EFFECTS OF AN ACUTE BOUT OF MODERATE-INTENSITY AEROBIC EXERCISE ON MOTOR LEARNING AND NEUROPLASTICITY

by

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Abstract

Aerobic exercise has been promoted as a possible adjunct therapy to neurorehabilitation practice, given its positive effects on brain health. In healthy young adults, acute high-intensity cycling can enhance motor performance and learning of a complex motor task, and promote neuroplasticity in the motor system. However, clinical populations may not be able to participate in high-intensity exercise. To date there is inconsistent evidence for the efficacy of moderate-intensity aerobic exercise to alter motor learning and neuroplasticity in healthy young adults. Using two experiments, we aimed to determine how acute moderate-intensity cycling affects motor behavior and neuroplasticity in healthy young individuals.

First, 16 participants practiced a complex motor skill after 30 minutes of moderate-intensity cycling or seated rest, on separate occasions. Motor performance was assessed at baseline, immediately after, and 5 minutes after exercise or rest. Twenty-four hours later, we assessed motor learning at a no-exercise retention test. Under the exercise condition, participants maintained performance over time, whereas, performance diminished over time under the rest condition, and became worse than post-exercise performance. Conditions did not differ at retention.

Second, another group of 16 participants underwent paired associative stimulation (PAS) a transcranial magnetic stimulation (TMS) protocol known to induce neuroplasticity in the motor system. Effects of PAS were separately compared after a 30-minute bout of moderate-intensity cycling versus seated rest. At baseline, immediately after PAS, and 30 minutes post-PAS, we measured corticomotoneuronal excitability and excitability of intracortical neural circuits using TMS. We found that PAS increased corticomotoneuronal excitability when performed after

exercise, but not rest. Exercise and PAS modulated activity in specific neural circuits post-intervention, without similar results under the rest condition.

Moderate-intensity aerobic exercise can promote neuroplasticity in the motor system, but in this study similar effects did not transfer to behavioral measures of motor learning. In order to evaluate the clinical feasibility of this pairing moderate intensity exercise with skilled motor practice, we must first elucidate the dose-response effects of exercise on motor behavior, explore timing effects of exercise on motor learning, and examine how long-term pairing of exercise with practice impacts motor learning.

Preface

The present thesis contains two experiments that have been completed by the candidate Nicholas Jacob Snow, under the supervision of Dr. Lara A. Boyd, with the assistance of Mr. Cameron Scott Mang (PhD Candidate). Experimental design and conception, data acquisition and analysis, data interpretation, and documentation were primarily the work of the candidate.

Both experiments and all associated methods were approved by the University of British Columbia (UBC)'s Clinical Research Ethics Board (certificate # H14-01556).

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List of Abbreviations

[BLa]: Blood lactate concentration

ACSM: American College of Sports Medicine

APB: *Abductor pollicis brevis*

BDNF: Brain-derived neurotrophic factor

BLa: Blood lactate

CNS: Central nervous system

CS: Conditioning stimulus

CT Task: Continuous tracking task

cTBS: Continuous theta-burst stimulation

EEG: Electroencephalography

EMG: Electromyography

GABA: γ-aminobutyric acid

$GABA_A$: GABA receptor subtype A

$GABA_B$: GABA receptor subtype B

GXT: Graded maximal exercise test

HR: Heart rate

HRpeak: Peak HR

IPAQ: International Physical Activity Questionnaire

I-wave: Indirect-wave

ISI: Inter-stimulus interval

iTBS: Intermittent theta-burst stimulation

LICI: Long-interval intracortical inhibition

LTD: Long-term depression

LTP: Long-term potentiation

LSD: Least significant difference

M-wave: Compound motor unit action potential

M1: Primary motor cortex

MEP: Motor evoked potential

MET: Metabolic equivalent of task

Mmax: Maximal M-wave

MSO: Maximal stimulator output

NE: Norepinephrine

NMDA: N-methyl-D-aspartate, a glutamate receptor

PAR-Q: Physical Activity Readiness Questionnaire

PAS: Paired associative stimulation

PO: Power output

RER: Respiratory exchange ratio

rmANOVA: Repeated-measures analysis of variance

RMSE: Root-mean-square error

RMT: Resting motor threshold

RPE: Rating of perceived exertion

RPM: Revolutions per minute

rTMS: Repetitive transcranial magnetic stimulation

SEM: Standard error of mean

$SI_{1\ mV}$: Magnetic stimulus intensity to evoke a ~1 mV MEP

STDP: Spike timing-dependent plasticity

TBS: theta-burst stimulation

TMS: Transcranial magnetic stimulation

TS: Test stimulus

TSA: Time series analysis

$\dot{V}CO_2$: Carbon dioxide output

$\dot{V}_E$: Minute ventilation

$\dot{V}O_2$: Oxygen uptake

$\dot{V}O_{2peak}$: Peak $\dot{V}O_2$, peak aerobic capacity

VT: Ventilatory threshold

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Dedication

To Mom, Dad, and Kyle

1 Introduction and Purpose

1.1 Introduction

In 2013 it was estimated that approximately 405,000 Canadians were directly affected by stroke.[6] Among individuals who survive a stroke, many experience varying degrees of motor impairments – approximately 36% of persons with stroke have significant disabilities 5 years post-infarct[7] and over 40% of these individuals require assistance with activities of daily living.[8] At present stoke costs the Canadian economy roughly $3.6 Billion annually,[9] with a lifetime individual cost of over $100,000.[10] Given that there is an expected increase in stroke prevalence, up to 726,000 by 2038,[6] it is imperative that interventions be developed to increase independence and quality of life among persons with stroke-related motor impairments.

During the past several years principles of motor learning have been used to guide neurorehabilitation efforts for motor impairments after stroke.[11,12] Nearly every aspect of human behavior involves the execution of some learned motor skill;[13] and importantly, it is believed that the same principles apply to both the acquisition of novel motor skills and the re-learning of previously consolidated skills.[13] Motor learning involves the acquisition and refinement of movement sequences in a novel order,[14] and refers to a relatively permanent change in an individual's internal capability for movement that is acquired through practice.[15] The evolution of motor memories during motor learning is a type of procedural (non-declarative) memory process that can be accessed implicitly (i.e., without conscious awareness).[15,16] Improved motor performance over time can occur via both generalized improvements in motor control or via the formation of a motor memory that is specific to a movement sequence.[17,18] A recent meta-analysis reported a positive dose-response relationship between time spent receiving physical

therapy and improvements in motor function and impairment after stroke.[19] Motor rehabilitation practices rely on principles of implicit sequence-specific motor learning;[12,20] and meaningful, skilled motor practice is required to drive changes in the brain.[21] Despite the well-known fact that improved motor behavior is a function of increased motor practice,[14,15] increased time in therapy presents a significant financial burden on persons with stroke; and existing neurorehabilitation methods do not consistently lead to positive motor outcomes.[12,19] As a consequence, the dose of treatment required to induce lasting behavioral changes may not feasible in the present healthcare setting. This limitation to current practice has led to an interest in the development of adjunct therapies that may be paired with standard neurorehabilitation procedure. Thus, it is desirable to explore possible neuromodulators that have the potential to enhance the benefits of existing neurorehabilitation techniques.

Novel literature suggests that aerobic exercise may be beneficial to neurorehabilitation by priming the brain for enhanced motor learning.[22–26] Indeed, there is consensus that aerobic exercise is a robust intervention to globally promote brain health[22,27–30] and enhance various forms of cognition[31–33] and memory.[25] In healthy young adults an acute bout of aerobic exercise can improve both the acquisition[23] and retention[23,24] of an implicitly-learned complex motor skill; and more recent evidence points to effects of acute aerobic exercise on explicit movement sequences.[34] In the first study to demonstrate positive effects of acute aerobic exercise on motor learning, Roig and colleagues[24] showed that participants who completed a single session of high-intensity cycling intervals in close temporal proximity to a continuous visuomotor task displayed significantly lower root-mean-square error (RMSE) both 24 hours and 7 days after initial exposure to the task, compared to a resting control group. Furthermore, those who exercised after skilled motor practice performed greatest during the 7-day retention period.[24] The authors

suggested that the high-intensity aerobic exercise bout enhanced motor memory consolidation.[24] More recently, work in our laboratory by Mang and others[23] demonstrated that when participants completed high-intensity cycling intervals prior to practicing a continuous tracking (CT) task[18] they improved acquisition and 24-hour retention of the temporal portion of an implicitly learned movement sequence, compared to CT task practice under a rest condition. Finally, Rhee et al.[34] found that the completion of vigorous continuous cycling had a protective effect on an explicitly-learned discrete movement sequence. When exercise was performed just prior to the performance of a movement sequence designed to interfere with the to-be-learned target sequence, there were improvements in offline memory gains compared to a control condition.[34] However, when exercise occurred immediately after practicing the target sequence, this protective effect was not apparent.[34] Thus, there is evidence that high-intensity aerobic exercise promotes improvements in general motor skills, as well as implicit and explicit sequence-specific motor learning. Likewise, both single and repeated sessions of aerobic exercise are beneficial to various forms of declarative and non-declarative memory,[25,35–37] as well as motor performance (distinct from motor learning[38]).[23,39,40] Yet, at present there is no evidence to support the efficacy of lower exercise intensities to promote improvements in motor learning.[34]

Proposed mechanisms for aerobic exercise effects on memory and motor learning are myriad. Generally, these explanations can be characterized by modifications in behavior, up-regulation of neuroendocrine activity, and changes in brain structure or function. Behaviourally, the benefits of aerobic exercise are discussed with reference to cognitive function, and have been outlined in several meta-analyses and systematic reviews.[31–33,41–43] For example, an acute moderate-intensity aerobic exercise bout can enhance indices of attention;[44–47] response planning, preparation, and inhibition;[48–51] working memory;[50] and reasoning.[50] Furthermore,

electroencephalographic (EEG) experiments show that a single session of aerobic exercise significantly modulates electrophysiological indices of attention,[44–47,52–54] response inhibition and preparation,[47,52,55] and sensory gating.[47] These beneficial effects are present for nearly an hour post-exercise,[49] and are apparently unrelated to exercise-induced changes in arousal or emotional stress.[56] It appears that aerobic exercise affects the above processes by shifting allocation of cognitive resources to more implicit pathways,[45,55] thus reducing the cognitive load associated with performing the experimental tasks at rest. Given these effects on cognition, it is presumable that exercise also impacts the encoding of motor memories, and enhances online motor performance.[25,57]

From a neuroendocrine perspective, aerobic exercise upregulates a cascade of neurochemicals that are positively associated with improvements in brain health.[27] In comparison to the animal literature the mechanisms that underlie memory formation in humans are less well understood. This is mainly due to the inability to invasively and directly measure central levels of hormones and neurochemicals. Although measuring biomarkers that are associated with exercise peripherally in humans is becoming increasingly recognized, strong conclusions regarding the direct relationships between behavior and central neural mechanisms cannot be drawn (e.g., due to lack of blood-brain barrier permeability to certain molecules,[58] or due to short molecular half-life[59]). Taken together studies of animals and peripheral changes in humans suggest that exercise-induced increases in neurochemicals or hormones in relation to changes in behavior represent specific molecular pathways that may be involved in motor learning and memory formation.[60] Accordingly, evidence indicates that high-intensity exercise influences on motor learning are related to increases in circulating levels of catecholamines, growth factors, and a milieu of other neurochemicals involved in brain recovery.[30,35,61–63] Specifically, recent

work has shown that high-intensity exercise-induced up-regulation of systemic norepinephrine (NE),[61] dopamine,[35,61] and epinephrine[35] is associated with increased long-term memory. Increased serum brain-derived neurotrophic factor (BDNF) after high-intensity exercise has also been related to memory consolidation.[61,64] In addition, high intensity exercise induces increases in blood lactate (BLa, which correlates with motor memory),[61] and significantly increases serum endocannabinoid concentration.[63] Likewise, increases in BLa modulate are associated with changes in primary motor cortex (M1) excitability,[65] while changes in serum dopamine[66] and epinephrine[67] are linked to positive effects on human memory. Although moderate-intensity bouts of exercise reportedly increase circulating BLa, BDNF,[30,62] catecholamines,[35,36] and endocannabinoids,[68] such changes in circulating neurochemicals occur to a lesser degree than after high-intensity exercise.[35] Nonetheless, there is strong overall evidence that exercise-induced changes in various hormones and neurochemicals leads to positive effects on memory consolidation and motor learning.

Neuroimaging studies have provided evidence for regional specificity of exercise effects. For instance, acute aerobic exercise at moderate intensities has been shown to increase brain activity in sensorimotor regions[69] and areas implicated in cognitive processing and working memory.[70–72] Likewise, aerobic exercise has acutely been shown to globally increase cerebral blood volume[73] and cerebral blood flow in white matter.[74] Long-term aerobic exercise has also been attributed to increases in hippocampal volume, which correlates with enhancements in memory.[75] Finally, in recent transcranial magnetic stimulation (TMS) studies acute aerobic exercise influenced the activity of intracortical brain networks.[76,77] Specifically, a single bout of moderate-intensity cycling reduces short-interval intracortical inhibition (SICI)[76,77] and increases intracortical facilitation (ICF),[76] and could reduce long-interval intracortical inhibition (LICI)[76]

in M1 representations for non-exercised upper-limb muscles. Briefly, SICI is measured by paired-pulse TMS when a sub-threshold stimulus is followed 1-5 ms later by a supra-threshold TMS pulse.[78] SICI measured using a 1 ms ISI presumably assesses intracortical inhibition modulated by extra-synaptic levels of the inhibitory neurotransmitter γ-aminobutyric acid (GABA),[79] while longer ISIs examine $GABA_A$-receptor-mediated inhibition.[80] ICF is collected in a similar manner to SICI (i.e., using a sub- and supra-threshold stimulus conditioning-test paradigm), except that ICF employs ISIs between 8 and 30 ms.[78] ICF is thought to be functionally related to the excitatory neurotransmitter glutamate and its receptor, *N*-methyl-$_D$-aspartate (NMDA).[81] LICI involves two supra-threshold TMS stimuli separated by 50-200 ms, and is believed to be an index of the effects of the $GABA_B$ receptor subtype.[82] Exercise effects on these outcomes are noteworthy due to the role of the above neurochemicals in neuroplasticity and stroke recovery.[83,84] After stroke, motor recovery is hampered by a substantial degree of intracortical inhibition in the lesioned brain hemisphere;[85] and thus, by releasing inhibition aerobic exercise has the capacity to create a fertile brain environment in which learning can occur.[22,85,84,86]

Motor learning involves the acquisition and refinement of movement sequences in a novel order.[14] Learning is temporally biphasic, characterized by distinguishable early and late phases: early learning involves rapid improvements in skill,[87] where brain activity is altered as a pattern necessary for optimal performance is selected[87] and changes begin to occur at a synaptic level;[88] late learning is more prolonged,[87] consisting of larger structural changes and neuronal reorganization.[87–89] Long-term potentiation (LTP) is believed to be a key mechanism underlying early learning,[90–93] and is of interest in the present thesis; long-term depression (LTD) is thought to predominate in late learning.[93] A unique feature of the central nervous system (CNS) is its

inherent capability to adapt and reorganize its function and structure in response to experiential reinforcement.[13,28,94,95] This capacity, termed neuroplasticity, encompasses molecular, cellular, and systems-level changes in the brain,[13,28,88] which can manifest as modified behavioural outputs.[13,88,89,94,95] During LTP repeated stimulation of a neural pathway results in sustained increases in resting synaptic excitability,[92,93] alterations in synaptic structure and function,[92,96] and eventually cortical reorganization.[88–91] Distinct aspects of skill learning are encoded by functional brain networks,[97] depending on task and practice structure.[98] Several brain regions including M1 and prefrontal cortices,[14,98–103] cerebellum,[100–102,104] and basal ganglia[100,105] contribute to specific aspects of motor memory formation and proliferation.[100,106] In human research TMS is used to noninvasively study changes in brain excitability and inhibition that accompany learning. Particularly, measuring changes in corticomotoneuronal excitability and intracortical networks using single- and paired-pulse TMS over M1 can provide valuable insight into various neurophysiological mechanisms underlying human behavior.[23,76,77,98,107–111]

In addition to studying neuroplastic changes in the human motor system that accompany motor learning, TMS can also be used to transiently induce neuroplasticity and change the cortical environment to promote learning.[78,112–115] The ability to excite or inhibit M1 non-invasively is particularly useful for individuals with neurological disorders such as stroke.[85] Paired associative stimulation (PAS) is a TMS intervention commonly used to modulate plasticity in M1.[78,112,116] Briefly, PAS involves combining peripheral nerve electrical stimulation to a target muscle in close temporal proximity (approximately 10-25 ms) to supra-threshold single-pulse TMS over the M1 representation of the homologous target muscle.[78,116] This intervention can be used to up- or down-regulate corticomotoneuronal excitability by adjusting the inter-stimulus interval (ISI).[78,108] During excitatory PAS protocols[116] the ISI is set (closer to

25 ms) such that the afferent volley arising from the peripheral nerve stimulation reaches M1 at the same time as the TMS pulse, resulting in increased excitability in corticospinal projections from M1 (**Figure 1-1**). In inhibitory PAS[117] the ISI is adjusted (closer to 10 ms) such that a corollary of the afferent volley reaches M1 after the TMS pulse, resulting in decreased corticomotoneuronal excitability.

