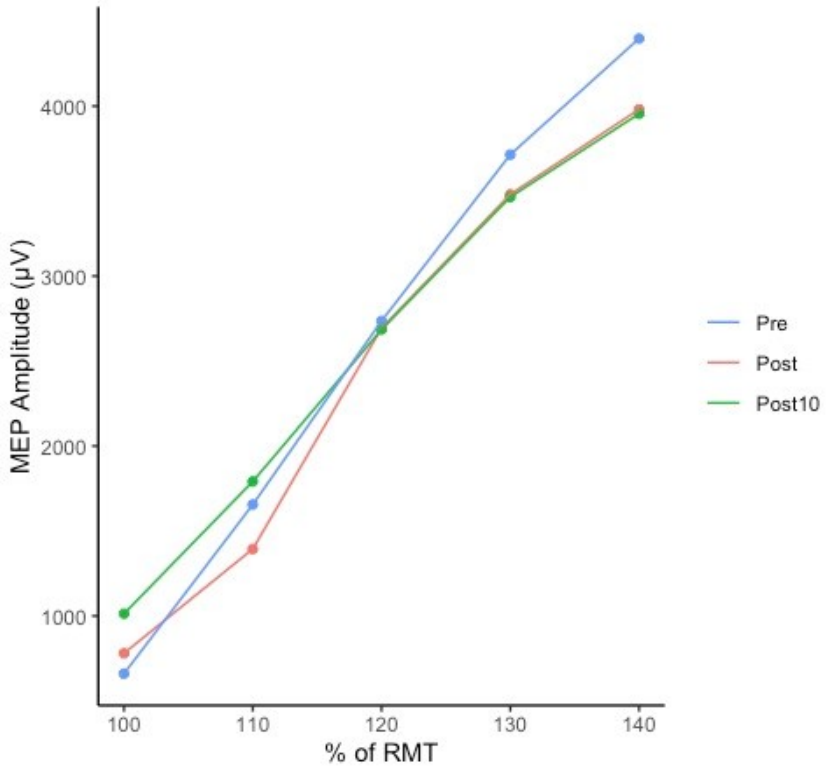

Figure 16. S-R curve at for each timepoint.
This plot depicts the S-R curve obtained at Pre (blue), Post (red), and Post10 (green) by calculating the average MEP obtained at each intensity (100, 110, 120, 130, 140% respectively)

Figure 17 highlights the range of AUC values obtained across participants at the Pre, Post, and Post10 timepoints. Specifically, the figure includes the frequency of participants within a range of AUC values. At Pre, 8 participants AUC value fell between 5000 and 10 000 (a.u); at Post, 10 participants AUC values ranged from 5000 to 10 000 (a.u.); and at Post10, 10 participants AUC values ranged from 5000 to 10 000 (a.u.).

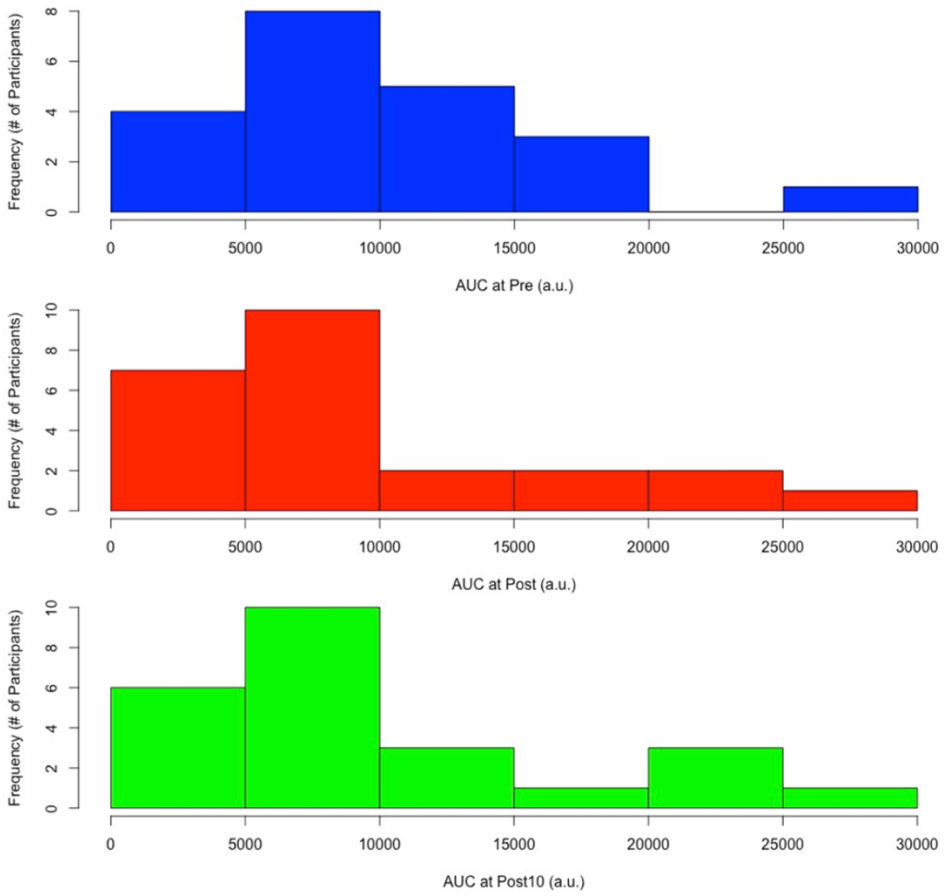

Figure 17. Frequency of AUC values at Pre, Post, and Post10
Note: where Pre (blue), Post (red), and Post10 (green). AUC refers to area under the curve, and a.u., indicates arbitrary units. The x-axis denotes range CSE values (AUC) and the y-axis is the frequency of participants who experienced a specific range of CSE.

5.6 Relationship Between Blood Lactate Concentration and CSE

The relationship between blood lactate concentration and CSE was assessed through a simple linear regression. The model included the change scores of blood lactate concentration from Pre-Post, Pre-Post10 and Post-Post10 as predictors of the change in CSE between the same time points (expressed as change in AUC values). The appropriate assumptions were met prior to execution of the analysis by determining the linearity of the data, removing outliers, assuming independence of observations, assuming

homoscedasticity, and if the residual errors were normally distributed, using Shapiro-Wilks test. The plots associated with each are included in Appendix H, I and J.

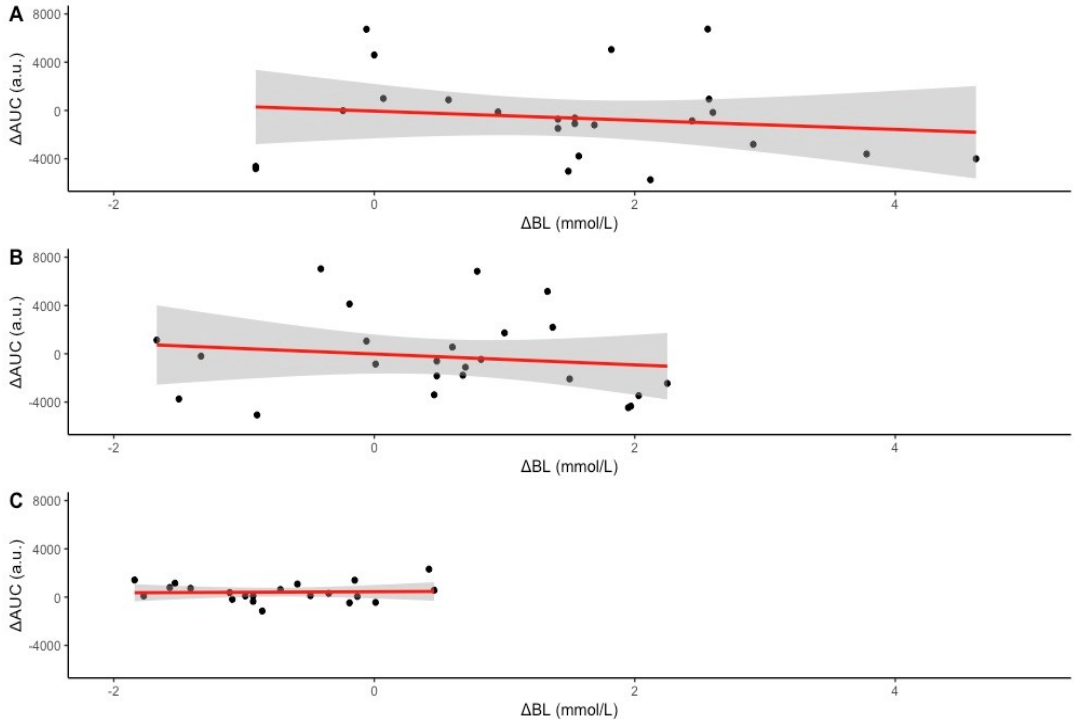

Figure 18. Linear regression model of blood lactate and CSE.
This figure depicts the results of a change (Δ) in blood lactate concentration (BL) plotted against a change (Δ) in CSE. (For A) Pre-Post AE; B) Pre-Post10 AE; and C) Post-Post10. Note the y-axis represents the change in AUC in arbitrary units (a.u.) and the x-axis represents the change in blood lactate concentration in mmol/L.

Figure 18 (A) shows the relationship between change in lactate concentration and CSE (AUC values) from Pre-Post exercise. The fitted regression model was: CSE = -54.99 ± -379.02×(change in BL concentration) and the overall model was not statistically significant (R^2 = 0.02184, F(1, 22) = 0.4913, p = 0.4907). Figure 18 (B) shows the relationship between change in lactate concentration and CSE (AUC values) from Pre to Post10. The regression model was CSE = -19.51 ± -448.69×(change in BL concentration) and the overall model was not statistically significant (R^2 = 0.02121 F(1, 22) = 0.4767, p = 0.4971). Finally, Figure 18 (C) depicts relationship between change in lactate

concentration and CSE (AUC values) from Post to Post10. The fitted regression model was: CSE = 492.7 ± 131.5×(change in BL concentration) and the overall model was not statistically significant (R^2 = 0.02163 F(1, 22) = 0.4864, p = 0.4992). Overall, the regression models showed no relationship between change in blood lactate concentration and change in CSE between any of the timepoints following a moderate-intensity AE intervention.

CHAPTER 6: Discussion

6.1 General Results

The current evidence suggests that AE may play a faciliatory role in enhancing CSE in healthy participants, however the mechanisms underlying this effect continue to be explored. From the work performed to date it is clear there is variability in the response to AE across participants and studies. To understand how AE may influence CSE, it is important to understand the mechanisms of AE and how it changes the concentration of metabolites in the body, in-turn altering excitability. By exploring the role of blood lactate concentration on CSE, we were able assess if lactate is involved in altering CSE. In randomly sampling participants we aimed to have a range of fitness levels and thus a range of responses in relation to lactate concentration (i.e., we suspected that some participants would be exercising at intensities in which lactate would accumulate and others may not), thus allowing us to assess whether lactate is a factor in altering CSE and possibly providing further explanation for the variability seen in previous literature.

We suspected that following a single-bout of moderate-intensity AE participants would experience an increase in CSE assessed via a S-R curve (expressed as an upward shift of the S-R curve from Pre-Post and Pre-Post10). Our results do not support our hypothesis and indicates that there is no relationship between blood lactate concentration and CSE. Further, as no significant change in CSE was observed across timepoints our results do not support our secondary research question. Our findings related to lactate concentration oppose that of work previously conducted in this field of research; the potential reasoning for these findings is discussed in the following sections.

