The Influence of Acute Aerobic Exercise on Excitability and Rapid Plasticity in the Primary Motor Cortex

by

Amaya Singh

A thesis
presented to the University of Waterloo
in fulfillment of the
thesis requirement for the degree of
Doctor of Philosophy
in
Kinesiology

Waterloo, Ontario, Canada, 2016

Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

The aim of this thesis was to explore the influence of a single session of aerobic exercise on excitability changes and markers of short-term plasticity in the human primary motor cortex (M1). To that end, this thesis consists of 4 studies. In the first experiment, we explored whether acute exercise alone could modulate resting excitability in M1. We demonstrated that 20 minutes of moderate-intensity stationary biking could suppress intracortical inhibition and enhance intracortical facilitation in a non-exercised upper limb muscle for up to 30 minutes following exercise completion. Since decreases in inhibition are a necessary precursor to neuroplastic changes, we then investigated whether exercise could enhance the induction of rapid plasticity. We used paired-associative stimulation (PAS), a technique that reliably induces long-term potentiation (LTP)-like plasticity in M1 and found that a preceding bout of exercise enhanced the effectiveness of the intervention. Next, we examined whether these cortical changes were consistent across the entire cortical representation of the target muscle and if they were related to any measurable changes in motor performance. We paired exercise with a bimanual motor training task and observed that while performance was not enhanced compared to training alone, exercise facilitated training-related cortical excitability increases throughout the representation of the trained muscle. Finally, we demonstrated that exercise has opposite effects on the induction of long-term depression (LTD), suggesting that exercise is biased towards increasing excitability, and that this influence is evident even when exercise is performed following, rather than prior to, plasticity induction.

This thesis demonstrates that aerobic exercise may optimize the conditions for experience-dependent plasticity to occur and provides a rationale for the use of exercise as an adjunct to interventions that aim to induce LTP in human motor cortex.

Acknowledgments

A PhD can be described as many things, but a solitary effort is not one of them. Every thesis is a collaboration, and I am fortunate to have had some of the best and brightest minds contributing to this one.

First, I would like to thank Dr. Richard Staines, my supervisor, mentor and friend. You have been the driving force behind my graduate studies, and I can hardly believe all of the years that have passed since I first met you as a bewildered undergrad. You shatter the myth that brilliant researchers can't be outstanding supervisors and have contributed in countless ways to my development as a researcher. Thank you for your guidance and support, your insight, and your willingness to talk about anything, at any time. I have never stopped learning from you, and I hope our collaborations continue beyond this thesis, because I think I still have so much to learn.

To my committee members, Dr. Bill McIlroy and Dr. Laura Middleton: I am deeply grateful for your contributions to this thesis. Every suggestion and comment you have offered has made my research stronger, and I thank you for challenging me and pushing me. I would also like to thank Dr. Robin Duncan and her students Ryan Bradley, Philip Marvyn and Ashley Patterson for their assistance with genetic analyses, and for the crash course in genotyping. Also thanks to Dr. Michelle Ploughman and Dr. Ben Thompson for kindly donating their time and expertise to serve on my thesis examining committee, and also to Denise Hay for helping to navigate the administrative labyrinth.

To my labmates, past and present: Meaghan Adams, Danielle Andrew, Carla Arasanz, Dave Bolton, Kate Brown, Matt Brown, Robyn Ibey, Maran Ma, Matteo Masucci, Jason Neva, Christina Popovich, Jake Tennant, Jon Thacker, and Mike Vesia. I wish I could tell you what your support has meant to me, but that would take up another volume. So I'll just say that our times together have been some of the happiest of my life. From cottage vacations and dancing the night away on Bourbon Street, to scientific debates and existential crisis management, my wonderful memories and deep friendships are some of the most valuable things I will take away from graduate school. I would also like to thank our numerous research assistants and co-op students for their help with data collection, especially Courtney Ellis, Jenna Gilbert, Alicia Page and Maryem Sidarous.

To Matt, the brightest star in my sky: Thank you for every laugh, every encouraging word, and every moment in between. There are no words to describe the person that you are, and the ways in which you are wonderful - you are simply extraordinary, and it is a privilege to know you and to love you. We can never know what the future holds, but I know that with you beside me, I am not afraid of it anymore.

And finally, to my family: my parents, my sister Nita and my brother-in-law Rob. Whenever I am adrift at sea, you are my anchors of unconditional love and unwavering support. You have shown me in a thousand ways how to live a life of truth and integrity, of discipline, of kindness and selflessness. To my parents, who worked so relentlessly hard and sacrificed so much to educate and empower their daughters: Thank you for teaching us to never stop challenging ourselves, to never take education for granted, and that nothing worth having comes easily. Without you, none of this would be possible. It is an incredible gift to be able to follow one's dreams, and I owe that gift to you.

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5-HT	Serotonin
APB	Abductor pollicis brevis
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMT	Active motor threshold
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic AMP
CBF	Cerebral blood flow
CMR	Cerebral metabolic ratio
COMT	Catechol-o-methyl transferase
CREB	cAMP response element binding protein
cTBS	Continuous theta-burst stimulation
CST	Corticospinal tract
DA	Dopamine
ECR	Extensor carpi radialis
EEG	Electroencephalography
EMG	Electromyography
FDI	First dorsal interosseus
GABA	Gamma-aminobutyric acid
HPA	Hypothalamic-pituitary-adrenal
iTBS	Intermittent theta-burst stimulation
ICF	Intracortical facilitation
IGF-1	Insulin-like growth factor-1
KIBRA	Kidney and brain associated protein
LC	Locus coeruleus
LICI	Long-interval intracortical inhibition
LT	Lactate threshold
LTD	Long-term depression
LTP	Long-term potentiation
M1	Primary motor cortex
MCA	Middle cerebral artery
MEP	Motor-evoked potential
NMDA	N-methyl d-aspartate
NE	Norepinephrine
NTS	Nucleus tractus solitarius
PAS	Paired associative stimulation
PCR	Polymerase chain reaction
PET	Positron emission tomography
PFC	Prefrontal cortex
PMC	Premotor cortex

PVN	Paraventricular nucleus
RMT	Resting motor threshold
SICI	Short-interval intracortical inhibition
S1	Primary somatosensory cortex
SMA	Supplementary motor area
SSRI	Selective serotonin reuptake inhibitor
TBS	Theta-burst stimulation
TMS	Transcranial magnetic stimulation
TrkB	Tyrosine kinase receptor B
TCD	Transcranial Doppler
TDCS	Transcranial direct current stimulation
VEGF	Vascular endothelial growth factor
VO_2max	Maximal oxygen consumption
VTA	Ventral tegmental area

1 Background and general methods

1.1 Organization and general objectives of thesis

This thesis is organized into five chapters. Chapter 1 provides a brief description of the techniques used and a review of the relevant background literature. Chapters 2 to 5 consist of the four completed studies that addressed the specific research objectives. Finally, Chapter 6 contains a general discussion of the research findings and possible future directions, as well as some limitations of the methods and techniques used throughout the experiments.

The general objective of this thesis is to probe the response of the primary motor cortex (M1) to an acute bout of aerobic exercise. Specifically, we aim to investigate whether acute exercise can modulate motor cortical excitability and the induction of plasticity, and consequently influence motor behaviour. M1 is a highly adaptable region and changes in neuronal excitability underlie both the early and late stages of plasticity. Acute exercise has been consistently shown to enhance cognitive performance and prefrontal cortical activity, but whether such benefits extend to motor regions is unknown. The potential benefits of exercise will be examined primarily from a neurophysiological perspective, and also with regard to motor performance in order to establish a relationship between these two critical measures.

Short-term excitability changes in motor areas are a necessary precursor to more lasting neuroplastic changes. Indeed, the induction of plasticity in M1 is a fundamental goal of both motor learning interventions and neurorehabilitation strategies following a brain injury. In healthy populations, we aimed to explore whether exercise could enhance

experience-dependent plasticity and the cortical markers of motor learning. From a clinical perspective, this investigation is particularly relevant for determining the feasibility of exercise as an adjunct therapeutic technique in the treatment of motor impairments.

At a mechanistic level, exercise may directly influence muscle activity by exerting its effects on corticospinal tract (CST) neurons. Alternatively, exercise may modulate the intracortical networks that drive and regulate CST excitability. Determining the neural targets of exercise will help to establish a specific role for exercise in the interconnected domains of motor learning and rehabilitation.

1.2 Organization of the primary motor cortex (M1)

M1 is the cortical region associated with the execution of voluntary movements. Also known as Brodmann area 4, it is located on the precentral gyrus anterior to the central sulcus, which separates the parietal lobe from the frontal lobe. It is bordered posteriorly by the primary somatosensory cortex (S1) and anteriorly by the premotor cortex (PMC). As is typical of the cortex, M1 can be stratified into six cortical layers numbered from the most superficial (layer 1) to the deepest (layer 6). Since M1 is primarily an output region, it lacks the granular layer 4 that receives input from the thalamus and is thus considered agranular cortex. Cells found in M1 can be generally divided into pyramidal neurons, or output cells, and interneurons, which are restricted to the cortex. Layers 1 to 3 contain horizontal cortico-cortical connections that are critical to the activity of output neurons. These interneurons account for 20-30% of the neurons in the neocortex and are responsible for intracortical communication. Interneurons comprise many cell types, of which approximately 50% are inhibitory basket cells [1] . Interneurons can release either

excitatory or inhibitory neurotransmitters onto pyramidal neurons, thereby regulating the gain of synaptic inputs. However, the majority are inhibitory and utilize gamma-aminobutyric acid, or GABA, as a neurotransmitter. The cell bodies of the main output neurons from M1 are located in layer 5 and include a specialized population of large pyramidal neurons known as Betz cells. These neurons are highly interconnected, as a single pyramidal cell receives approximately 60,000 inputs [2]. Pyramidal neurons use the excitatory amino acid glutamate as a neurotransmitter and send motor commands to the spinal cord to be executed. The corticospinal tracts (CSTs) are formed by the axons of cortical neurons and represent the main output pathway for the execution of voluntary movements of the limbs. The CSTs descend from M1 through the internal capsule, midbrain, pons, and medulla to synapse on the spinal cord. Before reaching the spinal cord, approximately 80-90% of fibres decussate and continue as the lateral corticospinal tract. The remaining uncrossed fibres form the ventral corticospinal tract [3]. Axons of M1 neurons comprise approximately one-third of the corticospinal and corticobulbar tracts, with the other two-thirds deriving from the supplementary motor area (SMA), the PMC, and S1.

The groundbreaking work of Penfield and Rasmussen [4] was instrumental in identifying the somatotopy of body regions within M1. Using electrical stimulation, they demonstrated the topographic representation of body parts along the motor strip. This motor homunculus revealed a medial-lateral organization of leg, arm and face representations. However, these areas are not represented equally, as a disproportionate amount of space is devoted to regions requiring greater precision of movement, such as the hand, face and digit representations. Furthermore, there is considerable overlap between

areas, and multiple representations of a given muscle can be found throughout M1. Thus, this general pattern of organization does not imply a precise topography. Overlapping representations, as well as extensive connections between representations, likely form the basis for both the astonishing variety and complexity of movements that can be performed, as well as the relative ease with which such representations can be modified. Indeed, functional linkages between synergistic muscle groups are thought to underlie the performance of complex movements [5]. M1 is also highly interconnected with other brain regions, including the bilateral S1 and premotor cortices, supplementary motor area, thalamus, basal ganglia and cerebellum.

1.3 Plasticity in M1

Far from having a fixed and immutable arrangement, it is now known that the brain is constantly evolving, adapting and reorganizing throughout the lifespan. The ability of neurons to alter their structure and function in response to new experiences is known as neuroplasticity, or simply plasticity [6]. These processes can be inhibitory or excitatory and are fundamental to learning and development, as well as the recovery from brain injury. There are multiple types of both synaptic and non-synaptic plasticity, including neurogenesis, Hebbian plasticity, axonal and dendritic remodeling, and receptor plasticity. This thesis will focus on two of the main processes underlying synaptic plasticity, namely long-term potentiation and long-term depression.

1.3.1 Long-term potentiation

Long-term potentiation, or LTP, is a primary mechanism by which learning and memory processes occur. It was first identified in the hippocampus and subsequently in a number of cortical regions including the motor cortex [7], cerebellum [8,9], and amygdala [10,11]. LTP is based on Hebbian principles of neuronal plasticity that state that the repeated stimulation of a post-synaptic cell by a pre-synaptic neuron causes an increase in the strength of that synapse. The hallmarks of LTP are: a) cooperativity, which requires the synchronous activation of multiple pre-synaptic neurons ; b) associativity, meaning that a weakly activated synapse can undergo LTP if triggered by a stronger input to a nearby pathway; c) specificity, since LTP at one synapse does not spread to neighbouring inactive synapses; and d) persistence, lasting anywhere from minutes to weeks [12–15]. Thus, LTP is characterized by a long-lasting enhancement in synaptic transmission and can be divided into two distinct phases. In the early phase of LTP, also known as rapid or short-term plasticity, increased synaptic efficacy is thought to be due to greater pre-synaptic neurotransmitter release coupled with an upregulation of post-synaptic receptor activity. Importantly, such changes are independent of protein synthesis. In contrast, late LTP is marked by changes in gene expression and transcription as well as protein synthesis that results in long-lasting structural changes.

LTP is mediated by glutamate transmission, which exerts its effects via two main classes of receptors: N-methyl d-aspartate (NMDA) receptors, and non-NMDA receptors, namely α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainite receptors. As fast ionotropic receptors, AMPA receptors (AMPARs) can mediate rapid excitatory neurotransmission. Upon glutamate binding, AMPA receptor channels open to permit the flow of sodium (Na^+) into the post-synaptic cell, thereby increasing the

membrane potential. The change in voltage triggers the activation of NMDA receptors (NMDARs), a unique class of ionotropic glutamate receptors that are permeable to both Na^+ and calcium (Ca^{2+}). NMDARs are unique in that they are ligand-gated, as activation requires the binding of both glutamate and glycine, and also voltage-gated. At resting membrane potentials, a magnesium (Mg^{2+}) ion remains in the receptor channel, blocking the flow of Ca^{2+} into the cell. However, with sufficient depolarization of the post-synaptic membrane, the Mg^{2+} is ejected and Ca^{2+} is free to enter the cell.

Once inside, Ca^{2+} binds to calmodulin (CaM), forming a Ca^{2+}-CaM complex that activates a number of downstream signaling pathways. Several protein kinases are thought to be involved in LTP induction, including protein kinsase A, p42/44 mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K). However, the two most critical second messengers appear to be calcium-calmodulin kinase 2 (CamKII) and protein kinase C (PKC) [12,16]. In early LTP, these kinases are thought to phosphorylate AMPARs, thereby increasing their sensitivity to glutamate, and are also involved in activating previously silent AMPA receptors and trafficking them to the post-synaptic membrane. In particular, the phosphorylation of the ser831 site on the GluR1 receptor subunit appears to be a key process underlying LTP [17–19]. Intraneuronal pools of AMPARs are stored near the synapse to allow for rapid transport to the membrane [20,21]. A number of proteins also bind directly to AMPARs to regulate their trafficking [20,22]. AMPARs may also move laterally along the plasma membrane, with the speed of movement regulated by intracellular Ca^{2+} [22]. Once activated, CamKII undergoes autophosphorylation, thus accounting for its sustained activity after Ca^{2+} levels have returned to baseline [16]. Early LTP is evidenced by an increase in the amplitude of excitatory post-synaptic potentials

(EPSPs) evoked in the post-synaptic cell by an equivalent stimulus. With continued increases in intracellular levels, Ca^{2+} can activate cyclic AMP (cAMP) and form a complex that enters the nucleus of the cell and phosphorylates the nuclear transcription factor CREB (cAMP response element-binding protein), resulting in the subsequent increase in transcription and translation of genes necessary for enhancing synaptic strength, such as new receptors on the post-synaptic membrane. Thus, the activation of CREB and subsequent protein synthesis are the hallmarks of late LTP, which results in the structural modification of the synapse. In addition, LTP has been demonstrated in dendritic spines that show Ca^{2+}-dependent enlargement following stimulation [23].

1.3.2 Long-term depression

In contrast to LTP, LTD describes a long-lasting decrease in neuronal excitability. Although multiple receptor types can mediate LTD, NMDAR-mediated induction is the most typical [24], indicating that NMDAR activity alone does not determine the direction of plasticity [25]. Likewise, LTD is mediated by both pre- and post-synaptic modifications. LTD is thought to involve a reduction in the probability of glutamate release from the pre-synaptic neuron, and also a decrease in the sensitivity of post-synaptic glutamate receptors. In addition, LTD may involve the removal of AMPARs from the synapse and decreases in ion channel conductance [24]. Whereas LTP is mediated by the activity of protein kinases, the trigger for LTD appears to be the activation of protein phosphatases [25], in particular protein phosphatase 1 (PP1) and protein phosphatase 2B (PP2B). Calcium entry via NMDARS binds to CaM, which can then activate PP2B, which in turn activates PP1. PP1 then targets and dephosphorylates the ser845 site on AMPARs, stimulating receptor

internalization [14,25]. LTD has been demonstrated in the hippocampus, cerebellum, striatum and multiple cortical regions, including M1 [24,26].

It is widely believed that the question of whether a given stimulus will induce LTP or LTD is determined by the rate and magnitude of Ca^{2+} entry [22,27]. Low and moderate increases in Ca^{2+} favour LTD, while a larger increase favours LTP. The coincident activation of both pre and post-synaptic cells in repeated excitatory transmission allows a greater rise in intracellular Ca^{2+} that leads to the phosphorylation of CamKII, while moderate levels activate phosphatases that lead to the dephosphorylation of CamKII and a decrease in synaptic activity [27].

1.4 Basics of transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive technique used to modulate and measure local cortical excitability. TMS works on the principle of electromagnetic induction and operates through a coil placed on the scalp. An electrical current passing through a copper wire inside the coil generates a magnetic field that can pass unimpeded through the skull. The strength of the magnetic field is approximately 2 Tesla with a duration of 100 μs [28]. The current can both directly and trans-synaptically activate superficial cortical neurons to a depth of approximately 2 cm [29]. The targets of TMS are typically the cortical association fibres or horizontal connections found in layers 1-3 that synapse onto motor neurons [30]. The direction of the induced current is determined by the current flowing in the coil and the orientation of the coil on the head. The largest responses in M1 are obtained when the coil is placed at a 45° angle to the mid-sagittal line to induce a posterior to anterior current in the underlying neural tissue [31,32]. When current is

generated in the lateral-medial direction, it is possible to directly activate descending corticospinal neurons, producing what is known as a D wave (direct wave). However, because TMS current travels parallel to the horizontal fibres in the superficial cortical layers, TMS preferentially generates indirect, or I waves, which result from the trans-synaptic activation of descending neurons. I waves are produced at approximately 1.5 ms intervals and are termed the I1, I2 and I3 waves according to their latency [28,30] or categorized as early (monosynaptic) I waves and late (polysynaptic) I waves. Sufficient stimulation of the corticospinal tract produces a motor-evoked potential (MEP), which is recorded from muscles using surface electromyography (EMG). Owing to the somatotopic representation of body regions in M1, focal MEPs in target muscles can be observed in response to stimulation of a particular M1 area. Measurement of both the latency and amplitude of MEPs reflects the excitability of the corticospinal tract. Excitability is also reflected in the measurement of the resting motor threshold (RMT), which is defined as the lowest stimulation intensity required to generate an MEP amplitude of >50 μV on 5 of 10 consecutive trials. The RMT is thought to reflect membrane excitability, as it is markedly altered by drugs that interfere with the activity of Na^+ and calcium Ca^{2+} channels, but unaffected by those related to neurotransmitter activity [33]. Threshold can be determined at rest, or while maintaining a slight contraction of the target muscle, referred to as the active motor threshold (AMT). As contraction increases the excitability of motor neurons, AMT is always lower than RMT. When single pulses of TMS are delivered, the peak-to-peak amplitude of the EMG response at a given intensity reflects the excitability of the output neurons within M1. Changes in excitability are generally measured as either a change in the RMT, or an increased MEP amplitude for a given stimulus intensity. Alternatively, an

input-output curve can be generated by incrementally increasing the stimulator intensity and calculating the resulting MEP amplitude at each intensity. For hand muscles, the I-O curve is generally sigmoidal, with an initial steep increase and a plateau as maximal MEP levels are approached [34,35]. Changes in the area under the curve or the slope of the line can be interpreted as a change in excitability. Lastly, grid mapping can be used to assess the spatial extent of cortical representations before and after training. Excitability changes can be observed either through changes in the size of the cortical map, or the measurement of MEP amplitudes within the map.

1.4.1 Paired-pulse TMS

In addition to the delivery of single pulses, paired-pulse TMS (PP-TMS) allows the direct investigation of intracortical circuitry. PP-TMS is performed by delivering two stimuli in rapid succession. The subthreshold conditioning stimulus (CS) is delivered first and alters the excitability state of the output neurons, while the response to the subsequent suprathreshold test stimulus (TS) reflects that altered state. The amplitude of the conditioned test stimulus is compared to that of the unconditioned single pulse, and the percent inhibition or facilitation is calculated. Since the TS on its own is too weak to activate descending tracts, its effects are confined to local intracortical neurons [36], and thus the relative amplitude of conditioned stimuli can be taken as a measure of intracortical activity. Circuits commonly probed using PP-TMS include short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI), and intracortical facilitation (ICF).

The different stimulation parameters for PP-TMS reflect the different properties of the receptors that mediate these processes. Both SICI and ICF can be probed by the delivery of a subthreshold CS followed by a suprathreshold TS. At inter-stimulus intervals (ISIs) between 2-5 ms, the CS predominantly activates rapid ionotropic $GABA_A$ receptors, reflecting SICI. In contrast, between 12-15 ms, glutamatergic interneurons are activated and ICF leads to an increase in MEP amplitude. LICI can be assessed using two identical suprathreshold pulses delivered approximately 100 ms apart, activating slower, metabotropic $GABA_B$ receptors and subsequently suppressing the TS amplitude.

1.4.2 Theta-burst stimulation

In addition to probing cortical excitability, TMS can also be used to temporarily alter excitability by varying the pattern of stimulation. The technique of repetitive TMS (rTMS) involves the delivery of a train of high-frequency stimuli over a short time frame. Depending on the frequency used, rTMS can induce a local facilitation or suppression of activity that outlasts the stimulation by minutes or hours. The mechanisms underlying the effects of rTMS are not clear, but are thought to involve processes similar to LTP and LTD. A landmark study by Huang and colleagues in 2005 [37] marked the first human use of the theta-burst stimulation (TBS) protocol, a variant of rTMS that is now widely used to modulate cortical activity. It was discovered that by manipulating the pattern of magnetic stimulation, transient (up to 60 minutes) changes in cortical excitability could be induced. Based on the pattern of stimulation, these techniques are described as either continuous (cTBS) or intermittent (iTBS). The most common protocols for both cTBS and iTBS comprise 600 pulses delivered in bursts of triplets ("theta bursts") at 80% of the AMT. In

cTBS the pulses are delivered continuously for 40 seconds, leading to a suppression of excitability, while in iTBS, a pattern of 2 s of stimulation and 8 s of rest is carried out over a 190 s period, leading to an enhancement of excitability. These changes are thought to be due to LTP and LTD-like processes, and theta bursts appear to mimic the naturally occurring rhythms that are thought to be critical to synaptic plasticity [38,39]. The primary mechanism of TBS appears to be Ca^{2+}-mediated signalling via NMDA receptors, with the rate of Ca^{2+} entry determining the effect [40]. Indeed, the blockade of either Ca^{2+} or NMDA receptors abolishes the effects of both cTBS and iTBS [41]. A model proposed by Huang and colleagues [40] suggests that LTP depends on the rate of Ca^{2+} entry, while LTD is determined by the amount of Ca^{2+} entry. It is generally agreed that LTP and LTD are triggered simultaneously, and that Ca^{2+} influx triggers both inhibitory and excitatory signalling pathways [40]. The increase in excitatory signals is related to the rate of change of Ca^{2+}, while the inhibitory substances are related to the absolute level. Thus, the sustained level of Ca^{2+} release during cTBS promotes inhibition, while in iTBS, the absolute level remains low and thus inhibition is not triggered [40]. Spinal epidural recordings have demonstrated that cTBS appears to specifically suppress early I-waves, while later I-waves are largely unaffected [42].

1.4.3 Paired associative stimulation

A different but related technique to modulate cortical excitability is known as paired-associative stimulation (PAS), a well-established method of inducing rapid plasticity within M1. PAS involves the delivery of repeated pairings of peripheral nerve stimulation coupled

with single TMS pulses over the contralateral M1, and relies on principles of temporal and spatial summation to alter excitability levels.

The PAS protocol used in this thesis was previously described by Stefan and colleagues [43]. In this model, afferent signals arrive in S1 approximately 20 ms after median nerve stimulation and in M1 3-5 ms later. Thus, a TMS pulse delivered over the APB representation 25 ms after peripheral nerve stimulation should result in the near-synchronous arrival of central and peripheral inputs in M1. The typical PAS protocol consists of 180 pairs of stimuli delivered at 0.1 Hz, and the repeated pairings of stimuli generate increases in local excitability that outlast the experimental intervention.

PAS can induce anywhere from a 5% to 185% increase in baseline MEP amplitudes, with effects lasting a minimum of 30-60 min but reversible within 24 hours [43]. PAS-induced plasticity has been shown to occur at the level of the cortex, as it modulates the cortically generated silent period in contracting muscles, and neither brainstem stimulation nor the spinal F-wave is affected by PAS. The effects of PAS display the hallmarks of LTP, and are abolished by NMDAR blockade [44,45], supporting the view that PAS induces LTP-like plasticity. While PAS can also be used to induce inhibition by varying the inter-stimulus interval between central and peripheral stimulation, the descriptions used in this thesis always refer to excitatory PAS.

1.5 Mechanisms of experience-dependent plasticity in M1

1.5.1 Cortical reorganization and use-dependent plasticity

LTP and LTD are two of the processes that may underlie use-dependent plasticity in M1. It is well-established that M1 can undergo reorganization in response to training, reflecting a fundamental component of motor learning [46]. Motor learning is a general term that can refer to either a) the acquisition of new movement skills, reflected by novel sequences of muscle activation, or b) the adaptation of existing skills; however, skill retention appears to be a defining characteristic of learning. Motor adaptation encompasses both motor practice, or repetition, and adapting movement patterns to novel environments or stimuli [46,47].

In a seminal study, Nudo et al. [48] demonstrated that the acquisition of fine motor skills dramatically alters motor maps within M1. Intracortical microstimulation of primate brains following training revealed that representations of trained muscles expanded into the cortical territory occupied by neighbouring, untrained muscles. Similarly, work in humans has shown that motor training ---ranging from learned sequences of finger tapping to playing a musical instrument-- modulates excitability in M1 via both a cortical expansion and a decreased firing threshold of trained neurons [49–54]. The mechanisms underlying motor learning include both synaptogenesis and the reorganization of cortical maps [55]. Indeed, rapid motor learning enhances the excitability of corticospinal tracts [56] and blocks subsequent LTP induction, suggesting that LTP may mediate motor skill leaning in M1 [57]. This is supported by the finding that NMDAR blockade prevents motor learning and the induction of LTP in M1 [7,58].

While longer-lasting structural changes occur with repeated training, rapid reorganization and enhanced excitability in M1 is also evident following short-term motor training (≤30 min) [58–62]. This is thought to reflect a fundamental structural

characteristic, as M1 also undergoes adaptive plasticity within hours of peripheral motor nerve injury, with neighbouring regions expanding their territory into damaged areas [63,64]. The time course of rapid reorganization suggests that synaptic plasticity, rather than neurogenesis, underlies these changes [5,64]. The key to this unique property appears to be the extensive intracortical connections between M1 regions. The removal of inhibition allows the unmasking of latent horizontal connections and promotes reorganization [64,65]. However, reorganization does not occur between regions with limited or no intracortical connectivity, indicating that early plasticity only occurs within a pre-existing network of horizontal connections [63].