When PAS is used to increase corticomotoneuronal excitability (excitatory PAS) the mechanisms of these effects are believed to be similar to LTP (**Figure 1-1**),[92] given that the excitatory response to PAS evolves rapidly, is reversible, and persists beyond the period of stimulation;[118] and NMDA receptor blockade drugs can suppress the excitatory effects of PAS.[118] With evidence from other pharmacological studies, neuroplastic changes in M1 after PAS have also been related to GABA-ergic intracortical networks – excitatory effects on corticomotoneuronal excitability are blocked when research participants are administered drugs known to enhance $GABA_A$[119] and $GABA_B$[120] receptor activity. Other mechanisms implicated through pharmacological studies include voltage-gated sodium[119] and calcium channels,[117] cholinergic receptors,[121] and dopaminergic pathways.[122] Responses to PAS are reported to last for periods up 120 minutes post-intervention;[116,123] and relevant findings suggest that LTP-like effects evoked by PAS share common neural pathways with motor learning.[108,112,124]

Several studies in humans have examined exercise effects on TMS-evoked changes in corticomotoneuronal excitability. Cirillo and others[125] found that highly active healthy adults demonstrated greater effects of excitatory PAS on corticomotoneuronal excitability of a small hand muscle M1 representation, compared to sedentary controls. The results prompted speculation that engagement in long-term exercise might offer global benefits to M1, such that physically active individuals could have an increased capacity to undergo neuroplastic change in

response to motor learning or neurorehabilitation.[125] With regards to acute sessions of aerobic exercise there is evidence that low-, moderate-, and high-intensity exercise impact M1 plasticity. Firstly, McDonnell et al.[109] found that, compared to a moderate-intensity exercise bout and a period of passive rest, low-intensity aerobic exercise promoted LTD-like changes in corticomotoneuronal excitability of a non-exercise upper-limb muscle representation when administered before continuous theta-burst stimulation (cTBS; a repetitive TMS protocol used to suppress corticomotoneuronal excitability).[113] More recently, Singh and colleagues[110] showed that moderate-intensity cycling performed prior to excitatory PAS resulted in significantly greater corticomotoneuronal excitability in a resting hand muscle representation. While the authors measured corticomotoneuronal excitability up to 30-minutes post-PAS, LTP-like effects were shown only immediately post-PAS.[110] The study authors also found significantly reduced SICI under the exercising condition compared to rest. Finally, Mang et al.[23] demonstrated that an acute bout of high-intensity cycling intervals significantly enhanced LTP-like plasticity evoked by PAS, in the M1 representation of a non-exercised hand muscle, compared to PAS alone. Thus, there appear to be robust effects of aerobic exercise on M1 plasticity. However, beneficial effects of acute exercise on M1 plasticity are not a ubiquitous finding.[109] As such, more work is necessary to understand how exercise intensity modulates the capacity of M1 to undergo neuroplastic change.

In evaluating the potential for aerobic exercise to prime the brain for enhanced learning and neuroplasticity, establishing a dose-response relationship for these effects is crucial. Given the exciting prospect of translating the beneficial learning and neuroplastic effects of exercise to clinical populations,[22] further research is necessary to elucidate whether “clinically feasible”

exercise intensities can be prescribed as a suitable adjunct therapy to existing neurorehabilitation techniques.

Currently, aerobic exercise is recommended as part of best practice guidelines for lifestyle and secondary prevention after stroke.[126,127] For persons with stroke, participation in a long-term aerobic exercise intervention has been shown to enhance cardiovascular function and cardiorespiratory fitness,[128–131] reduce depressive symptoms,[132] improve cognitive function,[133] and increase health-related quality of life.[134] A major shortcoming of applying current evidence for exercise effects on neuroplasticity and learning is that high exercise intensities may not be achievable for persons with stroke, who have a markedly reduced peak aerobic capacity ($\dot{V}O_{2peak}$) compared to healthy controls.[135] Likewise, persons with stroke are highly susceptible to fatigue, due in part to the presence of motor impairments;[136] and they may experience poor self-efficacy in relation to exercise abilities.[137] Fortunately, engagement in a community-based exercise intervention has been shown facilitate independent exercise for persons with neurological disorders.[134] Moreover, two recent systematic reviews have suggested that moderate-intensity aerobic exercise may be optimal for driving neuroplastic change and brain recovery after stroke.[29,30] Thus, for persons with stroke, moderate exercise intensities could be the most suitable in an aerobic exercise-based adjunct therapy for promoting motor learning and neuroplasticity during neurorehabilitation. Nonetheless, it is first necessary to elucidate these effects in healthy young adults.

1.2 Motivation, aims, and hypotheses

The primary motivation for the present thesis was to examine the effects of a single bout of moderate-intensity aerobic exercise on motor learning and PAS-evoked neuroplasticity in a sample of healthy young adults. This thesis was designed with the intention to build upon findings from high-intensity exercise interventions, to help establish a dose-response relationship for exercise effects on the human motor system, and to inform future research in both healthy elders and individuals with stroke. There were two major aims:

Aim 1: To determine whether 30 minutes of moderate-intensity cycling would improve motor performance and motor learning in a CT task compared to a seated rest period of equal duration.

Hypothesis 1: We hypothesized that undergoing an acute bout of moderate-intensity cycling prior to performing the CT task would improve both performance and learning of the complex motor skill, compared to rest. This experiment is described in **Chapter 2**.

Aim 2: To examine how a single bout of moderate-intensity cycling would impact LTP-like changes in corticomotoneuronal excitability, SICI, LICI, and ICF elicited by PAS, compared to PAS alone.

Hypothesis 2: We hypothesized that engaging in an acute bout of moderate-intensity cycling prior to the administration of PAS would significantly increase corticomotoneuronal excitability and ICF, and reduce SICI and LICI, relative to PAS alone. This experiment is described in **Chapter 3**.

1.3 Rationale

The priming effects of aerobic exercise on brain health and the human motor system give promise to the use of this intervention as a possible adjunct to typical neurorehabilitation practice.[22] To date, however, the only evidence for aerobic exercise effects on motor learning comes from studies using high-intensity exercise interventions; at present the evidence for positive effects of moderate-intensity exercise on neuroplasticity is equivocal. In order to inform clinical research studies, as well as to translate these findings to practice, it is necessary to solidify the effects of moderate-intensity aerobic exercise on the above outcomes. Likewise, it is necessary to determine a dose-response relationship of exercise on these effects for prescribing exercise to optimize motor learning and neuroplasticity. The present thesis contributes to the extant research literature by providing an analysis of the effects of moderate-intensity aerobic exercise on motor learning and neuroplasticity in M1.

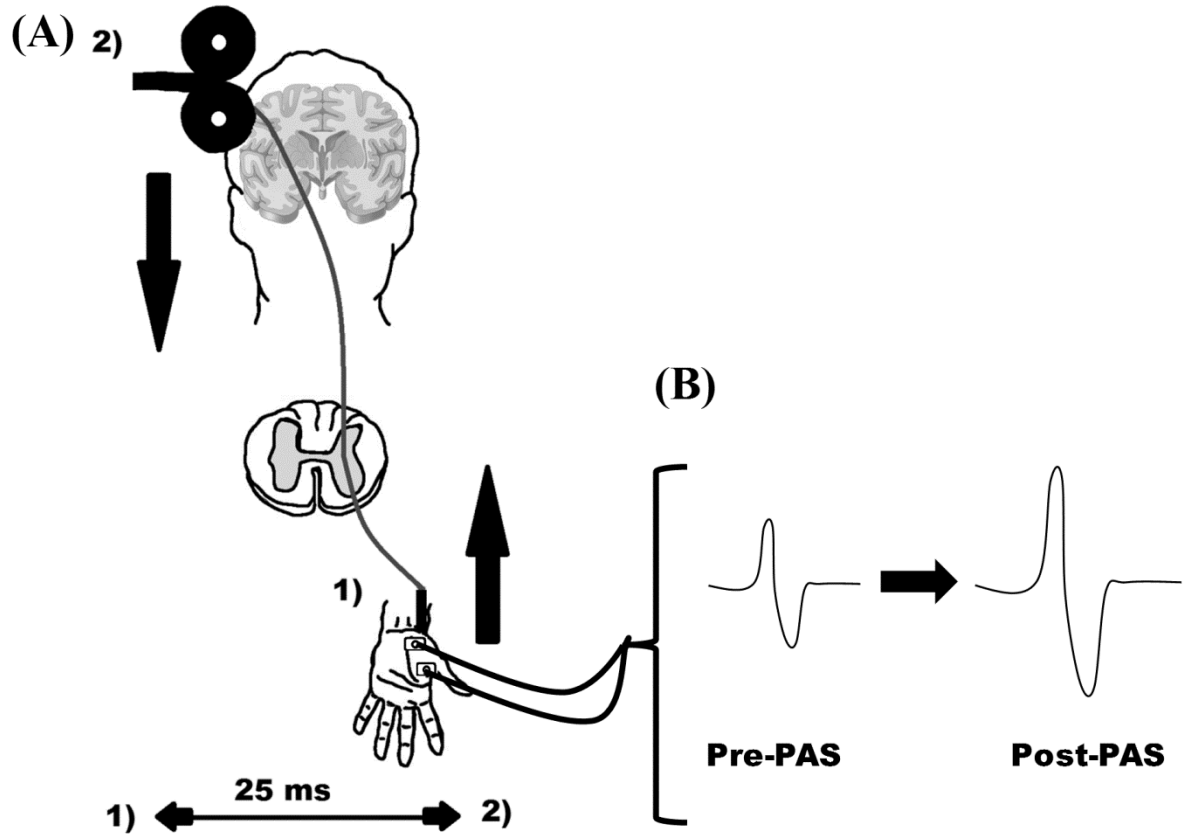

Figure 1-1. Depiction of long-term potentiation (LTP)-like plasticity effects elicited by paired associative stimulation (PAS). A) During excitatory PAS peripheral nerve electrical stimulation is applied to a target muscle **(1)** in close temporal proximity (e.g., 25 ms) to supra-threshold single-pulse transcranial magnetic stimulation (TMS) over the motor cortical (M1) representation of the homologous target muscle **(2)**.[78,116] The afferent volley arising from the peripheral nerve stimulation reaches M1 at the same time as the TMS pulse, resulting in increased excitability in corticospinal projections from M1. The mechanisms of these PAS effects are believed to be similar to LTP,[92] given that the excitatory response to PAS evolves rapidly, is reversible, and persists beyond the period of stimulation;[118] and *N*-methyl-$_{D}$-aspartate (NMDA) receptor blockade drugs can suppress the excitatory effects of PAS.[118] **B)** Increases in corticomotoneuronal excitability elicited by PAS can be quantified by comparing changes in the peak-to-peak amplitude of motor evoked potentials (MEPs) delivered by single-pulse TMS over the target muscle M1 representation, before PAS versus after PAS.

1.4 Significance

Despite improvements in standard neurorehabilitation techniques, in part due to the influx of motor learning research to inform practice,[12] existing methods do not consistently lead to positive motor outcomes.[12,19] Aerobic exercise has recently been promoted as a possible adjunct therapy to existing neurorehabilitation practice,[22,86] given its positive effects on motor learning,[23,24] neuroplasticity,[23,109,110] and brain health.[27–30] Yet the clinical application of exercise to enhance neurorehabilitation is undermined by a misalignment between present research findings in healthy young adults and exercise capacity in persons with stroke.[135] As such, we must fully elucidate the effects of aerobic exercise at various intensities on motor and neurophysiological outcomes. If moderate-intensity exercise can promote motor learning and neuroplasticity in healthy young adults, there will be greater impetus to test these effects in persons with stroke and to further the progress towards clinical application of this candidate adjunct therapy.

2 The Effect of an Acute Bout of Moderate-intensity Aerobic Exercise on Motor Learning in a Continuous Tracking Task.

2.1 Introduction

The acquisition and retention of complex motor skills is crucial to the execution of most human motor behaviors, both throughout the lifespan as well as during recovery from neurological insult.[11] Converging evidence indicates that both single and repeated sessions of aerobic exercise are beneficial to both cognitive[32,43] and memory outcomes.[25,33] Moreover, recent work has demonstrated that an acute aerobic exercise bout can facilitate the acquisition[23] and retention[23,24] of an implicitly learned complex motor skill, in healthy young adults, and enhance neuroplasticity in motor pathways believed to be implicated in skill learning.[23,109,110] However, existing evidence showing that pairing aerobic exercise with skilled practice can improve motor learning has, to date, focused exclusively on acute bouts of high-intensity exercise. Firstly, Roig et al.[24] showed that performing 20 minutes of high-intensity cycling intervals at 90% peak power output (PO) facilitated the 24-hour and 7-day retention of a visuomotor accuracy-tracking task, compared to a resting control condition. Moreover, it was also found that exercise performed after motor practice had a greater benefit to long-term retention than exercise prior to practice.[24] More recently, a study in from our laboratory by Mang et al.[23] noted that 20 minutes of high-intensity cycling intervals (90% peak PO) performed before practicing a CT task,[17] improved acquisition and 24-hour retention of the CT task, compared to a resting control condition. Specifically, participants showed significantly greater temporal precision in an implicitly learned sequence under the exercise condition.[23]

Studies highlight that the learning-oriented benefits of single and repeated bouts of aerobic exercise are both biological, affecting neuroendocrine processes,[23,36,61,62,109] and behavioral, manifesting through increases in cognitive processing, executive function, and attention.[25,32,33,48,52] Theoretically, acute bouts of high-intensity exercise stimulate the secretion of multiple neurochemicals that positively affect learning and neuroplasticity, and lead to enhanced motor memory consolidation.[57,61] For instance, Skriver et al.[61] found that elevated serum levels of BLa and NE after high-intensity cycling intervals related to the magnitude of change associated with motor skill acquisition and retention, in the visuomotor accuracy-tracking task reported by Roig et al.[24] Further, increased circulating BDNF was related to the amount of motor skill change at retention testing.[61]

Presently, there is a paucity of research literature describing the influence of low- to moderate-intensity aerobic exercise on motor memory. There are numerous studies showing that acute and consistent participation in moderate-intensity aerobic exercise benefits aspects of cognitive[42] and executive functioning,[54] including attention and[52] reaction time;[48] stimulates the up-regulation of neurochemicals such as BDNF and NE;[36,62] and enhances neuroplasticity in the human motor system.[109,110] However, no published work has examined how an acute bout of moderate-intensity aerobic exercise impacts the performance and learning of a complex motor skill. Moderate-intensity aerobic exercise may be more feasible and relevant in a rehabilitation setting for patients with mobility or other impairments, due to concerns about safety or an inability to physically reach higher exercise intensities.

It has been established that the acquisition and retention of complex motor skills is crucial to recovery from neurological insult;[11] and long-term aerobic exercise training has been shown to improve motor performance in adults with chronic stroke.[39] Novel literature suggests

that aerobic exercise can prime the motor system, with potential for improving existing motor rehabilitation paradigms.[22,86] Yet, it is unclear whether high-intensity aerobic exercise is a feasible practice for older adults with neurological disorders or other co-morbid conditions (e.g., cardiovascular diseases). Particularly, persons with stroke have a reduced peak work rate and aerobic capacity, have a diminished tolerance for prolonged high-intensity exercise, and may be at a heightened risk for cardiovascular events during exercise, compared to healthy adults.[126,127,135] As a result of these limitations, moderate-intensity aerobic exercise may be more feasible for individuals who have a neurological disorder such as stroke. Indeed, moderate-intensity aerobic exercise has been promoted as part of an overall program for secondary prevention after stroke,[126,127] and may therefore have promise to promote learning and neuroplasticity in these individuals. Nevertheless, to establish the viability of this approach, it is necessary to first investigate the effects of moderate-intensity aerobic exercise on motor skill performance and learning in healthy adults.

In the present study we examined how performing a single bout of continuous moderate-intensity aerobic exercise would impact the acquisition and retention of a motor skill in healthy young adults. Participants practiced a CT task, similar to those previously reported,[17,18,23,115] after either 30 minutes of moderate-intensity cycling, or a rest period of equal duration, in a crossover fashion. During CT task practice (i.e., motor skill acquisition/motor memory encoding) we assessed motor performance. Motor learning occurs offline, during the consolidation phase; 24 hours after CT task practice, we assessed motor learning using a no-exercise retention test.[38,138,139] We hypothesized that engaging in an acute bout of moderate-intensity cycling prior to performing the CT task would improve both performance and learning of the complex motor skill, compared to rest.

2.2 Materials and methods

The present study was approved by UBC's Clinical Research Ethics Board. All participants independently provided written and verbal informed consent, in accordance with the principles outlined by the Declaration of Helsinki.

2.2.1 Participants

Sixteen healthy young adults were recruited from UBC and the surrounding community of Vancouver, British Columbia, Canada (see **2.3 Results**, **Table 2-1**). We included right-handed (**Appendix A**)[1] volunteers who reported participating in ≥ 1500 metabolic equivalent of task [MET]-minutes•week^{-1} of physical activity, based on the long-form International Physical Activity Questionnaire (IPAQ, **Appendix B**).[2] Participants were also included if they were non-smokers, possessed an ability to read and understand English, and could maintain a seated, upright position for a prolonged period of time. Smokers were excluded on the basis that nicotine has been shown to influence memory performance.[140] Additional exclusion criteria included: a history of any neurological or psychiatric diagnoses (e.g., clinical depression); use of medication known to influence CNS activity; acute or chronic cardiorespiratory, musculoskeletal, or hormone-related (e.g., diabetes mellitus; eating disorders; obesity) conditions; a history of alcoholism or illicit drug dependency; visual or hearing impairment; acute or chronic contraindications to upper-extremity use; and contraindications to exercise (assessed via the Physical Activity Readiness Questionnaire [PAR-Q],[3] **Appendix C**). Participants were also excluded if they drank an excess of six cups of coffee per day,[35] due to the possible effect of caffeine intake on memory performance.[141] Upon initial contact, participants received a written copy of the informed consent form, and were asked to self-report the above criteria.

2.2.2 Experimental design

The present study utilized a crossover design with repeated measures (**Figure 2-1**). During the initial experimental session all participants completed a GXT to exhaustion. Participants were then pseudo-randomized to complete one of two experimental conditions, prior to crossover: 1) moderate-intensity aerobic exercise; or 2) seated rest. The order of participation under each condition was counter-balanced across the study sample.

