

**A MULTIFACETED INVESTIGATION INTO THE EFFECTS OF  
ACUTE EXERCISE ON INDICES OF BRAIN FUNCTION**

by

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## Abstract

Participation in regular exercise is important for the maintenance and improvement of brain health across the life span. These beneficial effects are realized almost immediately, as a single bout of exercise transiently improves cognitive function after cessation of exercise. This post-exercise time period represents an opportunity to strategically prescribe cognitively stimulating activities for enhancing brain plasticity and function. Mechanistically, acute exercise is proposed to upregulate brain-derived neurotrophic factor (BDNF) expression and increase regional activation and arousal of brain areas involved in cognitive control; however, the specific mechanisms underlying this facilitation are poorly understood.

Purpose: 1) To investigate BDNF responses to small muscle mass exercise in order to probe a potential mechanism of BDNF release during exercise. 2) To create and validate an equipment-free exercise protocol for use in a magnetic resonance imaging (MRI) scanner, with the eventual goal of investigating brain responses during exercise that may underlie improved cognition. 3) To determine the effect of a very brief bout of high-intensity interval exercise (HIIE) on neuroelectric indices of reinforcement learning in young adults.

Methods: 1) serum BDNF, platelet, and the amount of BDNF per platelet responses were measured following short-duration, maximal effort and long-duration submaximal effort forearm handgrip exercise. 2) We assessed the magnitude and reliability of metabolic responses to a novel whole-body isometric contraction (WBI) exercise protocol. We also characterized the amount of head movement created by WBI. 3) The event-related potential component associated with reward-based learning was assessed before and after a bout of HIIE.

Results: 1) Forearm handgrip exercise significantly increased serum BDNF, platelets, and BDNF per platelet, suggesting the possibility of splenic and cellular contribution of BDNF in response to handgrip exercise. 2) WBI reliably evokes metabolic responses that are similar in magnitude to previous in-MRI studies, and creates head movement suitable for MRI scanning. 3) HIIE

abolishes neuroelectric indices of reward learning, likely due to incomplete recovery from exercise.

Conclusions: These findings advance our understanding of neurochemical and neuroelectric responses to acute exercise and introduce a novel tool that stands to further elucidate the regional brain responses to exercise that may underlie enhanced cognition post-exercise.

## Co-Authorship

This dissertation is the work of Jeremy J. Walsh, in collaboration with his supervisor Dr. Michael E. Tschakovsky.

**Chapter 3 has been accepted for publication with *Frontiers in Exercise Physiology* as: Walsh, J.J., Bentley, R.F., Gurd, B.J., and Tschakovsky, M.E.** Short-duration maximal and long-duration submaximal effort forearm exercise achieve elevations in serum brain-derived neurotrophic factor.

JW and BG were responsible for performing biochemical assay optimization.

JW was responsible for the collection, processing, and analysis of all experimental data. BG oversaw biochemical analysis. JW and RB were responsible for running experimental sessions. JW, BG, and MT were responsible for the conception, design, and interpretation of the experiments. JW and MT wrote the manuscript, and JW, BG, RB, and MT revised the manuscript. All authors approved the final version of the manuscript.

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JW was responsible for the recruiting participants, running experimental sessions, designing and building the model MRI headpiece and bed, and analysis of all data from Study #1. PC was responsible for the technical design and guidance for head motion measures in Study #2. JW and PC were responsible for analysis of data from Study #2. JW and MT were responsible for the conception, design, and interpretation of all experimental data. JW and MT wrote and revised the manuscript. All authors approved the final version of the manuscript.

**Chapter 5 is prepared for submission to *Physiology and Behavior* as: Walsh, J.J.<sup>1</sup>, Colino, F.L.<sup>2</sup>, Krigolson, O.E.<sup>2</sup>, Luehr, S.<sup>2</sup>, Gurd, B.J.<sup>1</sup>, and Tschakovsky, M.E.<sup>1</sup>.** High-Intensity Interval Exercise Impairs Neuroelectric Indices of Reinforcement-Learning

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## List of Abbreviations

ACC = anterior cingulate cortex	MRI = magnetic resonance imaging
BBB = blood-brain barrier	MVC = maximal voluntary contraction
BDNF = brain-derived neurotrophic factor	N = newton
BMI = body mass index	N•s = newton second
BOLD = blood oxygen-level dependent	NO = nitric oxide
CBF = cerebral blood flow	OSC = open sound control
CNS = central nervous system	PaCO <sub>2</sub> = partial pressure of arterial carbon dioxide
CO = cardiac output	PBMC = peripheral blood mononuclear cells
CV = coefficient of variation	PET = positron emission tomography
DBP = diastolic blood pressure	PO <sub>2</sub> = partial pressure of oxygen
EEG = electroencephalography	RAH = reticular activating hypofrontality
ERN = error-related negativity	model of acute exercise
ERP = event related potential	rCBF = regional cerebral blood flow
fMRI = functional magnetic resonance imaging	RewP = reward positivity
fNIRS = functional near-infrared spectroscopy	RPE = rating of perceived exertion
gCBF = global cerebral blood flow	rpm = revolutions per minute
FRN = feedback-related negativity	SBP = systolic blood pressure
HIIE = high-intensity interval exercise	SE = submaximal effort exercise
HR = heart rate	SNA = sympathetic nerve activity
ICC = intraclass correlation coefficient	TPR = total peripheral resistance
MAP = mean arterial pressure	VEC = vascular endothelial cells
ME = maximal effort exercise	$\dot{V}O_2$ = rate of oxygen consumption

$\dot{V}O_{2\max}$  = maximal rate of oxygen  
consumption

$\dot{V}O_{2\text{peak}}$  = peak rate of oxygen consumption

W = watts

WBI = whole-body isometric contraction  
exercise

# Chapter 1

## General Introduction

### **1.1 The intimate coupling of movement and cognition: a (very) brief evolutionary perspective**

The shift from jungle life, where food was abundant and in close proximity, to life on the open plains presented as a massive shift in lifestyle for early human beings (36). Over the course of the next 2 million years, human survival depended on the ability to successfully forage for food while traveling over large distances across heterogeneous landscapes. The act of foraging required the integration of complex cognitive functions including strong spatial memory, the effective allocation of attention, and the ability to quickly learn and adapt behaviour based on the outcome of previous actions (36). This, coupled with the high computational load of bipedal movement sustained at a moderate intensity during foraging (37) would have carried with it a large metabolic cost (1, 19). The central nervous system (CNS) evolved to increase its capacity to perform foraging-related tasks (i.e., cognitive function) when exposed to exercise-related stress in order to minimize the perturbation of subsequent stressors and to increase the likelihood of successful foraging (15, 24). As such, higher levels of physical activity elicit greater adaptation in the brain, thereby facilitating a larger capacity for successful interaction with the environment at a lower metabolic cost.

The responses of the brain to exercise consist of both acute and chronic adaptations (8, 36). Acutely, exercise transiently potentiates executive functions via neurotransmitters and neurotrophic factor signalling, which leads to enhanced task performance (8). These acute events initiate a cascade of cellular signalling that promotes metabolic and structural plasticity of brain tissue for long-term adaptation with repeated exposure to exercise (45). Interestingly, the combination of exercise and cognitive stimulation appears to have an additive effect on brain plasticity and function compared to either performed alone (11, 12, 26, 27, 36, 46). Analogous to

skeletal muscle anabolism being augmented by the timing of protein intake (9), there appears to be a temporal sensitivity of brain tissue to cognitive stimulation following a bout of exercise (6, 12, 27, 46). From an evolutionary perspective, this interaction would ensure increased allocation of cognitive resources dedicated to foraging behaviour and an enhancement of learning and memory processes to guide future behaviour.

Despite a drastic shift towards a sedentary lifestyle, the integral link between physical activity and cognitive function is conserved in the modern human brain, and exercise represents one of the most effective strategies for improving brain function and resisting neurological disease across the lifespan (16). The exact mechanisms that underlie this acute upregulation and priming of brain function in humans are not fully understood. As such, the aim of this dissertation is to investigate potential mechanisms, propose new methodology for understanding this phenomenon, and to investigate brain responses to exercise from an applied perspective.

The investigatory approaches taken in this dissertation are diverse and represent efforts to understand the acute effect of exercise on the brain in new ways. Given that the early potentiation of function is driven by neurochemical mechanisms, primarily through the action of brain-derived neurotrophic factor (BDNF), Study #1 investigates mechanisms of peripheral BDNF release in response to exercise. Advances in neuroimaging, specifically functional magnetic resonance imaging (fMRI) affords high spatial resolution of neuronal function in response to various stimuli and stands to advance our understanding of how the brain responds to exercise; however, technical constraints of fMRI scanning limit the ability to perform exercise in a scanner without a large investment in specialized equipment. Study #2 presents a novel exercise protocol to be used in an MRI for the purpose of examining the antecedent neural events that may underlie the priming of cognitive function post-exercise. Finally, despite the mounting evidence implicating the importance of exercise for brain health and staving off neurological disease, there is generally poor adherence to regular exercise. Commonly cited barriers include time and access to equipment. High-intensity interval exercise (HIIE) represents a time-effective,

equipment free exercise strategy that has been shown to improve indices of cognitive function. We assert that an individual could strategically perform HIIE prior to a cognitively demanding activity in order to boost their brain power. As such, Study #3 investigates the effect of HIIE on neuroelectric indices of early reinforcement learning. *For the purposes of this dissertation, cognitive function refers specifically to executive functions unless stated otherwise. Executive functions are higher-order top-down processes that govern goal-directed behaviour in changing environments* (16).

## **1.2 BDNF responses to exercise**

BDNF is recognized as one of the most important signalling factors underlying exercise-induced brain plasticity. While it exerts pleiotropic effects throughout the body, BDNF is chiefly recognized for its integral role in orchestrating these exercise-induced neural adaptations through participation in acute and long-term exercise (8, 31). Acute exercise is a potent stimulus for transiently increasing circulating levels of BDNF in humans (25) and upregulating BDNF transmission and gene expression in animals (20, 38). While the functional significance of this response in humans is unknown, it has been speculated that each bout of exercise elicits a ‘dose’ of BDNF, which may signal neurogenic events underlying the priming effect (35, 42). In support of this, some human studies have found positive associations between improvements in cognition and levels of circulating BDNF following a bout of exercise (28, 41, 44); however, the exact role of circulating BDNF with exercise on brain health is unknown. Regardless of the exact role, circulating BDNF is important for brain health, and investigations into BDNF responses to exercise remain highly relevant. However, the majority of studies examining this response act under the assumption that the brain is the main source of circulating BDNF during exercise without consideration of known, alternate mechanisms.

Specifically, the spleen is likely a major contributor to the BDNF response with acute exercise. The majority of blood-borne BDNF is bound to platelets and at rest, 30% of platelets

are stored in the spleen (5). Instances of increased sympathetic nerve activity (SNA), such as exercise, causes the spleen to constrict and release a portion of its platelet pool into the circulation in an intensity-dependent manner (exercise-induced thrombocytosis) (5, 14, 18, 32). *While the link between thrombocytosis and BDNF has been suggested elsewhere (7, 32), no other study has considered the strategic provocation of this response in order to increase circulating BDNF.*

*We assert that an exercise protocol involving minimal muscle mass but evoking a large SNA response (22), such as forearm handgrip exercise, should be able to achieve meaningful increase in BDNF via mechanisms of thrombocytosis.* Such an exercise protocol could stand as a viable means of transiently increasing a dose of BDNF without performing whole-body exercise or can be performed concurrently while in sedentary, occupational setting or for individuals with mobility constraints.

### **1.3 New methodology for examining the brain during exercise - a novel, equipment-free whole-body isometric contraction exercise protocol for fMRI-based studies**

Advances in neuroimaging techniques over the past 25 years have allowed for investigation of the deepest regions of the brain. In particular, the blood oxygen-level dependent (BOLD) response measured by fMRI has provided unprecedented resolution of neural firing characteristics via the hemodynamic responses in cortical and subcortical structures of the brain. Advances in fMRI technology and methodology have produced the ability to regionally quantify cerebral blood flow (CBF) via arterial-spin labeling, assess cerebrovascular function via hypercapnic challenges, assess cerebral metabolic rate for O<sub>2</sub> via quantified MRI, and multiple other analytical approaches that provide a wealth of network-related information.

The spatial resolution afforded by fMRI coupled with powerful analytical techniques make it an ideal measurement tool for examining responses of the brain during exercise; however, given the strict head movement limits on fMRI scans, performing exercise inside of an MRI is a

challenge. To overcome this limitation, previous researchers have engineered specialized exercise equipment to be used for both small and large muscle-mass exercise while being scanned (13, 17, 21, 30, 34, 39, 40). Through the use of head and body bracing techniques (13, 34), as well as specialized scanning sequences (21), these groups have produced viable MRI scans during exercise that provide insight into the brain areas involved in the regulation of exercise. *Despite their utility, the exercise equipment used in these studies are very expensive, rendering these types of investigations relatively inaccessible to a larger cohort of researchers. We propose that a novel equipment-free exercise protocol consisting of rhythmic, whole-body isometric (WBI) contractions could overcome the barrier of requiring expensive, specialized equipment to study responses of the brain during exercise.*

#### **1.4 Reward-learning and high-intensity interval exercise**

The ability of a single bout of exercise to improve cognition represents a useful strategy for boosting brain function in situations where it may be compromised and/or better function would lead to better task performance. A particularly applicable demographic is university students, as a majority of the academic activities are sedentary in nature (i.e., studying, sitting in lecture, working on a computer, etc.) and prolonged bouts of sedentary activity is associated with a decline in arousal and a subsequent deterioration of cognitive functions (3). Despite acute exercise's ability to boost cognitive function, time is one of the greatest perceived barriers preventing university students from partaking in regular bouts of physical activity (10).

HIIE represents an accessible exercise strategy that elicits similar metabolic and fitness benefits to traditional exercise modalities in a fraction of time (4, 33). Emerging evidence demonstrates that HIIE improves indices of cognitive function, especially in attentional domains in a range of populations (2, 29, 43, 44). In support of this, a recent study found that HIIE increases neuroelectric indices of attention and inhibitory control (23). While improved attention would certainly contribute to better task performance in a learning context, *whether HIIE*

*directly improves neuroelectric mechanisms of reinforcement learning is currently unknown.*

Such an investigation is warranted given that exercise: 1) upregulates dopaminergic pathways that are proposed to underlie reinforcement learning and, 2) positively modulates anterior cingulate cortex (ACC) function, which is the neural generator of this reinforcement learning signal. *As such, we propose that a bout of HIIE could be an effective means of improving reinforcement learning in university students.*

## **1.5 Purposes**

The overarching goal of this dissertation is to use unique approaches to investigate the effect of acute exercise on indices of brain function in the context of utilizing the post-exercise period for enhancing cognitive function and brain plasticity. This work uses mechanistic and application-based approaches, as well as proposing new methodology for exploring the effect of acute exercise on the brain.

The purpose of Chapter 3 was to:

- 1) Determine the effect of short-duration, maximal effort and longer-duration, submaximal effort forearm handgrip exercise on serum BDNF.
- 2) Determine the thrombocytosis response to each exercise protocol and compare these response to BDNF responses

The purpose of Chapter 4 was to:

- 1) Determine the magnitude of metabolic and heart rate responses of our novel WBI exercise protocol to progressively increasing exercise intensities
- 2) Demonstrate retest reliability of WBI exercise using perceptually guided exercise intensity prescription
- 3) Characterize the amount of head movement associated with WBI in a simulated MRI environment

The purpose of Chapter 5 was to:

- 1) Determine the effect of HIIE on neuroelectric indices of reinforcement learning (reward-positivity) in university students

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## **Chapter 2**

### **Literature Review**

#### **2.1 Part 1 – Circulating BDNF responses to exercise**

##### **2.1.1 Introduction**

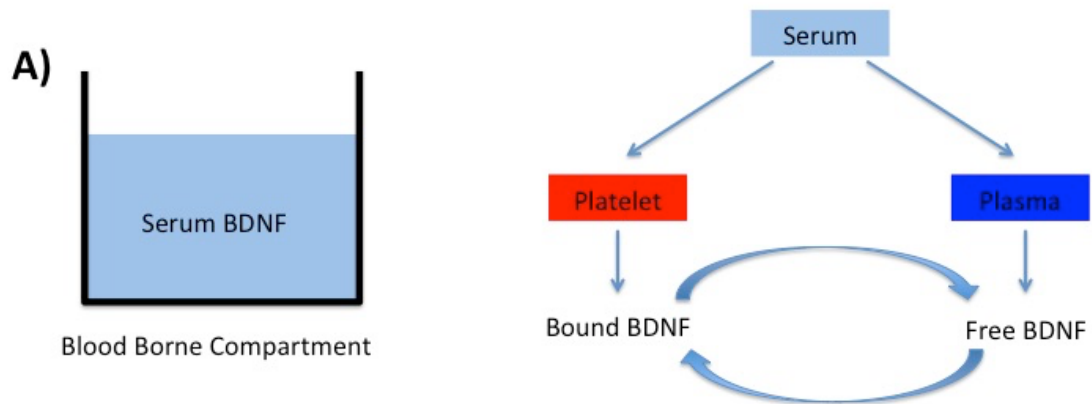
BDNF is emerging as a critical neurotrophin that exerts pleiotropic effects that extend well beyond the brain. In fact, a host of peripheral tissue produce (86) and in some cases, secrete BDNF into systemic circulation (117, 163). BDNF exerts beneficial neuro- and metabotropic effects, with some calling it a ‘master regulator of whole-body energy homeostasis’ (92). Acute exercise is a potent stimulus for increasing blood-borne BDNF (75) and has been suggested as an important mechanism by which exercise improves brain function (147, 159). A number of studies have examined the acute response of BDNF to various modalities of whole-body exercise (primarily aerobic, but some resistance exercise) and a majority have reported transient increases in serum or plasma BDNF. A consistent theme in emerging studies is a focus on understanding how specific exercise parameters, such as intensity (75) or duration (35), influence the BDNF response to exercise. Further, within these studies, there is a general consensus that the brain is the main source of increased circulating BDNF observed with exercise (53, 75, 120). However, the probable mechanism that would explain the bulk of increased BDNF with exercise (increased circulating platelets) is often overlooked and as such, has been relatively unexplored. In Part 1 of this literature review, I will provide a brief account of the circulating BDNF system and a rationale for why studies ought to consider this mechanism of BDNF release with exercise when designing future studies.

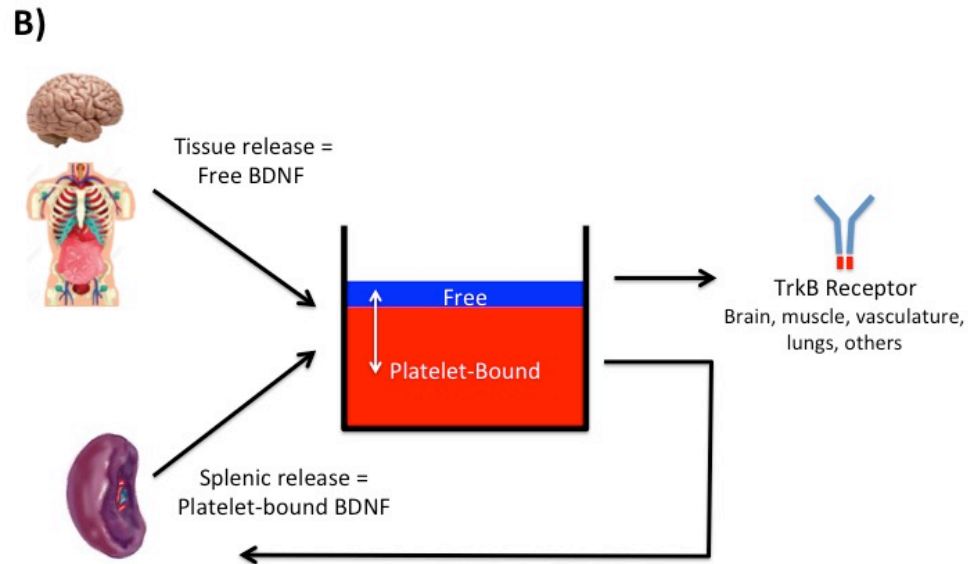
##### **2.1.2 Defining BDNF pools within the blood**

Total blood-borne BDNF can be divided into two pools: 1) BDNF that is bound to platelets, and 2) BDNF that freely circulates (unbound). The bound pool has ~200x greater BDNF content

compared to the unbound pool (124). A small fraction of BDNF is not bound to platelets and is considered to be ‘biologically active’ as it is free to associate with TrkB or p75 receptors (92, 127). However, BDNF can move between bound and unbound states depending on conditions in the local physiological milieu such as low PO<sub>2</sub> and high shear stress.

BDNF is typically measured via anti-body based sandwich ELISA’s and the portion of blood (serum or plasma) that is analyzed has critical implications for interpretation of data (110). Serum represents the total blood-borne BDNF (bound and unbound) while plasma represents the free (unbound) portion. Fujimura et al. (49) demonstrated that serum BDNF content closely matches BDNF content of washed platelet lysates (platelets that had been ‘emptied’ so to speak) in human blood, suggesting that almost all blood-borne BDNF is platelet bound. As such, the contribution of plasma (free) BDNF to overall blood-borne levels is minimal at best (Figure 2-1).





**Figure 2-1.** Model of circulating A) as a compartment and its division into pools, and B) the sources of BDNF inflow and outflow from the blood.

Figure 2-1 depicts a model of circulating BDNF and its sources of inflow and outflow. **A)** The entire blood-borne pool of BDNF (platelet bound + free = total BDNF) can be quantified in serum, whereas free (unbound) BDNF can be quantified in plasma. BDNF can move between the bound and unbound state, which has implications for the measurement of plasma but not serum. **B)** There are multiple sources of circulating BDNF. Tissue releases *de novo* BDNF into the free portion of the pool, whereas the spleen releases platelet-bound BDNF into the bound portion of the pool via the release of platelets. Free BDNF has two fates: 1) binding to platelets within the blood, thereby having no effect on total BDNF, or 2) binding to the TrkB receptor located on a host of different tissues, thereby reducing the total blood-borne pool. Platelet-bound BDNF also has two fates: 1) releasing BDNF into the free portion of the blood, thereby having no effect on total BDNF in the blood, or 2) being re-sequestered by the spleen, thereby reducing the total blood-borne pool.

### 2.1.3 Serum vs. Plasma – what BDNF can be used?

Within the BDNF and exercise literature, serum is the most commonly measured blood parameter, followed by plasma, and then platelets (75). The importance of which blood parameter is selected for a study is based on the fact that BDNF can move between pools without addition to or removal from the total BDNF pool (49). Specifically, the offloading of BDNF from platelets would increase plasma BDNF without affecting serum BDNF (49). Consequently, if plasma is the only blood parameter measured in a study, an observed increase in plasma BDNF does not necessarily reflect the addition from a cellular source (120). Historically, plasma is thought to represent the free, bioavailable portion of BDNF and therefore, has been argued as a more meaningful measure of BDNF in the context of increasing the neurotrophin for improving brain and metabolic health. However, this is an over simplification, as platelets release BDNF as within the vasculature of metabolically active tissue in response to shear stress and other agonist stimuli (49). High levels of shear stress are present in the vasculature of active muscle and the brain during exercise, suggesting that platelet release of BDNF would increase under these conditions. As such, serum is not an inert BDNF reservoir. Rather, by extension of platelet-BDNF dynamics, serum should also be viewed as a bioavailable pool of BDNF under physiological conditions such as exercise.

In support of this, serum BDNF levels are significantly lower in individuals with major depressive disorder compared to healthy controls (15, 71, 137) and there is a significant negative correlation between serum BDNF and depression scores (137). Treatment with antidepressant drugs significantly increases serum BDNF levels, the magnitude of which is significantly associated with improvements in depression scores (15). These findings are supported by murine models of depression, wherein peripheral infusion of exogenous BDNF significantly improves indices of depressive behaviour and stimulates antidepressant cellular adaptations in the brain (129).

The discrepancies between serum BDNF levels in depressed versus healthy individuals appears not to be due to differences in total blood content, but rather differences platelet release

of BDNF (71). Karege et al. (71) found that both serum and plasma levels were significantly lower in depressed patients compared to healthy controls, and that these differences can be explained by alterations to mechanisms of release from platelets rather than a reduction in total blood content. Interestingly, pharmacological treatment of healthy human (156) and rat (164) platelets with antidepressants significantly increases platelet release of BDNF *ex vivo*, which may be a mechanism to explain increase serum BDNF following treatment with antidepressants in individuals with depression (15). In summary, finding supports the notion that *serum represents a bioavailable pool of BDNF through the action of platelets*.

#### **2.1.4 BDNF responses to exercise**

An acute bout of exercise increases both serum and plasma BDNF in a range of populations (35, 75, 147). In a recent meta-analysis, Szuhany et al. (147) determined that a single bout of exercise (predominantly aerobic) has a moderate effect on plasma and serum BDNF (Hedges'  $g = 0.46$ ), and that this acute effect is potentiated by a preceding period of regular exercise training (Hedges'  $g = 0.59$ ). The factors by which exercise transiently increases BDNF have yet to be confirmed, as one systematic review suggests that exercise intensity may drive BDNF responses (75), whereas a recent meta-analysis found that exercise duration is positively associated with increases in BDNF (35). Regardless of the exercise-specific factors driving this response, aerobic (27, 45, 53, 95, 120, 154) and resistance exercise (28, 93, 162, 168) increase serum and plasma BDNF. This effect, however, is short lived following cessation of exercise as BDNF returns to baseline levels within 30 minutes for serum (162, 168) and 60 minutes for plasma (53).

Given its transient nature, the functional significance of the peripheral BDNF response to a bout of exercise is not fully understood. It has been suggested that each burst of circulating BDNF that accompanies a bout of exercise is a 'BDNF dose' and the accumulation of these BDNF doses have a positive impact on the brain (112, 147), possibly priming the brain for learning (76). Some studies have reported positive associations between circulating BDNF and

improvements in cognitive function following a bout of exercise (83, 139, 166), whereas others have reported no association between these variables, despite both being positively influenced by exercise (45, 154). It is possible that increases in BDNF with exercise contributes to acute improvements in cognitive function, given that greater blood content would mean greater availability for transport across the blood-brain barrier (BBB) (107, 114). However, this is speculative and the role of exercise-induced BDNF on the brain health has yet to be fully elucidated.

### **2.1.5 Sources of BDNF - Inflow to the blood pool**

The addition of BDNF to the blood at rest and during exercise is likely derived from a number of tissue sources that produce and release the neurotrophin into circulation. A host of tissues produce and release BDNF, including the brain, lungs, bladder, intestinal tissue, vascular endothelial cells, skeletal and cardiac muscle, peripheral neurons, mononuclear cells, and platelets (14, 49, 86, 92, 95, 107, 117, 161). The role of BDNF in these tissues appears to be related to neural growth and survival (86), modulation of smooth muscle tone (2, 117), tissue remodeling (20, 141), and energy regulation (95, 161). An important similarity between a number of these tissue sources is that BDNF is released under conditions of physiological stress, such as exercise. While it is very difficult to isolate the relative contribution of tissue to total circulating BDNF in humans, it is important to understand the potential sources and the factors that contribute to the release of BDNF. In the context of exercise, the candidate tissues for the addition of BDNF to circulation are the brain, skeletal muscle, vascular endothelial cells, and platelets.

#### **2.1.5.1 The brain**

Neurons in the brain produce and release BDNF (92, 107). The production and release of BDNF from neurons is regulated by excitatory synaptic activity as well as the presence of specific hormones and neuropeptides (92). Following its production, BDNF is stored in vesicles,

transported to areas of synaptic activity where it is released in response to excitatory signaling (111). BDNF increases neuron survival, growth, and development, and has neuromodulatory effects on both pre- and post-synaptic neurons (92, 111).

BDNF has been shown to cross the BBB in a bi-directional fashion via a saturable transport system (106, 114). This is supported by indirect evidence showing that peripheral infusion of exogenous BDNF stimulates brain plasticity in mice (129) and by others who have demonstrated that circulating and cerebral levels of BDNF are closely aligned across different species (72, 74). These findings, however, are met by opposition from other studies that assert that BDNF does not cross the BBB (109, 126). Infusion of BDNF as a therapeutic drug for stroke rehabilitation in rats has proven relatively ineffective unless a conjugated form of BDNF is administered (169), challenging the notion that unaltered (naturally occurring) BDNF crosses the BBB.

Despite these contradictory findings, evidence from human studies provide further support for the release of BDNF from the brain (77, 120, 135). Through the comparison of plasma BDNF from cerebral venous effluent from the internal jugular vein to peripheral plasma BDNF in the radial artery, it has been suggested that 70-85% of plasma BDNF is of cerebral origin at rest and during exercise (120, 135). An argument can be made that the pituitary gland is the source of the plasma BDNF observed in these studies, given that it exists outside of the BBB and is able to release BDNF directly into the circulation (49). However, circulating BDNF is unaltered in hypophysectomised rats suggesting that BDNF crosses the BBB and/or that peripheral sources of BDNF also have a significant contribution to circulating levels. Indeed, while the brain is a likely contributor to circulating BDNF, peripheral tissue such as the urinary bladder, lungs, colon, and vascular endothelial cells have comparable levels of BDNF mRNA to brain tissue and present as a probable source of circulating BDNF (86, 117, 119).

#### 2.1.5.2 Skeletal muscle and BDNF

Skeletal muscle produces BDNF under conditions of energetic stress such as prolonged exercise (95) and fasting (161). Matthews et al. (95) propose that BDNF is involved in the regulation of fat metabolism in an AMPK dependent manner. Interestingly, this same group found that skeletal muscle is not a secretory organ for BDNF, but rather uses the neurotrophin in an autocrine fashion. Further, the upregulation of BDNF mRNA displays a 3-hour lag relative exercise cessation, making skeletal muscle an unlikely tissue source of circulating BDNF during and immediately after exercise.

#### 2.1.5.3 Vascular endothelial cells and BDNF

Endothelial function is a marker of vascular health and factors such as hypertension impair endothelial function while exercise improves it. Endothelial BDNF production is linked to endothelial function, as hypertensive rats have attenuated endothelial BDNF expression compared to their sedentary, normotensive counterparts (117). Exercise, however, significantly mitigates the effect of hypertension on endothelial BDNF production, as 7 consecutive days of 30 min/day treadmill running drastically increases BDNF production in aortic and coronary artery endothelium in hypertensive rats (117). Interestingly, this effect is not localized to the peripheral vasculature, as the same exercise regime induced BDNF expression in cerebrovascular endothelium in hypertensive rats (119). In fact, Prigent-Tessier et al. (117) report that the BDNF levels in cardiac and aortic endothelium were in the same range as those measured in the hippocampus (119), challenging the prevailing notion that circulating BDNF is primarily of neuronal origin. As such, the vascular endothelium (as a unit) is a likely contributor to circulating levels of BDNF following an exercise stimulus. The primary stimuli for the production and release of BDNF by endothelial cells are shear stress and hypoxemia (low blood  $PO_2$ ) (56, 117). The release of BDNF by endothelial cells is proportional to the magnitude of the shear stimulus imposed and chronic exposure to shear stress *in vitro* upregulates endothelial BDNF to the same

degree as exercise training (117). Interestingly, the release of BDNF appears to be non-genomic, implicating the storage and release of BDNF in instances of high shear stress.

Given the provocation of BDNF release by shear stress and hypoxemia, it seems intuitive that BDNF plays a role in the modulation of vasomotor tone. Aortic endothelial cells prominently express TrkB, which in turn stimulates the production of nitric oxide (NO) in a dose-dependent manner (99, 148). Meuchel et al. (99) found that BDNF rapidly induces NO production in human pulmonary artery endothelial cells within 6 – 8 minutes of BDNF exposure and the magnitude of vasodilation induced by signalling was comparable to (but less than) that of acetylcholine, the gold standard agonist for evaluating endothelial-dependent vasodilation. Further, removal of the endothelium from human pulmonary arteries abolishes the vasorelaxant effects of BDNF (99), implicating endothelium as the vascular target of BDNF.

While a number of other tissues express BDNF, the vascular endothelium is a strong candidate for a cellular source that contributes *de novo* BDNF during exercise, given that shear stress is the primary stimulus for its production and release, and the ubiquitous distribution of endothelial cells in the cardiovascular system.

#### 2.1.5.4 Platelets and BDNF

Platelets are the primary transporter of BDNF in the blood and contain 99% of total blood-borne BDNF; however, extremely low levels of BDNF mRNA have been detected in platelets, suggesting that they do not endogenously produce the neurotrophin (49). Instead, platelets sequester free BDNF from the plasma via receptors located on the outside of platelets (49). Platelets release BDNF in a dose-response manner to both pharmacological (i.e., Riluzole) (156) and physiological stimuli (49). Fujimura et al. (49) report that low shear stress stimulates 16% release of platelet bound BDNF, while high shear evokes a release of 32%. As discussed in Section 2.1.3, platelet function appears to be the main mechanism underlying low serum and plasma BDNF in depressed patients and treatment with antidepressants significantly increases the

release of BDNF from platelets (71, 156, 164). As such, platelets not only act as a BDNF reservoir, but also their function can directly affect BDNF bioavailability.