While motor training is often performed unilaterally, bimanual movements can take advantage of interhemispheric connections between homologous muscles in order to enhance learning effects. Transcallosal fibres linking M1 regions are thought to synapse primarily onto inhibitory interneurons whose primary role is to suppress activation of the ipsilateral M1 during voluntary unilateral movements. Thus, coupling movements of the upper limbs together can remove this larger, surround inhibition and permit direct facilitation between homologous regions [66]. Interhemispheric facilitation has been shown to be maximized when homologous muscles are active simultaneously [67–71]. Thus, synchronous bimanual movements facilitate interhemispheric communication, promote disinhibition of M1 [72,73], and result in a greater activation of M1 relative to unimanual tasks [74].

1.5.1.1 Role of GABA

The induction of plasticity depends on the net state of inhibitory and excitatory activity within M1 [46]. GABA, the chief inhibitory neurotransmitter in the brain, is synthesized from glutamate via the enzyme glutamic acid decarboxylase (GAD). There are two main classes of GABA receptors: the $GABA_A$ receptor is the most abundant in the CNS and represents a class of fast-acting ionotropic receptors that gate the direct passage of chloride ions into the cell [75]. In contrast, the $GABA_B$ receptors are slower, G-protein coupled metabotropic receptors that mediate potassium (K^+) and Ca^{2+} transport via the activation of second messenger systems [76]. A third class of GABA receptors, previously known as $GABA_C$ but now classified as $GABA_A$-ρ, a subclass of $GABA_A$, is also expressed throughout the brain but is found in particularly high concentrations in the retina [75,77]. Decreases in GABA activity appear to be a key feature of plastic reorganization in M1. Pharmacological blockade with the GABA antagonist bicuculine induces a rapid expansion of M1 representations into neighbouring regions, likely by unmasking latent connections [78]. Indeed, activation of the functional linkages in M1 that allow the performance of complex movements requires a release of GABA-mediated inhibition [5], and this release is a necessary precursor to the induction of plasticity [79,80]. While decreased GABA levels are associated with improved motor learning [79–81], no such modulation is seen during identical movements that do not involve a learning component [79]. These findings suggest that interventions aimed at reducing GABA activity might be a useful adjunct to the induction of plasticity in M1.

1.5.2 Neurotransmitter mediated excitability

Rapid excitability changes can also be mediated by alterations in receptor activity, and in particular NMDARs. The term *LTP-like plasticity* is often used to describe interventions used in the lab that are thought to increase excitability via the same mechanisms that generate LTP. The activity of NMDARs in M1 can be regulated by a number of different neurotransmitters and neuromodulators, several of which are relevant to exercise. The characteristics of these neurotransmitters and their potential role in M1 excitability are described in the following section.

1.5.2.1 Dopamine

Dopamine (DA) is a catecholamine neurotransmitter synthesized from the amino acid tyrosine. As DA does not cross the blood-brain barrier, it must be synthesized in the brain from the DA precursor levodopa (L-DOPA) via the enzyme aromatic L-amino acid decarboxylase (AAAD). The most often-discussed role of DA within the context of motor activity is the basal ganglia-M1 circuitry that modulates motor output, and indeed 80% of the brain's dopamine is found within the striatum [82]. However, there is extensive innervation of dopaminergic terminals within M1, particularly in the deep layers [83]. Neurons originating in the ventral tegmental area (VTA) form the mesolimbic and mesocortical pathways that project to cortical regions, including M1. Dopaminergic receptors are G-protein-coupled receptors (GPCRs) and thus do not produce rapid post-synaptic currents [84], but DA can both inhibit and promote motor activity depending on the receptors involved. D1, D2 and D5 receptors are all expressed in M1 [85]. When activated, D1 receptors have excitatory effects on post-synaptic cells, while D2 receptors are associated with inhibitory activity. However, Vitrac et al. [83] have recently

demonstrated that M1 activity is enhanced by the activation of D2 receptors, while Guo et al. [86] report that D2 activation is required for dendritic spine formation. Although both receptors appear to regulate synaptic plasticity in M1 [86], activity of the D1 receptor is critical for the generation of LTP [86,87]. Indeed, DA loss induces rapid structural plasticity in layer V pyramidal neurons and impairs motor performance [86]. Conversely, administration of L-DOPA enhances and prolongs the effects of PAS-induced increases in excitability [88,89]. DA can also influence GABAergic interneurons, activating or inhibiting them via D1 and D2 receptors, respectively [90]. At rest, the firing of M1 neurons and subsequent movement generation are significantly impaired by dopamine receptor blockade [91]. It is not yet known what triggers the release of DA in M1 neurons [92], although a connection to the reward system that DA is commonly associated with has been hypothesized. In addition to the influence of basal ganglia-M1 circuits on movement [93], the ablation of either the mesolimbic pathway carrying dopaminergic signals from the VTA to the cortex, or of dopaminergic terminals in M1 prevents the learning of a new motor skill, although performance of previously learned skills is unaffected [94,95].

Kirschner et al. [96] observed an increase in both intracortical facilitation and intracortical inhibition when DA reuptake was blocked. Importantly, DA can enhance the induction of cortical inhibition but does not induce inhibition itself [97]. Similarly, the DA antagonist haloperidol decreases ICI and increases ICF but does not alter resting or active motor thresholds, and L-DOPA modulates plasticity induction but has no effect on MEP amplitude [89]. These results indicate that DA does not alter M1 excitability itself, but modulates intracortical excitability to enhance plasticity. Monte-Silva et al. [89] propose that DA may increase Ca^{2+} influx during plasticity-inducing interventions via the

phosphorylation of NMDA receptors. Indeed, application of DA rapidly increases the surface expression of NMDAR subunits and their trafficking to dendritic regions [98]. In addition, DA rapidly increases current flow through NMDA receptors [99], suggesting a modulatory role in the induction of plasticity. DA concentration appears to be a determining factor of its post-synaptic effects [89,92,100], likely due to the differential activation of D1 and D2 receptors. Yet it appears that the co-activation of D1 and D2 receptors is required for rapid plasticity induction, likely via an increase in extracellular kinase activity [101]. Indeed, mild-to-moderate Parkinson's disease patients fail to display a response to theta-burst simulation delivered to M1 [102]. Additionally, DA stimulates the expression of several genes involved in the formation of LTP [90,92].

1.5.2.2 Serotonin

Serotonin, or 5-hydroxytryptamine (5-HT) is a neurotransmitter found throughout the brain and also throughout the body, particularly in the gastrointestinal tract. 5-HT is derived from the amino acid tryptophan, which is converted to L-5 hydroxytryptophan (5-HTP) by tryptophan hydroxylase, and further broken down to 5-HT by aromatic L-amino decarboxylase. Unlike its precursor tryptophan, 5-HT does not cross the blood brain barrier and thus must be synthesized in the brain. Serotonergic neurons are localized to the raphe nucleus in the brainstem but their axons project to virtually every brain region. The majority of 5-HT receptors are GPCRs, which allows 5-HT to play a key role in the modulation of post-synaptic neurons [103]. The localization of 5-HT receptors on the apical dendrites and axon hillock of pyramidal cells suggests a strong ability to regulate the activity of these output neurons [103]. 5-HT can have inhibitory or excitatory effects on

pyramidal cells depending on the receptor that is activated. Indeed, the serotonergic system represents the largest behavioural modulatory network in the brain [104]. In particular, the 5-HT2_A receptor promotes neuronal depolarization via inwardly rectifying K^+ channels and is localized to the axon hillock, indicating a role in the generation of action potentials [103]. In addition, independent of neuronal firing, 5-HT may have a general excitatory effect on network activity, as it promotes large-scale depolarization of neuronal networks as measured by cortical oscillations [103].

In M1, administration of the selective serotonin reuptake inhibitor (SSRI) sertraline significantly increases cortical excitability, resulting in a steeper TMS recruitment curve that remains elevated above baseline levels for at least 24 hours [105]. Similarly, a single dose of the SSRI citalopram shows a strong facilitatory effect on the induction of LTP-like plasticity in M1 [106]. Correspondingly, when administered prior to cathodal transcranial direct current stimulation (TDCS), a technique that mimics LTD, citalopram reverses this effect and converts inhibition into facilitation [106]. Batsikadze et al. [107] were able to confirm these findings using PAS, an intervention that induces the same excitability changes but whose effects are more localized than those of TDCS. A dose of citalopram enhanced excitatory PAS, and reversed the effects of inhibitory PAS [107]. Thus, in its modulation of cortical excitability, 5-HT appears to lean towards facilitation, at least in the motor cortex. The effects of both TDCS and PAS are thought to be dependent on the activation of NMDA receptors, representing a potential substrate for serotonergic neurons. Outside of the motor cortex, 5-HT also appears to facilitate the induction of LTP in the amygdala [108], primary visual cortex [109], hippocampal-PFC pathways [110], and spinal cord [111].

1.5.2.3 Norepinephrine

Norepinephrine (NE) is a catecholamine neurotransmitter with multiple roles in the CNS. The first step in the synthesis of catecholamines is the hydrogenation of phenylalanine, which is then converted to tyrosine. Tyrosine is the precursor of L-DOPA and subsequently dopamine, from which NE is synthesized via the enzyme dopamine β-hydroxylase in the locus coeruleus (LC). The axons of noradrenergic neurons in the LC have widespread terminations to many regions of the brain including the cerebral cortex.

In the motor cortex, pharmacological blockade of NE receptors suppresses the induction of LTP-like plasticity [87]. Similarly, the NE reuptake inhibitor reboxetine enhances both M1 excitability and intracortical facilitation in the absence of changes in motor threshold or spinal excitability [112,113]. NE agonists such as methylphenidate increase MEP amplitudes as well as intracortical facilitation [114], indicating an effect of NE on glutamatergic transmission. Paulus et al. [114] propose that NE may upregulate cortical excitability via a reduction of outward K^+ currents and an increase in Na^+ currents.

1.5.2.4 Brain-derived neurotrophic factor

In the brain, the growth, differentiation and survival of neurons is regulated by a variety of neurotrophins, the most critical being brain-derived neurotrophic factor (BDNF). BDNF is essential for neural development and is widely expressed in the primate and human brain, including the cortex and hippocampus [115]. BDNF is unique in that it is not constitutively active; rather, its expression and release are activity-dependent. The role of BDNF in use-dependent plasticity is well-established, particularly in the hippocampus. BDNF exists in two main forms: the precursor pro-BDNF, and the mature form of BDNF. Pro-BDNF

primarily binds to the p75 neurotophin receptor, while mature BDNF binds with high affinity to the tyrosine kinase type B receptor (TrkB). While the p75 pathway is typically associated with apoptosis, binding to TrkB mediates the synaptic effects of BDNF via the activation of pathways that regulate gene transcription and promote neuronal survival, outgrowth and differentiation. Although not classified as a neurotransmitter, BDNF exerts a strong influence on the excitability of cortical neurons.

BDNF has long been known to play a critical role in the formation of LTP. In excitatory neurotransmission, BDNF release is triggered by the influx of Ca^{2+} into post-synaptic cells through NDMA receptor channels. In late LTP, BDNF stimulates the formation of CaMKII, Ca-CaM, PKC, and phosphorylation of the mitogen-activated protein (MAP) kinase ERK (extracellular signal-regulated protein kinase), which then phosphorylates CREB and upregulates a variety of genes critical for LTP formation. BDNF triggers the synthesis of a number of proteins associated with dendritic remodelling and mitochondrial biogenesis [116–118]. In addition, CREB stimulates the transcription of more BDNF, creating a positive feedback loop [118]. In the hippocampus, BDNF knockout mice exhibit severe impairments in LTP induction, an effect that is completely reversed by the application of exogenous BDNF [119]. However, recent research indicates a critical role for BDNF in early LTP, independent of changes in gene expression. BDNF released at the synapse can affect neuronal transmission both pre-and post-synaptically. In early LTP, BDNF acutely stimulates glutamate release in neuronal cells through the phosphorylation of synaptic proteins [120], and via the TrkB receptor, rapidly increases neuronal firing rates [121]. Post-synaptically, TrkB activates a range of second-messenger pathways that can phosphorylate NMDARs, thus directly enhancing postsynaptic currents [121]. In

addition, BDNF acting through TrkB directly depolarizes neurons, with a much greater potency than glutamate [122,123]. This was later found to be due to the interaction of TrkB with Na^+ channels, particularly AMPA and NMDA receptors. BDNF can rapidly phosphorylate both the GluR1 subunit of AMPA receptors [124] and the NR1 and NR2B subunits of NMDARs [122,125], increasing conductance and providing a putative link to LTP. BDNF increases the open probability of NMDARs, and the effects of BDNF on glutamate-induced NMDAR current is rapid, with a 3-fold increase after 20 minutes of BDNF exposure [122]. However, BDNF can only exert its effects in the presence of concurrent glutamatergic activity, indicating a strong relationship between TrkB and NMDARs [126]. This is supported by the finding that the depolarization of neurons using a glutamate agonist increases BDNF mRNA [127]. BDNF can be secreted both pre- and post-synaptically, which accounts for the highly localized modification of synaptic structure and activity [128]. In addition, emerging evidence suggests that BDNF may also interact with GABA during early LTP. The application of BDNF rapidly reduces $GABA_A$ receptor activity at the synapse and the amplitude of mini inhibitory post-synaptic currents [129], and conversely, BDNF is downregulated in the presence of GABA [127].

1.6 Determinants of resting M1 excitability

The induction of plasticity in M1 is strongly influenced by the excitability state of the target neurons at the time of induction. This section outlines some of the determinants of resting excitability in the motor cortex.

1.6.1 Individual characteristics

There can be substantial inter-individual variability in resting excitability levels in M1 as measured by TMS. A number of factors can influence the capacity for plasticity induction, including anatomical characteristics. Increased cortical thickness is associated with greater facilitation following paired-associative stimulation [130], and individual differences in intracortical circuitry appears to be a key source of variability in the response to TBS. Differences in which neuronal pools are recruited by single TMS pulses may determine the response to TBS. Specifically, those individuals that demonstrate late rather than early I-waves in response to single TMS pulses are more likely to show the expected responses to cTBS and iTBS [131]. Fibre orientation in pyramidal tracts appears to contribute substantially to variance in motor thresholds, as does the skull-to cortex distance [132].

In addition to structural differences, there are a number of state-related determinants of excitability. MEP amplitudes are influenced by the phase of cortical oscillations, particularly in the alpha and beta frequency bands, and strong cortico-muscular coherence at the time of stimulation increases MEP amplitude [133]. High pre-stimulation activity in the beta band is correlated with decreased MEP amplitude [133,134]. Similarly, responses to TMS are stronger when alpha power is low (i.e. desynchronized) prior to stimulus delivery [135]. As M1 forms a key component of motor networks, it is also influenced by activity in connected regions, such as the dorsal premotor cortex [136].

The ability of a given technique to induce plasticity is dependent on a number of different characteristics, such as gender, age, time of day, attention and diet. In female participants, excitability levels may be dependent on phase of menstrual cycle, as high

circulating estradiol is associated with increased excitability [137]. In addition, females display a prolonged response to the induction of LTD-like plasticity [138]. Females exhibit a linear relationship between testosterone levels and PAS-induced plasticity while in males, levels of circulating insulin-like growth factor 1 (IGF-1) are more predictive [139]. Muller-Dalhaus et al. [140] report that the response to PAS decreases linearly with age, possibly due to the degeneration of neuronal circuits or a decline in neurotransmitter levels. The response to PAS also appears to be influenced by circadian rhythms, as plasticity induction is greater in the afternoon than in the morning [141], likely due to lower cortisol levels [142,143]. Multiple reports also suggest that plasticity is enhanced by directing attention to the stimulated limb [45,144,145]. In contrast, high trait-level anxiety is associated with decreased intracortical inhibition [146]. While chronic nicotine usage is associated with a reduction in M1 excitability [147], neither caffeine nor sleep deprivation appear to influence the resting motor threshold [148–150]. Finally, there is strong evidence that the individual response to plasticity is influenced by genetic factors, and particularly by variation in the BDNF gene. This is discussed in more depth in Section 1.9.5.

1.6.2 History of synaptic activity

The concept of metaplasticity is used to describe "higher order" plasticity, or a change in the state of the neurons that generate LTP and LTD. Homeostatic metaplasticity refers to a mechanism by which synaptic strength can be adjusted. According to the Bienenstock-Cooper-Munro (BCM) theory of synaptic plasticity, synapses that have recently undergone LTP are resistant to further LTP induction and are more likely to undergo LTD [151]. It is now well-established that the ability to induce plasticity at a given synapse is strongly

influenced by the prior activity of the synapse. Thus, the threshold for the induction of LTP and LTD is not fixed, but instead varies according to the integrated post-synaptic activity [152–154]. Low levels of prior activity favour the induction of LTP, whereas higher levels make LTD induction more likely [154–156]. The role of homeostatic metaplasticity appears to be to prevent the saturation of LTP/LTD and to stabilize neuronal networks [156,157]. At the neuronal level, metaplasticity is likely governed by a range of mechanisms, including presynaptic neurotransmitter release, postsynaptic glutamate receptor trafficking, and the secretion of modulators such as BDNF and cell adhesion proteins [158]. The activity of NMDA receptors and their downstream signalling molecules are also likely to play a key role [157].

The BCM principle can be demonstrated quite readily using non-invasive brain stimulation. While PAS reliably induces LTP in M1, this effect is abolished if two consecutive PAS sessions are administered [159]. Indeed, a number of studies have shown a suppression of excitability when two LTP-inducing interventions are delivered sequentially [160–164]. Evidence suggests that rather than a complete inhibition, priming instead elevates the threshold for subsequent induction [165,166]. In contrast, this principle can be used to enhance plasticity. Applying iTBS prior to cTBS results in a greater suppression of cortical excitability than cTBS alone [167,168], and iTBS-induced excitability is enhanced when primed with cTBS [161,162]. Using quadripulse stimulation, Hamada et al. [169] demonstrated that priming the motor cortex with brief high-frequency (excitatory) repetitive TMS increases the threshold for further LTP induction. This study was the first to demonstrate homeostatic metaplasticity using a priming technique that did not affect cortical excitability on its own.

Metaplasticity can also be demonstrated behaviourally, as multiple studies have reported that following motor learning, the trained M1 is much more likely to undergo LTD than LTP [170–172]. Homeostatic effects appear to be critically dependent on the time interval between stimuli [163,164], which may depend on the specific protocols that are administered [154].

1.7 Aerobic exercise and brain function

Given the numerous ways in which exercise positively impacts body systems, it is perhaps not surprising that a wealth of evidence is now demonstrating beneficial effects of exercise on brain function. Early explorations of the relationship between acute exercise and the brain produced anecdotal evidence of the "runner's high", a phenomenon later found to be due to the exercise-related release of endorphins and increased opioid receptor binding [173]. There have since been numerous studies documenting the positive effects of exercise on cognition, particularly on executive functions such as selective attention, response inhibition, movement planning, and working memory. With regard to electrophysiology, acute exercise has been shown to increase the amplitude and decrease the latency of the P300 (or P3), an event-related potential associated with decision-making and a commonly-used marker to assess cognitive function [174–181]. A decreased P3 latency indicates faster processing speed, while increased P3 amplitude indicates greater allocation of attentional resources. Acute exercise improves performance on the Stroop colour-matching task [182] as well as visual choice reaction time tasks [183], working memory assessments [184], and tests of executive function such as the Flanker task [176,177]. In addition, physical activity is linked to increased cognitive reserve [185] and

an increase in resting state activity [186]. Indeed, brain activity across all frequency bands (alpha, beta, delta and theta) has been shown to increase following exercise [187].

Regular exercise is also associated with a proliferation of growth factors that stimulate the development of new neurons and new synaptic connections [185]. In particular, exercise increases BDNF protein levels in the dentate gyrus of the hippocampus [188–192], which is the primary site of ongoing adult neurogenesis. Exercise stimulates the production of not only BDNF, but also vascular endothelial growth factor (VEGF)[193] and IGF-1 [194], which are critical for long-term brain health. Exercise also stimulates CREB-mediated pathways which trigger the transcription of genes thought to be related to learning and memory. Benefits are evident throughout the lifespan, as six months of exercise training in older adults reverses age-related hippocampal volume loss [195], and children who are physically active demonstrate alterations in brain structure, enhanced cognitive function and greater academic achievement relative to lower-fit peers [196–198].

Importantly, aerobic exercise has also been shown to enhance LTP. Mice given access to a running wheel for 10 days display increased LTP in the hippocampus, along with increased cell proliferation and improved spatial navigation [199]. In the dentate gyrus, voluntary exercise also decreases the threshold for synaptic plasticity and triggers increased expression of NMDAR subunits [200].

As most of the research in this area has focused on the hippocampus and the prefrontal cortex, the aim of this thesis was to investigate exercise-related changes in motor regions. As the site of voluntary movement execution, M1 is not only critical for motor learning, but given its involvement in exercise performance, seems a likely candidate to be influenced by acute physical activity.

1.8 Exercise and the primary motor cortex

1.8.1 Acute exercise and motor function

Only a limited number of studies have examined changes in M1 during and after exercise. Using positron emission tomography (PET), Christensen et al. [201] observed increased activity in M1 during active, voluntary cycling compared to passive cycling, suggesting a contribution of M1 to rhythmic locomotion [201]. Animal work suggests that glucose uptake in M1 increases by approximately 40% during intense running exercise [202]. Interestingly, Subudhi et al. [203] report that deoxygenation of the motor cortex also occurs during maximal exercise. This is supported by Brummer et al. [204], who observed altered M1 activity in the stages leading up to exhaustion. In addition, Sidhu et al. [205] report increased intracortical inhibition in the vastus lateralis muscle during cycling exercise. Interestingly, fatiguing lower limb resistance exercise decreases excitability not only in the exercised muscle, but in non-exercised upper limb muscles as well [206]. In contrast to resistance exercise, a single session of aerobic cycling has been shown to decrease intracortical inhibition in the leg region of M1, indicating increased excitability [207]. Thus, given these two observations, the first aim of this thesis was to investigate whether an exercise-induced decrease in inhibition could also be observed in a non-exercised upper limb muscle.

1.8.2 Exercise prescription used in this thesis

The exercise prescription used in this thesis is based on the standard exercise prescription for aerobic fitness provided by the American College of Sports Medicine [208]. Participants

performed 20 minutes of steady-state, moderate-intensity aerobic activity. The intensity was determined using a combination of age-predicted maximal heart rate (HR) and subjective ratings of perceived exertion (RPE) using the Borg scale [209]. Maximal HR was calculated as 220 beats per minute-age. Participants were given a range between 65-70% of this value as a target but were instructed to keep their exertion level in the "moderate" range (ratings of 3 to 4 on the modified Borg scale). A number of additional metrics exist with which to prescribe exercise intensity, including percentage of maximal oxygen uptake (VO_2 max), heart rate reserve, ventilatory threshold (VT), and lactate threshold. There are multiple reasons for an increased emphasis on perceived exertion, which integrates signals from the periphery as well as the CNS, including: a) VO_2 measures require trained personnel, and are costly and time-consuming to collect; b) measures of VO_2, while being the gold standard for cardiovascular fitness, are not linked to any specific central processes and thus may not be as useful for prescribing exercise for neurological benefits; c) there is no consensus as to what percentage of VO_2 might be ideal for brain function; d) measurements of lactate threshold are invasive; e) there is a very strong correlation between measures of RPE and blood lactate, as well as RPE and HR, and RPE and anaerobic threshold [210,211]; f) perceived exertion reflects the psychological component of exercise as well as the physiological; and g) our participants were a relatively homogeneous group of young, healthy, active university-age students. In studies with clinical populations, while RPE remains a useful adjunct, a more precise measure of fitness may be recommended. The psychological component of exercise is one that is often overlooked when designing protocols, yet research indicates that the sensation of fatigue is associated with a modulation of activity in multiple brain centres [212–214], and indeed

RPE is correlated with increases in prefrontal cortical activity [215]. There is increasing evidence that factors such as motivation and self-belief play a role in the perception of fatigue, which is a multi-factorial emotion not linked to any particular event in the periphery [216].

The majority of studies examining exercise for brain health employ moderate-intensity exercise, likely influenced by the U-shaped arousal hypothesis first proposed by Yerkes and Dodson [217], which suggests that cognitive performance suffers at low and high levels of arousal. From a neurochemical perspective, Kashihara et al. [183] hypothesize that at high intensities, neurotransmitters detrimental to cognitive performance, such as stress-related hormones (eg. cortisol and excessive serotonin) dramatically increase. In the motor cortex, this is supported by the work of Sale and colleagues [141,142], who found that high cortisol levels suppress the induction of LTP. Indeed, higher exercise intensities are associated with a greater release of plasma catecholamines such as norepinephrine [218,219], although it should be noted that most studies employ incremental studies where fatigue is increasing, rather than using independent groups. However, entering the anaerobic range is associated with multiple processes that induce fatigue, including the rapid accumulation of lactate in working muscles, and makes exercise more likely to be terminated [220]. As the VT varies substantially among individuals, ratings of perceived exertion are a useful and practical marker [220]. There is recent evidence that high-intensity interval training may be slightly more effective than continuous aerobic training at increasing circulating BDNF levels [221,222], and this is likely to be an emerging area of research in the near future.

From a practical standpoint, it is useful to examine the benefits of the exercise using the parameters that are already being prescribed to the general public, particularly since there is no standardized intensity, duration or mode of exercise recommended to provide maximal acute neurological benefits. However, while these guidelines are based on cardiovascular benefits, there is evidence that these parameters are also beneficial for brain function. Dopamine levels appear to be increased after 20 minutes of running activity [223], and BDNF levels are enhanced after 20 minutes of both moderate and high-intensity exercise when exercise is prescribed using heart rate reserve [224].

1.9 Potential mechanisms of acute exercise-induced M1 excitability changes

While the mechanisms that may contribute to exercise-induced modulations of cortical excitability are unclear, in recent years the neurochemical hypothesis has received much attention. It is clear that acute exercise involves altered neurotransmitter activity, but such changes are difficult to accurately measure and the correlation with behavioural outcomes is speculative at best. Yet, this hypothesis is well-supported in the literature, and receives much attention due to the known time course of changes and the established effects of neurotransmitters on cortical excitability. Candidate neurotransmitters are reviewed below, as well as the potential contribution of cerebral blood flow changes and activation of the stress response. While much of this work remains hypothetical, it is the foundation of a model describing how M1 may benefit from acute aerobic exercise.

1.9.1 Cerebral metabolism

1.9.1.1 Global and regional CBF

There is conflicting evidence concerning changes in CBF as a result of exercise. Exercise is clearly associated with a shift in regional CBF (rCBF) to areas involved in maintaining activity. On the other hand, measurements of global CBF have been confounded by differences in techniques, timing of measurements, and location of measurements. The multitude of outcome measures include total CBF, rCBF, hemoglobin, deoxyhemoglobin, mean flow velocity, glucose uptake, and blood oxygenation, either during or following exercise. At rest, blood flow through the brain is approximately 750 ml/min, or 15% of total cardiac output and due to cerebral autoregulation, this is not thought to change unless mean arterial pressure drops below 60 mmHg or exceeds 150 mmHg [225–227]. While early studies supported the idea that CBF did not change during exercise, the theory of constant blood flow likely flourished due to the limitations of the existing neuroimaging techniques [228]. The Kety-Schmidt technique [229], which measures arterial-venous differences in inhaled nitrous oxide, was found to underestimate CBF due to its assumption that hemispheric drainage always occurs through the right internal jugular vein [230]. Since then, a variety of techniques have been developed, including single photon emission computed tomography (SPECT) with the radioactive tracer Xenon-133, arterial spin labelling, measurement of mean flow velocity, and transcranial Doppler ultrasound (TCD). TCD has become a popular technique in recent years and calculates blood flow velocity from the frequency of the Doppler shift [231]. Using such techniques, global CBF has been shown to increase [230,232–239] or remain unchanged [240,241] during aerobic exercise;

however, the majority of evidence points to an increase in CBF (reviewed by Querido & Sheel [231]). In particular, flow velocity through the middle cerebral artery (MCA) increases at the onset of exercise [230,233–235,239,242] and remains elevated until exercise completion [236,243].