2.2.3 Exercise protocol

2.2.3.1 GXT

All participants completed a GXT, to determine their $\dot{V}O_{2peak}$ for subsequent exercise prescription. Before attending this laboratory visit, participants were instructed to refrain from engaging in vigorous physical activity for ≥ 48 hours, ingesting alcohol for ≥ 6 hours, and eating for ≥ 2 hours. Upon arrival at the laboratory, participants completed several pre-screening questionnaires (see **2.2.1 Participants**), after which measurements of height and body mass were recorded in one layer of light clothes, with shoes removed. For the GXT, participants were outfitted with a silicone mouthpiece, a nose clip, and a one-way air valve (Hans Rudolph Inc., Shawkee, KS, USA). Participants' heart rate (HR) was continually monitored via a Polar Wearlink®+ wireless HR transmitter and FS1 HR monitor watch (Polar Electro, Oy, Kempele, Finland). Throughout the GXT, measurements of $\dot{V}O_2$, CO_2 output ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), and respiratory exchange ratio (RER) were continuously monitored (5-second resolution) using a ParvoMedics TrueOne 2400 metabolic cart system (Sandy, UT, USA). The reliability and validity of this metabolic cart system have been established in previous research.[142]

The GXT was completed on an electronically-braked Ergoline Ergoselect 200 cycle ergometer (Ergoline GmbH, Bitz, Germany). Briefly, exercise began at a PO of 50 Watts, for

females, or 100 Watts for males – there was no formal warm-up period. For both females and males cycling resistance was incrementally increased by 30 Watts every 2 minutes, until the termination of the GXT. During cycling participants were instructed to maintain a pedaling cadence of 70-90 revolutions per minute (RPM). Participants had visual feedback of pedaling cadence, via a display mounted on the handlebars of the cycle ergometer. We also provided verbal feedback for the maintenance of cadence. At the end of every test stage (i.e., every 2 minutes), we recorded participants' HR and rating of perceived exertion (RPE, 6-20 ratings; **Appendix D**).[4] Immediately after exercise cessation BLa concentration ([BLa]) was measured via finger-stick and an automated portable BLa analyzer and test strips (Lactate Pro, Arkray Inc., Kyoto, Japan); the validity of this device has been previously reported.[143] The GXT was terminated at volitional exhaustion, inability to maintain desired cadence, or participant request to stop. Achievement of maximal $\dot{V}O_2$ was determined *post hoc* under the following conditions: HR > age-predicted maximal value, a plateau in $\dot{V}O_2$ and HR with further increases in workload, RER > 1.15, RPE > 17.[23,144,145] From the GXT, peak values of $\dot{V}O_2$, PO, HR, and RER were extracted (**Table 2-1**). $\dot{V}O_{2peak}$ was considered the peak $\dot{V}O_2$ value extracted from the GXT.

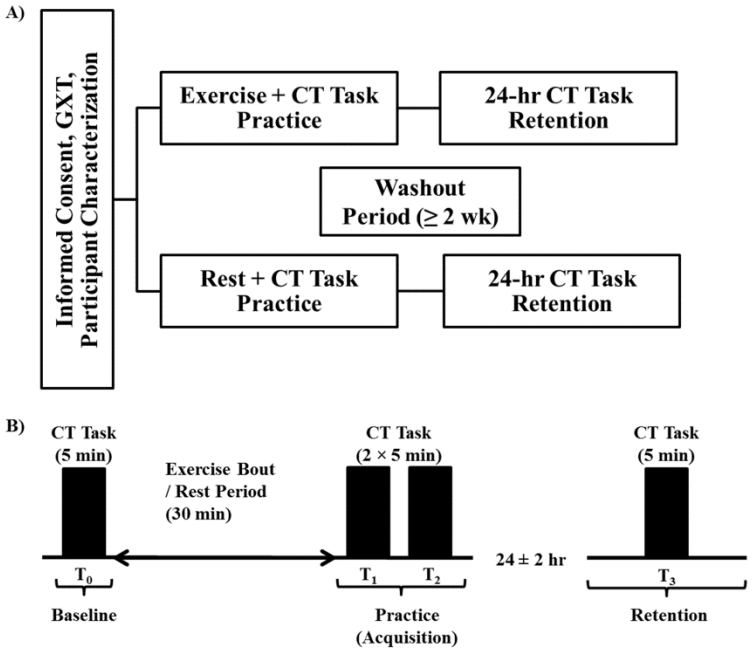

Figure 2-1. Diagrammatic representation of study design. The present study utilized a crossover design with repeated measures. **A)** Participants provided informed consent, underwent a graded exercise test (GXT) to exhaustion, and completed several screening and characterization questionnaires during the first experimental session. Participants were then pseudo-randomized to two experimental conditions including moderate-intensity aerobic exercise (based on GXT results) or seated rest prior to continuous tracking (CT) task practice. The CT task practice sessions were each followed by no-exercise retention test 24 ± 2 hours later. Experimental conditions were separated by a washout period of ≥ 2 weeks. **B)** During CT task practice sessions participants completed a single 5-minute tracking block (10 × 30-second trials) at baseline (T_0). Thereafter, participants completed either 30 minutes of moderate-intensity cycling (power output [PO] corresponding to 60% $\dot{V}O_{2peak}$) or seated rest, followed by two consecutive 5-minute tracking blocks at T_1 and T_2. Performance on practice blocks was used to index motor skill acquisition. Twenty-four ± 2 hours later, a 5-minute retention test was used to assess motor skill learning (T_3).

Table 2-1. Participant characteristics

	Demographic					Exercise Test					Exercise Bout				
ID	**Age**	**Sex**	**Height**	**Body Mass**	**IPAQ Category**	**$V\dot{O}_{2peak}$**	**Peak PO**	**HR_{peak}**	**RPE**	**[BLa]**	**60% $V\dot{O}_{2peak}$**	**PO**	**HR**	**RPE**	**[BLa]**
s01	31	M	186.0	94.1	High	42.0	280	199	19	12.8	25.2	160	149	12	6.8
s02	26	M	177.0	74.9	Moderate	51.2	310	186	20	10.3	30.7	190	139	13	6.7
s03	25	F	168.0	63.1	High	36.3	200	178	16	11.2	21.8	110	152	13	4.1
s04	32	F	176.0	63.0	High	42.7	260	174	18	6.6	25.6	140	147	12	1.7
s05	29	F	171.0	54.9	High	53.6	260	179	19	16.4	32.2	140	141	12	1.8
s06	28	M	178.5	60.5	High	60.9	310	184	17	12.4	36.5	160	159	10	7.7
s07	25	M	186.0	73.2	Moderate	50.2	310	184	20	17.2	30.1	190	158	14	8.0
s08	25	M	184.0	75.0	Moderate	42.8	250	179	18	15.2	25.7	160	153	14	8.7
s09	23	M	188.2	81.4	High	48.4	310	178	19	13.0	29.0	160	127	12	4.6
s10	26	M	182.4	92.9	High	36.3	280	196	18	14.8	21.8	160	154	12	4.7
s11	27	F	162.2	56.2	High	32.9	170	166	18	7.9	19.7	80	126	14	3.2
s12	21	F	162.1	52.2	High	49.5	260	185	14	10.7	29.7	140	155	11	2.8
s13	25	F	165.0	60.5	High	45.1	230	178	18	11.8	27.1	110	126	11	2.6
s14	24	M	189.2	77.8	High	49.8	310	196	18	8.1	29.9	190	170	10	7.6
s15	22	M	181.1	71.9	Moderate	46	250	197	17	13.9	27.6	130	154	14	2.6
s16	22	F	167.0	57.5	High	44.9	260	197	20	12.9	26.9	110	152	13	3.1
Mean	25.7	–	176.5	69.3	–	45.8	265.6	184.8	18.1	12.2	27.5	145.6	147.5	12.2	4.8
SEM	0.8	–	2.4	3.2	–	1.8	10.3	2.4	0.4	0.8	1.1	8.0	3.2	0.4	0.6

Age recorded in years; height recorded in cm; body mass recorded in kg. IPAQ, International Physical Activity Questionnaire, long-form version; V̇O2peak, peak O2 uptake (mL•min-1•kg-1); PO, power output (Watts); HR, heart rate (beats•minute-1); RPE rating of perceived exertion (6-20 scale); [BLa], blood lactate concentration (Mmol). IPAQ categories: "moderate", ≥ five days with combination of walking or moderate-to vigorous-intensity physical activity, achieving ≥ 600 metabolic equivalent of task [MET]-minutes•week-1; "high", ≥ seven days with any combination of walking or moderate-to vigorous-intensity physical activity achieving ≥ 3000 MET-minutes•week-1.[2]

2.2.3.2 Standardized exercise bout

For 48 hours prior to each laboratory visit, participants were asked to refrain from vigorous exercise and alcohol consumption and were advised to get a normal night's sleep. Each participant was tested at approximately the same time of day, to attenuate any diurnal fluctuations in motor memory processes.[37] Under the exercise condition, participants completed a 30 minute bout of cycling on a stationary cycle ergometer, at a PO corresponding to 60% $\dot{V}O_{2peak}$ (determined from the GXT)[146] and a pedaling cadence of 70-90 RPM.[23] Every 5 minutes HR and RPE were recorded. To ensure that the exercise bout was perceived as moderately intense to participants, PO was adjusted online to maintain a RPE value under 15.[145] Upon completion of exercise, [BLa] was assessed using finger-stick. Under the exercise condition, this cycling bout immediately preceded CT task practice; whereas, under the resting condition CT task practice was preceded by 30 minutes of seated rest. Participants were asked to remain seated and relaxed for the entire rest period.

2.2.4 CT task

To examine the effect of a single bout of moderate-intensity aerobic exercise on motor skill performance and learning, participants practiced the CT task immediately following both exercise and rest conditions (**Figure 2-1**). After each condition, participants returned 24 ± 2 hours later, to complete a no-exercise retention test. Conditions were separated by a ≥ 2 week washout period, to prevent any order effect on subsequent practice. The CT task required the manipulation of a modified joystick (Logitech, Newark, CA, USA) via abduction and adduction movements of the non-dominant thumb (**Figure 2-2**). All participants wore ear plugs and a noise-canceling headset during CT task practice and at the retention test.

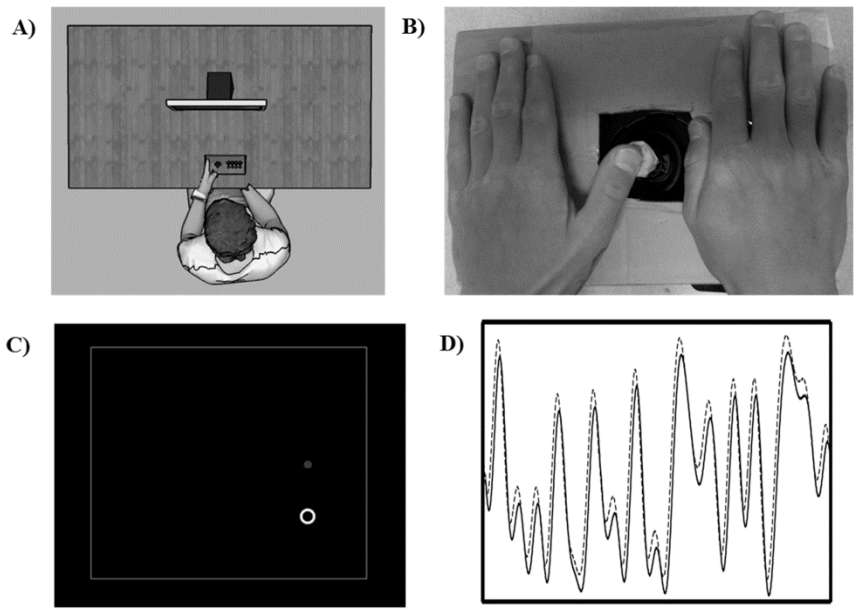

Figure 2-2. Schematic of the continuous tracking (CT) task used throughout study protocol. A) Participants were seated at a desk, in front of a computer monitor. **B)** A modified joystick was manipulated via abduction and adduction movements of the non-dominant hand. **C)** Participants' view of the target (white ring) and cursor (red dot) presented on the computer monitor during CT task performance. **D)** A sample waveform used during a single CT task trial (30 seconds). The solid line represents a sample target sequence, whereas the dashed line depicts a participant's movement trajectory during target tracking.

The joystick was interfaced with a custom software program, developed using the LabVIEW platform (v. 9.0, National Instruments Corporation, Austin, TX, USA).[102] Joystick position sampling and all stimuli were presented at 50 Hz. Participants were seated in front of a computer monitor, and used joystick movements to control a cursor (a red dot), to track a moving target (a white ring which encircled the cursor) presented on a black background. Throughout tracking the target oscillated vertically, while moving right-to-left across the screen at a constant horizontal velocity.

The duration of a single trial (i.e., the amount of time it took the target to scroll across the screen) was 30 seconds. Each subsequent trial was preceded by a 2-second normalization period, during which the target (i.e., the white ring) and cursor (i.e., the red dot) were zeroed to their initial starting positions. One block of movements was 5 minutes in duration, consisting of 10 × 30-second trials; participants completed: 1) one block at baseline, prior to the exercise bout or rest period (T_0); 2) two blocks immediately after exercise or rest (T_1 and T_2); and 3) one block at the no-exercise retention session (T_3). The purpose of the practice blocks T_1 and T_2 was to assess motor skill acquisition during early (T_1) and late practice (T_2), whereas the retention block (T_3) examined motor skill learning. No rest was taken between acquisition blocks. Each trial was presented as a visual representation of a trigonometric series, constructed using the polynomial equation previously described by Wulf and Schmidt.[147] We have previously reported this method elsewhere.[17] Each trial consisted of a movement sequence that was identical across participants and conditions, to ensure uniform difficulty. Difficulty was controlled based on target movement range and velocity.

Prior to CT task practice we instructed participants to track the target with the cursor as accurately as possible at all times. For each participant, the direction of joystick control was reversed between exercise and rest conditions, such that left/right joystick movements corresponded to up/down cursor movements for one condition and down/up cursor movements for the other. Additionally, the order of sequence presentation (i.e., regular presentation, reversed presentation) was reversed between conditions. Participants were explicitly informed of the direction of joystick control at the beginning of each session. Movement directionality was the same for practice and retention sessions under each condition; and directionality across

conditions was pseudo-randomized and counterbalanced across the sample. Participants were not provided error feedback during or after tracking practice.

2.2.5 Data analyses

All CT task data were processed using a custom MATLAB script (Version R2013b, The Mathworks, Inc., Natick, MA, USA). Data from each individual trial were collapsed to provide a measure of tracking performance within each block, and to make comparisons across tracking blocks.

Participants' motor performance was evaluated based on changes in spatial accuracy and temporal precision. To accomplish this, participants' absolute RMSE[148] of tracking was separated into temporal and spatial components using a time series analysis (TSA).[17,18] In the TSA, participants' tracking patterns from each trial were cross-correlated with the target pattern until a maximum correlation coefficient (R^2) was reached. The cross-correlation coefficients reflect the spatial accuracy of participants' tracking performance, while the distance (number of samples, multiplied by 5 ms) that tracking data are shifted along the target data sequence to achieve the maximum R^2 represents participants' temporal precision. Spatial accuracy is reported as "shifted RMSE" and temporal precision is reported as "time lag". Lower shifted RMSE score indicates greater spatial tracking performance. Time lag scores in larger negative numbers indicate greater time lag of tracking, while a zero value represents no tracking time lag between participant movements and the target; any trial including a positive time lag value was omitted. Thus, measures of temporal precision (time lag) and spatial accuracy (shifted RMSE) were calculated separately, to evaluate tracking error across practice and at retention.[17] Tracking performance was decomposed into temporal and spatial dimensions because these aspects of procedural memory have been shown to evolve distinctly from one another,[106] involve separate

neural pathways,[17,106] and have been shown to be differentially impacted by an acute bout of aerobic exercise.[23] These outcome measures were compared across experimental conditions and time-points. To account for possible differences in tracking performance at baseline (T_0), all data from acquisition (T_1, T_2), and retention (T_3) blocks were analyzed as a change score from T_0. Additionally, a change score was calculated between performance at T_2 and T_3, to assess offline motor memory consolidation.[115,138]

2.2.6 Statistical analyses

Statistical tests were performed with SPSS (V23.0, IBM Corporation, Armonk, New York, USA). Data distributions and assumptions were tested using the Shapiro-Wilk test and visual inspection of histogram plots. Omnibus statistical tests were conducted via repeated-measures analyses of variance (rmANOVAs) and paired-samples *t*-tests. Motor skill acquisition was assessed using a two-way (Condition [exercise, rest] × Time [T_0-T_1, T_0-T_2]) rmANOVA for change score values of time lag and shifted RMSE. Motor skill learning was evaluated via a separate paired-samples *t*-tests on time lag and shifted RMSE change scores, calculated between T_0 and T_3. Additionally, offline motor memory consolidation was tested using paired-samples *t*-tests on participants' change-score in time lag and shifted RMSE, calculated between T_2 and T_3. Pairwise comparisons were made *post hoc*, using the Bonferroni correction. Statistical significance was set at $p \leq 0.05$. Results are reported as mean ± standard error of mean (SEM).

2.3 Results

2.3.1 Participants

Of the 16 participants, nine were male and seven were female, with an overall mean age of 25.7 (0.8) years (**Table 2-1**). Participants reported an average of 4136.3 (413.2) MET-

minutes•week^{-1} of moderate- to-vigorous leisure time physical activity. No participants achieved all criteria for maximal $\dot{V}O_2$, during the GXT; however, all participants achieved at least one criterion, with the exception of one individual (s15). Mean $\dot{V}O_{2peak}$ for males was 47.5 (2.3) mL•min^{-1}•kg^{-1} and 43.6 (2.7) mL•min^{-1}•kg^{-1} for females, corresponding to "excellent" average fitness for both males and females.[145] The mean PO, HR, RPE and post-exercise [BLa] readings for the continuous exercise bout were 167 (7) Watts, 151 (4) beats•minute^{-1}, 12 (1), and 6.4 (0.7) Mmol for males; and 119 (9) Watts, 143 (5) beats•minute^{-1}, 12 (0), and 2.8 (0.3) Mmol for females, respectively.