Previous work has highlighted changes in CSE following a bout of moderate-intensity AE (Baltar et al., 2018; El-Sayes, Harasym, et al., 2019; Garnier et al., 2017; Lulic et al., 2017a; MacDonald et al., 2019) however, metabolic by-products of exercise have not been assessed in these studies. Understanding how metabolic by-products such as blood lactate accumulate during AE could be useful in determining underlying mechanisms associated with changes in CSE following AE and could help to provide specific reasoning for variability we see among participants and in the previous literature. The previous work examining the effects of a change in blood lactate concentration on CSE included exercise interventions that were exhaustive in nature (Coco et al., 2010a), showing that in the presence of increased blood lactate concentration, CSE is increased (while in other instances, no change in CSE was observed). To our knowledge, blood lactate concentration has not been assessed as a potential factor contributing to changes in CSE during bouts of steady-state moderate-intensity AE. This is despite the previously mentioned evidence supporting moderate-intensity AE's role in increasing CSE. Therefore, this study sought to examine the effects of altered blood lactate concentration following a single bout of moderate-intensity AE and its relationship with CSE. Secondly, the study sought to determine if moderate-intensity AE in general increased CSE. Our hypothesis suggests that with an increase in blood lactate concentration an increase in CSE (i.e., from Pre-Post and Pre-Post10) would be seen, while in some instances CSE may remain unchanged due to lower levels of lactate concentration in the blood.

There was no relationship between CSE and an AE-induced alteration in blood lactate concentration. The lack of a relationship between lactate concentration in the

blood and CSE suggests that lactate may not be involved in driving changes in excitability as seen in the prior literature, and that there are other potential mechanisms that could be influencing those changes. Some potential mechanisms for driving these cortical changes have been explored by (Singh & Staines, 2015) who report that alterations in CSE may be occurring due to changes in cerebral blood flow and, altered levels of neurotransmitters, stress hormones, and alterations in BDNF.

6.2 Does a Relationship Between CSE and Blood Lactate Exist?

The primary question explored in this study was the relationship between blood lactate concentration and CSE. Our results did not show significance and indicate that the null hypothesis was accepted (Figure 18). Previous literature has shown variability among results wherein the presence of increased blood lactate concentration, CSE tends to increase, while in other instances changes in CSE are not seen (Coco et al., 2010a, 2014; Moscatelli, Valenzano, Petito, Triggiani, Ciliberti, et al., 2016). Our results show variability amongst participants exists, similar to what has been shown in the literature to date. One noteworthy discrepancy between the current study and the previous work completed in this field is the type of exercise intervention used. The literature exploring the effects of AE on the brain typically has used steady-state moderate-intensity exercise as an intervention. However, in exercise studies examining the impact of blood lactate concentration on CSE, exhaustive exercise interventions have been used (Coco et al., 2010a, 2014; Moscatelli, Valenzano, Petito, Triggiani, Ciliberti, et al., 2016). The use of exhaustive exercise allows for a greater accumulation of lactate in the blood, whereas with moderate-intensity AE, the accumulation of lactate may vary. In understanding that moderate-intensity AE may modulate CSE, a moderate-intensity AE intervention was

selected for the current study as we wanted to determine if blood lactate is potentially a driving factor involved in the CSE alterations observed in previous literature.

In Coco et al., (2010), an exhaustive exercise intervention was used to increase blood lactate concentration. As a result, the participants experienced increases in blood lactate concentration up to 11.56 mmol/L from pre-post intervention (Coco et al., 2010a). The BL increase reported by Coco et al., (2010a) was considerably larger than that of the current study, wherein participants blood lactate concentration increased to an average of 4.1 mmol/L during exercise, and 3.08 mmol/L immediately after the exercise intervention. It is unlikely to have lactate concentration increase exponentially during moderate-intensity AE. We expected our participants to come from a diverse background of training experience, meaning that their physiological response to moderate-intensity AE would be variable across participants. It is possible that a 20-min bout of moderate-intensity AE was not enough to produce a change in blood lactate concentration that could alter CSE in some participants, while inducing a rise in others due to exercising at an intensity at, or higher than their lactate threshold.

Notably, within (Coco et al., 2010a), participants were also injected with an intravenous dose of a lactate solution, which increased the concentration of lactate in their blood to 2.65 mmol/L immediately post infusion, and reached a peak (average) value of 4.45 mmol/L 5-min following the infusion. Despite the low concentration of blood lactate following the infusion, the study showed that with increases in blood lactate concentration, motor threshold values decreased (indicating an increase in CSE). It is worth noting that an intravenous dose of lactate is not synonymous with accumulation of lactate in the blood observed during an exercise intervention. These findings are of course

contradictory to those of the present work, as even with low concentrations of blood lactate no change in CSE was observed. Our current findings suggest that there is little to no relationship between blood lactate concentration and CSE and using AE.

There are a multitude of reasons for why blood lactate concentration may not influence CSE. In the current study, participants completed a maximal exercise test to determine their PPO, however no measurement of lactate concentration was obtained at this time as lactate was only collected during the second session. Given that AE is primarily completed through aerobic energy systems, it is likely that some participants were not accumulating lactate in the same capacity as they would during exhaustive exercise, which further explains the low blood lactate concentration observed in our study in other words, our study lacked participants who were less aerobically fit. It is likely that, depending on how aerobic or anaerobically trained our participants were, the accumulation of lactate in the blood varied. Given this, some participants could have been exercising below their lactate threshold, resulting in little to no accumulation in lactate, whereas individuals who are less aerobically fit, may rely more heavily on other energy systems and as a result, may accumulate more blood lactate in their system. Table 7 highlights the average blood lactate concentration at each timepoint for each participant. At 15-min into AE, 14 participants had a blood lactate concentration that was > 4.0 mmol/L, and immediately at the cessation of AE (Post), 3 participants blood lactate concentration remained around 4.0 mmol/L. By 10-min post AE (Post10) all participants blood lactate concentration had returned to resting levels. There is potential that given the time between the cessation of exercise and the Post testing, participants blood lactate levels began to return to resting levels through metabolic processes prior to the delivery

of the single-pulse TMS measures. As a result, we were unable to capture the true lactate values following AE.

As previously discussed, Lulic and colleagues (2017) found that participants who self-reported as more physically active experienced increases in CSE compared to those who self-reported as less physically active. However, one caveat to Lulic et al., (2017) is that the physically active group was not further separated into type of activity (i.e., runner vs. weightlifter), which would ultimately affect the quantity of blood lactate a participate would accumulate. Though, based on understanding how blood lactate accumulates in the body (i.e., through anaerobic work) participants who reported themselves as less physically active would likely accumulate more lactate than those who are more physically active. The participants who reported themselves in the low physically active category did not experience changes in CSE. Interestingly, previous work from our laboratory has shown that aerobic fitness (assessed via $\dot{V}O_{2max}$) is not a predictor in CSE (MacDonald et al., 2019), which supports the idea that individual variability in regards to the percentage of energy systems used (i.e., the ratio between aerobic and anaerobic energy systems during AE) and should be considered when questioning the effects of AE on CSE and is discussed in more detail in subsequent sections.

Given that blood lactate concentration does not appear to be directly involved in increasing CSE, it poses the question as to what mechanism is underlying the change in CSE resulting from a bout of AE. Firstly, we question if the relationship between lactate and CSE is linear, or whether it is non-linear in nature. Given that we did not see a relationship that was linear, we question if these is an optimal concentration of lactate that may be involved in driving changes in CSE. To our knowledge, whether a non-linear

relationship between lactate and CSE exists has yet to be examined. Secondly, emerging evidence suggests that other metabolites or trophic factors may be driving changes in CSE. For instance, BDNF can enhance neuroplasticity through various means including synaptogenesis, neurogenesis, and long-term potentiation (Müller et al., 2020). Physical activity and exercise are associated with an upregulation of BDNF. These changes have been seen following acute, and long term participation in exercise and like lactate, an increase in the concentration of BDNF is dependent on the duration and intensity of the exercise (Müller et al., 2020). Though not fully understood, it is hypothesized that BDNF may interact with lactate through increased NMDA receptor activity (Müller et al., 2020; Yang et al., 2014b). Based on the results of the current study, increased lactate concentration may not be the linking step between AE and increased CSE, however its potential interaction with BDNF may indirectly drive changes in plasticity. Therefore, future studies should examine the interaction between lactate and BDNF during AE, and if there is a parallel in relation to CSE.

6.3 Blood Lactate and CSE Following Moderate-Intensity AE

Our results indicate that a significant change in blood lactate concentration was observed from Pre-Post exercise and between the two post-exercise timepoints. This finding suggests that, at least at the group level, the participants in the study did experience a significant increase in blood lactate concentration following the AE intervention. On average, participants blood lactate concentration at baseline (Pre) was 1.5 ± 0.7 mmol/L, increasing to 3.0 ± 1.2 mmol/L immediately following AE, with a decrease to 2.0 ± 0.9 mmol/L at the 10-min mark following AE. These results suggest that, at least on average, lactate was accumulating in the blood during AE, however it was

at a concentration below what would be expected if our participants were surpassing their lactate threshold, which is likely to occur when blood lactate concentration exceeds 4.0 mmol/L. Given the variability in lactate concentration values among our participants however, it is likely that some had surpassed their lactate threshold, while others were below it. Individuals who appear to be exercising at an intensity above their lactate threshold support the idea that blood lactate concentration can increase in regards to time despite no change in work rate (Bentley et al., 2001; E. W. Smith et al., 1998). Despite a statistically significant increase in blood lactate following AE, it is possible that the increase was not substantial enough for all participants to truly induce changes in the brain on a chemical level, further denoting the inter-individual variability found within the current study. Regardless, even in participants who accumulated lactate at a greater intensity than others, no change in CSE was observed.

Despite our efforts to obtain a sample that included participants with varying levels of aerobic fitness, it is likely most of our participants were not exercising at an intensity or duration where lactate accumulated and suggests that our participants as a group were more aerobically fit than anticipated. Within our dataset, only one participant experienced a change in lactate concentration greater than 4 mmol/L from Pre- to Post-exercise. Figure 19 highlights the change in blood lactate concentration from Pre to Post and the frequency these changes occurred across participants. While there is spread in the data related to change in blood lactate concentration, the highest frequency of change was found in the 1-3 mmol range, which is considered below the concentration to be considered exceeding LT.

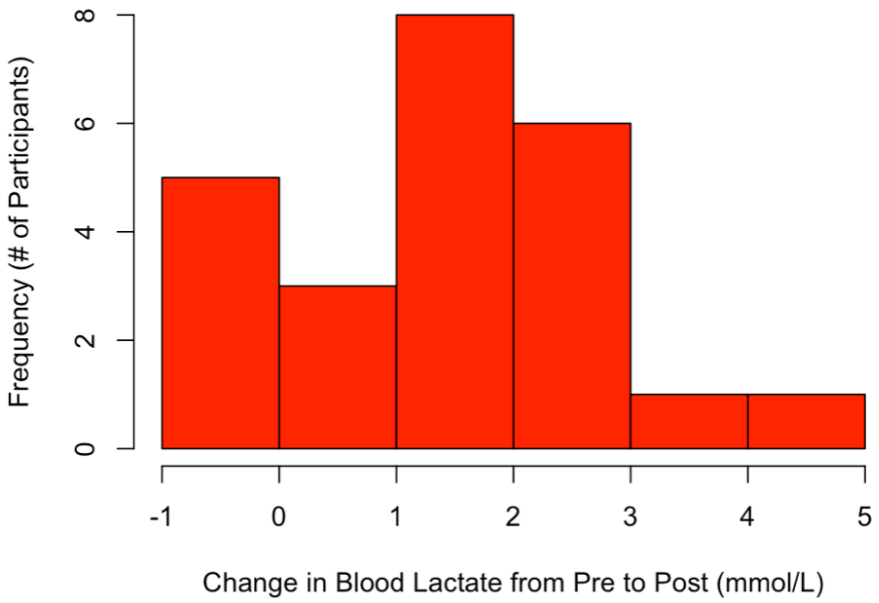

Figure 19. The frequency of the change in blood lactate concentration from Pre – Post. Note: The x-axis denotes range in blood lactate scores in mmol/L and the y-axis is the frequency of participants who experienced a change in blood lactate.

Similarly, we experienced a spread of participants regarding changes in CSE from Pre to Post timepoints. Figure 20 shows the change in CSE and the frequency of participants to experience these changes across participants. As observed in Figure 20, 17 participants experienced a decrease in CSE following a bout of moderate-intensity AE, with only 7 participants experiencing an increase in CSE.