Principles of neurovascular coupling apply during exercise, where CBF increases to several areas associated with movement production, such as vestibular areas and cardiovascular centres in the brainstem, as well as the cortex [235]. Using PET scanning, Christensen et al. [201] report increased rCBF in bilateral M1 regions following cycling exercise, with M1 activity increasing in proportion to exercise intensity. Indeed, activity in M1 may increase according to task demands in order to maintain intensity [204]. Exhaustion endpoint is preceded by a decrease in cerebral oxygenation in the prefrontal cortex, possibly as a result of increased carbon dioxide (PCO_2), indicating that regions upstream of M1 may mediate the decreased cortical output associated with fatigue [244].

1.9.1.2 Determinants of CBF

The major factors contributing to increased blood flow appear to be mean arterial pressure (MAP), PCO_2, and cardiac output (CO) [231]. The increase in MCA velocity appears to be dependent on the ability to increase CO [226]. In addition, local vasodilation in part contributes to an increase in cerebral blood volume during activity [228]. It is well-established that PCO_2 is a strong regulator of cerebral blood flow. Increased $PC0_2$ causes substantial increases in CBF [226]; indeed, inhaling a mixture of 5-7% CO_2 increases CBF by about 75% [245]. As Kety and Schmidt [245] describe, CO_2 is a potent vasodilator and is also the primary byproduct of cerebral metabolism. As such, PCO_2 appears to be a key

mediator of exercise-induced changes in CBF [241,242,246,247]. In a state of increased oxidative metabolism, modulations of CBF serve to clear the brain of excess CO_2 and maintain homeostasis. While hypercapnia is indeed associated with vasodilation and increased blood flow, it is unclear whether it is the CO_2 itself, the change in pH, or the bicarbonate ions that alter cerebral blood flow [231]. Regardless of the mechanism, at higher intensities, it appears that a hyperventilation-induced decrease in PCO_2 causes decreased CBF [231,242]. As a result, continued decreases in PCO_2 may lead to inadequate energy production, possibly resulting in exhaustion [227].

Due to its links with energy metabolism, it is not known if changes in CBF are strictly centrally mediated, or whether they occur in response to peripheral changes. Intravenous infusion of epinephrine (resulting in increased circulating epinephrine) causes a significant increase in CBF [248] and cerebral O_2 consumption. Yet, as indicated above, there is evidence that central changes in PCO_2 and blood flow may mediate central fatigue and determine the endpoint of exercise.

1.9.1.3 Lactic acid

The cerebral metabolic ratio (CMR) is calculated as [oxygen uptake/(glucose uptake+lactate uptake)/2] and gradually decreases with increasing exercise intensity [227]. Thus, there is a corresponding decrease in brain glucose uptake with increasing exercise intensity [214,249]. In contrast, brain uptake of lactate increases with intensity [250], indicating a shift in cerebral fuel usage. Lactate passage to the brain is transporter-mediated, and under conditions of high plasma lactate, there is a linear increase in its cerebral metabolism [249–251]. Membrane passage is achieved via the activity of

monocarboxylate transporters (MCTs), three of which have been identified in the brain [252]. Of these, MCT2 is the predominant form expressed in neurons and co-localizes with AMPA receptors. The lactate receptor HCAR1 (hydroxycarboxylic acid receptor 1) is also co-localized with MCT2, suggesting a link to rapid excitatory neurotransmission [252]. Indeed, neurons can be fueled by lactate for hours in the absence of glucose [253], and lactate is not confined to active areas of brain activity, as it diffuses to neighbouring regions [254]. While metabolic substrates may differ between brain regions, high lactate levels correspond to increased M1 excitability following a bout of maximal cycling [255], possibly indicating a shift to lactate as a fuel. M1 excitability displays a linear relationship with blood lactate levels, as increased lactate concentrations, whether as a result of exhaustive exercise or when administered intravenously, rapidly decrease motor thresholds [255]. Interestingly, the intravenous infusion of lactate into resting participants increases both circulating BDNF and the expression of BDNF, and lactate potentiates both current flow and Ca^{2+} influx through NMDARs [256,257]. While the net contribution of lactate to cerebral metabolism at rest is estimated to be relatively low (approximately 7%), this can increase to up to 25% during exercise [258].

1.9.2 Neurotransmitters

1.9.2.1 GABA

There is limited information available on GABA levels immediately following exercise; however, a downregulation of GABA signaling on baroreceptor neurons is thought to contribute to post-exercise hypotension [259]. In addition, up to a 76% increase in striatal GABA levels has been reported following 60 minutes of treadmill running, although it

should be noted that these data did not reach statistical significance [260]. Given the inherent difficulties in measuring changes in GABA levels in human participants, the assessment of intracortical inhibition can be taken as an indirect measure of GABA activity. An acute, seven-minute session of stationary biking has been shown to decrease SICI in the leg region of M1 [207].

1.9.2.2 Exercise and DA

Although the number of studies is limited, a recent review reports that DA is consistently shown to increase during acute exercise [261]. Gerin and Privat [262] report increased brain DA turnover during exercise, and region-specific changes have also been observed. In hippocampal neurons, DA levels have been shown to increase during a 60-minute session of treadmill running [263], and both hypothalamic [264] and basal ganglia [265] DA levels also increase during exercise. Indeed, twenty minutes of treadmill running is sufficient to cause a 30% increase in extracellular DA in the striatum [223], while Meeusen et al. [260] report a 50% increase in striatal DA release following 60 minutes of running. The number of human studies is limited; however, Skriver et al. report increased plasma DA levels following 20 minutes of intense cycling activity, while Winter and colleagues observed increases following both moderate and intense running [266,267]. In contrast, Wang et al. [268] observed no change in striatal DA release after 30 minutes of intense treadmill running as measured by PET scanning, although the authors acknowledge the poor sensitivity of the radionuclide to small changes in DA levels.

1.9.2.3 Exercise and 5-HT

Evidence regarding the relationship between exercise and 5-HT is mixed. Previous studies have found either an increase, decrease, or no change after acute exercise. Dey et al. [269] report increased 5-HT in the brainstem, but not cortex, after one hour of swimming. In contrast, Lukaszyk et al. [270] observed a decrease in cortical 5-HT levels after 20 minutes of treadmill running, while Chauloff et al. [271] showed increases in 5-HT after similar exercise. While early studies were limited by the techniques available at the time, the advent of microdialysis has made it possible to collect accurate measures of neurotransmitter levels in real-time. Using this technique, Kurosawa et al. [272] reported an increase in 5-HT in the cortex of rats after just 5 minutes of walking. In their 1999 review, Jacobs and Fornal [273] describe why 5-HT is a likely candidate to mediate the non-specific exercise-induced effects on motor activity. Specifically, the activity of 5-HT neurons remains consistently elevated during the execution of a motor behaviour, as opposed to cyclical periods of activity that correspond to muscle contractions. Further, they describe the distribution of 5-HT neurons in the brainstem and spinal cord, wherein greater connectivity is seen to areas involved in the control of lower limb and axial muscles, while connections to areas involved with fine motor control are more sparse. Such an arrangement corresponds to a greater involvement of 5-HT in gross motor movements. Lastly, 5-HT neurons project to areas of the spinal cord involved in both motor control and autonomic functions [273], and thus are ideally suited to mediate the cortical response to exercise. In particular, the aerobic nature of the exercise appears to be of fundamental importance, as it is the mobilization of free fatty acids in the blood during exercise that liberates tryptophan from its carrier, albumin. This unbound tryptophan can then cross the BBB and synthesize 5-HT. In support of this, increases in 5-HT appear to be

time-dependent, and thus, as exercise progresses, 5-HT synthesis correlates with increases in FFA metabolism [82].

Perhaps the best known link between 5-HT and exercise is the well-established role of 5-HT in central fatigue. Central fatigue refers to an apparent loss in neural drive and can be defined as a failure to maintain the required force output that is not due to dysfunction in contracting muscles [274]. Early on, 5-HT was linked to central fatigue, as increased levels are known to cause lethargy and low motivation. Indeed, 5-HT receptor agonists significantly decrease time to exhaustion and endurance performance during intense exercise in both animal and human subjects [275–277], although other studies have found no effect of increased 5-HT levels on performance or perceived exertion [260,278,279]. Similarly, administration of SSRIs may reduce aerobic capacity, although this has not been consistently replicated in human studies. Parise et al. [280] observed no effect of SSRIs on aerobic or anaerobic performance. Similarly, Strachan et al. [281] showed no effect of administration on RPE or time to fatigue. The apparent conflict between the excitatory and inhibitory effects of 5-HT may be resolved by viewing brain neurotransmitter relationships as a continuum. In their 2001 review, Struder and Weicker [104] suggest a dose-response relationship exists wherein the 5-HT network stabilizes and regulates movement until the system becomes overwhelmed, at which point performance may deteriorate. This corresponds with both the U-shaped arousal curve proposed by Yerkes and Dodson [217] and the known dual function of 5-HT.

1.9.2.4 Exercise and NE

In the peripheral circulation, simultaneous activation of the sympathoadrenal system with the hypothalamic-pituitary-adrenal (HPA) axis stimulates the release of catecholamines [179]. Feedback to the autonomic nervous system (ANS) regarding the intensity of exercise, such as cardiorespiratory stress and glycogen depletion, then triggers further increases in catecholamines [82]. Circulating NE levels have been consistently shown to increase during and after acute exercise. This is not surprising given the role of NE in the regulation of heart rate, circulation, and glucose metabolism and during physical activity [282]. In addition, there is a psychological component, as plasma levels are in part determined by emotional strain and perception of effort [82]. Blood and plasma NE levels are significantly upregulated following both incremental and steady-state cycling activity [234,283–285]. Additionally, plasma NE increases proportionally with heart rate [286]. Although catecholamines are produced both centrally and peripherally during exercise, it has been argued that measuring levels of plasma levels may be of limited usefulness given that catecholamines do not cross the blood-brain barrier. However, in vivo microdialysis has confirmed central increases in NE following exercise. Aerobic activity significantly upregulates extracellular NE levels in the striatum [260,264], hypothalamus [264], and frontal cortex [287]. However, Goekint et al. [263] report no significant changes in hippocampal NE following 60 minutes of exercise, indicating that such changes may be region-specific. In addition, treadmill running increases NE turnover in the cortex [262,287] and levels remain elevated following exercise completion [287]. Interestingly, fitness does not appear to be a factor, as the magnitude of the NE increase during exercise is similar for trained and untrained individuals [286,288]. These findings indicate that

sympathetic responses at a given intensity are relatively fixed, likely to maintain the sensitivity of the SNS to disruptions in homeostasis.

1.9.3 Cortisol and the HPA axis

The HPA axis is a well-known pathway involved in the regulation of the stress response. The initial stimulus is provided by threat-detection systems in the amygdala, PFC and hippocampus. Together, these regions send excitatory projections to the paraventricular (PVN) nucleus in the hypothalamus, prompting the release of corticotropin-releasing hormone (CRH). CRH then stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH), which then travels to the adrenal cortex where it stimulates the synthesis and release of cortisol. Cortisol, a steroid hormone, is primarily associated with gluconeogenesis and immune suppression but has widespread effects throughout the body. The activation of the HPA axis during exercise provides strong evidence that exercise elicits a generalized stress response within the body. This response differs from traditional models of stress in that while exercise is a threat to homeostasis, there is no corresponding psychological threat and thus exercise is not perceived as a harmful stressor [289].

Acute exercise and cortisol

The relationship between exercise and cortisol is not a straightforward one. While chronic physical activity is associated with inhibition of the HPA axis [290–293], acute exercise displays a complex and intensity-dependent relationship to cortisol production and HPA axis activity. Voluntary exercise appears to increase excitatory drive from both the PFC and the hypothalamus to inhibitory neurons of the PVN, decreasing HPA axis activity [289]. Yet plasma, serum and salivary cortisol have been shown to significantly

increase after exercise [294–298]. There appears to be a particularly strong correlation between intense exercise and cortisol, as many of the studies reporting such increases used protocols involving high-intensity or exhaustive exercise. Indeed, cortisol has been shown to decrease following low-intensity [299] and moderate-intensity [179] exercise. In general, circulating cortisol levels exhibit a linear relationship with exercise intensity [179,300,301]. However, a sharp increase in cortisol secretion appears to occur at the transition from aerobic to anaerobic activity, indicating extreme stress upon the aerobic system [286,301]. Indeed, there is likely a threshold for the release of cortisol via the HPA axis, but it is unclear where this occurs [293]. As the lactate threshold is also associated with a shift towards pain and discomfort, it is unclear as to what extent the increase in HPA axis activity reflects a physiological or a perceptual stressor. It has been proposed that the stress response (and activation of the HPA axis) is identical whether the stress is physiological or perceived [82]. However, in the perceptual model, one would expect variability in fitness levels to correspond with the level of perceived stress, yet this intensity threshold does not differ between highly active and very sedentary subjects [302]. Indeed, Deuster et al. [286] report that adrenal secretion at a given intensity is independent of fitness and aerobic capacity, indicating that fitness may alter sensitivity to, but not release of, hormones such as cortisol. Plasma cortisol appears to return to pre-exercise levels within 2 hours of exercise completion [294,302].

Cortisol, performance and M1

The harmful effects of chronic stress on neuroplasticity are well-known. Chronic stress inhibits the transcription of growth factors such as BDNF and inhibits hippocampal and prefrontal neurogenesis (reviewed by Dranvosky and Hen [303]). Cortisol activity

appears to follow the Yerkes-Dodson [217] U-shaped hypothesis, as both excessively high and low levels of cortisol are damaging to the brain [304]. In the acute exercise literature, similar evidence supports the existence of a dose-response relationship between cortisol levels and cortical excitability. A single intravenous dose of hydrocortisone significantly increases M1 excitability and decreases intracortical inhibition within 15 minutes of injection time [305]. In contrast, McMorris et al. [306] report that a substantial increase in plasma ACTH concentration during high-intensity exercise may be linked to a slowing in reaction times. Echoing these findings, McDonnell et al. [299] report that higher-intensity exercise that increases circulating cortisol impairs plasticity, while lower-intensity exercise elicits a decrease in cortisol and facilitates the induction of LTP-like plasticity. Indeed, Sale et al. [142] report that high circulating cortisol strongly inhibits the induction of rapid plasticity in M1. Additionally, the decrease in cortisol levels following repetitive theta-burst stimulation suggests a link between low cortisol and neuroplasticity [307]. Thus, current evidence seems to suggest there is a point at which stress changes from beneficial to detrimental, and it is likely that this is reflected in the brain. It is not known if cortisol has direct effects on neuronal transmission or if it acts as a neuromodulator [142]. Cortisol release appears to follow a circadian rhythm [308,309], and correspondingly, there is strong evidence that hippocampal LTP exhibits circadian rhythms, presumably under the control of circulating hormones [310]. There are strong connections between the amygdala and the PFC, and exposure to acute stress blocks plasticity induction in this pathway, likely via the suppression of NMDA receptor activity [311]. Finally, while the mechanisms underlying a dose-response relationship have yet to be identified, this shift may be mediated by a modulation in receptor activity. At low cortisol levels, binding to

mineralocorticoid receptors may promote LTP, while at higher concentrations, receptor saturation occurs and the subsequent binding of cortisol to glucocorticoid receptors may impair LTP [312].

1.9.4 BDNF and acute exercise

Given its role in early LTP, BDNF has emerged as a potential mediator of the plasticity-promoting effects of exercise. Increases in circulating (serum or plasma) BDNF are consistently shown following an acute bout of moderate to intense aerobic exercise [182,224,267,313–317]. The magnitude of the BDNF increase appears to correspond with intensity [224,267] and may be partially dependent on mental state [318,319] and racial background [320]. A potential confound in such measurements is that platelets, immune cells and skeletal muscle cells are all capable of producing BDNF [315] [321,322]. While it does not appear that skeletal muscle-derived BDNF is released into circulation [322], the immune and circulatory systems remain potential sources. Evidence suggests that exercise of a sufficiently high-intensity may stimulate release of BDNF from B and T cells, while shear stress may activate the release of BDNF from platelets [323]. Direct measurements of brain levels are rare; however, Rasmussen et al. [317] report up to a five-fold increase in brain BDNF mRNA expression following two hours of treadmill exercise. Although the time course of BDNF release is unclear, Schmidt-Kassow et al. report that plasma levels peak after 20 minutes of exercise, and in general, systemic BDNF is increased for 10-60 min following aerobic activity [324]. This is assumed to reflect brain levels, as there is bidirectional transport across the blood-brain barrier [325]. Based on measurement of the arterial –to-internal jugular venous difference, Rasmussen et al. [317] report that the brain

is the source of 70-80% of circulating BDNF during exercise. Interestingly, lactate infusion at rest will result in a linear increase in plasma BDNF, suggesting a potential trigger for activity-dependent release [257]. In one of the few studies examining the impact of exercise-induced BDNF changes on cortical excitability, McDonnell et al. [299] report no correlation between BDNF and enhancement of neuroplasticity. Despite hypothesizing that increases in circulating BDNF may mediate cortical adaptations, the authors in fact found a decrease in BDNF levels over time during both low- and moderate-intensity exercise. However, it is possible that much of the BDNF produced in the brain does not enter the peripheral circulation and thus remains undetected. BDNF remains in circulation for less than 60 minutes after release [324] and can be taken up by both working muscles and blood platelets [326].

1.9.5 Influence of the Val66Met polymorphism

Even before the discovery of a common variant in the BDNF gene, it was suspected that genetic factors might influence the brain's capacity for plasticity. Studies of dizygotic and monozygotic twins indicate a strong genetic influence on plasticity induction in M1, specifically in CST excitability, intracortical inhibition and intracortical facilitation following PAS [327,328], with estimates that up to 90% of the response in M1 is genetically determined [327,328]. The ideal candidate genes for testing have an established role in neuronal function or cognition, and have at least one allelic variant that occurs with relative frequency in the general population. Cheung et al. [329] recently identified a number of gene expression changes during the course of motor skill acquisition, including expected increases in a number of synaptic proteins and receptors linked to

synaptogenesis and LTP. However, due to its established role in learning and plasticity and the existence of a relatively common genetic variant, the BDNF gene is a leading candidate to account for some of the variability in the response to non-invasive brain stimulation. The valine-to-methionine substitution at codon 66 of the BDNF gene (Val66Met polymorphism) underlies a single nucleotide polymorphism (SNP) that occurs in approximately 30% of the population, though some estimates are as high as 50%. Val66Met is associated with decreased activity-dependent BDNF release and has been consistently, though not unequivocally, linked to diminished short-term motor cortical plasticity. Homozygous Met carriers seem to display more impairments than Val/Met individuals in tests of verbal episodic memory [330], and show abnormal hippocampal activation patterns [330]. Furthermore, Met carriers demonstrate lower levels of hippocampal n-acetyl aspartate (NAA), a marker related to neuronal integrity and synaptic activity. In the primary motor cortex, Met carriers display decreased task-related M1 activation [331], reduced responses to the induction of experience-dependent plasticity [332], and impaired synaptic transmission [333]. In two of the earliest exploratory studies in the motor cortex, Cheeran et al. [334] reported impaired responses to a number of plasticity-inducing interventions in Met carriers, including cTBS, iTBS, and conditioning TDCS, while Kleim et al. [332] reported decreased motor map size in Met carriers following motor training relative to the Val/Val group, suggesting impaired use-dependent plasticity in these individuals. Met carriers also have decreased activation volumes during performance of a motor task relative to Val/Val individuals, and while both groups demonstrate a reduction in map volume following motor training, this response is significantly attenuated in Met carriers [331]. These findings were supported by Cirillo et al. [335], who demonstrated

that carriers of the SNP failed to show a modulation of M1 excitability following PAS. In addition, Met carriers display impaired responses to both PAS- and TDCS-induced plasticity, demonstrating greatly reduced or even reversed changes in MEP excitability [334]. Interestingly, Met carriers also display lower susceptibility to priming techniques. Lee et al. [336] observed an enhancement of motor learning when training was preceded by excitatory iTBS in Val-Val individuals, while Met carriers did not demonstrate this additive benefit. It is not yet known whether Met carriers lack the ability to respond to such interventions or whether the threshold for plasticity induction is simply higher. Indeed, despite not responding to PAS directly, Cirillo et al. [335] report that Met carriers show no impairment when PAS is followed by a motor training task. Antal et al. [337] suggest that Met carriers may actually respond more effectively to facilitatory TDCS than Val/Val individuals, indicating that not all cortical networks are affected equivalently by the polymorphism and that the optimal method of plasticity induction may differ between the groups. Despite strong evidence of impaired plasticity, this is not a universal finding, as Li Voti et al. report no effect of Val66Met on the response to rTMS, iTBS, or motor learning in a sample of healthy volunteers [338]. This is supported by Mastroeni et al. [162], who observed no differences in homeostatic metaplasticity between Val/Val and Val/Met individuals. These discrepancies may be due to differences in the time of measurement [338], current flow [162], or hormonal cycle [162]. It has also been suggested that the effect of BDNF is dependent on the co-expression of other related genes [339] . Although a large-scale study involving exercise has not been undertaken, in Chapter 2 of this thesis we observed that Met carriers and Val/Val individuals displayed a similar reduction in SICI and increase in ICF following a single exercise session.

1.9.6 Determination of genotype with polymerase chain reaction (TaqMan assay)

The polymerase chain reaction, known as PCR, or real-time quantitative PCR (RT-qPCR), is a commonly used technique to amplify and identify DNA sequences. Using a small sample of DNA from sources such as blood, serum or saliva, PCR is capable of exponentially amplifying sequences of interest, using repeated cycles of heating and cooling (thermal cycling). For all relevant studies in this thesis, genotype analysis will be performed using the TaqMan assay for identifying single nucleotide polymorphisms (SNPs). A brief overview of this process is given below.

PCR can be generally divided into 3 stages. In the initial stage, a sample containing the DNA of interest is added to a solution containing buffer, two primers for the genes of interest (one for the wild-type allele and one for the polymorphism), DNA polymerase, and free nucleotides. For genotype analysis, two probes containing fluorescent reporter dyes are also added to distinguish between the alleles following PCR, specifically VIC dye for allele 1 and FAM dye for allele 2. The reporter dyes are linked to non-fluorescent quencher dyes that suppress reporter fluorescence until they are cleaved from the probe. The solution is then heated to 95°C in order to denature the DNA, resulting in the unwinding of DNA from its double helix formation and the breaking of the hydrogen bonds between base pairs. In step 2, the solution is rapidly cooled to between 50-65°C to allow the primers to anneal to their target regions on the two, newly-single strands of DNA, indicating the region to be amplified. In step 3, at a temperature between 75-80°C, a DNA polymerase, typically *Taq* polymerase, moves along each strand, attaching free nucleotides to the 3' end

at an approximate rate of 1000 nucleotides/minute and synthesizing two new strands of DNA. These daughter strands are identical to the template DNA and are thus amplified themselves in the next cycle. These three steps are typically repeated 20-40 times, with a doubling in the amount of DNA with each cycle. During PCR, the probes will have annealed to their complementary sequence on the amplified DNA. Probes are then cleaved to release the reporter dye, which generates a fluorescent signal. Amplification continues until the concentrations of substrates/reagents are depleted. The strength of the fluorescent signal is quantified once PCR is complete and indicates which sequences are present in the sample: a VIC only signal indicates homozygosity for allele 1; a FAM only signal indicates only allele 2 is present; and a combination of VIC and FAM fluorescence indicates a heterozygous sample containing both alleles.

1.10 Specific research objectives

In this thesis, we aimed to investigate the response of M1 to an acute bout of aerobic exercise. We explored the influence of both exercise alone and exercise as an adjunct intervention on measures of excitability and plasticity induction in M1. The specific hypotheses of this thesis are listed below.

1) To determine if acute, lower-limb aerobic exercise can modulate the cortical excitability of an upper limb muscle.

Hypothesis: Exercise will enhance cortical excitability of the target muscle.

2) To investigate whether aerobic exercise can facilitate the induction of M1 plasticity by paired associative stimulation (PAS).

Hypothesis: The combination of exercise and PAS will increase cortical excitability to a greater extent than PAS alone.

3) To determine whether aerobic exercise can enhance cortical and behavioural adaptations to a motor learning task.

Hypothesis: The combination of exercise and motor training will improve motor performance and enhance cortical excitability to a greater extent than training alone.

4) To investigate the relationship between acute exercise and the timing and magnitude of long-term depression (LTD) induced by continuous theta-burst stimulation (cTBS).

Hypothesis: Exercise will impair the duration or the magnitude of cTBS after-effects.

2 Aerobic exercise modulates intracortical inhibition and facilitation in a non-exercised upper limb muscle

Adapted from: Singh et al., *BMC Sports Sci Med Rehabil*. 2014 Jun 21;6:23

2.1 Introduction

The benefits of exercise on brain function have been widely documented. However, little is known about the direct effects of exercise on motor cortical excitability. In clinical settings, aerobic exercise is commonly prescribed to improve cardiovascular function following brain injury and can successfully improve aerobic capacity in neurological patient populations [340–343]. In addition to secondary cardiovascular disease prevention and improved quality of life, emerging evidence suggests exercise may also promote beneficial cortical adaptations. Plasticity in the motor cortex is a primary goal of rehabilitation programs following brain injury, and much attention has been focused on the ability of exercise to act as a potential primer for subsequent task-specific changes in cortical excitability associated with learning-based rehabilitation. However, the mechanisms underlying such modulation are not yet known. While chronic physical activity is associated with increased metabolic capacity and increased angiogenesis in the primary motor cortex (M1) [344,345], little is known about how aerobic exercise modulates cortical excitability, and the effects of an acute bout of aerobic exercise on the motor cortex are unclear.

Recently, pedaling exercise has been shown to decrease intracortical inhibition in the leg region of M1 [207], which suggests that such an intervention may be effective in increasing excitability. However, in clinical settings, spasticity and muscle weakness are seen frequently in the upper limbs, particularly following a stroke. Up to 85% of stroke patients present with hemiparesis of the upper limbs [346,347], and thus the upper limb musculature is often the focus of rehabilitation. Yet, the majority of clinical aerobic

exercise interventions, such as walking, running and cycling, predominantly involve the lower limbs. Pedaling exercise is frequently used in rehabilitation settings for patients who exhibit gait disturbances, or who present with balance or stability issues. Recent evidence indicates that acute cycling modulates intracortical inhibition in the cortical representations of active muscles; however, it is not known if this response is limited to muscles involved in the exercise or if such responses can be observed in nonexercised limbs. Here, we use transcranial magnetic stimulation (TMS) to probe both the excitability of descending tracts of nonexercised muscles following exercise and the intracortical inhibitory and facilitatory networks within M1. We assessed the effect of aerobic exercise on corticospinal excitability by using single pulses to generate a stimulus-response (S-R) curve at varying intensities. Three paired-pulse paradigms were used to probe the effect of exercise on the intracortical networks within M1: short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI) and intracortical facilitation (ICF).

The primary aim of this study was to investigate the effects of a brief session of lower-limb aerobic exercise on the cortical excitability of an upper-limb muscle representation. In addition, we investigated whether the presence of a common single nucleotide polymorphism (a valine-to-methionine substitution at codon 66, or Val66Met) of the brain-derived neurotrophic factor (BDNF) gene would impact the cortical response to exercise. BDNF is a growth factor secreted by the brain that is critical for the growth and survival of neurons and plays a key role in the development of long-term potentiation (LTP). The Val66Met polymorphism is associated with decreased activity-related BDNF release and has been linked to diminished motor cortical plasticity, with Met carriers displaying decreased task-related M1 activation [331], reduced responses to the induction

of experience-dependent plasticity[332], and impaired synaptic transmission [333]. Thus, we examined whether genetic variability in the BDNF gene would affect the response of M1 to aerobic exercise. We found that while the input-output curve and LICI were not significantly affected by exercise, lower-limb exercise induced a significant decrease in SICI and increase in ICF in a non-exercised muscle. None of the above measures were significantly affected by the presence of the BDNF polymorphism. These findings may have important implications for the use of aerobic exercise in treating upper limb motor deficits.