2.3.2 Data inspection

All CT task data were deemed normally distributed on the basis of non-significant Shapiro-Wilk statistics ($W_{(16)}$ = 0.900-0.977, p = 0.081-0.934), as well as upon visual inspection of histogram plots.

2.3.3 Temporal precision (time lag)

Group plots of time lag by time-point (T_0, T_1, T_2, T_3), under the exercise and rest conditions, are depicted in **Figure 2-3A**. Group plots of time lag change score by time-point (T_0-T_1, T_0-T_2, T_0-T_3) are illustrated in **Figure 2-3B**.

2.3.3.1 Acquisition

The two-way (Condition [exercise, rest] × Time [T_0-T_1, T_0-T_2]) rmANOVA on change score values of time lag demonstrated a trend towards a significant main effect of Time ($F_{(1, 15)}$ = 3.919, p = 0.066). *Post hoc* inspection of this trending main effect of Time indicated that temporal performance on the CT task tended to worsen from T_1 to T_2 regardless of condition. Otherwise, there was neither a significant main effect of Condition ($F_{(1, 15)}$ = 0.101, p = 0.756), nor a significant Condition × Time interaction ($F_{(1, 15)}$ = 0.003, p = 0.956).

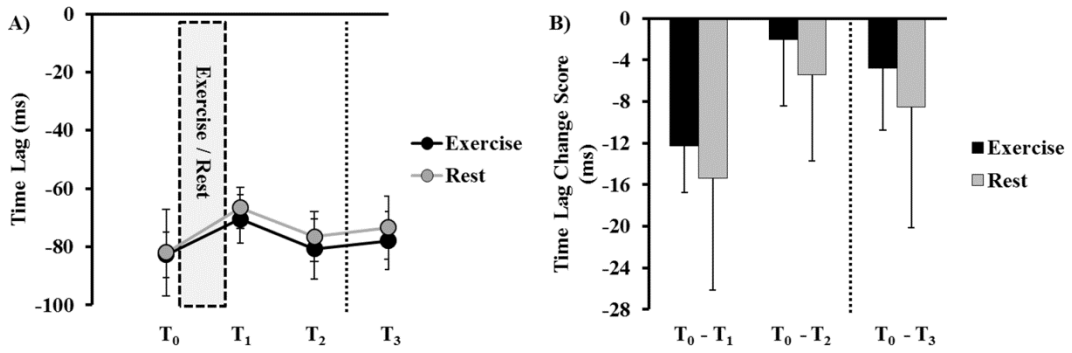

Figure 2-3. Temporal precision (time lag) performance on the continuous tracking (CT) task. A) Raw time lag values at baseline (T_0), acquisition (T_1, T_2), and retention (T_3) under exercise (black line) and rest (gray line) conditions. Less negative time lag values indicate greater temporal precision. The inlaid box represents the 30-minute exercise bout or rest period. **B)** Time lag change scores between baseline, acquisition (T_0-T_1, T_0-T_2), and retention (T_0-T_3) blocks, under exercise (black bars) and rest (gray bars) conditions. More negative change scores indicate greater temporal precision. There was no significant difference between conditions during acquisition and retention measurements ($p > 0.05$). The vertical dotted lines in **A** and **B** represent the 24 ± 2 hours between CT practice and retention days. Error bars in **A** and **B** represent mean ± standard error of mean (SEM).

2.3.3.2 Retention

The paired-samples *t*-test highlighted that there was no main effect of Condition ($t_{(15)} = 0.310$, $p = 0.761$). There was no difference in temporal precision between exercise and rest conditions at retention.

2.3.3.3 Offline consolidation

In terms of offline motor memory consolidation, the paired-samples *t*-test demonstrated no main effect of Condition ($t_{(15)} = 0.043$, $p = 0.966$). Thus, offline consolidation of temporal precision in the CT task did not differ between exercise and rest conditions.

2.3.4 Spatial accuracy (shifted RMSE)

Group plots of shifted RMSE by time-point (T_0, T_1, T_2, T_3), under the exercise and rest conditions, are shown in **Figure 2-4A**. Group plots of time lag change score by time-point (T_0-T_1, T_0-T_2, T_0-T_3) are displayed in **Figure 2-4B**.

2.3.4.1 Acquisition

The two-way (Condition [exercise, rest] × Time [T_0-T_1, T_0-T_2]) rmANOVA on motor skill acquisition data showed no significant main effects of Condition ($F_{(1, 15)} = 1.292$, $p = 0.274$) or Time ($F_{(1, 15)} = 0.916$, $p = 0.354$). However, the rmANOVA revealed a significant Condition × Time interaction effect ($F_{(1, 15)} = 4.396$, $p = 0.050$). Pairwise comparisons showed that, under the rest condition, spatial accuracy worsened from T_1 to T_2 ($p = 0.050$), but performance was stable from T_1 to T_2 under the exercise condition ($p = 1.00$). Furthermore, spatial accuracy in the exercise condition, at both T_1 ($p = 0.003$) and T_2 ($p = 0.002$), was greater than that of the rest condition at T_2. However, there was no significant difference between the rest condition at T_1 and the exercise condition at T_1 ($p = 0.421$) or T_2 ($p = 0.375$). These results indicate that participants were able to maintain tracking performance for a longer time, under the exercise condition; whereas under the rest condition, there was decay in the spatial aspect of tracking performance.

2.3.4.2 Retention

At retention (change score at T_3), the paired-samples *t*-test indicated that there was no difference in spatial accuracy ($t_{(15)} = 0.640$, $p = 0.532$) between exercise and rest conditions.

2.3.4.3 Offline consolidation

The paired-samples *t*-test on offline consolidation change scores showed that participants' motor memory consolidation of spatial performance did not differ between exercise and rest conditions ($t_{(15)} = 1.208$, $p = 0.246$).

2.4 Discussion

The primary aim of the present study was to determine the effect of a single 30-minute bout of moderate-intensity cycling (PO corresponding to 60% $\dot{V}O_{2peak}$) on the acquisition and retention of a complex motor skill (CT task), in a sample of healthy young adults. Based on our previous findings using high-intensity interval exercise,[23] we hypothesized that exercising at a moderate intensity before practicing the CT task would also lead to significantly improved motor skill acquisition and retention, compared to a rest period of equal duration. We discovered that, compared to rest, exercise appeared to facilitate the maintenance of motor performance throughout the acquisition phase; however, contrary to our primary hypothesis, we found that moderate-intensity exercise did not influence indices of motor skill learning, nor did it affect offline motor memory consolidation. These data suggest that intensity modulates the effects of exercise on motor memory processes.

As an increasing amount of exercise-motor learning research literature has begun to accrue, it is evident that there is a complex interaction between exercise intensity and the distinct motor memory processes – namely, encoding, consolidation, and retrieval.[38,138,139] To date, three published reports have examined the role of acute aerobic exercise in modifying these processes.[23,24] Two of these studies have shown that performing high-intensity intermittent aerobic exercise in close temporal proximity to motor skill practice enhanced measures indicative of both motor performance (Mang et al.[23]) and motor learning (Roig et al.[24], Mang et al.[23]). More recent work shows that vigorous continuous cycling can help stabilize an explicit motor memory against interference, without improving learning.[34] The present findings add to our understanding of how single bouts of exercise affect skill acquisition, showing that moderate-intensity efforts appear to stabilize performance during practice, but that when

delivered as a single session have little effect on changes in performance associated with motor learning. In other words, motor memory encoding may be stabilized after moderate-intensity aerobic exercise, without impacting consolidation. With the present task and participant characteristics, we believe that high-intensity exercise may be necessary to drive lasting changes in motor behavior, when delivered acutely, in close temporal proximity to skilled motor practice.

The present findings suggest that a single bout of moderate-intensity cycling allows for sustained motor performance over a practice period, but may be insufficient to drive changes in motor memory consolidation. The observed effect of moderate-intensity exercise on online performance agrees with previous literature examining the cognitive and neural effects of acute moderate-intensity aerobic exercise.[32] For instance, meta-analyses have concluded that acute and long-term participation in moderate-intensity exercise can enhance executive function,[32,43] working memory,[33] and short- and long-term (non-motor) memory, when provided in conjunction with behavioral tasks.[25] Studies involving multiple neuroimaging modalities have provided connections between these observed behavioral enhancements of acute moderate-intensity aerobic exercise and underlying neural correlates. For example, single bouts of continuous moderate-intensity aerobic exercise can modulate event-related potentials related to: improved attentional resources allocated to task performance[45,52]; altered response inhibition and gating of irrelevant stimuli[45,47,52]; enhanced motor planning and response selection processes; and increased selective attention.[47,55] Likewise, continuous moderate-intensity cycling can modify brain activation patterns associated with executive control and working memory, solving complex tasks, attentional control and conflict resolution, and semantic processing.[70,74] Such work supports the idea that stabilized motor performance after moderate-intensity aerobic exercise could be related, in part, to exercise-induced enhancements in cognitive processes and

underlying neural correlates. Here we provide evidence that acute moderate-intensity aerobic exercise can influence online human motor behavior.

While we demonstrated a relative improvement in motor skill acquisition after exercise, compared to rest, we found no differences at the retention test, indicating no effect on motor learning. Likewise, we found no between-condition differences in offline motor memory consolidation. It is possible that these observations are related to differences in the neurochemical and hormonal consequences of moderate- and high-intensity aerobic exercise protocols. Evidence indicates that high-intensity exercise may influence motor memory consolidation by increasing circulating levels of catecholamines, growth factors, and a milieu of other substrates.[35,61–63] Specifically, recent work has shown that high-intensity exercise-induced up-regulation of systemic NE,[61] dopamine,[35,61] and epinephrine[35] has been related to increased long-term memory. Increased serum BDNF after high-intensity exercise has also been related to memory consolidation.[61,64] In addition, high intensity exercise has been shown to induce increases in BLa which correlate with motor memory,[61] and significantly increased serum concentration of endocannabinoids,[63] which are reported to modify synaptic plasticity.[149] Although moderate-intensity bouts of exercise reportedly increase circulating BLa, BDNF,[62] catecholamines,[35,36] and endocannabinoids,[68] such changes in circulating neurochemicals occur to a lesser degree than after high-intensity exercise.[35,150] Therefore, there is likelihood that these transient increases in neurochemical secretion underlie acute cognitive benefits of moderate-intensity aerobic exercise, without affecting offline memory consolidation processes. In line with this belief, one study showed that both online learning rate (i.e., memory encoding) and 1-week retention of a vocabulary learning task were significantly greater after high-intensity exercise, compared to moderate-intensity exercise and rest, and that there were no differences in learning

between moderate-intensity exercise and rest.[35] High-intensity exercise-induced increases in systemic BDNF, dopamine, and epinephrine were significantly correlated with retention scores immediately, 1 week, and > 8 months, respectively, after the intervention; yet no effects or correlations were associated with moderate-intensity aerobic exercise, despite significant increases in circulating catecholamines after this exercise bout. Although there is presently negligible evidence to indicate whether exercise-induced changes in endogenous neurochemicals can impact learning (i.e., memory consolidation) after a single session of moderate-intensity exercise, it is evident that a single bout of moderate-intensity aerobic exercise is associated with within-session improvements in cognitive and neuroendocrine processes. We thus provide evidence that moderate-intensity aerobic exercise can also enhance online motor performance (i.e., motor memory encoding), when compared to passive rest.

In the present study we found that 30 minutes of cycling at a PO corresponding with 60% $V\dot{O}_{2peak}$ resulted in improved motor memory encoding at the end of the acquisition period, relative to a rest period of equivalent duration. Specifically, improved encoding came as a result of maintained motor skill performance after exercise, while performance decreased over time after rest. In the current work we utilized two blocks of CT task practice, consisting of a total of 20, 30-second trials. Albeit we previously[23] used a similar task with an equivalent practice dose, other work from our laboratory has prescribed a much larger practice dose, in terms of block duration, number of blocks, and number of practice days.[17,18,115] While we found that acute moderate-intensity aerobic exercise was insufficient to improve learning of the CT task, despite an improvement in both performance and change associated with learning after high-intensity exercise,[23] it is possible that with more sustained practice after moderate-intensity exercise could have a beneficial effect on motor learning. Here, we consider a low practice dose a potential

limitation of the present study. Previous literature has described improvements in online motor performance after a long-term exercise intervention in the absence of continued motor practice;[39,40] yet, there is presently insufficient evidence to support the possibility that moderate-intensity exercise will enhance motor learning in the long-term. Future work must examine the impact of moderate-intensity aerobic exercise on motor behavior, in the presence of a larger acute practice dose, or multiple practice sessions.

2.5 Conclusions

We have shown that a single bout of moderate-intensity aerobic exercise can enhance motor skill performance relative to a period of rest, but in isolation has no effect of motor skill learning. Based on existing studies employing similar exercise protocols we speculate that improved online performance may be related to enhanced cognitive function, arousal, and attention; and a lack of learning effect may be attributed to an inability to sufficiently up-regulate neuroendocrine processes to support offline motor memory consolidation. At present, it appears that exercise intensity is a key modulator in driving enhancements in motor memory, after a single session. However, further research efforts should include measurements of cognitive function, attention, and arousal (e.g., through validated inventories), as well as the assessment of neurochemicals (e.g., through serum or saliva), to fully understand how moderate-intensity aerobic exercise influences motor memory processes. Furthermore, additional work should assess how exercise affects the evolution of motor skills over longer, or multiple, acquisition periods. Finally, in order to design and explore novel interventions that can augment existing rehabilitation practice, we must elucidate the appropriate dose-response relationship (i.e., intensity, duration, mode, and frequency), between aerobic exercise and motor learning.

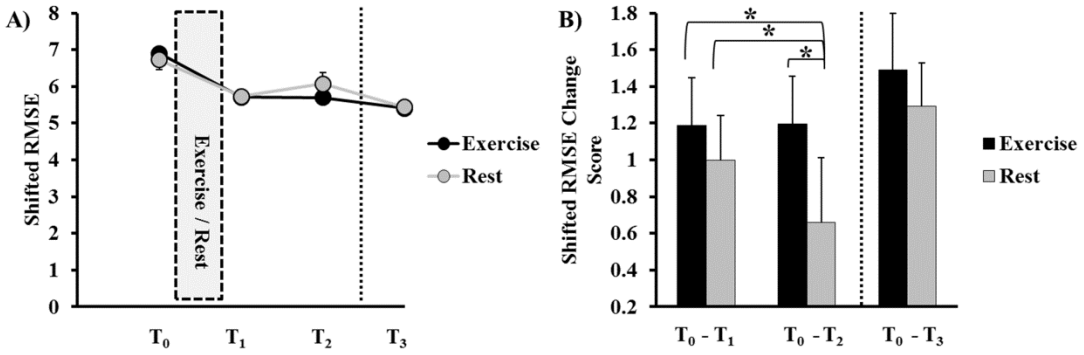

Figure 2-4. Spatial accuracy (shifted root-mean-square error [RMSE]) performance on the continuous tracking (CT) task. A) Raw shifted RMSE values at baseline (T_0), acquisition (T_1, T_2), and retention (T_3) under exercise (black line) and rest (gray line) conditions. Smaller shifted RMSE values indicate greater spatial accuracy. The inlaid box represents the 30-minute exercise bout or rest period. **B)** Shifted RMSE change scores between baseline, acquisition (T_0–T_1, T_0–T_2), and retention (T_0–T_3) blocks, under exercise (black bars) and rest (gray bars) conditions. Greater change scores indicate greater spatial accuracy. Performance on both acquisition blocks under the exercise condition was significantly greater than the second acquisition block under the rest condition ($p < 0.05$). Additionally, performance was significantly reduced from the first to the second acquisition block under the rest condition ($p = 0.05$). Spatial accuracy did not differ between conditions at retention ($p > 0.05$). The vertical dotted lines in **A** and **B** represent the 24 ± 2 hours between CT practice and retention days. Error bars in **A** and **B** represent mean ± standard error of mean (SEM). * statistically significant, $p \leq 0.05$.

2.6 Bridging summary

We show in **Chapter 2**, that a single session of moderate-intensity aerobic exercise does not enhance motor learning for the CT task in a sample of healthy young participants. However, prior to the motor memory consolidation period (i.e., after practice), we show that acute moderate-intensity exercise maintained tracking performance on the spatial dimension of the task, while performance diminished over time under a resting control condition. These results suggest that there may be a benefit of multiple sessions of aerobic exercise paired with motor practice. Alternately it may be that a more prolonged practice period is required to enhance and extend these effects.

While observable motor behavior is necessary for future translation and application of aerobic exercise as an adjunct therapy to standard neurorehabilitation, it is crucial to understand the underlying neurophysiological effects of this intervention on the motor system. Previous work using PAS and motor learning paradigms has shown that similar pathways are affected by both protocols.[108,124] Likewise, a recent study performed in our laboratory[23] demonstrated beneficial effects of high-intensity aerobic exercise on motor performance and learning, as well as PAS-evoked LTP-like plasticity, compared to a resting condition.

Chapter 3 describes an experiment that explores the influence of an acute bout of moderate-intensity aerobic exercise (the same as used in **Chapter 2**) on LTP-like plasticity elicited by an excitatory PAS protocol.[23] We aimed to extend the findings of our previous study, and to work towards building a dose-response relationship of exercise effects on neuroplasticity in the motor system, by examining the effects in response to a more "clinically feasible" exercise intensity, using a sample of healthy young adults.

3 Effects of an Acute Bout of Moderate-intensity Aerobic Exercise on Long-term Potentiation-like Plasticity Elicited by Paired Associative Stimulation.