#### 2.1.5.5 The spleen stores and releases platelets

At rest, the red pulp of the spleen stores 30% of the body's platelets in a pool that is freely exchangeable with circulating platelets (22, 160). Pools of splenic and circulating platelets exist in a dynamic equilibrium such that  $\alpha$ -adrenergic stimulation causes the release of platelets from the spleen (thrombocytosis) and  $\beta$ -agonists stimulate reuptake of circulating platelets into the spleen (22, 100). The spleen is encapsulated by a sheath of collagen fibres, smooth muscle cells, and a tight mesh of elastic fibres and has the capacity to actively constrict in response to sympathetic activation (8). As well, splenic volume is highly dependent on splenic blood flow and therefore adrenergic modulation of splenic artery tone directly affects splenic volume (160). Release of norepinephrine from sympathetic nerves causes splenic constriction and thrombocytosis in an activity-dependent fashion (140, 152). As well, epinephrine evokes thrombocytosis, as low dose infusion reduces splenic volume by 43%, resulting in a 31% increase in circulating platelets (5).

#### 2.1.5.6 Splenic responses to exercise

Given that an increase in SNA evokes splenic constriction, and that exercise increases SNA, it is not surprising that exercise increases circulating platelets in an intensity-dependent manner (exercise-induced thrombocytosis) (22, 67). Following cessation of exercise, these platelets are re-sequestered by the spleen (67), the time course of which parallels serum BDNF reductions following a bout of exercise (95, 162). In the context of BDNF and exercise, ***thrombocytosis presents as a strong candidate for the exercise-induced increase in serum BDNF as platelets are the primary carrier of blood-borne BDNF and their temporal responses to exercise are tightly aligned.***

This is an important consideration for exercise interventions that are designed to increase blood-borne BDNF. Since an increase in sympathetic outflow is the primary mechanism for thrombocytosis, any intervention that can sufficiently increase sympathetic activation and circulating catecholamines should be able to evoke a meaningful increase in serum BDNF, independent of metabolic cost or the size of mass muscle involved. For instance, small muscle mass exercise, such as forearm handgrip exercise stimulates large increases in SNA (70) and circulating catecholamines (143) in an exercise intensity-dependent manner. In support of this, 1-minute of isometric handgrip exercise performed at 40% maximal voluntary contraction (MVC) causes splenic constriction and a small thrombocytosis (47). As such, it is hypothesized that an exercise modality that is capable of increasing SNA and eliciting a thrombocytosis response should, by extension of an increase in circulating platelets, increase levels of serum BDNF independent of the size of muscle mass or the metabolic cost of exercise.

#### **2.1.6 Platelets or tissue – what is the source of exercise-induced BDNF?**

Cho et al. (27) were the first to characterize the response of serum, plasma, and platelet BDNF following a treadmill  $VO_2$ max test in young males. Blood was sampled before and immediately after exercise cessation. Consistent with other findings, serum and plasma BDNF increased following the bout of exercise. Importantly, both platelet count and the amount of BDNF per platelet (calculated as *serum BDNF / platelet count*) also increased, suggesting that a) contribution from a cellular source and/or b) platelets released by the spleen contain higher concentrations of BDNF, thereby increasing the amount of BDNF per platelet. This is a critical finding, as it suggests that **the BDNF system as a whole is upregulated** by an acute bout of exercise.

First, an increase in BDNF per platelet likely represents addition from cellular sources rather than from splenic platelets with a higher BDNF content than circulating platelets. This is because circulating and splenic platelets are constantly undergoing exchange and do not differ in age or

cellular content (22). **This suggests that increased BDNF per platelet following exercise likely represents the contribution from cellular sources.**

Current convention holds that the brain is responsible for 70-85% of plasma BDNF released at rest and during exercise (120), which has apparently been translated by other authors to mean ‘circulating’ BDNF (75). This notion is based off of the work by Rasmussen et al. (120) who observed a significant increase in plasma BDNF in the jugular vein compared to the radial artery at rest and during 4 hours of moderate intensity rowing exercise in young men (15% below lactate threshold). The observed venous-arterial difference in plasma BDNF led to the conclusion that the majority of exercise-derived BDNF was of cerebral origin.

Given that platelets sequester and release BDNF in the presence or absence of physiological stressors such as shear stress, it is possible that higher plasma BDNF obtained from the jugular vein are, in part, due to offloading of BDNF from platelets with an increase in cerebral blood flow during exercise. Further, given the ubiquitous distribution of vascular endothelial cells in the body, the associated increase in shear stress and oxygen offloading at the cell could stimulate the release of BDNF from endothelial cells, thus further increasing BDNF levels exiting the brain. In contrast, as blood returns to the heart from the brain, there is a reduction in shear forces on blood constituents, thus providing the opportunity for BDNF to be uptaken by platelets and mix with blood returning from the periphery, ultimately diluting the concentration of free BDNF measured at the radial artery.

### **2.1.7 Summary**

While it is generally accepted that an increase in platelets (thrombocytosis) contributes to the exercise-induced BDNF response, it is often overlooked as an important, if not the primary means by which exercise increases BDNF. The main reason for this is likely that platelet-bound BDNF is considered to be inaccessible to target tissue, and as such, serum represents an inert pool that acts as a storage unit for BDNF. However, the dynamic release and uptake of BDNF from

platelets under physiological conditions present during exercise provides an argument for serum being an important source of bioavailable BDNF (71, 87). As such, we propose that an exercise intervention that targets mechanisms of thrombocytosis via increases in SNA could evoke meaningful increases in serum BDNF, independent of the amount of muscle mass used.

#### **2.1.8 Purpose and Hypothesis for Study #1**

The purpose of Study #1 is to investigate the effect of two different forearm handgrip exercise modalities (short duration, maximal effort and longer duration, submaximal effort) on serum BDNF and platelets. We hypothesized that maximal effort exercise (ME) would result in significant elevations of BDNF and platelets compared to submaximal effort exercise (SE), given the larger effect of ME on SNA thereby evoking a greater thrombocytosis compared to SE.

## **2.2 Part 2 – A novel exercise protocol for fMRI-based studies**

Multimodal cognitive interventions that combine exercise and cognitive training appear to have an additive effect on cognitive function and indices of brain plasticity compared to either modality performed alone (82). The mechanistic basis for this phenomenon may be rooted in both the acute, transient facilitation of cognitive function during a bout of exercise, and the divergent pathways by which exercise and cognitive training stimulate neuroplasticity (31, 158). This acute enhancement of cognitive function following exercise must be accompanied by a potentiation and/or reallocation of neural resources, which may be a product of regional brain activation during exercise (125, 138). However, how and which brain regions respond during exercise are currently unknown.

### **2.2.1 Acute exercise improves cognitive function**

Acute bouts of exercise facilitate transient improvements in cognitive function. Meta-analyses reveal small positive effects for a single bout of aerobic exercise improving cognitive function, specifically executive functions and reaction time up to an hour following the cessation of exercise (25, 38). There are many moderators of this phenomenon, including exercise duration, intensity, type of cognitive test administered, and fitness of participants (25). In terms of exercise duration, Chang et al. (24) found an inverted-U dose-response relationship between exercise duration and cognitive task performance, as 20 minutes of cycling at 65% heart rate reserve resulted in significantly greater accuracy and response time on the Stroop task compared to shorter (10 min) and longer (45 min) exercise bouts (24). The acute facilitation of cognitive function may also be intensity dependent, as Chmura, Nazar, and Kaciuba-Uscilko (26) reported that improvements in choice reaction time mirrored exercise intensity up to 75%  $\dot{V}O_{2peak}$ , above which reaction time worsened by 18% compared baseline scores. Conversely, Davranche, Brisswalter, and Radel (33) found that mild, moderate, and high intensity exercise similarly improved measures of reaction time (Simon Task), suggesting that cognitive control is not

compromised by high intensity exercise. A consistent and interesting finding is that low and moderate intensity exercises produce similar improvements in cognitive function, (25) suggesting that lighter and more enjoyable modalities of exercise can serve to benefit an individual's cognition.

#### 2.2.1.1 Mechanistic basis for cognitive improvement following exercise

The mechanism driving this transient improvement in cognition is postulated to be a function of potentiated neuronal activity due to heightened arousal rather than increased CBF experienced during exercise (34, 104). Initially proposed by Cooper in 1973 (30), the 'Arousal Theory' states that exercise engages the reticular activating system located in the brainstem, which is a major hub for the integration of bidirectional neural transmission during exercise and is the main locus for the regulation of wakefulness and arousal in the brain. The reticular activating system contains the primary nuclei for the serotonergic and noradrenergic systems, each of which modulates arousal through diffuse projections from the reticular formation to the entire brain (30, 34). Current convention holds that exercise increases arousal through the release of monoamines via the reticular activating system, which in turn drives the potentiation of cognitive function following a bout of exercise (6). ***Generally speaking, these arousal systems modulate regions of the brain responsible for attentional, sensory, and motor processes by reducing the signal-to-noise ratio and decreasing neural transmission time for some motor reflexes*** (34). Based on this evidence, it appears that through arousal exercise 'primes' the brain for subsequent cognitive challenges, which may be driven by activation of specific brain areas during exercise (9, 42, 76).

#### 2.2.2 Previous work examining brain responses to exercise

Previous studies have sought to identify and characterize the underlying neural substrate for this exercise-induced cognitive enhancement using a variety of methods, including electroencephalography (EEG), functional near-infrared spectroscopy (fNIRS), and fMRI. Studies involving EEG and fNIRS demonstrate that there is a diffuse increase in neural activity

during exercise (4, 32, 37, 123), which may underlie this apparent priming of cognitive function. Recently, Enders et al. (37) observed increased neural activity during cycling within the domain of the prefrontal cortex using EEG. The prefrontal cortex is responsible for the facilitation of higher ordered functions, and during exercise it is important for communicating higher ordered information related to motor planning and execution to motor areas (37, 122). In support of prefrontal activation during exercise, Fumoto et al. (50) observed a significant increase in ventral prefrontal cortex oxygenation during 15 minutes of light-to-moderate intensity cycling (93 Watts; W). The activation of prefrontal areas during exercise may extend to improved cognitive performance following exercise, as Yanagisawa et al. (167) observed an improvement in Stroop performance that was coupled with enhanced oxygenation of the dorsolateral prefrontal cortex following 10 minutes of moderate intensity exercise (50%  $\dot{V}O_{2peak}$ ). **However, despite having excellent temporal resolution, these imaging techniques lack the spatial resolution required to fully characterize neural activation evoked during exercise that may contribute to the subsequent priming of post-exercise cognitive function.**

fMRI and arterial spin labeling provide excellent spatial resolution of the functional (neuronal activity via BOLD signal) and CBF events in the brain, respectively. However, in-MRI exercise studies are significantly constrained due to the strict limitations on head movement in order to achieve viable scans. As such, research groups have purchased or engineered specialized non-ferrous MRI-compatible exercise equipment in an attempt to overcome these limitations, which in effect has opened up new avenues of exploration into regional responses of the brain during exercise. These devices include cycle ergometers (46, 61, 98), a supine treadmill (94), a stepping robot (69), and in other cases, rhythmic movement of the lower limbs without equipment (128, 153). However, only 3 investigations have specifically examined brain responses to aerobic exercise (46, 61, 98), whereas others were concerned with motor activation patterns associated with lower-limb movement (62, 69, 94, 153). Nonetheless, important lessons can be gleaned

from these works, specifically that head movement can be effectively controlled with specialized head pieces, specialized scanning approaches (69), and *post-hoc* digital motion correction (46).

### **2.2.3 Studies examining brain responses to in-MRI exercise**

In the context of exercise during functional scanning, Hiura et al. (61) examined CBF response to cycling exercise using oxygen-15-labeled water ( $H_2^{15}O$ ) during positron emission tomography (PET) imaging. The authors investigated the global (gCBF) and regional (rCBF) response of CBF during the initial and the late phase of exercise, with a particular interest in whether the CBF response corresponded with changes in physiological variables such as MAP, CO, PaCO<sub>2</sub>, etc. Healthy males performed steady-state supine cycling in the PET scanner for 15 minutes at 50-60% age-predicted maximal heart rate (HR) at a cycling cadence of 60 rpm, which coupled with a custom head piece, maintained a stable head position. The resultant exercise intensity achieved in this study was considerably light, as the average cycling work rate was  $71 \pm 11$  W and the average HR was 104 bpm at the end of exercise. Nonetheless, the cycling ergometer protocol created a sufficient exercise stimulus to evoke regional changes in gCBF and rCBF without any significant head movement. As such, the use of a custom head piece and coupled with low-intensity exercise was an effective tool for measuring the brain during exercise with functional imaging.

Mehta et al. (98) were the first group to examine brain responses associated with multi-joint exercise (cycling) using fMRI technology. The main purpose of this work was to determine the viability of brain scans during exercise using a custom-made cycle ergometer. The protocol consisted of 4 rounds of cycling for 30 s followed by 30 s of rest while laying supine in the scanner and participants were instructed to pedal in a manner that minimized head movement while maintaining a 'natural', comfortable cadence. As such, the exercise intensity was not controlled. The authors report that the exercise protocol resulted in  $< 1$  mm of head movement during cycling and that the signal-to-noise ratio during cycling was the same as a scan using a

silicone head (phantom scan). This work demonstrated that given access to specialized equipment it is feasible to perform multi-joint exercise involving large muscle mass in an MRI.

Fontes et al. (46) built on this study by investigating the neural circuitry involved in ratings of perceived exertion (RPE) during exercise using fMRI. Using a novel cycle ergometer, these authors had young males perform 2 minute blocks of cycling exercise at 60 rpm separated by 16 s of rest, which was repeated for 6 trials at a constant workload of  $\sim 1.25$  W/Kg. At the end of the 1st and 2nd minute of each exercise trial, participants reported their respective RPE via a microphone. The work rates achieved during this exercise ranged from 95 – 135 W (based on body weight) and the range of reported RPE was 11 – 19, with an increasing trend in RPE over the course of the 6 trials. Head movement limits for a viable scan are typically  $< 3$  mm, and following digital motion correction the authors report that head movement was maintained below the 3 mm limit. In addition to the technical aspects of this research, the fMRI data revealed an increased BOLD signal in the posterior cingulate gyrus and precuneus during exercise, two structures important for both cognitive function and conscious awareness. Since the study by Fontes et al. (46) there have been no published studies that have examined brain responses during exercise, possibly due to the significant limitations of performing exercise in an MRI.

The use of specialized MRI exercise equipment does indeed stand to advance our knowledge of neural responses to exercise; however, with a price tag of upwards of \$70,000 (i.e., MRI Ergometer Pedal device, Lode, Groningen, the Netherlands), in-MRI ergometers are inaccessible to a large number of researchers. As such, alternate approaches to in-MRI exercise are needed in order to provide greater access for the exploration of regional brain responses to whole-body exercise. Currently, such an approach does not exist.

#### **2.2.4 Proposed solution**

The biggest barrier associated with performing conventional multi-joint exercise modalities (cycling, walking, running, etc.) in an MRI is that there is an inherent large range of motion over

which the exercise takes place. In effect, these movement patterns translate into movement of the torso and the head, rendering functional scans unusable (91). To overcome this limitation, we envision an exercise modality consisting of rhythmic whole-body isometric (WBI) contractions, which could stimulate metabolic responses similar to previous in-MRI exercise modalities and minimize head movement, thereby eliminating the need for specialized equipment.

#### 2.2.4.1 Prescribing exercise intensity with ratings of perceived exertion

One anticipated limitation of such a protocol is the inability to quantify work rate given the lack of equipment. To overcome this barrier, we propose the use of RPE using the Borg scale (81), which was developed to perceptually guide exercise intensity. The Borg scale is a 15-point visual analog scale ranging from 6-20 that is coupled with affective descriptors of exertion (i.e., RPE 13 - somewhat hard). The scale descriptors range from RPE 6 – no exertion at all, to RPE 20 – maximal exertion. The use of RPE during submaximal exercise has been shown to have good retest reliability on measures of  $\dot{V}O_2$  and HR across multiple testing sessions for both treadmill and cycling exercise (40, 59). Further, submaximal graded exercise guided by RPE has good predictive validity for estimating  $\dot{V}O_{2max}$  (39, 41, 43). There is a strong relationship between RPE and exercise intensity across multiple exercise modalities, including treadmill exercise (39), continuous and intermittent cycling (36), and resistance exercise (12). RPE is also positively correlated with a number of physiological variables with increasing exercise intensity, including HR (11, 36, 113),  $\dot{V}O_2$  (36, 39), blood lactate (36, 113), and ventilation (36). Indeed, the physiological perturbations evoked by increases in exercise intensity are coupled with a graded conscious perception of exertion (103). Importantly, these perceptions of exertion are stable over time as evidenced by good retest reliability when RPE is used in either a passive (reporting RPE at a set work rate) or active manner (self-paced exercise based on RPE) (1, 39, 41). There are a number of factors that can influence the perception of exertion during exercise, including temperature, knowledge of exercise endpoint, music, and many others (103); however,

in the context of the proposed WBI exercise protocol, the relationship between RPE and the novelty / familiarity of an exercise modality is the most relevant consideration.

The reliability of exercise intensity at a given RPE during submaximal exercise appears to be sensitive to familiarity with a given exercise modality, as highly trained runners, generally fit non-cyclists, and sedentary individuals display higher RPE at a given HR during cycling compared to treadmill exercise, whereas trained cyclists show no differences in RPE between exercise modalities (54, 55). This may be due to the fact that sensations of exertion are localized to the legs during cycling and may produce a stronger RPE in individuals who are unfamiliar with cycling exercise. In support of this, cycling at a lower cadence yields higher RPE compared higher cadence at the same work rate with no differences in ventilation, oxygen uptake, and muscle activation (18, 142). Additionally, higher RPE is reported during eccentric compared to concentric exercise despite no differences in  $\dot{V}O_2$  or HR (58, 108). Taken together, these results suggest that localized muscle strain may contribute to perceived exertion during exercise independent of metabolic stress, and that this may affect modality-specific exercise performance in naïve participants. Importantly, the reliability of predicting  $\dot{V}O_{2peak}$  from RPE during cycling exercise is significantly improved following a full familiarization trial (40). Another strategy that improves reliability using RPE-based exercise is ‘anchoring’, wherein a subject is introduced to an RPE scale through memorizing and/or experiencing the sensations associated with a given RPE with exercise (41, 44, 80). As such, in order to ensure reliable performance with WBI exercise, it seems imperative to expose participants to the exertional sensations associated with rhythmically co-contracting major muscle groups at different RPEs through the use of anchoring and full familiarization trials.

### **2.2.5 Previous head movement validation trials**

Given the high cost associated with MRI scanning, previous studies that have used motion capture technology to assess head movement associated with various protocols prior to use in the

scanner (68, 91, 128, 136). Motion capture technology using multiple infrared cameras to track the movement of reflective markers that are placed on landmarks of bodily structures of interest, and through the use of processing software, translational and rotational movement characteristics can be derived. Using this technology, we propose that evaluation of head movement created by WBI could be effectively characterized using motion capture technology in a simulated MRI setup. A particularly important consideration for WBI is the movement caused by the initiation of exercise. Jaeger (68) reported that the initiation of stepping (referred to as initial offset) using a robotic stepper created the most drastic movement during the stepping trial. This may be due to the relatively large range of motion over which the stepping activity took place and that movement of the legs would translate to movement of the torso and head. In support of this, MacIntosh et al. (91) found that rhythmic dorsiflexion over a large range of motion produced significantly more head movement compared to rhythmic dorsiflexion performed over a small range of motion. Despite WBI being isometric in nature, we still anticipate movement being produced by the initiation of exercise, as strong contraction of the gluteal muscles can cause displacement. To overcome this, we propose that participants progressively work up to the prescribed RPE over the course of multiple contractions rather than beginning exercise at the prescribed exercise intensity.

#### **2.2.6 Purpose and hypothesis**

The proposed WBI exercise protocol stands to provide cost-free, universal access to researchers investigating neural responses to exercise using fMRI technology. However, before such an exercise modality can be used in an MRI, it must first be validated as an effective and reliable exercise stimulus and must control for head movement within the acceptable limits of an fMRI scan. Given that such an exercise modality does not yet exist, the purpose of this work is to create a WBI protocol capable of stimulating reliable metabolic responses similar to previous in-MRI exercise modalities and that can effectively minimize head movement. We hypothesize that

through use of RPE, WBI exercise will be capable of reliably evoking increases in metabolism that are similar in magnitude to previous in-MRI studies. Further, given the isometric nature of the exercise, we hypothesize that participants will effectively be able to limit head movement within the MRI limits while performing WBI exercise.

## **2.3 Part 3 – High-intensity interval exercise and reinforcement learning**

### **2.3.1 Hierarchical reinforcement learning**

Predicting the outcome of future events based on the outcome of previous events is one of the most prevalent types of learning in biological and artificial intelligence systems, alike (145). We affix a predicted value to an action, either positive or negative, and this predicted value governs decision-making for future events (78). Ultimately, humans seek to maximize behaviours that are intrinsically rewarding and minimize those that are negative (146). As such, the human brain has developed error-processing systems that provide feedback about the consequences of a particular action and through updating the prediction value of an action, reinforce those behaviours that are adaptive or rewarding.

Reinforcement learning theory purports that humans learn from the outcomes of previous actions such that behaviours resulting in reward are more likely to be repeated and those that result in punishment are more likely to be avoided, providing a framework to explain how humans behave and make decisions in a constantly changing, unpredictable environment (66, 146). In brief, reinforcement learning consists of an *actor-adaptive critic* framework wherein the actor is involved in the execution of an action based on a prediction of a resulting reward and the adaptive critic is responsible for monitoring the outcome of this action. The application of reinforcement learning to real world scenarios is elegantly summarized by Krigolson et al. (78). In brief, when in a state of Q and faced with options A and B, we seek to make a decision that will give us the highest probability of receiving a reward. In a situation where the value of options A and B are unknown, we cannot make a prediction regarding the likelihood of which

option is most rewarding. This is referred to as a prediction error, where the actual value of an option is different from the predicted value. As such, the first time we select option A or B, we will experience a prediction error in the direction of the actual value. If the selection produces a rewarding outcome, we would experience a positive prediction error whereas a negative outcome would produce a negative prediction error, and through this recursive mechanism, the predicted value of an action approaches the true value (63, 78, 102, 146).

#### 2.3.1.1 Reward predictions optimize behaviour and decision making

Rewards serve a vital function for wellbeing, reproduction, and the survival of a biological organism. Autoregulatory reward pathways have evolved in mammals to provide intrinsic reward that serves to reinforce behaviours that are vital for survival and discourage those that threaten an organism (131). In the case higher ordered mammals such as humans, these reward pathways drive both biological (survival, homeostatic) and social (cognitive) behaviours with the same basic premise: reinforce those behaviours that optimize positive outcomes and discourage those that are dangerous, aversive, or less valuable than other options (130). According to Schultz (130, 131), rewards serve three main functions: 1) they increase approach behaviour, in that an organism will divert attention from a given task towards the rewarding stimuli, 2) they increase the frequency and intensity of a behaviour to a rewarding stimulus, thereby consolidating learning, and 3) rewards produce subjective hedonic (pleasurable) feelings and induce positive emotional states. In contrast, aversive stimuli have the opposite effect, wherein negative reinforcement drives learning of avoidance behaviour to the aversive stimuli and produces negative emotional states such as fear, anger, and panic. Importantly, the value of a reward or what an organism perceives to be rewarding is dependent on their current state when the stimuli is encountered (134). For example, finding a bush full of berries would be highly rewarding to an individual with scarce food resources; however, that same stimulus would be viewed as neutral

by someone with secure food resources. As such, the current biological and cognitive needs of an individual determines the subsequent value affixed to the outcome of an action (131).

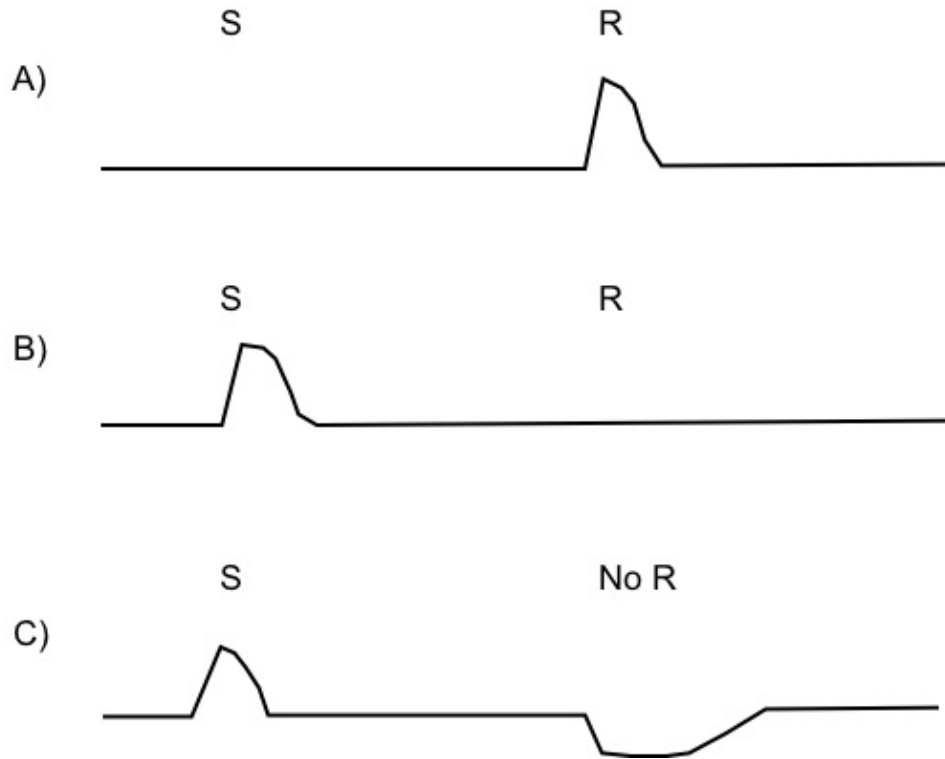
In the context of the reinforcement learning theory framework, predictions are made prior to selection and execution of an action using information from previous experiences (130). In turn, predictions afford the individual an opportunity to compare multiple options and select an action that has the highest probability of success given the current goal / state, or select an action that minimizes the probability of a potentially harmful or adverse outcome. Importantly, predictions afford an individual the ability to improve future outcomes and provide more time to select a behavioural reaction (130, 131). These predictions, more specifically prediction errors, are communicated through the mesencephalic dopamine system to the ACC and the cortex in order to reinforce adaptive behaviours and discourage those that are maladaptive.

### **2.3.2 Dopamine and the mesencephalic reward pathway**

In the human brain, the mesencephalic dopamine pathway originates in nuclei of the basal ganglia and has diffuse projections of dopaminergic neurons to the cortex (10, 51). Early evidence for a reward pathway in the brain and its subsequent influence on behaviour comes from the work of Olds and Milner (105). It was found that rats engaged in self-stimulating behaviour by pressing a lever that delivered an electrical current via electrodes implanted in specific areas of the rats' brain. The frequency of lever pressing behaviour drastically increased over time in these animals to the point where some rats were pressing the lever at a frequency of every two seconds. The authors concluded that electrical stimulation of the 'reinforcing structures' of the brain produces rewarding effects; however, the mechanisms underlying the reward sensation were not elucidated by this work (105). Since this study, dopamine has been identified as the candidate mechanism signalling reward (85).

The main evidence for the mesencephalic dopamine system underlying reward signals comes from studies examining the firing of dopaminergic neurons in monkeys following receipt of an

unexpected reward. In a series of studies by Schultz et al. (133, 134), the activity of dopaminergic neurons was continuously monitored during trials where monkeys were required to make a response (i.e., push a lever) following the presentation of a simple stimulus (i.e., green light). If the monkey correctly responded to the stimulus by pushing a lever, they were rewarded with appetitive fruit juice. Early in these trials when the pairing between stimulus and reward was unknown, and therefore unpredicted, there was a phasic burst of dopaminergic neuron firing upon receiving the reward (Figure 2-2 A). This phasic burst in firing represents a positive prediction error for the preceding action, in that the outcome of the selected action was better than expected. After numerous trials, the monkeys learned the pairing between stimulus and reward, and upon receipt of reward the phasic dopamine response was no longer present (Figure 2-2 B). Instead, dopamine firing occurred upon stimulus presentation, suggesting that this reinforcement occurs in anticipation (prediction), rather than receipt, of a reward. In later trials, after the stimulus had been learned and the monkeys had practice performing the task, researchers removed the reward following stimulus presentation. In these manipulations, there was a predictable phasic dopamine response upon presentation of the learned stimulus; however, at the time when reward was expected but not received, there was a complete suppression in dopamine firing indicating that the actual outcome was worse than predicted (Figure 2-2 C). When the conditioned stimulus was repeatedly followed by no reward, the associated dopamine response to the stimulus-reward mapping was extinguished, suggesting that dopamine response is highly adaptive and continually updates the contexts based on the outcome of new events (131, 133, 134).



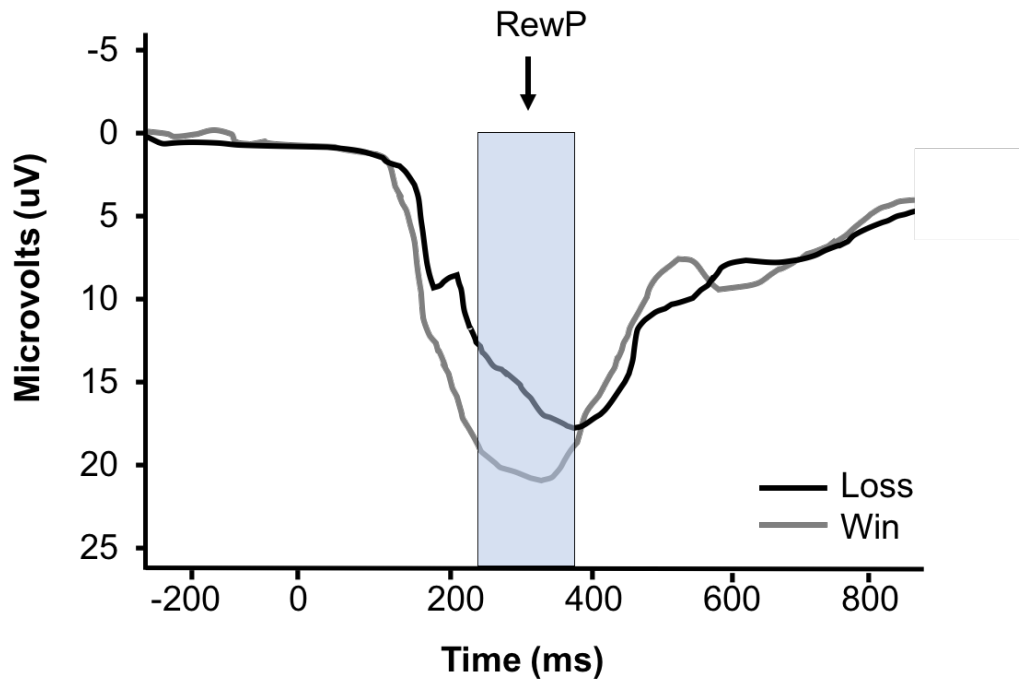
**Figure 2-2.** Dopamine response to reward. A) When reward is unexpected following a stimulus, a burst of phasic dopamine firing is observed upon receipt of reward, signalling a positive prediction error. B) Once the stimulus-reward association has been learned, the phasic dopamine response occurs upon stimulus presentation rather than after receiving the reward. C) After learning the task there is a predictable dopamine response upon stimulus presentation; however, when a reward is not given there is a suppression of tonic dopamine firing at the time when reward is expected, thereby signalling an negative prediction error. The straight black line represents tonic dopamine firing, which can be suppressed when a negative error has been detected (i.e., no reward). Adapted from Schultz (131). S = stimulus, R = reward, No R = no reward

Mechanistically, the phasic adjustments in dopamine signalling that accompany a prediction error modulates neural pathways involved in the previous behaviour and can induce long-term potentiation or long-term depression of these networks (19, 121). Dopaminergic neurons form a synaptic triad with local synapses within the cortex and can modulate the strength of neurotransmitter release by the pre-synaptic neuron and the sensitivity of the post-synaptic neuron (19, 121, 132). In the case of a positive prediction error (i.e. unexpected reward), an increase in

phasic dopamine firing will have an excitatory effect on local synapses of the active network and reinforce that pattern of neural firing. The opposite occurs in situations of a negative prediction error, wherein temporary suppression of dopamine signalling will reduce the excitability of a given network and weaken the associated pattern of neural firing (131). Given that dopaminergic neurons diffusively project to the cortex and striatum in a homogenous manner (10, 51), the accompanying error signal, be it positive or negative, will have the greatest effect on neurons involved in the behaviour, whereas those not involved will not be affected (121, 132). As such, despite the dopamine network being broad and demonstrating non-specific release patterns, it acts to selectively reinforce those networks that are active at the time of release (132).