2.2 Methods

2.2.1 Subjects

Twelve young, healthy, moderately active individuals were recruited (7 males; 1 left-handed, 11 right-handed; average age = 28 years). All subjects had prior experience with TMS. Informed consent was obtained from all participants prior to undergoing the experimental protocol. Participants were screened for any contraindications to TMS using a standard screening form. All experimental procedures received clearance from the University of Waterloo Office of Research Ethics.

2.2.2 Exercise protocol

Heart rate (HR) and rate of perceived exertion (RPE) were collected at rest prior to exercise. During exercise, heart rate was monitored using a wrist-mounted heart rate monitor. Participants were instructed to work at 65-70% of their age-predicted maximal heart rate [average =125-135 beats per minute (bpm)]. After a brief warm-up to elevate

HR into the target zone, participants performed 20 minutes of continuous stationary biking on a recumbent bicycle in an isolated room. The duration and intensity were intended to mimic a standard aerobic workout. HR was carefully monitored and maintained throughout the session. Participants were seated comfortably with their feet strapped to the pedals and their backs against the backrest. RPE was verbally reported using the modified Borg scale every five minutes, and HR was continuously monitored throughout the exercise period. Instructions were given to work at a moderate intensity (RPE of 3 -4), and participants could adjust either the pedaling resistance or the rate of pedaling to maintain the target heart rate. All participants reported intensity rates in the moderate range, with no individual exceeding an RPE of 4. The experimenters remained with the participant throughout the exercise and insured that arms were resting comfortably by their sides and not gripping the handlebars during the session. The arms, and particularly the forearms, remained stationary during pedaling exercise. Participants were given free access to water. Immediately following exercise completion, subjects returned to the TMS testing room for the collection of post-exercise measures. Post 1 occurred immediately following exercise and lasted approximately 5 minutes. Participants then rested in an upright chair in a quiet room for the remainder of the rest period. Post 2 was collected 30 minutes following exercise completion. In all cases, heart rate had returned to resting or near-resting levels (within 5bpm) by the 30 minute mark post-exercise.

2.2.3 BDNF genotyping

The brain-derived neurotrophic factor (BDNF) Val66Met polymorphism (rs6265) was genotyped from saliva samples by qPCR on an ABI7500 using a TaqMan SNP

genotyping assay (Applied Biosystems) with 10 ng of saliva genomic DNA isolated by standard procedures from anonymized samples. Random duplicate analyses showed 100% concordance with runs.

2.2.4 TMS protocol

Focal TMS was performed using a MagPro x 100 stimulator (Medtronic, Minneapolis, MN, USA) and a 'figure of eight' coil (MCF-B65; 70 mm). BrainSight Neuronavigation (Rogue Research, Canada) was used to guide the placement of the coil to the target motor region using a template MRI for all participants. Anatomical co-registration was performed prior to baseline collection and subsequent coil positioning was tracked using reflective markers affixed to custom-fitted glasses. The coil was placed at a 45° angle to the mid-sagittal line to induce a posterior to anterior current in the underlying neural tissue. EMG recordings of motor-evoked potentials (MEPs) were obtained using surface electrodes placed over the right extensor carpi radialis muscle (ECR). Raw EMG signals were recorded and stored in a customized Labview (National Instruments, Austin, TX, USA) program for offline analysis. The motor hotspot of the right ECR muscle was identified as the left M1 location that consistently elicited a maximal MEP in the resting muscle, as assessed by EMG amplitude, while producing a visible muscle twitch. The resting motor threshold (RMT) was determined by the stimulation intensity required to elicit a peak-to-peak MEP amplitude of >50 μV on 5 out of 10 trials. After localization of the hotspot, a stimulus-response curve was generated by assessing the cortical response to single-pulse TMS at a range of intensities. Ten single pulses were delivered with a minimum of 2-second intervals at stimulus intensities of 100%, 110%,

120%, 130%, and 140% of RMT. Three paired-pulse measures were also assessed using the following parameters for the conditioning stimulus (CS), test stimulus (TS) and inter-stimulus interval (ISI): a) SICI (CS=80% and TS=120% of RMT, 2.5 ms ISI); b) LICI (CS=120% and TS=120% of RMT, 100 ms ISI); and c) ICF (CS=80% and TS=120% of RMT, 12 ms ISI). Ten pairs of stimuli were delivered in each paired-pulse protocol with an ISI of 2 seconds between stimulus pairs. Thus, the following four measures were randomized across participants but the order remained consistent throughout each individual experiment: i) S-R curve, ii) SICI, iii) LICI, and iv) ICF. Measures were collected just prior to exercise, immediately following exercise, and again 30 minutes following exercise completion.

2.2.5 Statistical analysis

In all paired-pulse measures, the degree of inhibition or excitation was normalized to the single pulse amplitude at 120% RMT for each timepoint. Participants in whom intracortical inhibition could not be induced pre-exercise using standard protocols were excluded from the corresponding analysis. For SICI and ICF, the average amplitude elicited by the conditioned stimulus was expressed as a percentage of the average unconditioned MEP amplitude at 120%. For LICI, the amplitude of the MEP evoked by the test pulse was expressed as a percentage of the conditioning stimulus amplitude, and the average of 20 trials was taken. For the S-R curves, 10 MEPs were averaged at each intensity and the average values were compared. To assess changes in resting single-pulse excitability within the S-R curves, a two-way repeated measures ANOVA was conducted with time (pre, post 1 and post 2) and stimulus intensity (100%, 110%, 120%, 130% and 140% RMT) as

factors. Paired-pulse measures were analyzed using three separate one-way repeated measures ANOVAs for SICI, LICI and ICF data using time as the main factor. Post hoc testing was performed using Tukey's HSD. To test the effect of the BDNF polymorphism, subjects were divided into Met carriers (n=6) or non-Met carriers (n=6). A mixed 2 x 3 x 5 ANOVA was conducted to assess differences in S-R curves between Met carriers and non-Met carriers using stimulus intensity and time as the within-subjects factors and genotype as the between-subjects factor. The response to paired-pulse measures within each group was assessed using separate two-way mixed ANOVAs for SICI, LICI, and ICF, with time as the within-subjects factor and genotype as the between-subjects factor. Significant main effects in the ANOVA were followed with post hoc tests using Tukey's HSD. The significance level for all tests was set at $p<0.05$.

2.3 Results

Although EMG was not collected continuously during the exercise session, offline testing was conducted to monitor upper limb muscle activity during an identical biking task. There was no detectable muscle activity in right ECR, flexor carpi radialis (FCR), or first dorsal interosseus (FDI) during biking. For all measures, pre-exercise responses were taken as baseline values. Figure 2.1 displays the S-R curve, with the average MEP amplitude evoked in response to varying stimulus intensities at each timepoint. Not surprisingly, a two-way repeated measures ANOVA showed a significant main effect of intensity ($F_{4, 44} = 9.70$, $p<0.001$, Fig.2.1). Post-hoc testing using Tukey's HSD revealed that MEP amplitude differed significantly between 100% RMT and 120%, 130% and 140% RMT. In addition, evoked responses at 110% differed significantly from those at 130% and

140%. There was no main effect of timepoint relative to exercise ($F_{2, 22}$=1.59, p=0.23) and no interaction between intensity and timepoint ($F_{8, 88}$ =1.11 p=0.36).

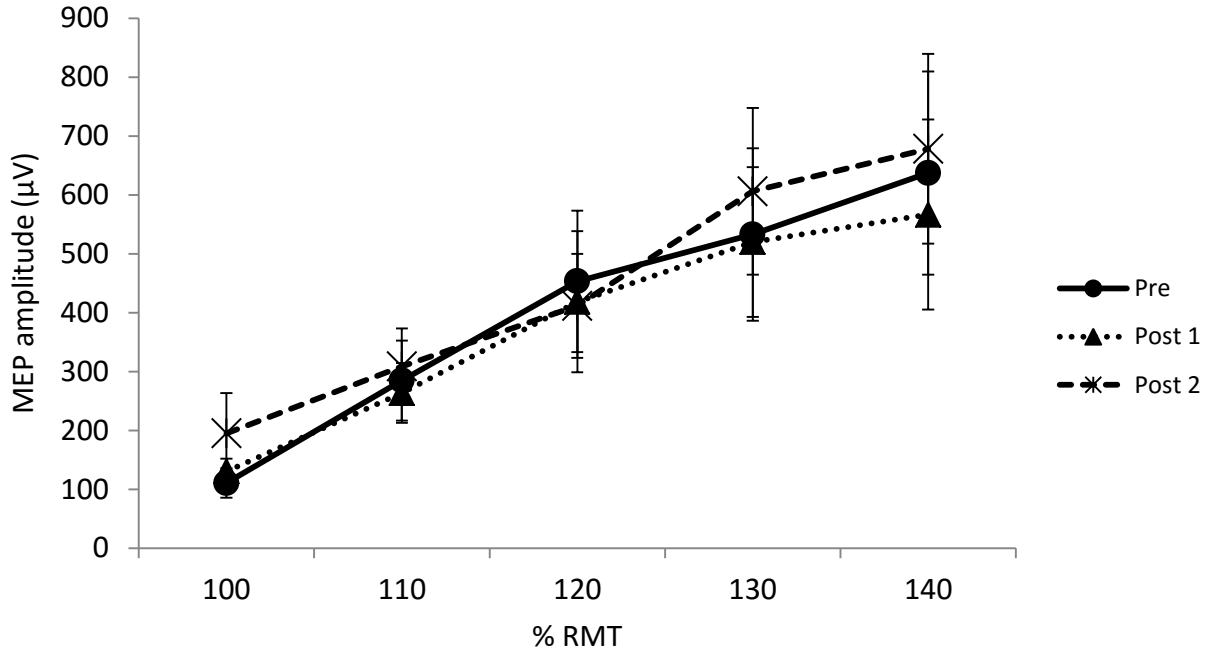
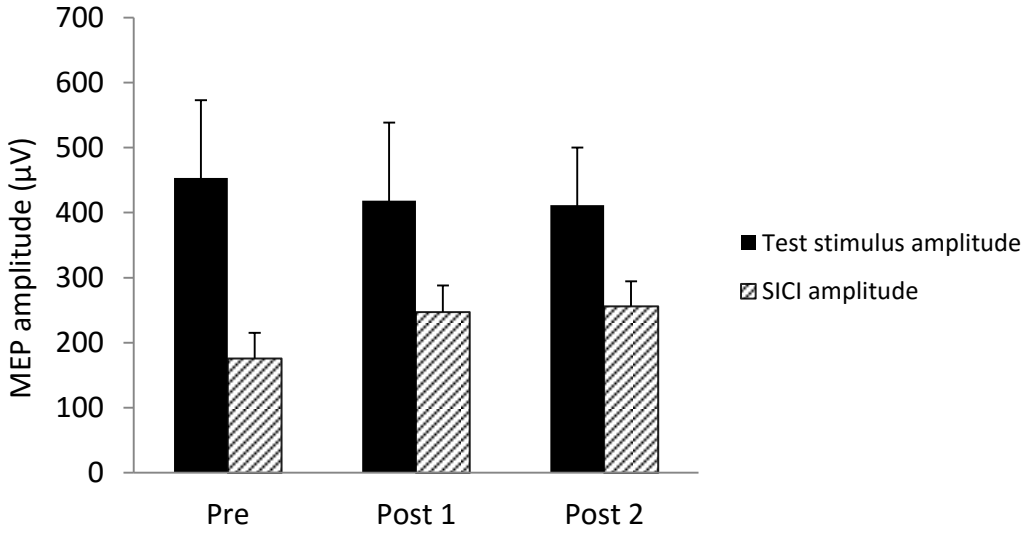


Figure 2.1 Recruitment curves before and after exercise. Stimulus-response curves pre- and post-exercise in response to stimulation at increasing percentages of RMT (n=12). Bars represent SEM.

Average paired-pulse responses across all subjects are shown in Figures 2-4. The above-noted exclusion criteria resulted in one participant being removed from the SICI analysis, and one participant from the LICI analysis. Figure 2.2a displays the consistency of SICI induction across each timepoint. Results of a one-way repeated measures ANOVA showed that following exercise, SICI was significantly decreased ($F_{2, 20}$ =4.30, p=0.028, Fig. 2.2b). Prior to exercise, SICI induced an average of 53.8±8.8% inhibition of the unconditioned stimulus. Immediately after exercise (post 1), SICI levels decreased to

21.8±18.5% and remained at 19.4±15.1% 30 minutes following exercise (post 2). Post-hoc testing using Tukey's HSD revealed a significant decrease in SICI from pre to post 2. Results from the LICI analysis demonstrate a similar trend: pre-exercise levels of LICI showed 54.2±9.6% inhibition of test stimulus amplitude. Following exercise, this decreased to 25.0±20.3% and increased slightly to 36.3±21.5% at post 2; however, these differences were not statistically significant ($F_{2, 20}$ =1.36, p=0.28, Fig. 2.3b). Correspondingly, a one-way repeated measures ANOVA of ICF revealed that following exercise, ICF was significantly elevated ($F_{2, 22}$ =5.29, p=0.013, Fig.2.4b). Baseline values showed a 140.1±11.2% increase relative to unconditioned stimulus amplitudes. At post 1, ICF values increased to 224.8±31.1% of unconditioned test pulses. ICF levels remained elevated at post 2, with an average of 193.7±23.6% facilitation. Post-hoc testing revealed significant differences between pre and post 1, and while ICF levels remained elevated at post 2 relative to pre, this difference was not statistically significant.

a)



b)

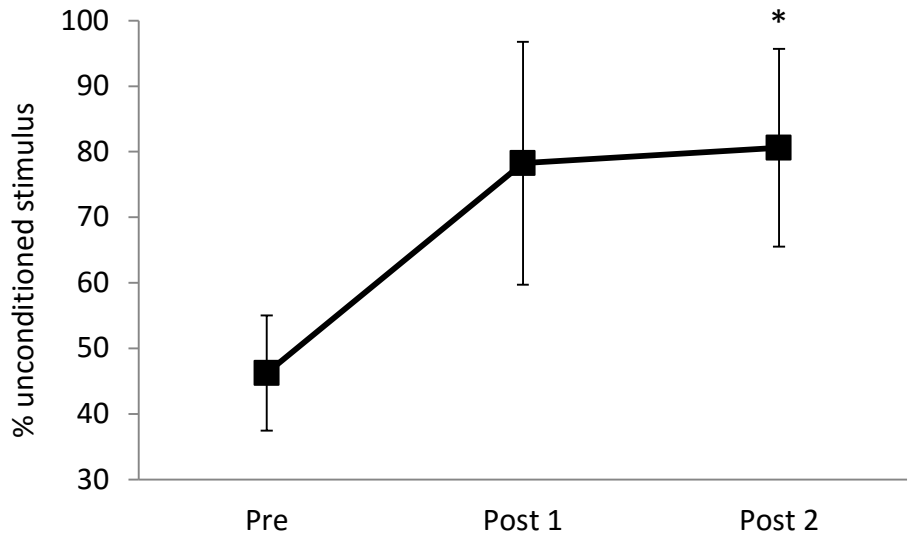
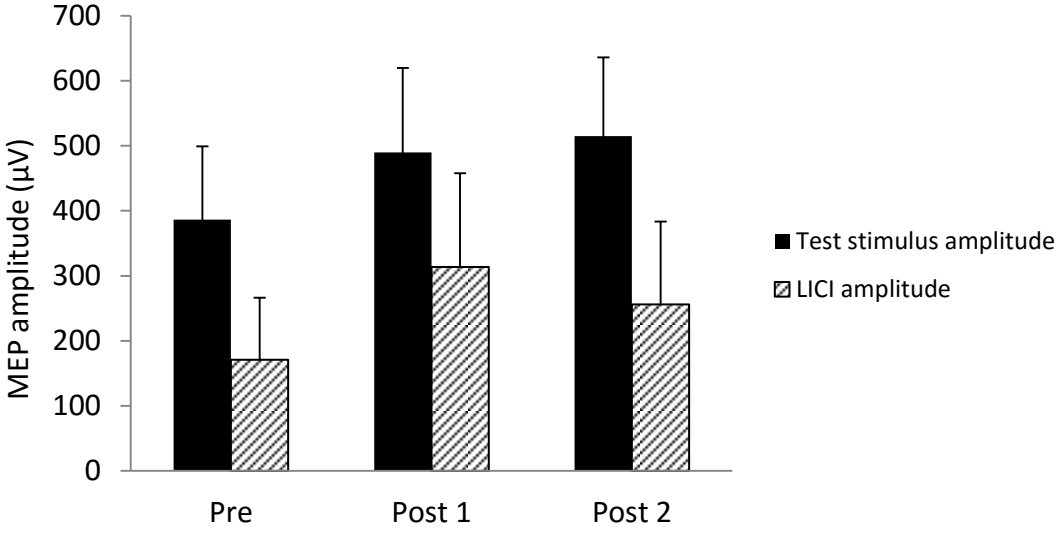


Figure 2.2 Modulation of SICI following exercise. Induction of SICI across all participants (n=11) at each timepoint (a) and % of test stimulus amplitude (b). Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SEM. Asterisks indicate values significantly different from pre-exercise ($p<0.05$).

a)



b)

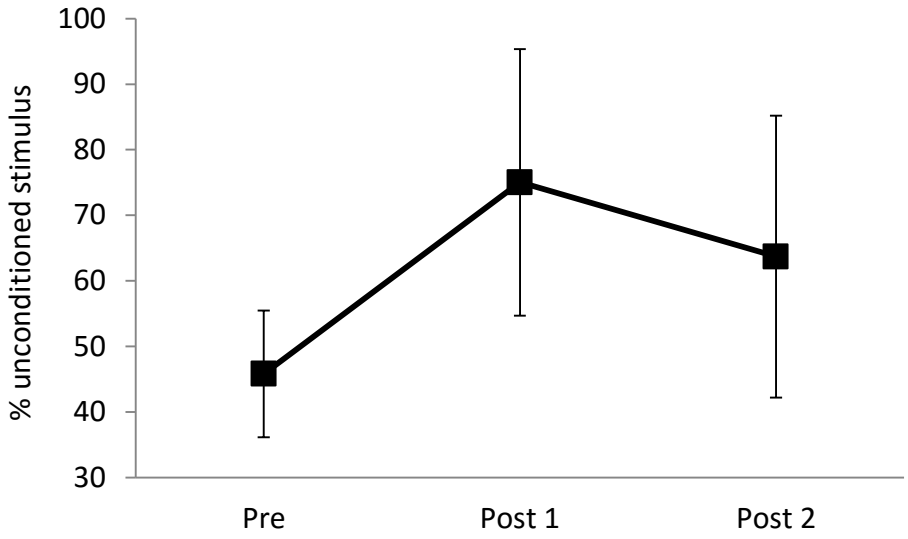
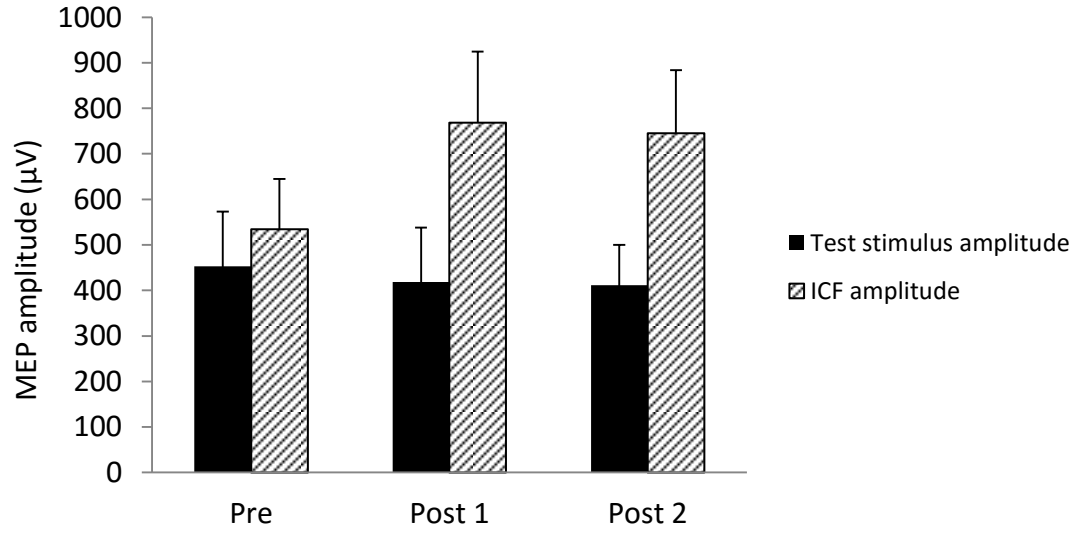


Figure 2.3 Modulation of LICI following exercise. Induction of LICI across all participants (n=11) at each timepoint (a) and % of test stimulus amplitude (b). Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SEM.

a)



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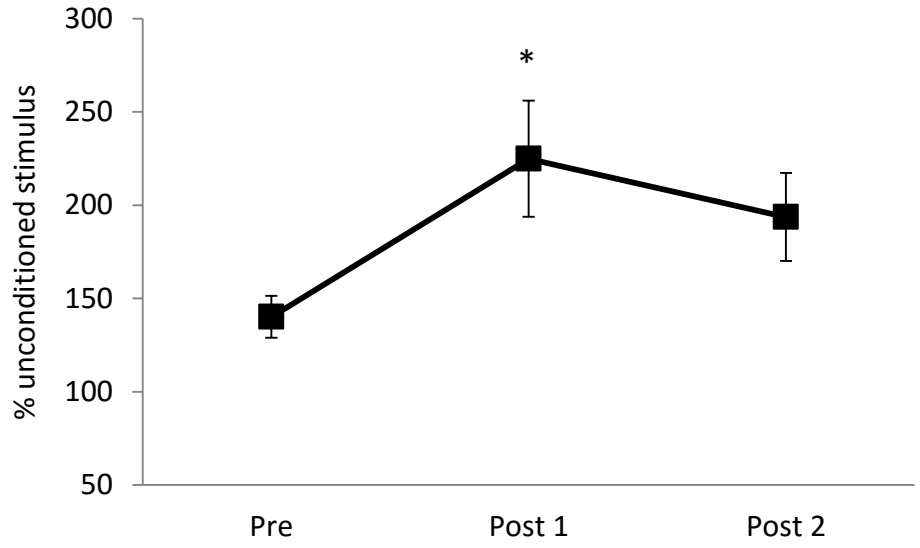


Figure 2.4 Modulation of ICF following exercise. Induction of ICF across all participants (n=12) at each timepoint (a) and % facilitation of test stimulus (b). Unconditioned single pulse amplitudes at 120% RMT are compared to conditioned stimulus amplitudes. Bars represent SEM. Asterisks indicate values significantly different from pre-exercise ($p<0.05$).

Results from BDNF genotyping indicated that six of twelve subjects were Met carriers (two homozygous and four heterozygous). Results from a 2 x 3 x 5 mixed ANOVA performed on S-R curves indicated no significant differences between Met carriers and Val/Val subjects in single-pulse excitability at any timepoint or any stimulus intensity ($F_{1, 7} = 0.14$, $p=0.71$, Fig.2.5).

Results from separate two-way mixed ANOVAs revealed no main effect of BDNF for SICI ($F_{1, 9} = 2.71$, $p=0.13$), LICI ($F_{1, 9} = 2.66$, $p=0.14$), or ICF ($F_{1, 10} = 0.00035$, $p=0.95$), and no BDNF x time interaction for SICI ($F_{2, 18} = 0.3$, $p=0.74$), LICI ($F_{2, 18} = 1.3$, $p=0.30$), or ICF ($F_{2, 20} = 0.5$, $p=0.62$) (Fig. 2.6).

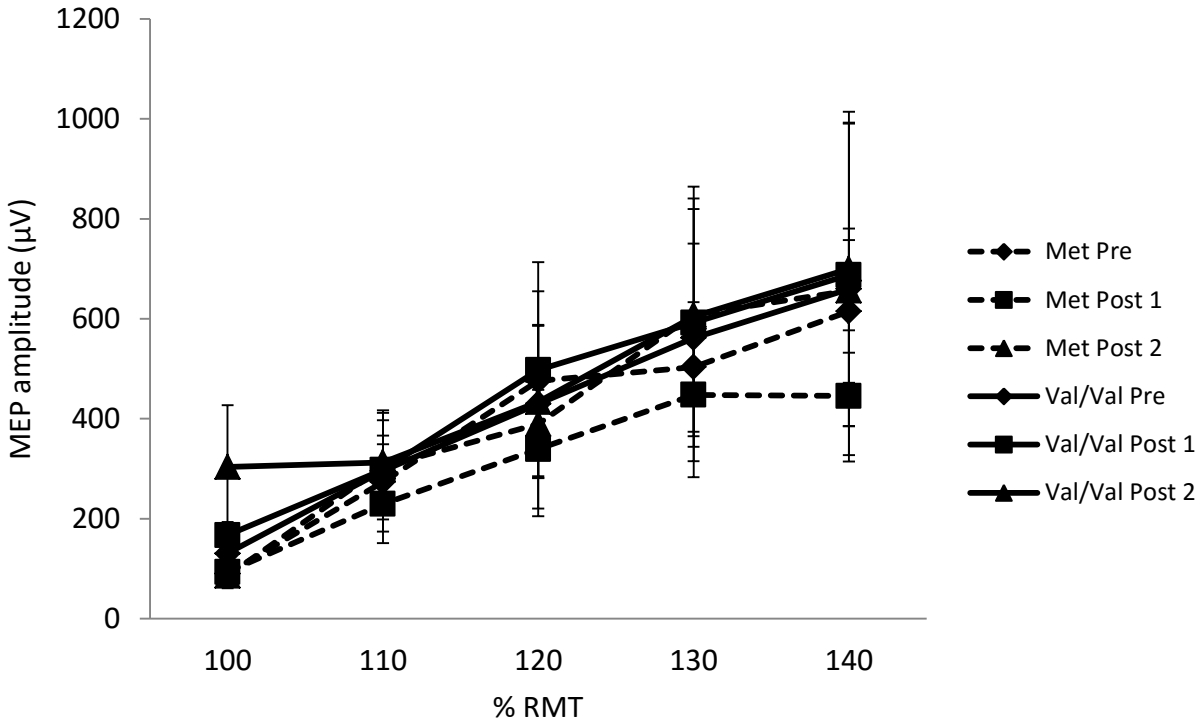
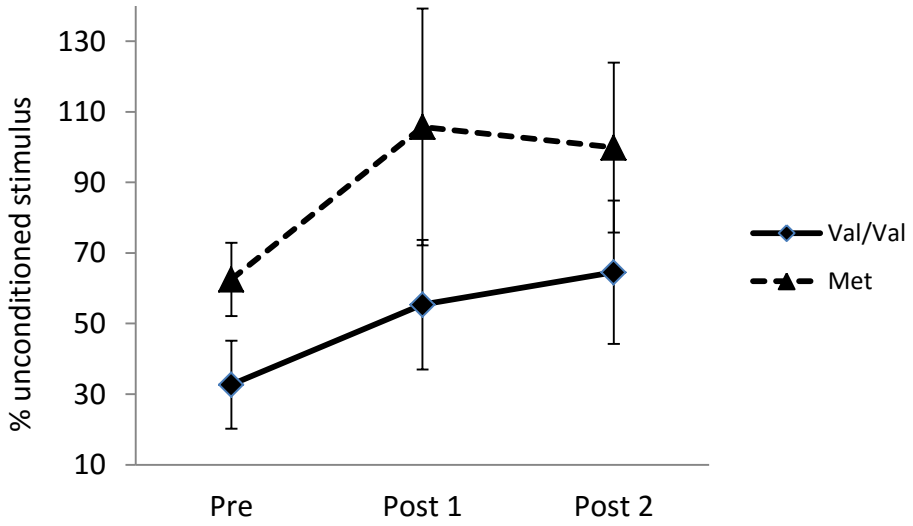


Figure 2.5 Effect of BDNF genotype on recruitment curves. Group differences between Met carriers (n=6) and non-Met carriers (n=6) in S-R curve outputs at each timepoint. Bars represent SEM.

a)



b)

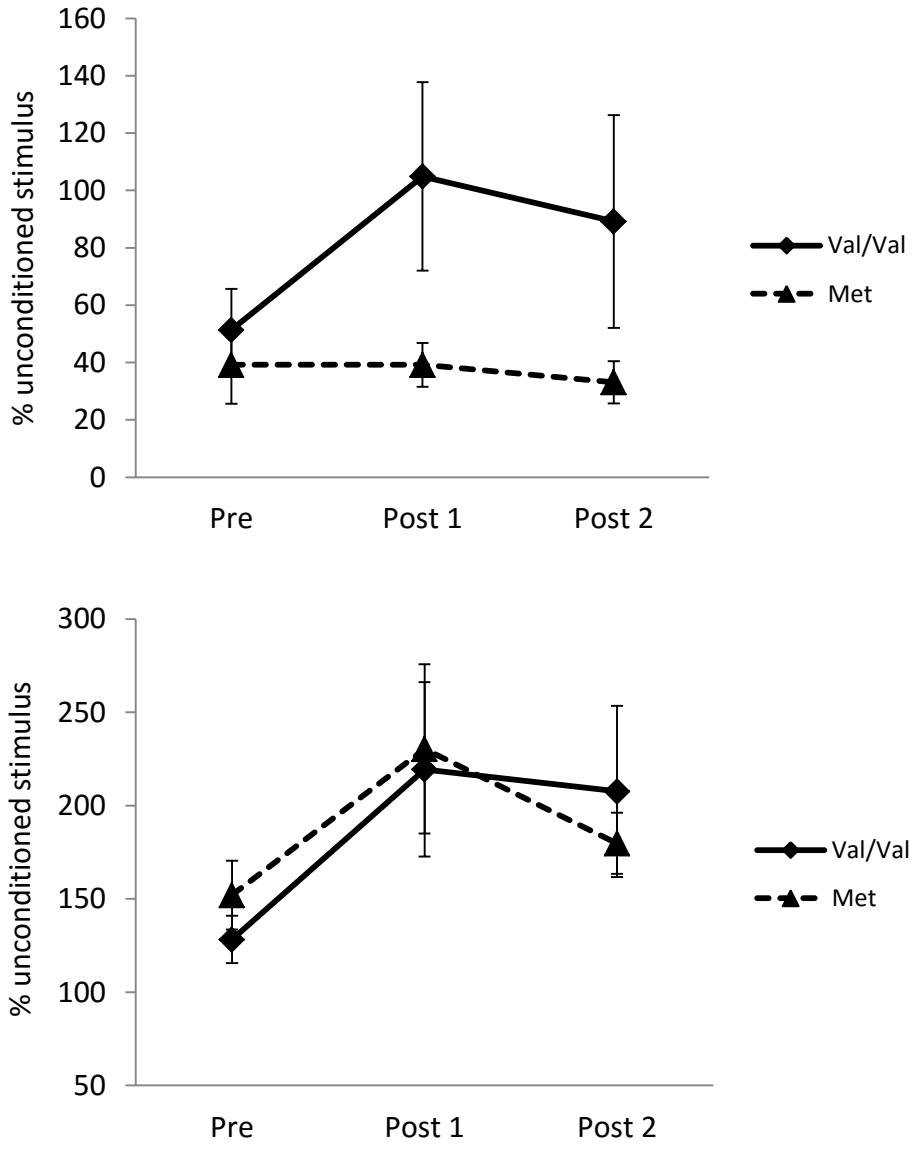


Figure 2.6 Effect of BDNF genotype on intracortical inhibition and facilitation. Group differences between a) Met carriers (n=6) and non-Met carriers (n=5) for SICI; b) Met carriers (n=5) and non-Met carriers (n=6) for LICI, and c) Met carriers (n=6) and non-Met carriers (n=6) for ICF. Bars represent SEM.