3.1 Introduction

Research literature supports the benefits of aerobic exercise on brain health.[22,27,28] Converging evidence in healthy young adults indicates that both single and repeated sessions of aerobic exercise are beneficial for cognition,[32,43] memory,[25,33] and motor performance and learning.[23,24,39,40] Concurrent work has demonstrated that a single bout of aerobic exercise can promote neuroplastic change in M1, as assessed with TMS techniques.[23,109,110] Likewise, cross-sectional evidence highlights that physically active individuals have an enhanced capacity for neuroplastic change induced by TMS, compared to sedentary controls.[125] These aerobic exercise-induced alterations in the capacity for M1 to undergo neuroplastic change are thought to partly underlie reports of behavioral improvements.[23]

In a sample of healthy young adults we recently demonstrated that high-intensity cycling intervals significantly enhanced LTP-like plasticity in M1, versus a resting control condition.[23] In this work we employed PAS, a TMS protocol that can modulate corticomotoneuronal excitability via spike timing-dependent plasticity (STDP) principles[83] (**Figure 1-1**).[78,116] Using a similar study design Singh and others[110] found that 20 minutes of continuous cycling at 65-70% age-predicted maximal HR also enhanced LTP-like responses to PAS, compared to PAS alone. Nevertheless, the use of moderate-intensity aerobic exercise to facilitate neuroplastic change has not been consistently shown. McDonnell et al.[109] found that 15 minutes of moderate-intensity aerobic exercise (~75% maximal HR) did not promote neuroplastic change in M1 after cTBS, a

repetitive TMS protocol used to suppress corticomotoneuronal excitability;[113] while 30 minutes of low-intensity cycling (~55% maximal HR) prior to the same cTBS protocol significantly depressed corticomotoneuronal excitability.[109]

Evidence that aerobic exercise can enhance motor learning in healthy young adults suggests that this intervention could be used to foster improvements in motor behavior after neurological insult[22,39,86] and during healthy aging.[40] However, much of the existing data demonstrating aerobic exercise effects on motor learning have, to date, focused on acute bouts of high-intensity exercise.[23,24] It is unlikely that the exercise intensities employed in this past work [23,24] will be feasible for older adults or individuals with neurological disorders and co-morbid conditions (e.g., cardiovascular diseases). Particularly, persons with stroke have a reduced exercise capacity, a diminished tolerance for prolonged high-intensity exercise, and may be at a heightened risk for cardiovascular events during exercise.[126,127,135]

Moderate-intensity aerobic exercise has been promoted as part of an overall program for secondary prevention after stroke,[126,127] and may have promise to promote learning and neuroplasticity in these individuals.[22] However, contradictory findings related to the effects of moderate-intensity aerobic exercise on M1 plasticity call into question the potential of it to be used as an intervention to promote neuroplasticity, and consequently enhance motor skill learning. As such, replicability of relationships between moderate-intensity aerobic exercise and M1 plasticity after TMS interventions is important to advance the clinical application of this field of research.

In the present study we examined how a single bout of continuous moderate-intensity aerobic exercise impacted LTP-like changes in corticomotoneuronal excitability evoked by PAS. Given the implications for intracortical brain networks in underscoring neuroplastic effects of

aerobic exercise on M1,[76,77,151] we also measured SICI, LICI and ICF. A single group of participants completed both an exercise and rest condition in a repeated-measures crossover fashion, to discriminate between effects of exercise and PAS versus PAS alone. Corticomotoneuronal excitability, SICI, LICI, and ICF were measured at baseline, immediately after and 30 minutes following PAS. We hypothesized that engaging in an acute bout of moderate-intensity cycling prior to the administration of excitatory PAS would significantly increase corticomotoneuronal excitability and ICF, and reduce SICI and LICI, relative to PAS alone.

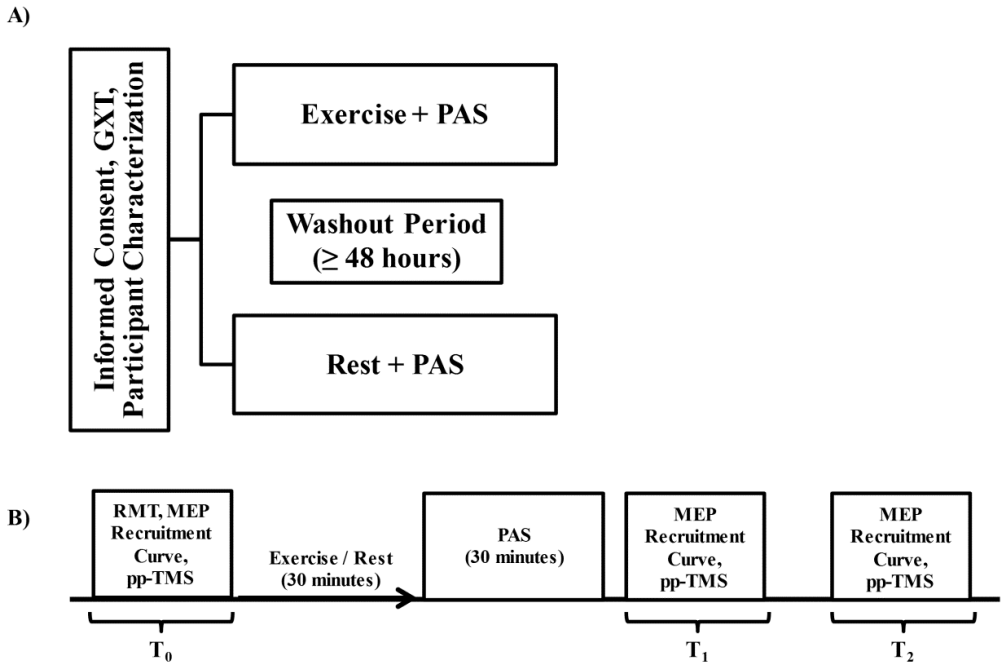

Figure 3-1. Schematic of experimental design and protocol. (A) Diagram representing the study design. (**B)** Illustration of within-session study protocol, including neurophysiological assessments at baseline (T_0) immediately after paired associative stimulation (PAS; T_1), and 30 minutes following PAS (T_2). RMT, resting motor threshold; MEP, motor evoked potential; pp-TMS, paired-pulse transcranial magnetic stimulation assessment, including unconditioned test stimulus (TS) MEPs, short- (SICI) and long-interval intracortical inhibition (LICI), and intracortical facilitation (ICF).

3.2 Materials and methods

The present study was approved by UBC's Clinical Research Ethics Board. All participants independently provided written and verbal informed consent, in accordance with the principles of the Declaration of Helsinki.

3.2.1 Participants

Sixteen healthy adults were recruited from UBC and the surrounding community of Vancouver, British Columbia, Canada (**Table 3-1**, **Table 3-2**). We included right-handed volunteers (handedness assessed as per the Edinburgh Handedness Inventory[1]), who reported participating in ≥ 1500 MET-minutes•week^{-1} of physical activity, based on the long-form IPAQ.[2] Smokers were excluded from the study on the basis that nicotine administration has been shown to abolish the effects of PAS on corticomotoneuronal excitability.[152] All participants were screened for contraindications to exercise (assessed as per the PAR-Q[3]) and TMS (assessed as per Rossi et al.[5]; **Appendix E**).

3.3.2 Experimental design

The current study utilized a crossover design with repeated measures (**Figure 3-1**). During the initial experimental session all participants completed a GXT to exhaustion, to determine $\dot{V}O_{2peak}$ for subsequent exercise prescription. After a period of ≥ 48 hours participants were pseudo-randomized to complete one of two experimental conditions, prior to crossover: 1) moderate-intensity aerobic exercise and PAS; or 2) seated rest and PAS. The order of participation under each condition was counter-balanced across the study sample. To prevent any interaction between repetitive bouts of PAS in close succession,[153] respective PAS sessions were separated by ≥ 48 hours. To attenuate any confounding diurnal fluctuations in PAS response,[154]

respective PAS sessions conducted at approximately the same time of day (within ± 2 hours), after 10:00 am.

3.2.3 Exercise protocol

3.2.3.1 GXT

Before attending this laboratory visit, participants were instructed to refrain from engaging in vigorous physical activity for ≥ 48 hours, ingesting alcohol for ≥ 6 hours, and eating for ≥ 2 hours. Upon arrival at the laboratory participants' height and body mass were measured in one layer of light clothes, with shoes removed. Participants were next outfitted with a silicone mouthpiece, a nose clip, and a one-way air valve (Hans Rudolph Inc., Shawkee, KS, USA). Throughout the GXT, HR was continually monitored via a Polar Wearlink®+ wireless HR transmitter and FS1 HR monitor watch (Polar Electro, Oy, Kempele, Finland), and measurements of $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and RER were continuously monitored (5-second resolution) using a ParvoMedics TrueOne 2400 metabolic cart system (Sandy, UT, USA).[142] The GXT was completed on an electronically-braked Ergoline Ergoselect 200 cycle ergometer (Ergoline GmbH, Bitz, Germany). Briefly, exercise began at a PO of 50 W, for females, or 100 W for males. Cycling resistance was incrementally increased by 30 W every 2 minutes, until the termination of the GXT. Participants were instructed to maintain a pedaling cadence of 70-90 RPM. Every 2 minutes we recorded participants' HR and RPE (6-20 ratings).[4] Immediately after exercise cessation [BLa] was measured using finger-stick and an automated portable BLa analyzer and test strips (Lactate Pro, Arkray Inc., Kyoto, Japan).[143] The GXT was terminated at volitional exhaustion, inability to maintain desired cadence, or participant request to stop. Achievement of maximal $\dot{V}O_2$ was determined *post hoc* under the following conditions: HR > age-predicted maximal value, a plateau in $\dot{V}O_2$ and HR with further increases in workload, RER

> 1.15, RPE > 17.[23,144,145] From the GXT, peak values of $\dot{V}O_2$, PO, HR, and RER were extracted (**Table 3-1**). $\dot{V}O_{2peak}$ was considered the peak $\dot{V}O_2$ value extracted from the GXT.

3.2.3.2 Standardized exercise bout

Under the exercise condition participants completed a single 30-minute session of cycling on a stationary cycle ergometer, at a PO corresponding to 60% $\dot{V}O_{2peak}$[146] and a pedaling cadence of 70-90 RPM. The exercise bout immediately preceded the excitatory PAS intervention, after baseline neurophysiological measurements (see section **3.2.4 Neurophysiology** for details). Every 5 minutes HR and RPE were measured and recorded. Upon completion of exercise, [BLa] was assessed by finger stick. Throughout the exercise bout participants were instructed not to grip the handlebars of the cycle ergometer, in order to prevent any possible influence on responses to PAS.[155] Under the resting condition PAS was preceded by 30 minutes of seated rest, equal in duration to the exercise bout. Participants were asked to remain seated and relaxed for the entire rest period. Two participants (s15, s16) had difficulty maintaining the prescribed PO, which gradually reduced by 5 W increments until their RPE was within the "Moderate Intensity" range (11-14).[145]

3.2.4 Neurophysiology

Assessments of compound motor unit action potentials (M-wave), as well as single- and paired-pulse TMS at were conducted at the following time-points under both conditions (i.e., six total): T_0) prior to the exercise bout or rest period; T_1) immediately following PAS; and T_2) 30 minutes post-PAS. During all procedures participants were seated in a relaxed position with their hands rested on a pillow on their lap.

Table 3-1. Participant characteristics.

	Demographic					Exercise Test					Exercise Bout				
ID	Age	Sex	Height	Body Mass	IPAQ Category	$V\dot{O}_{2peak}$	Peak PO	HR_{peak}	RPE	[BLa]	60% $V\dot{O}_{2peak}$	PO	HR	RPE	[BLa]
s01	31	M	186.0	94.1	High	42.0	280	199	19	12.8	25.2	160	149	12	6.8
s02	26	M	177.0	74.9	Moderate	51.2	310	186	20	10.3	30.7	190	139	13	6.7
s03	25	F	168.0	63.1	High	36.3	200	178	16	11.2	21.8	110	152	13	4.1
s04	32	F	176.0	63.0	High	42.7	260	174	18	6.6	25.6	140	147	12	1.7
s05	29	F	171.0	54.9	High	53.6	260	179	19	16.4	32.6	140	141	12	1.8
s06	28	M	178.5	60.5	High	60.9	310	184	17	12.4	36.5	160	159	10	7.7
s07	25	M	186.0	73.2	Moderate	50.2	310	184	20	17.2	30.1	190	158	14	8.0
s08	25	M	184.0	75.0	Moderate	42.8	250	179	18	15.2	25.7	160	153	14	8.7
s09	23	M	188.2	81.4	High	48.4	310	178	19	13.0	29.0	160	127	12	4.6
s10	24	M	189.2	77.8	High	49.8	310	196	18	8.1	29.9	190	168	10	7.4
s11	29	M	180.0	69.8	High	63.4	310	179	18	14.1	38.0	190	146	14	4.4
s12	29	M	180.0	83.4	High	39.2	220	193	19	17.1	23.5	130	145	13	2.8
s13	35	F	171.0	66.8	Moderate	31.8	170	196	19	12.7	19.1	80	150	14	2.6
s14	23	M	173.5	69.2	High	60.1	310	185	19	11.4	36.1	160	113	12	1.9
s15	23	F	159.0	49.6	High	40.5	170	200	18	10.9	24.3	100	171	14	9.4
s16	28	F	172.0	64.1	High	46.9	230	178	18	12.7	28.1	100	113	13	3.2
Mean	27.2	–	177.5	70.1	–	47.5	263.1	185.5	18.4	12.6	28.5	147.5	144.7	12.0	4.0
SEM	0.9	–	2.1	2.8	–	2.3	12.9	2.1	0.3	0.7	1.4	8.9	4.1	0.3	0.7

Age recorded in years; height recorded in cm; body mass recorded in kg. IPAQ, International Physical Activity Questionnaire, long-form version; $V\dot{O}_{2peak}$, peak O_2 uptake ($mL \cdot min^{-1} \cdot kg^{-1}$); PO, power output (Watts); HR, heart rate ($beats \cdot minute^{-1}$); RPE rating of perceived exertion (6-20 scale); [BLa], blood lactate concentration (Mmol). IPAQ categories: "moderate", ≥ five days with combination of walking or moderate-to vigorous-intensity physical activity, achieving ≥ 600 metabolic equivalent of task [MET]-$minutes \cdot week^{-1}$; "high", ≥ seven days with any combination of walking or moderate-to vigorous-intensity physical activity achieving ≥ 3000 $MET\text{-}minutes \cdot week^{-1}$.[2]

3.2.4.1 Electromyography (EMG)

For the duration of the experimental sessions, participants were fitted with a bipolar electrode configuration (1 cm × 1 cm Kendall™ Ag^{+}/AgCl Foam Electrodes with Conductive Adhesive Hydrogel, Covidien™, Mansfield, MA, USA) over the belly of the non-dominant *abductor pollicis brevis* muscle (APB).[116] A ground electrode was placed over the dorsal surface of the left hand. EMG activity was sampled and monitored using a PowerLab 8/30 data acquisition system and BioAmp biological amplifier (AD Instruments Inc., Colorado Sprinds, CO, USA). Surface EMG was collected using LabChart software (LabChart 7.0, AD Instruments Inc., Colorado Springs, CO), and was pre-amplified at 1000 ×, band-pass filtered at 10-1000 Hz, and sampled at 2000 Hz. EMG collection was triggered by an external stimulus (either TMS or electrical simulator) and recorded in a 300 ms time window relative to the stimulus (100 ms pre- to 200 ms post-stimulus). All EMG data were stored on a personal computer for offline analysis.

3.2.4.2 Median nerve stimulation

After EMG electrode placement a bar electrode (Digitimer Ltd., Welyn Garden City, Hertfordshire, UK) was positioned over the median nerve at the non-dominant wrist,[116] with conducting paste (Ten20® Conductive, Weaver and Co., Aurora, CO, USA). The electrode was secured with a cuff and connected to a constant-current stimulator (DS7AH HV, Digitimer Ltd., Welyn Garden City, Hertfordshire, UK). Electrical stimulation over the median nerve (0.2-ms square-wave pulse) was provided during the M-wave assessment, as well as throughout the PAS protocol.

3.2.4.2.1 M-wave

Immediately before respective TMS assessments, median nerve electrical stimulation intensity was gradually increased from below motor threshold to 1.5 × the minimum current to

evoke a maximal M-wave (Mmax) in the resting APB. Mmax was considered the largest peak-to-peak amplitude M-wave evoked in APB throughout these stimuli, and is a stable measure of muscle activity during maximal muscle fiber recruitment.[156]

3.2.4.3 TMS

Monophasic TMS stimuli were delivered from two 200^2 Magstim magnetic stimulators connected by a BiStim^2 unit, via a 70 mm diameter P/N 9790 figure-of-eight coil (Magstim Co. Ltd., Whitland, Carmarthenshire, UK), at a frequency of 0.25 Hz. Coil location and trajectory for the APB M1 representation were plotted and monitored using a Brainsight^{TM} neuronavigation system and a standard anatomical image template (Rogue Research Inc., Montreal, QC, Canada). Coil and participant localization in space were calibrated during each experimental session. For all procedures the TMS coil was held tangentially to the participant's skull, with the handle pointing laterally and posteriorly at 45° to the mid-sagittal plane.[157] After plotting the APB M1 representation, we determined participants' resting motor threshold (RMT), the percent maximal stimulator output (% MSO) required to produce a 50 μV motor evoked potential (MEP) in the relaxed APB, in at least five out of 10 consecutive TMS stimuli.[157] The order in which each TMS protocol was delivered was randomized across the study sample, but kept consistent between sessions for each participant.