### **2.3.3 RewP: a neuroelectric substrate for reinforcement learning**

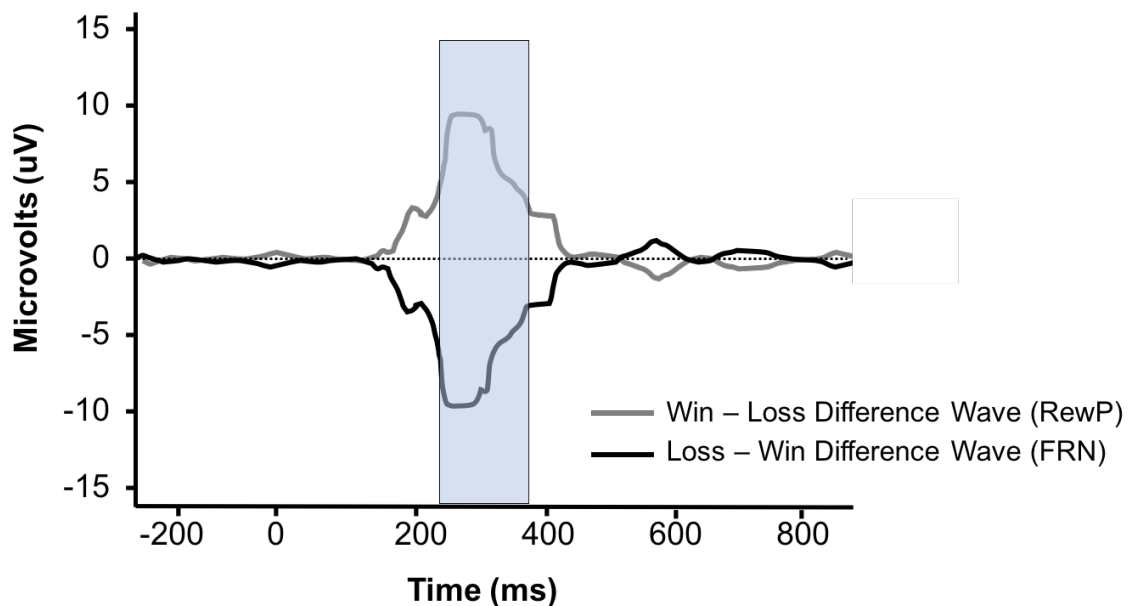
EEG studies have identified an event-related potential (ERP) component called the reward positivity (RewP) that is likely the neuroelectric substrate of dopamine firing underlying mechanisms of reinforcement learning (118). RewP is a positive going wave that occurs approximately 250 ms following both response- and feedback-locked commission of a 'win' (positive prediction error) on a task (Figure 2-3) (63, 65, 118). RewP is typically derived by calculating a difference wave from 'win' minus 'loss' trials (118); however, RewP is a relatively new ERP component.



**Figure 2-3.** Representative conditional waveform of RewP response to 'WIN' vs. 'LOSS' stimuli. Shaded area represents the RewP time frame. Adapted from Proudfit (118).

Historically speaking, RewP represents a paradigm shift in the reinforcement learning community, as this phenomenon was more commonly known as error-related negativity (ERN), and later, feedback-related negativity (FRN) (63). Originally, these components were thought to represent neural indexing of error detection and error processing within the midbrain dopamine system (63); however, recent research has shown that reward feedback drives this response, whereas neuroelectric events following negative and neutral feedback are the baseline responses of this system (65, 118, 165). An important point for the reader is to differentiate RewP, FRN, and ERN. Given the paradigm shift in the reinforcement learning ERP field, different names are used to describe the same phenomena depending on the year of publication (118). FRN refers to the ERP component elicited by task performance **feedback**, which can be either positive or negative feedback. The manner in which difference waves between positive (win) and negative (loss) feedback responses are analyzed affects the resultant naming of the ERP component.

When the wave is processed as loss – win, the ERP component is referred to as FRN and represents the response of the error-processing system to ‘loss’ related feedback. Whereas processing difference waves as win – loss produces the RewP component (Figure 2-4) (118). The ERN component represents error-processing related to **response-locked** error commission on a task where the rules are known (a learned task) (63, 118). For consistency, RewP will be used to describe the pertinent research, except for cases where describing FRN and ERN are more appropriate (i.e., error-processing at different stages of learning).



**Figure 2-4.** Representative difference waves for RewP and FRN. Approaches to analysis (WIN – LOSS vs. LOSS – WIN) affects the subsequent naming of the ERP component. Shaded area represents the RewP time frame. Adapted from Proudfit (118).

Despite the recalibration of the ERP nomenclature, RewP, FRN, and ERN are manifestations of a “generic neural system for error detection” (101), which detects discrepancies between predicted and actual outcomes (win or loss) of an action (63). The manner in which performance is evaluated (external feedback vs. internal recognition), the nature of the error (win vs. loss), and approaches to analysis will affect the resultant ERP component. It is important to reiterate that an

error is not a negative outcome *per se*, rather, this error detection system is concerned with monitoring differences in the predicted outcome vs. the actual outcome of an action. This is especially important for decision making, as the anticipated value of an action is constantly being updated to reflect the most recently available information, thereby increasing the number of sampling points such that the predicted value of an actions approaches the true value.

The characterization of a “generic neural system for error detection” was put forward by Holroyd and Coles (63) based on the following observations. The magnitude of an error is reflected by RewP amplitude, suggesting that the error-processing system has the sensitivity to discriminate the severity of an error (52). For example, RewP is consistently larger when participants have an opportunity to win actual money on a gambling task compared to receiving ‘win’ feedback without any tangible reward (97, 165). As well, the size of the reward drives the RewP response, as larger rewards (i.e. winning \$.25 vs. \$.05) are accompanied by a larger RewP magnitude compared to lesser outcomes (52). The generic descriptor of the error-processing system is suitable given that it is highly flexible and can detect errors in a number of different contexts, which is supported by the homogeneity of dopamine projections and firing characteristics to the cortex and striatum (10, 51). Specifically, this system has the flexibility to detect errors in a wide range of contexts including task-output modality (i.e., hand vs. foot manipulations) (64) and error-feedback modality (i.e., visual vs. auditory vs. tactile feedback) (101). Finally, the ‘high-level’ component described by Holroyd and Coles refers to the fact that this error-processing system is intimately associated with higher-ordered executive control processes, specifically within the ACC, the prefrontal cortex, and the basal ganglia (13, 66, 134). In the early stages of learning, when a prediction about a possible outcome cannot be made, the error detection system relies on performance feedback. As such, in the earliest stages of learning RewP is observed upon receipt of performance feedback as opposed to when the selection is made; however, as an individual learns the value mapping of an action, RewP is observed upon response selection rather than when feedback is given (78).

### 2.3.3.1 Does performance feedback actually affect learning?

Learning a new skill or set of rules requires knowledge of performance through feedback. That is, the earliest stages of learning require information about performance from external sources in order to optimize future performances. Once a skill has been learned and indicators of successful performance are known, internal representations of performance are relied upon to monitor and adjust subsequent action. From ERP research, two main conclusions have been drawn regarding the relationship between RewP and learning. The first point is that as learning increases, the magnitude of feedback-locked RewP / FRN decreases (7, 57, 79, 88), which is to be expected given the understanding of dopamine responses to conditioned stimuli (134). Once the stimulus-response mapping has been learned, the error detection system shifts from relying on feedback (external information) as a performance indicator to relying on response-locked error detection (RewP or ERN).

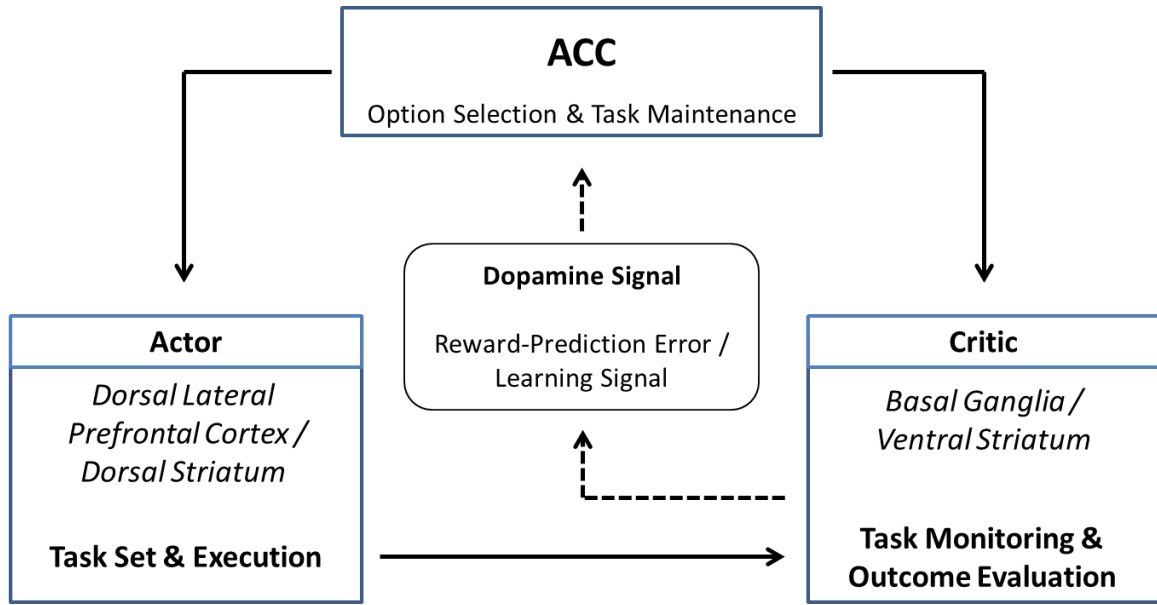
The second point is that RewP predicts good learning (79, 88). Krigolson et al. (79) examined FRN and ERN during a learning-based visual discrimination task, wherein subjects were required to learn differences between computer-generated shapes ('blobs') over a repeated number of trials and were provided with negative (error) performance feedback. Post-hoc, subjects were categorized as either high or low learners based on their learning rate on the task. High learners displayed larger FRN compared to low learners during the early stages of learning; however, there was a shift from FRN to ERN as the high group learned the task. Conversely, by the end of the task the low learners had yet to make the switch from FRN to ERN, suggesting that they were still actively engaged in trying to learn the task rules through performance feedback. These results demonstrate that an individual's ability to make rapid and accurate decisions on a learned tasks depends on early reinforcement learning through performance feedback (79).

Fromer et al. (48) recently demonstrated that win feedback (RewP) is important for learning new tasks. Specifically, participants learned a complex motor task (throwing) where the goal was to hit a target, and feedback about performance was provided after each trial. Results from this

work show that RewP was larger following more accurate performances (larger reward) and that the frequency of accurate performance ('hits') was higher following a hit trial compared to a miss trial. This suggests that the large reinforcement learning signal that accompanies positive performance feedback helps to guide subsequent behaviours, which is in line with predictions about reinforcement learning and RewP (63, 78). As well, in agreement with Krigolson et al. (79) and others (57), there was a reduction in feedback RewP with an accumulation of successful trials indicating a shift from external to internal performance metrics; however, in situations where performance declines (i.e., increased frequency of misses), the magnitude of feedback RewP subsequently increases. Collectively, these and other findings demonstrates that successful learning is dependent on reward feedback at the earliest stages of learning and that this error-processing system is highly adaptive, constantly monitoring and updating actual performance outcome vs. expected for the optimization of performance (48).

#### **2.3.4 RewP and the ACC**

RewP indexes mechanisms of reinforcement learning, such that when an action leads to a better than expected outcome it results in a phasic burst in dopaminergic neuron firing from the basal ganglia to the ACC (13, 63, 134). It is proposed that the ACC uses this learning signal to reinforce adaptive behaviour (13, 66). That is, the dopamine firing transmits a prediction error signal and teaches the ACC about behavioural outcomes. While the exact role of the ACC in task performance has been debated, evidence suggests that it is more concerned with the execution of an overall task rather than the individual component parts that make up said task (66, 157). Evidence from lesion studies demonstrates that ACC deficits impairs the reinforcement learning and the execution of actions despite fully intact motor system (66). As such, modulation of the ACC can have a direct impact on learning and behavioural performance.



**Figure 2-5.** Theoretical framework of reinforcement learning in the context of brain structure and their respective roles in monitoring and adapting behaviour. RewP is proposed to be generated by the dopamine signal projecting to the ACC via the ventral striatum in response to unexpected reward feedback. ACC = anterior cingulate cortex. Adapted from Holroyd and Yeung (66).

### 2.3.5 Exercise, ACC, and reward pathways

A number of studies have shown that the ACC is a target structure benefiting from participation in regular exercise, such that older adults with higher aerobic fitness demonstrated a reduction in ACC activation that is associated with greater executive control during a cognitive task (29). These findings are further supported by ERP studies examining the relationship between the ERN and aerobic fitness (115, 149–151). In one such study, it was demonstrated that higher fit young adults had significantly greater ERN amplitude compared to their less fit counterparts on tasks that emphasize response accuracy over speed (151). Further, these neuroelectric differences corresponded with greater post-error correction behaviour for those with higher fitness. Interestingly, when task instructions emphasized speed over accuracy, higher fit individuals displayed a corresponding reduction in the ERN magnitude following error commission, given that speed, not accuracy, was the most salient feature of the task (149, 151). Additionally, these findings were mirrored in another study where higher fit older and younger

adults had significantly better measures of action-monitoring compared to their less fit counterparts, as indicated by a decrease in ERN amplitude during a task-switching paradigm where speed was emphasized over accuracy (150). Together, these findings suggest that individuals with higher fitness (likely garnered through regular participation in physical activity) have greater top-down executive flexibility and control.

Acute exercise also appears to modulate ACC activity, as greater deactivation of the ACC during a working memory task was observed following a bout of submaximal intensity cycling exercise (84). Importantly, ACC deactivation facilitates improved performance on attentional tasks requiring executive control, such as the Stroop task (21), implicating the ACC as a target structure involved in exercise-induced enhanced cognitive function. In addition to the ACC, the midbrain dopamine system is upregulated by a bout of exercise (73, 144) and contributes to enhanced post-exercise cognitive function via mechanisms of arousal (34, 89, 96). Through the upregulation of the dopamine system, acute exercise increases cortical excitability and enhances neuroplasticity in the motor cortex in humans (138).

Taken together, this leads us to propose that performing a preceding bout of exercise may also augment mechanisms of feedback-based reinforcement learning leading to greater early-stage learning performance (48, 79, 88). Given that acute exercise modulates ACC activation and upregulates the midbrain dopamine system, one could hypothesize that RewP response characteristics may also be impacted; however, only two studies have examined ERPs related to reinforcement learning (ERN) following a bout of aerobic exercise, and only one in young adults. Pontifex et al. (116) found that a 20-minute bout of moderate intensity treadmill exercise did not affect ERN during an inhibitory control task in school-aged children. Interestingly, these authors found that at baseline, children with ADHD had reduced ERN compared to their classmates without ADHD; following exercise, however, ERN amplitude in children with ADHD was not different compared to children without ADHD. These findings suggest that acute exercise may reallocate neural resources devoted to inhibition and cognitive control in children with ADHD

(116). Similar to the findings from children without ADHD, Themanson and Hillman (149) found that 30 minutes of treadmill exercise did not impact ERN amplitude in young adults on a Flanker task where speed was emphasized over accuracy. However, it is important to note that researchers waited on average 40 minutes following cessation of exercise before taking post-exercise EEG measures. Aside from these studies, no other ERP studies have further investigated the effect of acute exercise on the ERN, FRN, or RewP components.

Another important gap in the current research is the context in which this error-processing system was evaluated. The above studies examined paradigms of action-monitoring on tasks where subjects already knew the rules and indicators of good performance (ERN), and in the acute exercise studies, speed was emphasized over accuracy. To date, no study has assessed the effect of an acute bout of exercise on the earliest stages of learning through reward feedback as indicated by RewP. Given that acute exercise impacts ACC function (84), which may affect myriad of executive control processes (23, 60, 167), it stands to reason that a single bout of exercise may modulate RewP. This leads us to propose that performing a preceding bout of exercise may also augment mechanisms of reinforcement learning leading to greater early-stage learning performance (48, 79, 88).

In the context of using exercise to improve cognition it is important to consider the accessibility and usability of a prescribed exercise protocol. One of the biggest cited barriers to performing regular physical activity is lack of time. As such, an exercise modality that can overcome this barrier and improve cognition could prove to be a useful tool in situations where an individual could benefit from enhanced cognitive function but has a limited amount of time to exercise. HIIE elicits metabolic and fitness adaptations to the same magnitude as traditional continuous aerobic exercise but in a fraction of the time (17), and emerging evidence suggests that a bout of HIIE improves cognitive function (3, 16, 90, 155, 166). As such, we assert that a single bout of HIIE may have a modulatory effect of the RewP, which would have implications

for learning and provide an time-effective tool for doing so; however, the effect of HIIE on RewP is currently unknown.

### **2.3.6 Study #3 – purpose and hypothesis**

The purpose of Study #3 is to examine the effect of a single bout of HIIE on RewP responses to reward feedback in university students. We hypothesize that RewP amplitude would be enhanced following a bout of HIIE compared to rest.

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## **Chapter 3**

### **Short-Duration Maximal and Long-Duration Submaximal Effort**

### **Forearm Exercise Achieve Elevations in Serum Brain-Derived**

### **Neurotrophic Factor**

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Neurotrophic Factor**

### 3.1 Abstract

Brain-derived neurotrophic factor (BDNF) is a major orchestrator of exercise-induced brain plasticity and circulating (peripheral) BDNF may have central effects. 99% of circulating BDNF is platelet-bound, and at rest ~30% of circulating platelets are stored in the spleen. Interestingly, forearm handgrip exercise significantly elevates sympathetic outflow and has been shown to induce splenic constriction, suggesting that small muscle mass exercise could stand as a viable strategy for increasing circulating BDNF; however, the BDNF response to handgrip exercise is currently unknown. **Purpose:** This study examined BDNF and platelet responses to short-duration maximal (ME) and prolonged submaximal (SE) effort handgrip exercise. **Methods:** Healthy males (n=18) performed 10 minutes of ME and 30 minutes of SE. Blood was sampled for the determination of serum BDNF and platelet count at rest and during the last minute of exercise. **Results:** Compared to rest, serum BDNF significantly increased during ME (21.2%) and SE (11.2%), which displayed a non-significant trend towards an intensity-dependent response. Platelets increased in an intensity-dependent fashion compared to rest with an 8.0% increase during ME and 3.1% during SE, and these responses were significantly correlated with diastolic blood pressure responses to handgrip exercise. Further, the amount of BDNF per platelet significantly increased compared to rest during ME (13.4%) and SE (8.7%). **Conclusions:** Handgrip exercise evokes physiologically meaningful increases in serum BDNF and platelets, implicating splenic constriction as a key mechanism and confirming efficacy of this exercise model for elevating circulating BDNF.

### **3.2 Introduction**

Brain-derived neurotrophic factor (BDNF) is chiefly recognized for its role in orchestrating activity-dependent brain plasticity (9). At the central nervous system level, BDNF is critical for learning and memory processes, and is a key regulator of neuronal growth and integrity (32). Interestingly, there is an association between central nervous system (CNS) and circulating levels of BDNF, suggesting that peripheral levels may be important for CNS function (2). In support of this are findings from studies on depression and aging. In a murine model of depression it was demonstrated that chronic peripheral infusion of BDNF resulted in antidepressant behavioral and neuronal adaptations (43). Human studies reveal that individuals with depression have significantly lower circulating BDNF compared to healthy controls. Treatment with antidepressant drugs significantly elevates circulating BDNF and these changes are significantly correlated with improved depression scores (5). Finally, the age-associated decline in circulating BDNF (27, 55) is thought to mediate hippocampal shrinkage and worsening of spatial memory (11). Given this evidence, exploration into means by which circulating BDNF can be augmented has important implications for brain health in both clinical and healthy populations, alike.

Physical exercise is one particularly effective strategy for increasing circulating levels of BDNF (26, 49). It has repeatedly been demonstrated that an acute bout of aerobic exercise transiently increases both serum and plasma BDNF in an intensity-dependent manner (26, 49). While the means by which exercise evokes this transient BDNF response is poorly understood, the current dogma accepts the brain as the primary source of plasma BDNF at rest and during exercise (36, 44); however, the plasma BDNF accounts for only 1% of total circulating BDNF (35). Given that previous studies have observed increases in serum BDNF greater than 30% following aerobic exercise (7, 12, 15), and that serum has approximately 200 times greater BDNF content than plasma (39), serum responses to exercise are not explained by a cerebral source. As such, consideration must be given to additional sources of BDNF during exercise, especially

when considered within the context of developing interventions to significantly elevate circulating BDNF.

The spleen is likely a significant contributor to elevated BDNF with exercise via the release of platelets (7, 29) and the magnitude of this release appears to be exercise intensity-dependent (exercise-induced thrombocytosis) (6, 19). Given that nearly 99% of blood-borne BDNF is platelet-bound (35), the addition of splenic platelets to the blood via thrombocytosis could account for a majority of the increased BDNF observed in response to exercise. Importantly, this increase in platelet-bound BDNF is not an inert pool; rather, this BDNF should be considered bioavailable as platelets release BDNF under conditions of increased shear stress (14), which is present in active muscle (25) and the cerebral vasculature during exercise (20). Further, the increase in circulating catecholamines that accompanies exercise increases platelet activation, thereby potentiating the release of BDNF with exercise (1, 29).

In support of the dynamic contribution of platelets to bioavailable BDNF, platelet release of BDNF is significantly blunted in individuals with depression and this is evidenced by significantly lower levels of serum BDNF in this population despite having similar whole-blood levels to healthy controls (22). As such, platelets should be considered an important contributor of bioavailable BDNF and any exercise modality that can increase circulating platelets may have the potential to improve brain health. Traditional exercise modalities (i.e. aerobic or resistance exercise) may be a potent stimulus for augmenting platelets and BDNF (7, 29); however, the large muscle mass recruited with such exercise may not be requisite to evoke a meaningful platelet and serum BDNF response.

A closer look at the mechanism of thrombocytosis supports the potential for forearm handgrip exercise as a modality to achieve increases in serum BDNF. Thrombocytosis is the result of splenic constriction, caused by sympathetic stimulation of  $\alpha$ -adrenergic receptors within the walls of the spleen (3, 6). Norepinephrine infusion causes splenic constriction and increases circulating platelets in a dose-dependent manner (46, 51). Similarly, low-dose infusion of epinephrine

significantly increases circulating platelets due to splenic constriction in humans (3). Stewart et al. (47) found that following exercise spleen volume was inversely correlated with levels of circulating catecholamines. Therefore, an exercise modality that evokes sufficient sympathetic outflow could stimulate exercise-induced thrombocytosis and subsequently increase circulating BDNF.

This may not require large muscle mass exercise modalities, as small muscle mass exercise increases circulating catecholamines (48), as well as muscle sympathetic nerve activity (21) in an intensity-dependent manner. Frances et al. (13) have provided preliminary evidence for splenic constriction and an associated small thrombocytosis response following 1 minute of 40% maximal voluntary contraction (MVC) isometric forearm contraction. As such, it is plausible that performing small muscle mass exercise, such as forearm handgrip exercise, could stimulate thrombocytosis and consequently achieve significant elevations in serum BDNF. However, no work to date has investigated this possibility.

Therefore, the purpose of this study was to determine the effect of two different forearm exercise modalities (maximal effort, short duration and submaximal effort, longer duration) on circulating BDNF and platelet levels. We hypothesized that maximal effort exercise (ME) would significantly elevate serum BDNF and platelet levels compared to rest and compared to submaximal effort exercise (SE), as ME would result in greater sympathetic activation, and therefore thrombocytosis, compared to SE.

### **3.3 Methods**

#### **3.3.1 Subjects**

18 healthy males ( $21.4 \pm 2.1$  years; BMI  $25.0 \pm 1.0$  kg/m<sup>2</sup>) were recruited for this study with no history of cardiovascular disease, hypertension, asthma, depression, smoking, or forearm-specific training. Participants were not taking prescription medication at the time of the study. This study was carried out in accordance with the recommendations of the Health Sciences

Research Ethics Board at Queen's University, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Health Sciences Research Ethics Board at Queen's University.

### **3.3.2 Experimental protocols**

This study examined the response of serum BDNF to short-duration maximal and long-duration submaximal forearm handgrip exercise. Experimental protocols were performed on separate days and a one week washout period was enforced to prevent potential crossover effects from the previous session. Participants were instructed to refrain from exercise for 24 hours, caffeine and alcohol 16 hours, and food 8 hours prior to experimental visits. All experimental sessions took place in the morning, between the hours of 8:00 and 10:00am, to avoid variability in serum BDNF levels due to diurnal fluctuations (24).

### **3.3.3 Exercise protocols**

Forearm handgrip exercise consisted of rhythmic isometric contractions of the forearm at a 2:2 sec contraction:relaxation duty cycle. Handgrip exercise was performed using an electric handgrip dynamometer (ADInstruments, Colorado Springs, USA) with visual feedback of real-time force output displayed on a computer screen for participants.

The maximal effort (ME) protocol was 10 minutes in duration, during which participants performed MVCs for each contraction. Participants performed 3 MVC efforts separated by 1 minute of rest prior to commencement of the ME protocol. Following completion of the MVC trial, a researcher set a visual target that participants were asked to attempt to reach with every contraction. The ME exercise protocol is characterized by an exponential decline in force production that eventually plateaus at what is termed critical impulse for such isometric exercise (25). Critical impulse is the force analog of critical power (i.e. is equivalent to critical power) which represents the maximal work rate supported by aerobic metabolism at which a metabolic steady state can be achieved (25). For our purposes, force output during the last minute of

exercise was analyzed and used for the determination of the submaximal exercise (SE) protocol exercise intensity. Participants received continual verbal encouragement from researchers to ensure maximal effort on every contraction.

For the SE protocol, participants performed 30 minutes of handgrip exercise at an intensity set at 15% below their estimated critical impulse. A visual target was displayed on a computer screen and participants were instructed to hit the target on every contraction. In both exercise protocols, venous blood draws were taken following 30 minutes of quiet rest (rest sample) and during the last 30 seconds of exercise (exercise sample). Exercise was well tolerated and all participants were able to complete the exercise sessions.

#### **3.3.4 Hemodynamic monitoring as a surrogate measure of autonomic activation**

Hemodynamic variables, including arterial blood pressure, total peripheral resistance (TPR), cardiac output (CO), and heart rate (HR) responses to the experimental protocols were continually monitored on a beat-by-beat basis using finger photoplethysmography using the ModelFlow method (Finometer MIDI, Finapres Medical Systems, Amsterdam, Netherlands) on the non-exercising hand. These measures were used as a surrogate for sympathetic activity, as muscle sympathetic nerve activity parallels diastolic blood pressure (DBP) during handgrip exercise (41, 42).

#### **3.3.5 Catheterization and blood sampling/treatment**

A vein of the exercising forearm was catheterized using a 20-gauge Teflon catheter (BD Insyte Autoguard, Oakville, Canada) upon entry into the lab. In all experimental protocols participants rested in a supine position for at least 30 minutes to avoid variability in plasma volume associated with postural changes (23). Blood for platelet determination was collected in EDTA K2 3 mL vacutainers and was analyzed by complete blood count by the CORE Lab at Kingston General Hospital (Kingston, Canada). Blood for serum BDNF determination was collected in serum collector tubes and left to clot at room temperature for 30 minutes and then at

4°C for an additional 30 minutes. Following the clotting period, samples were centrifuged at 2000 x g for 15 minutes at 4°C, after which the supernatant was aliquoted and stored at -80°C.

### **3.3.6 Biochemical analysis**

Serum BDNF was analyzed using the BDNF Emax Immunoassay System (Promega, Madison, WI, USA). The kit detection range was 7.8 pg/mL – 500 pg/mL and the intra-assay coefficient of variation was 2.9%. The coating buffer was set to a pH of 9.7 and pipetted in each well of an Immulon 4 HBX Extra High Binding 96-well plate (Thermo Scientific, Rochester, NY, USA) and incubated overnight at 4°C. Serum samples were diluted by a factor of 1/128 with the prepared 1x block and sample buffer and run in duplicate. All incubation times and washing steps were followed in accordance with the manufacturer's instructions. Following the addition of TMB substrate (color development step), samples were incubated at room temperature for 10 minutes, upon which 1 mmol HCl was added to stop color development. Following this, the plate was immediately read at 450 nm using an automated plate reader (Synergy 2, BioTek Instruments, Winooski, VT, USA). A standard curve was generated using a linear regression curve-fit approach with the plate reader software and all standard curves  $r^2$  values were between 0.99 – 1.00. All samples were subsequently multiplied by a dilution factor of 128. BDNF samples were analyzed within 4 months of collection.

### **3.3.7 Handgrip data acquisition and analysis**

Handgrip exercise was performed using an electronic handgrip dynamometer (ADInstruments, Colorado Springs, USA) and force output was recorded via data acquisition software on a lab computer (Powerlab, ADInstruments, Colorado Springs, USA). MVC peak force was determined as the highest point of a handgrip contraction impulse performed during MVC testing at the beginning of the ME session. All contractions performed during the ME and SE conditions were quantified using a time-tension integral of the force tracing and an average

impulse force output was obtained by dividing total impulse force by the number of contractions performed, and further dividing by two given each contraction was two seconds in duration.

### **3.3.8 Statistical analysis and sample size determination**

A two-way repeated measured analysis of variance was used to determine differences in BDNF, platelet, and hemodynamic variables at rest and during exercise in ME and SE testing sessions. Given that changes in plasma volume that occur with exercise can directly impact the concentration of measured blood constituents, we calculated a 5% and 3% decrease in plasma for ME and SE, respectively based on the method of Dill and Costill (10). Blood samples from one participant were misplaced by the hematological lab that ran our platelet analysis, as such we report platelets values for 17 participants in response to ME and SE. A Bonferroni correction for multiple comparisons was performed where appropriate. A two-tailed paired *t*-test was used to compare the total amount of work performed between ME and SE. Further, a two-tailed paired *t*-test was also used to compare average handgrip impulse force between conditions. Pearson product-moment correlation coefficients were calculated to determine relationships between DBP (surrogate for sympathetic activation), platelets, and BDNF. All analysis was performed using SigmaPlot Statistical Analysis and Scientific Graphing Software version 12.0. All data are expressed as mean  $\pm$  standard deviation (SD) with statistical significance set at  $p \leq 0.05$ . Sample size was calculated using a two-tailed paired *t*-test with  $\alpha$  set at 0.05 and desired power of 0.95. Using the baseline serum BDNF values reported by Ferris et al. (12) ( $18,168 \pm 1193$  pg/mL) with a desired minimum increase of 10%, we determined the required sample size for this study was  $n = 18$ .

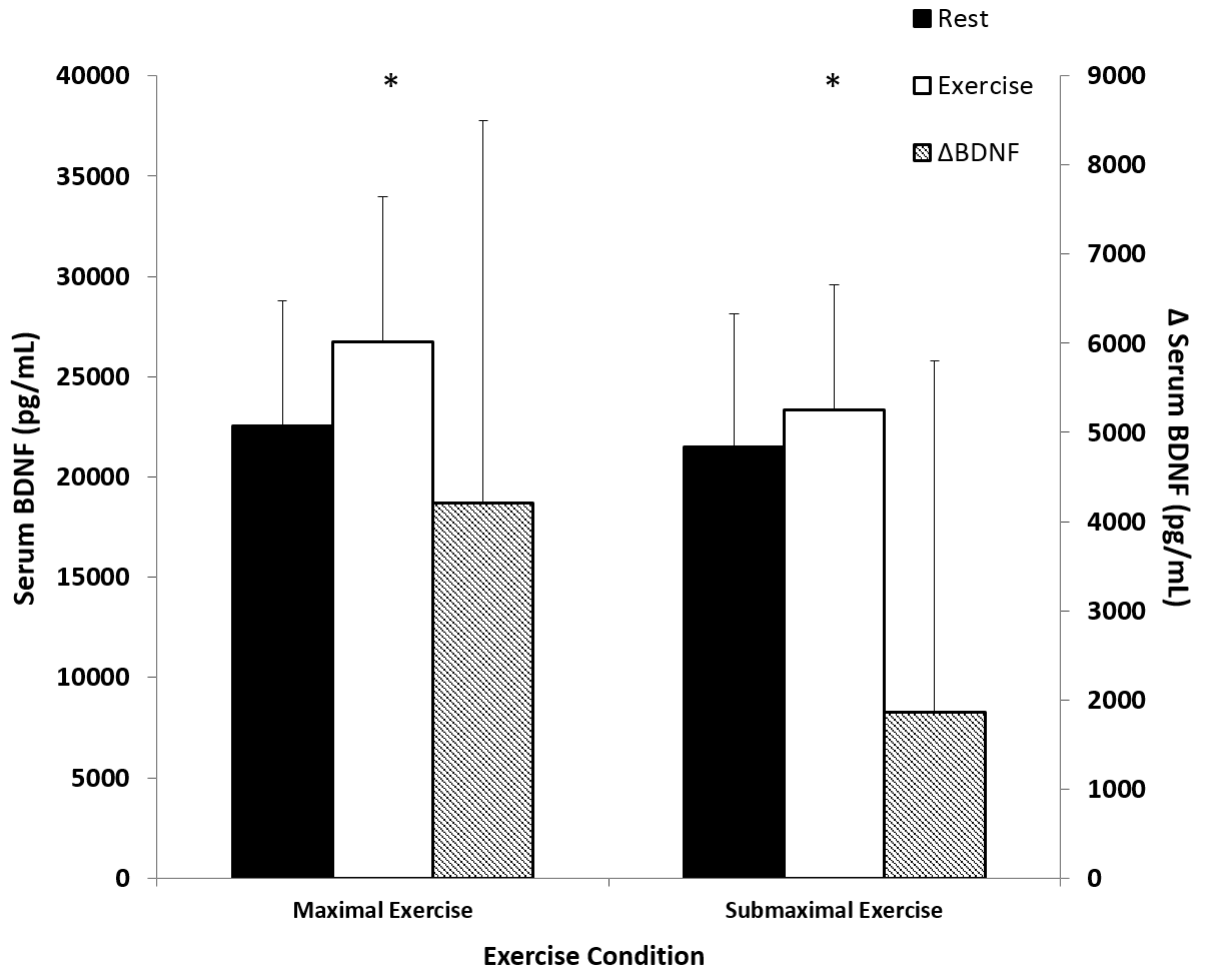
## **3.4 Results**

### **3.4.1 BDNF responses to forearm handgrip exercise**

There was a main effect of time as serum BDNF significantly increased in response to exercise compared to rest in both exercise conditions (Figure 3-1). Specifically, BDNF increased

by  $21.2 \pm 24.5\%$  in response to ME; and  $11.2 \pm 21.2\%$  in response to SE;  $F_{(1,16)} = 14.59$ ,  $p < 0.05$ .