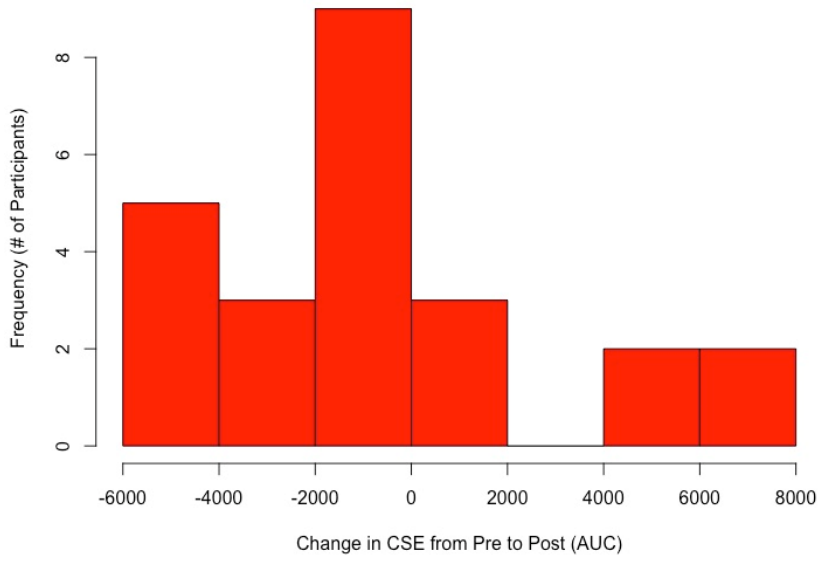

Figure 20. The frequency of the change in CSE from Pre – Post.
Note: The x-axis denotes range CSE values (AUC) and the y-axis is the frequency of participants who experienced a change in CSE.

It is expected that individuals who are aerobically trained would have higher lactate thresholds compared to individuals who are less aerobically trained. For instance, a more aerobically trained individual would not accumulate lactate at the same rate as someone who is less aerobically trained. A primary finding from (Lulic et al., 2017a) indicated that individuals who reported higher physical activity (in METs per the IPAQ) experienced an increase in CSE following a 20-min bout of moderate-intensity AE, while those reported as having low levels of physical activity reported no changes in CSE. This finding is contradictory to our *a priori* hypothesis that an increase in blood lactate concentration would result in increased CSE, as the findings of Lulic indicate it is those who are less likely to accumulate lactate who showed an increase in CSE. One caveat to Lulic et al., (2017) was their use of the IPAQ for determining physical activity level. The IPAQ only quantifies the amount of physical activity participants completed in the last 7 days and fails to quantify the types of activities, such as aerobic vs. anaerobic activities.

As detailed above, prior work from our laboratory, considered aerobic fitness in relation to the effect of AE on CSE, finding that fitness level ($\dot{V}O_{2max}$) was not a predictor of change in CSE (MacDonald et al., 2019). This result is somewhat contradictory to that of Lulic et. al., (2017a) and led to the question of whether there is individual variability that may contribute to the changes in CSE across studies examining the effects of AE on CSE. Certainly these findings coupled with those of the present work beg the question as to whether lactate is truly involved in increasing CSE as previously shown in the literature (Coco et al., 2020), or if there are other factors that could be driving these changes.

6.4 Exploring a Change in CSE following AE

We sought to explore if moderate-intensity AE caused a global increase in CSE however, per our results no change in CSE was observed (Figure 16). Previous literature primarily supports the use of moderate-intensity AE for increasing CSE, however between-study variability exists. Our study indicates that there is variability in the response to AE between our participants. In some instances, participants experienced an increase in CSE following exercise, while other participants experienced decreases in CSE. As a result, our data showed no significant findings regarding CSE following AE.

In studies using similar exercise protocols to that used here, increases in CSE were observed (Lulic et al., 2017a; MacDonald et al., 2019), however in others, no change was documented (McDonnell et al., 2013; Neva et al., 2017). In work completed by McDonnell et al., (2013), no change in CSE was observed following bouts of low-, moderate-, and vigorous-intensity AE. Similarly, Neva et al., (2017) found that following

a 20-min bout of moderate-intensity cycling, no change in MEP amplitude curves was observed, suggesting that no change in CSE occurred following the exercise intervention.

As mentioned, studies with similar AE protocols to ours experienced increases in CSE. For example, (El-Sayes, Turco, et al., 2019) examined the effects of sex hormones on CSE. Participants completed a 20-min bout of moderate-intensity AE (65-70% of HR_{max}), and MEP recruitment curves (S-R curves) were used prior to exercise and 10-min following the cessation of exercise. The results of the study indicate that moderate-intensity AE increased CSE following exercise. Blood samples were obtained during baseline testing to determine sex hormone and BDNF (plasma and serum) concentrations (El-Sayes, Turco, et al., 2019). As no differences in the response to AE were detected based on sex hormone concentrations (estradiol, progesterone, and testosterone) or BDNF concentration at baseline, neither appear to significantly influence the brain's response to AE. Given that BDNF levels were not measured following exercise, it is difficult to know if BDNF played a mediating role in increasing CSE (El-Sayes, Turco, et al., 2019). Given that the current literature hypothesizes that BDNF is involved in processes related to increasing CSE, future work could focus on this area of study (Müller et al., 2020).

In some instances, intracortical networks (i.e., excitatory/inhibitory changes which could manifest as changes in CSE) were examined using paired-pulse TMS (i.e., SICI, LICI, ICF) rather than single pulse measures (i.e., S-R curve). Measures of SICI, LICI and ICF allow for exploration of inhibitory and excitatory intracortical networks within M1. Single-pulse measures are typically used to assess cortical reactivity (i.e., how the cortex responds to stimuli) while paired-pulse measures examine the inhibition and facilitation of intracortical networks (i.e., receptor activity). Though the current study did

not examine intracortical networks, it is possible that changes in inhibitory and excitatory mechanisms could exist following moderate-intensity AE. For example, (Singh et al., 2014) followed a similar exercise protocol (65-70% of HR_{max} for 20-min of stationary cycling) and measured single- and paired-pulse TMS immediately following the AE intervention and then 30-min after. The results showed that change in S-R curve data were not significant between timepoints (Pre-Post1, and Post1-Post2), like the results of the present work. However, Singh et al., (2014) did show that SICI decreased significantly from pre-post exercise while ICF showed a significant increase from pre-post exercise. The single-pulse TMS measures do not support a change in CSE, however a decrease in SICI is indicative of a change in intracortical network activity. These results indicate that AE does not always manifest as a direct change in CSE, however can cause a change in intracortical networks through alteration of receptor activity in the brain signifying that exercise can be used to modulate neurotransmission (Singh et al., 2014).

Neurotransmitter concentration varies during acute bouts of exercise. In studies examining the effects of varying levels of dopamine, serotonin, norepinephrine, and blood lactate on CSE increases in M1 excitability have been observed (Mang et al., 2013). To this end, Singh and colleagues' findings indicate that the effects of AE modulate inhibitory processes at the level of the synapse given that SICI assesses GABA receptor activity. Specifically, with AE it is thought that $GABA_A$ receptor activity becomes downregulated, reducing the ability for GABA to bind thus reducing inhibition of the post-synaptic neuron. Reducing inhibition in this manner effectively increases the excitability of the neuron, moving its resting membrane potential closer to the threshold

for depolarization. As for ICF, it is suspected to be modulated through the neurotransmitter glutamate, the primary excitatory neurotransmitter. It is thought that increases in ICF are due to increases in glutamate activity following AE (Mang et al., 2016). Accordingly, upregulation of glutamate by AE results in a more excitable neuron and ultimately creates an environment that is most conducive for motor learning. In the case of our study, we chose to examine single pulse measures of TMS to assess cortical activity to see if exercise had a broad effect on the brain excitability. As our results show, no change in CSE was observed across the timepoints. Based on previous evidence, it may well be that AE impacted on cortical activity (at the synaptic level) and that these changes did not manifest as increased CSE, like that of the results of Singh and colleagues. This finding indicates that the inclusion of paired pulse measures is vital for future studies.

6.5 Limitations

There are several limitations that need to be considered when interpreting the findings of this study. Firstly, the participants in the study completed a GXT through which PPO was determined; however, given that $\dot{V}O_{2max}$ testing is the gold standard of exercise testing, it would have been beneficial to determine $\dot{V}O_{2max}$ to ascertain participants aerobic fitness level as this could have been used as a covariate in the analysis. Given that the premise of our study was to show individual variability following AE, we did not determine their fitness levels, rather we anticipated recruiting participants with a wide range of fitness levels. It would have been beneficial to know how their fitness level is related to their blood lactate concentration during exercise, and as a result could have provided more information about the variability seen across the data.

Secondly, we measured blood lactate concentration at various timepoints during our experimental protocol but did not determine participants lactate threshold. As a result, we were unable to determine if participants were truly exercising below, at, or above their lactate threshold. Determining lactate threshold could have been performed during $\dot{V}O_{2max}$ testing, which for reasons related to feasibility we were unable to perform. As above, through a random sample of individuals we anticipated varying levels of aerobic fitness. Given that our participants exercised at 60% of their PPO rather than a percentage of their HR, we expected some individuals to be exercising at, above, or below their lactate threshold. However, given that we did not measure lactate threshold, we can't know for certain where our participants were exercising in relation to their lactate threshold.

Lastly, we randomly sampled from a university aged population, and it is likely that all the participants were more aerobically trained than we had anticipated. As a result, many of our participants did not appear to be exercising at an intensity higher than their suspected lactate threshold. As a result, these participants were not accumulating lactate to a great extent. A caveat to conducting research involving physical activity is that individuals who self-refer tend to already be physically active. It is likely that we did not recruit enough participants who considered themselves to be untrained given the nature of completing an exercise study.

6.6 Future Directions

It is vital that future work include assessment of aerobic fitness via $\dot{V}O_{2max}$ testing and in-turn determination of lactate threshold. It would also be worthwhile to include the measurement of trophic factors, including BDNF, to explore the role their upregulation

following AE plays in increasing CSE. It would be worthwhile to have participants complete AE at varying intensities relative to their individual lactate threshold to determine if there is relationship between lactate accumulation and CSE. Secondly, other serum blood factors, such as BDNF should be analyzed given the recent evidence of their benefit in facilitating brain excitability and their role in neurorehabilitation. Specifically, if BDNF and lactate interact to facilitate changes in plasticity. Future research in this area should focus on including lactate threshold testing, and AE interventions at intensities above, below and at each participants threshold. Lastly, given that in studies where increases in CSE were not seen with single pulse TMS measures (i.e., S-R curve), but with paired-pulse (i.e., SICI, LICI, and ICF) it would be of benefit to include these as post measures following AE interventions moving forward.

6.7 Conclusion

While there is clear evidence that a bout of AE can result in increased CSE, the exact mechanisms through which this increase occur remains largely unknown. Exploring the effects of exercise by-products is one means of unraveling the mechanisms that may contribute to these alterations. Although prior work has shown lactate to be directly related to an increase in CSE, the present findings exploring the same following a bout of moderate-intensity AE do not, as the results indicate no relationship exists between CSE and blood lactate concentration. Though the results of our study were not significant, they have highlighted further gaps in the literature that once answered and help to create environments that are most beneficial for supporting neuroplasticity and motor learning.

Appendix A – Informed Consent

DALHOUSIE UNIVERSITY

CONSENT FORM

Project title: Does an Exercise Induced Alteration in Blood Lactate Concentration Affect Brain Excitability?

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Introduction

You have been invited to take part in a research study. A research study is a way of gathering information on a treatment, procedure, or medical device or to answer a question about something that is not well understood. Taking part in this study is voluntary. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study.
Please read this carefully. Take as much time as you like. Mark anything you don't understand or requires further explanation. After you have read it, please ask questions about anything that is not clear.
The researchers will:

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

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

Purpose and Outline of the Research Study

We learn new skills by practicing them over, and over again. This repetitive practice allows connections in our brain to strengthen, which is the basis for learning. Exercise has been shown to alter the strength of connections in our brain by increasing how excitable the brain is. When we exercise metabolic by-products such as, lactate can accumulate or 'build-up' in our body. What we don't understand is why bouts of moderate-intensity aerobic exercise (AE) may mediate brain excitability. Therefore, we want to explore the potential role of blood lactate concentration and AE and if it is a link between brain excitability.
The information gathered in this study will tell us about how exercise and lactate affect the brain, and ultimately how exercise can be used to make learning new skills more individualized. Therefore, the purpose of this study is to investigate if a steady-state bout of moderate intensity AE will induce a change in blood lactate concentration which will alter brain excitability when assessed using transcranial magnetic stimulation (TMS).