3.2.4.3.1 Single-pulse TMS

After RMT determination, single-pulse TMS was used to assess corticomotoneuronal excitability. Briefly, 10 single TMS pulses were delivered over the APB M1 representation at 100-160% RMT, in 10% increments (70 trials total). The order of stimulus intensities was randomized to attenuate any hysteresis effects induced by systematic MEP elicitation.[158]

3.2.4.3.2 Paired-pulse TMS

For SICI and ICF protocols the conditioning stimulus (CS) was set at 80% RMT, while the test stimulus (TS) was the stimulator intensity required to evoke a ~1 mV MEP in the resting APB ($SI_{1\ mV}$). The ISIs for SICI and ICF were 2 ms and 12 ms, respectively. During measurement of LICI the CS and TS were both set at $SI_{1\ mV}$, and the ISI was 100 ms. Given that the degree of intracortical inhibition and facilitation has been shown to depend on the magnitude of the TS,[159] the TS stimulator intensity was adjusted to maintain a MEP of ~1 mV throughout the entire experiment. Twenty unconditioned TS MEPs were delivered at each time-point; and these were used as a reference to determine the degree of inhibition or facilitation. To ensure that the standardized CS intensity for SICI and ICF (80% RMT) did not evoke a MEP after PAS, test pulses were sent at 90% RMT after PAS.[110]

3.2.4.3.3 PAS

After the exercise bout or rest period, we assessed the magnitude of the TS used throughout paired-pulse TMS (i.e., $SI_{1\ mV}$). Thereafter, median nerve stimulation was used to determine participants' perceptual threshold (PT).[116] For the duration of the PAS protocol, single-pulse TMS was delivered over the APB M1 representation at $SI_{1\ mV}$, and electrical stimulation was delivered over the median nerve at the wrist at 300% PT.[116] Electrical stimulation preceded single-pulse TMS by 25 ms,[116] and 450 total pairs of stimuli were delivered at a frequency of 0.25 Hz (~30 min total stimulation).[23] Throughout PAS participants were instructed to remain relaxed, and were provided verbal feedback if background EMG activity was observed in online trials. Given that attention modulates the effect of PAS on M1,[160] participants were instructed to count the number of stimuli received at the wrist, and report this number at the end of the protocol.[110,161]

3.2.5 Data analyses

All raw MEP data were first pre-processed and inspected using a custom script on the MATLAB platform (Version R2013b, The Mathworks, Inc., Natick, MA, USA). A MEP was excluded from further analysis in the presence of pre-stimulus (100 ms) EMG activity.

3.2.5.1 Single-pulse TMS

All single-pulse TMS data were normalized to Mmax at each respective time-point, to adjust for possible changes in MEP amplitude induced by exercise-related changes in body temperature.[109,162,163] For each participant these normalized values were averaged to provide the mean MEP amplitude at each stimulus intensity (100%-160% RMT) in the MEP recruitment curve. In order to assess corticomotoneuronal excitability after exercise and PAS versus PAS alone, MEP recruitment curve plots of stimulus intensity (% RMT) by normalized MEP peak-to-peak amplitude were constructed for each participant at each measurement (six total).[23] The slope of the linear regression line through each MEP recruitment curve was calculated for each individual MEP recruitment curve.[23,108]

3.2.5.2 Paired-pulse TMS

Paired-pulse TMS data were normalized to the mean of the 20 unconditioned TS MEPs to provide a mean percent-inhibition (for SICI and LICI) or facilitation (for ICF) measure at each time-point.

3.2.6 Statistical analyses

Statistical tests were performed using SPSS (V23.0, IBM Corporation, Armonk, New York, USA). Data are expressed as mean ± SEM. Statistical significance was set at $p \leq 0.05$.

Table 3-2. Baseline neurophysiological measures during paired associative stimulation (PAS) experiments.

	Exercise	**Rest**
RMT (% MSO)	48.7 (2.8)	48.1 (2.3)
Baseline MEP Recruitment Curve Slope	0.19 (0.04)	0.27 (0.06)
Baseline SICI (% Unconditioned TS MEP)	40.3 (6.4)	32.3 (4.1)
Baseline LICI (% Unconditioned TS MEP)	18.9 (3.8)	15.1 (3.3)
Baseline ICF (% Unconditioned TS MEP)	168.7 (28.5)	155.4 (17.1)
$SI_{1\ mV}$ (% MSO; Used During PAS)	64.1 (3.2)	62.9 (3.5)
300% PT (mA; Used During PAS)	11.7 (0.6)	12.0 (0.8)
Number of Stimuli Counted During PAS	431.4 (10.2)	436.6 (7.2)

Values recorded as mean (SEM). For MEP recruitment curve slope, SICI, LICI, and ICF, statistical tests were performed on square root transformed values. RMT, resting motor threshold; % MSO, percent-maximal stimulator output; MEP, motor evoked potential; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation; TS, test stimulus amplitude; $SI_{1\ mV}$, supra-threshold stimulator intensity to elicit a ~1 mV motor evoked potential; PAS, paired associative stimulation; PT, perceptual threshold; SEM, standard error of mean.

3.2.6.1 Data inspection

Data normality was tested using the Shapiro-Wilk test and visual inspection of histogram plots. Omnibus statistical tests were conducted via rmANOVAs. In the event of a violation of sphericity (significant Mauchly's test, $p < 0.05$), the Greenhouse-Geisser correction was applied. Pairwise comparisons were completed using Fischer's Least Significant Difference (LSD) test. Additional *post hoc* pairwise comparisons were conducted post-rmANOVA, to investigate our hypothesized effects of exercise and PAS on the single- and paired-pulse TMS measures of interest.

Baseline (T_0) TMS and PAS parameters were tested for any potential differences to ensure that these measures were well matched between exercise and rest conditions. To compare Mmax

(mV), unconditioned TS MEP amplitudes (μV), MEP recruitment curve slope, and percent inhibition and facilitation (% unconditioned TS MEP), paired-samples *t*-tests were conducted on each of these measures, across exercise and rest conditions at T_0. Additionally, separate paired-samples *t*-tests were used to detect differences in the number of PAS stimuli participants counted across conditions (exercise, rest). Separate paired-samples *t*-tests were also used to assess potential differences in RMT intensity (% MSO), $SI_{1\ mV}$ intensity (mV; used during PAS), and 300% PT intensity (mA; used during PAS) across conditions. To examine whether Mmax (mV) and unconditioned TS MEP amplitude (μV) changed across time-points within the exercise and rest conditions, separate one-way rmANOVAs were conducted using the factor Time (T_0, T_1, T_2). Here, the Bonferroni correction was applied for multiple comparisons.

3.2.6.2 Single-pulse TMS

To examine changes in corticomotoneuronal excitability across time-points and conditions a two-way (Condition × Time) rmANOVA was conducted on MEP recruitment curve slope values at each time-point (T_0, T_1, T_2).

3.2.6.3 Paired-pulse TMS

To test for changes in intracortical inhibition and facilitation across time and conditions, separate two-way (Condition × Time) rmANOVAs were run using SICI, LICI, and ICF data at each time-point (T_0, T_1, T_2), expressed as percent inhibition (SICI, LICI) and facilitation (ICF).

3.3 Results

3.3.1 Participants

Of the 16 participants, 10 were male and six were female, with an overall mean age of 25.2 ± 0.9 years (**Table 3-1**). Participants were highly physically active, reporting an average of 4204.7 ± 416.6 MET-minutes•week^{-1} of moderate- to-vigorous leisure time physical activity.[2]

Only one participant (s12) achieved all criteria for maximal $\dot{V}O_2$, during the GXT; however, all participants achieved at least one criterion. The mean $\dot{V}O_{2peak}$ for males was 50.8 ± 2.7 mL•min^{-1}•kg^{-1} and 42.0 ± 3.2 mL•min^{-1}•kg^{-1} for females, approximately corresponding to "excellent" fitness.[145] The mean PO, HR, RPE and post-exercise [BLa] readings for the continuous exercise bout were 169 ± 6 Watts, 143 ± 5 beats•minute^{-1}, 12 ± 1, and 4.8 ± 0.9 Mmol for males; and 112 ± 10 Watts, 147 ± 8 beats•minute^{-1}, 13 ± 0, and 4.0 ± 1.2 Mmol for females, respectively. On average, participants cycled at 75% age-predicted maximal HR, or 78% measured HR_{peak}, resulting in an average HR of 145 ± 1 beats•minute^{-1} across the entire sample. Average HR and RPE values confirm that the participants were exercising at a moderate intensity.[145]

3.3.2 Data inspection

After inspecting MEP recruitment curve data, two participants were omitted from the entire data set (s05, s07). One participant exhibited excessive background noise in EMG trials, while the other was deemed a statistical outlier with MEP recruitment curve slope, SICI, and ICF measures exceeding 2 standard deviations above the mean. Additionally, we were unable to evoke LICI in one participant (s08), who was subsequently eliminated from the LICI data set. Thus, 14 participants were included in the final MEP recruitment curve slope, SICI, and ICF data sets, while 13 participants were included in the LICI data set. After accounting for the above participants, < 3% of possible EMG trials were eliminated from the remaining data set due to the presence of background or pre-stimulus EMG artefact.

Upon inspecting the normality of data distributions, both Mmax and unconditioned TS MEP values were considered normally distributed (Mmax, $W_{(13)} \geq 0.914$, $p \geq 0.208$; TS MEP, $W_{(13)} \geq 0.898$, $p \geq 0.144$). However, during at least one time-point MEP recruitment curve slope, SICI, LICI, and ICF values were deemed non-normal ($W \geq 0.776$, $p \geq 0.004$). As such, these

measures were square root transformed and statistical tests were performed on the transformed values. Raw data are presented in the figures and tables.

3.3.3 Baseline measurements

Baseline neurophysiological data are shown in **Table 3-2**. Between conditions RMT ($t_{(13)}$ = 0.651, p = 0.526), $SI_{1\ mV}$ ($t_{(13)}$ = 0.594, p = 0.563), and 300% PT ($t_{(13)}$ = -0.372, p = 0.716) were not significantly different. Likewise, baseline (T_0) measures of Mmax ($t_{(13)}$ = -0.451, p = 0.660), unconditioned TS MEP ($t_{(13)}$ = -0.295, p = 0.772), MEP recruitment curve slope ($t_{(13)}$ = -0.855, p = 0.408), SICI ($t_{(13)}$ = 1.738, p = 0.106), LICI ($t_{(13)}$ = 1.523, p = 0.154), and ICF ($t_{(13)}$ = 0.361, p = 0.724) were similar across conditions. Finally, neither Mmax (exercise, $F_{(2, 26)}$ = 0.424, p = 0.659; rest, $F_{(2, 26)}$ = 2.647, p = 0.128) nor unconditioned TS MEP values (exercise, $F_{(2, 26)}$ = 0.096, p = 0.909; rest, $F_{(2, 26)}$ = 0.832, p = 0.378) changed significantly over time (T_0, T_1, T_2) in either the exercise or rest condition. Across conditions, there was no significant difference in the number of PAS stimuli counted by participants ($t_{(13)}$ = -0.722, p = 0.454), suggesting that attention was not significantly affected by exercise.

3.3.4 Single-pulse TMS

See **Figure 3-2** for individual- and group-level plots of corticomotoneuronal excitability. A larger slope of the MEP recruitment curve indicates an increase in corticomotoneuronal excitability. Under the exercise condition 11/14 participants showed an increase in MEP recruitment curve slope from T_0 to T_1. Under the rest condition 10/14 participants demonstrated increases in MEP recruitment curve slope from T_0 to T_1. The two-way rmANOVA on MEP recruitment curve slope indicated a significant main effect of Time ($F_{(2, 26)}$ = 6.264, p = 0.006). Pairwise comparisons revealed that corticomotoneuronal excitability was higher at T_1 (mean ± SEM MEP recruitment curve slope, collapsed across conditions = 0.31 ± 0.04) compared to T_0

(mean ± SEM = 0.23 ± 0.04, p = 0.010), as well as T_2 (mean ± SEM = 0.28 ± 0.05) versus T_0 (p = 0.006); however there was no significant difference between T_1 and T_2 (p = 0.246). The rmANOVA did not show a significant main effect of Condition ($F_{(1, 13)}$ = 1.501, p = 0.242) or a significant Condition × Time interaction effect ($F_{(2, 26)}$ = 0.455, p = 0.639).

Post hoc pairwise comparisons were performed to investigate our hypothesis that the LTP-like effect of PAS on corticomotoneuronal excitability would be enhanced under the exercise condition. Pairwise comparisons revealed that the main effect of Time, observed above, was driven by the exercise condition. Specifically, under the exercise condition MEP recruitment curve slope was greater at T_1 (mean ± SEM = 0.30 ± 0.05) than both T_0 (mean ± SEM = 0.19 ± 0.04, p = 0.012) and T_2 (mean ± SEM = 0.23 ± 0.04, p = 0.048). There was no significant difference between T_0 and T_2 (p = 0.126), indicating that corticomotoneuronal excitability returned to baseline levels after 30 minutes post-PAS. Meanwhile, under the rest condition MEP recruitment curve slope was not significantly different between T_0 (mean ± SEM = 0.27 ± 0.06) and T_1 (mean ± SEM = 0.32 ± 0.05, p = 0.204) or T_1 and T_2 (mean ± SEM = 0.33 ± 0.07, p = 0.820); however, there was a trend towards significance for MEP recruitment curve slope to be greater at T_2 compared to T_0 (p = 0.090). There were no significant differences between conditions (p = 0.125-0.609).

3.3.5 Paired-pulse TMS

3.3.5.1 SICI

Figure 3-3 depicts group-level plots of SICI. Increasing conditioned MEP amplitude, relative to the unconditioned TS MEP, indicates a release of inhibition. The two-way rmANOVA showed that there was neither a significant main effect of Condition ($F_{(1, 13)}$ = 2.803, p = 0.118), nor a significant Condition × Time interaction effect ($F_{(2, 26)}$ = 1.118, p = 0.342). However, there

was a trend towards a main effect of Time ($F_{(2, 26)} = 3.190$, $p = 0.058$). Here, pairwise comparisons demonstrated that SICI was reduced at T_2 (mean ± SEM % inhibition, collapsed across conditions = 48.19 ± 7.15% unconditioned TS MEP) compared to T_0 (mean ± SEM = 36.35 ± 5.01% unconditioned TS MEP, $p = 0.043$), with no differences between either T_0 and T_1 (mean ± SEM = 38.70 ± 4.89% unconditioned TS MEP, $p = 0.431$) or T_1 and T_2 ($p = 0.122$).

To examine our hypothesis that there would be a significant reduction in SICI after exercise and PAS, compared to rest and PAS, *post hoc* pairwise comparisons were conducted. Pairwise comparisons revealed that under the exercise condition there was a significant reduction in SICI at T_2 (mean ± SEM = 61.38 ± 10.55% unconditioned TS MEP) versus T_0 (mean ± SEM = 39.15 ± 6.07% unconditioned TS MEP, $p = 0.027$), as well as a trend towards a significant reduction in SICI at T_2 compared to T_1 (mean ± SEM = 44.86 ± 6.97% unconditioned TS MEP, $p = 0.059$). There was no difference in SICI between T_0 and T_1 under the exercise condition ($p = 0.744$). Under the rest condition SICI did not significantly differ at any time-point ($p = 0.170$-0446). Between conditions, there were no differences at any time-point ($p = 0.093$-0.471).

3.3.5.2 ICF

Figure 3-4 illustrates group-level plots of ICF. Greater conditioned MEP amplitude, relative to the unconditioned TS MEP, indicates an increase in facilitation. The two-way rmANOVA showed that there no significant main effects of Condition ($F_{(1, 13)} = 0.962$, $p = 0.345$) or Time ($F_{(2, 26)} = 0.057$, $p = 0.945$). Likewise, there was no significant Condition × Time interaction effect ($F_{(2, 26)} = 0.174$, $p = 0.842$).

To assess our hypothesis that there would be a significant increase in ICF under the exercise condition compared to the rest condition, *post hoc* pairwise comparisons were conducted. Pairwise comparisons revealed that under the exercise condition ICF was similar at

all time-points (p = 0.828-0.892). Similarly, ICF did not significantly change between time-points under the rest condition (p = 0.507-0.895). ICF was not significantly different between conditions at any time-point (p = 0.361-0.724).

3.3.5.3 LICI

Figure 3-5 shows group-level plots of LICI. Increased conditioned MEP amplitude, relative to the unconditioned TS MEP, represents a release of inhibition. Results of the two-way rmANOVA revealed that there was no significant main effect of or Time ($F_{(2, 24)}$ = 0.183, p = 0.834) and there was no significant Condition × Time interaction effect ($F_{(2, 24)}$ = 0.015, p = 0.985). However, there was a near-significant trend towards a main effect of Condition ($F_{(1, 12)}$ = 4.701, p = 0.051), indicating that LICI was reduced under the exercise condition (mean ± SEM % inhibition, collapsed across time-points = 18.00 ± 3.30% unconditioned TS MEP) compared to the rest condition (mean ± SEM % = 14.45 ± 2.88% unconditioned TS MEP).

We conducted *post hoc* pairwise comparisons to examine our hypothesis that LICI would be reduced under the post-PAS time-points for the exercise condition, versus the rest condition. Pairwise comparisons showed that LICI did not change over time under either the exercise (p = 0.567-0.799) or rest condition (p = 0.683-0.916). Between conditions, exercise and rest conditions did not significantly differ at any one time-point (p = 0.114-0.181).

3.4 Discussion

The aim of the current study was to examine the effects of a single bout of moderate-intensity continuous cycling on changes in corticomotoneuronal excitability evoked by PAS. Our primary finding was that when PAS was preceded by 30 minutes of cycling at a PO corresponding to 60% $\dot{V}O_{2peak}$, there was a significant increase in corticomotoneuronal

excitability, not observed under the resting condition. When PAS is used to increase corticomotoneuronal excitability the mechanisms of these effects are believed to be similar to LTP,[92] given that the excitatory response evoked by PAS evolves rapidly, is reversible, and persists beyond the period of stimulation;[118] NMDA receptor blockade drugs can suppress the excitatory effects of PAS;[118] and the observed increases in corticomotoneuronal excitability reflect LTP induction in reduced animal preparations, via STDP.[83] Thus, our current finding suggests that acute moderate-intensity exercise performed prior to PAS may enhance the LTP-like plasticity in M1, evoked by PAS. This induction of LTP-like plasticity under the exercise condition occurred immediately after PAS, but did not remain 30 minutes post-PAS.