There was a statistically non-significant trend towards a time x condition interaction with ME being greater than SE post-exercise BDNF;  $F_{(1,16)} = 4.06$ ,  $p = 0.06$ .

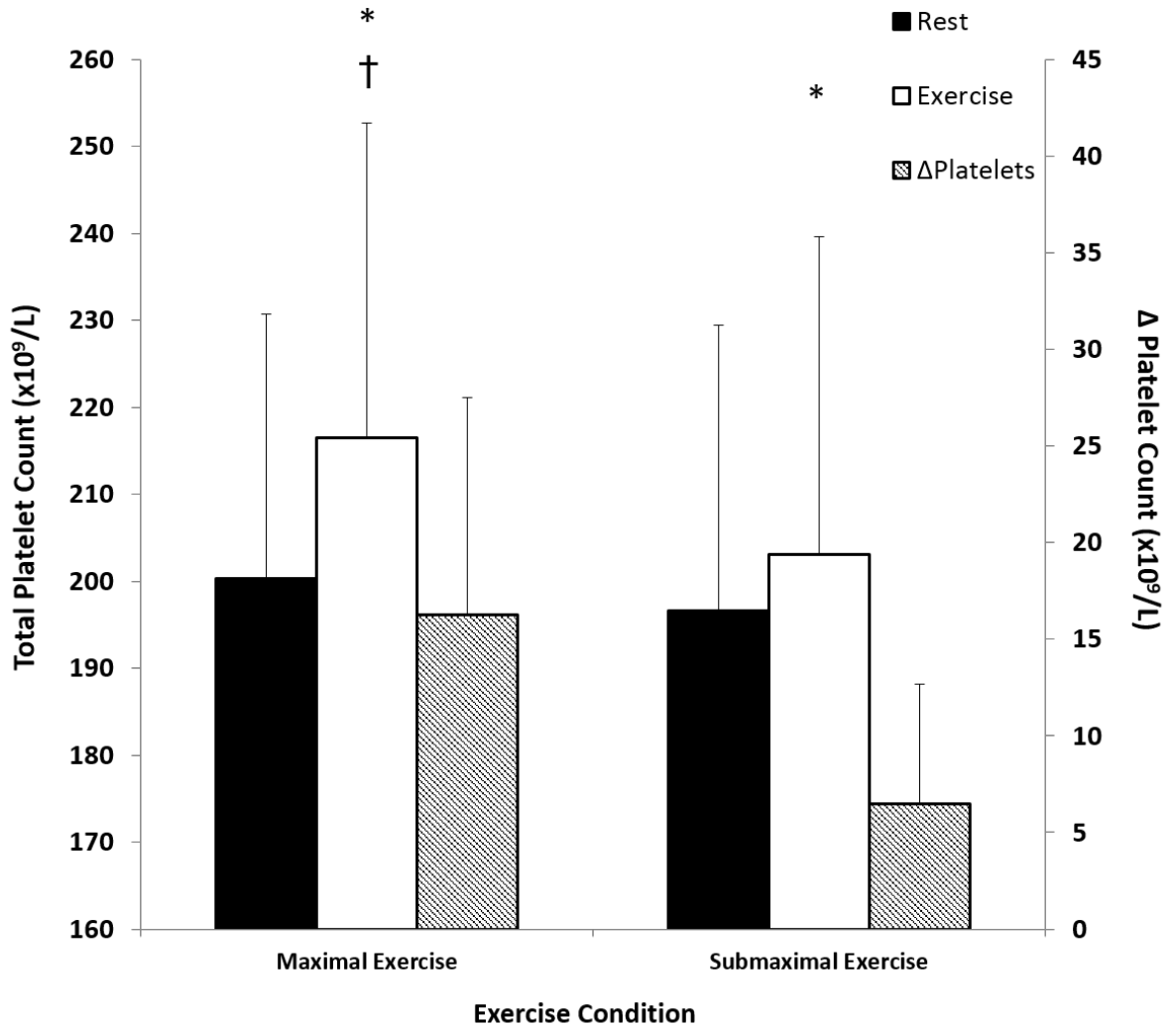


**Figure 3-1.** BDNF responses to small muscle mass exercise. Total serum BDNF at rest (black bars) and total serum BDNF during exercise (white bars) correspond with the left y-axis.  $\Delta$ BDNF represents the difference between exercise and rest BDNF (hatched bars) and correspond with the right y-axis. \* Significantly different compared to rest,  $p < 0.05$

### 3.4.2 Platelet responses to forearm handgrip exercise

There was a significant time x condition interaction as platelets significantly increased in response to both exercise conditions compared to pre-exercise measures,  $F_{(1,15)} = 17.75$ ,  $p < 0.05$

(Figure 3-2). Specifically, ME stimulated an  $8.0 \pm 5.2\%$  increase in platelets, which was a significantly greater response compared to SE, which stimulated a  $3.1 \pm 3.5\%$  increase in platelets;  $F_{(1,15)} = 34.73, p < 0.05$ .

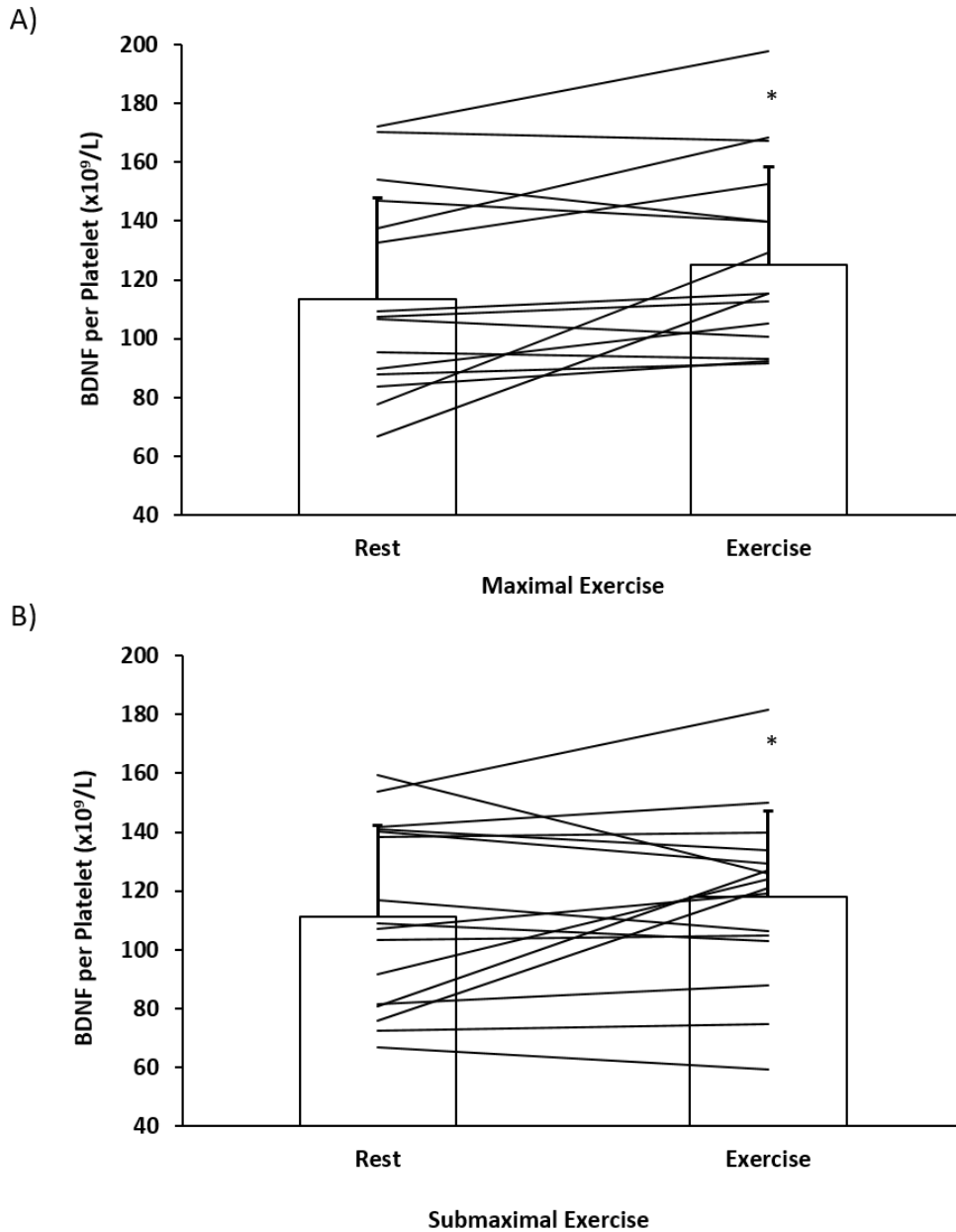


**Figure 3-2.** Platelet responses to small muscle mass exercise. Total platelets at rest (black bars) and total platelets during exercise (white bars) correspond with the left y-axis.  $\Delta$ Platelets represents the difference between exercise and rest platelets (hatched) and corresponds with the right y-axis.

\* Significantly different compared to rest,  $p < 0.05$ . † Significantly different compared to SE,  $p < 0.05$ .

### 3.4.3 BDNF per platelet analysis

The calculated amount of BDNF per platelet significantly increased by  $13.4 \pm 23.6\%$  following ME and a  $8.7 \pm 23.2\%$  increase in BDNF per platelet following SE;  $F_{(1,15)} = 4.60$ ,  $p < 0.05$  (Figure 3-3).



**Figure 3-3.** BDNF per platelet response to A) Maximal effort exercise and B) Submaximal effort exercise. Individual responses denoted by straight lines and group means represented by bars.

\* Significantly different compared to rest,  $p < 0.05$ .

### 3.4.4 Hemodynamic responses to exercise

Table 3-1 displays the hemodynamic responses to ME and SE protocols. There was an effect of time in both exercise conditions for all variables except for total peripheral resistance (TPR),  $p < 0.05$ . There was also an effect of condition, as ME was significantly greater than SE across all variables except for cardiac output (CO) and TPR,  $p < 0.05$ .

**Table 3-1.** Hemodynamic responses to exercise.

	ME		SE	
	Rest	Exercise	Rest	Exercise
<b>SBP (mmHg)</b>	125 (12)	153 (12)*†	121 (7)	140 (8)*
<b>DBP (mmHg)</b>	72 (7)	97 (10)*†	66 (10)	82 (6)*
<b>MAP (mmHg)</b>	90 (9)	121 (10)*†	84 (9)	105 (8)*
<b>CO (L/min)</b>	6.0 (1.2)	8.6 (1.5)*	6.0 (1.2)	7.5 (1.2)*
<b>TPR (mmHg/mL/min)</b>	0.95 (0.20)	0.91 (0.19)	0.88 (0.20)	0.86 (0.14)
<b>HR (bpm)</b>	59.5 (10.5)	95.3 (15.9)*†	55.9 (7.4)	70.2 (7.4)*

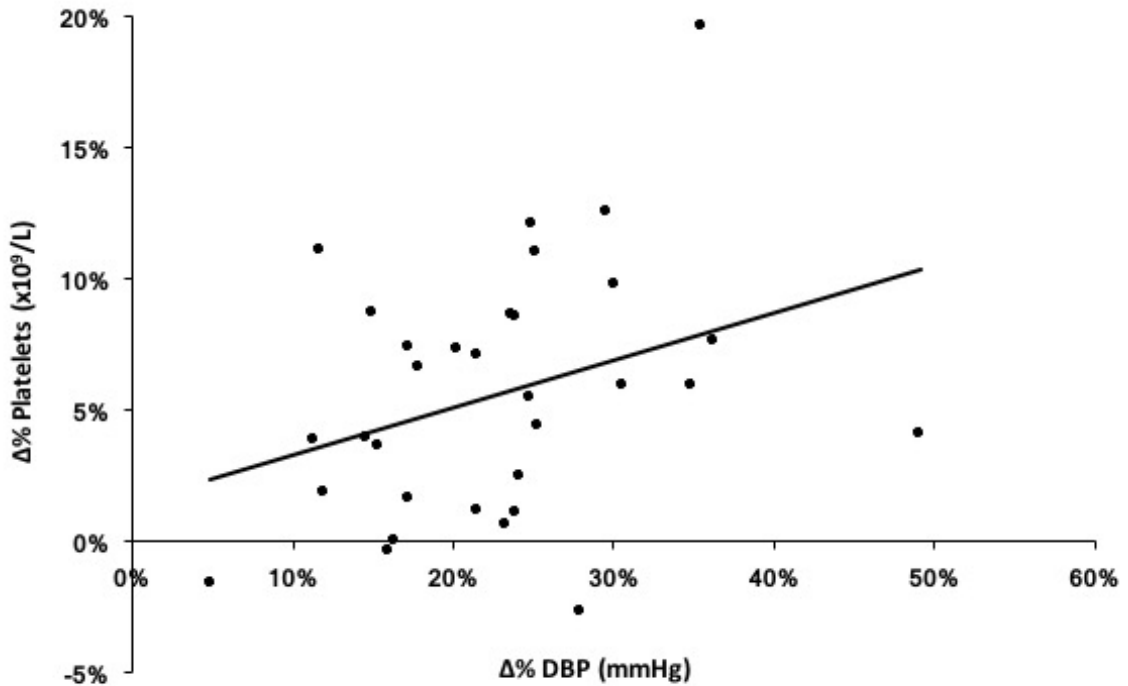
**SBP** = systolic blood pressure, **DBP** = diastolic blood pressure, **MAP** = mean arterial pressure, **CO** = cardiac output, **TPR** = total peripheral resistance, **HR** = heart rate. Data are mean (SD).

\* Significantly different than Rest of the same condition. † Significantly greater than SE.

### 3.4.5 Correlations between DBP, platelets, and BDNF

Pearson product-moment correlation coefficients were calculated for DBP, platelets, and BDNF for each exercise condition and pooled data from both exercise protocols (Figure 3-4).

There was a significant association between DBP and platelets in the pooled exercise conditions,  $r = 0.363$ ,  $p < 0.05$ . There were no associations found between BDNF and platelets or DBP in response to exercise.



**Figure 3-4.** Exploratory correlation between percent change ( $\Delta\%$ ) in diastolic blood pressure (DBP) and platelets in response to both ME and SE (pooled exercise condition).

### 3.4.6 Handgrip exercise performance and total work performed

Given the differences in both intensity and duration of the respective exercise protocols, the total volume of work performed was analyzed. The SE protocol resulted in significantly greater total work performed ( $154,508.4 \pm 21,673.4$  N) compared to ME ( $75,282.2 \pm 15,342.9$  N),  $p < 0.05$ . The average critical power impulse for the group was  $192.5 \pm 35.5$  N•s, from which an average target of  $163.6 \pm 30.2$  N•s corresponding to 15% below critical power was set as the SE intensity. The average handgrip impulse force during ME was  $225.2 \pm 72.0$  N•s, which was significantly greater than SE ( $164.4 \pm 25.1$  N•s),  $p < 0.05$ .

## 3.5 Discussion

While it has been established that large muscle mass exercise can transiently increase circulating BDNF (26), it is unknown whether and to what degree circulating BDNF is elevated by small muscle mass exercise. The guiding rationale for this study was that the robust increase

in serum BDNF following aerobic exercise of sufficient intensity is in part due to the addition of splenic platelets, via sympathetically mediated splenic constriction. As such, we hypothesized that forearm handgrip exercise could increase serum BDNF in an intensity-dependent manner due to increased sympathetic activation evoked by exercise (21, 48). The major findings from this study were a) serum BDNF significantly increased in response to both exercise protocols, b) platelets significantly increased in an intensity-dependent manner, with ME evoking a larger response compared to SE, and c) the amount of BDNF per platelet significantly increased in both exercise conditions. These findings support our hypothesis and to our knowledge are the first to demonstrate a significant serum BDNF response can be achieved with as little exercising muscle mass as a single forearm.

### **3.5.1 BDNF response to exercise: intensity and muscle mass considerations**

10 minutes of ME and 30 minutes of SE elicited 21.2% and 11.2% increases in serum BDNF, respectively. Our small muscle mass exercise protocols were designed based on the rationale that elevated sympathetic nervous activity was a required effect of exercise in order to induce thrombocytosis and thereby achieve serum BDNF elevation. Prior studies that did use a small muscle mass resistance exercise model did not consider the nature of exercise in this context. This may explain why these studies did not observe an increase in either serum (37) or plasma (8) BDNF in young adults.

Specifically, Rojas Vega (37) found a non-significant trend towards increased serum BDNF following 50 continuous repetitions of either low or high intensity (40 and 110% 1 repetition max, respectively) isokinetic leg extension exercise in a sample of 8 young adults. The resistance exercise protocol used by this group is considered high volume for a single set and would require considerable effort to complete; however, total duration of this protocol would be very brief, taking an estimated 3 minutes. Similarly the study by Correia et al. (8) found no response of plasma BDNF following either maximal effort elbow flexor and extensor or knee flexor and

extensor resistance exercise consisting of 5 sets of 10 repetitions. Each set was 30 seconds in duration, followed by 40 seconds of rest, and that total exercise time was 5.1 minutes including rest.

Potential explanations for the differences between these and our findings reveal themselves when we consider exercise protocols in the context of activating specific mechanisms of BDNF addition to the blood. Specifically, insufficient intensity and/or duration could explain the lack of findings in these studies compared to the robust serum BDNF increase in our forearm handgrip exercise model. This is because of the need for sufficient magnitude and duration of peripheral stimuli like shear stress or deoxygenation for endothelial or brain BDNF production and release (33, 36), and sympathetic splenic vasoconstriction for thrombocytosis. The former was recently proposed by Marston et al. (28) to explain the finding that only a strength training bout for the purpose of hypertrophy provided enough stimulus to achieve elevations in serum BDNF. In support of the latter, we did obtain blood samples at 3 min in a small subset ( $n = 5$ ) of subjects and found at that time point that there was a limited although non-statistically significant ( $p = 0.053$ ) increase in platelets compared to baseline for ME ( $188.3 \pm 23.3$  vs.  $196.3 \pm 23.1 \times 10^9/L$ ) and no evidence of an increase in SE ( $190.3 \pm 25.8$  vs.  $189.3 \pm 21.7 \times 10^9/L$ ). By comparison, the full exercise protocols in our study required participants to complete 300 contractions for ME for a duration of 10 minutes and 900 contractions over a period of 30 minutes for SE, at which time we saw robust serum BDNF increases. Furthermore, we (53) and others (28, 54) have reported significant increases in serum BDNF following resistance exercise protocols consisting of multiple exercises and lasting longer than 3 minutes (i.e., 45 minutes including rest). We would emphasize the need for activation of specific BDNF elevating mechanisms to be a prime consideration when assessing the potential efficacy of an exercise modality.

The body of evidence with regard to aerobic exercise as summarized by Knaepen et al. (26) suggests that circulating BDNF increases in an intensity-dependent fashion, with the largest BDNF responses occurring at higher aerobic exercise intensities in healthy populations. The

magnitude of the BDNF response observed in our study using a single forearm handgrip exercise was expectedly lower than whole-body high intensity exercise protocols, such as Cho et al. (7) who observed a 39% increase in serum BDNF at the group level following a VO<sub>2</sub>max treadmill test, and Gilder et al. (15) and Ferris et al. (12) who noted a 32% and 30% increase in serum BDNF in response to a maximal exercise test on a cycle ergometer, respectively. We have demonstrated that 10 minutes of ME forearm exercise achieved an ~21% increase in serum BDNF, which is 2/3 of the response observed by Gilder et al. (15) and Ferris et al. (12). Based on previous work, and without considering splenic constriction as a key mechanism for achieving elevated serum BDNF, this would seem a remarkable effect given that forearm muscle mass is a mere fraction of that used during maximal cycling or running exercise. Thus, we believe our work is the first to demonstrate that stimulation of thrombocytosis independent of muscle mass activation is an important consideration for an exercise modality's efficacy in elevating circulating BDNF.

Interestingly, the work by Ferris et al. (12) alludes to the possibility of an intensity threshold for achieving circulating BDNF elevation. They found that cycling exercise at 75% VO<sub>2</sub>max evoked a 13% increase in serum BDNF, whereas cycling at 55% VO<sub>2</sub>max did not have an effect on BDNF following exercise. This finding is supported by those of Rojas Vega et al. (38) and Hötting et al. (18) and recently emphasized by Marston et al. (28) for resistance exercise. In the present study, although not statistically significant between ME and SE (P=0.06) the serum BDNF response, in conjunction with the significantly greater platelet response in ME, encourage consideration of a relationship between exercise intensity and serum BDNF elevation that, when viewed in conjunction with the findings of Ferris et al. (12) may be somewhat independent of muscle mass. The potential for this independence was in fact the basis for our hypothesis that forearm handgrip exercise could achieve meaningful elevations in circulating BDNF, given that the magnitude of local skeletal muscle metabolic stress appears to be a critical driver of sympathetic nerve activity independent of muscle mass (40) (i.e. lots of exercising muscle with

little metabolic stress does little to sympathetic activity relevant for thrombocytosis, whereas a small muscle mass with considerable metabolic stress is a potent stimulus).

### **3.5.2 Platelet source of BDNF in response to exercise**

To reiterate, the rationale of the current study was that sympathetic activity evoked by handgrip exercise would cause splenic constriction, thereby resulting in the ejection of stored platelets. Given that approximately 99% of circulating BDNF is platelet-bound, this thrombocytosis could account for a large part of the robust serum BDNF increase following acute exercise, even if only 30-40% of this BDNF is releasable (14, 50). In support of this relationship, we found a significant correlation between platelet and DBP responses to exercise, given that DBP and muscle sympathetic nerve activity respond in parallel to handgrip exercise (41, 42). There are two interesting parallels between BDNF and platelet responses to exercise that suggest a primary role for thrombocytosis in BDNF elevation. The first is that both are exercise intensity-dependent phenomena (16, 26) and the second commonality is that both share similar post-exercise characteristics (4, 15). While we did not include post-exercise measures, previous works have independently shown a return to baseline within 30 minutes of exercise cessation for both platelets (4) and serum BDNF (15, 29, 38), a response that is likely the result of splenic reuptake of platelets due to sympathetic withdrawal (6, 47).

Serum BDNF and platelet count are strongly linked (14, 29, 30); however, correlational analysis revealed no relationship between changes in platelets and BDNF for either of our exercise conditions, despite both moving in the same direction. Nonetheless, both variables significantly increased with exercise, suggesting that the relationship may be more complex than simple addition or removal of platelets from the blood. In support of this statement, an important finding from our work is that the amount of BDNF per platelet significantly increased following both exercise protocols, which raises questions regarding the potential source(s) of this BDNF. An increase in BDNF per platelet cannot be the function of endogenous platelet BDNF

production, as platelets do not synthesize the neurotrophin (14). Therefore, another possibility is that the splenic platelets added to the circulation in our study contained higher amounts of BDNF relative to their circulating counterparts. Arguing against this are findings that mature platelets exist in a dynamic equilibrium between the circulating and splenic pools, with constant exchange between these pools occurring (52) and splenic platelets are the same age and contain the same cellular contents as circulating platelets (6). Regardless, determining whether splenic platelet BDNF content differs from circulating platelet content would seem an important avenue of future investigation.

### **3.5.3 Tissue sources of BDNF in response to exercise**

Another explanation for increased BDNF per platelet with ME and SE exercise is *de novo* production and release from tissues. The seminal study by Rasmussen et al. (36) implicating the brain as the primary source of circulating BDNF dominates the current framework by which exercise-induced elevation of blood borne BDNF via tissue source is understood. However, their conclusions that 70-80% of circulating BDNF is of cerebral origin may overestimate the brain's contribution to circulating BDNF as these authors only measured plasma and it was specifically venous effluent immediately downstream of the brain. The major issue with tissue effluent plasma as the lone measure of BDNF, and its interpretation with regard to tissue production and release is that BDNF can move between platelets and plasma in response to local vascular bed elevations in shear-stress (14). As this is elevated in the cerebral circulation during exercise (45), there exists the conditions for increased platelet release of BDNF in the cerebral microcirculation. This suggests that caution should be used in interpreting tissue venous effluent plasma-only measures of BDNF, as both tissue and platelet release contributions are possible.

Other tissues that produce and release BDNF in response to exercise-like stimuli have also been identified, including the vascular endothelial cells (VEC) (33) and peripheral blood mononuclear cells (PBMC) (4). VEC have recently been shown to rapidly secrete BDNF in

proportion to the magnitude of shear stress stimuli (33) and reductions in PO<sub>2</sub> (17), both of which occur in considerable measure in exercising skeletal muscle. In fact, Prigent-Tessier et al. (33) report that levels of pro and mature BDNF in cardiac and aortic endothelium were in the same range as those measured in the hippocampus (34), highlighting that the cardiovascular system is a likely contributor to exercise-induced elevation in circulating BDNF. To what extent this source was activated by forearm handgrip exercise remains unclear, given the small exercising muscle mass. In summary, both splenic-specific elevated platelet BDNF content and release and tissue *de novo* BDNF production remain potential contributors to the observed increase in BDNF per platelet in our study.

#### **3.5.4 Perspectives**

This is the first study to demonstrate that it only takes a single forearm exercising muscle mass to stimulate increases in serum BDNF similar to those levels seen in high to moderate intensity traditional aerobic paradigms. In fact, the 10 minutes of ME forearm exercise resulted in 2/3 of the serum BDNF response evoked by a whole-body graded exercise test to exhaustion as seen by Ferris et al. (12). The important implications of these findings are two-fold. First, these results provide insight into potential mechanisms by which exercise may increase circulating BDNF. Whole-body aerobic exercise creates a substantial multi-faceted physiological perturbation (i.e. increased shear stress, tissue deoxygenation, increased neuronal firing, and increase sympathetic outflow), that could evoke a coordinated response from multiple systems that are potential sources of BDNF. Evidence from cell studies suggests the magnitude of tissue BDNF release is proportional to the physiological perturbations such as shear stress (33), tissue deoxygenation (17), and chemical signaling on PBMCs (4); conditions which would be present in exercising muscle. In this context, the exercising forearm muscle mass would represent but a fraction of such an endothelial source compared to cycling or running. Therefore, it would seem at first glance that such small muscle mass exercise could not significantly elevate serum BDNF.

However, based on our findings it would appear that constriction of the spleen and the subsequent thrombocytosis is an important mechanism for elevating BDNF during exercise and forearm exercise provides ample stimulus for this. The major differentiator between forearm and whole-body exercise may therefore be the contribution of cellular sources to total circulating BDNF. We found a 13.5% increase in BDNF per platelet following maximal effort forearm exercise compared to a calculated 59.7% increase following a treadmill VO<sub>2</sub>max test (7). In the context of the current body of literature, our findings are important because they identify the potential for exercise modality-specific mechanisms of BDNF elevation, and direct follow up research investigation into issues of platelet-bound BDNF bioavailability at tissue exchange sites and its importance, and the potential of sympathetic activation interventions to contribute.

The second important aspect of this study pertains to developing strategies for increasing circulating BDNF in populations that stand to benefit most from this transient response. The relationship between circulating BDNF and brain function has been demonstrated in populations with depression and in aging populations (5, 55). In both cases, exercise has been touted as an effective strategy for restoring and improving function and BDNF appears to be a central mediator of this relationship. It follows that finding easy yet effective ways of transiently increasing BDNF may have important functional implications for such populations and warrants further investigation.

We also believe it important to recognize that, while serum BDNF elevation is transient following exercise, this does not mean that significant impact on neuroplasticity cannot be achieved. We have previously put forward the notion that transient exercise-induced elevations in circulating neurotrophic factors could be taken advantage of by performing cognitive training immediately following exercise (53). The concept is that the increased cerebral perfusion due to neurovascular coupling with brain activation would benefit from having increased blood borne BDNF as a means of increasing the BDNF “dose” to brain areas important in cognitive function. Again it is important to stress that platelet-bound BDNF should be considered bioavailable at the

site of tissue uptake as platelets release BDNF under conditions of increased shear stress (14), which would be the case in areas of increase cerebral perfusion. As well, elevated epinephrine accompanying adrenergic activating exercise intensities increases such a release response. To draw an analogy; greater hemoglobin content accompanied by decreased affinity of Hb for oxygen, ensures a higher tissue microvascular availability of oxygen for concentration dependent diffusion. Likewise greater platelet content accompanied by increased reactivity to tissue exchange site stimuli may serve to enhance the microvascular plasma concentration of BDNF and thereby the transporter dependent uptake by neural tissue (31). It may be the case that such transient but periodic increases in BDNF uptake as a result of regular exercise bouts are an important contributor to neuroplasticity.

### **3.5.5 Limitations**

We do not report plasma measures of BDNF in this study. We had intended to do so as samples were collected and processed for the determination of plasma BDNF; however, the optical density signal in these plasma samples was below that of the lowest standard, rendering these samples unmeasurable. Unfortunately, this is not an uncommon occurrence, as Brunelli et al. (4) and Gilder et al. (15) both report difficulties in the measurement of plasma samples. However, given that the overwhelming majority of blood borne BDNF is platelet bound and the established interchange of BDNF between plasma and platelets, the measurement of serum and BDNF/platelet provide a meaningful characterization of the BDNF system that captures both splenic and tissue sources of BDNF. It should be noted that in contrast to plasma samples the optical density signal of serum samples was in the optimal kit detection range and there was therefore no difficulty in measuring serum BDNF.

We report that the amount of BDNF per platelet increased following both exercise protocols; however, this measure is calculated from serum BDNF divided by platelet count rather than a direct measurement from lysed platelets. While this measure may limit direct quantitative

comparison between exercise conditions, it does allow for the identification of a direction of change in platelet bound BDNF.

Our study exercise modalities were not originally intended to control for exercise volume across exercise intensities and therefore it must be acknowledged that greater platelet and BDNF elevation in ME than SE may also be explained by the response dwindling over time with continued steady state forearm exercise in SE rather than because of intensity differences between ME and SE. This seems unlikely given that exercise intensity has a greater impact on the magnitude of thrombocytosis than exercise duration (16). It is also possible that with longer duration, increased tissue uptake might begin to reduce circulating BDNF (28). Regardless, our findings are the first to support the efficacy of single forearm exercise to elevate circulating BDNF to levels similar to those observed in moderate to high intensity whole-body aerobic exercise (26) and encourage further investigation into muscle mass independent exercise intensity effects on mechanisms of circulating BDNF elevation.

Finally, this study only examined males, which limits the generalizability of our findings given that males appear to have stronger BDNF responses to exercise than females (49). As such, follow-up work should be undertaken to examine the response in BDNF response to handgrip exercise in both males and females.

### **3.6 Conclusions**

In conclusion, this is the first study to demonstrate that exercise with as little muscle mass as contained in a forearm can significantly increase circulating BDNF. Furthermore, this appears to be sensitive to exercise intensity ( $p = 0.06$  for differences between ME and SE). This substantial BDNF response is associated with an intensity-dependent increase in circulating platelets indicative of thrombocytosis that we interpret as being a major source of exercise-induced BDNF in a forearm handgrip model. As well, the observation that calculated BDNF per platelet significantly increased could implicate the contribution of a cellular source of BDNF in response

to handgrip exercise and/or higher BDNF content of splenic-derived platelets. These findings support the potential for forearm handgrip exercise as an intervention capable of achieving significant increases in serum BDNF. They also provide the rationale for pursuing new lines of research into the role of sympathetic activation in BDNF bioavailability, and introduce an emphasis on thrombocytosis as an important response for an exercise modality that is accessible to most populations, requires minimal equipment (i.e. stress ball), and requires considerably less effort than conventional exercise.

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## **Chapter 4**

### **Demonstrating the Utility of a Novel, Equipment-Free Whole-Body Isometric Exercise Protocol for fMRI-Based Studies**

**Chapter 4 has been submitted to Psychology in Sport and Exercise as:**

**Walsh, J.J., Costigan, P.A., and Tschakovsky, M.E.** Demonstrating the utility of a novel, equipment-free whole-body isometric exercise protocol for fMRI-based studies

#### 4.1 Abstract

**Background:** Current MRI-compatible exercise modalities allowing brain imaging are limited by the need for expensive MRI-compatible exercise equipment and strict head movement limitation. **New Method:** To address these limitations we developed a novel exercise modality consisting of rhythmic whole-body isometric contractions (WBI) that achieves repeatable  $\dot{V}O_2$  responses via targeted Borg 20-point scale ratings of perceived exertion (RPE). Study 1: participants performed WBI exercise laying supine at 3 RPE intensities (13, 15, and 17) on each of 3 separate visits.  $\dot{V}O_2$  response was measured via metabolic cart. On a fourth visit participants performed a submaximal upright cycling ramp test to characterize their  $\dot{V}O_2$ -to-power relationship allowing estimation of the power output equivalent for WBI exercise  $\dot{V}O_2$ . Study 2: participants performed WBI laying supine on a base and head stabilization set up simulating fMRI standard of care and head movement was quantified (3-D motion-capture). **Results:**  $\dot{V}O_2$  responses were similar in magnitude to previous in-MRI exercise studies. Specifically, RPE and cycling Watt  $\dot{V}O_2$  equivalents were 13,  $65.3 \pm 28.2$ ; 15,  $95.4 \pm 34.0$ ; 17,  $134.5 \pm 44.4$ .  $\dot{V}O_2$  demonstrated good-to-excellent reliability at each RPE stage. 81.4% of head movement was below 1 mm and 99.7% was below 3 mm, (maximum allowable for fMRI scanning).