2.4 Discussion

The aim of this study was to test whether the modulation of the cortical excitability of a specific muscle representation in M1 following aerobic exercise is dependent on the involvement of that muscle in the exercise itself. Specifically, we sought to investigate whether aerobic exercise involving the lower limbs could modulate upper limb motor excitability and also to determine the time course of this modulation and potential mechanisms that contribute to it. Thus, both S-R curves and paired-pulse measures of SICI, LICI and ICF were used to probe the excitability changes in a wrist extensor muscle following a single session of stationary biking. Immediately after exercise completion, there was a significant decrease in short intracortical inhibition and a significant increase in intracortical facilitation. These levels remained elevated relative to pre-exercise values at 30 minutes after exercise completion. While LICI displayed a similar trend to SICI, in this case the decrease in inhibition was not statistically significant. In contrast, the S-R curves indicate that the resting motor threshold was not modulated by exercise. There were no significant differences observed in MEP amplitudes pre- and post-exercise at any intensity. Thus, resting motor thresholds of inactive muscles appear unchanged by exercise. However, the current results indicate that aerobic activity using the lower limbs causes an immediate and sustained modulation of intracortical facilitation and inhibition of an upper limb muscle. Such excitability changes are a necessary precursor to the relatively more permanent changes in synaptic strength seen in the processes of long-term potentiation (LTP) and long-term depression (LTD). It is likely that the altered excitability state of these interneuronal pools will render them more receptive to strategies aimed at inducing

plasticity, such as skilled motor training or targeted rehabilitation, when they are preceded by an exercise session. Furthermore, interventions that directly target the mechanisms of LTP/LTD, such as repetitive theta-burst stimulation (rTBS), may benefit from the addition of exercise. It should be noted, however, that the benefits of such interventions will not necessarily be additive. The emerging principles of homeostatic metaplasticity suggest that the probability of LTP induction depends on prior synaptic activity, and that when LTP has been recently induced, subsequent facilitatory interventions will be suppressed or even reversed in order to maintain a balance between LTP and LTD [152,159,161].

As indicated, previous research has demonstrated a decrease in SICI in exercising muscles [207]. The current results extend this finding to non-exercised muscles and indicate that such changes are not a direct consequence of preceding muscle activity. These results are in line with the findings of Takahashi et al. [206], who report that lower limb resistance exercise influences cortical excitability in nonexercised hand muscles. Takahashi and colleagues [206] propose several potential mechanisms for their findings, including facilitatory cortical pathways between synergistic arm and leg representations, and a spread of cortical excitability from active muscles to non-active muscles in proximal M1 areas. Neither of these possibilities can be ruled out here. However, the observed changes were seen at the motor hotspot of the ECR and not on the periphery of the representation, which would indicate a modulation of the ECR representation itself. Furthermore, the lack of an effect on single-pulse amplitude after exercise argues against a spread of excitability from active muscle representations. Nor do the current results address the contribution of spinal circuits. Although decreases in H-reflex amplitude following prolonged aerobic exercise have been reported in lower limb muscles [348],

upper limb muscles are unaffected, indicating that such changes do not represent a generalized decrease in spinal excitability but rather are specific to those muscles involved in locomotion [349]. Additionally, one would expect a change in spinal networks to be reflected in the single pulse excitability. In contrast, emerging evidence suggests that aerobic exercise is uniquely suited to cause a more generalized increase in intracortical excitability following exercise [186,238,350–352]. Indeed, a model of a more widespread neural effect of exercise is well-supported. Chronic physical activity is associated with increased activation of regions as diverse as the superior parietal cortex and the dentate gyrus [186], and can modulate everything from pain perception [350] to mood [351]. Further, it is clear that lower limb aerobic exercise can affect vascular functioning in upper limb muscles [352]. Indeed, a single bout of moderate intensity stationary biking can induce a 20% increase in global cerebral blood flow (CBF) [238]. Yet, it has been hypothesized that with limited metabolic resources, exercise may upregulate those regions involved with maintaining exercise [353] which, it is assumed, includes movement-related cortical regions such as M1. Such a global response could be mediated by the supplementary motor area (SMA) or the prefrontal cortex (PFC), both of which have shown increased activity with exercise [201,354,355].

Role of GABA and clinical significance

The mechanisms that may underlie a more widespread response to exercise are not entirely clear; however, there is strong evidence that exercise can modulate neurotransmission. Acute aerobic exercise has been shown to upregulate the activity and/or release of serotonin (5HT) [356,357], dopamine (DA) [260,263,356], and

norepinephrine (NE) [260,358], all of which can modulate the excitability of M1 neurons [95,105,112,113]. Exercise-induced increases in blood lactate have shown corresponding increases in M1 excitability [255], while increased uptake of the trophic factor insulin-like growth factor 1 (IGF-1) appears to mediate an increase in neuronal sensitivity and firing rates post-exercise [359]. Both the time course of the exercise-induced changes in excitability and the optimal exercise parameters for stimulating the release of neurotrophic factors remain under investigation. While the potential contribution of such excitatory neurotransmitters cannot be discounted here, the current results point to modulations in GABA (γ-aminobutyric acid) as a primary outcome of exercise. GABA is the principal inhibitory neurotransmitter in the CNS and exerts its effects via multiple receptors, particularly in cortical inhibitory networks. SICI is thought to be mediated by $GABA_A$ receptors [360], which are ligand-gated chloride channels, while LICI is believed to activate $GABA_B$ receptors [361], which are coupled to G-protein complexes that activate downstream K^+ ion channels. Although the cortical mechanisms of ICF are not fully understood, it appears to be mediated by glutamatergic interneurons, and possibly NMDA receptors [362,363]. While both LICI and SICI directly affect the excitability of corticospinal neurons, there are also interactions between them, as LICI appears to reduce SICI, likely via GABA-mediated inhibition of GABA-release [360,364]. The current results indicate that SICI is more sensitive to the effects of aerobic exercise than LICI. This is perhaps not surprising given that there appears to be little correlation between SICI and LICI measures [361,364]. Indeed, it has been suggested that $GABA_A$ and $GABA_B$ receptors may differ in their activation thresholds, with $GABA_A$ receptors requiring greater levels of exposure to the neurotransmitter [365]. Another potential reason for this disconnect is the

variation in test stimulus intensities, in that SICI and ICF both employ a subthreshold conditioning pulse that is assumed to activate intracortical connections, while LICI requires two suprathreshold pulses, and may therefore be activating a different pool of neurons.

Such intracortical networks are critical to the modulation of cortical output and are implicated in cortical plasticity and reorganization [366]. The release of GABA at inhibitory synapses directly modulates the excitability of pyramidal cells and the current results suggest this process may be sensitive to exercise. There is limited information available on GABA levels immediately following exercise; however, a downregulation of GABA signalling on baroreceptor neurons is thought to contribute to post-exercise hypotension [259]. Further, mRNA levels of a key $GABA_A$ receptor subunit are reduced after only 3 days of exercise training [367]. Meeusen et al. [260] report up to a 76% increase in striatal GABA levels following 60 minutes of treadmill running, although their data did not reach statistical significance. There are considerable clinical implications of an exercise-induced modulation of GABA activity. Decreases in GABA are critical for motor learning and M1 plasticity [79,80]. Indeed, excessive inhibition is a key cause of post-stroke motor impairment [368–370]. GABA blockade removes tonic inhibition and promotes plasticity [371], and indeed, a decrease in GABA levels is key to functional recovery after stroke [371–373]. It is clear that motor reorganization following a brain injury is dependent on functional plasticity. As GABA levels were not directly measured in this study, we cannot determine whether exercise results in changes in GABA release, uptake, or activity, or alters the sensitivity of $GABA_A$ receptors. However, these results indicate that there is a reduction in short intracortical inhibition following aerobic exercise, which is likely mediated by exercise-induced changes in $GABA_A$ activity.

Our results, taken together with previously observed increases in excitatory neurotransmission, indicate that the net effect of exercise appears to be a decrease in M1 inhibition that may facilitate the induction of plasticity. In the current study, these effects are seen immediately after exercise and persist at 30 minutes after exercise completion. Thus, it is possible that the intracortical network changes seen here are a necessary precursor for cortical plasticity, and that exercise creates the conditions under which more permanent plastic changes may occur. The current results indicate that in non-active muscles, exercise alone does not directly affect the resting motor threshold of pyramidal cells, but instead modulates the balance of inhibitory and excitatory inputs to these cells. This is supported by the findings of Smith et al. [238], who despite observing a global increase in CBF following exercise, did not see an observable modulation in M1 until a subsequent motor task was performed. In addition, McDonnell et al. report no changes in MEP amplitude in the FDI muscle following cycling exercise, but instead demonstrate that the effects of theta-burst stimulation (TBS) are potentiated when preceded by exercise [299] . Thus, while exercise may not modulate CST excitability in and of itself, it can potentially create favourable conditions for the induction of cortical plasticity with subsequent motor training. Indeed, aerobic exercise training has been shown to improve motor arm function after stroke [374,375], and the combination of exercise and skilled motor training improves motor recovery to a greater extent than training alone [376].

Thus, in this context, it is perhaps not surprising that the paired-pulse measures here do not correlate with the single-pulse data, in which we observed a decrease in SICI and an increase in ICF, but no concomitant increases in single-pulse MEP amplitude. This would seem to indicate that there is not a direct correlational relationship between these

two measures. Previous studies have reported a similar disconnect between single and paired-pulse measures of CST excitability [105,364,377]. Indeed, Ilic et al. [105] propose that single and paired-pulse measures may reflect substantially different mechanisms. The final corticospinal output reflects the summation of all inhibitory and excitatory inputs to the descending neuron, and can be influenced by many factors, both cortical and subcortical. The paired-pulse measures taken here reflect the activity of particular cortical interneuron pools whose activity may be modulated by exercise, but which are only one of a multitude of inputs on the descending motor neuron.

BDNF

As a neurotrophic factor, the relationship between acute exercise and BDNF is not clear. Although increases in levels of serum BDNF have been reported following acute aerobic exercise [182,313,314,317,378], BDNF is known to exert its effects primarily over longer time frames and is correlated with the induction of LTP and postsynaptic modification [379]. Thus, it is unlikely that BDNF levels significantly influenced the response to exercise seen here.

Although not the principal aim of this study, we were interested in exploring the relationship between a relatively common single nucleotide polymorphism of the BDNF gene and exercise-related changes in cortical excitability. The valine-to-methionine substitution at codon 66 of the BDNF gene occurs in approximately 30% of the population [380] and is associated with decreased activity-dependent BDNF release and impaired synaptic and cortical plasticity [331,332,334]. Here, as in the majority of the literature, Val/Met and Met/Met individuals were grouped together and compared to Val/Val

subjects. There was no difference between the groups in the S-R curves before or at either time point following exercise. Nor was there any interaction between BDNF and time, indicating that genotype did not influence the response to exercise. Previous studies investigating the response to facilitatory intermittent TBS have reported impairments [334,337] or no difference [162,338] in Met carriers, but methodological differences prevent direct comparisons of these studies. While the current sample size is smaller than in the above studies, a key difference is their use of a technique known to induce LTP-like plasticity. The neurological response to exercise is not well-understood, and as such is it not clear how such changes relate to the mechanisms underlying LTP. In the current study, two interesting trends are evident, in that Met carriers, on average, display a complete abolition of SICI following exercise (Fig. 2.6a). Secondly, Met carriers appear to be more resistant to the modulation of LICI following exercise (Fig. 2.6b). Indeed, the lack of response in this group is likely the reason the overall group effect for LICI failed to reach significance. While preliminary, these trends suggest modulations in $GABA_B$ receptor activity or sensitivity may contribute to the impaired short-term plasticity frequently observed in Met carriers, and warrants further investigation with a larger subject pool. Our aim was to investigate whether Met carriers would still display these exercise-induced effects, and these results suggest that Met carriers display no impairment in the response to exercise-induced modulations in SICI and ICF.

2.5 Conclusions

The present results suggest that lower-body focused aerobic activity can modulate cortical excitability in an upper limb muscle and that at the cortical level, exercise may

prime the motor cortex for the induction of plasticity. While these findings have potential clinical utility, further research will be required to determine how the relationship between exercise and cortical excitability may be altered by disruptions to the balance of cortical inhibition and facilitation following a brain injury, and how the response to exercise is affected by characteristics such as the location, severity and type of brain injury. However, the current findings support the use of aerobic training as an adjunct to traditional rehabilitation methods. A potential limitation of this study is that EMG data was not collected during the exercise session. Although a lack of upper limb activity was confirmed with offline EMG, there nevertheless remains the slight possibility of upper limb muscle activation during the biking session. A second limitation of this study is the investigation of only one upper limb muscle. However, given that changes in upper limb excitability following lower body aerobic exercise are not well-studied, our goal was to create a comprehensive profile of excitability changes that would be sensitive to modulations in both motor neurons and interneurons. This, combined with the time-sensitive nature of the post-exercise measures, made it difficult to test additional muscles. The generalizability of our findings across the upper limb is an interesting direction for future studies.

Acknowledgements

The authors thank Philip Marvyn and Ashley Patterson for technical assistance with genotyping. This work was supported by a research grant to WRS from the Natural Sciences and Engineering Research Council of Canada (NSERC).

3 Acute exercise enhances the response to paired associative stimulation-induced plasticity in the primary motor cortex

Adapted from: Singh et al. *Exp Brain Res*. 2014 Nov;232(11):3675-85

3.1 Introduction

There is widespread interest in the use of aerobic exercise as a potential modulator of cortical excitability. To date, much of the literature in this area has focused on the relationship between acute exercise and the prefrontal cortex. A single session of exercise has been shown to improve cognitive function [182,381], cortical processing speeds [177], and the extent of cortical activation [201,354,355] when followed by a cognitive task. Yet, such benefits are not limited to prefrontal areas; when coupled with a novel motor learning task, exercise improves the long-term retention of motor skills [382]. However, the effects of exercise on other cortical areas, particularly motor regions, are not clear. Plasticity in the primary motor cortex (M1) is of particular importance for motor learning, and is also a key goal of neurorehabilitation strategies. In clinical settings, the use of exercise as an adjunct therapy along with traditional rehabilitation interventions may promote motor recovery following neurological injury [6]. Yet, the mechanisms underlying the beneficial effects of short-term exercise on neuroplasticity are unclear. In particular, the effects of acute aerobic exercise on M1 excitability are not well-known. While the role of M1 in movement execution during exercise is well-established, it is not known if this region may benefit from continued excitability changes following exercise cessation.

An enhancement in corticospinal tract (CST) excitability is often an early marker of more lasting neuroplastic changes. Within M1, local neuronal activity is primarily regulated by the intracortical networks of excitatory and inhibitory interneurons that surround pyramidal cells. Such networks are responsible for the generation of both short- and long-interval intracortical inhibition (SICI and LICI) as well as intracortical facilitation (ICF). In

addition to their critical role in the modulation of cortical output, these networks are strongly implicated in cortical plasticity and reorganization [366]. In exercising (lower-limb) muscles, SICI decreases immediately following aerobic exercise [207]. However, such modulations in SICI do not appear to be restricted to muscles involved in the exercise and may translate to the upper limb. Indeed, fatiguing lower limb contractions decrease SICI and modulate excitability in resting upper limb muscles [206], and an immediate release of SICI has been observed in the upper limb following acute stationary biking (Smith et al. 2014). A reduction of intracortical inhibition is a necessary precursor to the induction of short-term plasticity [26,33,64]. The release of gamma-aminobutyric acid (GABA) at inhibitory synapses directly modulates the excitability of pyramidal cells [46], and motor learning is associated with a rapid reduction of GABA levels [79]. Thus, an exercise-induced reduction in intracortical inhibition may create favourable conditions for neuroplastic change and ultimately increase the effectiveness of interventions that target M1. One such technique is paired-associative stimulation (PAS), a well-established method of inducing excitability changes within M1. PAS is based on Hebbian principles of neuronal plasticity that state that the repeated stimulation of a post-synaptic cell by a pre-synaptic neuron causes an increase in the strength of that synapse. PAS involves the delivery of repeated pairings of peripheral nerve stimuli coupled with single transcranial magnetic stimulation (TMS) pulses over M1 and is thought to induce long-term potentiation (LTP)-like plasticity. LTP involves a relatively permanent enhancement of synaptic transmission and efficacy, and is a primary goal of both motor learning interventions and neurorehabilitation following a brain injury. Thus, the aim of this study was to investigate the influence of a single session of aerobic exercise on PAS-induced plasticity in M1. We

tested the hypotheses that a bout of lower limb cycling exercise would enhance the response to a subsequent PAS intervention in an upper limb muscle, as demonstrated by an increase in cortical motor neuronal excitability, and that exercise would be associated with a release of SICI.

3.2 Methods

3.2.1 Participants

Eleven young, healthy, right-handed individuals were recruited (5 males; age range 20-32 years; average age = 28 years). All participants had prior experience with TMS, and informed consent was obtained prior to the experimental protocol. Each participant completed the control and the experimental sessions. One participant reported ill effects of the peripheral nerve stimulation following the first PAS session and did not complete the second session. Thus, the final experimental group consisted of ten individuals. All participants self-reported being moderately active and were screened for any contraindications to TMS. All experimental procedures received clearance from the University of Waterloo Office of Research Ethics.

3.2.2 Study design

Each participant completed two experimental sessions: one with PAS alone (control session) and one in which PAS was preceded by cycling activity (exercise session). The order of the sessions was randomized across participants, with the two collections

separated by one week. As cortisol levels have been shown to affect the response to PAS [142], all collections were performed in the afternoon (2pm) in order to minimize variability in cortisol levels. The timeline for both sessions is shown in Figure 3.1.

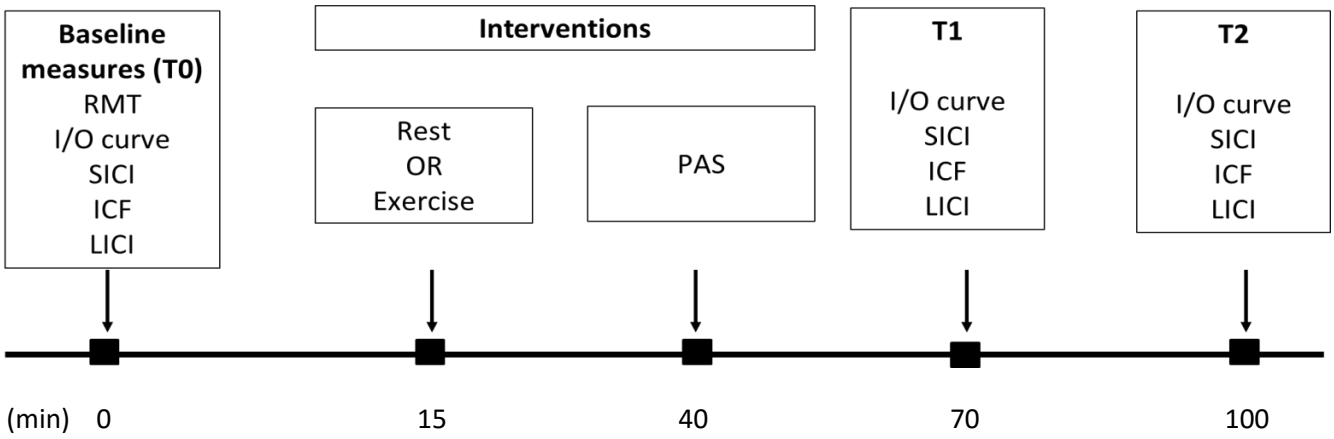


Figure 3.1: Timeline of TMS measures. RMT, resting motor threshold; I/O curve, recruitment curve; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation; LICI, long-interval intracortical inhibition. Measurements were taken at baseline (TO), immediately following PAS (T1), and again 30 minutes following PAS completion (T2).

Baseline measures were collected at the beginning of each session. In the control condition, participants then rested comfortably in a chair in the collection room for 25 minutes, following which PAS was delivered. In the exercise condition, following baseline measures, participants performed 20-25 minutes of stationary biking before the delivery of PAS. PAS was performed as soon as possible following the exercise/rest periods, which varied from 3-7 minutes depending on the time required to determine the perceptual threshold and attach electrodes for nerve stimulation. Post measures were collected at two timepoints: one immediately following PAS (T1), and one 30 minutes after PAS completion (T2).

3.2.3 Exercise protocol

Prior to testing, offline EMG analysis was conducted to ensure that the biking protocol used did not require or generate activity in the APB or FDI muscles. Heart rate (HR) and rate of perceived exertion (RPE) were collected at rest prior to exercise. During cycling, heart rate was monitored using a wrist-mounted pulse rate sensor. Participants were instructed to work at 65-70% of their age-predicted maximal heart rate (APMHR) [average =125-135 beats per minute (bpm)]. After a brief (approximately 5 minutes) warm-up to elevate HR into the target zone, participants performed 20 minutes of continuous stationary biking on an upright bicycle in an isolated room. The duration and intensity were intended to mimic a standard aerobic workout. HR was carefully monitored and maintained throughout the session. Participants were seated comfortably with their feet strapped to the pedals and the seat height adjusted for comfort. RPE was verbally reported using the modified Borg scale every five minutes, and HR was continuously monitored throughout the exercise period. Instructions were given to work at a moderate intensity (RPE of 3-4), and participants could adjust either the pedaling resistance or the rate of pedaling to maintain the target heart rate. All participants reported intensity rates in the moderate range, with no individual exceeding an RPE of 4. The experimenters remained with the participant throughout the exercise and ensured that arms were resting comfortably by their sides or on top of the handlebars and not gripping the handlebars during the session. Participants were given free access to water. Immediately following exercise completion, subjects returned to the TMS testing room to undergo PAS.

3.2.4 TMS protocol – Recruitment curves

Focal TMS was performed using a MagStim 200[2] stimulator (Magstim, Whitland, UK) connected to a figure eight coil (50 mm inner diameter). BrainSight Neuronavigation (Rogue Research, Canada) was used to guide the placement of the coil to the target motor region using a template MRI for all participants. The coil was placed at a 45° angle to the mid-sagittal line to induce a posterior to anterior current in the underlying neural tissue. EMG recordings of motor-evoked potentials (MEPs) were obtained using surface electrodes placed over the right abductor pollicis brevis (APB) muscle and the right first dorsal interosseous (FDI). Raw EMG signals were amplified (1000x), band-pass filtered (2Hz-2.5kHz; Intronix Technologies Corporation, Model2024F, Canada), digitized (5 kHz, Micro1401, Cambridge Electronics Design, Cambridge, UK), and then recorded by a computer using SIGNAL software (Cambridge Electronic Devices, Cambridge, UK) and stored for off-line analysis. The motor hotspot of the right APB muscle was defined as the left M1 location that consistently elicited an optimal MEP in the resting muscle, as assessed by both MEP amplitude and a visible muscle twitch. The resting motor threshold (RMT) was determined by the stimulation intensity required to elicit a peak-to-peak MEP amplitude of >50 μV on 5 out of 10 trials. After localization of the hotspot, a recruitment curve was generated by assessing the cortical response to single-pulse TMS at a range of intensities. Ten single pulses were delivered with a 5 second inter-stimulus interval at intensities set to 100%, 110%, 120%, 130%, and 140% of RMT. The order of intensities tested was randomized across participants, but remained consistent within each session.