Our finding that moderate-intensity exercise promotes LTP-like plasticity when performed prior to PAS is in agreement with work by Singh and others.[110] The authors demonstrated that 20 minutes of moderate-intensity cycling at 65-70% age-predicted maximal HR significantly increased area under the MEP recruitment curve after PAS, compared to PAS alone.[110] Similarly, previous work in our laboratory demonstrated that when PAS followed 15 minutes of high-intensity cycling intervals at 90% peak PO, larger increases in the slope of the MEP recruitment curve were observed than when 20 minutes of seated rest preceded PAS.[23] On the contrary, McDonnell et al.[109] showed that when participants completed 15 minutes of moderate-intensity cycling at ~75% maximal HR before a cTBS protocol, the suppressive effects of the intervention on MEP amplitude were not present. One possibility underscoring the inconsistencies surrounding the effects of moderate-intensity aerobic exercise on M1 plasticity elicited by TMS could involve a preferential modulation of specific intracortical mechanisms after exercise. For instance, evidence form epidural spinal recordings in humans show that the effects of PAS and cTBS are likely enacted on distinct populations of corticospinal neurons –

PAS effects have been shown to modulate late indirect waves (I-waves), specifically I3-I5,[164] whereas cTBS effects are present in early I-waves, namely I1.[165] However, McDonnell et al.[109] also found that low-intensity cycling at ~55% maximal HR modulated the LTD-like effects of cTBS in the expected direction. This prospect that exercise intensity moderates neuroplasticity in specific cortical circuits must be examined in subsequent work.

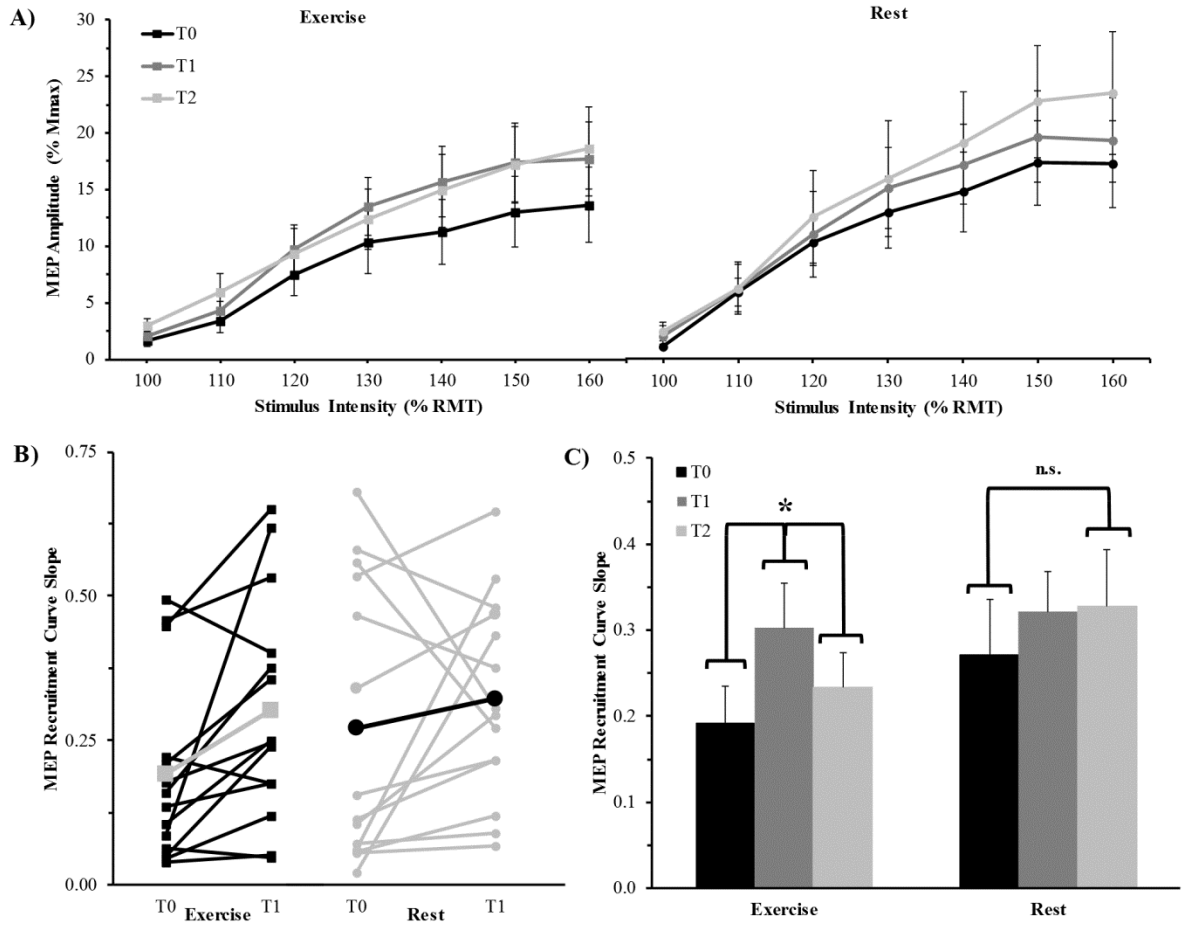

Figure 3-2. Motor evoked potential (MEP) recruitment curve data. (A) Group-level MEP recruitment curves under the exercise (left) and rest (right) conditions. MEP amplitude was normalized maximal M-waves (Mmax) at each time-point (T_0-T_2). The slope of linear regression line plotted through individual MEP recruitment curves was used to characterize corticomotoneuronal excitability. **(B)** Individual plots of MEP recruitment curve slope at T_0 and T_1, under the exercise (left) and rest (right) conditions. In the exercise plot, the gray bar represents the group mean; in the rest plot, the black bar represents the group mean. **(C)** Group-level plots of MEP recruitment curve slope. Error bars represent the standard error of the mean (SEM). *, statistically significant at $p \leq 0.05$. n.s., non-significant trend.

Acute sessions of aerobic exercise are believed to impact M1 through multiple neural pathways, including reductions in SICI[76,77] and increases in ICF.[76] It is also possible that LICI is influenced by aerobic exercise.[76] Aerobic exercise is thus influential on the activity of $GABA_A$, NMDA, and possibly $GABA_B$ receptors in M1 intracortical circuits, as these receptor types are believed to underlie the effects of SICI, ICF, and LICI, respectively.[80,82,81] With evidence from pharmacological studies in humans, changes in M1 plasticity after PAS have been related to GABA-ergic intracortical networks – facilitatory effects on corticomotoneuronal excitability are blocked when research participants are administered drugs known to enhance $GABA_A$[119] and $GABA_B$[120] receptor activity. Likewise, excitatory PAS effects are nullified when human participants are given NMDA receptor blockade drugs.[118] Conversely, excitatory PAS has not reliably been shown to modulate SICI, ICF, or LICI.[78] Thus, effects of exercise and PAS on these paired-pulse TMS measures may conceivably be owed to an interaction between the exercise bout and PAS protocol, or merely a carry-over effect from the exercise bout.

In the present study, we observed a non-significant trend whereby SICI tended to be reduced 30 minutes after PAS. Pairwise comparisons showed that this trend was driven by changes in SICI under the exercise condition. A similar result was found by Singh et al.,[110] who showed a significant reduction of SICI across a 30-minute time-period after excitatory PAS primed by 20 minutes of moderate-intensity cycling, compared to rest and PAS. Our lack of statistical significance could be owed to the high degree of variability inherent in SICI;[166] however, our result could also be limited by the fact that we used only a 2 ms ISI to examine SICI; Singh et al.[110] found an effect of exercise on PAS using a 2.5 ms ISI. Several ISIs ranging from 1-5 ms have been employed for SICI,[78] where a 1 ms ISI presumably assesses intracortical inhibition modulated by extra-synaptic levels of GABA,[79] while longer ISIs probe into $GABA_A$-

receptor-mediated inhibition.[80] Consequently, our work and others'[110] may have overlooked specific effects of exercise and PAS on SICI. Moreover, due to the fact that we examined a single TS and CS intensity, this may have impacted our observed results.[159] Nevertheless, we support the existing evidence that exercise and PAS can reduce SICI in healthy young adults. By reducing intracortical inhibition, aerobic exercise may provide a fertile cortical environment, in which neuroplasticity can occur in response to behavioral or non-invasive brain stimulation paradigms.[22,86] Accordingly, this release of intracortical inhibition could facilitate improvements in brain recovery after stroke.[85]

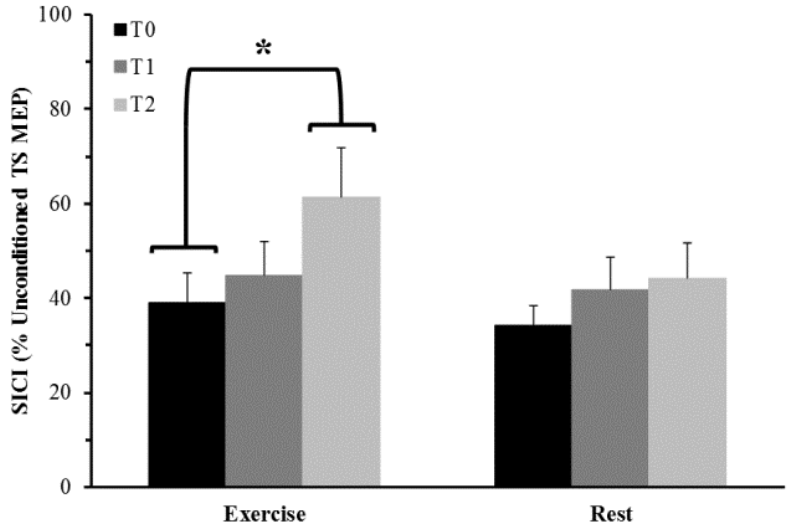

Figure 3-3. Group-level short-interval intracortical inhibition (SICI). Using paired-pulse transcranial magnetic stimulation (TMS), SICI was measured at baseline (T_0), immediately after paired associative stimulation (PAS; T_1), and 30 minutes after PAS (T_2), using a conditioning stimulus (CS) intensity of 80% resting motor threshold (RMT), a test stimulus (TS) intensity evoking a ~1 mV motor evoked potential (MEP; $SI_{1\ mV}$), and an inter-stimulus interval (ISI) of 2 ms. SICI is expressed as a percentage of the unconditioned TS MEP at each time-point. Increasing conditioned MEP amplitude, relative to the unconditioned TS MEP, indicates a release of inhibition. Error bars represent standard error of the mean (SEM). *, statistically significant at $p \leq 0.05$.

We found no effect of PAS on ICF under the exercise or rest condition. This finding is similar to that of existing work;[110] although, aerobic exercise in isolation can modulate ICF.[76] It is possible that the effect of aerobic exercise on facilitatory intracortical brain networks may be short-lasting, and did not endure beyond the duration of the PAS protocol. However, this interpretation is limited by the fact that we did not assess paired-pulse TMS immediately after exercise or rest, so as to minimize to time delay between exercise and PAS.

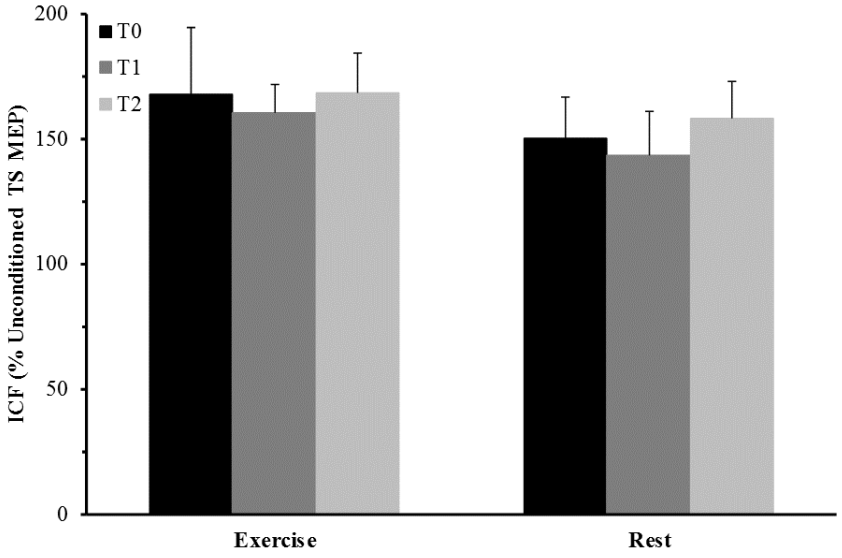

Figure 3-4. Group-level intracortical facilitation (ICF). Using paired-pulse transcranial magnetic stimulation (TMS), ICF was measured at baseline (T_0), immediately after paired associative stimulation (PAS; T_1), and 30 minutes after PAS (T_2). using a conditioning stimulus (CS) intensity of 80% resting motor threshold (RMT), a test stimulus (TS) intensity evoking a ~1 mV motor evoked potential (MEP; $SI_{1\ mV}$), and an inter-stimulus interval of 12 ms. ICF is expressed as a percentage of the unconditioned TS MEP at each time-point. Increasing conditioned MEP amplitude, relative to the unconditioned TS MEP, indicates increased facilitation. Error bars represent standard error of the mean (SEM).

Presently, we showed that 30 minutes of cycling at a PO corresponding to 60% $\dot{V}O_{2peak}$ resulted in LTP-like plasticity in M1 after the administration of excitatory PAS. Pairwise comparisons revealed that our significant main effect of time (i.e., effect of PAS on corticomotoneuronal excitability) was driven by changes in MEP recruitment curve slope under the exercise condition only. Nevertheless, there was a trend towards significantly increased MEP recruitment curve slope under the rest condition as well. Our findings are not consistent with others[23,110] and may be due to methodological differences. For example, we employed a markedly greater dose of PAS stimuli as compared to Singh et al.[110] The former authors utilized 180 pairs of stimuli delivered at 0.1 Hz,[110] while our protocol involved 450 paired stimuli delivered at 0.25 Hz. Changes in corticomotoneuronal excitability induced by PAS may have been masked under the rest condition in our study, due to homeostatic plasticity-like mechanisms, whereby LTP-like plasticity elicited by the early component of the PAS protocol was subsequently down-regulated.[114,167] Previous work shows that providing successive excitatory PAS interventions of 225 paired stimuli each (450 total pairs of stimuli) can suppress the LTP-like plasticity induced by this protocol.[153] However, we consider this unlikely, as previous work from our laboratory used the same PAS protocol in a similar group of healthy young individuals to evoke LTP-like plasticity at rest.[23]

Alternatively, LTP-like plasticity evoked by PAS under the exercise condition could also be due to homeostatic plasticity effects. For instance, prior work indicates that aerobic exercise can result in a non-significant reduction in corticomotoneuronal excitability in the APB M1 representation.[23] Similarly, fatiguing leg-press exercise has been shown to significantly reduce corticomotoneuronal excitability in the M1 representation for a non-exercised upper-limb muscle during the recovery period post-exercise.[151] Accordingly, corticomotoneuronal excitability for

the APB M1 representation would be expected to increase following excitatory PAS, if corticomotoneuronal excitability were reduced following exercise alone.[114] Because we did not measure corticomotoneuronal excitability prior to performing PAS, after the exercise bout/rest period, this is speculation.

It is likely that the non-significant effect of PAS on corticomotoneuronal excitability under the rest condition is a result of day-to-day differences in response to PAS, or a high degree of inter-individual variability in PAS responses. For example, previous work indicates that LTP-like plasticity in response to PAS is not consistent evoked across multiple experimental sessions,[168,169] and that responses to PAS lack test-retest reliability.[168] Likewise, the variability of PAS responses has been subject to several investigations (see Ridding and Ziemann[170] for review), which attribute differences to age,[171] sex,[172] time of day,[154] and BDNF genotype,[173] among other factors. Indeed, in 3/14 participants MEP recruitment curve slope decreased after exercise and PAS, while under the rest condition 4/14 participants showed a decrease in corticomotoneuronal excitability post-PAS. Moreover, no individual demonstrated a consistent decrease in post-PAS MEP recruitment curve slope across conditions. Yet, due to accruing evidence in favor of the beneficial effects of aerobic exercise on M1, this possibility is not likely.

The present results show that a single session of cycling at a PO equivalent to 60% $\dot{V}O_{2peak}$ enhances LTP-like changes in corticomotoneuronal excitability after PAS, compared to PAS alone. Since there were no significant differences in MEP recruitment curve slope across conditions, and in lieu of evidence that moderate-intensity aerobic exercise may impair changes in M1 plasticity,[109] there is potential that our exercise prescription may not optimize the motor system for enhanced neuroplasticity. Although we consider this unlikely, we also wish to highlight the long-term benefits of aerobic exercise on M1 plasticity. For example, Cirillo at

al.[125] found that highly physically active healthy adults demonstrated greater effects of excitatory PAS on corticomotoneuronal excitability compared to sedentary controls. Likewise, compared to baseline both healthy elders[40] and persons with stroke[39] exhibit enhanced motor performance on complex tasks after an 8-week aerobic exercise intervention. Physiologically, the primary difference between prescribing acute and long-term exercise interventions is that a single session provides a single stimulus, whereas long-term exercise provides several repeated stimuli. The effects of long-term exercise depend on the cumulative effects of repeated exposure to exercise; and such interventions may impact brain structures responsible for motor behavior.[75] As such, long-term moderate-intensity exercise prescription may be necessary to realize benefits on neural repair, while the acute benefits may more achievable through high-intensity exercise bouts.