Who Can Take Part in the Research Study

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

1. Individuals aged 18-40.
2. Suitability to perform exercise safely (as assessed by the PAR-Q+)
3. Suitability to engage in non-invasive brain stimulation (TMS)
4. No self-reported history of neurological, cardiovascular, or pulmonary disorders

Exclusion criteria:

1. *Having respiratory disorders, hypertension or other cardiovascular diseases that would preclude participating in exercise*
2. *Having any contraindications to TMS (as assessed by the TMS) screening form).*
3. *Having a body mass index* $\geq$ 30kg/m^2 and a waist circumference >102 cm for men and >88 for women
4. Is a regular smoker.

What You Will Be Asked to Do

<u>Screening</u>

Once you have expressed interest in this study, you will be asked to complete a series of questionnaires to see if you are eligible to take part in the study. This is called screening. It is possible that the screening results will show that you are ineligible to participate. The research team will discuss these with you. Screening will take place over phone or via email prior to the first session.

The first screening task is a questionnaire to determine if you can participate in physical activity and exercise (called the PAR-Q+). The PAR-Q+ will take approximately three minutes to complete. The second questionnaire is to determine if you are eligible to receive a non-invasive form of brain stimulation, called transcranial magnetic stimulation (TMS; described in the next section). We will be using this technique to assess brain excitability. This set of questions will take about 5 minutes to complete. The answers to the questions will determine whether or not you have any conditions that could possibly cause you harm if you were to have brain stimulation (TMS). The third questionnaire is a Health History Questionnaire which will record your age, height, weight, smoking history and exercise history. This questionnaire will take approximately 3 minutes to complete. Following screening, if you are eligible to participate, you will be asked to attend 2 sessions within a period of 1 week for a total time commitment of approximately 4 hours. All sessions will take place in the Laboratory for Brain Recovery and Function at Dalhousie University.

<u>During the Maximal Exercise Test Session (Session 1, 90 minutes):</u>

Overview of Session:

You will be asked first to complete the screening forms as mentioned above (PAR-Q+, TMS screening form and Health History Questionnaire) to verify the information provided during screening. The International Physical Activity Questionnaire (IPAQ) will be completed during session one. The IPAQ will ask you about the time you spent being physically active in the last 7 days. We will then review this document and ask you to sign it.

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.

Prior to completing the exercise test, the investigator will administer TMS as per the procedure explained below. This will be to ensure that your brain is excitable (i.e., we can get a response). If you are NOT responsive to TMS you will be determined as ineligible for the study. If you ARE responsive to TMS you will be set-up to complete the graded exercise test (GXT).

You will begin the exercise test portion of session one by sitting on a stationary bike quietly for 5 minutes to measure your resting heart rate. Then, you will start cycling on the stationary bike and your heart rate will be monitored continuously and your peak power output (PPO) will be determined for exercise protocol in session 2. Your peak power output is defined as the highest wattage recorded during the graded exercise test. The cycling portion 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 60 to 90 minutes.

During the Brain Activity Assessment Session (Session 2, 150 minutes):

Overview of Session 2:

As you arrive, you will be asked to sit in a reclined position on a chair and the TMS coil will be positioned on your head. During this part of the study, we will record muscle activity from your hand as we have described below. You will be asked if you would like to wear disposable earplugs (which we will provide) while you receive the magnetic stimulation to protect your hearing from the clicking noises.

After you finish the TMS session, you will complete the 20-minute exercise intervention at 60% of your PPO (plus 5-minute warm-up and 5-minute cool-down). Throughout, we will monitor your heart rate using a wrist mounted HR monitor (outlined below). As you finish cycling, you are going to transfer back to the TMS chair to let us take the brain measurements again. This time we will take brain measurements twice, immediately after you finish the exercise intervention, and again 10-minutes after exercise. After this is done, the testing is completed. In total, this second session will last approximately 150 minutes.

Study Procedures

Each of the participants will perform the following testing procedures:
Transcranial Magnetic Stimulation (TMS)

A TMS machine 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. This current can evoke a small muscle twitch, when the pulse is delivered over the part of your head that corresponds to movement. In this study we will use single pulse TMS (where we a single stimuli is delivered resulting in a small muscle movement in your hand) to measure excitability of your brain. No permanent changes to your brain will result from TMS.

You will be comfortably seated in a chair with your hands resting on your lap. The TMS coil will be positioned on your head. During this time, you will be asked to sit quietly and keep your head as still as possible. 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. You will hear the same clicking noises as the current flows through the coil.

Muscle Activity

Activity in your muscles will be measured using electromyography (EMG). EMG involves attaching two non-invasive electrodes (like stickers) to the skin over the muscles of the hand.

Aerobic Exercise on a Stationary Bike

An exercise intervention will be performed on a stationary cycle; you will be asked to cycle at a moderate-intensity relative to your own physiological parameters (the PPO, established in session one). This intensity is described as 60% of the individuals PPO. The intervention will be 20-minutes in duration, plus a 5-minute warm-up and 5-minute cool-down. The warm-up involves participants cycling at a 50 W (watts; power output on the cycle) workload for 2 min, then 75 W for minutes 3-5. There will also be a 5-minute cool-down period, where participants will cycle at 50W for 3-5 minutes, or until your heart rate returns to its resting level.

Watch to Monitor your Heart Rate ('Polar HR Verity')

The wrist mounted HR monitor is simply a device that allows us to measure your heart rate in real time or record the heart rate. Heart rate information will only be recorded.

Blood Lactate Measurements

To measure blood lactate your finger will be pricked with a small needle (lancet) and a sample of ~1-2 drops of blood will be collected. Band-aids® will be made available as required. Blood samples will be collected before the exercise intervention (resting value), during the exercise intervention at minute 5 and minute 15, at the end of the intervention, and 10-minutes following the intervention. At each time point, 3 finger pricks will be obtained in order to determine the average blood lactate measure. Therefore, a total of 15 finger pricks will be obtained during the second study session. These pricks will be obtained by the qualified investigators. The finger pricks will be obtained when the participant is in a seated position at pre, post and post-10-minutes following the exercise intervention. While the participant is exercising their hands will remain at their sides, allowing for easy access to obtain the finger pricks as only their lower body will be moving during the test and their upper body will remain relatively still.

Possible Benefits, Risks and Discomforts

There are risks associated with this, or any study. We do not want to alarm you, but we do want to make sure that if you decide to participate in the study, you have had a chance to think about the risks carefully. Please also be aware that there may be risks in participating in this study that we do not know about yet.

Potential Risks of TMS

TMS has been approved in Canada for both therapeutic and research use and has been used in numerous 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 or ask your physician to contact us if you experience any effects that you feel may be a result of your participation in the study.
TMS is painless, although it can cause tingling or twitching of muscles in the face, which may lead to soreness.
Common risks (1 or more out of every 100 people but less than 1 out of every 10 people have experienced the following):

- 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.
- Neck stiffness and pain. This is believed to be due to the straight posture of the head and neck during the application rTMS, which involves a continuous train of pulses vs. single pulses delivered at a time as in the current study. In the case of such an event, you will be advised to take whatever pain medication you usually take for mild headaches, which in most cases promptly resolves the discomfort. You should advise a member of the research team at the first opportunity if you experience any neck stiffness or soreness. In this situation, you may opt to withdraw from the study or to rest and change posture for several minutes before the procedures are resumed.

Rare risks (1 or more out of every 10,000 people but less than 1 out of every 1000 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 and is related to a type of TMS that invovles a continuous train of pulses (vs. single pulses as employed in the current study). You will be asked to complete a TMS screening form, and precautions will be taken to ensure your safety such as removal of metallic objects from your body. 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. The forms of magnetic

stimulation that will be used during this study are well within the limits recommended by the safety guidelines.

- In the event a participant does experience a seizure, one of the two investigators will remain with the study participant at all times while the other contacts Dalhousie Security Services at extension 4109 to inform campus police of the location of the incident to facilitate the arrival of emergency personnel (Security Services coordinates with external emergency services and thus there is no requirement for lab personnel to contact 911).

TMS produces a loud clicking noise when the current passes through the handle of the machine. This loud click can result in ringing in the ears and temporary hearing problems if no ear protection is used. To prevent this, you will wear earplugs which we will provide for you. Animal and human 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.

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 Recording Muscle Activity (EMG)

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 (1 or more out of every 10,000 people but less than 1 out of every 1000 people) 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.

Potential Risks of Collecting Blood Lactate Measures

A tiny needle will 'prick' a hole in your skin on one of your fingers. There will be 15 finger 'pricks' performed during session two. This may be slightly painful and feel like a quick pinch. We also may need to apply a slight squeezing pressure on the finger to retrieve 2-3 drops of blood.

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

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

Compensation / Reimbursement

You will be paid $20/visit, regardless of whether you complete the session or not. This compensation is intended as an honorarium — a gesture of appreciation for volunteering your time — and not as a form of employment or fee for service.

How Your Information Will be Protected

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

- Age
- Biological sex
- Information from the study questionnaires

Confidentiality: In order to protect your privacy and keep your participation in the study confidential, you will be de-identified using a study code. For the purpose of data analyses, all participants will only be identified by their study code (e.g. s001). This identification code will only be used during the analysis of the data obtained during the experimental procedure. The hard copy data (i.e., the screening forms) will not be utilized in the analysis and will be stored in a locked cabinet in a secured laboratory that is accessible only to lab personnel via personalized pin codes and who are trained in confidentiality. All data collected will be stored on a secure, password-protected server in the Laboratory for Brain Recovery and Function. No documentation will exist (hard copy or electronic) that links your name with your study code.

Data retention: Information that you provide to us will be kept private. Only the research team at Dalhousie University will have access to this information. We will describe and

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

If You Decide to Stop Participating

You may choose not to continue your participation in the study at any time, (i.e. during the TMS portion or during the exercise test). If you decide not to take part in the study or if you leave the session early, your data will be automatically withdrawn from the study. Once you complete the session, your data can no longer be withdrawn from the study, as this data is automatically added to the database and entered into the analysis.

How to Obtain Results

If you would like a description of the results at the end of the study, you can obtain a short description of these results by visiting boelab.com in approximately 12 months. No individual results will be provided.

Questions

We are happy to talk with you about any questions or concerns you may have about your participation in this research study. For further information about the study, you may call the principal investigator, who is the person in charge of this study.

The principal investigator is Dr. Shaun Boe, telephone: (902) 494-6360

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 Research Ethics, Dalhousie University at (902) 494-1462, or email: ethics@dal.ca (and reference REB file # 2020-XXXX).

Other

Neither the Principal Investigator nor any other individuals associated with the administration of this study have any financial interest in its outcome.

In the next part you will be asked if you agree (consent) to join this study. If the answer is "yes", you will need to sign the form.

Signature Page

Project Title: Does an Exercise Induced Alteration in Blood Lactate Concentration Affect Brain Excitability?

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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. My participation is voluntary and I understand that I am free to withdraw from the study at any time, prior to data analysis. I understand I will be given a copy of this consent form.

_____	_____	
Name of Participant	Signature of Participant	Date
_____	_____	
Name of Investigator	Signature of Investigator	Date

Appendix B – Physical Activity and Readiness Questionnaire (2021)

2021 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	**YES**	**NO**
1) Has your doctor ever said that you have a heart condition ☐ **OR** high blood pressure ☐?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity?	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.
Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow Global Physical Activity Guidelines for your age (https://www.who.int/publications/i/item/9789240015128).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

PARTICIPANT DECLARATION

If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

 1 / 4

01-11-2020

2021 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. Do you have Arthritis, Osteoporosis, or Back Problems?