3.2.5 TMS Protocol – Paired-pulse measures

Following the calculation of RMT, the stimulator intensity required to generate an MEP of approximately 1 mV in the resting APB was determined. This intensity was used as the test stimulus (TS) in paired-pulse trials and also for single-pulse stimulation during PAS. The amount of SICI, LICI and ICF has been shown to be dependent on the size of the TS amplitude (Sanger et al. 2001), and thus the TS intensity at each timepoint was adjusted in order to produce MEPs of equivalent magnitude (0.5 – 1mv). For all paired-pulse measures, the conditioning stimulus (CS) was set to 80% of RMT, and the TS was set to the intensity required to evoke a 1 mV contraction in the APB. At both post-PAS timepoints (T1 and T2), test pulses were delivered at 90% RMT to confirm that the CS intensity of 80% RMT would not evoke an MEP. For those participants in which a 1 mV MEP in the APB was difficult to generate, or occurred at very high percentages of stimulator output (>75%), TS intensities were set to 0.5-0.7 mV. Ten unconditioned TS pulses were delivered at the 1 mV (or equivalent) intensity to determine percent inhibition/facilitation. The three paired-pulse measures were assessed using the following parameters for the CS, TS and inter-stimulus interval (ISI): a) SICI (CS=80% RMT and TS=1 mV%, 2.5 ms ISI); b) LICI (CS=1mV and TS=1 mV, 100 ms ISI); and c) ICF (CS=80% RMT and TS=120% of RMT, 12 ms ISI). Ten pairs of stimuli were delivered in each paired-pulse protocol with an ISI of 5 seconds between stimulus pairs. The order of the four TMS measures (recruitment curve, SICI, LICI, and ICF) was randomized across participants but remained consistent throughout each individual experiment. In both sessions, all TMS measures were collected

at baseline and at both post-PAS timepoints. In all cases, heart rate had returned to resting or near-resting levels (within 5bpm) by the second post-collection (T2).

3.2.6 PAS protocol

Electrical stimuli were delivered to the right median nerve via bipolar surface electrodes placed at the wrist with the anode distal. Stimuli were square-wave pulses of 0.5 ms duration (GRASS S88 stimulator with SIU5 stimulus isolation unit; West Warwick, Rhode Island, USA) set to an intensity of 300% of perceptual threshold (PT). The TMS pulse was adjusted to the intensity required to evoke an average MEP of 1 mV in the APB in the absence of preceding nerve stimulation. PAS consisted of 180 pairs of stimuli delivered at 0.1 Hz with an inter-stimulus interval of 25 ms. Since attention has been shown to strongly influence the response to PAS [45], participants were instructed to count the number of stimuli and report this number at the end of the session. All participants reported the correct number ±3 stimuli. In the subsequent session, participants were required to count each 10^{th} stimulation aloud to avoid simple recall of the number of stimuli from the previous session.

3.2.7 Statistical analysis

Paired t-tests were used to examine any differences in baseline measures between sessions and changes in APB excitability during PAS. For recruitment curves, the peak-to-peak MEP amplitude was averaged across 10 pulses at each intensity. Any trials where EMG recordings indicated pre-stimulus activity in the target muscle were discarded. The average raw MEP amplitude was plotted as a function of intensity, and the area under the

curve (AUC) was calculated using the trapezoidal integral method. A two-way repeated measures analysis of variance (RM ANOVA) was conducted using time (T0, T1, T2) and session (exercise vs. control) as the main factors. Specific *a priori* contrasts were performed to test the hypothesis that exercise would enhance PAS-induced excitability. Given that there is variability regarding the time course of maximal PAS effects, planned contrasts were carried out between T0 and T1 and between T0 and T2. For SICI and ICF, average conditioned stimulus amplitudes were expressed as a percentage of the unconditioned test stimulus amplitude. Conditioned stimulus pulses for LICI were expressed as a percentage of the preceding unconditioned stimulus. For all paired-pulse measures (SICI, LICI and ICF), separate two-way RM ANOVAs were carried out with time (T0, T1, T2) and session (exercise vs. control) as the main factors. The hypothesis that exercise+PAS would modulate SICI was tested using a planned contrast. Although previous literature indicates that SICI is suppressed for at least 30 minutes following exercise, there is no indication of when levels might return to baseline and thus, for the SICI analysis, the two post-PAS timepoints were combined and compared to baseline values. Specific hypotheses for ICF and LICI were not possible and thus any significant main effects were further analyzed using Tukey's HSD test. Prior to running the ANOVAs, the residual errors were plotted and inspected to confirm normality of distribution and homogeneity of variances required by the assumptions inherent in ANOVA. Sphericity was calculated using Mauchly's test, with the Huynh-Feldt correction automatically applied if the assumption was violated. For all tests, statistical significance was set at $p<0.05$. Values are presented as mean +/- standard error (SE).

3.3 Results

Baseline measurements between the two sessions are shown in Table 3.1. There were no significant differences in RMT, average MEP amplitude at RMT, baseline AUC, perceptual thresholds, or the stimulator intensity used to deliver PAS between sessions. There were also no differences in baseline levels of SICI, ICF or LICI between sessions.

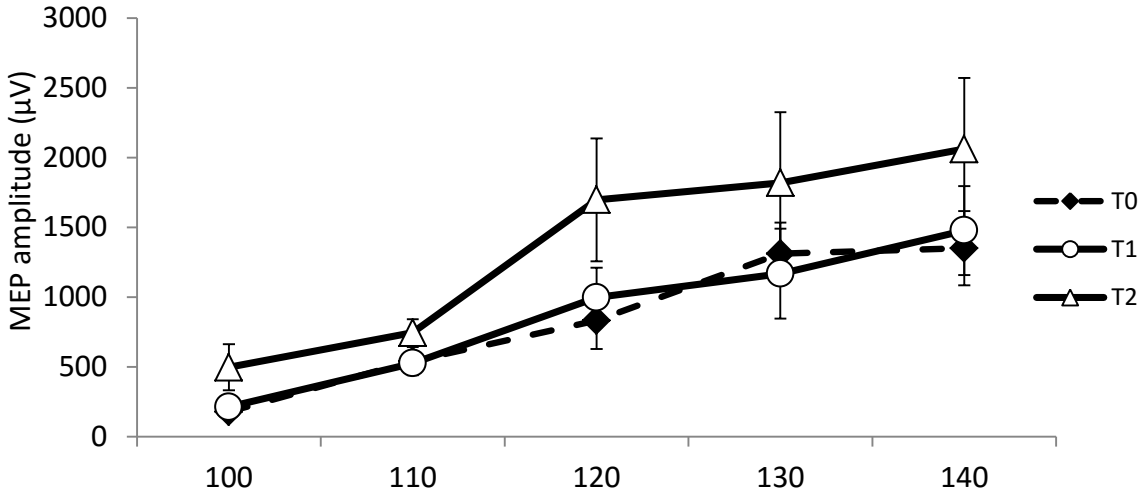
Table 3.1 Baseline measures in exercise and control sessions

	SESSION	
	PAS (mean ±SE)	**Ex + PAS (mean ±SE)**
RMT (% stimulator output)	50.0 ± 1.5	47.7 ± 1.9
Mean MEP amplitude at RMT (uV)	175.8 ± 39.0	109.4 ± 33.8
Baseline AUC (amplitude/%RMT)	3167.1 ± 556.3	3293.7 ± 811.9
Perceptual threshold (uV)	2.21 ± 0.48	2.13 ± 0.44
Stimulator intensity during PAS (%)	59.8 ± 2.9	55.3 ± 2.08
Baseline SICI (% CS)	30.4 ± 7.5	25.0 ± 9.4
Baseline ICF (% CS)	183.4 ± 45.8	160.8 ± 17.8
Baseline LICI (% CS)	31.4 ± 17.2	27.4 ± 10.2

Figures 3.2 and 3.3 show changes in the area under the recruitment curve following PAS. Figure 3.2 displays raw MEP amplitude changes at each intensity and timepoint for the APB, and AUC values are shown in Figure 3.3 for both APB and FDI muscles. For the APB, results of a 2-way RM-ANOVA indicated a main effect of time ($F_{2,18}$=7.28, p<0.005) but no effect of session ($F_{1,9}$=0.62, p=0.45) and no session x time interaction ($F_{2,18}$=1.48, p=0.25). The hypothesis that exercise would enhance the effects of PAS was tested by planned contrasts between the sessions at timepoints T1 and T2 and revealed an increased AUC following exercise + PAS compared to PAS alone at T1 ($F_{1,18}$=5.96, p<0.026, Figure 3.3a)

but no difference at T2 ($F_{1,18}$=0.43, p=0.52). In the non-target FDI, a 2-way ANOVA revealed no effect of time ($F_{2,18}$=0.27, p=0.76) or session ($F_{1,9}$=1.9, p=0.2), and no session x time interaction ($F_{2,18}$=0.23, p=0.79) (Fig. 3.3b).

a)



b)

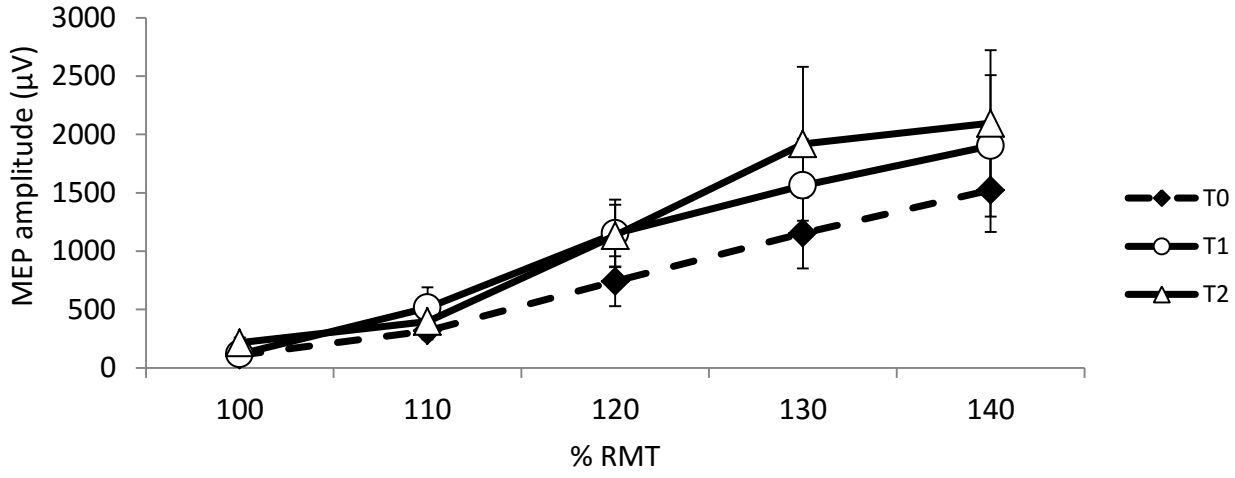


Figure 3. 2 Effect of PAS on cortical excitability. Changes in APB muscle following PAS are shown in a) control and b) exercise sessions. T0=baseline; T1=immediately post-PAS; T2=30 minutes post-PAS. Bars represent SEM.

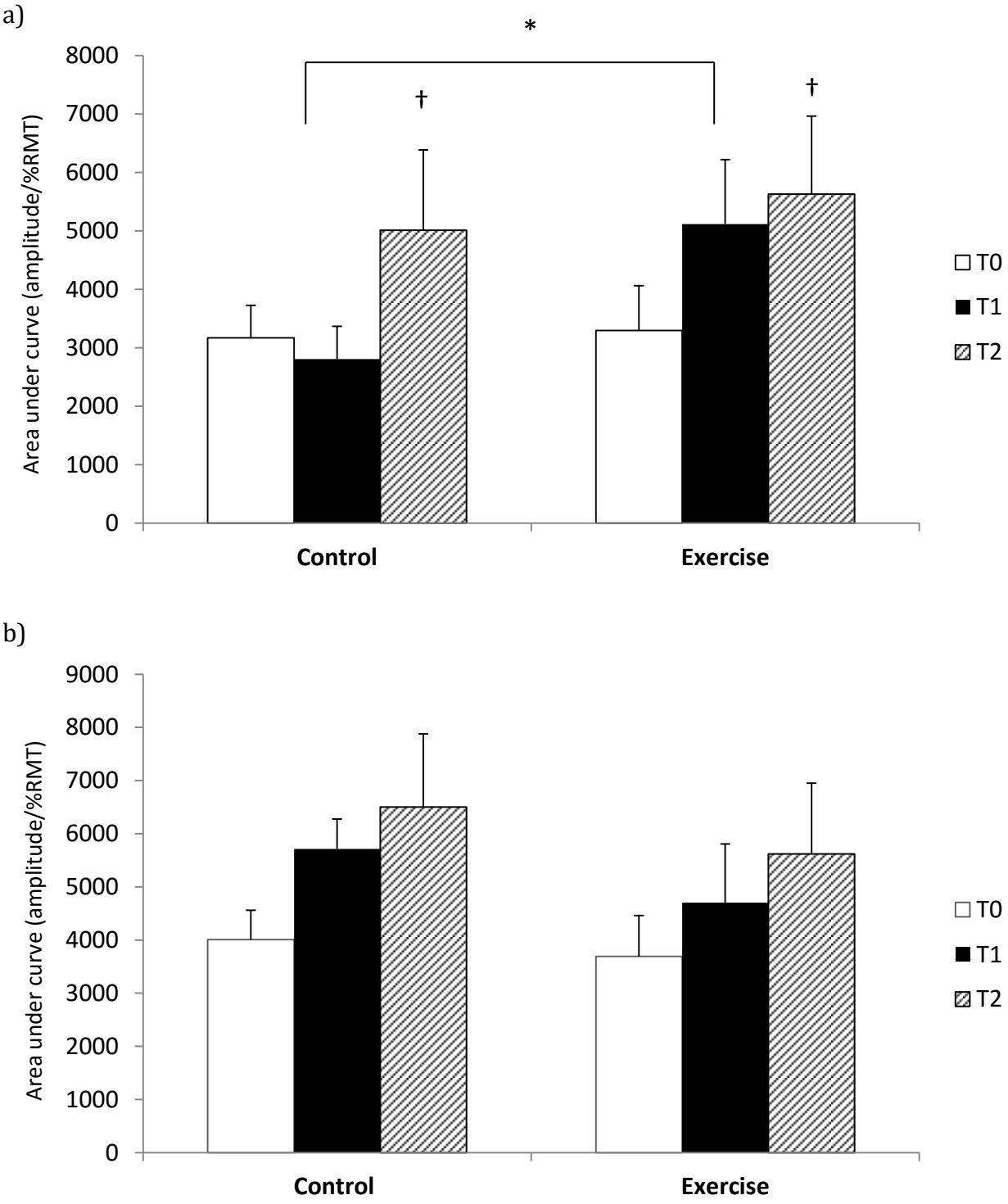


Figure 3.3 Area under the curve (AUC). Average area under recruitment curves for the a) APB and b) FDI muscles are shown at each timepoint for control and exercise sessions (n=10). Bars represent SEM; *=p<0.05; † indicates significant difference from T0.

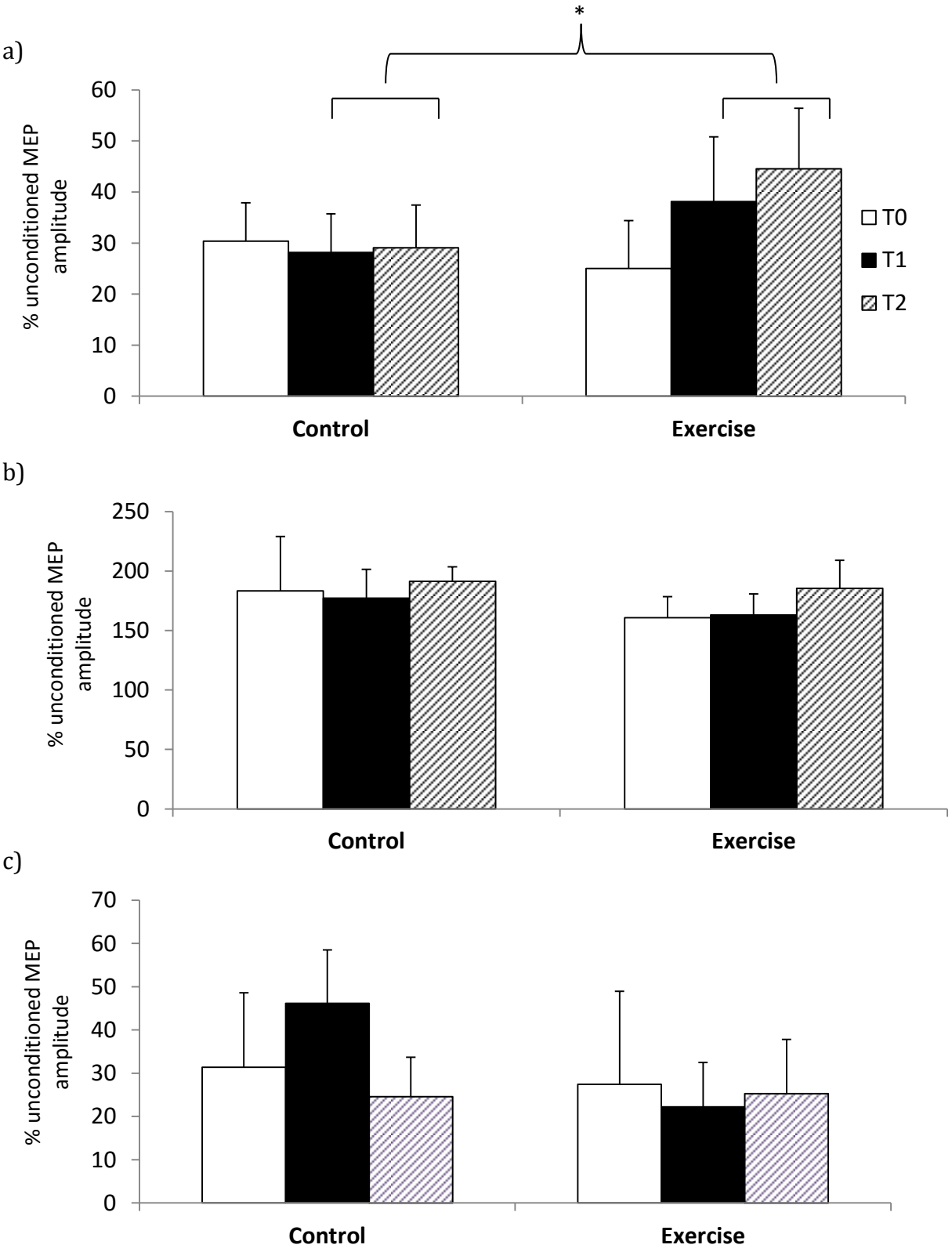


Figure 3. 4 Changes in a) SICI, b) ICF and c) LICI in the APB muscle representation before and after PAS. Raw values are expressed as % of unconditioned MEP amplitude at each timepoint. T0=baseline, T1=immediately following PAS, T2=30 minutes post-PAS. Bars represent SEM; *=$p<0.05$.

As shown in Figure 3.4a, a two-way RM-ANOVA revealed a main effect of session for SICI ($F_{1,9}$=5.30, p<0.047), but no effect of time ($F_{2,18}$=0.23, p=0.79) and no session x time interaction ($F_{2,18}$=0.64, p=0.54). An *a priori* contrast was carried out between pre (T0) and the combination of both post-timepoints (T1+T2) and revealed that SICI was decreased at both time points following exercise + PAS compared to following PAS alone ($F_{1,18}$=5.35, p<0.03). A two-way ANOVA revealed no effect of session for ICF ($F_{1,9}$=0.33, p=0.58), no effect of time ($F_{2,18}$=0.21, p=0.81) and no session x time interaction ($F_{2,18}$=0.03, p=0.98) (Figure 3.4b). One participant did not display LICI during baseline testing and was thus excluded from further analysis. Results from a two-way ANOVA indicated no effect of session for LICI ($F_{1,8}$=1.2, p=0.30), no effect of time ($F_{2,16}$=1.99, p=0.17) and no session x time interaction ($F_{2,16}$=1.55, p=0.24) (Figure 3.4c).

3.4 Discussion

PAS-induced plasticity is modulated by exercise

In this study, we sought to establish a baseline response to PAS in a group of individuals and then investigate whether the addition of a preceding bout of aerobic exercise would modulate this response. While both sessions resulted in a change in excitability following PAS, the magnitude of the change differed between sessions. The current results indicate that PAS-induced increases in M1 excitability are enhanced when preceded by an acute bout of aerobic exercise. In addition, SICI is differentially modulated between the two sessions, with greater decreases in SICI observed in the exercise session.

Exercise and LTP-like plasticity in M1

The effects of exercise on M1 plasticity are not well-studied. A single session of exercise has been shown to improve motor skill retention in M1, but without any corresponding enhancement of motor skill acquisition [383]. Yet, motor learning involves a complex interaction of attentional, motivational, neurological and performance factors, and thus it is important to identify the specific processes upon which exercise may exert its effects. Recently, McDonnell and colleagues [299] reported that a bout of acute, low-intensity cycling improves the response to subsequent continuous theta-burst stimulation (cTBS), an intervention thought to induce long-term depression (LTD). However, to our knowledge, no previous studies have examined the effect of acute exercise on LTP-like plasticity. LTP is a key process in learning and memory that involves the long-term (>30 minutes) strengthening of synaptic efficacy. A hallmark of LTP is the involvement of N-methyl D-aspartate (NMDA) receptors and the activation of Ca^{2+}-dependent signalling cascades which, in late LTP, result in the structural modification of the synapse. PAS appears to mimic the early stages of LTP in which synaptic enhancement is thought to be due to Ca^{2+}-dependent phosphorylation of AMPA receptors and their subsequent trafficking to the post-synaptic membrane. The PAS protocol used here was previously described by Stefan et al. [43]. In this model, afferent signals arrive in the primary somatosensory cortex (S1) approximately 20 ms after median nerve stimulation, and in M1 3-5 ms later [43]. Thus, a TMS pulse delivered over the APB representation 25 ms after peripheral nerve stimulation should result in the near-synchronous arrival of central and peripheral inputs in M1. PAS can induce anywhere from a 5% to 185% increase in baseline MEP amplitudes, with effects lasting a minimum of 30-60 min but reversible within 24 hours [43]. In the current study, participants displayed a greater area under the recruitment curve in response to PAS when

PAS was preceded by exercise, in the absence of any difference in baseline values between sessions. In contrast, there were no changes in cortical excitability of the FDI muscle, confirming the input specificity of PAS and indicating that, rather than inducing a general spread of excitability, exercise specifically enhanced the response to PAS.

Potential mechanisms of enhanced PAS-induced excitability

Evidence suggests that LTP-like plasticity can be induced in M1 neurons quite readily, both due to reorganization following injury [384] or when learning a new motor task [48,385]. Importantly, in the current study, excitability in the target upper limb muscle was altered following predominantly lower limb-driven aerobic exercise. These results, combined with those of McDonnell et al. [299] suggest an effect on M1 beyond the muscles that are involved in the exercise. Indeed, Takahashi et al. [206] report that lower limb resistance exercise influences cortical excitability in nonexercised hand muscles. Further, lower limb aerobic exercise can affect vascular functioning in upper limb muscles [352] and stationary biking has been shown to induce a global increase in cerebral blood flow (CBF) [238], all of which suggest a non-specific modulation of M1 excitability. While certainly not the only explanation, such findings correspond with a neurochemical model of exercise. Similarly, while the mechanisms behind PAS are not fully established, the limited time frame indicates that chemical, and not structural changes, are driving neuronal plasticity [43]. With regard to neurotransmitters, acute exercise is associated with increases in dopamine (DA)[260,263,356], serotonin (5-HT) [356,357] and norepinephrine (NE) [260,358], all of which can increase M1 excitability [95,105,112,113]. Indeed, drugs that impair neuromodulation, such as DA, NE and acetylcholine antagonists, lead to significant

impairments in PAS-induced plasticity [87]. Batsikadze and colleagues [107] recently demonstrated that the selective serotonin reuptake inhibitor citalopram significantly increases PAS-induced excitability, indicating that 5-HT may be a key regulator of early neuroplastic changes. Similarly, L-DOPA facilitates both the degree and duration of PAS [88]. Thus, in the current study, it is possible that an exercise-induced increase in circulating neurotransmitters facilitated the subsequent PAS intervention. In contrast, cortisol levels have been shown to negatively correlate with excitability changes, as high circulating cortisol markedly impairs the response to PAS [142]. Here, all collections were performed in the afternoon in order to minimize variability in cortisol levels, which are highest in the morning [141]. Cortisol levels also appear to be affected by exercise intensity, with lower workloads associated with a decrease in cortisol levels, while increases are seen following moderate-to-high [299,301] or high [386,387] intensity exercise. Taken together, such a model suggests that by minimizing circulating cortisol and increasing levels of neurotransmitters known to modulate M1 excitability, exercise may act to prime M1 for neuroplastic change. The current results suggest that exercise alters the time course of PAS effects, as between-session differences in excitability are evident only immediately following PAS, and no differences are detected between sessions at 30 minutes post-PAS. Given the current model of PAS-induced LTP, this suggests an exercise-related enhancement of NMDA receptor (NMDAR) activity, which could potentially be due to neurochemical changes. Dopamine in particular has been shown to rapidly activate NMDARs in the striatum [98] and prefrontal cortex [388,389], indicating that increases in circulating DA could potentially stimulate NMDAR activity prior to, or during, the PAS session. Alternatively, elevated levels of BDNF may allow PAS-induced changes to occur

more rapidly by directly promoting NMDAR activity [122,333,379]. The timing of plasticity induction may be especially relevant for interventions involving skilled motor tasks, where more rapid changes may enhance learning and performance. The potential behavioural benefits of such interventions should be explored in future studies.

Factors affecting the response to PAS

The ability of PAS to induce plasticity can be highly variable across individuals and is related to several different factors. In late LTP, one of the key cytokines activated by the calcium cascade is brain-derived neurotrophic factor (BDNF). Carriers of a common polymorphism of the BDNF gene, a valine-to-methionine substitution at codon 66, display an 18-30% reduction in activity-dependent BDNF release [330] and severe impairments in PAS-induced plasticity [335]. Responsiveness to PAS declines with age [140] and is negatively correlated with RMT [140]. Additionally, Cirillo et al. [390] report that physically active individuals demonstrate greater responsiveness to PAS, though it is unclear why this is the case. Finally, the effectiveness of PAS is increased in individuals with greater cortical thickness in the sensorimotor region [130].

It is somewhat curious that as a group, there was no immediate increase in excitability following PAS (T1) in the control condition. However, this is in line with multiple studies that report greater enhancement at 30 minutes post vs. immediately after PAS [334,391,392]. In addition, most studies reporting changes immediately after PAS record MEP amplitude at a single intensity, while the AUC reflects changes at 5 different intensities. While AUC measurements are more sensitive to changes in cortical excitability, they are also affected by a lack of response at any particular intensity. Thus, as there were

no participants who failed to show an increase in excitability at any of the timepoints measured, none were classified as non-responders, which may comprise anywhere from 25% - 35% of the population [45,130,140]. Yet, in the majority of studies reporting the percent of non-responders, such characterizations are typically based on immediate responses to PAS. While Müller-Dahlhaus et al. [140] did test multiple timepoints following PAS, they did not observe changes beyond 30 minutes. Overall, in the current study, exercise-related enhancements occurred consistently despite some variability in the immediate response to PAS.

Role of intracortical networks in LTP-like plasticity

Intracortical networks are potent mediators of CST excitability, often via the release of inhibitory gamma-aminobutyric acid (GABA) or excitatory glutamate onto post-synaptic cells. SICI is thought to be mediated by ionotropic $GABA_A$ receptors [360], while LICI likely reflects the activity of metabotropic $GABA_B$ receptors [361]. Although the cortical mechanisms of ICF are not fully understood, it appears to involve activation of glutamatergic interneurons, and possibly NMDA receptors [362,363]. In the current study, there were no changes in ICF or LICI following PAS in either session and no effect of session. This echoes the previous findings of Sale et al. [141]and Russman et al. [393] who found no change in ICF following a similar PAS protocol, but contrasts with the decrease in LICI reported by Russman et al. [393]. The observation that SICI was not altered after PAS alone is in line with the findings of several authors [44,141,393–396], despite the fact that SICI likely contributes to plasticity during PAS [44]. In the current study, there were no significant changes in SICI at any timepoint following PAS, yet there was a significant

difference in SICI modulation between sessions. One possible interpretation is that the activity-induced decreases in SICI are most prominent immediately following exercise, and contribute to the enhancement of the subsequent PAS intervention, but are no longer detectable by 30 minutes post-exercise. The finding that SICI was modulated to a greater extent in the exercise condition is in line with the findings of Yamaguchi et al. [207], who report a decrease in SICI in exercising muscles following cycling, and also with both a recent study by Smith et al. [397] and previous findings from our lab [398] that demonstrate a decrease in SICI in an upper limb muscle immediately following lower limb cycling activity. Thus it is possible that an exercise-induced decrease in SICI may contribute to the enhanced effectiveness of PAS. However, it should be noted that in the current study, SICI was only measured following exercise + PAS, and not following exercise alone. Thus, it cannot be determined whether the decrease in SICI is a result of the exercise alone, or whether the exercise increased the likelihood that PAS would lead to a suppression of SICI. Similarly, we did not evaluate whether PAS would augment the decrease in SICI following exercise, or whether the suppression of SICI serves only to facilitate the effects of PAS on CST neurons.