**Comparison to Existing Methods:** WBI achieved similar, repeatable  $\dot{V}O_2$  responses as previous studies while maintaining head movement within requirements for fMRI imaging, all without requiring exercise equipment. **Conclusion:** WBI provides a cost-free, reliable exercise protocol for MRI-based studies allowing universal access to investigations of the brain responses during exercise.

KEY WORDS: Novel exercise; fMRI; head movement

## 4.2 Introduction

Participation in regular exercise exerts myriad beneficial effects on the brain that span across structural and physiological realms, and converge on improvements in cognitive function. Studies continue to emerge that support the utility of exercise training for improving indices of brain health, as well as reducing the risk for neurological disease (13). Interestingly, positive effects of exercise on cognitive function are observed immediately after exercise, as a single bout of aerobic exercise transiently improves measures of executive function (3). Executive functions refer to a swath of cognitive functions that coordinate goal-directed behaviour in an ever-changing environment (5). One of the mechanisms driving this transient improvement in cognitive function is postulated to be a function of potentiated neuronal activity due to heightened arousal rather than increased cerebral blood flow experienced during exercise (2, 6, 22).

Previous studies using electroencephalography (EEG) and functional near-infrared spectroscopy (fNIRS) have sought to identify and characterize the antecedent neural activity to this post-exercise cognitive enhancement. These collective works indicate an increase in brain activity during exercise, as evidenced by increases in frontal lobe cortical oxygen during submaximal exercise (23) and a shift in neural firing frequency across a range of exercise intensities (1, 4). Interestingly, EEG studies suggest that changes in frequency bands are not localized to a specific hemispheric site during exercise, suggesting diffuse coordination of neural activity during exercise (1, 4, 11). Despite these revealing findings, EEG and fNIRS do not afford the spatial resolution requisite for investigating regional activation of supraspinal structures during exercise like that of functional magnetic resonance imaging (fMRI). However, performing dynamic multi-joint exercise in an MRI is particularly challenging due to spatial constraints and strict limits on head movement during scanning.

Despite these limitations, variations of MRI-compatible exercise equipment have been used to examine the BOLD response associated with aerobic exercise (10) and lower-limb motor

activation (15, 17, 20, 21, 28). These devices include cycle ergometers (10, 14, 21), a supine treadmill (20), a stepping robot (17), and in other cases, rhythmic movement of the lower limbs without equipment (24, 28). Given that the common challenge of performing such experiments is limiting head movement, these researchers have found effective strategies that restrict head movement during exercise within the acceptable range of fMRI scanning and have also employed fMRI scanning methods, such as sparse sampling, to reduce head motion artifact during scanning (17). Indeed, the use of specialized MRI exercise equipment stands to advance our knowledge of neural responses to exercise; however, with a price tag of upwards of \$70,000 (i.e. MRI Ergometer Pedal device, Lode, Groningen, the Netherlands), MRI-compatible exercise equipment can be inaccessible to a large number of researchers. As such, alternate approaches to performing exercise in an MRI scanner should be sought out in order to provide greater access to researchers endeavouring to explore regional brain responses to whole body exercise. Currently, such an approach does not exist.

To address these limitations, we envisioned an exercise modality consisting of rhythmic whole-body isometric (WBI) contractions, which could stimulate metabolic responses similar to previous in-MRI exercise modalities and that is effective at limiting head movement. Such an exercise modality would eliminate the need for expensive, specialized exercise equipment. However, one aspect of such an exercise modality is the inability to directly quantify power output. As a result, modulation of exercise intensity using a WBI protocol would need to be perceptually regulated using ratings of perceived exertion (RPE). The use of RPE may overcome the inability to quantitatively monitor exercise intensity in an MRI, as it has strong utility in predicting  $\dot{V}O_2\text{max}$  from submaximal bouts of treadmill exercise (7). Further, the prescription of submaximal exercise intensity using RPE results in reliable metabolic responses ( $\dot{V}O_2$ ) across multiple treadmill or cycling exercise sessions (12). In terms of the head movement limitations, a WBI exercise protocol could effectively reduce the largest source of head movement during exercise, which is rhythmic multi-joint movement over a large range of motion (10, 19, 21).

Therefore, we propose that the isometric nature of WBI exercise could effectively minimize head movement whilst maintaining the ability to evoke metabolic responses that are similar to previous in-MRI exercise protocols. However, the viability of such an exercise modality has yet to be established.

Therefore, the main objectives of this study were: a) to identify the magnitude and reliability of  $\dot{V}O_2$  and heart rate (HR) responses to WBI at a given RPE across multiple testing sessions, b) to contextualize the equivalent metabolic cost of WBI exercise through a power output estimation, and c) to assess and quantify the amount of head movement produced by WBI exercise.

## **4.3 Methods**

### **4.3.1 Experimental protocol**

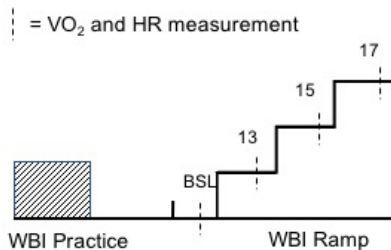
The current study consisted of two separate experimental protocols: Study #1 - characterization of test-retest reliability and metabolic responses to WBI, and Study #2 - characterization of head motion during WBI (**Figure 1**). The WBI protocol consisted of participants laying supine with slightly bent knees performing rhythmic contractions of major muscle groups of the upper (back, chest, abdominals) and lower body (gluteal, quadriceps, hamstrings) at a 1:1 sec, contraction:relaxation duty-cycle using a metronome for auditory cues. For Study #1, exercise was performed while laying supine on a bed and participants were instructed to maintain a stable head position throughout exercise. For Study #2, WBI exercise was performed on a custom MRI bed simulator with subjects in the same body position as Study #1.

## Study #1

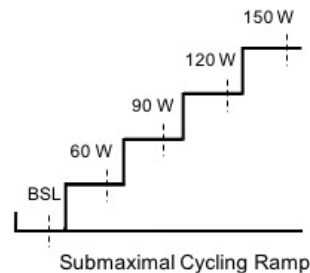


## Study #1 Protocols

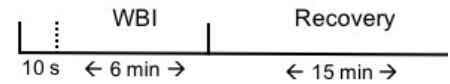
A)



B)



## Study #2 Protocol



### WBI Intensities for Head Motion Trial

1. 13 – SWH 6 min
2. 15 – Hard 6 min
3. Ramp Protocol
  - I. SWH – 3 min
  - II. Hard – 3 min

**Figure 4-1.** Protocol schematic. Study #1 shows protocol A (WBI Ramp) or B (Submaximal Cycling Ramp) specific to visit day as well as details of exercise increments specific to exercise modality. Study #2 Protocol shows the duration of WBI and Recovery and number of trials and the target RPE's used. For the WBI ramp protocol and the cycling ramp protocol, each stage was 4 minutes in length.

BSL = Baseline, 13 SWH = Somewhat Hard RPE, 15 = Hard RPE, 17 VH = Very Hard RPE.

### 4.3.2 Study #1

#### 4.3.2.1 Participants

19 healthy participants ( $21.6 \pm 2.1$  years; 17 males) who were deemed safe to perform exercise volunteered for this study. Participants provided written consent after receiving verbal and written descriptions of experimental procedures and the associated risks. This study was approved by the Health Sciences Research Ethics Board at Queen's University in accordance with the terms of the Declaration of Helsinki.

#### 4.3.2.2 WBI exercise intensity prescription using RPE

Given the inability to measure work using this exercise protocol, exercise intensity for the WBI protocol was prescribed via RPE using the Borg Scale (18). Previous works have shown

that participants reliably achieve similar metabolic responses at a given submaximal RPE across multiple testing sessions for either treadmill or cycling exercise (12) and that submaximal exercise using RPE is a good predictor of  $\dot{V}O_2\text{max}$  (7). As such, we believe that the use of RPE would serve as the best tool for WBI exercise intensity prescription. For the purpose of this study, we selected three exercise intensity: 1) 13 Somewhat Hard, 2) 15 Hard, and 3) 17 Very Hard.

#### 4.3.2.3 Familiarization session

Given that all participants were naïve to the WBI protocol, the first visit to the lab was a familiarization session, where participants were introduced to the WBI protocol. During this session, participants practiced the WBI exercise and then performed a 12-minute WBI ramp protocol. To begin, researchers detailed a number of strategies for co-contracting large muscle mass in a supine position with bent knees during each 1 second contraction. In order to gauge the relative intensity of each stage, participants performed 45 seconds of WBI exercise at Very Hard (17 RPE) and were encouraged to try different contraction strategies during the trial. Following this period, researchers prompted participants to discuss which strategies they attempted and which strategies worked best (i.e., felt strongest) for approximately 2 minutes, which was used as a rest period. This approach was repeated for Somewhat Hard (13 RPE) and Hard (15 RPE) intensities, respectively. The rationale for performing Very Hard first during the familiarization session was to allow participants to experience the sensations associated with sustained effort at the highest intensity of WBI exercise, thereby providing a perceptual ‘ceiling’ reference point when performing WBI at lower intensities. Indeed, participants reported performing contractions at a maximal intensity during the Very Hard stage and that they could not increase their relative intensity if asked; as such, we interpret Very Hard as the maximal intensity achievable for WBI exercise. Before the commencement of each stage, a visual copy of the Borg scale was presented and participants were cued as to which stage they would be working at. Following the

familiarization session, participants were given 10 minutes to recover before performing the 12-minute WBI ramp protocol (described below).

#### 4.3.2.4 Characterization of metabolic responses to WBI

The experimental trials for characterizing metabolic response to WBI took place over two separate visits. The purpose of these visits was twofold: 1) characterize the metabolic response to increasing intensity of WBI, 2) evaluate the trial-to-trial repeatability of the exercise protocol, and 3) to determine each participant's  $\dot{V}O_2$ -to-power slope in order to estimate power output during WBI.  $\dot{V}O_2$  and HR responses to WBI were measured during a ramp protocol that consisted of three 4-minute stages at progressively harder work rates: Somewhat Hard (13 RPE), Hard (15 RPE), and Very Hard (17 RPE), respectively. The ramp protocol was repeated on two separate days to determine the retest reliability of the RPE-based WBI exercise (Figure 4-1).  $\dot{V}O_2$  and HR were continuously measured using a metabolic cart (Moxus, AEI Technologies, Pittsburgh, PA).

#### 4.3.2.5 Submaximal cycling for the determination of $\dot{V}O_2$ -to-power slope

At the beginning of the trial 2 (the third visit), participants performed a submaximal incremental ramp test on a cycle ergometer (Monark, Ergomedic 874E, Varberg, Sweden) in order to determine their  $\dot{V}O_2$ -to-power slope. The  $\dot{V}O_2$ -to-power slope was used to contextualize the equivalent metabolic cost of WBI exercise. Gas exchange and HR were continuously measured during the cycling ramp test, which consisted of four 4-minute stages at workloads for 60, 90, 120, and 150 Watts (W), respectively. Participants were instructed to maintain a pedaling cadence of 60 rpm for the duration of the trial. During the last 30 seconds of each stage, participants were shown a copy of the Borg scale and were asked to indicate their RPE by pointing to the number that best represented their perceived exertion. Following completion of the cycling ramp test, participants were given 30 minutes of recovery time before performing the final WBI ramp test.

#### 4.3.2.6 Statistical analysis

A one-way repeated measured analysis of variance (ANOVA) was used to determine differences in relative  $\dot{V}O_2$  and HR variables at rest and at each stage of the WBI ramp protocol. A Bonferroni correction for multiple comparisons was performed to compare differences between stages of WBI protocol. An estimation of metabolic-equivalent Watts was calculated for each participant at each stage of the WBI using the  $\dot{V}O_2$ -to-power slope determined from the submaximal cycling ramp test. The  $\dot{V}O_2$ -to-power slope equation was determined using the graphing function in Excel (Microsoft Corporation, WA, USA) and was re-arranged to solve for X (Watts) using measures of  $\dot{V}O_2$  obtained during WBI exercise. This measure is recognized as an estimate and is intended to provide descriptive detail about the absolute power output equivalent achieved at each stage of WBI. For measures of retest reliability, a coefficient of variation (CV) was calculated for each stage by expressing the typical error of the two trials as a percentage of the grand mean. An intraclass correlation coefficient (ICC) with 95% confidence limits was calculated to determine the degree to which measures of metabolic responses to WBI tracked between Trial 1 and Trial 2.

### **4.3.3 Study #2 - Characterization of head motion during WBI exercise**

#### 4.3.3.1 Participants

14 participants (20.6  $\pm$  1.2 years; 13 males) who participated in Study #1 volunteered for this study. Of the original 19 participants from Study #1, 3 participants were no longer living in Kingston and 2 were unable to complete the protocol as they were too big for the simulated MRI bed. Participants provided written consent after receiving verbal and written descriptions of experimental procedures and the associated risks. This study was approved by the Health Sciences Research Ethics Board at Queen's University in accordance with the terms of the Declaration of Helsinki.

#### 4.3.3.2 Experimental Protocol

In order to determine whether WBI could be a feasible exercise strategy for MRI-based experiments, we sought to characterize the head movement produced by WBI exercise using 3D motion capture technology. A model MRI headpiece was constructed using foam insulation that was carved to the dimensions of a standard headpiece associated with a Siemens 3 Tesla whole-body MRI (Siemens, Munich, Germany). The model headpiece was affixed to a rigid spinal board (200 cm x 42.5 cm) using Velcro. The purpose of this experiment was to characterize the amount of head movement produced by WBI exercise in a MRI-like environment. As such, we followed the standard of care for head immobilization used by the Centre for Neuroscience Studies MRI facility at Queen's University, which was tightly packing the space between the head and the headpiece with soft dishtowels and restraining the body with straps. A BOSU Ball was placed under participants' knees to optimize leg position and improve comfort. The same body position was used by participants for Study #1. Participants performed three separate WBI exercise trials: 1) Somewhat Hard (13 RPE) for 6 minutes, 2) Hard (15 RPE) for 6 minutes, and 3) a ramp test consisting of 3 minutes at Somewhat Hard followed by 3 minutes of Hard (**Figure 1**). Importantly, participants were instructed to progressively work up to the desired RPE over the course of 5 contractions in order to minimize the large movement associated with the initiation of exercise (16).

#### 4.3.3.3 Instrumentation: motion capture details

Head motion was determined using a 14 Oqus infrared light-emitting camera system (Qualisys, Gothenburg, Sweden) in a motion capture laboratory. 11 mm reflective markers were placed on landmarks on the head, specifically on either temple and the middle of the forehead at an individual's hairline. All markers were sampled at 50 Hz. Calibration of the camera system was performed prior to experimentation and calibration was deemed acceptable if values were below 0.5 mm.

#### 4.3.3.4 Data analysis

Head motion data was analyzed for both translation and rotation of the head. For translational and rotational data, the average, minimum, maximum, and SD were calculated for each 1 second time point (50 data points) for each directional axis. From this, the following metrics were derived to assess task-related head motion during WBI exercise. The Range was calculated as Maximum – Minimum, as described by Seto et al., (26). The Offset associated with the initiation of exercise was calculated as the first 3 seconds of exercise minus the last 3 seconds of baseline. Finally, in order to further characterize the suitability of this exercise for use in an MRI, we quantified the number of instances (1 second time bins) where head movement exceeded the 3 mm threshold for translation and 3 degrees for rotation. A one-way repeated measures ANOVA was used to compare differences in the number of instances where head movement exceeded these thresholds between trials.

### 4.4 Results

#### 4.4.1 Study #1

##### 4.4.1.1 $\dot{V}O_2$ and HR responses to WBI

There was a significant effect of RPE intensity on relative  $\dot{V}O_2$ ,  $F_{(2, 113)} = 233.22$ ,  $p < 0.001$  (Figure 4-2). There was a significant effect of RPE intensity on HR responses to WBI,  $F_{(2, 113)} = 174.85$ ,  $p < 0.001$  (Figure 4-2). There was no difference between testing sessions for either relative  $\dot{V}O_2$  or HR.

##### 4.4.1.2 Estimated watts to WBI

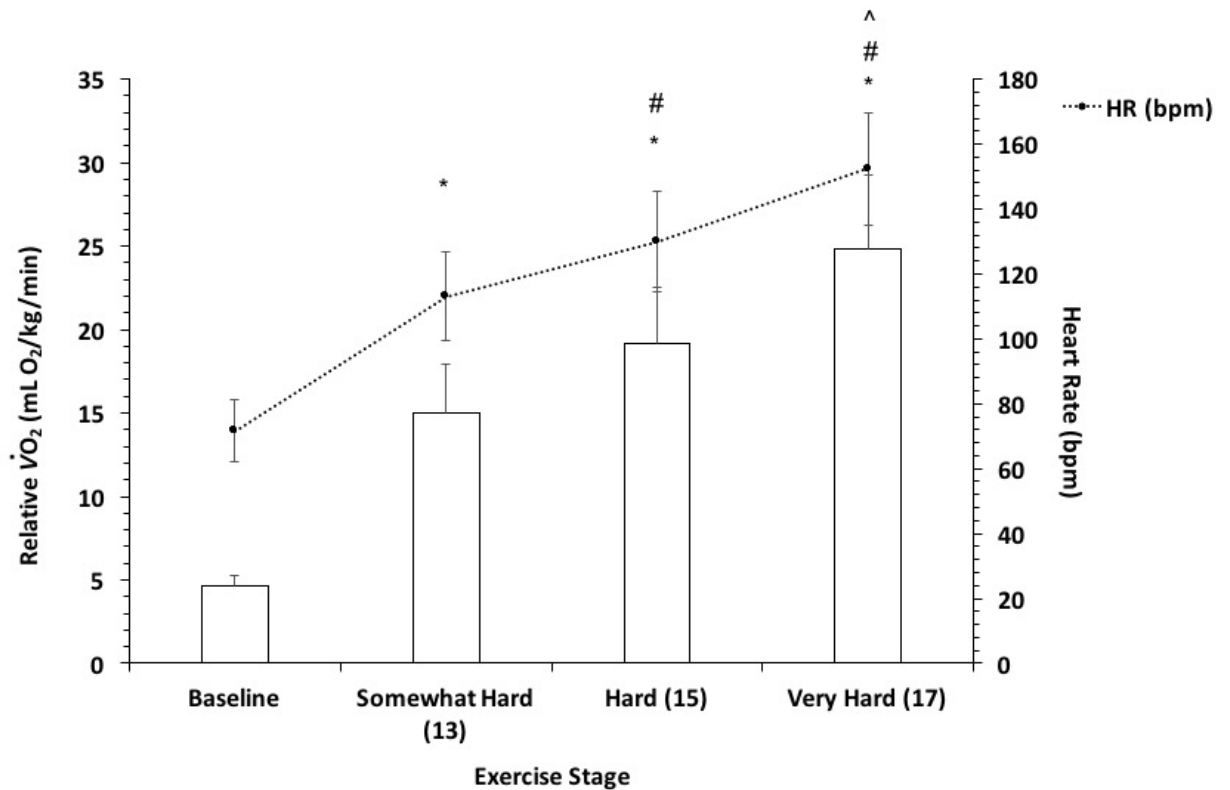
There was a significant effect of RPE intensity on the estimated Watts performed during WBI exercise (Figure 4-3),  $F_{(2, 113)} = 147.65$ ,  $p < 0.001$ . There was no difference in estimated Watts between testing sessions.

##### 4.4.1.3 WBI repeatability of HR responses

Repeatability of HR responses to WBI was assessed by comparing Trial 1 to Trial 2 of the WBI ramp protocol. Somewhat Hard ICC = 0.82 [0.61, 0.91], CV = 6.4%, Hard ICC = 0.84 [0.69, 0.93], CV = 5.6%, Very Hard ICC = 0.93 [0.85, 0.97], CV = 3.3%.

#### 4.4.1.4 WBI repeatability of relative $\dot{V}O_2$ responses

Repeatability of relative  $\dot{V}O_2$  responses to WBI was assessed by comparing Trial 1 to Trial 2 of the WBI ramp protocol. Somewhat Hard ICC = 0.75 [0.52, 0.88], CV = 12.6%, Hard ICC = 0.83 [0.63, 0.91], CV = 9.3%, Very Hard ICC = 0.93 [0.87, 0.97], CV = 5.4%.

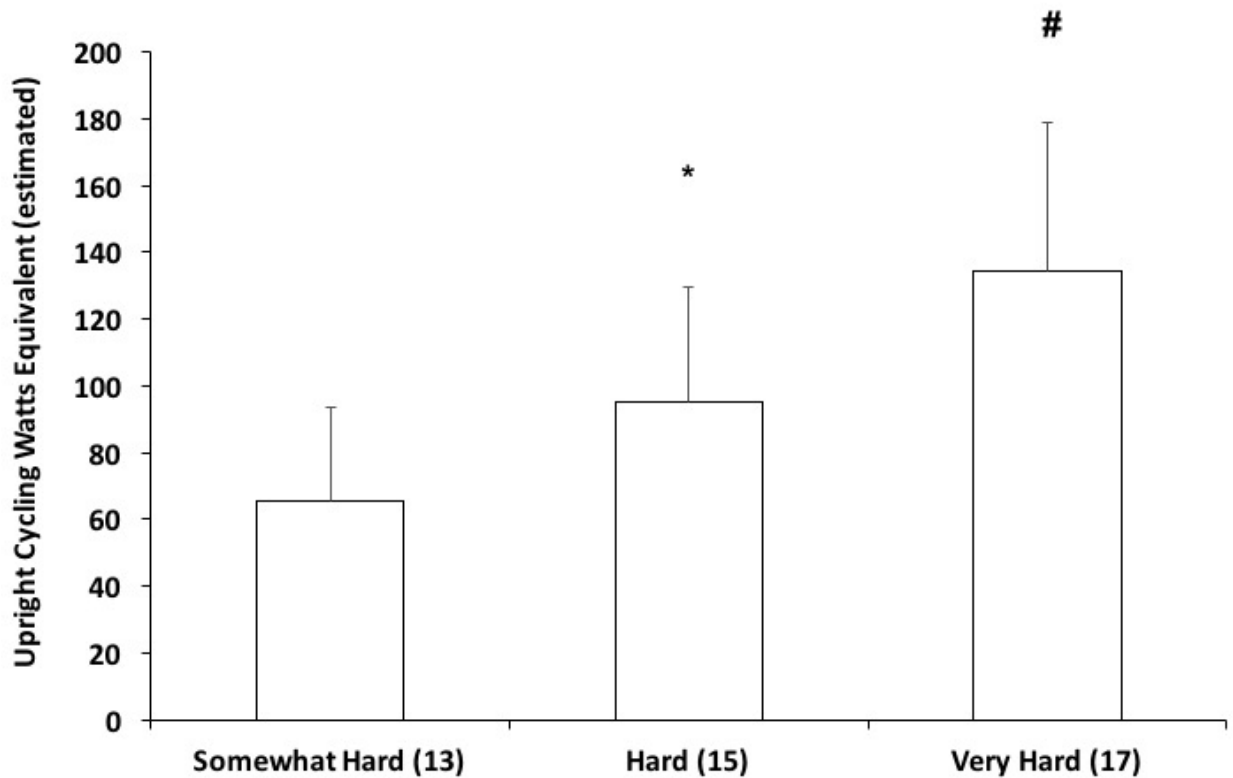


**Figure 4-2.**  $\dot{V}O_2$  (open bars) and HR (closed circles) responses to the WBI exercise ramp protocol measured during the last minute of each stage during WBI exercise.

\* Significantly greater than Baseline,  $p < 0.05$

# Significantly greater than Somewhat Hard,  $p < 0.05$

^ Significantly greater than Hard,  $p < 0.05$



**Figure 4-3.** The  $\dot{V}O_2$  obtained in the final 30 seconds of each stage of WBI exercise as converted into the watts in upright cycling that would elicit the given  $\dot{V}O_2$  for each RPE.

\* Significantly greater than Somewhat Hard,  $p < 0.05$

# Significantly greater than Somewhat Hard and Hard,  $p < 0.05$

#### 4.4.2 Study #2

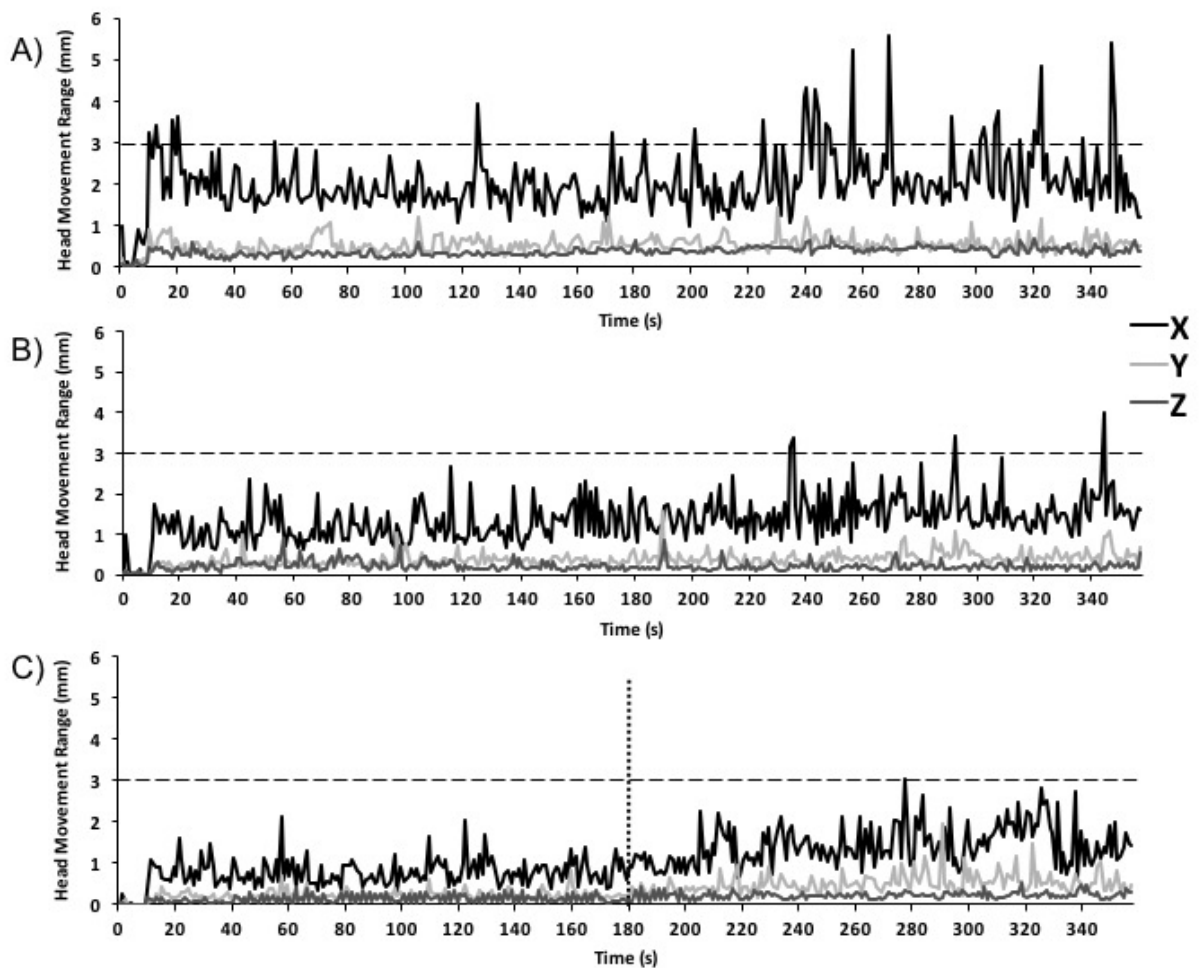
##### 4.4.2.1 Head translation and WBI

We report translation data for the X-axis (superior-inferior), which experienced the largest translation of head movement during WBI exercise. Time course data for the range of translation is available in Figure 4-4. Translational offsets associated with the initiation of exercise in trial 1 was significantly higher than trials 2 and 3,  $F_{(8, 113)} = 17.01$   $p < 0.05$  (Figure 4-4). To understand the number of times the threshold of 3 mm for head translation was exceeded, we quantified these events using 1 second bins. For trial 1, 0.7% of events exceed 3 mm (31 / 4654 seconds) and 62.7% of events were below 1 mm (2916 / 4654 seconds). For trial 2, 0.1% of events exceed 3

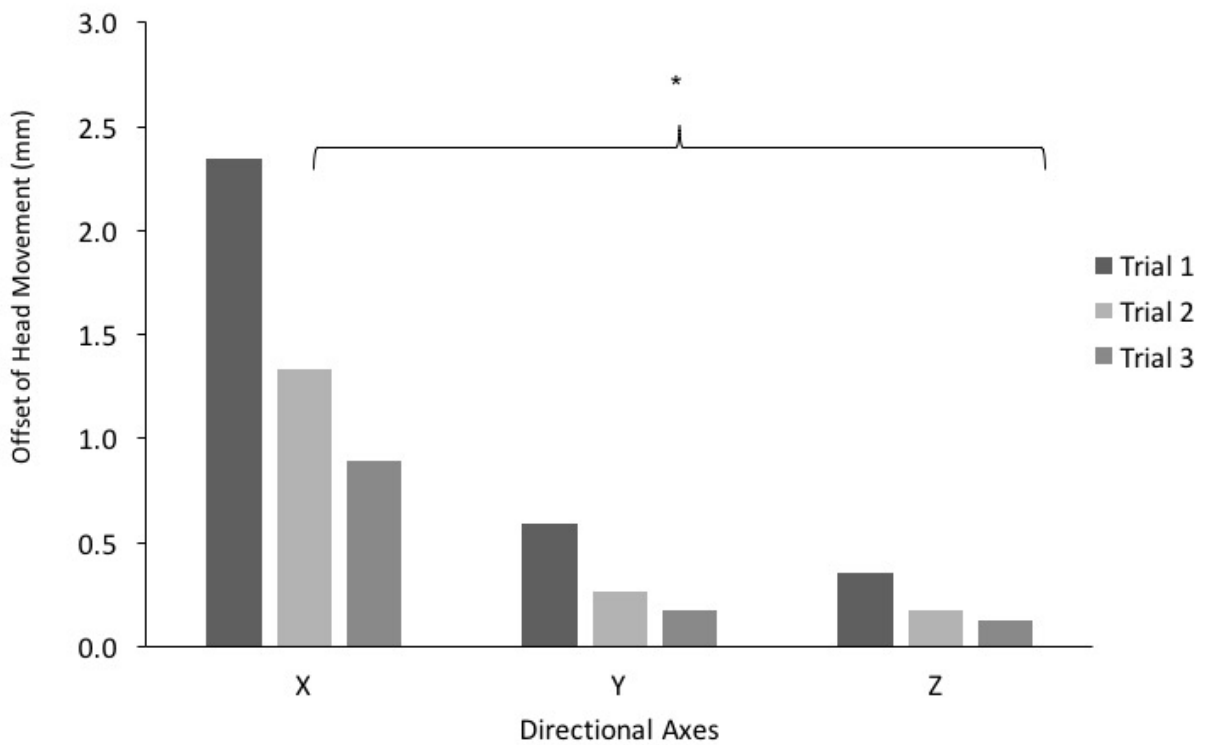
mm (4 / 4654 seconds) and 88.4% were below 1 mm (4155 / 4654 seconds). For trial 3, <0.1% of events exceed 3 mm (1 / 4654 seconds) and 93.3% of events were below 1 mm (4340 / 4654 seconds).

#### 4.4.2.2 Head rotation and WBI

We report rotation data for the X-axis (neck flexion), which was determined to be the dominant axis of rotation. For trial 1, average rotation was  $0.83 \pm 0.14$  degrees with a maximal range of 3.78 degrees. Trial 2, average rotation was  $0.97 \pm 0.20$  with a maximal range of 6.08 degrees. Trial 3 average rotation was  $1.01 \pm 0.24$  degrees with a maximal range of 4.28 degrees. The rotational offsets associated with the initiation of exercise for trials 1, 2, and 3 in the neck-flexion axis were 0.73, 0.66, and 0.29 degrees, respectively. In order to determine individual performance and relate these measures to group performance, we quantified the number of instances based on 1 second bins where head rotation exceeded certain thresholds. For trial 1, 79.2% of exercise time was spent below 1 degree of rotation (3688 / 4654 seconds), 19.0% was spent between 1-2 degrees (885 / 4654 seconds), and 1.7% was spent between 2-3 degrees (81 / 4654 seconds). For trial 2, 77.7% of exercise time was spent below 1 degree of rotation (3618 / 4654 seconds), 13.5% was spent between 1-2 degrees (628 / 4654 seconds), and 4.7% was spent between 2-3 degrees (218 / 4654). For trial 3, 79.1% of exercise time was spent below 1 degree of rotation (3680 / 4654 seconds), 18.9% was spent between 1-2 degrees (878 / 4654 seconds), and 2.1% was spent between 3-3 degrees (96 / 4654).