If the above condition(s) is/are present, answer questions 1a-1c — If **NO** ☐ go to question 2

1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	YES ☐ NO ☐
1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	YES ☐ NO ☐

2. Do you currently have Cancer of any kind?

If the above condition(s) is/are present, answer questions 2a-2b — If **NO** ☐ go to question 3

2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck?	YES ☐ NO ☐
2b.	Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)?	YES ☐ NO ☐

3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm

If the above condition(s) is/are present, answer questions 3a-3d — If **NO** ☐ go to question 4

3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
3b.	Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction)	YES ☐ NO ☐
3c.	Do you have chronic heart failure?	YES ☐ NO ☐
3d.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	YES ☐ NO ☐

4. Do you currently have High Blood Pressure?

If the above condition(s) is/are present, answer questions 4a-4b — If **NO** ☐ go to question 5

4a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
4b.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure)	YES ☐ NO ☐

5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes

If the above condition(s) is/are present, answer questions 5a-5e — If **NO** ☐ go to question 6

5a.	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	YES ☐ NO ☐
5b.	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	YES ☐ NO ☐
5c.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	YES ☐ NO ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)?	YES ☐ NO ☐
5e.	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	YES ☐ NO ☐

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6. **Do you have any Mental Health Problems or Learning Difficulties?** This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome

If the above condition(s) is/are present, answer questions 6a-6b If **NO** ☐ go to question 7

6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
6b.	Do you have Down Syndrome **AND** back problems affecting nerves or muscles?	YES ☐ NO ☐

7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d If **NO** ☐ go to question 8

7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
7b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	YES ☐ NO ☐
7c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	YES ☐ NO ☐
7d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	YES ☐ NO ☐

8. **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c If **NO** ☐ go to question 9

8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
8b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	YES ☐ NO ☐
8c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	YES ☐ NO ☐

9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c If **NO** ☐ go to question 10

9a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	YES ☐ NO ☐
9b.	Do you have any impairment in walking or mobility?	YES ☐ NO ☐
9c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	YES ☐ NO ☐

10. **Do you have any other medical condition not listed above or do you have two or more medical conditions?**

If you have other medical conditions, answer questions 10a-10c If **NO** ☐ read the Page 4 recommendations

10a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months?	YES ☐ NO ☐
10b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	YES ☐ NO ☐
10c.	Do you currently live with two or more medical conditions?	YES ☐ NO ☐

PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE: _____

_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

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2021 PAR-Q+

If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

Delay becoming more active if:

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

Citation for PAR-Q+
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The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

Key References

1. Jamnik VK, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the effectiveness of clearance for physical activity participation; background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.
3. Chisholm DM, Collis ML, Kulak LL, Davenport W, and Gruber N. Physical activity readiness. British Columbia Medical Journal. 1975;17:375-378.
4. Thomas S, Reading J, and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). Canadian Journal of Sport Science 1992;17:4 338-345.

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01-11-2020

Appendix C – TMS Screening Form

Version: August 2021

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. Do you have any hearing problems or ringing in your ears?		
3. Do you have cochlear implants?		
4. Are you pregnant or is there any chance that you might be?		
5. Do you have an implanted neurostimulator (e.g., DBS, epidural/subdural, VNS)?		
6. Do you have cardiac pacemaker or intracardiac lines?		
7. Do you have a medication infusion device?		
8. Have you ever had a fainting spell or syncope (loss of consciousness)? If yes, please describe on which occasion:		
9. Have you ever had a head trauma that was diagnosed as a concussion or was associated with a loss of consciousness?		

10. Are you taking any medications? (please list):		
11. Do you have metal in the brain, skull or elsewhere in your body? (e.g., splinters, fragments, clips, etc.)? If so, please specify:		
12. Did you ever undergo TMS in the past? If yes, were there any problems:		
13. Did you ever undergo MRI in the past? If yes, were there any problems:		

If you answered "yes" to any of the first 7 questions you are not eligible 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.

Please bring a list of your medications to the first study visit.

***** TMS screening form is from the International Consensus Guidelines:
Rossi S et. al. (2021). Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. *Clin Neurophysiol* 132: 269-306.

Appendix D – Health History Questionnaire

Health History Questionnaire

Participant ID: _____

1. Age: _____

2. Sex
    a. Male
    b. Female

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

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

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

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

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

Appendix E – International Physical Activity Questionnaire (IPAQ)

Participant ID: _____

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE
(August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

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

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.
Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

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

   _____ **days per week**

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

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

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe

somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

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

_____ **days per week**

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

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

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

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

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

_____ **days per week**

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

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

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

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

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

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

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

Appendix F – Borg Scale for Rating of Perceived Exertion

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

Appendix G – Guidelines for Interpreting the IPAQ

Revised April 2004

APPENDIX 1

At A Glance
IPAQ Scoring Protocol (Short Versions)

Categorical Score- three levels of physical activity are proposed

1. *Inactive*

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

2. *Minimally Active*

   Any one of the following 3 criteria

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

3. *HEPA active*

   Any one of the following 2 criteria

   - Vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week **OR**
   - 7 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 3000 MET-minutes/week

Continuous Score

Expressed as MET-min per week: MET level x minutes of activity x events per week

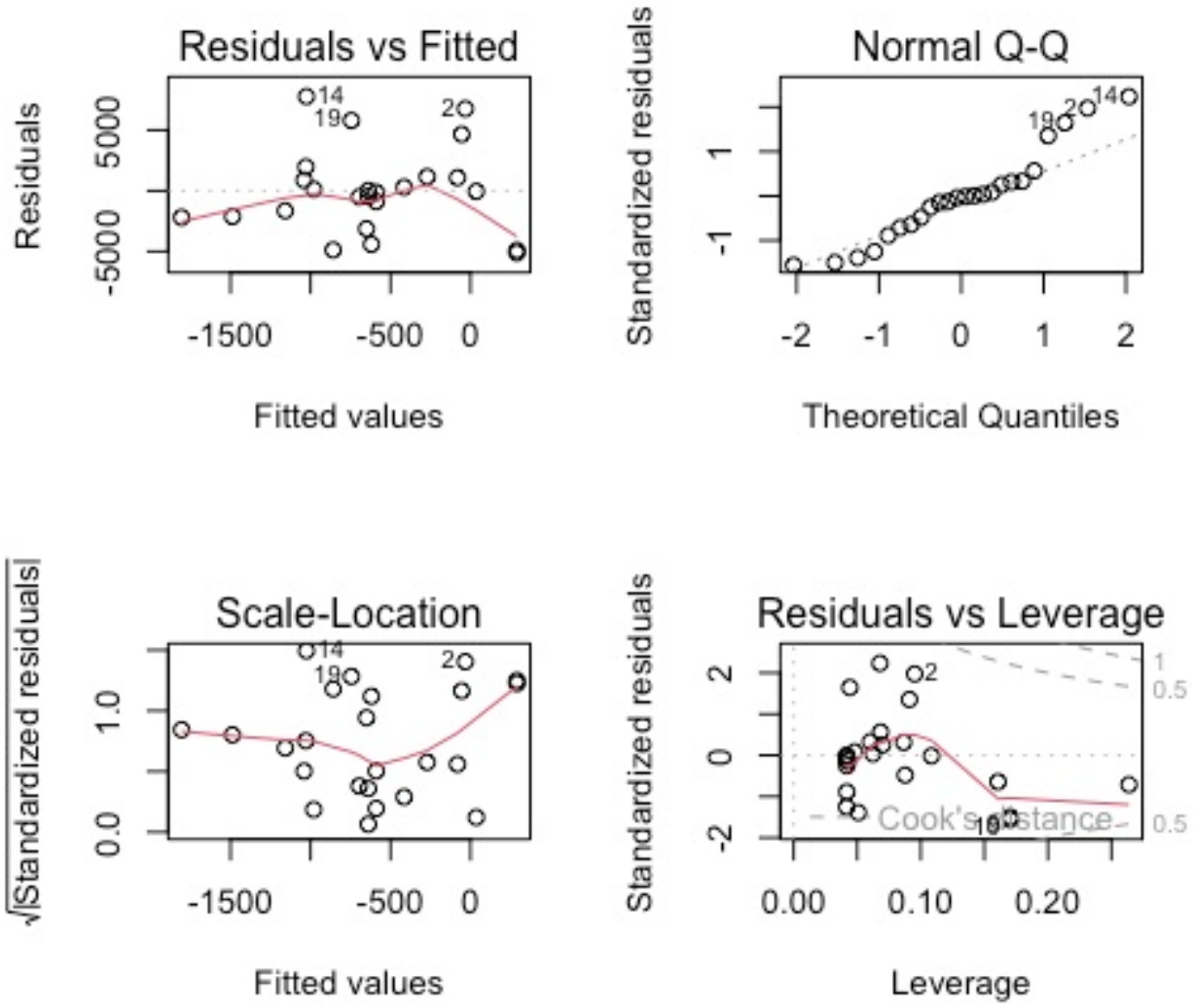
Sample Calculation

MET levels	**MET-min/week for 30 min episodes, 5 times/week**
Walking = 3.3 METs	3.3*30*5 = 495 MET-min/week
Moderate Intensity = 4.0 METs	4.0*30*5 = 600 MET-min/week
Vigorous Intensity = 8.0 METs	8.0*30*5 = 1,200 MET-min/week
	TOTAL = 2,295 MET-min/week

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

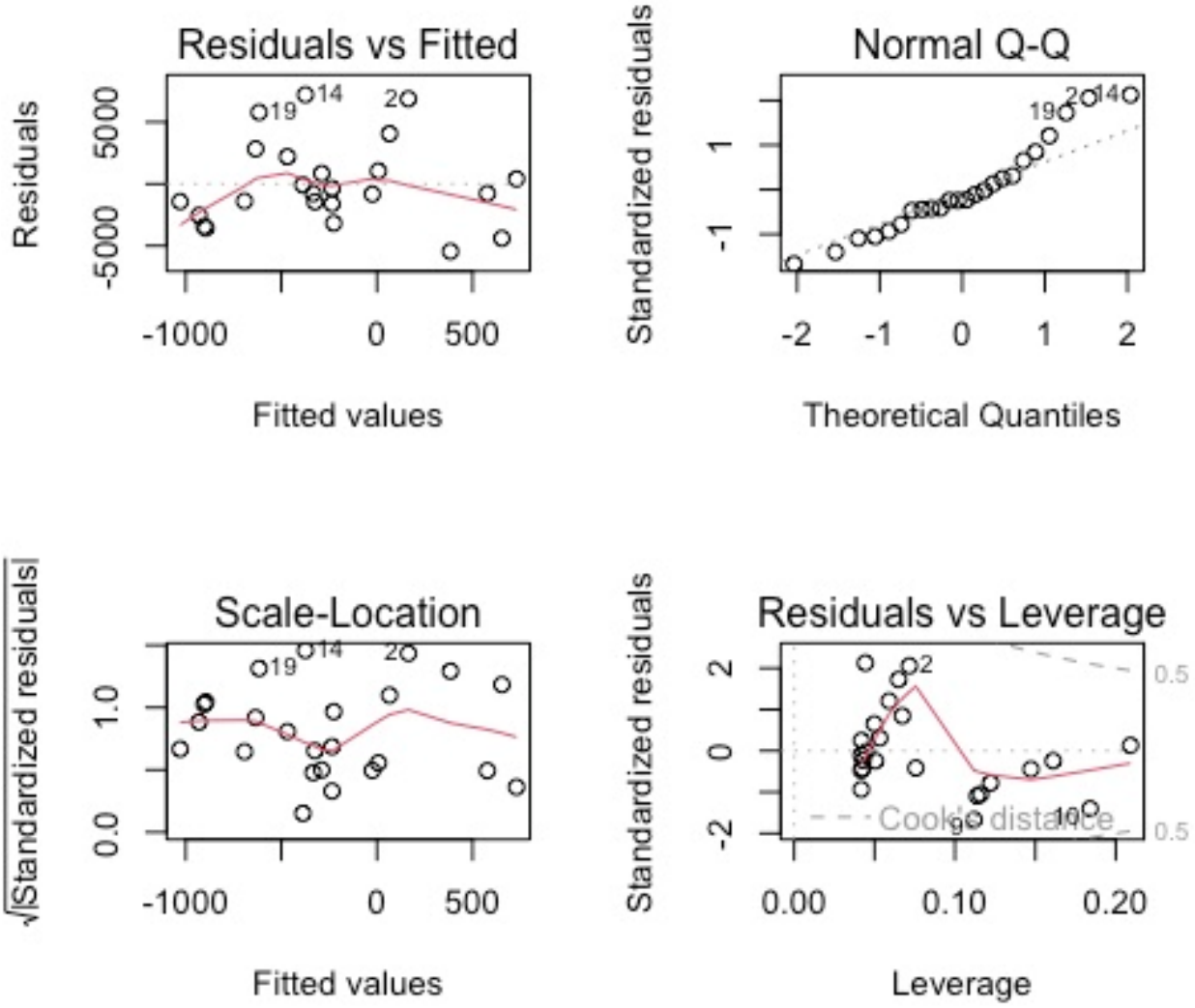
Please review the document "Guidelines for the data processing and analysis of the International Physical Activity Questionnaire (Short Form)" for more detailed description of IPAQ analysis and recommendations for data cleaning and processing [www.ipaq.ki.se].