Interestingly, an enhancement of ICF has been reported in the upper limb immediately following an identical cycling session [398], but was not observed in the current study. This discrepancy may simply be due to timing, since such changes were previously observed immediately following exercise, while testing in the current study did not begin until 30 minutes post-exercise. Alternatively, PAS may have induced a suppression of ICF in order to maintain excitability levels within a physiological range, as has been demonstrated for intracortical inhibitory circuits [161], although this cannot be assessed

without testing post-exercise levels of ICF. Finally, this may reflect differing contributions of facilitatory and inhibitory circuits to plasticity induction, since, unlike SICI, ICF is only minimally modulated following interventions such as theta-burst stimulation [37]. Indeed, while the link between exercise and inhibitory circuits has only recently been investigated, there is a well-established association between inhibitory circuits and LTP. LTP in the motor cortex is promoted in the presence of $GABA_A$ blockade [26,399]. Furthermore, high GABA levels may block the induction of LTP, as administration of either $GABA_A$ [400] or $GABA_B$ [361] agonists interferes with PAS and prevents the induction of plasticity. Indeed, when SICI networks are activated during the delivery of PAS, plasticity is significantly impaired [401]. Correspondingly, the NMDAR-mediated Ca^{2+} currents typical of LTP reduce GABA conductance, likely via the dephosphorylation of $GABA_A$ receptors [402–404]. Thus, the extent of inducible plasticity in M1 may be determined by the degree of GABA-mediated intracortical inhibition. This was confirmed by Ziemann et al. [33], who examined M1 plasticity after ischemic nerve block, a procedure associated with a rapid drop in GABA levels. Deafferentation significantly enhanced practice-dependent plasticity, indicating that a decrease in inhibition creates a more favourable environment for the induction of plasticity [33]. As a result, similar interventions that can induce early LTP, such as anodal transcranial direct current stimulation and intermittent theta-burst stimulation, may also benefit from the addition of exercise.

Limitations

A potential limitation of this study is that, similar to McDonnell et al. [299], we did not include a third session where participants performed exercise without undergoing PAS.

However, the goal of the study was not to observe exercise on its own, but to assess its ability to enhance the effectiveness of a plasticity-inducing intervention. Indeed, it has previously been shown that exercise alone does not alter CST excitability [397]. In addition, we did not re-test the RMT after exercise. It is well-established that PAS does not change either active or resting motor thresholds of the target muscle [43,141,142,390,393,395,401]. Nor is there any evidence that aerobic exercise can alter RMT. RMT was used at the beginning of each session to determine the testing intensities, which necessarily remained consistent within each session in order to accurately compare changes in AUC. Thus, the determination of RMT in this study was largely for the purposes of calculating the intensity of the CS in paired-pulse conditions. Even in the unlikely event of a transient change in RMT, SICI is evoked quite readily with a range of CS intensities from 60-90% RMT [405]. In addition, test pulses were delivered at 90% RMT at T1 and T2 to ensure that the subthreshold intensity did not produce any detectable MEPs. Finally, there is some question surrounding the intra-individual variability between repeated PAS sessions. While Fratelllo et al. [406] report high test-retest variability among the same subjects tested on different days, this may be dependent on time of day, as reproducibility is much higher when sessions are conducted in the afternoon [141]. In addition, given that the response to PAS is highly variable among individuals [130,140,335,339,390,407], having each participant act as their own control was considered the optimal way to reduce inter-individual variability when comparing between sessions. However, individual characteristics may have contributed additional sources of variability. Participants were all non-smokers and were instructed not to exercise the day of the collection or to consume caffeine within four hours of the experimental session. However, a number of other

variables were not controlled for, including gender, phase of menstrual cycle, and genetic variability, particularly in the BDNF genotype.

3.5 Conclusion

In conclusion, the current results demonstrate that the combination of acute aerobic cycling and PAS modulates cortical excitability to a greater extent that PAS alone. While the mechanisms driving such changes are not clear, it is possible that local decreases in intracortical inhibition may contribute to this effect.

Acknowledgements

This work was supported by funding to WRS from the Natural Sciences and Engineering Research Council of Canada, the Canada Research Chairs Program and the Ontario Research Fund.

4 Aerobic exercise enhances neural correlates of motor skill learning

Adapted from: Singh et al. *Behav Brain Res*. 2016 Mar 15;301:19-26

4.1 Introduction

There is growing interest in the potential ability of aerobic exercise to enhance cortical excitability. A single session of moderate-intensity exercise has been shown to transiently increase cortical activity and cognitive function in frontal and motor regions, changes that persist following exercise cessation. Given its role in movement execution, it is not surprising that excitability in the primary motor cortex (M1) may be enhanced following an acute exercise bout. Although little is known about the direct effects of aerobic exercise on motor cortical neurons, emerging evidence suggests that a single session of moderate-intensity cycling activity can suppress short-interval intracortical inhibition (SICI) and enhance intracortical facilitation (ICF) for at least 30 minutes following exercise completion [397,398], suggesting that the post-exercise environment may be ideal for inducing experience-dependent plasticity. Indeed, early markers of long-term potentiation (LTP) are enhanced when induction is preceded by acute exercise. Exercise has been shown to enhance the response to paired-associative stimulation (PAS), a technique thought to induce LTP-like plasticity in the motor cortex, and this effect is observed following both moderate and high-intensity exercise [408,409]. One limitation of these studies is that excitability changes have only been examined at the motor hotspot. Motor learning often involves not only changes at the central site, but an outward expansion of the excitable area [48,61,384,410,411]. Whether and to what extent exercise affects the overall M1 representation is unclear. Additionally, it is not known whether this benefit extends beyond passive stimulation to tasks involving active motor learning. Although the retention of motor skills appears to be enhanced by subsequent aerobic activity [383], and

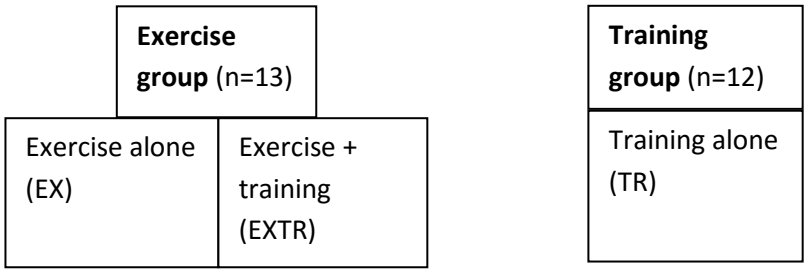
neurorehabilitation outcomes are improved by the addition of aerobic exercise [375,412], enhancements in motor performance following acute exercise in healthy individuals have not yet been investigated. Such investigations are critical for determining the potential mechanisms and clinical utility of aerobic exercise as an adjunct therapy to improve motor function and motor skill training in neurological patient populations. In-phase bimanual movements can increase both cortical excitability and the spatial representation of target muscles [61,413]. Such movements can exploit interhemispheric connections between homologous muscles in order to enhance learning effects. Motor learning comprises skill acquisition and motor adaptation [47] and requires a cortical environment that is receptive to experience-dependent plasticity. Facilitatory interventions that target the motor cortex, such as intermittent theta-burst stimulation, or anodal transcranial direct current stimulation, have been shown to effectively prime the brain for subsequent motor learning [414–416]. In the current study, we investigate whether exercise may have a similar effect when performed prior to a bimanual visuomotor learning task. We used single-pulse transcranial magnetic stimulation to generate a cortical map of the extensor carpi radialis (ECR) muscle representation before and after training. We hypothesized that a) exercise would enhance the cortical response to training, both in terms of the spatial extent of the cortical map and the excitability changes within the map; and b) that exercise would improve motor learning as measured by response time, accuracy and movement trajectory on the motor task.

4.2 Methods

4.2.1 Subjects and experimental setup

Twenty-five young, healthy, self-reported right-handed individuals were recruited (14 males; average age = 27 years). Participants were screened for any contraindications to TMS and informed consent was obtained prior to undergoing the experimental protocol. All experimental procedures received clearance from the University of Waterloo Office of Research Ethics. Participants were divided into an exercise group (n=13) and a training only group (n=12). The exercise group underwent two experimental sessions at least one week apart: exercise alone (EX) and exercise followed by training (EXTR). The training group underwent one session of training alone (TR). Two participants in the exercise group were unable to return for a follow-up visit and thus this group consisted of 11 individuals who completed both the EX and EXTR sessions, one who completed EX alone, and one who completed EXTR alone. For the participants who completed both sessions, collections were scheduled one week apart and were collected at the same time of day.

a)



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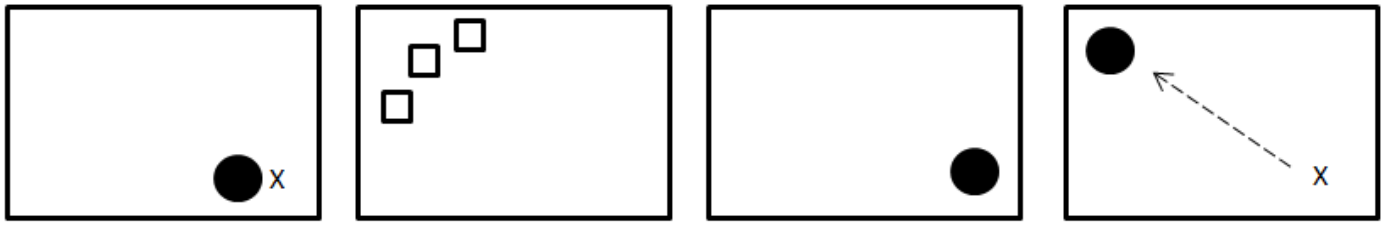


Figure 4.1: a) Experimental setup and b) training task. Trial begins by moving cursor over the "x". "x" disappears and target appears in one of 3 locations for 1000 msec then disappears. Cursor reappears and participant moves quickly to the target location.

4.2.2 Exercise protocol

Heart rate (HR) and rate of perceived exertion (RPE) were collected at rest prior to exercise. During exercise, heart rate was monitored using a wrist-mounted heart rate sensor. Participants were instructed to work at approximately 65-70% of their age-predicted maximal heart rate [average =120-130 beats per minute (bpm)] but to keep their perceived exertion level in the moderate range. After a brief warm-up to elevate HR into the target zone, participants performed 20 minutes of continuous stationary biking on a recumbent bicycle in an isolated room. The duration and intensity were intended to mimic a standard aerobic workout. Participants were seated comfortably with their feet strapped to the pedals and their backs against the backrest. RPE was verbally reported using the modified Borg scale every five minutes, and HR was continuously monitored throughout the exercise period. Instructions were given to work at a moderate intensity (RPE of 3 -4), and participants could adjust either the pedaling resistance or the rate of pedaling to

maintain the target heart rate. All participants reported intensity rates in the moderate range, with no individual exceeding an RPE of 4. The experimenters remained with the participant throughout the exercise and ensured that arms were resting comfortably by their sides and not gripping the handlebars during the session. The arms and forearms remained stationary during pedaling exercise. Participants were given free access to water. Immediately following exercise completion, subjects returned to the TMS testing room for the collection of post-exercise measures. In all cases, heart rate had returned to resting or near-resting levels (within 5bpm) by the 30 minute mark post-exercise.

4.2.3 Bimanual training task

For performance of the motor task, participants were seated in front of a computer monitor with their elbows supported on a table. A custom-built device was secured to the table and consisted of two handles attached to potentiometers that controlled the position of a cursor on the monitor. The right and left handles were calibrated to each participant's range of motion between wrist flexion and extension. The left handle moved the cursor in the horizontal direction, while the right controlled the vertical displacement. Participants were required to perform simultaneous in-phase wrist extension movements to move a visual stimulus to a target location on the screen. At the start of each trial, a box would flash indicating the location of the target for the next trial (stimulus duration=1000 msec). Two seconds later, the cursor became visible in the bottom right quadrant and the participants used their wrist extension movements to rapidly move the cursor to the previously indicated target location. The targets were set to appear randomly at one of three locations in the upper left quadrant of the screen (set at 30°, 45°, and 60° from the y-

axis, Fig. 1b). Thus, while each target required simultaneous wrist extension movements, the 30° and 60° targets required slightly different endpoint positions for the two hands. In-phase movements were required to reach the target, and participants were instructed to move as quickly and accurately as possible. After each trial, feedback appeared indicating the response time. Any trials where the target was missed, or was not reached within 2000 milliseconds, was considered a missed trial and no response time feedback was given. The participant then initiated the next trial by placing the cursor over an X in the bottom right corner of the screen. The training session consisted of 160 self-paced trials with an equivalent number of trials for each target position.

4.2.4 TMS measures and grid mapping

Focal TMS was performed using a MagPro x 100 stimulator (Medtronic, Minneapolis, MN, USA) and a figure of eight coil (MCF-B65; 70 mm). BrainSight Neuronavigation (Rogue Research, Canada) was used to guide the placement of the coil to the target motor region using a template MRI for all participants. Anatomical co-registration was performed prior to baseline collection and subsequent coil positioning was tracked using reflective markers affixed to custom-fitted glasses. The coil was placed at a 45° angle to the mid-sagittal line to induce a posterior to anterior current in the underlying neural tissue. EMG recordings of motor-evoked potentials (MEPs) were obtained using surface electrodes placed over the right extensor carpi radialis muscle (ECR). Raw EMG signals were recorded and stored in a customized LabVIEW (National Instruments, Austin, TX, USA) program for offline analysis. The motor hotspot of the right ECR muscle was identified as the left M1 location that consistently elicited a maximal MEP in the resting muscle, as assessed by EMG amplitude,

while producing a visible muscle twitch. The resting motor threshold (RMT) was determined by the minimum stimulation intensity required to elicit a peak-to-peak MEP amplitude of >50 μV on 5 out of 10 trials. After localization of the hotspot, a 9x9 rectangular grid was generated around the hotspot with sites spaced 10 mm apart [61,410,411]. Grid mapping was performed according to the method described by Wassermann and colleagues [417]. Ten stimuli were delivered at each location with an interstimulus interval (ISI) of 2 seconds. A site was considered active if a minimum of 2 of 10 stimuli produced MEPS greater than 30 μV. The sites where MEPS could no longer be elicited were considered to be the borders of the representation. Following grid mapping, participants in the exercise group performed 20 minutes of biking followed by either a rest period (EX) or training (EXTR), with post-grid collection occurring approximately 60 minutes after the pre-grid. For participants in the training only group (TR), post-grid measures were collected immediately after training (approximately 30 minutes following pre-grid).

4.2.5 Data analysis: TMS

For each site, the maximal peak-to-peak MEP amplitude was calculated and averaged over the 10 pulses. Any trials with evidence of preceding muscle activity were discarded. Changes in cortical excitability from pre to post were quantified by a) the number of active sites; b) the global average MEP amplitude within the map; and c) the average MEP amplitude in the sites immediately adjacent to the hotspot (central zone). Within each session, paired t-tests were used to detect cortical changes from pre to post for each dependent measure. For the between-group analysis, ANOVAs were performed to compare

the changes in excitability across sessions. *A priori* contrasts were used to test the specific hypothesis that exercise would enhance the cortical response to training. In-phase bimanual training has been previously shown to increase cortical excitability [61], while aerobic exercise can suppress intracortical inhibition and increase intracortical facilitation for at least 30 minutes post-exercise [397,398]. In addition, acute exercise facilitates the induction of experience-dependent plasticity when performed prior to induction [408,409]. The significance level for all tests was set to $p<0.05$.

4.2.6 Data analysis: Performance

For behavioural data, the response time, accuracy, and movement trajectory to the target were analyzed for each block of 10 trials. The trajectory was measured by the deviation from a straight (ideal) path to the target. Deviations were measured at the moment of peak velocity, and minimal deviations from a straight line were considered markers of good motor performance. Between-session effects were analyzed using a 2-way ANOVA, with group (training alone vs. exercise+training) and block (first 10/last 10) as factors. Due to a software error, behavioural data was not available for one participant in the exercise and training group.

4.3 Results

4.3.1 Baseline characteristics

One-way ANOVAs of baseline measures revealed significant differences between the number of active sites ($F_{2, 33}=4.09$, $p<0.026$) and the MEP amplitudes in central sites ($F_{2,}$

$_{33}$=2.98, p<0.037), and a near-significant difference in global MEP amplitude ($F_{2, 33}$=2.98, p<0.065) across groups. Consequently, MEP amplitudes were expressed as a percent of baseline and subsequent between-group analyses were performed on normalized data.

Table 4.1: Baseline characteristics between sessions (mean ± standard deviation)

	EX	**TR**	**EXTR**
Number of active sites	19.5 ± 7.1	12.8 ± 4.3	18.5 ± 6.7
Average MEP amplitude (μV)	161 ± 115.1	85.6 ± 32.0	113 ± 57.1
Average central zone amplitude (μV)	220 ±159.1	107 ± 58.5	135 ± 62.1
Average response time (first 10 trials) (msec)	---	1141 ± 249.3	1004 ± 332.7
Average accuracy (first 10 trials)	---	57% ± 1.8	53% ± 1.9

4.3.2 Within- and between-session effects

Results of the TMS measures are displayed in Figure 4.2. For the number of active sites, an increase was observed in the TR group [t(11)=3.26, t<0.008], with a trend towards an increase in the EXTR group([t(11)=2.10, t=0.059], but no change in the EX group [t(11)=1.25, t=0.24]. However, a one-way ANOVA of change scores revealed no main effect of session ($F_{2,33}$=2.14, p=0.94) on the increase in active sites. For global MEP amplitude, an increase was observed in the EXTR group [t(11)=3.40, t<0.006], with a trend towards an increase in the TR group [t(11)=1.95, t=0.077], but no change in the EX group [t(11)=0.54, t=0.60]. Again, one-way ANOVAs of change scores revealed no main effect of session for global MEP amplitude ($F_{2,33}$=2.14, p=0.13). For central zone amplitude, a significant increase was detected in the EXTR group [t(11)=4.24, t<0.001], but no changes were observed in the EX alone group [t(11)=0.71, t=0.49], or the TR alone group [t(11)=0.85,

t=0.41]. A one-way ANOVA of change scores revealed no main effect of session for central zone amplitude ($F_{2,33}$=2.12, p=0.14).

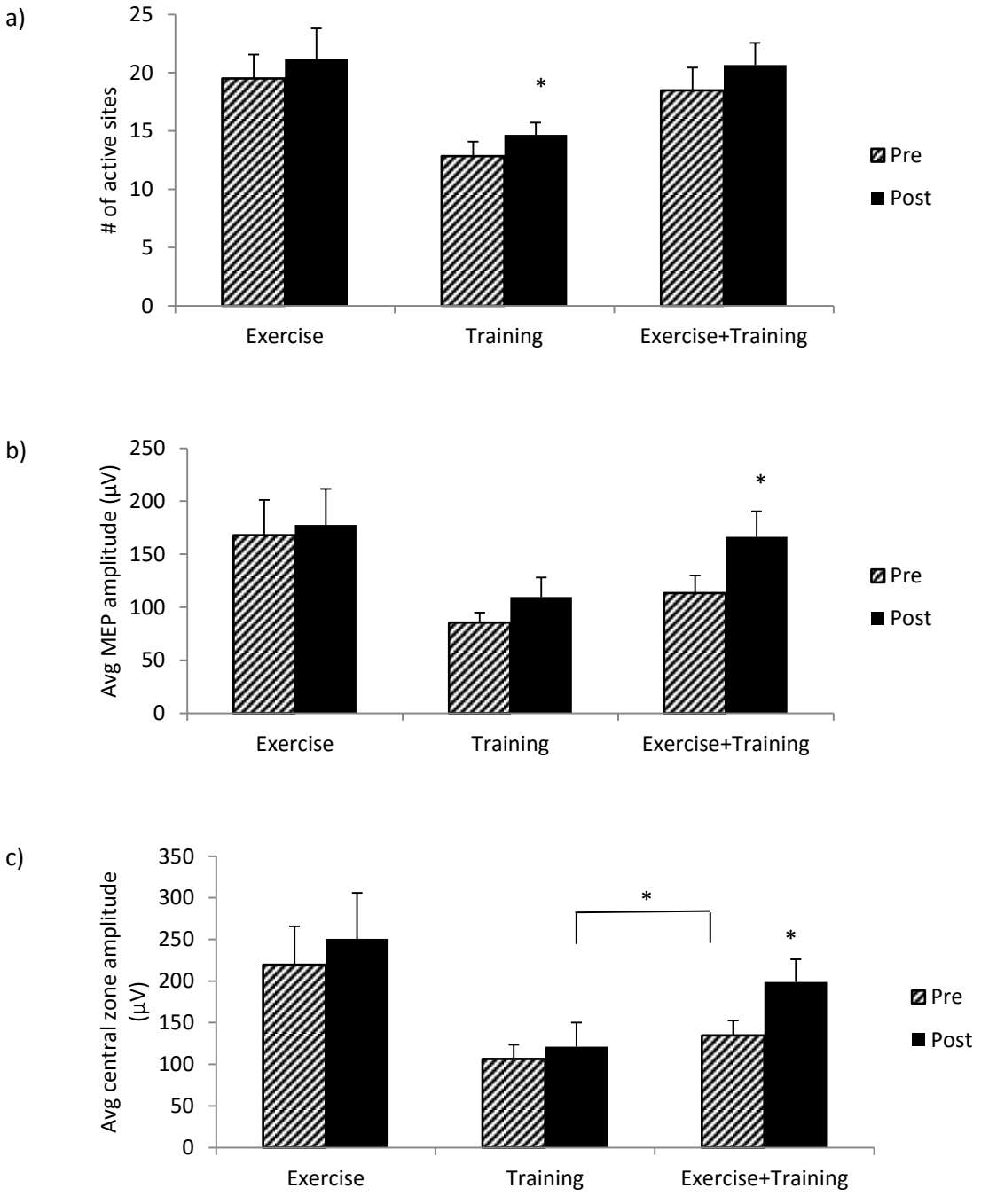


Figure 4.2: Within-session excitability changes in the ECR representation for a) number of active sites, b) average global MEP amplitude, and c) average central zone MEP amplitude. *=p<0.05

Effects of training

A priori contrasts were used to test the hypothesis that training would be necessary to cause an expansion in grid size or MEP amplitude by comparing training (TR and EXTR groups) with no training (EX group). Training induced a significant increase in the both global MEP amplitude ($F_{1,33}$=5.17, p<0.030) and the central zone amplitude ($F_{1,33}$=6.56, p<0.015). However, we did not detect any significant differences between training vs. no training groups ($F_{1,33}$=0.02, p=0.90, Fig.4.2a) with regard to the increase in active sites.

Effects of exercise

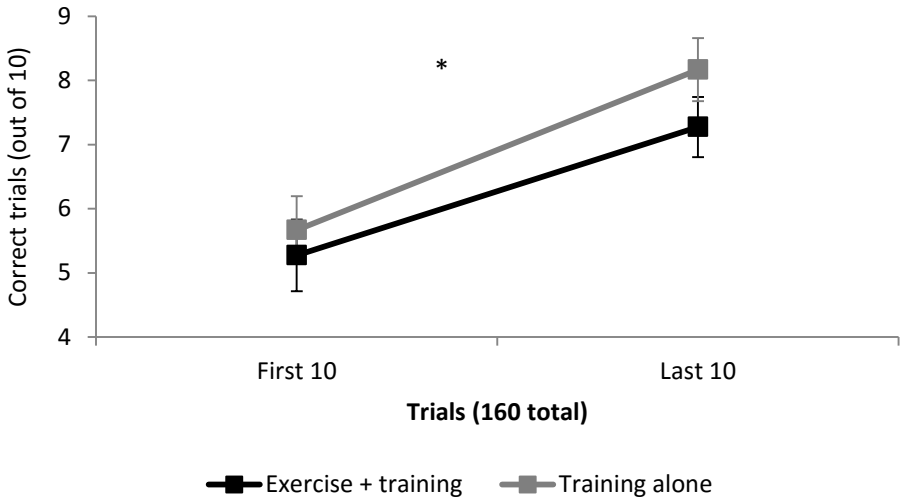
A priori contrasts were used to test the hypothesis that exercise would enhance the response to training. A comparison of TR vs EXTR groups revealed no difference in the increase in active sites ($F_{1,33}$=0.05, p=0.82, Fig.4.2a) or global MEP amplitude ($F_{1,33}$=2.68, p=0.11, Fig.4.2b), but central zone amplitudes were significantly increased when training was preceded by exercise ($F_{1,33}$=4.22, p<0.048, Fig.4.2c).

Behavioural performance

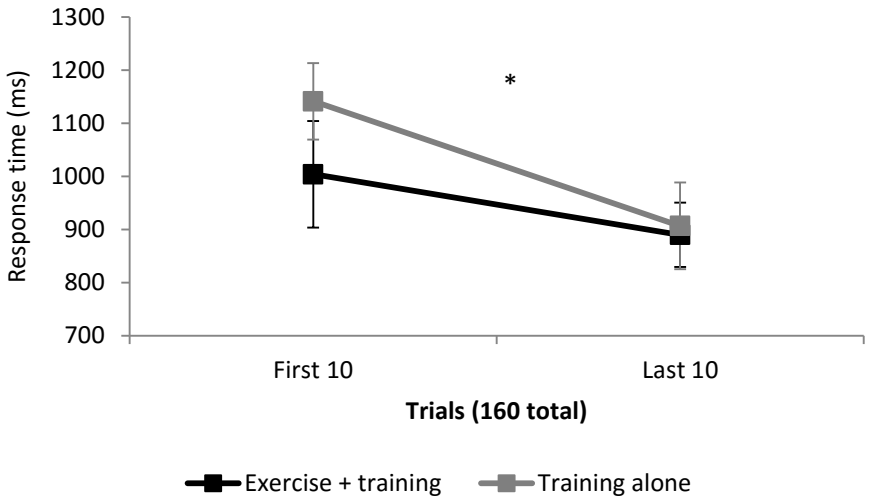
Training data is displayed in Figure 4.3. A comparison of the first 10 trials indicated no baseline differences in accuracy, response time or trajectory between groups. Learning was quantified by differences between the first 10 and last 10 trials. Two-way ANOVAs revealed a significant effect of time on both response time ($F_{1,43}$=5.19, p<0.03, Fig. 4.3a) and accuracy ($F_{1,43}$=19.84, p<0.0001) but no effect of session ($F_{1,43}$=0.5, p=0.49 and $F_{1.43}$=1.61, p=0.22, respectively) and no session by time interactions ($F_{1,42}$=0.04, p=0.84 and $F_{1,42}$=0.24,

p=0.63, respectively). A two-way ANOVA revealed no main effect of time ($F_{1,43}$=0.07) but a main effect of session ($F_{1,43}$=6.96, p<0.012, Fig. 4.3c) for trajectory, and no session by time interaction ($F_{1,42}$=3.01, p=0.09).

a)



b)



c)

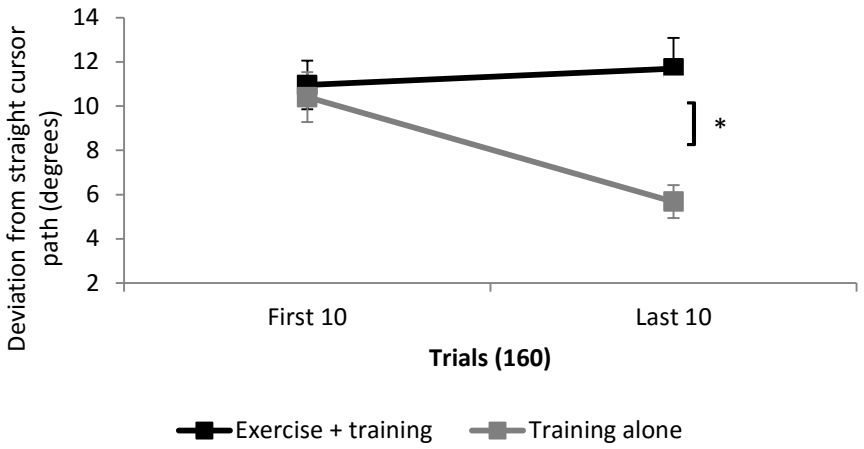


Figure 4.3: Performance on training task during first 10 and last 10 trials reflecting a) accuracy, b) average response time, and c) average deviation from straight cursor path. *p<0.05

4.4 Discussion

Summary of main findings

The primary aim of this study was to investigate whether the addition of exercise could enhance the cortical and behavioural responses to a motor training task. M1 is a highly plastic region and with repeated training, use-dependent reorganization in M1 is associated with skill acquisition [48,385] and improvements in performance markers such as reaction time [385]. Single sessions of training have also been demonstrated to alter cortical representations [50,59,60]. TMS is a reliable method of mapping muscle representations within M1 [418] and these maps show good stability over time and high reproducibility in individual participants [419,420]. Previously observed changes in excitability following exercise have been assessed only at the motor hotspot. The technique

of grid mapping is a more comprehensive method of assessing cortical excitability and is ideal for measuring changes following experimental interventions [421].