**Figure 4-4.** The maximal range of the head movement produced by WBI exercise in Study #2. The separate trials are: A) Somewhat Hard, B) Hard, C) 3 min Somewhat Hard, 3 min Hard (indicated by the dashed vertical line). The dashed horizontal line represents the 3 mm threshold for acceptable head movement during an fMRI scan. Line type representing specific plane of movement (X, Y, Z) as indicated in the figure.



**Figure 4-5.** The maximal observed offset of head movement associated with the initiation of exercise. Offset calculated as the difference between the first 5 seconds of exercise and 5 seconds during baseline.

\* Significantly less than Trial 1 in the X-axis,  $p < 0.05$

#### 4.5 Discussion

This study sought to identify the magnitude and repeatability of the  $\dot{V}O_2$ , HR, and head motion responses of a novel whole-body exercise protocol across a range of perceptually regulated exercise intensities to assess its applicability for use in fMRI-based studies. The key novel findings of the study were: 1) the magnitude of metabolic and HR responses to WBI were comparable to previous in-MRI exercise studies, evidenced by estimates of power output using WBI  $\dot{V}O_2$  data, 2) metabolic and HR responses demonstrated good-to-excellent test-retest reliability at each perceptually regulated stage, and 3) the head movement produced during WBI

exercise was within the movement threshold for fMRI scans. The results from this study suggest that WBI exercise can evoke significant increases in metabolism which demonstrate good repeatability and effectively controls head movement within the acceptable range of an fMRI scan.

#### **4.5.1 Metabolic responses to WBI exercise**

The original conception of WBI exercise was born out of a desire to provide a universally accessible exercise modality for fMRI investigations into neural firing during exercise that may be an antecedent the post-exercise enhancement of cognitive function observed following large muscle mass exercise (3). Indeed, this transient facilitation of cognitive function is likely in part due to mechanisms of arousal evoked by exercise (2, 6), and therefore require a sufficient metabolic stimulus (3). As such, an important factor in creating an exercise protocol intended for use in an MRI is the ability to elicit a metabolic response that is similar to previous in-MRI exercise studies that used specialized equipment. We observed that participants were effective at significantly increasing their  $\dot{V}O_2$  and HR with each subsequent increase in RPE-guided exercise stage during the WBI ramp test (Figure 4-2). In an attempt to contextualize what  $\dot{V}O_2$  achieved during WBI represents in Watts equivalent, we had participants perform a submaximal upright cycling exercise protocol to determine their  $\dot{V}O_2$ -to-power slope. From this, we identified that  $65.3 \pm 28.2$ ,  $95.4 \pm 34.0$ , and  $134.5 \pm 44.4$  Watt equivalents for  $\dot{V}O_2$  were achieved during 13, 15, and 17 RPE, respectively (Figure 4-3). The estimated Watt range in our study is similar to that performed by Fontes et al., (10), who had individuals perform six 2-minute intervals at work rates corresponding to 95-135 W using an MRI-adapted cycle ergometer. Similarly, Hiura et al., (14) prescribed exercise intensity using a target HR between 50-60% of participants' age predicted maximal HR during a bout of cycling exercise in a PET scanner, which corresponded to an average of 72 W. By comparison, the average HR for 13 and 15 RPE during WBI exercise correspond with 57 and 66% of participants' maximal HR, respectively.

Interestingly, the metabolic responses and therefore estimated power output to WBI exercise are considerably less than those observed during the upright cycling trial at a given RPE. At the group level, the average  $\dot{V}O_2$  achieved during WBI at Hard is similar to 90 W of cycling; however, the average reported RPE at 90 W cycling was 10.5, which is considerably lower than 15 during WBI. Likewise, the group average  $\dot{V}O_2$  achieved during WBI at Very Hard was similar to 120 W cycling, and the average reported RPE for cycling was 12 compared to the 17 during WBI. As such, it appears that the perceived effort required to perform WBI is considerably higher for a given metabolic cost compared to cycling exercise. Despite these differences, the metabolic responses evoked by WBI exercise are similar to those reported in previous in-MRI exercise studies (10, 14). This is important for two reasons: 1) WBI presents as an equipment-free exercise protocol that can achieve similar metabolic responses as MRI-compatible exercise equipment, and 2) it has been previously demonstrated that exercise performed at similar submaximal intensity evokes significant increases in regional cerebral blood flow, suggesting that WBI could be a viable exercise stimulus for investigating responses of the brain to whole-body exercise, and possibly provide insight into the events that precede improved cognitive function post-exercise.

The metabolic response to Very Hard was predictably the highest of the three stages; however, it also created a large amount of head movement that could not be eliminated. Given that participants reported going “all-out” during the Very Hard stage and that they would not be able to increase their contraction intensity if asked, we interpret this as the maximal effort achievable during WBI exercise. WBI is an irregular exercise protocol and increasing contraction intensity without the resistance of an external load performed over a larger range of motion is difficult. As such, Very Hard represents the intensity ceiling of the WBI exercise protocol but is likely unsuitable for MRI-based studies. Importantly, the metabolic responses achieved during the Somewhat Hard and Hard stages are within the same range as those seen in previous in-MRI exercise studies (10, 14), and this, coupled with the demonstration of good-to-excellent reliability

and minimal head movement, supports the utility of WBI as an exercise protocol in MRI-based studies.

#### **4.5.2 Reliability**

The advantage of this newly developed WBI exercise protocol is that reliable metabolic responses can be achieved without the need for exercise equipment, subsequently providing an accessible tool for other research groups. The potential limitation is that it therefore does not afford measurement of power output (energy requirement and therefore cost). To overcome this barrier, we elected to use RPE to prescribe intensity, which has strong test-retest reliability for progressive treadmill (7, 18) and cycling exercise (8). To assess reliability, we calculated the CV and ICC of trials 1 and 2 for measures of HR and relative  $\dot{V}O_2$  at each stage of WBI exercise and our findings indicate that WBI has good-to-excellent test-retest reliability for these measures.

An important finding, and recurring theme of this protocol, was that a familiarization session is necessary in order to teach participants how to perform the exercise, especially for lower intensities. Pilot work for this study demonstrated the necessity of the familiarization session and repeatability analysis from the current study confirmed this finding as the ICC and CV for relative  $\dot{V}O_2$  measures from the familiarization session and trial 1 during Somewhat Hard were 0.63 and 15%, respectively. Following a familiarization session, however, these measures were improved as the ICC and CV for  $\dot{V}O_2$  between trial 1 and 2 were 0.75 and 12.6%, respectively. These findings are in agreement with others who report that reliability measures are strengthened by a familiarization session for exercise performed at lower RPE (i.e., 13) (7, 8). We therefore emphasize that a familiarization session with the WBI exercise is necessary to ensure reliable within-subject performance.

#### **4.5.3 Head movement in simulated MRI environment**

Given that WBI was created for use in an MRI and that controlling head movement is paramount for obtaining viable fMRI scans, we sought to characterize the head movement

associated with WBI exercise in a follow-up study. For this study, we purposely did not make attempts to brace the head or body beyond the standard of care used at the Queen's University Centre for Neuroscience Studies' MRI facility, as we were interested in characterizing the amount of head movement that is produced by performing this exercise without specialized restraints (i.e., vacuum pillows, chin-straps, etc.).

From previous research, we interpret the threshold for digital motion correction to be 3 mm (9, 10) and used this as the movement threshold for WBI. The main finding was that the large majority of movement produced during WBI exercise was below 1 mm, which has been suggested as unavoidable during any fMRI scan (9). We quantified the number of excursions above 3 mm committed by the entire sample during our three trials and results indicate that there was a total of 36 / 13,962 excursions above 3 mm committed across all 14 participants over the three trials of WBI exercise (Figure 4-4). Said another way, 81.4% of total WBI exercise was under 1 mm of movement (11,371 / 13,962 seconds) and 99.7% (13,926 / 13,962 seconds) was under 3 mm of movement. Importantly, there was a significant increase in the percentage of time spent below 1 mm from trial 1 (63%) to trials 2 and 3 (88% and 93%, respectively), reinforcing the benefit of a practice effect. Simply stated, with practice and using the standard of care for head bracing, WBI exercise can effectively minimize head movement within the acceptable limits of an fMRI scan.

The largest source of movement was caused by the initiation of exercise, which we refer to as the offset. The largest offset occurred during trial 1 and significantly improved with subsequent trials (Figure 4-5), which further supports the need for a familiarization trial given the obvious learning effect associated with repeated trials of this novel exercise protocol. A similar finding was reported by Jaeger (16), who observed an average deviation of 1.2 – 1.5 mm at the initiation of a stepping task using a robot assisted stepping device (MACROS). By comparison, we observed an offset of 2.35 mm during trial 1, which was significantly reduced to 1.33 mm during trial 2 and 0.89 mm during trial 3.

The issue of movement during scanning is not reserved for tasks involving large muscle mass, as this issue transcends most MRI-based studies, including those involving small motor tasks (19, 26). For example, despite that fact that Seto et al., (26) and MacIntosh et al., (19) observed head translation in the range of < 1 mm for young adults during handgrip and ankle dorsiflexion tasks, this movement was further reduced by familiarization sessions. By comparison, Fontes et al., (10) reported a range of 2-8 mm for head translation during cycling exercise but were able to effectively realign the head to constrain motion to be less than 1 mm. Similarly, in order to effectively maintain head movement below 1 mm during very low cadence (30 rpm) cycling in an MRI, Mehta et al., (21) performed a familiarization session and braced the head of participants with a vacuum pillow. Given the success of previous strategies reducing head movement, we expect that using specialized restraints (i.e., chin strap, vacuum pillow) would further minimize the amount of movement created by WBI exercise.

#### **4.5.4 Potential application**

The purpose of creating this WBI exercise modality was to establish a whole-body exercise protocol for MRI-based studies that does not require expensive equipment. There are a number of questions related to regulation of exercise which could be explored using such a protocol. Specifically, we are interested in understanding neural activity during exercise that may explain the facilitation of improved cognitive function post-exercise. Indeed, structures involved in cognitive control, such as the posterior cingulate gyrus, anterior cingulate cortex, hippocampus, and others, have also been demonstrated to be involved in the regulation of exercise (10, 27). However, these types of investigations are limited, likely in part due to the need for expensive exercise equipment that is MRI-compatible.

An overarching theme repeatedly demonstrated by our data is that practice improves WBI performance. Therefore, it is imperative for researchers endeavouring to use this protocol to provide opportunities for structured practice and familiarization with WBI exercise in order to

ensure reliability of performance and minimization of head movement. Further, as suggested by MacIntosh et al., (19) the familiarization session allows for screening for suitable participants, even if motion tracking software is not available. Anecdotally speaking, we found that one can visually discriminate a participant's suitability while performing WBI, as some participants were quite obviously unable to minimize head movement in our simulator, such that 2 participants were excluded from the study prior to data collection.

#### **4.5.5 Experimental considerations**

It is recognized that whole-body contractions are not an analog for cycling or running exercise. The motor patterns associated with WBI are likely entirely different than traditional aerobic exercise, which may have implications for the generalizability WBI to other forms of exercise, especially in the context of exercise and cognitive function. However, WBI exercise can elicit a significant metabolic stress that is comparable exercise protocols using MRI-compatible equipment and could stand as a useful, inexpensive tool for investigating responses of the brain during exercise.

While it is beyond the scope of the present study to discuss specific technical considerations regarding MRI scan acquisition, we feel it is important to at least acknowledge that there are a number of factors beyond head movement per se, that can impact an fMRI scan (i.e., magnetic field distortion due to movement) (25). As such, the next steps for WBI exercise will involve addressing these technical considerations for fMRI scanning

Finally, the applicability of WBI exercise to a larger population is currently unknown, as younger adults may be more effective at minimizing head movement compared to healthy older adults and stroke patients (26). However, while this might constrain populations of interest that can be investigated, there is much to be learned investigating BOLD responses during exercise from the population sampled in the present study. Further down the road it will be important to examine the feasibility of this research tool for other populations.

## **4.6 Conclusion**

Investigation of the brain during exercise using fMRI is significantly limited by the physical constraints of an MRI scanner and the need to minimize head movement. While specialized exercise equipment exists that can effectively minimize head movement, the cost of this equipment renders these types of investigations relatively inaccessible to researchers at large. As such, we have developed a WBI exercise protocol that is the first equipment-free whole-body exercise protocol intended for use in an MRI. We have effectively developed a cost-free in-MRI exercise modality that is capable of eliciting metabolic responses that are similar to previous studies and can effectively minimize head movement well within the acceptable range for fMRI scans without the use of specialized head restraints. Simply put, the WBI protocol has the potential to be accessed by a large range of researchers for the examination of cerebral responses during exercise using fMRI.

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## **Chapter 5**

# **High-Intensity Interval Exercise Impairs Neuroelectric Indices of Reinforcement-Learning**

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High-Intensity Interval Exercise Impairs Neuroelectric Indices of Reinforcement-Learning

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## 5.1 Abstract

A single bout of high-intensity interval exercise (HIIE) improves behavioural measures of cognitive function; however, investigations into the response of event-related potentials (ERPs) that underlie these cognitive improvements are lacking. The reward positivity (RewP) is a positive-going ERP component that indexes reward prediction errors following unexpected 'win' feedback on novel tasks and is a candidate mechanism underlying reinforcement learning. While HIIE improves behavioural measures of learning, it is unknown how HIIE affects the RewP. Therefore, the purpose of this study was to investigate how HIIE affects RewP in response to unexpected reward feedback in university students. Using a single-group randomly assigned counterbalanced crossover design, 25 healthy university students performed HIIE and control visits on separate days. RewP was assessed before (pre-intervention) and 10 minutes after (post-intervention) the intervention period. Participants performed a novel gambling task on a laptop and RewP was determined as the difference wave between win-loss feedback occurring ~250 ms post-feedback. The HIIE intervention consisted of 4 separate body-weight exercises totaling 11 minutes in duration, including rest. The control visit intervention consisted of quietly watching a nature documentary for 11 minutes. Heart rate (HR) was measured at the same time intervals in both trials. Analysis revealed that HIIE significantly diminished the amplitude of RewP in response to 'win' feedback, whereas it remained unaffected in the control condition. HR was significantly higher following HIIE compared to control during post-intervention testing. These findings suggest that mechanisms of reinforcement learning are impaired shortly after HIIE cessation, possibly due to persistent autonomic arousal as evidenced by elevated HR post-HIIE.

## 5.2 Introduction

Participation in regular physical activity elicits beneficial structural and physiological adaptations in the brain across the lifespan (23). In addition to the benefits of habitual exercise, a single bout of aerobic exercise has been shown to be an effective stimulus for improving behavioural measures of cognitive function for up to an hour following cessation from exercise (11). In particular, higher-order top-down processes that govern goal-directed behaviour in changing environments (executive functions) appear to be especially sensitive to acute exercise (3, 13). Mechanistically, exercise increases arousal through the reticular activating system, which has been shown to augment the amount of neural resources allocated to attentional tasks and may act to facilitate improvements in cognitive task performance (7, 14, 45).

These exercise-induced behavioural improvements are supported by underlying changes in neuroelectric activity, as evidenced by event-related potential (ERP) studies (3, 10, 48). ERPs reflect electrophysiological underpinnings of cognitive function, and as a measurement tool, ERPs afford high temporal resolution in characterizing these neuroelectric events (24). The majority of studies examining ERPs post-exercise have focused on the P300 (24, 30, 31, 37), which is an endogenous component that is the neural substrate for processes of stimulus discrimination and information-processing governed by mechanisms of attention, working memory, and decision-making (29, 47). The general consensus in this literature is that continuous aerobic exercise positively modulates P300 amplitude (30, 31) and decreases latency (31, 37), suggesting that greater allocation of neural resources devoted to a cognitive task and faster task-related processing speed occur post-exercise, respectively. We believe that this period of transiently improved cognitive function presents an opportunity to strategically prescribe tasks that require considerable cognitive control, such as learning a new skill (e.g., piano, free-throw shooting); however, aside from the P300, few studies have examined exercise effect on other ERP components involved in executive-control (9, 59).

An important ERP component that has yet to be explored with respect to exercise, and is relevant in the context of learning, is the reward-positivity (RewP) (26, 34, 49). The RewP is a positive deflection that occurs approximately 250 ms following unexpected reward feedback and reflects neural activity associated with early reward evaluation by a reinforcement learning system within the medial frontal cortex (25, 57). Mechanistically, one prominent theory proposes that RewP reflects a phasic burst in dopamine firing within the mesencephalic reward system that projects to the anterior cingulate cortex (ACC) when the outcome of an action is better than predicted (i.e., reward prediction error) (16, 25, 52). Originally, processing of error feedback was associated with a negative deflection that called feedback related negativity (FRN) (44); however, in recent years it has become accepted that this response reflects the modulation of a positive waveform, RewP. This recognition of a positive waveform comes from changes in analytical approaches using difference waveform subtraction, as well as evidence from experimental manipulations demonstrating that negative or breakeven feedback appears to elicit a baseline response for error processing (26, 49). Accordingly, RewP represents the addition of neural processes devoted to processing reward-related feedback and serves as biomarker for early learning, such that the magnitude of response following reward-related feedback (RewP) predicts successfully learning a variety of tasks (2, 17, 34, 40, 41). As such, given the positive impact of exercise on cognitive function it makes sense that the amplitude of the RewP would be increased following exercise.

Justification for the possibility of acute exercise modulating RewP is two-fold: first, dopamine signalling on the ACC is a proposed mechanism underlying RewP (25). Exercise increases dopamine release in the brain (32, 56), which contributes to enhanced cognitive function following exercise through mechanisms of arousal (14, 43, 53). Second, the ACC is a target structure for a myriad of higher ordered cognitive processes including reinforcement learning and attentional functions (27, 39), the latter of which are positively modulated following a dose of exercise (9, 23, 63). As such, given the upregulation of the dopaminergic pathway

during exercise and the convergence of effect on the ACC, it stands to reason that a single bout of exercise may also enhance mechanisms of reward learning.

This leads us to propose that learning a new skill may benefit from performing a preceding bout of exercise that augments reinforcement learning systems in order to improve performance (34). Accordingly, investigation into accessible and time-effective exercise modalities that boost learning is warranted. High intensity interval exercise (HIIE) is a time-effective exercise stimulus that elicits metabolic and fitness adaptations to the same magnitude as traditional continuous aerobic exercise but in a fraction of the time (5), and emerging evidence suggests that a bout of HIIE improves selective attention, inhibition, and learning (1, 4, 42, 60). Importantly, these functions are facilitated by the ACC (6, 8), suggesting that HIIE modulates ACC activation in a manner that facilitates improved executive control. As such, we assert that a single bout of HIIE may have a modulatory effect of the RewP, which would have implications for learning; however, the effect of HIIE on RewP is currently unknown. Therefore, the purpose of this study was to examine the RewP response to a gambling task following an acute bout of whole-body HIIE in young adults. We hypothesized that RewP amplitude would be enhanced following a bout of HIIE compared to rest.

## **5.3 Methods**

### **5.3.1 Participants**

Healthy young male and female young adults were invited to volunteer in this study ( $n = 25$ , 16 females; age =  $22.4 \pm 3.5$  years; BMI =  $22.5 \pm 2.4$  kg/m<sup>2</sup>). Prior to acceptance in this study participants were deemed suitable to perform high-intensity, whole-body exercise as determined by an exercise screening questionnaire. Participants were excluded from this study if they were diagnosed with ADHD or major depression. All participants read and signed consent forms that explicitly detailed study procedures and the associated risks. All experimental procedures were in accordance with the Declaration of Helsinki, and subsequent revisions, and this study was

approved by the Health Sciences Research Ethics Boards at Queen's University and University of Victoria.

### **5.3.2 Experimental protocol**

This study consisted of two experimental visits (exercise and control) that were separated by a one week washout period. A counter-balanced crossover design was used, wherein participants were randomized to either exercise or control for their first visit, and subsequently performed the other experimental visit following the washout period. For both visits, heart rate (HR) measures were obtained using HR monitors (Polar Electro, Kempele, Finland). Participants were instructed to abstain from exercise for 24 hours and consumption of alcohol or caffeine for 12 hours prior to the experimental visits. Otherwise, participants were instructed to maintain their regular activities in between visits to the lab.

### **5.3.3 Exercise visit**

For the exercise visit, participants were asked to wear a Muse EEG headband and performed the pre-EEG testing session. Following completion of the EEG testing participants performed the HIIE protocol, which was a modified Tabata (58) protocol and was 11 minutes in duration including rest periods, with a total exercise time of 5.5 minutes. The protocol consisted of four exercise blocks, each consisting of four 20 s exercise intervals separated by 10 s rest intervals. Each exercise block consisted of a different whole-body exercise, which were: 1) burpees, 2) jumping jacks, 3) mountain climbers, and 4) squat-jumps. A one-minute rest period was provided following the completion of each exercise block (Figure 5-1). Participants were instructed to perform each exercise as fast as possible while maintaining proper form. Researchers provided continual verbal encouragement and feedback regarding movement technique throughout the exercise session. Following completion of the HIIE bout, participants were given ten minutes to recover prior to performing the post-exercise EEG testing. HR measures were obtained

immediately before commencement of the pre- and post-EEG testing, and during the one minute rest periods following an exercise block.

#### **5.3.4 Control visit**

For the control visit, participants performed the pre-EEG testing, after which they watched a nature documentary for 11 minutes to account for the elapsed time of the HIIE intervention, followed by a 10-minute recovery period prior to performing the second cognitive testing session. Participants remained seated for the duration of the documentary and did not have access to electronic devices. HR measures were obtained at the same time points as the exercise visit.

#### **5.3.5 Cognitive task**

Participants were seated in a quiet room and performed a reward learning task on an 11" MacBook Air laptop (Apple, Inc., California, USA) with participants wearing the MUSE EEG headband (InteraXon, Inc., Toronto, Ontario, Canada). Participants performed a novel gambling task where they were instructed to make a choice between two squares that were linked to probabilities of winning. Participants made responses to the feedback (see Figure 1) with the "f" and "j" keys ("f" key for square appearing to the left and "j" key for square appearing to the right) on the laptop keyboard. On each trial participants viewed a black fixation cross against a grey background (MATLAB RGB value = [108 108 108]). Participants were instructed to fixate on the black fixation cross and to keep eye blinks to a minimum. This cross was presented for 300 to 500 ms and followed by a pair of squares on either side of the fixation cross. Participants were asked, on each trial, to select one of the squares and feedback was presented 300 to 500 ms after square selection and remained visible for 1,000 ms ("WIN" for wins, "LOSE" for losses). The next trial began immediately after feedback offset. Each square was assigned a win probability such that one square would "win" more often than the other (60% vs. 10% win/loss ratio). The location of each square (left, right) was randomly determined for each trial and win/loss ratio to

colour mapping did not change throughout the experiment. New colours were randomly selected for every block. Participants completed 5 blocks of 20 trials.

### **5.3.6 Data acquisition**

For data acquisition, the experimenter ensured laptop batteries were fully charged. MacBook Air laptops were disconnected from power outlets as, found in pilot testing, 60 Hz noise could be introduced into EEG channels. Therefore, unplugging the laptops prevented electrical noise contamination. Data were recorded from a MUSE EEG headband with research preset AD (500 Hz sampling rate, no onboard data processing; InteraXon, Toronto, Ontario, Canada) (see <http://developer.choosemuse.com/hardware-firmware/hardware-specifications> for full technical specifications). The MUSE EEG system has electrodes located analogous to Fpz, AF7, AF8, TP9, and TP10 with electrode Fpz used as a reference electrode. Data were streamed from the MUSE device via open sound control (OSC) protocol (see <http://www.neuroeconlab.com/muse.html> for all configuration, setup, methods and software). We sampled 1,000 ms of data into MATLAB for every trial – from 200 ms before stimulus onset to 800 ms after. Data were subject to a small, variable Bluetooth transmission delay measured elsewhere (35) (also see <http://developer.choosemuse.com/protocols/data-streaming-protocol>).

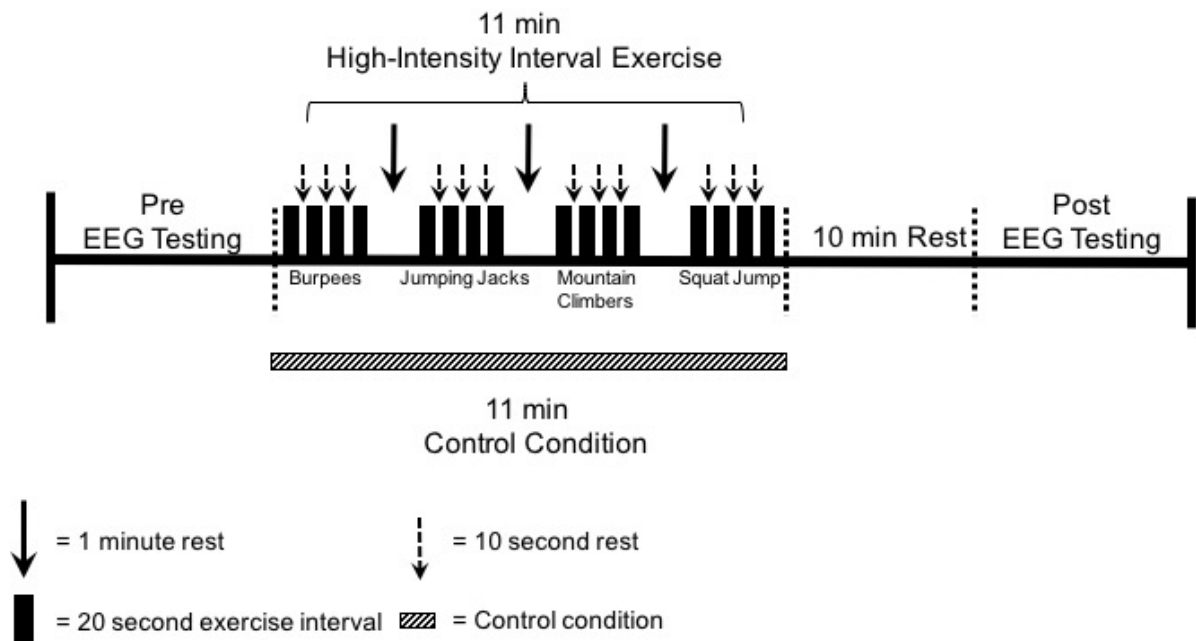
### **5.3.7 MUSE data processing**

The Muse EEG was processed in the same manner as done previously (35). The raw Muse EEG data were converted to a format suitable for BrainVision Analyzer (available at <http://neuroeconlab.com/muse-analysis.html>) (35). Following the analysis of the Muse data, the ERP components were quantified. Data were re-referenced to the average of electrodes TP9 and TP10 and filtered with a dual pass Butterworth filter with a passband of 0.1 Hz to 15 Hz in addition to a 60 Hz notch filter (35). Next, the data were segmented from stimulus onset to 600 ms after. A baseline correction was applied using a time window from stimulus onset (0 ms) to 50 ms after. This was done as data before stimulus onset were not collected. We shortened each

trial epoch and then applied an artifact rejection algorithm in which voltage gradients larger than 10  $\mu\text{V}/\text{ms}$  and/or an absolute voltage difference greater than 100  $\mu\text{V}$  were removed. Segmented data were separated according to condition (win or loss). Electrodes AF7 and AF8 were pooled and ERP averages were calculated for each condition for every participant. Finally, a difference waveform for each condition (i.e., pre-HIIE, post-HIIE, pre-control and post-control) was calculated by subtracting loss waveforms from the win waveforms (i.e., win – loss) for each participant. Next, we calculated grand average condition waveforms and difference waveforms across all participants and found the grand average RewP difference wave peak was 297 ms.

### 5.3.8 Data analysis

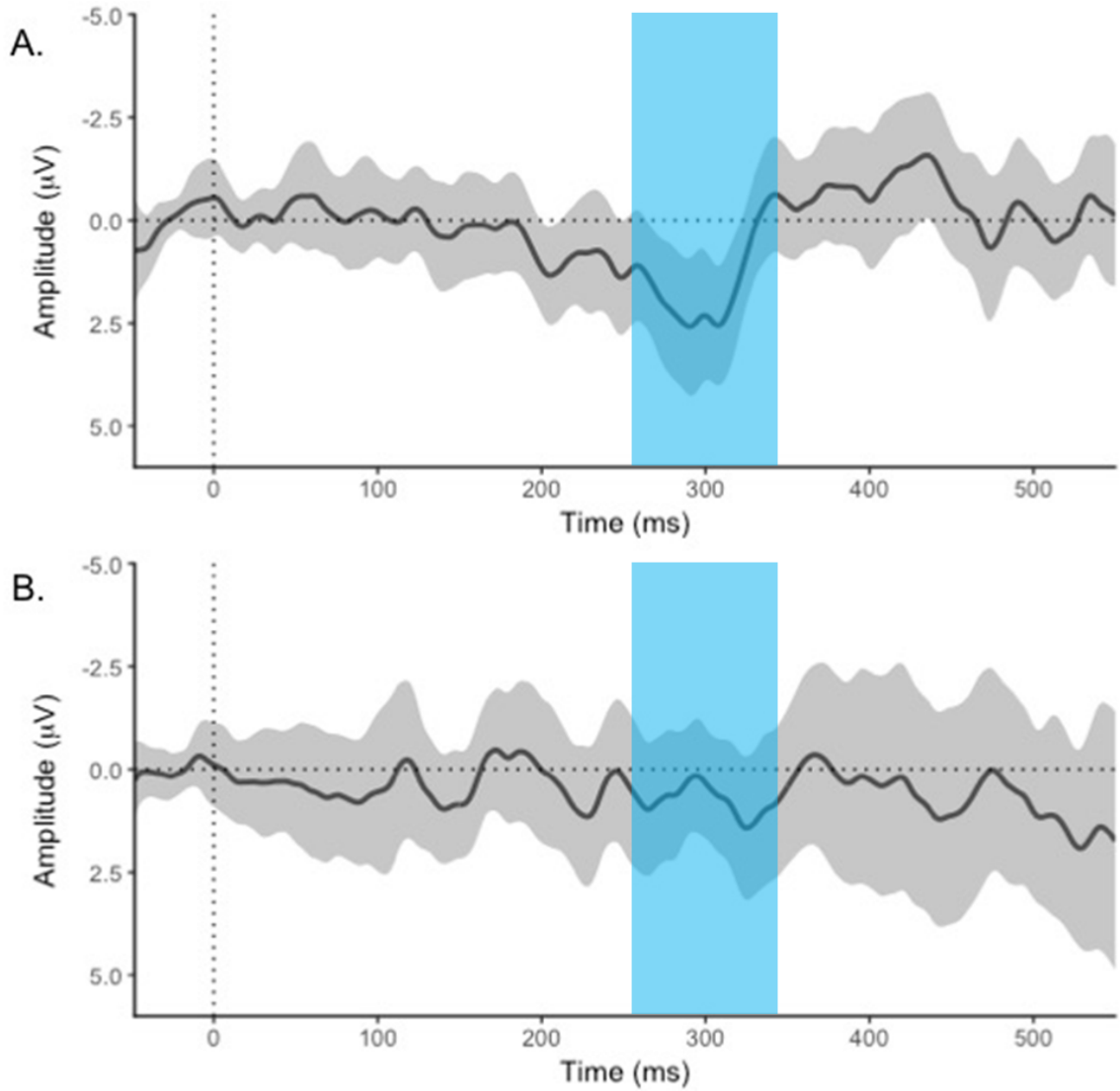
For the RewP, mean peak amplitudes were extracted from difference waves (i.e., pre-HIIE – post-HIIE; pre-control – post-control). To confirm statistically significant differences 95% highest density Bayesian credible intervals were conducted within pre-exercise, post-exercise, pre-control and post-control difference waves (i.e., win – loss). This test was performed on pre-exercise and post-exercise difference waves, and pre-control and post-control difference waves.