3.5 Conclusions

In conclusion, we support the existing evidence showing favorable effects of a single session of aerobic exercise on LTP-like plasticity in M1. Future work must further explore these effects longitudinally, as well as in healthy elders and persons with neurological impairment such as stroke. Moreover, it is imperative to establish the dose-response effects of exercise on changes in M1 plasticity, as well as to further examine the role of aerobic exercise in influencing motor behavior. Continuing efforts must also examine other biomarkers for neuroplastic change including hormones (e.g., cortisol) and neurochemicals (e.g., BDNF), and using additional TMS (e.g., short-interval ICF) and neuroimaging techniques to probe into the effects of aerobic exercise on specific neural circuits and brain structures.

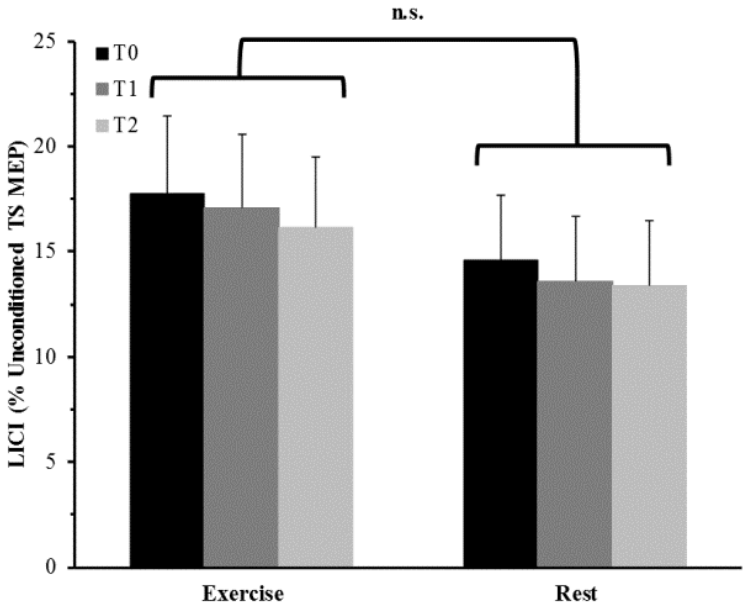

Figure 3-5. Group-level long-interval intracortical inhibition (LICI). Using paired-pulse transcranial magnetic stimulation (TMS), LICI was measured at baseline (T_0), immediately after paired associative stimulation (PAS; T_1), and 30 minutes after PAS (T_2), using conditioning (CS) and test stimulus (TS) intensities evoking a ~1 mV motor evoked potential (MEP; SI1 mV), and an inter-stimulus interval of 100 ms. LICI is expressed as a percentage of the unconditioned test stimulus (TS) motor evoked potential (MEP) at each time-point. Increasing conditioned MEP amplitude, relative to the unconditioned TS MEP, indicates a release of inhibition. Error bars represent standard error of the mean (SEM). n.s., non-significant trend

4 Conclusions and General Discussion

4.1 Introduction

The purpose of the present thesis was to determine the effects of a single bout of moderate-intensity aerobic exercise on motor performance and learning in a CT task, and LTP-like plasticity in M1 elicited by PAS. Exercise consisted of 30 minutes of cycling at a PO corresponding to 60% $\dot{V}O_{2peak}$; the rest condition was comprised of 30 minutes of seated rest. Participants completed both conditions, in a pseudo-randomized and counterbalanced order.

In the first experiment (**Chapter 2**), 16 healthy adults completed a GXT, followed by exercise and CT task practice, or rest and CT task practice, ≥ 48 hours later. During CT task practice, motor performance was assessed at baseline, as well as immediately and 5 minutes after exercise or rest. Twenty-four hours after CT task practice, we assessed motor learning with a no-exercise retention test. We also quantified changes in offline motor memory consolidation after practice. Tracking error was separated into indices of temporal precision and spatial accuracy.

In the second experiment (**Chapter 3**), 16 healthy adults completed a GXT, followed ≥ 48 hours later by exercise and PAS or rest and PAS. At baseline (i.e., pre-exercise or rest), immediately after PAS, and 30 minutes following PAS we measured corticomotoneuronal excitability, SICI, LICI, and ICF.

We hypothesized that undergoing acute moderate-intensity cycling prior to performing the CT task would improve both online performance of the skill and motor learning, measured in a 24-hour no-exercise retention test. Likewise, we hypothesized that a single session of moderate-intensity cycling performed prior to excitatory PAS would significantly increase

corticospinal excitability and ICF, and reduce SICI and LICI, relative to PAS alone. The results of these experiments are summarized and discussed in the current chapter.

4.2 Summary of findings

4.2.1 The effect of an acute bout of moderate-intensity aerobic exercise on motor learning in a continuous tracking task.

Existing work from our laboratory[23] and elsewhere[24] describes the benefits of high-intensity aerobic exercise for promoting improvements in motor learning. These results in healthy young adults indicate that high-intensity cycling intervals can both prime the motor system[22,26] for improved skill acquisition and online performance,[23] impacting the encoding of motor memories,[25,38] and enhance motor learning[23,24] by influencing motor memory consolidation.[25,38] Aerobic exercise effects on motor learning have been linked to up-regulation of systemic BDNF, NA, and BLa.[61]

Despite these findings, translation of this work to stroke populations may require the use of lower exercise intensities. Moderate-intensity aerobic exercise is commonly used in secondary prevention after stroke,[126,127] and has been shown to promote neuroplasticity and brain recovery in animal models.[29,30] Moreover, long-term moderate-intensity aerobic exercise interventions can improve indices of memory and cognitive function, as well as online motor performance in both healthy elders[31,40] and persons with stroke.[39] The present work (**Chapter 2**) aimed to examine the effects of an acute bout of moderate-intensity cycling on motor skill acquisition and motor learning of a CT task,[17,18,23,115] in a sample of healthy young adults.

Results from the present experiment (**Chapter 2**) demonstrated that moderate-intensity exercise, performed prior to CT task practice did not improve motor learning, tested using a 24-

hour no-exercise retention test, compared to a period of seated rest. However, during CT task practice there was a decrease in tracking performance over time, observed only under the rest condition. We interpreted this result as being due to a potential ability of moderate-intensity aerobic exercise to facilitate the maintenance of online motor skill performance, perhaps due to targeted effects on cognitive processes.[32,33,43] Thus, an acute bout moderate-intensity aerobic exercise may be effective to modulate processes underlying motor memory encoding, without the capacity to up-regulate motor memory consolidation.[25,38]

4.2.2 Effects of an acute bout of moderate-intensity aerobic exercise on long-term potentiation-like plasticity elicited by paired associative stimulation.

In addition to examining the effects of aerobic exercise on motor behavior, a growing body of work in healthy young adults has used TMS to investigated how acute[23,109,110] and long-term[125] exercise impacts neurophysiological processes underlying motor learning. Namely, a single bout of aerobic exercise has been shown to impact LTP-like changes in corticomotoneuronal excitability in non-exercised M1 representations, when performed at a moderate[110] or high intensity;[23] and low-intensity exercise appears to influence LTD-like changes in M1.[109] Additional work demonstrates that the neuroplastic effects of aerobic exercise on M1 may be underscored by influences on facilitatory[76] and inhibitory intracortical networks,[76,77,110] in M1 representations of non-exercising upper-limb muscles.

However, due to discrepancies in research findings on the effects of moderate-intensity exercise on M1 plasticity,[109,110] further work is required before these findings can be translated to clinical populations. Thus, in the current experiment (**Chapter 3**), we aimed to investigate the

effects of a single bout of moderate-intensity cycling on LTP-like changes in corticomotoneuronal excitability, SICI, LICI, and ICF, elicited by PAS.

This study (**Chapter 3**) shows that a single bout of moderate-intensity cycling performed before PAS[116] results in LTP-like changes in corticomotoneuronal excitability, and a reduction in SICI, in the absence of such effects after rest and PAS. The present results are similar to that observed in prior work employing moderate-intensity exercise prior to PAS.[110] We suggest that discrepancies between findings supporting the use of moderate-intensity exercise to promote PAS-evoked LTP-like plasticity in M1, and those showing negative effects of this intervention on LTD-like plasticity elicited by cTBS, may involve an effect of moderate-intensity exercise bouts on specific populations of neurons, as evidenced by differential effects of PAS and cTBS on I-waves.[164,165]

4.3 Synopsis

The overarching message of the present thesis is that an acute bout of aerobic exercise has beneficial effects on human motor behavior and underlying neurophysiological processes, but that intensity may be a key factor in modulating these effects. We show that moderate-intensity aerobic exercise can promote LTP-like effects in M1, but that similar effects do not transfer to behavioral measures of motor learning. Nevertheless, moderate-intensity exercise has been shown to affect various cognitive processes[32] and other declarative forms of memory;[25] and similarly, exercise effects on motor memory may translate to motor tasks with different characteristics than the CT task employed here.[34]

Given the relative infancy of this body of literature, there are ample opportunities to elucidate the dose-response effects of exercise on motor learning, to explore the nature of timing

effects of exercise bouts relative to the phases of motor memory formation, and to examine how long-term exercise impacts motor learning, compared to an acute bout. In order to evaluate the clinical effectiveness of this intervention, we must first unpack the above effects in low-risk populations such as healthy young adults or healthy elders.

4.4 Limitations

There were several major limitations inherent in the current thesis, and barriers to translating the current research findings to a clinical or field setting. Firstly, the exercise intensity prescribed here (PO corresponding to 60% $\dot{V}O_{2peak}$) may have negatively influenced our findings. Although exercise has been routinely prescribed relative to $\dot{V}O_{2peak,}$[174] this method can result in large inter-individual variability, in terms of metabolic,[175] and hormonal[176–178] responses to an acute exercise stimulus. In the present thesis we show a wide range of BLa responses (1.7-9.4 Mmol), to the same "relative" exercise intensity. In lieu of evidence linking systemic BLa accumulation to motor learning[61] and changes in M1 excitability after acute exercise,[65] it is possible that this inter-individual variability may have undermined potential benefits of our moderate-intensity exercise prescription on motor learning and neuroplasticity. Other methods have been proposed to mitigate the variability in participant responses to exercise, including exercise prescription relative to RPE, $\dot{V}O_2$ or HR reserve (taking resting levels into account), PO, or ventilatory threshold (VT).[23,24,127,145,174] Additionally, exercise prescription based on $\dot{V}O_2$ would be difficult and expensive to administer in a field setting. However, no "gold standard" method for moderate-intensity exercise prescription has been established.[174,175,179]

A second major limitation surrounds the use of PAS to promote LTP-like plasticity in M1. Albeit the effects of this TMS protocol are believed to reflect LTP (or LTD, depending on the ISI employed),[78,112,118] there is wide inter-[125,170,171,173] and intra-individual[168,169] variability

inherent in responses to PAS. Moreover, while the LTP-like effects of PAS are thought to involve similar pathways to those involved in motor learning,[108,112,124] previous work from our laboratory and elsewhere has found no relationship between these outcomes after an acute bout of high-intensity cycling,[23] or at rest,[111] respectively. Similarly, other work employing cTBS,[111] intermittent TBS (iTBS),[180] and 5 Hz repetitive (rTMS)[180] has shown no relationship between TMS-elicited changes in M1 plasticity and motor learning. Nevertheless, high variability is commonly reported after TMS protocols shown to modulate plasticity, including TBS and rTMS;[111,181,182] this large degree of variability is not unique to PAS. Given the complex nature of motor learning and the various brain structures involved (e.g., M1 and prefrontal cortices,[14,98–101] cerebellum,[100,101,104] and basal ganglia[100,105]) it is somewhat short-sighted to assume that LTP-like responses to PAS in M1 projections will fully explain changes in motor behavior. As such, it will be important for future research to more closely examine exercise effects on a broader range of brain regions, in relation to motor learning.

Finally, in the present thesis we exclusively employed a CT task, similar to that used in prior work.[17,18,23,115] Consequently, the interpretation of our results is constrained to tasks with similar characteristics to the CT task. Evidence indicates that the brain regions involved in motor memory formation depend on the nature of the task involved (e.g., discrete versus continuous movements;[17,18,34,102,103,183,184] temporal versus spatial elements;[106] implicit versus explicit information[23,24,34]), as well as the structure of the practice schedule (e.g., varied/random versus consistent[98]). As such, it is possible that moderate-intensity exercise may be more effective to improve the learning of a task distinct from the CT task. The effects observed in **Chapter 2** involved spatial accuracy, while our previous work showed high-intensity exercise-induced improvements in motor learning targeted temporal precision. Conversely, if exercise effects on

motor learning are intensity-[23,24] or timing-dependent,[25,34] then it is unlikely that moderate-intensity aerobic exercise will be a sufficient stimulus to modulate motor memory consolidation, but may have targeted effects on motor memory encoding.[25,34,38,57] Further work is required to unpack the influence of moderate-intensity aerobic exercise on motor memory processes.

4.5 Future directions

The present thesis has described multiple avenues for future research interventions. In particular, continuing efforts should address issues surrounding a dose-response relationship for exercise intensity effects on motor learning; the timing effects of moderate-intensity aerobic exercise on motor memory processes; effects of acute versus long-term exercise interventions; and the translation of this work in healthy elder and stroke populations. In addition to these areas, it is important to determine whether exercise effects on motor learning are indeed intensity-dependent, or whether these effects depend on the characteristics of the motor task and practice schedule employed. In terms of neurophysiological correlates of motor learning, it is appropriate for future research to examine how different intracortical brain networks, corticomotoneuronal populations, and brain structures are influenced by acute aerobic exercise at various intensities; and it remains undetermined whether lower-limb aerobic exercise interacts with homeostatic plasticity mechanisms in upper-limb M1 representations. Finally, additional work is required to unpack interactions between various biomarkers for neuroplastic change in the human motor system, including cognitive and motor behavior, neurophysiological and electrophysiological outcomes, neurochemicals, and genetic variation.

Presently, stroke-related disabilities contribute to a major reduction in quality of life, and a major economic burden. Despite improvements in standard neurorehabilitation techniques,

existing methods do not consistently lead to positive motor outcomes. The potential priming effects of aerobic exercise on brain health and the human motor system give promise for application to neurorehabilitation practice as an adjunct therapy. In order to inform clinical research studies, as well as to translate these findings to practice, it is necessary to solidify the effects of moderate-intensity aerobic exercise on the above outcomes. Indeed, the opportunities for progress in this burgeoning field of work are abundant and promising.

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Appendices

Appendix A: Edinburgh Handedness Inventory.[1]

Participant Code: _____

Please indicate with a check (✓) your preference in using your left or right hand in the following tasks.

Where the preference is so strong you would never use the other hand, unless absolutely forced to, put two checks (✓✓).

If you are indifferent, put one check in each column (✓ | ✓).

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

Task / Object	Left Hand	Right Hand
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking a Match (match)		
10. Opening a Box (lid)		
Total checks:	LH =	RH =
Cumulative Total	CT = LH + RH =	
Difference	D = RH – LH =	
Result	R = (D / CT) × 100 =	
Interpretation: (Left Handed: R < -40) (Ambidextrous: -40 ≤ R ≤ +40) (Right Handed: R > +40)		

Appendix B: International Physical Activity Questionnaire (IPAQ) Long-form Version.[2]

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

(October 2002)

LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

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

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

Background on IPAQ

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

Using IPAQ

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

Translation from English and Cultural Adaptation

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

Further Developments of IPAQ

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

More Information

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

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

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

Think about all the **vigorous** and **moderate** 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. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ **days per week**

☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**

_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**

_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**

_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**

_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**

_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**

_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**

_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**

_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities

like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?

_____ **hours per day**

_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while 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. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**

_____ **minutes per day**

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

_____ hours per day

_____ minutes per day

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

Appendix C: Physical Activity Readiness Questionnaire (PAR-Q).[3]

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

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

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

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

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

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

If you answered

YES to one or more questions

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

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

NO to all questions

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

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

→

DELAY BECOMING MUCH MORE ACTIVE:

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

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

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

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

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

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

NAME _____

SIGNATURE _____ DATE _____

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

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

CSEP | SCPE

Appendix D: Borg's Rating of Perceived Exertion (RPE) Scale (6-20 Ratings).[4]

Participant Code: _____

Borg Rating of Perceived Exertion (RPE) Scale

6	**No Exertion At All**
7	**Extremely Light**
8	
9	**Very Light**
10	
11	**Light**
12	
13	**Somewhat Hard**
14	
15	**Hard (Heavy)**
16	
17	**Very Hard**
18	
19	**Extremely Hard**
20	**Maximal Exertion**

Appendix E: Screening Questionnaire Before TMS: An Update.[5]

BRAIN BEHAVIOR LAB
TRANSCRANIAL MAGNETIC STIMULATION (TMS) SCREENING FORM

Below is a questionnaire used to exclude participants considered not suitable for transcranial magnetic stimulation (TMS). This information, as well as your identity, will be kept confidential.
PLEASE COMPLETE FORM BELOW:

Participant Code: _____

Please **CIRCLE ONE**:

Neurological or Psychiatric Disorder	YES	NO	Multiple Sclerosis	YES	NO
Head Trauma	YES	NO	Depression	YES	NO
Stroke	YES	NO	Clinical Depression	YES	NO
Brain surgery	YES	NO	Treatment with amitryptiline and haloperidol	YES	NO
Metal in cranium	YES	NO	Implanted medication pump	YES	NO
Brain Lesion	YES	NO	Intracranial Pathology	YES	NO
Pacemaker	YES	NO	Albinism	YES	NO
History of seizure	YES	NO	Intractable anxiety	YES	NO
Family history of epilepsy	YES	NO	Pregnant	YES	NO
History of epilepsy	YES	NO	Headaches or Hearing problems	YES	NO
Intracorporal electronic devices	YES	NO	Family History of Hearing Loss	YES	NO
Intracardic lines	YES	NO	Other medical conditions	YES	NO

If you answered "yes" to any of the above questions, please provide details below.

_____
_____