Appendix H – Linear Regression Assumptions for Pre-Post Data



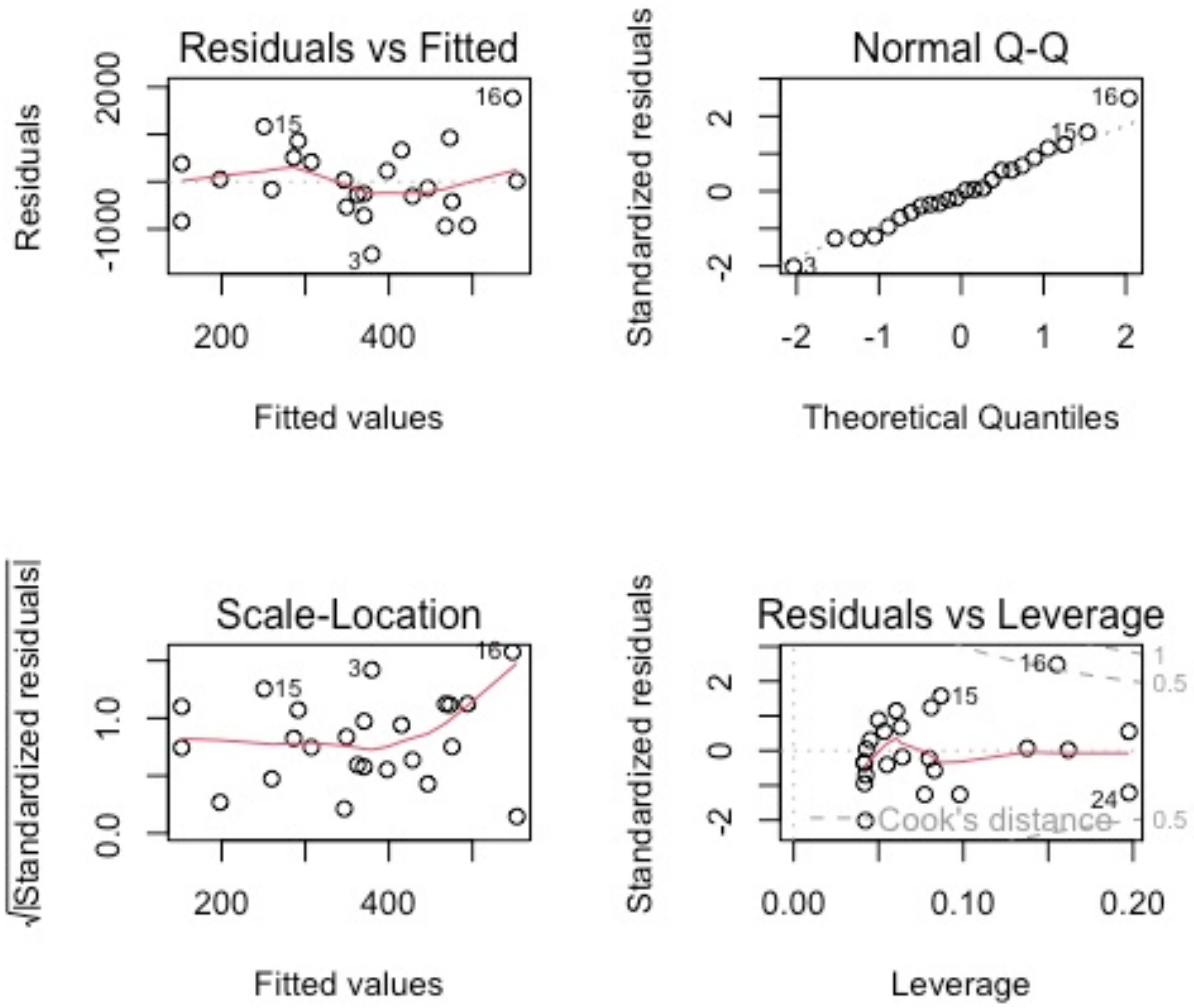
Note: The following graphs denote the assumptions for a linear regression model. Top left: plots the regression residuals vs. the fitted values. The red line shows a relatively even distribution between the predictor (blood lactate) and outcome (CSE) variables. Top right: The Q-Q plot shows if the residuals are normally distributed and indicate that there is no obvious deviation from the distribution. Bottom left: denotes a scale-location plot to confirm homoscedasticity. With a roughly horizontal red line. Bottom right: plots the residuals vs. leverage with Cook's distance. This plot denotes that there are no influential cases within the data.

Appendix I – Linear Regression Assumptions for Pre-Post10 Data



Note: The following graphs denote the assumptions for a linear regression model. Top left: plots the regression residuals vs. the fitted values. The red line shows a relatively even distribution between the predictor (blood lactate) and outcome (CSE) variables. Top right: The Q-Q plot shows if the residuals are normally distributed and indicate that there is no obvious deviation from the distribution. Bottom left: denotes a scale-location plot to confirm homoscedasticity. With a roughly horizontal red line. Bottom right: plots the residuals vs. leverage with Cook's distance. This plot denotes that there are no influential cases within the data.

Appendix I – Linear Regression Assumptions for Post-Post10 Data

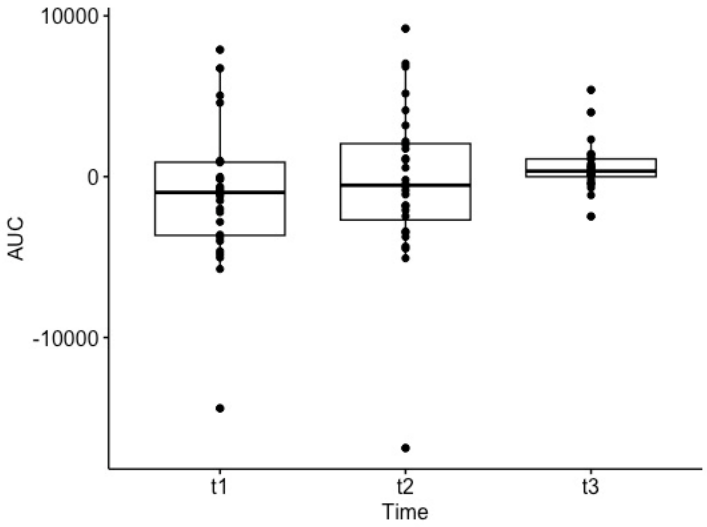


Note: The following graphs denote the assumptions for a linear regression model. Top left: plots the regression residuals vs. the fitted values. The red line shows a relatively even distribution between the predictor (blood lactate) and outcome (CSE) variables. Top right: The Q-Q plot shows if the residuals are normally distributed and indicate that there is no obvious deviation from the distribution. Bottom left: denotes a scale-location plot to confirm homoscedasticity. With a roughly horizontal red line. Bottom right: plots the residuals vs. leverage with Cook's distance. This plot denotes that there are no influential cases within the data.

Appendix J – Removal of Outliers Plots and Tables

Timepoint	ID	Outlier	Extreme Outlier
Pre	P025	True	False
Pre	P032	True	False
Post	P025	True	False
Post	P032	True	False
Post10	P031	True	True
Post10	P032	True	False
Post10	P035	True	False

Note: Participants considered outliers were removed from analysis.



Note: The boxplot was used to provide visual aid of outliers within the data. Participants considered outliers were removed from analysis.

REFERENCES

Abbruzzese, G., & Trompetto, C. (2002). Clinical and Research Methods for Evaluating Cortical Excitability. *Journal of Clinical Neurophysiology*, *19*(4), 307–321.

American College of Sports Medicine. (n.d.). *Benefits and Risks Associated with Physical Activity* (p. 21).

American College of Sports Medicine, Riebe, D., Ehrman, J. K., Liguori, G., & Magal, M. (Eds.). (2018). *ACSM's guidelines for exercise testing and prescription* (Tenth edition). Wolters Kluwer.

Andrews, S. C., Curtin, D., Hawi, Z., Wongtrakun, J., Stout, J. C., & Coxon, J. P. (2020). Intensity matters: High-intensity interval exercise enhances motor cortex plasticity more than moderate exercise. *Cerebral Cortex*, *30*(1), 101–112. https://doi.org/10.1093/cercor/bhz075

Badawy, R. A. B., Loetscher, T., Macdonell, R. A. L., & Brodtmann, A. (2013). Cortical excitability and neurology: Insights into the pathophysiology. *Functional Neurology*, *27*(3), 131–145.

Baltar, A., Nogueira, F., Marques, D., Carneiro, M., & Monte-Silva, K. (2018). Evidence of the homeostatic regulation with the combination of transcranial direct current stimulation and physical activity. *American Journal of Physical Medicine & Rehabilitation*, *97*(10), 727–733. https://doi.org/10.1097/PHM.0000000000000956

Barros, L. F. (2013). Metabolic signaling by lactate in the brain. *Trends in Neurosciences*, *36*(7), 396–404. https://doi.org/10.1016/j.tins.2013.04.002

Belman, M., & Gaesser, G. (1991). Exercise training below and above the lactate threshold in the elderly. *Medicine and Science in Sports and Exercise*, *23*(5), 562–568.

Beltz, N. M., Gibson, A. L., Janot, J. M., Kravitz, L., Mermier, C. M., & Dalleck, L. C. (2016). Graded exercise testing protocols for the determination of VO2max: Historical perspectives, progress, and future considerations. *Journal of Sports Medicine*, *2016*. https://doi.org/10.1155/2016/3968393

Beneke, R., Leithäuser, R. M., & Ochentel, O. (2011). Blood lactate diagnostics in exercise testing and training. *International Journal of Sports Physiology and Performance*, *6*(1), 8–24. https://doi.org/10.1123/ijspp.6.1.8

Bentley, D., McNaughton, L., & Batterham, A. (2001). Prolonged stage duration during incremental cycle exercise: Effects on the lactate threshold and onset of blood lactate accumulation. *European Journal of Applied Physiology*, *85*(3), 351–357. https://doi.org/10.1007/s004210100452

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*, *14*(5), 377–381.

Brooks, G. A. (1986a). Lactate production under fully aerobic conditions: The lactate shuttle during rest and exercise. *Federation Proceedings*, *45*(13), 2924–2929.

Brooks, G. A. (1986b). The lactate shuttle during exercise and recovery. *Medicine and Science in Sports and Exercise*, *18*(3), 360–368. https://doi.org/10.1249/00005768-198606000-00019

Canadian Society for Exercise Physiology. (2013). *CSEP-PATH*. Canadian Society for Exercise Physiology.

Carmichael, S. T. (2012). Brain excitability in stroke. *Archives of Neurology*, *69*(2), 161–167. https://doi.org/10.1001/archneurol.2011.1175

Coast, J. R., Cox, R. H., & Welch, H. G. (1986). Optimal pedalling rate in prolonged bouts of cycle ergometry. *Medicine and Science in Sports and Exercise*, *18*(2), 225–230.