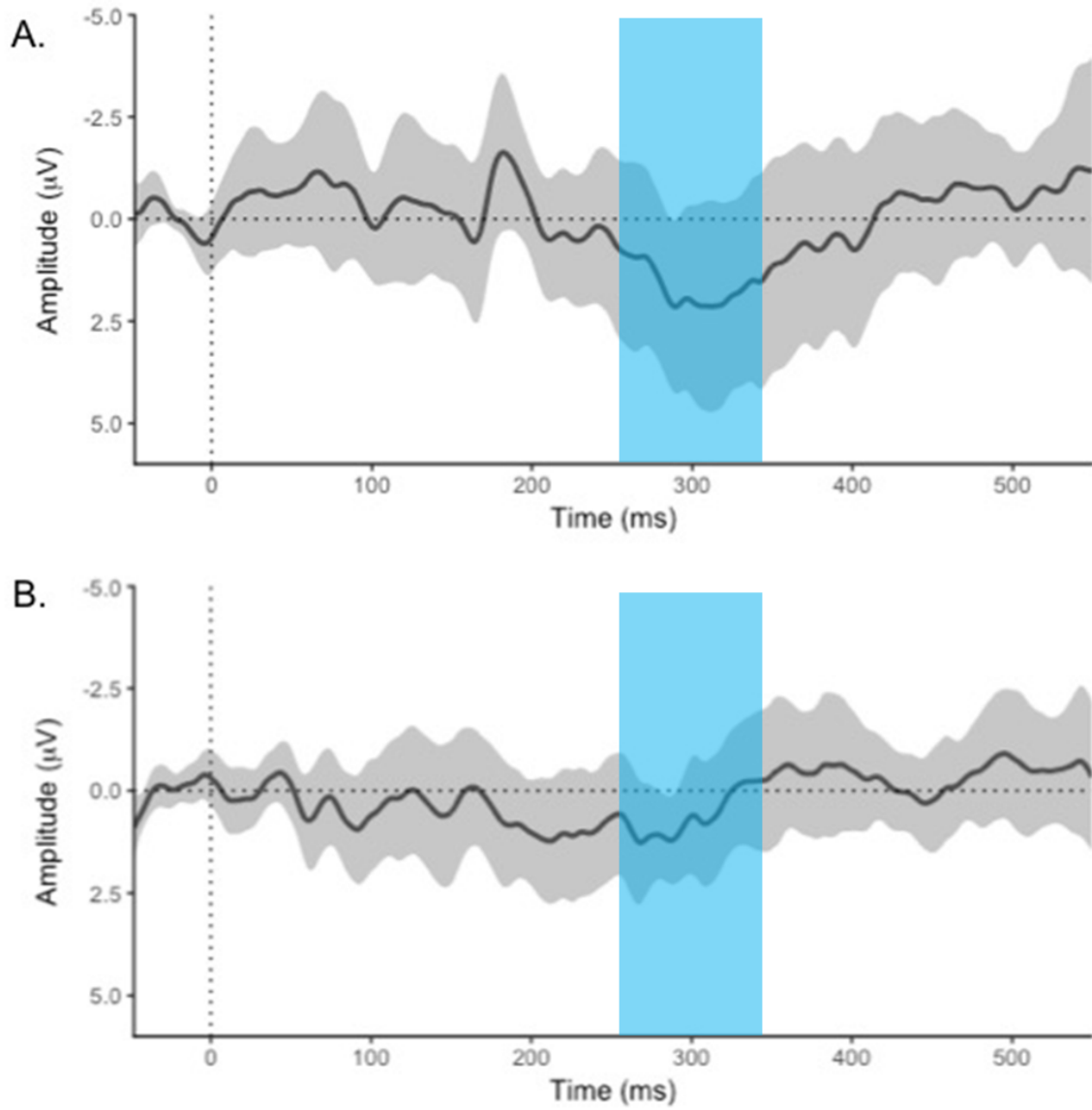
**Figure 5-1.** Protocol schematic for the HIIE and Control conditions.

## 5.4 Results

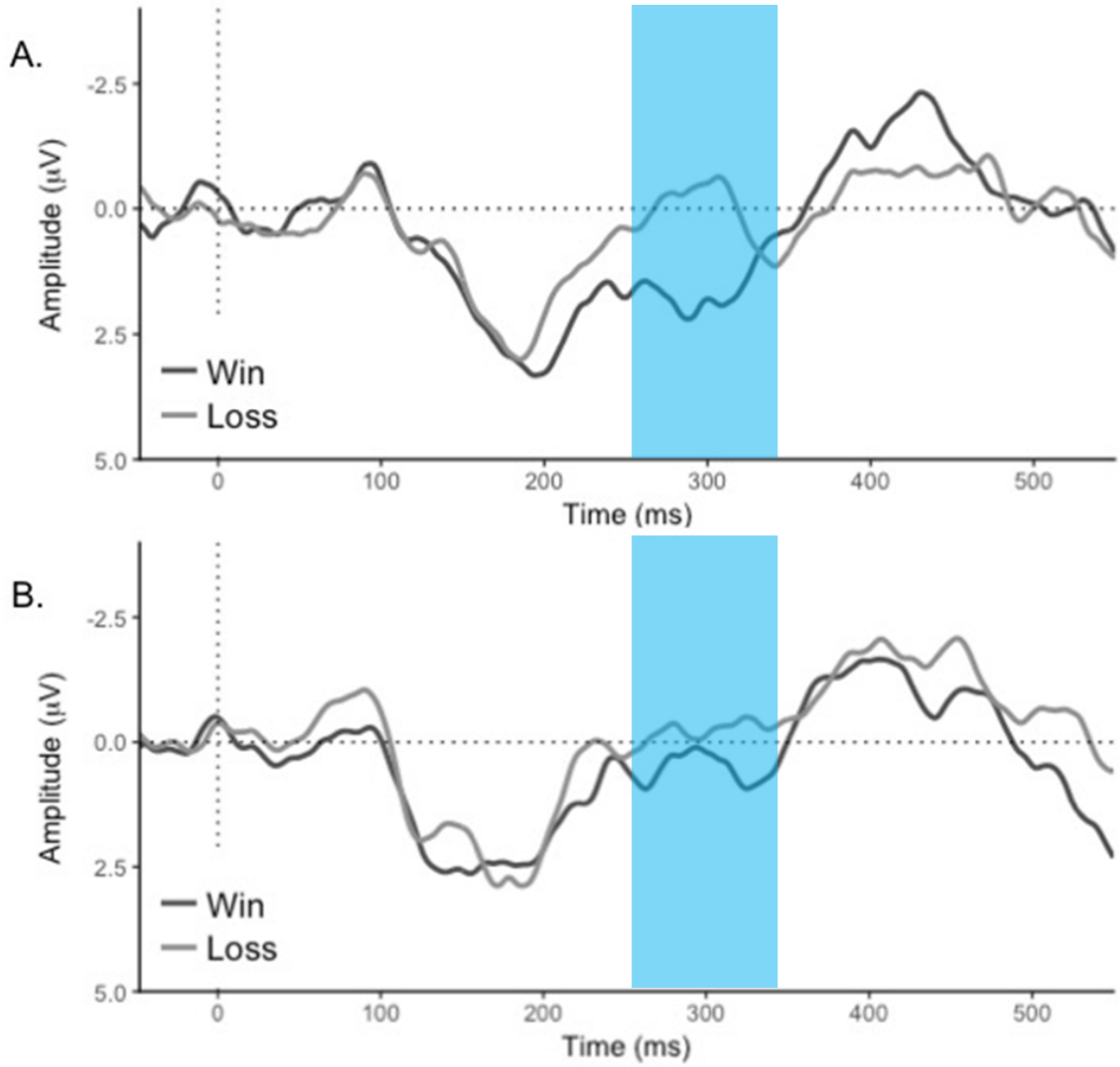
Analysis of each difference wave revealed a positivity consistent with the timing of the RewP. In other words, the “win” feedback stimuli modulated the amplitude of the reward positivity relative to that generated by “loss” stimuli in both pre-HIIE (Bayesian HDI:  $M_d = 1.17 \mu\text{V}$  [0.95  $\mu\text{V}$  1.4  $\mu\text{V}$ ]; Figure 5-2 A) and pre-control difference waves (Bayesian HDI:  $M_d = 1.30 \mu\text{V}$  [1.11  $\mu\text{V}$  1.48  $\mu\text{V}$ ]; Figure 5-3 A). However, the reward positivity effect was diminished post-HIIE (Bayesian HDI:  $M_d = 0.01 \mu\text{V}$  [-0.15  $\mu\text{V}$  0.18  $\mu\text{V}$ ]; Figure 5-2 B) but remained unchanged post-control (Bayesian HDI:  $M_d = 1.6 \mu\text{V}$  [1.47  $\mu\text{V}$  1.72  $\mu\text{V}$ ]; Figure 5-3 B). Additional analysis was conducted on the difference waves quantifying the difference between pre-exercise and post-exercise, as well as the difference between pre-control and post-control conditions. These analyses found that pre-exercise reward positivity difference wave was larger than post-exercise reward positivity difference wave (Bayesian HDI:  $M_d = 0.69 \mu\text{V}$  [0.28  $\mu\text{V}$  1.11  $\mu\text{V}$ ]; Figure 5-4). Curiously, pre-control reward positivity was larger than post-control reward positivity (Bayesian HDI:  $M_d = 0.37 \mu\text{V}$  [0.11  $\mu\text{V}$  0.64  $\mu\text{V}$ ]; Figure 5-5).



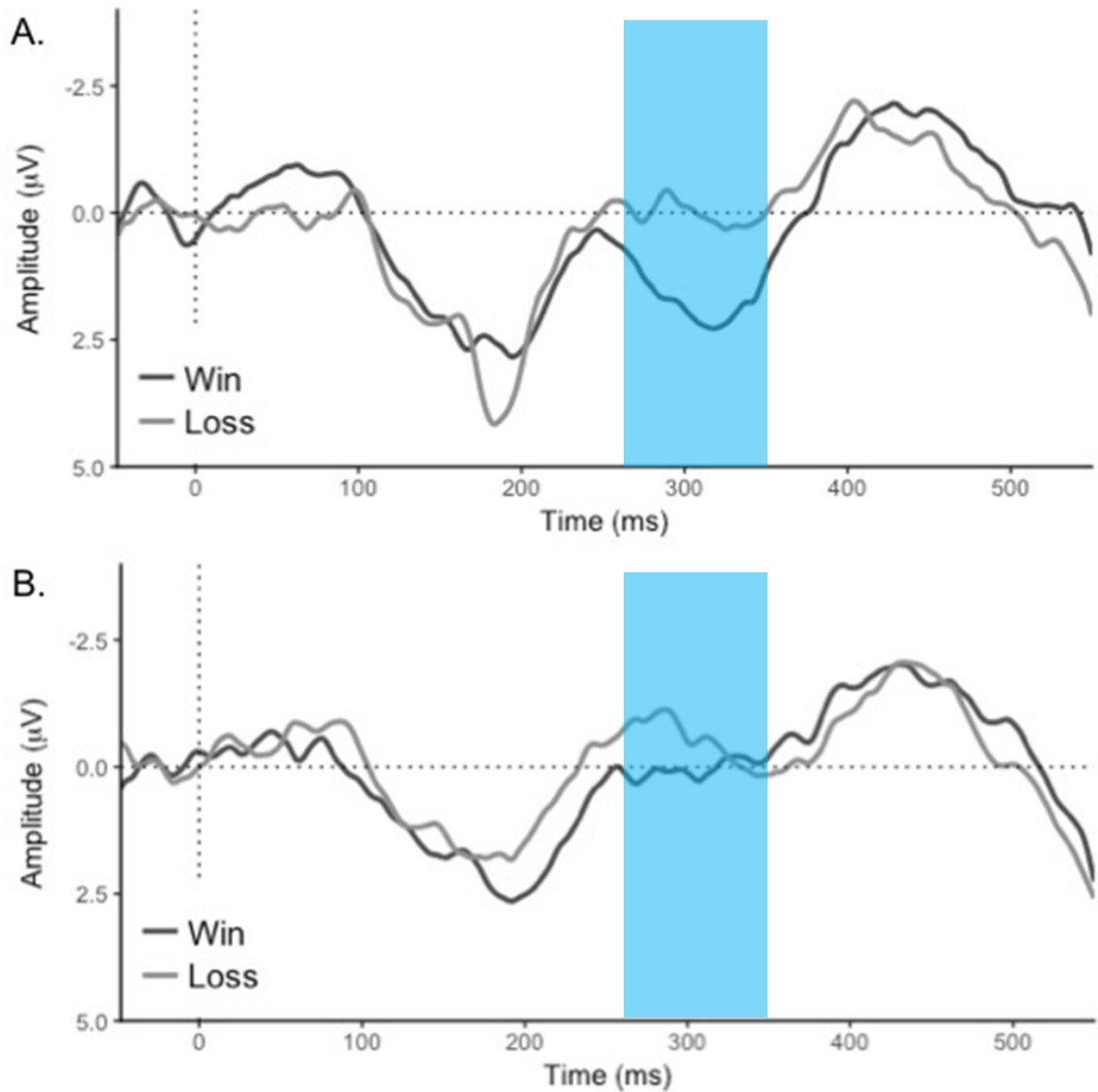
**Figure 5-2.** Difference waveform (win – loss) for A) pre-HIIE, and B) post-HIIE trials. Gray shaded region represents 95% confidence intervals encompassing the difference waveform. The vertical blue shaded region between 250 ms – 350 ms represents RewP.



**Figure 5-3.** Difference waveform (win – loss) for A) pre-control, and B) post-control trials. Gray shaded region represents 95% confidence intervals encompassing the difference waveform. The vertical blue shaded region between 250 ms – 350 ms represents RewP.



**Figure 5-4.** Grand average conditional waveforms in response to the reward-learning task for A) pre-HIIE, and B) post-HIIE. The vertical blue shaded region between 250 ms – 350 ms represents RewP.

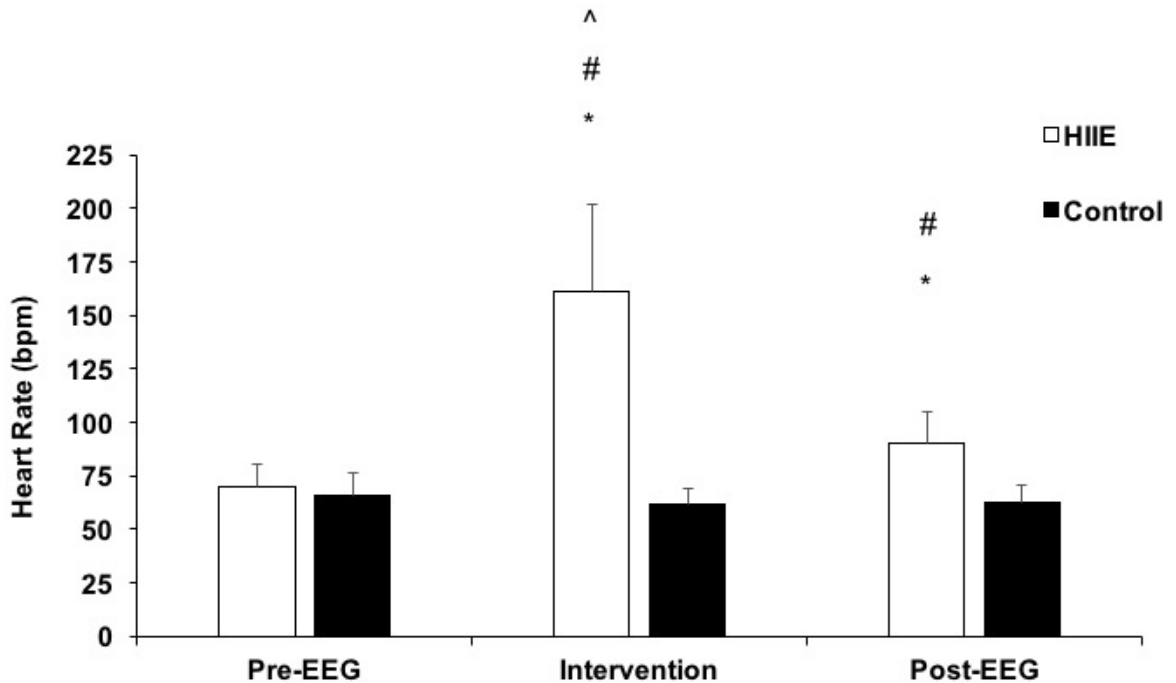


**Figure 5-5.** Grand average conditional waveforms in response to the reward-learning task for A) pre-control, and B) post-control trials. The vertical blue shaded region between 250 ms – 350 ms represents RewP.

#### 5.4.1 HR responses

There was a significant time x condition interaction on measures of HR (Figure 5-6). HIIE significantly elevated HR and HR remained significantly elevated during the Post-EEG testing session,  $F_{(2, 149)} = 106.8$ ,  $p < 0.05$ . Significant effect of time, with HIIE HR being significantly

higher than Pre and Post-EEG testing, and Post-EEG testing being higher than Pre-EEG testing,  $F_{(2, 149)} = 73.9, p < 0.05$ .



**Figure 5-6.** HR responses throughout the experiment.

\* Significantly different compared to Control condition at the same time point

# Significantly different compared to both pre-EEG HIIE and Control conditions

^ Significantly different compared to both post-EEG HIIE and Control conditions

## 5.5 Discussion

In the present study, we sought to investigate the effect of HIIE on RewP, an ERP component that indexes reward prediction errors related to processes of reward-learning. To the best of our knowledge, we are the first group to investigate the effect of exercise on RewP. We instructed participants to make a choice between two alternatives that were linked to a particular probability to produce a win (0.6 compared to 0.1 win probabilities). Each participant's aim was to learn the association between each alternative and win probability by trial and error. Results from this work demonstrate that a preceding bout of HIIE abolishes RewP in response to 'win' feedback on

a reward-learning task, suggesting that HIIE temporarily impairs reward learning mechanisms immediately after HIIE. This finding stands in contrast to reports that a bout of HIIE improves behavioural measures of executive function (1, 42, 60), and shed light on how HIIE affects mechanisms of reward-learning. Importantly, we interpret this effect to be solely due to exercise since RewP was unaffected following a period of rest in the control condition.

The present finding contradicts positive exercise effect on cognitive function observed elsewhere (11), as accumulating evidence supports the ability of acute exercise to transiently improve behavioural (11) and neuroelectric indices (24) of cognitive function for up to an hour post-exercise. While acute exercise positively modulates the P300 (24), only one previous study has investigated ERP componentry related to reinforcement learning following acute exercise in young adults. From this work, it was found that 30 minutes of submaximal treadmill exercise did not affect the response-locked ERP upon error commission (error-related negativity; ERN) during the flanker task (59). However, it is important to note that negative feedback (FRN) and response-related error commission (ERN) represents the baseline response of this high-level error-processing system as only reward conditions evokes an additional positivity, even in the situations where breaking even is the best possible outcome (36). The positivity observed in reward trials (calculated as win – loss ERP difference wave) represents the addition of neural processes related to reinforcement learning that is sensitive to the discrepancy between predicted and actual value of an outcome (26, 49). As such, the mechanisms underlying RewP might be differentially affected by acute exercise compared to ERN. Moreover, the resulting ERP from feedback-locked and response-locked tasks represent different aspects of the error-processing system (64) and therefore may have divergent responses to acute exercise. Recently, we (**unpublished observations**) and others (1, 60, 62) demonstrated that brief, HIIE improves behavioural measures of cognitive function, which led us to hypothesize that HIIE may also improve mechanisms of reward learning. However, the observed loss of RewP in response to ‘win’ feedback following HIIE stands opposed to this hypothesis. Mechanistically, we interpret

reduced RewP amplitude following HIIE as a reduction in the phasic dopamine learning signal transmitted from the striatum to the ACC following unexpected reward feedback (25, 52).

### **5.5.1 What may account for the present observations?**

RewP magnitude is sensitive to a number of different factors including the perceived value of the reward (61), an individual's motivation (15), inter-individual differences in reward processing (15), and task learning (34). However, these factors are likely not driving the observed effect, as RewP was unaltered in the control (i.e., no exercise) condition. Rather, the reduction in RewP is likely due to alterations in the dopamine reward pathway and/or competition for neural resources during recovery from HIIE as explained by the reticular activating hypofrontality (RAH) model of acute exercise (14). Acute exercise upregulates the midbrain dopamine system (20), increases cortical excitability (50), and enhances long-term potentiation in the motor cortex (53). The neuromodulatory effect of the dopamine system on cortical pathways following acute exercise may have an additive effect over time, as long-term exercise training evokes plasticity of the mesocorticolimbic reward pathway (18) and reduces the positive reinforcing effects of cocaine in rats (55). In humans, however, evidence suggests that reward-pathway activation is downregulated in response to reward stimuli following a bout of exercise, as evidenced by altered activation of the mesocorticolimbic reward-pathway in response to smoking-related images in cigarette-addicted individuals (28). However, acute exercise's effect on the midbrain dopamine system in humans is poorly understood given measurement limitations (54). Peripherally, exercise significantly increases levels of plasma dopamine (54, 62), which was found to predict aspects of learning in young adults (62). However, the relationship between peripheral (circulating) and central (brain) levels of dopamine has yet to be established. Nonetheless, if the midbrain dopamine system is in fact upregulated by exercise in humans, as suggested by rodent studies, one might expect an enhanced RewP response; however, our findings suggest that HIIE

may downregulate the reward pathway, which may be explained by competition for neural resources post-exercise.

A candidate mechanism for the apparent loss of RewP is the RAH model of acute exercise proposed by Dietrich and Audiffren (14), which asserts that exercise involving a large amount of muscle mass performed at higher intensities results in reallocation of cognitive resources from explicit to implicit systems in order to sustain the high computational output required for maintaining exercise and homeostasis. Conditions of high cognitive load blunt RewP relative to low cognitive load conditions due to competition for neural resources during task performance (33). Accordingly, the RAH model would predict that our whole-body HIIE protocol would significantly downregulate prefrontal areas due to reallocation of neural resources required to maintain and recover from exercise. In support of this, van Rensburg et al. (28) observed reduced activation of the dorsolateral prefrontal cortex and increased activation of the ACC and frontal areas responsible for maintenance of homeostasis in response to rewarding stimuli following exercise. As such, the apparent down-regulation of the reward-pathway by HIIE may be due to persistent autonomic arousal following cessation from exercise (12, 38), as evidenced by HR being elevated by  $21.0 \pm 15.9\%$  in post-HIIE testing compared to pre-HIIE in the present study (**Figure 2**). Interestingly, this impairment appears to be short-lived following exercise cessation (11, 14), which guided our rationale for prescribing a 10-minute recovery period following HIIE. We previously observed improved selective attention following the very same HIIE protocol (**unpublished observations**) and deemed this a suitable duration for a recovery period. However, it is possible that the 10 minutes provided in our sample was insufficient for adequate recovery period from HIIE, thereby impairing mechanisms of reward-learning. Accordingly, a relatively longer post-exercise recovery period and/or multiple post-exercise testing time points might reveal differential modulation of RewP post-HIIE due to a reduction in arousal and/or reallocation of neural resources to frontal areas of the brain (14).

### 5.5.2 Implications

In the earliest stages of learning, before action values are known, external feedback regarding performance is required for successful learning. RewP indexes the underlying mechanisms of reinforcement learning that accompany feedback regarding a better than expected outcome on task (25). Modulation of RewP magnitude reflects the discrepancy between the predicted and actual outcome of an action, as well as adjustments in learning mechanisms as an individual moves from external to internal sources of information for error-processing (17, 21, 34). For example, individuals categorized as high learners effectively integrate performance feedback in the early stages of learning, as evidenced by greater RewP magnitude compared to low learners. Importantly, high learners demonstrate a shift from feedback- to response-locked RewP by the end of a task demonstrating that they have learned the action-value mappings, whereas low learners remain dependent on performance feedback (17, 34). As such, it appears that the early RewP responses to feedback is integral for the successful facilitation of learning. While our findings suggest that mechanisms of reinforcement learning are impaired after HIIE, we emphasize that these results should not be extended to the entire post-exercise time period, as more testing time points beyond 10 minutes are required to clarify how HIIE affects RewP.

RewP has implications beyond learning, as individuals with major depression (15), anxiety (19), and addiction (22, 46) appear to have dysfunctional reward pathways. In individuals with depression, RewP is consistently smaller relative to people without depression (15), whereas RewP appears to be hypersensitive to unexpected reward in individuals with problem gambling behaviours (22). Both acute and chronic exercise reduce symptoms of depression and anxiety (51) and positively modulate responses to addictive stimuli (28); however, whether reward-pathways function (i.e., RewP) is improved via exercise is currently unknown. Given the wealth of evidence supporting the therapeutic utility of exercise for mood disorders and addiction, and findings from animal studies showing positive brain plasticity in the mesocorticolimbic reward pathway (18), there is good evidence that RewP may be positively implicated. As such,

characterizing the effect of acute exercise on RewP stands to advance our understanding of how exercise affects mechanisms of learning and could elucidate how exercise remedies dysfunctional neural pathways.

## **5.6 Future directions and conclusions**

We are the first group to investigate the effect of exercise on reward-positivity. Our finding of an abolished RewP in response to unexpected reward feedback 10 minutes after HIIE suggests that mechanisms of reinforcement learning are down-regulated during the initial post-exercise recovery period, possibly due to persistent autonomic arousal. This work represents preliminary exploration into the effect of exercise on RewP, and as such, there are a number of questions that remain to be answered. First, post-exercise measurements need to be extended beyond 10 minutes in order to fully characterize the RewP response. These measures should be coupled to indices of arousal, such as HR, in order to gain a greater understanding of potential mechanisms underlying the modulation of RewP post-exercise. Second, future research should compare differences between HIIE and continuous aerobic exercise, and systematically investigate the potential of an intensity dose-response relationship. Finally, the implications of an altered RewP on learning should also be investigated in individuals with normal and dysfunctional reward pathways, in order to further our understanding of how exercise can be used for enhancing learning.

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## Chapter 6

### General Discussion

#### 6.1 Study #1 – BDNF responses to forearm handgrip exercise

##### 6.1.1 Key findings and implications

Study 1 investigated the serum BDNF and platelet response to short-duration, maximal and long-duration, submaximal forearm handgrip exercise. Previous research has consistently demonstrated that large muscle mass aerobic exercise transiently increases serum BDNF (27); however, the general conclusion regarding the source of this BDNF is suggested to be of a tissue source (i.e., brain). The basis for this investigation was developed from the often overlooked knowledge that exercise induces thrombocytosis via sympathetic activation, and this may account for the large serum BDNF response to exercise. Given that the majority of circulating BDNF is platelet-bound and 30% of platelets are stored in the spleen at rest, we reasoned that an exercise protocol that is capable of evoking thrombocytosis by extension should be capable of increasing serum BDNF. Therefore, we hypothesized that handgrip exercise would increase serum BDNF in an intensity-dependent manner via exercise-induced thrombocytosis. The findings from this work are the first to demonstrate that exercise using muscle mass as small as a single forearm can evoke large, meaningful increases in serum BDNF. The magnitude of this response displayed a non-significant intensity-dependent trend, which was supported by an intensity-dependent thrombocytosis response to forearm handgrip exercise. We did not, however, find any meaningful association between the BDNF and platelet responses to exercise, which we interpret as the addition of *de novo* BDNF from tissue sources. In healthy adults, there is a strong correlation between TGF- $\beta$ 1 (a marker of platelet alpha granule release) and BDNF (24), whereas this relationship is uncoupled in situations of augmented tissue release of BDNF (i.e., allergic

airway inflammation), suggesting that platelets have the capacity for increasing their storage of BDNF in situations of increased tissue release (32).

A host of different tissues produce and release BDNF in an activity-dependent manner (3, 18, 36, 39). Exercise is an energetic and homeostatic stressor that creates physiological conditions conducive to the release of BDNF from the brain and periphery (34, 38, 39). It is therefore not surprising that the amount of BDNF per platelet significantly increases following a whole-body graded exercise test (9). Comparatively, the physiological perturbation created by maximal and submaximal forearm handgrip exercise is magnitudes of order smaller and very localized relative to a bout of maximal intensity treadmill running. For this reason, the observed increase in BDNF per platelet following both exercise protocols in our study is a particularly intriguing finding. This finding suggests that exercise performed with small muscle is sufficient enough of a stimulus to increase circulating BDNF through both thrombocytosis and *de novo* BDNF release from a tissue source, and could stand as a viable exercise strategy for the purpose of increasing circulating BDNF.

### **6.1.2 Limitations**

The purported mechanism driving the large BDNF release observed in our study was thrombocytosis caused by increased SNA during exercise; however, we have no measure of splenic volume in response to exercise. Previous studies have demonstrated that isometric handgrip exercise performed at 40% MVC for 1 minute was sufficient enough of a stimulus to cause splenic constriction, and a small non-significant thrombocytosis. Given that splenic constriction and thrombocytosis are exercise intensity-dependent phenomena (6, 16, 21), we reasoned that handgrip exercise performed at intensities above 40% MVC should evoke a greater splenic constriction and thrombocytosis. Importantly, the thrombocytosis hypothesis was confirmed. Nonetheless, without a direct measure of changes in splenic volume to couple with changes in platelets, we cannot directly establish the source of additional platelets with exercise.

While the spleen contains approximately 30% of the body's platelets at rest, other tissue such as that lungs have been implicated in the storage of platelets (26, 31, 44). However, the lungs appear to be a site of platelet biogenesis from megakaryocytes rather than a secretory organ of mature platelets (31), as evidenced by the significantly lower platelet response to epinephrine infusion in asplenic individuals compared to individuals with spleens (8% vs. 41%, respectively) (44). As such, despite not directly measuring splenic volume in response to handgrip exercise, it is most likely the case that the majority of platelets observed in our study were due to thrombocytosis.

We emphasize that full characterization the circulating BDNF via serum, plasma, and platelet measures is critical for understanding changes in BDNF due to exercise; however, we do not report plasma measures in this study. While blood samples were collected and processed for the determination of plasma, the subsequent analysis repeatedly yielded unmeasurable samples despite considerable time and energy spent troubleshooting and further optimizing the analysis process. This is not an uncommon occurrence, as others have also reported difficulties in the measurement of plasma samples (3, 15, 38). The determination of plasma BDNF, when coupled with serum and platelet measures, can be a useful surrogate for tissue and/or platelet release of BDNF draining the vasculature of active tissue. Nonetheless, the overwhelming majority of blood borne BDNF is platelet bound. Given the established interchange of BDNF between plasma and platelets, the measurement of serum and BDNF/platelet should provide a meaningful characterization of the BDNF system that captures both splenic and tissue sources of BDNF.

In the present study, our sample consisted of young healthy males which limits the generalizability of these findings given that sex differences have been reported in BDNF responses to exercise (42) and platelet BDNF content (33). The decision to only investigate males was made in an attempt to reduce response variability (i.e., menstrual cycle effects on BDNF levels) that was not due to exercise alone (33). While this study only characterized young

males, it provides early evidence for a thrombocytosis-related mechanism and establishes the need to investigate this response in females.

Finally, our study did not control for exercise intensity vs. duration, and differences between ME and SE may be due to these factors. For example, the larger BDNF response in ME may not be due to intensity-related mechanisms. Instead, it is possible that a reduction in circulating BDNF from peak values occurred in SE due to the longer duration of this exercise, as suggested by Marston et al. (35).

### **6.1.3 Future directions**

Study #1 was originally designed to compare plasma BDNF release from active skeletal muscle vasculature vs. systemic changes in serum BDNF via thrombocytosis. In this preparation, arterialized blood samples were drawn from the non-exercising arm to represent systemic BDNF (plasma and serum) and effluent from a deep vein draining active forearm muscle vasculature was collected to examine BDNF release from exercising forearm. The guiding rationale was that SNA evoked by forearm exercise would stimulate thrombocytosis and result in a systemic increase in serum BDNF, whereas the localized conditions within the active forearm muscle would stimulate offloading of BDNF from platelets and the release of BDNF from vascular endothelial cells, thereby increasing plasma BDNF in the venous effluent. However, given inability to measure plasma BDNF in our samples, we were unable to directly make these comparisons in the present study.

Nonetheless, we believe that the proposed model is well suited for investigating localized alteration in the circulating BDNF system, if the determination of plasma can be ensured. A future study that we propose is to replicate the seminal work by Rasmussen et al. (38), who compared plasma BDNF in systemic arterial blood to cerebral venous effluent during exercise and determined that the brain was the primary source of blood-borne BDNF during exercise. Using forearm handgrip exercise, we propose modifying their model by adding blood samples

from a deep vein catheter in the active forearm and characterizing shear stress in the forearm and cerebral vasculature using brachial artery and internal carotid artery ultrasound, respectively. This would allow for comparison of plasma and serum BDNF between active tissue beds (brain vs. skeletal muscle) and systemic circulation. The inclusion of serum provides a measure of total BDNF (platelet bound and free). If changes in plasma are observed in active tissue venous effluent, such as that reported by Rasmussen et al. (38), a measure of serum would provide information about the possible source of that plasma BDNF. If serum increased in localized sample relative to systemic samples, it would provide evidence that the measured plasma BDNF was from a tissue source. However, if serum remained constant with an increase in plasma, it would provide evidence for the possible offloading of BDNF from platelets given that serum represents both platelet-bound and plasma BDNF, suggesting a redistribution of BDNF within the same total pool.

In addition to the proposed model study above, we emphasize that the role of platelets in BDNF dynamics and overall brain health need to be examined more closely. The review of the BDNF literature in Chapter 2 and the extensive discussion in Chapter 3 detail the relationship between platelet-bound BDNF and plasma (free) BDNF. While current convention holds that plasma represents bioavailable BDNF and serum is primarily a storage compartment, we contend that serum also represents bioavailable BDNF by extension of platelet-BDNF dynamics under conditions of shear stress and agonist stimuli (14). Findings continue to emerge implicating serum BDNF as being important for brain health (4, 12, 40); however, the exact role of peripheral BDNF for brain health and the importance of the transient increase with exercise in mediating brain health is unknown. An interesting, yet often overlooked piece of this puzzle is the role of platelet function in modulating levels of serum BDNF and the subsequent bioavailability to central and peripheral tissue alike. Total platelet BDNF content does not determine serum levels *per se*, as dysfunctional mechanisms of platelet release are responsible for lower serum BDNF in depressed compared to non-depressed individuals, despite showing no difference in whole-blood

BDNF (24). The observation that platelet release mechanisms determines serum BDNF (24) and that serum levels reflect cortical levels in animal models (2, 25) led Karege et al. (25) to suggest that circulating platelets could be "...a peripheral mirror of brain BDNF". As such, given the integral role of platelets in peripheral BDNF regulation, future studies should determine the effect of exercise on platelet function and BDNF release to determine the subsequent impact on bioavailability.

## **6.2 Study #2: Demonstrating the utility of a novel, equipment-free whole-body isometric exercise protocol for fMRI-based studies**

Study #2 is our answer to a barrier that I encountered during the early stages of my PhD, which was performing exercise in an MRI. I had an opportunity to collaborate with a research group at the Queen's MRI facility that had access to a custom-made MRI-compatible cycle ergometer. However, after observing pilot work it was clear that the device was poor at controlling resistance and was not able to quantify work rate, and thus a discussion about alternate means of exercising in an MRI began. The specific protocol presented in Study #2 (i.e., duty cycle, RPE prescription, familiarization, etc.) was designed using information from pilot work, which consisted of considerable experimentation and modification of exercise parameters. Therefore, we feel that this, the first iteration of WBI, has been carefully refined and is ready to be used in an MRI setting.