Coco, M., Alagona, G., Perciavalle, V., Perciavalle, V., Cavallari, P., & Caronni, A. (2014). Changes in cortical excitability and blood lactate after a fatiguing hand-grip exercise. *Somatosensory & Motor Research*, *31*(1), 35–39. https://doi.org/10.3109/08990220.2013.834816

Coco, M., Alagona, G., Rapisarda, G., Costanzo, E., Calogero, R. A., Perciavalle, V., & Perciavalle, V. (2010a). Elevated blood lactate is associated with increased motor cortex excitability. *Somatosensory & Motor Research*, *27*(1), 1–8. https://doi.org/10.3109/08990220903471765

Coco, M., Alagona, G., Rapisarda, G., Costanzo, E., Calogero, R. A., Perciavalle, V., & Perciavalle, V. (2010b). Elevated blood lactate is associated with increased motor cortex excitability. *Somatosensory & Motor Research*, *27*(1), 1–8. https://doi.org/10.3109/08990220903471765

Coco, M., Buscemi, A., Ramaci, T., Tusak, M., Corrado, D. D., Perciavalle, V., Maugeri, G., Perciavalle, V., & Musumeci, G. (2020). Influences of Blood Lactate Levels on Cognitive Domains and Physical Health during a Sports Stress. Brief Review. *International Journal of Environmental Research and Public Health*, *17*(23), 9043. https://doi.org/10.3390/ijerph17239043

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. F., & Oja, P. (2003). International physical activity questionnaire: 12-country reliability and validity. *Medicine and Science in Sports and Exercise*, *35*(8), 1381–1395. https://doi.org/10.1249/01.MSS.0000078924.61453.FB

Cruz, R. S. de O., de Aguiar, R. A., Turnes, T., Penteado Dos Santos, R., Fernandes Mendes de Oliveira, M., & Caputo, F. (2012). Intracellular Shuttle: The Lactate Aerobic Metabolism. *The Scientific World Journal*, *2012*. https://doi.org/10.1100/2012/420984

Cuypers, K., Thijs, H., & Meesen, R. L. J. (2014). Optimization of the Transcranial Magnetic Stimulation protocol by defining a reliable estimate for corticospinal excitability. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0086380

Dalsgaard, M. K., Quistorff, B., Danielsen, E. R., Selmer, C., Vogelsang, T., & Secher, N. H. (2004). A reduced cerebral metabolic ratio in exercise reflects metabolism and not accumulation of lactate within the human brain. *The Journal of Physiology*, *554*(Pt 2), 571–578. https://doi.org/10.1113/jphysiol.2003.055053

Di Lazzaro, V., Pilato, F., Oliviero, A., Dileone, M., Saturno, E., Mazzone, P., Insola, A., Profice, P., Ranieri, F., Capone, F., Tonali, P. A., & Rothwell, J. C. (2006). Origin of facilitation of motor-evoked potentials after paired magnetic stimulation: Direct recording of epidural activity in conscious humans. *Journal of Neurophysiology*, *96*(4), 1765–1771. https://doi.org/10.1152/jn.00360.2006

Donovan, C. M., & Pagliassotti, M. J. (2000). Quantitative assessment of pathways for lactate disposal in skeletal muscle fiber types: *Medicine & Science in Sports & Exercise*, *32*(4), 772–777. https://doi.org/10.1097/00005768-200004000-00009

El-Sayes, J., Harasym, D., Turco, C. V., Locke, M. B., & Nelson, A. J. (2019). Exercise-induced neuroplasticity: A mechanistic model and prospects for promoting plasticity. *The Neuroscientist*, *25*(1), 65–85. https://doi.org/10.1177/1073858418771538

El-Sayes, J., Turco, C. V., Skelly, L. E., Locke, M. B., Gibala, M. J., & Nelson, A. J. (2020). Acute high-intensity and moderate-intensity interval exercise do not change corticospinal excitability in low fit, young adults. *PLoS One; San Francisco*, *15*(1), e0227581. http://dx.doi.org.ezproxy.library.dal.ca/10.1371/journal.pone.0227581

El-Sayes, J., Turco, C. V., Skelly, L. E., Nicolini, C., Fahnestock, M., Gibala, M. J., & Nelson, A. J. (2019). The effects of biological sex and ovarian hormones on exercise-induced neuroplasticity. *Neuroscience*, *410*, 29–40. https://doi.org/10.1016/j.neuroscience.2019.04.054

Garnier, Y. M., Lepers, R., Stapley, P. J., Papaxanthis, C., & Paizis, C. (2017). Changes in cortico-spinal excitability following uphill versus downhill treadmill exercise. *Behavioural Brain Research*, *317*, 242–250. https://doi.org/10.1016/j.bbr.2016.09.051

Gladden, L. B. (2004). Lactate metabolism: A new paradigm for the third millennium. *The Journal of Physiology*, *558*(Pt 1), 5–30. https://doi.org/10.1113/jphysiol.2003.058701

Goodwin, M. L., Harris, J. E., Hernández, A., & Gladden, L. B. (2007). Blood Lactate Measurements and Analysis during Exercise: A Guide for Clinicians. *Journal of Diabetes Science and Technology (Online)*, *1*(4), 558–569.

Griskova, I., Hoppner, J., Ruksenas, O., & Dapsys, K. (n.d.). Transcranial magnetic stimulation: The method and application. *Transcranial Magnetic Stimulation*, 7.

Hallett, M. (2000). Transcranial magnetic stimulation and the human brain. *Nature*, *406*(6792), 147–150.

Hariri, A. H. L. (2020). *Investigating the effect of aerobic exercise on corticospinal excitability: A scoping review*.

Harris, K. M., Fiala, J. C., & Ostroff, L. (2003). Structural changes at dendritic spine synapses during long-term potentiation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *358*(1432), 745–748. https://doi.org/10.1098/rstb.2002.1254

Hettiarachchi, I. T., Hanoun, S., Nahavandi, D., & Nahavandi, S. (2019). Validation of Polar OH1 optical heart rate sensor for moderate and high intensity physical activities. *PLoS ONE*, *14*(5), e0217288. https://doi.org/10.1371/journal.pone.0217288

Hubbard, I. J., Parsons, M. W., Neilson, C., & Carey, L. M. (2009). Task-specific training: Evidence for and translation to clinical practice. *Occupational Therapy International*, *16*(3–4), 175–189. https://doi.org/10.1002/oti.275

Jamnick, N. A., Botella, J., Pyne, D. B., & Bishop, D. J. (2018). Manipulating graded exercise test variables affects the validity of the lactate threshold and V˙O2peak. *PLoS ONE*, *13*(7). https://doi.org/10.1371/journal.pone.0199794

Kallioniemi, E., Pitkänen, M., Säisänen, L., & Julkunen, P. (2015). Onset latency of motor evoked potentials in motor cortical mapping with neuronavigated Transcranial Magnetic Stimulation. *The Open Neurology Journal*, *9*, 62–69. https://doi.org/10.2174/1874205X01509010062

Khan, F., Amatya, B., Galea, M. P., Gonzenbach, R., & Kesselring, J. (2017). Neurorehabilitation: Applied neuroplasticity. *Journal of Neurology*, *264*(3), 603–615. https://doi.org/10.1007/s00415-016-8307-9

Klomjai, W., Katz, R., & Lackmy-Vallée, A. (2015). Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Annals of Physical and Rehabilitation Medicine*, *58*(4), 208–213. https://doi.org/10.1016/j.rehab.2015.05.005

Krakauer, J. W. (2006). Motor learning: Its relevance to stroke recovery and neurorehabilitation: *Current Opinion in Neurology*, *19*(1), 84–90. https://doi.org/10.1097/01.wco.0000200544.29915.cc

Lamberts, R. P., Lambert, M. I., Swart, J., & Noakes, T. D. (2012). Allometric scaling of peak power output accurately predicts time trial performance and maximal oxygen consumption in trained cyclists. *British Journal of Sports Medicine*, *46*(1), 36–41. https://doi.org/10.1136/bjsm.2010.083071

Lanzi, S., Codecasa, F., Cornacchia, M., Maestrini, S., Capodaglio, P., Brunani, A., Fanari, P., Salvadori, A., & Malatesta, D. (2015). Long Maximal Incremental Tests Accurately Assess Aerobic Fitness in Class II and III Obese Men. *PLOS ONE*, *10*(4), e0124180. https://doi.org/10.1371/journal.pone.0124180

Lanzi, S., Codecasa, F., Cornacchia, M., Maestrini, S., Salvadori, A., Brunani, A., & Malatesta, D. (2014). Fat oxidation, hormonal and plasma metabolite kinetics during a submaximal incremental test in lean and obese adults. *PloS One*, *9*(2), e88707. https://doi.org/10.1371/journal.pone.0088707

Livingston, S. C., & Ingersoll, C. D. (2008). Intra-rater reliability of a Transcranial Magnetic Stimulation technique to obtain motor evoked potentials. *International Journal of Neuroscience*, *118*(2), 239–256. https://doi.org/10.1080/00207450701668020

Lulic, T., El-Sayes, J., Fassett, H. J., & Nelson, A. J. (2017a). Physical activity levels determine exercise-induced changes in brain excitability. *PloS One*, *12*(3), e0173672. http://dx.doi.org.ezproxy.library.dal.ca/10.1371/journal.pone.0173672

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

MacDonald, M. A., Khan, H., Kraeutner, S. N., Usai, F., Rogers, E. A., Kimmerly, D. S., Dechman, G., & Boe, S. G. (2019). Intensity of acute aerobic exercise but not aerobic fitness impacts on corticospinal excitability. *Applied Physiology, Nutrition, and Metabolism*, *44*(8), 869–878. https://doi.org/10.1139/apnm-2018-0643

Maddock, R. J., Casazza, G. A., Fernandez, D. H., & Maddock, M. I. (2016). Acute modulation of cortical glutamate and GABA content by physical activity. *The Journal of Neuroscience*, *36*(8), 2449–2457. https://doi.org/10.1523/JNEUROSCI.3455-15.2016

Magistretti, P. J., & Allaman, I. (2018). Lactate in the brain: From metabolic end-product to signalling molecule. *Nature Reviews. Neuroscience; London*, *19*(4), 235–249. http://dx.doi.org.ezproxy.library.dal.ca/10.1038/nrn.2018.19

Management of the upper extremity following stroke. (2019). Canadian Stroke Best Practices. https://www.strokebestpractices.ca/en/recommendations/stroke-rehabilitation/management-of-the-upper-extremity-following-stroke/

Mang, C. S., Brown, K. E., Neva, J. L., Snow, N. J., Campbell, K. L., & Boyd, L. A. (2016). Promoting Motor Cortical Plasticity with Acute Aerobic Exercise: A Role for Cerebellar Circuits. *Neural Plasticity*, *2016*, e6797928. https://doi.org/10.1155/2016/6797928

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

Mann, T., Lamberts, R. P., & Lambert, M. I. (2013). Methods of prescribing relative exercise intensity: Physiological and practical considerations. *Sports Medicine*, *43*(7), 613–625. https://doi.org/10.1007/s40279-013-0045-x

Mateos-Aparicio, P., & Rodríguez-Moreno, A. (2019). The impact of studying brain plasticity. *Frontiers in Cellular Neuroscience*, *13*. https://doi.org/10.3389/fncel.2019.00066

McArdle, W. D., Katch, F. I., & Katch, V. L. (2015). *Exercise Physiology: Energy, nutrition, and human performance* (Eighth). Wolters Kluwer Health/Lippincott Williams & Wilkins.

McDonnell, M. N., Buckley, J. D., Opie, G. M., Ridding, M. C., & Semmler, J. G. (2013). A single bout of aerobic exercise promotes motor cortical neuroplasticity. *Journal of Applied Physiology*, *114*(9), 1174–1182. https://doi.org/10.1152/japplphysiol.01378.2012

McDonnell, M. N., Orekhov, Y., & Ziemann, U. (2006). The role of GABAB receptors in intracortical inhibition in the human motor cortex. *Experimental Brain Research*, *173*(1), 86–93. https://doi.org/10.1007/s00221-006-0365-2

Mekari, S., Fraser, S., Bosquet, L., Bonnéry, C., Labelle, V., Pouliot, P., Lesage, F., & Bherer, L. (2015). The relationship between exercise intensity, cerebral oxygenation and cognitive performance in young adults. *European Journal of Applied Physiology*, *115*(10), 2189–2197. https://doi.org/10.1007/s00421-015-3199-4

Meyer, D., Dungs, C., & Senner, V. (2015). Estimating the Relationship between Heart Rate and Power Output for Short Term Cycling Exercises. *Procedia Engineering*, *112*, 237–243. https://doi.org/10.1016/j.proeng.2015.07.206

Meyer, T., Gabriel, H. H., & Kindermann, W. (1999). Is determination of exercise intensities as percentages of VO2max or HRmax adequate? *Medicine and Science in Sports and Exercise*, *31*(9), 1342–1345. https://doi.org/10.1097/00005768-199909000-00017

Mooney, R. A., Coxon, J. P., Cirillo, J., Glenny, H., Gant, N., & Byblow, W. D. (2016). Acute aerobic exercise modulates primary motor cortex inhibition. *Experimental Brain Research*, *234*(12), 3669–3676.

Morris, T. P., Fried, P. J., Macone, J., Stillman, A., Gomes-Osman, J., Costa-Miserachs, D., Muñoz, J. M. T., Santarnecchi, E., & Pascual-Leone, A. (2020). Light aerobic exercise modulates executive function and cortical excitability. *European Journal of Neuroscience*, *51*(7), 1723–1734. https://doi.org/10.1111/ejn.14593

Moscatelli, F., Valenzano, A., Petito, A., Triggiani, A. I., Cilibert, M. A. P., Luongo, L., Carotenuto, M., Esposito, M., Messina, A., Monda, V., Monda, M., Capranica, L., Messina, G., & Cibelli, G. (2016). Relationship between blood lactate and cortical excitability between taekwondo athletes and non-athletes after hand-grip exercise. *Somatosensory & Motor Research*. http://dx.doi.org/10.1080/08990220.2016.1203305

Moscatelli, F., Valenzano, A., Petito, A., Triggiani, A. I., Ciliberti, M. A. P., Luongo, L., Carotenuto, M., Esposito, M., Messina, A., Monda, V., Monda, M., Capranica, L., Messina, G., & Cibelli, G. (2016). Relationship between blood lactate and cortical excitability between taekwondo athletes and non-athletes after hand-grip exercise. *Somatosensory & Motor Research*, *33*(2), 137–144. https://doi.org/10.1080/08990220.2016.1203305

Müller, P., Duderstadt, Y., Lessmann, V., & Müller, N. G. (2020). Lactate and BDNF: Key mediators of exercise induced neuroplasticity? *Journal of Clinical Medicine*, *9*(4), 1136. https://doi.org/10.3390/jcm9041136

Neva, J. L., Brown, K. E., Mang, C. S., Francisco, B. A., & Boyd, L. A. (2017). An acute bout of exercise modulates both intracortical and interhemispheric excitability. *European Journal of Neuroscience*, *45*(10), 1343–1355. https://doi.org/10.1111/ejn.13569

Nicolini, C., Fahnestock, M., Gibala, M. J., & Nelson, A. J. (2020). Understanding the Neurophysiological and Molecular Mechanisms of Exercise-Induced Neuroplasticity in Cortical and Descending Motor Pathways: Where Do We Stand? *Neuroscience*, S030645222030782X. https://doi.org/10.1016/j.neuroscience.2020.12.013

Overgaard, M., Rasmussen, P., Bohm, A. M., Seifert, T., Brassard, P., Zaar, M., Homann, P., Evans, K. A., Nielsen, H. B., & Secher, N. H. (2012). Hypoxia and exercise provoke both lactate release and lactate oxidation by the human brain. *The FASEB Journal*, *26*(7), 3012–3020. https://doi.org/10.1096/fj.11-191999

Pallarés, J. G., Morán-Navarro, R., Ortega, J. F., Fernández-Elías, V. E., & Mora-Rodriguez, R. (2016). Validity and reliability of ventilatory and blood lactate thresholds in well-trained cyclists. *PloS ONE*, *11*(9), e0163389. https://doi.org/10.1371/journal.pone.0163389

Perciavalle, V., Coco, M., Alagona, G., Maci, T., & Perciavalle, V. (2010a). Gender differences in changes of motor cortex excitability during elevated blood lactate levels. *Somatosensory & Motor Research*, *27*(3), 106–110. https://doi.org/10.3109/08990220.2010.507102

Perciavalle, V., Coco, M., Alagona, G., Maci, T., & Perciavalle, V. (2010b). Gender differences in changes of motor cortex excitability during elevated blood lactate levels. *Somatosensory & Motor Research*, *27*(3), 106–110. https://doi.org/10.3109/08990220.2010.507102

Peri, E., Ambrosini, E., Colombo, V. M., Ruit, M. van de, Grey, M. J., Monticone, M., Ferriero, G., Pedrocchi, A., Ferrigno, G., & Ferrante, S. (2017). Intra and inter-session reliability of rapid Transcranial Magnetic Stimulation stimulus-response curves of tibialis anterior muscle in healthy older adults. *PLOS ONE*, *12*(9), e0184828. https://doi.org/10.1371/journal.pone.0184828

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

Pinot, J., & Grappe, F. (2014). *Determination of maximal aerobic power on the field in cycling*. *3*, 26–31.

Powers, S. K., & Howley, E. T. (2018). *Exercise physiology: Theory and application to fitness and performance* (Tenth edition). McGraw-Hill Education.

Proia, P., Di Liegro, C. M., Schiera, G., Fricano, A., & Di Liegro, I. (2016). Lactate as a Metabolite and a Regulator in the Central Nervous System. *International Journal of Molecular Sciences*, *17*(9). https://doi.org/10.3390/ijms17091450

Purves, D., Augustine, G. J., Fitzpatrick, D., Hall, W. C., LaMantia, A.-S., McNamara, J. O., & Williams, S. M. (Eds.). (2004). *Neuroscience* (Third Edition). Sinaur Associates, Inc.

Quistorff, B., Secher, N. H., & Van Lieshout, J. J. (2008). Lactate fuels the human brain during exercise. *The FASEB Journal*, *22*(10), 3443–3449. https://doi.org/10.1096/fj.08-106104

Rascon, J., Trujillo, E., Morales-Acuña, F., & Gurovich, A. N. (2020). Differences between males and females in determining exercise intensity. *International Journal of Exercise Science*, *13*(4), 1305–1316.

Riske, L., Thomas, R. K., Baker, G. B., & Dursun, S. M. (2017). Lactate in the brain: An update on its relevance to brain energy, neurons, glia and panic disorder. *Therapeutic Advances in Psychopharmacology*, *7*(2), 85–89. https://doi.org/10.1177/2045125316675579

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P. B., George, M. S., Hallett, M., Lefaucheur, J. P., Langguth, B., Matsumoto, H., Miniussi, C., Nitsche, M. A., Pascual-Leone, A., Paulus, W., Rossi, S., … Ziemann, U. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology*, *126*(6), 1071–1107. https://doi.org/10.1016/j.clinph.2015.02.001

Sada, N., Lee, S., Katsu, T., Otsuki, T., & Inoue, T. (2015). Epilepsy treatment. Targeting LDH enzymes with a stiripentol analog to treat epilepsy. *Science (New York, N.Y.)*, *347*(6228), 1362–1367. https://doi.org/10.1126/science.aaa1299

Saikaley, M., Pauli, G., Iruthayarajah, J., Mirkowski, M., Iliescu, A., Fragis, N., Alam, R., Harris, J., Dukelow, S., Chae, J., Knutson, J., Miller, T., & Teasell, R. (n.d.). Upper extremity motor rehabilitation interventions. In *Evidence Based Review Stroke Rehabilitation* (p. 366).

Sanghvi, S. (2013). Cardiac Rehabilitation. In S. B. Porter (Ed.), *Tidy's Physiotherapy (Fifteenth Edition)* (pp. 147–168). Churchill Livingstone. https://doi.org/10.1016/B978-0-7020-4344-4.00008-0

Schurr, A. (2008). Lactate: A major and crucial player in normal function of both muscle and brain. *The Journal of Physiology*, *586*(11), 2665–2666. https://doi.org/10.1113/jphysiol.2008.155416

Singh, A. M., Duncan, R. E., Neva, J. L., & Staines, W. R. (2014). Aerobic exercise modulates intracortical inhibition and facilitation in a nonexercised upper limb muscle. *BMC Sports Science, Medicine and Rehabilitation*, *6*, 23. https://doi.org/10.1186/2052-1847-6-23

Singh, A. M., Neva, J. L., & Staines, W. R. (2016). Aerobic exercise enhances neural correlates of motor skill learning. *Behavioural Brain Research*, *301*, 19–26. https://doi.org/10.1016/j.bbr.2015.12.020

Singh, A. M., & Staines, W. R. (2015). The Effects of Acute Aerobic Exercise on the Primary Motor Cortex. *Journal of Motor Behavior*, *47*(4), 328–339. https://doi.org/10.1080/00222895.2014.983450

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

Smith, A. E., Goldsworthy, M. R., Wood, F. M., Olds, T. S., Garside, T., & Ridding, M. C. (2018). High-intensity aerobic exercise blocks the facilitation of iTBS-induced plasticity in the human motor cortex. *Neuroscience*, *373*, 1–6. https://doi.org/10.1016/j.neuroscience.2017.12.034

Smith, E. W., Skelton, M. S., Kremer, D. E., Pascoe, D. D., & Gladden, L. B. (1998). Lactate distribution in the blood during steady-state exercise. *Medicine & Science in Sports & Exercise*, *30*(9), 1424.

Suzuki, A., Stern, S. A., Bozdagi, O., Huntley, G. W., Walker, R. H., Magistretti, P. J., & Alberini, C. M. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell*, *144*(5), 810–823. https://doi.org/10.1016/j.cell.2011.02.018

Teasell, R., Bayona, N. A., & Bitensky, J. (2005). Plasticity and reorganization of the brain post stroke. *Topics in Stroke Rehabilitation*, *12*(3), 11–26. https://doi.org/10.1310/6AUM-ETYW-Q8XV-8XAC

Todd, J. J. (2014). Lactate: Valuable for physical performance and maintenance of brain function during exercise. *Bioscience Horizons*, *7*(0), hzu001–hzu001. https://doi.org/10.1093/biohorizons/hzu001

Turco, C. V., & Nelson, A. J. (2021). Transcranial Magnetic Stimulation to assess exercise-induced neuroplasticity. *Frontiers in Neuroergonomics*, *2*. https://doi.org/10.3389/fnrgo.2021.679033

Valentini, M., & Parati, G. (2009). Variables Influencing Heart Rate. *Progress in Cardiovascular Diseases*, *52*(1), 11–19. https://doi.org/10.1016/j.pcad.2009.05.004

Wyss, M. T., Jolivet, R., Buck, A., Magistretti, P. J., & Weber, B. (2011). In Vivo evidence for lactate as a neuronal energy source. *Journal of Neuroscience*, *31*(20), 7477–7485. Scopus. https://doi.org/10.1523/JNEUROSCI.0415-11.2011

Yamazaki, Y., Sato, D., Yamashiro, K., Nakano, S., Onishi, H., & Maruyama, A. (2019). Acute low-intensity aerobic exercise modulates intracortical inhibitory and excitatory circuits in an exercised and a non-exercised muscle in the primary motor cortex. *Frontiers in Physiology*, *10*. https://doi.org/10.3389/fphys.2019.01361

Yang, J., Ruchti, E., Petit, J.-M., Jourdain, P., Grenningloh, G., Allaman, I., & Magistretti, P. J. (2014a). Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proceedings of the National Academy of Sciences*, *111*(33), 12228–12233.

Yang, J., Ruchti, E., Petit, J.-M., Jourdain, P., Grenningloh, G., Allaman, I., & Magistretti, P. J. (2014b). Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proceedings of the National Academy of Sciences*, *111*(33), 12228–12233. https://doi.org/10.1073/pnas.1322912111

Yousry, T. A., Schmid, U. D., Alkadhi, H., Schmidt, D., Peraud, A., Buettner, A., & Winkler, P. (1997). Localization of the motor hand area to a knob on the precentral gyrus. A new landmark. *Brain: A Journal of Neurology*, *120 (Pt 1)*, 141–157. https://doi.org/10.1093/brain/120.1.141