### **6.2.1 Key findings and implications**

The purpose of Study #2 was to validate WBI by characterizing the magnitude and repeatability of metabolic and HR response using RPE. Further, given that WBI was developed to be used in an MRI, we sought to characterize the head movement associated with performing WBI exercise. We found that WBI exercise can achieve significant increases in  $\dot{V}O_2$  and HR that are similar to previous in-MRI exercise studies, and importantly, these responses had good-to-

excellent repeatability using RPE. The other main finding was that WBI produced minimal head movement, suggesting that this exercise protocol would be a useful tool for fMRI scanning. The conception of WBI came from the recognition that traditional forms of aerobic exercise, when stripped down, involve rhythmic co-contraction of large muscle groups that produce marked increases in metabolic demand of the active muscle, as well as the brain (17, 22). Of course, the complex coordination involved in planning and executing locomotor patterns with an activity like running would be considerably different than static isometric contractions; however, in context of investigating how exercise facilitates cognitive function, we believe that increasing brain metabolism and arousal are of greater importance than the specificity of motor activation. We have demonstrated that WBI exercise can achieve metabolic and homeostatic responses that are similar to traditional exercise modality used in previous MRI studies. We view this as support for the potential application of WBI in MRI-based studies examining brain responses during exercise. As discussed earlier, one of the primary mechanisms underlying improved cognitive function following exercise is an optimization of arousal via the reticular activating system. The mechanisms of increased arousal do not discriminate against stimuli type nor exercise modality (10, 37). As such, WBI should be an effective exercise protocol for increasing arousal that facilitates augmented cognition.

### **6.2.2 Limitations**

Our findings provide us with confidence that WBI could be an effective exercise tool for use in an MRI; however, these findings do not exclude the possibility that alternate variations of this protocol could also achieve similar metabolic responses. Unpublished work from our lab has demonstrated that performing lower body-only WBI at different duty cycles (i.e., 2:3 vs. 2:1, contraction:relaxation) elicits similar metabolic and bulk leg blood flow responses at a given RPE. These findings provide additional support for the utility of RPE for prescribing WBI exercise intensity and also suggest that WBI exercise is modifiable to suit the specific needs of a

given study. A longer duty cycle may be an effective strategy for reducing head movement in populations that have difficulty controlling movement when performing the 1:1 duty cycle. For example, Seto et al. (41) found that stroke patients and aged-matched controls were less effective at controlling head movement during hand (finger tapping and grasping) and foot (plantar and dorsiflexion) tasks compared to younger controls. Therefore, being able to modify the exercise protocol for specific populations is an important feature of WBI. Building on this point, the present study demonstrated that healthy young adults were effective at increasing metabolism and controlling head movement during WBI; however, the applicability of WBI to a broader population is unknown. The work by Seto et al. (41) suggests that older adults are less effective at controlling task-related head movement and as such, future research should examine the feasibility of WBI in more diverse populations.

A consistent finding from this study was the importance of familiarization and practice for improving reliability and reducing head movement. We employed a number of familiarization and practice strategies that were effective for our study sample; however, whether these approaches would be similarly effective when used by another research group is unknown. We originally recruited 23 individuals for this study and 4 were not invited back to perform a second trial given their inability to increase metabolism with increasing RPE. As well, of the individuals who participated in the head movement portion of the study, 2 were removed from data collection because of an obvious inability to control head movement. It is possible that individual differences in fitness and athletic experience may explain why some individuals were unable to perform WBI effectively. However, anecdotally speaking, individuals who performed poorly on the WBI trials did not appear to have a consistent set of traits. For example, a nationally ranked varsity athlete was unable to increase  $\dot{V}O_2$  or HR at higher RPE, despite being highly fit and having considerable experience with resistance and aerobic training, whereas others with markedly less training experience were able to achieve substantial increases in these measures. As such, focused investigation into the role of individual characteristics affecting WBI

performance is needed. Of course, other research labs who use WBI will encounter participants that are unable to perform WBI well despite detailed instruction and familiarization sessions; however, poor physical task performance by a research participant transcends all types of human research. Accordingly, we emphasize the importance of carefully selecting participants during the training phase of WBI prior to performing fMRI scans.

### **6.2.3 Future direction**

The next steps for WBI exercise are fairly straightforward: perform this protocol during fMRI scanning and perform a detailed assessment of its suitability for this type of research. Developing a collaboration with fMRI specialists will be crucial, as they can advise scanning sequence parameters and approaches to analysis. During pilot work for this study, I had the opportunity to perform WBI at RPE 13 - Somewhat Hard while being scanned at the Queen's Centre for Neuroscience MRI facility. Following the trial, our collaborator provided a graphical display of head movement during WBI, which resulted in less head movement compared to that caused by deep breathing during a hypercapnic challenge. As such, these findings coupled with the results from the present study give us confidence that WBI will be a useable tool to perform during fMRI scanning.

Another important next step will be evaluating the effect of WBI on cognitive function, which is especially important given that WBI was created to investigate mechanisms of exercise-induced cognitive enhancement. In the context of the current study, the characterization of cognitive function post-WBI was not important given that the purpose was to develop and characterize an exercise protocol for MRI use; however, this is an important feature of WBI that needs to be investigated going forward.

### **6.3 Study #3 – High-intensity interval exercise and the reward-positivity: implications for learning**

Study #3 was the product of two earlier collaborations, one with my colleague and mentor Dr. Gurd from Queen's University and the other with Dr. Krigolson from the University of Victoria, who I met on a research expedition to Mount Everest Base Camp in 2016. The HIIE protocol used in this study was previously investigated by Dr. Gurd's lab and it was found to have a positive effect on measures of selective attention 10 minutes following HIIE. This piqued my interest to investigate the neuroelectric activity that may underlie this response after working with Dr. Krigolson in Nepal. These previous collaborations developed into the present study, which investigated the effect of HIIE on mechanisms of learning using EEG.

#### **6.3.1 Key findings and implications**

The main finding from this study was that a single bout of HIIE abolished RewP in response to reward feedback on a novel gambling task. These findings suggest that mechanisms of learning are impaired during recovery from brief, intense exercise. While exact functional role of RewP is still being elucidated, there is consensus that good learning is characterized by a strong RewP signal in response to feedback during the earliest stages of learning (13), such that the magnitude of RewP predicts successful learning (13, 19). The reliance on external sources of feedback regarding performance is critical in the earliest stages of learning, and as an individual begins to learn action-value mappings, there is a shift from reliance on explicit to implicit sources of performance information (20). Accordingly, the loss of RewP following HIIE suggests that these early stages of learning would be negatively impacted.

The action-monitoring system that is responsible for error-detection represents the intersection between exercise-induced improvements in executive function and the processes underlying reward-learning. In a naturalistic setting, the action-monitoring system guides goal-directed behaviour and does not hold a singular focus. In fact, cognitive resources are competitively partitioned, as other goals are concurrently being monitored, while ensuring that

enough attentional resources are devoted to the task at hand in order to adjust to sudden changes in the environment (19). As such, the human brain makes predictions about the valence of an outcome in an attempt to optimize behaviour and minimize the amount of energy required for successful completion of a task. However, in situations of increased cognitive load or homeostatic disturbance, there is a reduction in the availability of these cognitive resources that can be devoted to a given task (11, 29). In the context of the present study, we propose that the loss of RewP following HIIE is due to persistent autonomic arousal associated with recovery from exercise. According to the RAH of acute exercise, during exercise and the initial stages of recovery there is competition for neural resources that favour processes involved with homeostatic regulation rather than executive control (10, 11). As such, following an intense bout of exercise there is a downregulation of higher order cognitive processes, manifesting as temporarily impaired measures of cognitive function (10, 23, 30). In the context of our findings, one of the strongest pieces of evidence that supports incomplete recovery from HIIE is the finding that higher cognitive load negatively impacts RewP magnitude (28). Accordingly, future investigations are needed to fully elucidate the effect of HIIE, and exercise in general for that matter, on RewP at different stages of recovery.

### **6.3.2 Limitations and future direction**

The biggest limitation of the current work is the post-exercise time course for evaluating RewP, especially in the context of our observed downregulation of the reinforcement learning signal following reward feedback. Based on these findings, one may be tempted to conclude that exercise is detrimental for learning; however, additional time points are needed to fully elucidate the effect of HIIE on these processes. The decision to measure RewP 10-minutes post-exercise was based on the meta-analysis by Chang et al. (8), who observed that the greatest effect of exercise on cognitive function was observed when cognitive tests were administered within 15 minutes of exercise cessation; however, 10 minutes of recovery from our bout of HIIE was likely

insufficient to enhance processes of executive control. As such, this work provides an important first step in that it demonstrates that mechanisms of reinforcement learning are affected by exercise. Accordingly, future studies should extend the post-exercise evaluation period in order to better characterize the effect of exercise on these processes.

The decision to use HIIE as the exercise stimulus was based on a previous study that we conducted, wherein we observed the same HIIE protocol significantly improved performance on the d2 test of attention in a similar population. In particular, the rationale for investigating HIIE in this study was to find accessible means of boosting cognition prior to a cognitively challenging situation, such as a university lecture. From an accessibility stand point, this type of exercise can be performed by most able-bodied individuals with access to minimal space in less than 15 minutes. However, given that this is the first study to investigate the effect of exercise (in general) on RewP, future studies need investigate continuous aerobic exercise at different intensities and durations affects RewP post-exercise. Behavioural and neuroelectric studies suggest that exercise improves cognition in an intensity-dependent manner (7, 23); however, continually emerging evidence suggest that low and high intensity continuous exercise, as well as HIIE can improve cognition (1, 5, 43). As such, systematic investigation into the effect of exercise on RewP is warranted in an attempt to further understand ways in which exercise can boost cognition and learning.

## **6.4 Conclusion**

The overarching theme of this thesis was to further the understanding of how a single bout of exercise can augment brain function from a neurochemical and neuroelectric standpoint. Further, given the measurement limitations of certain neuroimaging techniques, we provide a novel, equipment-free exercise protocol to be used in an MRI in an attempt to further characterize brain responses during exercise. Collectively, these studies represent novel approaches to understanding the physiological and functional responses to exercise, and in doing so provide a

unique, multifaceted characterization of how exercise affects the brain. Exercise is a metabolic and homeostatic stressor that evokes a myriad of physiological responses that collectively protect and improve brain function. The long-term benefits of participation in regular exercise have been extensively demonstrated; however, there is considerable value in examining the acute effects, especially in the context of strategically timing the performance of cognitively demanding activities following a bout of exercise.

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**Appendix A**  
**BDNF Responses to Handgrip Exercise Ethics**



School of Kinesiology and Health Studies  
Queen's University

***Peripheral BDNF Project***

Human Vascular Control Laboratory  
Room 400B, Kinesiology and Health Studies Building  
Michael E. Tschakovsky, Ph.D. – Principal Investigator  
Jeremy J. Walsh, MSc., PhD Candidate – Investigator

**CONSENT FORM**  
**FOR RESEARCH PROJECTS ENTITLED:**  
***Investigation into Peripheral Sources of BDNF in Humans***

**This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.**

**Purpose of the Study:**

***The purpose of this study is to characterize the response of brain derived neurotrophic factor (BDNF) in venous blood during handgrip exercise.***

## Description of Experiment and Risks:

- HEART RATE MEASUREMENTS:** Heart rate is continuously monitored by an electrocardiogram (EKG) through 3 spot electrodes on the skin surface. The electrodes are normally placed in the lower portion of the chest and they can detect the electrical activity that makes your heart beat.  
**RISKS: This procedure is entirely safe. In a very small group of individuals, a skin rash might occur from the adhesive on the electrodes. There is no way of knowing this ahead of time. The rash, if it develops, will resolve itself within a day or so. Avoid scratching the rash and keep clean.**
  
- BLOOD PRESSURE MEASUREMENTS:**  
A small cuff is fit around your finger. This cuff inflates to pressures that match the blood pressure in your finger, so you feel the cuff pulsing with your heart beat. It shines infrared light through your finger to measure changes in the size of your finger with each heart beat.  
**RISKS: These techniques are non-invasive and pose no risk.**
  
- ARM BLOOD FLOW AND BLOOD VESSEL DIAMETER MEASUREMENTS:** The blood flowing through your brachial artery (above the elbow) can be detected and the diameter measured using Doppler and echo ultrasound, respectively. A probe will be placed on the skin over your artery and adjustments in its position controlled by hand by the investigator. High frequency sound (ultrasound) will penetrate your skin. The returning sound provides information on blood flow.  
**RISKS: This technique is non-invasive and poses no risk.**
  
- HANDGRIP EXERCISE:** You will be asked to perform rhythmic handgrip-squeezing exercise at an all-out intensity (~100% of your max strength) for 10 minutes on two separate occasions; the screening visit and an experimental visit (CP Day). You will be continuously encouraged to continue exercising for the full 10 minutes by the research personnel. On a separate experimental visit, you will be asked to perform rhythmic handgrip-squeezing exercise at a moderate-intensity (~50 – 60% of your max strength) for 30 minutes (Endurance Day). You will be continuously encouraged to continue exercising for the full 30 minutes by the research personnel. You have the right to declare that you are unable to continue without any consequence to you. **RISKS: When forearm muscle contractions are maintained, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no known/associated risk to your muscles in performing this exercise. You may experience muscle soreness in the muscles of your forearm for 24-72 hours after**

**performing the handgrip exercise, much as you would if you had been lifting weights.**

- **VENOUS BLOOD SAMPLING:** Blood samples from veins are used to measure the amount brain derived neurotrophic factor and the number of platelets in the blood. For this, a researcher trained and certified in venipuncture (catheter placement into a vein) will use sterile technique to draw blood samples. We will be taking multiple 4 ml samples of blood from a vein at the elbow of the exercising limb and from a vein at the hand on the non-exercising limb, on two occasions during the experiment. The researcher will place a teflon catheter into your vein using sterile technique. The catheter will be secured to your skin with tape and a self-sealing access attached to allow for drawing blood from the vein. This process will be repeated on the non-exercising arm, which will act as the control blood sample. We will take a volume of blood that is in total no more than ~60 ml per day. This represents approximately 1/8<sup>th</sup> of the volume of blood taken when you donate blood (400-450 ml). Periodically, the researcher will, after drawing some blood, inject (flush) sterile saline through the catheter into your vein. When the study is over, we will remove the catheter and secure sterile gauze over the puncture site.  
**RISKS: The most common complications of inserting a small catheter in the arm is a small bruise and pain at the site of catheter insertion. This might last several days after removal of the catheter. It is also possible that this pain may refer down the arm (a “shooting” pain sensation), if there has been nerve irritation in the catheterization process. When the catheter is removed pressure must be applied to the vein to prevent internal bleeding. If adequate pressure is not applied a bruise and some discomfort might result for a short period of time. The puncture site should be kept clean and covered with a sterile gauze pad while stopping the bleeding after catheter removal to prevent infection. There is very little risk of infection or injury to the vein. The amount of blood taken can result in at most a 0.5% reduction in the hemoglobin content in your blood (hemoglobin carries oxygen in your blood), in comparison to ~7.5% reductions experienced when you donate blood. Nevertheless, this 0.5% does constitute an extremely mild anemia, and in the case of a person with chronic hemoglobin disorders it could increase the risk of adverse health consequences.**
  
- **MUSCLE MASS:** Circumference and length measurements of segments of your arm will be taken via manual placement of a tape measure on your limb by the investigator. **RISKS: These techniques are non-invasive and pose no risk.**

- **FOREARM / HAND HEATING:** In order to increase the blood flow through your brachial artery and/or radial artery, your forearm and hand will be enclosed in an electric heating pad. Your hand will be wrapped in the heating pad for < 1 hour and the temperature of the pad will not exceed 40<sup>o</sup>C. The researcher will move the heating pad aside on two occasions in order to access the catheter port during the blood sampling portion of the experiment.  
**RISKS: Your skin may appear red after removal from the heating pad. This is due to increased skin circulation. The redness should resolve as your forearm and hand circulation decrease.**

#### **How long will it take?**

During the initial screening visit we will use ultrasound to get an image of your brachial artery to determine whether you are eligible to participate in the study. You will also be introduced to the principles of a Critical Power test and perform a full critical power handgrip test.

If you are eligible for the study, you will attend two separate testing sessions. During these sessions you will come into the lab following an overnight fast, without consuming alcohol or caffeine in the previous 12 hours and without performing physical exercise in the previous 24 hours. During this time you will perform a bout of handgrip exercise. Each visit will last ~1 – 1.5 hours.

#### **Talking and Movements:**

Talking or moving during the times that we are taking measurements will cause variations in the measurements we are making. If you have any discomfort, please let us know immediately and we can temporarily break from data collection. However, if everything is comfortable, please maintain a very quiet posture. Even very slight movements interfere with our experiments.

#### **Special Instructions:**

Participants are asked to not eat, drink alcohol or caffeine during the 12 hours prior to the study. Further, you are asked to avoid physical exercise for at least 24 hours prior to testing. You should empty your bladder immediately prior to starting the test. ***If these conditions are not met, the experimental session will be canceled.***

#### **Attached Medical Screening Form:**

This questionnaire asks some simple questions about your health. This information is used to guide us with your entry into the study. Current health problems indicated on this form which are related to cardiovascular diseases (including high blood pressure) and liver or kidney problems will exclude you from the study.

### **Safety Precautions:**

Safety precautions for the study will include the following:

- Subjects who enter the study will be identified as healthy
- Before entering the study you will be screened using a medical screening form. You will not be able to enter the study if anything is found which indicates that it is dangerous for you to participate.
- Trained personnel will be supervising every aspect of training. Any concerns or questions should be addressed immediately.

### **Confidentiality:**

All information obtained during the course of the study is strictly confidential and will not be released in a form traceable to you, except to you and your personal physician. Your data will be kept in locked files which are available only to the investigators and research assistants who will perform statistical analysis of the data. There is a possibility that your data file, including identifying information, may be inspected by officials from the Health Protection Branch in Canada in the course of carrying out regular government functions. The study results will be used as anonymous data for scientific publications and presentations, or for the education of students in the School of Physical and Health Education at Queen's University.

### **Study Compensation**

You will receive \$10/hour of volunteering upon completion of the study.

### **Freedom to Withdraw from the Study**

Your participation in this study is voluntary. You may refuse to participate or you may discontinue participation at any time during the duration of the study without penalty.

**Subject Statement and Signature Section**

I have read and understand the consent form for this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I am voluntarily signing this form. I will receive a copy of this consent form for my information.

If at any time I have further questions, problems or adverse events, I will contact:

Michael E. Tschakovsky, Ph.D.  
(Principal Investigator)  
KHS 306, Kinesiology and Health Studies Building  
Queen’s University, Kingston, ON, K7L 3N6  
Tel: (613) 533-6000, ext, 74697

Jeremy J. Walsh, MSc.  
(Investigator)  
KHS400, Kinesiology and Health Studies  
Queen’s University, Kingston, ON K7L 3N6  
Tel: (613) 533-6000 ext. 78425

Jean Cote, Ph.D.  
Director, School of Kinesiology and Health Studies  
KHS 206, Kinesiology and Health Studies Building  
Queen’s University, Kingston, ON, K7L 3N6  
Tel: (613) 533-3054

If I have any questions concerning research subject’s rights, I will contact:

Dr. Albert F. Clark, Chair  
Office of Research Services  
Fleming Hall, Jemmett Wing 301  
Queen’s University, Kingston, ON, K7L 3N6  
Tel: 533-6081

By signing this consent form, I am indicating that I agree to participate in this study.

\_\_\_\_\_  
Subject Signature

\_\_\_\_\_  
Signature of Person Obtaining Consent

\_\_\_\_\_  
Subject Name (please print)

\_\_\_\_\_  
Name of Person Obtaining Consent (please print)

\_\_\_\_\_  
Date (day/month/year)

\_\_\_\_\_  
Date (day/month/year)

**Appendix B**  
**WBI Exercise Ethics**



School of Kinesiology and Health Studies  
Queen's University

***Whole-body Isometric Contraction Project***

Human Vascular Control Laboratory  
Room 400B, Kinesiology and Health Studies Building  
Michael E. Tschakovsky, Ph.D. – Principal Investigator  
Jeremy J. Walsh, MSc., PhD Candidate – Investigator

**CONSENT FORM  
FOR RESEARCH PROJECTS ENTITLED:  
*Whole-body Isometric Contraction Project***

**This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.**

**Purpose of the Study:**

***The purpose of this study is to determine the metabolic responses and the reliability of a novel whole-body isometric contraction exercise protocol for the eventual use in an MRI.***

## Description of Experiment and Risks:

- HEART RATE MEASUREMENTS:** Heart rate is continuously monitored by a heart rate monitor. The heart rate monitor is a strap that is placed around the chest and detects the electrical activity that makes your heart beat.  
**RISKS:** This procedure is entirely safe. If the strap is uncomfortable, please notify a researcher and the strap length will be readjusted.
  
- CYCLING EXERCISE:** You will be asked to perform cycling exercise on an upright stationary bicycle. The cycling exercise protocol consists of four 4-minute stages that progressively increase in difficulty, but the intensity will remain submaximal. You will be asked to maintain a pedal rate of 60 rpm for the entire cycling protocol. Pedal rate will be visually displayed on the bike computer. The purpose of this protocol is to measure how much oxygen your body uses at known work rates. The cycling exercise will only take place during the first visit. **RISKS: as with any exercise, you may experience feelings of discomfort in your legs and an increase in breathing rate, especially at the higher work rates. However, the intensity of the cycling exercise is submaximal and for healthy individuals, there are no risks to performing this exercise. If you experience any joint or muscle pain that is not associated with the exercise, please notify a researcher immediately.**
  
- WHOLE-BODY SQUEEZING EXERCISE:** You will be asked to perform rhythmic contractions of your whole-body. This type of exercise is unique to this study and will require practice in order to become comfortable performing it. You will contract the large muscle groups of your body (legs, glutes, chest, back, core, etc.) for 1 second and relax for 1 second. An auditory metronome will be used as a cue for when to contract vs. when to relax. In order to gauge an intensity for performing the whole-body squeezing, you will be shown the Borg scale of Ratings of Perceived Exertion (RPE) to inform you of how hard you should be squeezing. A researcher will explain the Borg scale in detail prior to commencing exercise. This exercise protocol consists of three 4-minute stages that increase in intensity: 1) Somewhat Hard, 2) Hard, and 3) Very Hard. You will be continuously encouraged throughout the exercise protocol. You have the right to declare that you are unable to continue without any consequence to you. **RISKS: When muscle contractions are maintained, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no known/associated risk to your muscles in performing this exercise. You may experience muscle soreness in the muscles used in this protocol, much as you would if you had been lifting weights.**

- GAS EXCHANGE MEASURES:** The amount of oxygen that your body uses during exercise will be measured via gas exchange. In order to get these measures, you will be required to wear a specialized headpiece containing a rubber mouthpiece that you will breathe into throughout the exercise protocols. You will also wear a nose clip in order to prevent you from breathing out of your nose. It is important that you breathe normally when wearing the headpiece. **RISKS: There are no risks associated with this procedure.**

#### **How long will it take?**

There are 3 visits in total. The first visit is the longest and will take ~1.5 hours. During this visit you will practice the whole-body squeezing exercise, perform the cycling exercise, and finish by performing a session of the whole-body exercise. The second and third visits will be ~20 minutes in length, during which you will repeat a session of the whole-body exercise protocol.

#### **Special Instructions:**

Participants are asked to not drink alcohol or caffeine during the 12 hours prior to the study. Further, you are asked to avoid physical exercise for at least 12 hours prior to testing. You should empty your bladder immediately prior to starting the test. ***If these conditions are not met, the experimental session will be canceled.***

#### **Attached Medical Screening Form:**

This questionnaire asks some simple questions about your health. This information is used to guide us with your entry into the study. Current health problems indicated on this form which are related to cardiovascular diseases (including high blood pressure) and liver or kidney problems will exclude you from the study.

#### **Safety Precautions:**

Safety precautions for the study will include the following:

- Subjects who enter the study will be identified as healthy
- Before entering the study, you will be screened using a medical screening form. You will not be able to enter the study if anything is found which indicates that it is dangerous for you to participate.
- Trained personnel will be supervising every aspect of the study. Any concerns or questions should be addressed immediately.

#### **Confidentiality:**

All information obtained during the course of the study is strictly confidential and will not be released in a form traceable to you, except to you and your personal physician. Your data will be kept in locked files which are available only to the investigators and research assistants who will perform statistical analysis of the data. There is a possibility that your data file, including identifying information, may be inspected by officials from the Health Protection Branch in Canada in the course of carrying out regular government functions. The study results will be used as anonymous data for scientific publications and presentations, or for the education of students in the School of Physical and Health Education at Queen's University.

### **Freedom to Withdraw from the Study**

Your participation in this study is voluntary. You may refuse to participate or you may discontinue participation at any time during the duration of the study without penalty.

**Subject Statement and Signature Section**

I have read and understand the consent form for this study. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I choose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I am voluntarily signing this form. I will receive a copy of this consent form for my information.

If at any time I have further questions, problems or adverse events, I will contact:

Michael E. Tschakovsky, Ph.D.  
(Principal Investigator)  
KHS 306, Kinesiology and Health Studies Building  
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Jeremy J. Walsh, MSc.  
(Investigator)  
KHS400, Kinesiology and Health Studies  
Queen’s University, Kingston, ON K7L 3N6  
Tel: (613) 533-6000 ext. 78425

Jean Cote, Ph.D.  
Director, School of Kinesiology and Health Studies  
KHS 206, Kinesiology and Health Studies Building  
Queen’s University, Kingston, ON, K7L 3N6  
Tel: (613) 533-3054

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Office of Research Services  
Fleming Hall, Jemmett Wing 301  
Queen’s University, Kingston, ON, K7L 3N6  
Tel: 533-6081

By signing this consent form, I am indicating that I agree to participate in this study.

\_\_\_\_\_  
Subject Signature

\_\_\_\_\_  
Signature of Person Obtaining Consent

\_\_\_\_\_  
Subject Name (please print)

\_\_\_\_\_  
Name of Person Obtaining Consent (please print)

\_\_\_\_\_  
Date (day/month/year)

\_\_\_\_\_  
Date (day/month/year)

## **Appendix C**

### **High-Intensity Interval Exercise and Reward Positivity Ethics**



Principal Investigator: Olave Krigolson

Lab: McKinnon

Room 0021 Department of Exercise Science, Health & Physical Education

Phone: 250-721-8381

University of Victoria

Email:

krigolsonlab@gmail.com

### **Learning and Decision Making Systems and Exercise**

#### **Introduction**

We invite you to take part in a research study being conducted by Dr. Olave Krigolson, who is an Assistant Professor in the Department of Exercise Science, Health & Physical Education at the University of Victoria. Your participation in this study is voluntary and you may withdraw from the study at any time. If you are a student in one of the courses Dr. Krigolson teaches, your performance evaluation will not be affected if you decide not to participate. The study is described below. This description tells you about the risks, inconvenience, or discomfort that you might experience. You should discuss any questions you have about this study with the research assistant who will be testing you. Dr. Krigolson will not be aware of whether or not you participate in this study.

#### **Purpose of the Study**

You are being invited to take part in this study because it is important to study ways in which we can improve the basic mechanisms that underlie human learning and decision-making. At present, we are interested in studying how performing a bout of high intensity interval exercise affects these processes.

#### **Study Design**

In this study, you will be performing a simple computer-based task while we record event-related brain potentials – the electrical activity generated by your brain - from electrodes contained on a specialized headband that you will wear on your forehead. These simple computer tasks will be performed before and after a session of either exercise or quiet reading. The experiment will consist of a familiarization visit (<30 minutes) and 2 experimental visits (1.5 – 2 hours).

#### **Who Can Participate in the Study**

You may participate in this study if you are between 18-30 years old and are deemed suitable to perform exercise.

You may not participate for this study if you:

- have a history of any conditions that may affect your memory.
- are a recent (within past 6 months) or current smoker.
- have been diagnosed with a medical condition that affects your cardiovascular system.
- have been told not to engage in strenuous physical activity by a physician.
- have answered 'yes' to any questions on the Physical Activity Readiness-Questionnaire.

- suffer from joint problems or other physical limitations that will not permit you to exercise.

### **Who Will Be Conducting the Research**

The experiment you will be taking part in will be conducted by a graduate student or by a research assistant working for Dr. Krigolson.

### **What You Will Be Asked to Do**

The study will take place in Dr. Krigolson's laboratory (Room 0021 in the McKinnon building) and will take place over 3 visits, taking two or three hours in total.

In this study, you will be asked to perform a session of exercise on the second visit and a session of quiet reading on the third visit. Before and after the respective activity, you will perform computer-based tasks. In particular, you will be asked to play a game where possible reward and cognitive load is varied. Prior to the computer tasks, you will be fitted with a specialized headband which will record electrical activity at the scalp. Your task will be completed on a computer. You will participate in a learning and decision making task that will include a series of trials in which you will receive feedback on your performance. You will learn to select presented stimuli to maximize reward. You shall do this by choosing either a colour, shape, or other stimuli to determine what is most valuable. The amount won each trial may contribute to a monetary performance bonus but will not affect your participation incentive (whether monetary or course credit). At the end of the experiment, you may be paid a portion of the amount that was won in the game.

We will be recording event-related brain potentials (ERPs) while you complete the learning task. An ERP is a brain response to a particular event, such as seeing a picture. The ERP recording process will involve the application of recording electrodes via the specialized headband on which the 6 electrodes are mounted to your scalp. These electrodes will allow us to measure the activity in your brain while you perform the task. Application of the electrodes involves the use of a small amount of electrode gel. The gel is water soluble and you will be given an opportunity to wash remove the gel from your forehead and hair after the session. Following application of the headband, you will complete the learning and decision making task. This process will be performed before and after exercise.

The exercise sessions will consist of 4 different body weight supported exercises (4 sets of each exercise). The exercises will include burpees, jumping jacks, mountain climbers and jump squats. Each exercise will be performed with maximal effort for 20 seconds followed by a 10 second rest period, which constitutes one interval. You will perform 4 intervals of each exercise (a set). Following the completion of a set, you will be given a 1 minute rest period. Following this rest, you will begin another set with a different exercise for a total of 4 sets. Following completion of exercise, you will be given 10 minutes to rest before completed the post-exercise computer task.

### **Possible Risks And Discomforts**

**High-Intensity Interval Exercise:** Performing a maximal exercise is safe but can induce unpleasant side effects such as dizziness, light-headedness, nausea, vomiting or loss of consciousness. First aid qualified personnel will ensure your safety by helping

you manage these symptoms if they arise. We will have the ability to call for medical assistance if necessary. Performing at high intensities during the exercise sessions may also result in a heightened heart rate, shortness of breath, muscular fatigue and soreness. These feelings will start to decrease once exercise stops.

**Heart Rate Measurements:** A chest strap wireless monitor will be placed around your upper chest that will record the electrical activity of your heart. Redness may develop on the skin after you remove the strap but should disappear within a few hours.

### **Possible Benefits**

Your participation is beneficial to the scientific community as a whole, as the data we gain from testing you may help improve our understanding of how learning and decision making occurs in the human brain. There will be no specific benefit to you.

### **Compensation**

You will be compensated \$10 per hour depending. You may also have the opportunity to win additional money depending on your performance (\$2 dollars minimum, up to a maximum of \$20).

### **Anonymity and Confidentiality**

Your participation in this study will not be anonymous, however, neither your name nor any other personal identifier will be associated with the data we collect from you. Instead, your data will be identified with a code number. However, a file with your code number and initials will be kept by the researcher for 2 months after your participation such that your data can be removed from the study if you decide you do not want us to use it. Additionally, you will not be identified in any reports and publications that stem from this research.

Your confidentiality will be respected. All of our data will be stored in a locked room with limited access on a password protected computer. Only Dr. Francisco Colino, Dr. Brendon Gurd, Jeremy Walsh, Cameron Hassall, Chad Williams, or research assistants working for Dr. Krigolson will have access to your data. Following completion of the study, and publication of the results, your data will be kept on the same secure computer for five years. After five years, your data will be destroyed.

### **Questions**

If you have any questions about the study, at any time, you may contact us by phone at 250-721-8381 or by email at [krigolsonlab@gmail.com](mailto:krigolsonlab@gmail.com).

### **Problems or Concerns**

If you experience any discomfort with, or wish to voice concerns about, completing the questionnaires, you may contact:

UVic Counselling Services

By phone: 250-721-8341

If you have any difficulties with, or wish to voice concerns about, any aspect of your participation in this study, you may contact:

Dr. Krigolson directly at 250-721-7843 or

The Human Research Ethics Board at the University of Victoria:

By phone: 250-472-4545

By email: [ethics@uvic.ca](mailto:ethics@uvic.ca)



**Signature Page**

**Study Title: Learning and Decision Making Systems**

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

\_\_\_\_\_  
Participant Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Participant Name (printed)

If you would like to participate solely as an observer, then we will not utilize your data in our analysis – it will be destroyed immediately upon completion of this testing session. If you would like to participate solely as an observer, please check here:

\_\_\_\_\_  
Investigator Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Investigator Name (printed)

A copy of this consent form will be left with you and a copy will be taken by the researcher.