We mapped the cortical boundaries of the ECR representation before and after three different interventions: training alone, exercise alone, and exercise followed by training. We observed that cortical excitability was enhanced in the exercise and training group versus training alone. Specifically, the MEP amplitude in the central zone of the ECR representation was maximally increased following exercise and training. Despite this, there was no difference in motor learning effects between the two training groups.

Number of active sites

A somewhat unexpected finding was the lack of a significant difference in the expansion of the cortical map between sessions. Increases in the size of the cortical representation reflect short-term cortical adaptations and can be taken as a neural correlate of motor learning [48,49,384]. In addition, expansions in motor maps are associated with improved functional recovery in neurological patient populations [53,422]. At the neuronal level, these changes may involve the unmasking of latent horizontal connections [64] or LTP-type processes within the existing representation [170]. Intracortical microstimulation studies suggest that an increase in the cortical representation may induce atrophy of neighbouring motor representations [48,384,423], or alternatively, maps may extend anteriorly or posteriorly into the edges of premotor or primary somatosensory areas, respectively [53]. Although these changes are well-established following training, we also observed these changes following exercise alone. These changes were remarkably consistent across groups, with only one participant in the TR group, two in the EXTR group, and two in the

EX group not showing an expansion in the cortical map. The lack of a statistical within-group change in the number of active sites in the EX group is primarily due to a single participant who demonstrated a drastic reduction in active sites from pre to post. Although previous studies have suggested that exercise alone is insufficient to increase the excitability of output neurons in M1 [397,398], these changes have previously only been examined at the motor hotspot, with MEP amplitude as the sole outcome measure. Our current results suggest that both exercise and training can induce an expansion of the representation but that combining exercise and training does not confer any additional benefits in this aspect. The analysis of training effects suggests that cortical excitability changes are primarily reflected by enhancements in MEP amplitude within the trained muscle representation rather than an expansion of the cortical area.

Global and central MEP amplitude

In contrast, results from the analysis of MEP amplitudes are consistent with the notion that exercise does not directly modulate output motor neurons. Here, we confirm previous findings and extend them to the entire representation of the muscle. While the greatest increase in MEP amplitude was seen in the EXTR session, this did not significantly differ from the TR session, suggesting that training is the predominant mechanism driving the increase in excitability. This is not surprising given the generalized, whole-body nature of the exercise versus the targeted motor training of the wrist extensors in isolation.

However, in the central zone surrounding the ECR hotspot, the addition of exercise increased MEP amplitude to a greater extent than training alone. The central zone was designated by the hotspot and its 8 adjacent sites. Spatially, these sites largely correspond

to the top-third position sites (T3Ps) previously identified as the sites where two-thirds of the maximal MEP amplitude is elicited [59]. Central sites have been shown to be more reliable and less variable than those on the periphery of the representation [424]. In addition, motor skill acquisition is largely reflected by increases in excitability at the center of the representation rather than on the periphery [49]. Importantly, motor learning-related plasticity appears to be a highly specific process, with only neurons directly engaged in the trained movement undergoing adaptations, while adjacent neuronal populations are unaffected [425,426]. This restriction based on functional relevance suggests a framework wherein a global exercise effect may induce region-specific changes at the core of the target muscle representation and supports the use of exercise to prime the induction of plasticity.

Behavioural performance

Although evidence strongly suggests that motor learning induces LTP in M1 [170], and despite the ability of exercise to enhance LTP induction, it is unclear what, if any, benefit exercise may confer on motor learning and motor behaviour. A single session of exercise has been shown to improve motor skill retention in M1, but without any corresponding enhancement of motor skill acquisition [383]. However, skill acquisition is positively correlated with exercise-dependent increases in norepinephrine and lactate [427]. In the current study, we employed a bimanual visuomotor learning paradigm. Bimanual tasks require greater cortical control than unimanual movements in addition to interlimb coordination [73,428,429]. The transcallosal connections between homologous M1 regions promote interhemispheric facilitation and communication that may contribute to enhanced

excitability [430]. Cued, visuomotor bimanual training tasks engage not only the primary motor areas, but also motor preparation areas such as the premotor cortex [411,431]. In this study, in-phase movements were required to reach the target. Three targets were included to increase the task difficulty, as each target required a slightly different endpoint while still maintaining the requirement for simultaneous extension of the wrists. The inclusion of a skilled movement is critical for training, as simple movement is not sufficient to induce functional reorganization in the cortex [423,432]. While both TR and EXTR groups exhibited performance improvements between the first 10 and last 10 trials, we did not observe any additional benefit from exercise in terms of accuracy, movement time, or arm trajectory. The only significant difference between the two groups was observed with cursor trajectory, where we observed a dramatic improvement in the TR group and a slight increase in the EXTR group. While the TR group had only one participant who declined in performance between the first and last 10 trials, the EXTR group had three, two of whom declined dramatically. However, there were no other discrepancies in the behaviour of these two participants, and in both cases the large errors were consistent throughout the training session (i.e. did not appear only in the last 10 trials). Additionally, these participants did not differ from the group in RT or accuracy, so they did not appear to find the task more difficult, but rather, they likely employed different movement strategies, or consistently overshot the target. In the future, tasks that focus on one outcome measure may be preferable.

While the lack of a difference between EX and EXTR group may suggest that exercise has no measurable effect on motor learning, it is very likely that our task was not sensitive enough to reflect subtle changes in cortical excitability. Such benefits may be more evident

in clinical populations where excitability changes are limited by excessive inhibition, or in patients in whom movement initiation and execution are compromised. In addition, variability in attention and arousal levels, baseline differences in performance and prior experience with video games may all have affected learning. One limitation of this task is that we did not collect EMG data during the training. If exercise-induced changes are primarily seen at the cortical level, it is likely that reaction time, rather than response time, would have better reflected changes in cortical excitability. Consequently, a pure reaction time task may be a more appropriate measure, as participants in the current task had to focus on reaction time, accuracy and coupling of the hands, which may have led to shifting strategies and trial-to-trial variability.

Potential mechanisms of enhanced cortical plasticity with exercise

While it remains unclear which structures or processes may mediate exercise effects on cortical excitability, the neurochemical hypothesis has received much support. This model suggests that an acute increase in arousal-linked neurotransmitters may contribute to post-exercise changes in excitability.

Animal work has demonstrated that aerobic exercise is associated with increases in dopamine (DA) [260,263,356], serotonin (5-HT)[356,357], and norepinephrine (NE) [260,358], all of which can directly enhance M1 excitability [87,98]. Dopamine in particular can reduce inhibitory GABA activity [433] and enhances glutamate currents via direct interactions with n-methyl d-aspartate receptors (NMDARs) [99]. Furthermore, exercise has been shown to enhance the induction of LTP-like plasticity using PAS [408,409], which is thought to induce LTP via an alteration in NMDAR-mediated currents.

Such activity appears to be critical for motor learning, as selective knockdown of NMDARs in M1 abolishes LTP [434].

In addition to neurotransmitters, several other potential mediators have been identified, including brain-derived neurotrophic factor (BDNF), which has been consistently shown to increase following acute aerobic exercise [182,224,267,313–317,435]. BDNF acting through the TrkB receptor directly depolarizes neurons, with a much greater potency than glutamate [122,123], increases the open probability of NMDA receptors, and rapidly enhances glutamate-induced NMDAR currents [122].

Other substances that may contribute include lactic acid, as cortical lactate levels are increased following acute exercise [435], and cerebral metabolism of lactate increases when plasma levels are elevated [249–251]. Interestingly, high lactate levels correspond to increased M1 excitability following a bout of maximal cycling [255], possibly indicating a shift to lactate as a fuel, and M1 excitability increases in proportion to blood lactate levels [255].

Additionally, imaging studies reveal that regional cerebral blood flow (CBF) is increased in bilateral M1 regions following cycling exercise [201], and the movement-related perfusion of M1 is enhanced following exercise [238]. As blood provides essential neuronal energy substrates such as glucose, such a relationship is a putative mechanism for the relationship to cortical excitability [183]. Further, changes in CBF have been associated with both short-term [436–438] and long-term skill learning [439].

Alternatively, while excitatory neuromodulators may enhance the induction of early LTP in M1, exercise may induce a suppression of inhibitory activity. Reductions in SICI have

been observed in both upper and lower limb muscles for at least 30 minutes following cycling exercise [207,397,398], indicating a decrease in local $GABA_A$ production or receptor activity within M1. This suggests that exercise may primarily exert its effects on the lower-threshold interneurons rather than layer V output neurons. Such transient changes may indicate a state in which motor cortical areas are more responsive to subsequent plasticity inducing-interventions. The intracortical networks of excitatory and inhibitory interneurons that surround pyramidal cells are the primary regulators of neuronal activity and are strongly implicated in cortical plasticity and reorganization [366]. Decreases in inhibition are a necessary precursor to more lasting neuroplastic changes [79,80] as the release of GABA at inhibitory synapses directly modulates the excitability of pyramidal cells [46] and a release of inhibition is thought to be critical for cortical reorganization in M1 [33,64].

Thus, we hypothesize a theoretical model whereby an increase in arousal-related neurotransmitters and neuromodulators, combined with a suppression of GABA-mediated inhibition, combine to create an environment that is more responsive to experience-dependent plasticity. In this model, the exercise can be thought of having a general priming effect on the motor cortex, while the motor task provides more targeted training, leading to excitability changes in the cortical ECR representation.

4.5 Conclusion

This study demonstrates that the addition of aerobic exercise can enhance the cortical changes induced by a motor training task. Specifically, the combination of exercise and

training increases the excitability of the target muscle representation to a greater extent than training alone. Thus, exercise may be a potentially beneficial adjunct to motor learning.

5 Aerobic exercise abolishes cTBS-induced suppression of motor cortical excitability

5.1 Introduction

Recent evidence suggests that aerobic exercise can transiently enhance excitability in the primary motor cortex (M1). In addition to stimulating the release of excitatory neurotransmitters, a single session of acute, moderate-intensity exercise can suppress short-interval intracortical inhibition (SICI) and enhance intracortical facilitation (ICF) in M1 for up to 30 minutes following exercise cessation [207,397,398]. Importantly, these effects are not specific to muscles involved in the exercise, suggesting that a generalized response occurs within the cortex. As decreases in intracortical inhibition are a necessary precursor to both rapid and more permanent neuroplastic changes [33,79–81,395,440], these findings suggest that exercise has the potential to prime the induction of experience-dependent plasticity. Indeed, exercise has been shown to enhance the effectiveness of techniques that induce long-term potentiation (LTP), such as paired-associative stimulation (PAS) [408,409]. However, it is not clear whether exercise has the same influence on interventions that induce long-term depression (LTD). While motor learning primarily occurs through LTP-type mechanisms, bidirectional plasticity appears to be a key feature of motor skill learning [57,170]. LTD is required for the successful acquisition of novel information [441] and impaired LTD is linked to impairments in motor learning [442]. Using non-invasive brain stimulation, LTD-like plasticity can be induced by continuous-theta burst stimulation (cTBS), which has been shown to temporarily suppress cortical excitability in the target region for up to 60 minutes. In addition to a decrease in corticospinal tract excitability, cTBS also suppresses SICI and ICF [37].

While research on the link between exercise and rapid plasticity is limited, a study by McDonnell and colleagues demonstrated that a low-intensity bout of exercise enhanced the response to cTBS, while moderate-intensity exercise appeared to abolish its effects [299]. However, the authors note that an elevation in cortisol levels associated with venopuncture may have impaired the response to cTBS and influenced their findings. A further question surrounding the use of exercise relates to the timing of performance. Previous studies have used exercise as a priming technique and consequently, exercise has always been performed prior to the target intervention. It is unknown whether exercise can still influence excitability when performed after the induction of plasticity, and whether the timing of the exercise is critical to its effects. Thus, here we further examined the relationship between exercise and LTD. We investigated the effects of subsequent aerobic exercise on the duration and intensity of cTBS in a non-exercised upper limb muscle. We hypothesized that the response to cTBS would be diminished when followed by acute exercise.

5.2 Methods

5.2.1 Subjects and experimental setup

Ten young, healthy, self-reported right-handed individuals were recruited (6 females; average age = 25 years). Participants were screened for any contraindications to TMS and informed consent was obtained prior to undergoing the experimental protocol. All experimental procedures received clearance from the University of Waterloo Office of Research Ethics. Participants self-reported being moderately active but were required to

refrain from structured exercise on the days of testing. Each participant completed two experimental sessions, the order of which was randomized, and which were collected one week apart at the same time of day. The control session consisted of cTBS alone, while in the exercise session cTBS was followed by a bout of stationary biking. TMS measurements were collected at baseline, immediately post-cTBS (post 1), 30 minutes post-cTBS/immediately post-exercise (post 2), and 60 minutes post-cTBS/30 minutes post-exercise (post 3).

5.2.2 Exercise protocol

Heart rate (HR) and rate of perceived exertion (RPE) were collected at rest prior to exercise. During exercise, HR was continuously monitored using a wrist-mounted heart rate sensor. RPE was verbally reported every 5 minutes using the modified Borg scale. Participants were instructed to work at approximately 65-70% of their age-predicted maximal heart rate [average =120-130 beats per minute (bpm)] but to keep their perceived exertion level in the moderate range (between 3 and 4). After a 5-minute warm-up to elevate HR into the target zone, participants performed 20 minutes of continuous stationary biking on a recumbent bicycle in an isolated room. Participants could adjust either the pedaling resistance or the rate of pedaling to maintain the target heart rate and RPE. The duration and intensity were intended to mimic a standard aerobic workout. Participants were seated comfortably with their feet secured to the pedals and their backs against the backrest. All participants reported intensity rates in the moderate range, with no individual exceeding an RPE of 4. The experimenters remained with the participant throughout the exercise and ensured that arms were resting comfortably by their sides and

not gripping the handlebars during the session. The arms and hands remained stationary during pedaling exercise. Participants were given free access to water. Immediately following exercise completion, subjects returned to the TMS testing room for the collection of post-exercise measures. In all cases, HR had returned to resting or near-resting levels (within 5bpm) by the 30 minute mark post-exercise (post 3).

5.2.3 TMS protocols

Focal TMS was performed using a MagStim 2002 stimulator (Magstim, Whitland, UK) connected to a figure eight coil (50 mm inner diameter). BrainSight Neuronavigation (Rogue Research, Canada) was used to guide the placement of the coil to the target motor region using a template MRI for all participants. The coil was placed at a 45° angle to the mid-sagittal line to induce a posterior to anterior current in the underlying neural tissue. EMG recordings of motor-evoked potentials (MEPs) were obtained using surface electrodes placed over the right first dorsal interosseous (FDI) muscle. Additional electrodes were placed over the right abductor pollicis brevis (APB) muscle in order to monitor co-contractions. Raw EMG signals were amplified (1000x), band-pass filtered (2Hz-2.5kHz; Intronix Technologies Corporation, Model2024F, Canada), digitized (5 kHz, Micro1401, Cambridge Electronics Design, Cambridge, UK), and then recorded by a computer using SIGNAL software (Cambridge Electronic Devices, Cambridge, UK) and stored for off-line analysis. The motor hotspot of the right FDI muscle was defined as the left M1 location that consistently elicited an optimal and isolated MEP in the resting muscle, as assessed by both EMG amplitude and a visible muscle twitch. The resting motor threshold (RMT) of the FDI muscle was defined as the minimum stimulator intensity required to elicit a contraction

with a peak-to-peak MEP amplitude of >50 μV on 5 out of 10 trials. After localization of the hotspot and calculation of RMT, the stimulation intensity required to elicit an MEP with a peak-to-peak amplitude of approximately 0.5 mV was determined. The active motor threshold (AMT) was subsequently calculated as the minimum intensity required to elicit a peak-to-peak amplitude of >200 μV along with a visible silent period on 5 of 10 trials while maintaining a contraction equal to approximately 10% of the maximal voluntary contraction.

SICI and ICF were assessed using the following parameters for the conditioning stimulus (CS), test stimulus (TS) and inter-stimulus interval (ISI): a) SICI (CS=80% RMT and TS=0.5 mV intensity, 2.5 ms ISI); and b) ICF (CS=80% RMT and TS=0.5 mV intensity, 12 ms ISI). Ten pairs of stimuli were delivered in each paired-pulse protocol with an ISI of 5 seconds between stimulus pairs. Average values in each paired-pulse condition were compared to the average amplitude for the test stimulus alone in order to determine the percent inhibition/facilitation. At baseline, post 2 and post 3, test pulses were delivered at 90% RMT to confirm that the CS intensity of 80% RMT did not evoke a measurable MEP. For single pulse measures, ten stimuli were delivered with a 5 second inter-stimulus interval at 90% RMT, 100% RMT, and the 0.5 mV intensity.

cTBS was delivered according to the protocol described by Huang et al. (2005). Groups of 3 stimuli at 50 Hz were delivered at 5 Hz for 40 seconds (600 pulses in total) at 80% AMT. Participants remained seated and stationary for 5 minutes after the delivery of cTBS while Post 1 was collected and then either remained seated for the control session or moved to an adjacent room to perform exercise. Post 1 consisted of ten single pulses at the 0.5 mV intensity. At all other timepoints (baseline, post 2 and post 3), MEPs at 90% RMT,

100% RMT and the 0.5 mV intensity as well as SICI and ICF were recorded. The order of intensities tested was randomized across participants, but remained consistent within each session.

5.2.4 Statistical analysis

Within each session, one-way ANOVAs were used to assess the effects of cTBS on MEP amplitude and changes in SICI and ICF following cTBS. For the between-session analysis, MEP amplitude changes were normalized to baseline values and two-way ANOVAs (session x time) were conducted to determine the effect of exercise on cTBS-induced changes in cortical excitability. Raw SICI and ICF values were normalized to baseline values for each session and change scores were used to conduct the 2-way ANOVAs. A priori hypotheses were made based on existing evidence on the time course of MEP changes. Huang et al. report that MEP amplitudes are variable in the first few minutes following stimulation, but are then consistently suppressed for up to 60 minutes, although amplitudes approach baseline values at the 60-minute mark [37]. Thus, a priori contrasts were used to test the hypotheses that a) cTBS-induced suppression would be maximal at 30 minutes post-cTBS (post 2), and b) that the suppression of MEP amplitude at post 2 would be greater in the control session. The significance level was set to $p \leq 0.05$.

5.3 Results

MEPs

Changes in MEP amplitude are displayed in Figure 5.1. In the control session, a one-way ANOVA revealed a main effect of time on cortical excitability ($F_{3,27} = 2.93$, $p = 0.05$).

Results from planned contrasts revealed that cTBS led to a reduction in MEP amplitude at post 2 ($F_{1,27}$ =6.15, p<0.02). In the exercise session, there was no main effect of time indicating no significant differences in MEP amplitude following cTBS ($F_{3,27}$ =0.29, p=0.83). Results from a 2-way ANOVA performed on change scores revealed a main effect of session ($F_{1,9}$ =5.52, p<0.43), but no main effect of time ($F_{2,18}$ =0.39, p=0.68) and no session x time interaction ($F_{2,18}$ =0.74, p=0.49). Results from planned contrasts demonstrated that MEP suppression was significantly greater in the control session at 30 minutes post-cTBS (Control: -39%, Exercise: +75%; $F_{1,18}$ =10.03, p=0.005).

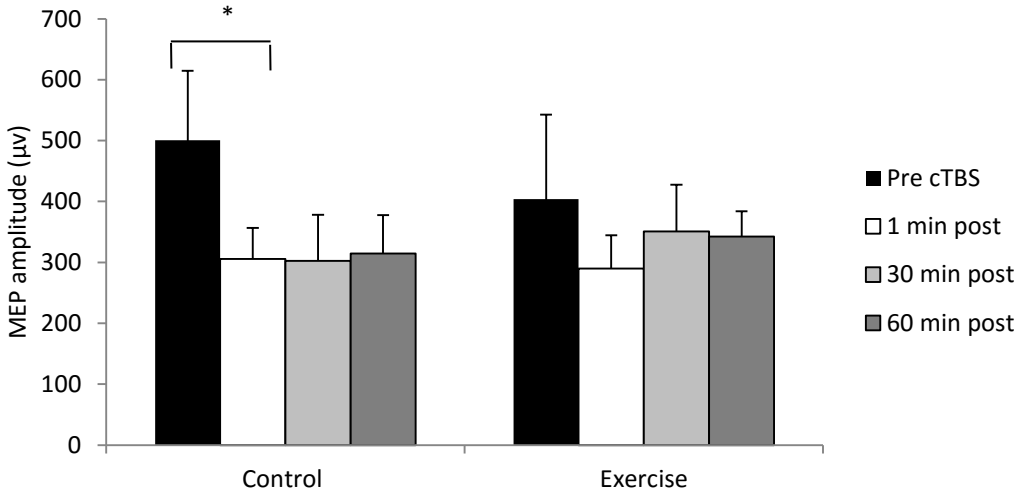


Figure 5.1: Raw MEP amplitude changes following cTBS in control session and exercise sessions. Bars represent SEM. *=p≤0.05

SICI and ICF

Figure 5.2 displays changes in intracortical inhibition and facilitation following cTBS. One participant was removed from the ICF analysis due to baseline values that differed from the mean by greater than 2.5 standard deviations. Results from separate one-way ANOVAs revealed no main effect of time on SICI in either the control ($F_{2,18}$ =1.21,

p=0.32) or exercise sessions ($F_{2,18}$ =0.03, p=0.96), and similarly, no effect of time on ICF in the control ($F_{2,18}$ =0.27, p=0.76) or exercise sessions ($F_{2,16}$ =1.43, p=0.27). A 2-way ANOVA of SICI change scores detected no main effect of session ($F_{1,36}$ =1.51, p=0.23) or time ($F_{1,36}$ =0.72, p=0.40), and no session x time interaction ($F_{1,36}$ =0.20, p=0.66). For ICF, a 2-way ANOVA of change scores revealed a main effect of session ($F_{1,36}$ =4.11, p=0.05) but no effect of time ($F_{1,36}$ =0.01, p=0.92) and no session x time interaction ($F_{1,36}$ =0.27, p=0.61).

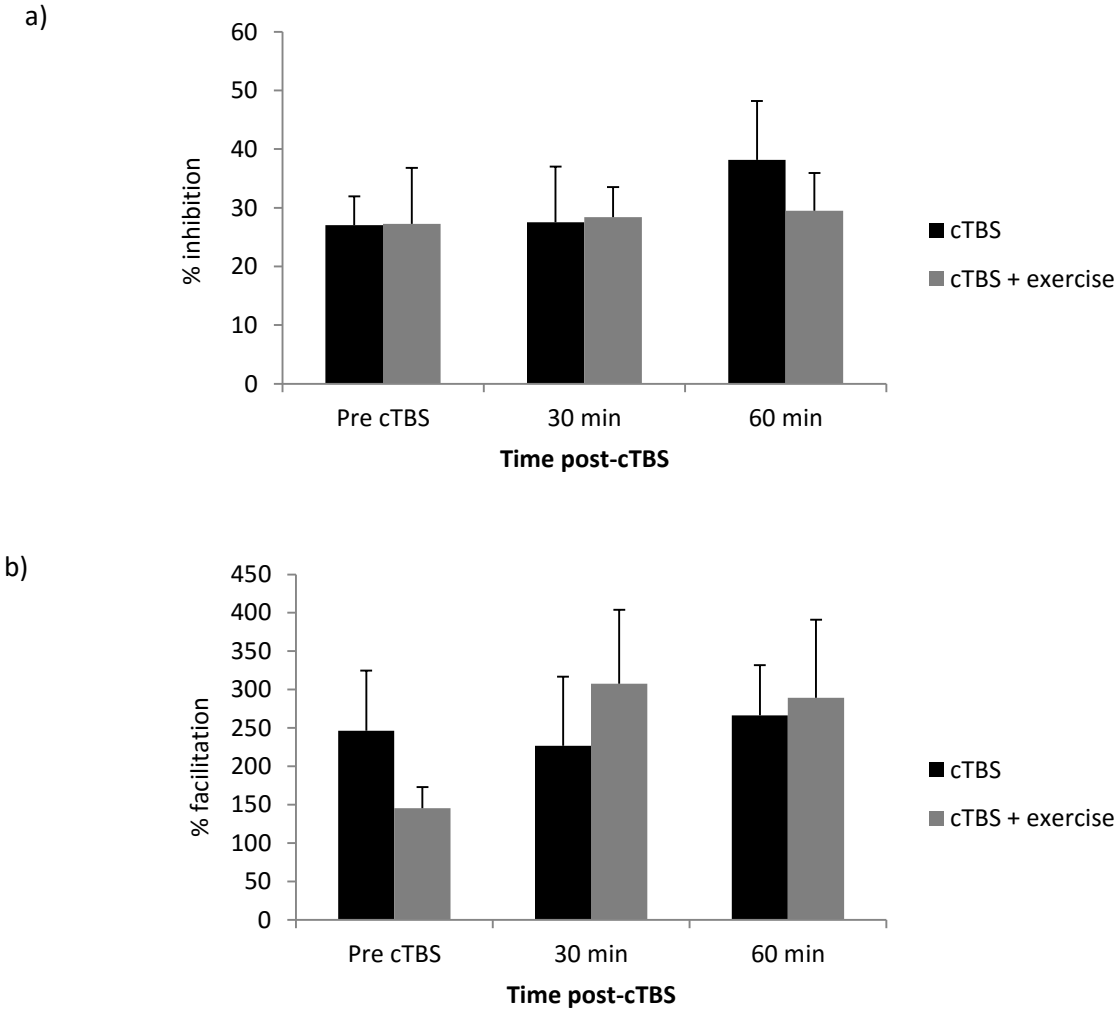


Figure 5.2: Changes in paired-pulse measures following cTBS. Group data of average values for a) SICI and b) ICF pre and post-cTBS . Values are expressed as a percent of single pulse amplitude. Bars represent SEM.

5.4 Discussion

Summary of results

Acute exercise appears to enhance the early markers of plasticity in healthy individuals and has shown promising potential as a primer for plasticity-inducing interventions such as PAS and cTBS. However, it is not known how exercise affects the properties of plasticity induction when performed after such interventions. In addition, it is unclear whether the priming effect of exercise is limited to LTP-like processes, or whether LTD induction is similarly facilitated. The main finding of this study is that an acute bout of exercise abolishes the response to cTBS when exercise is performed immediately after stimulation. We observed a maximal suppression of cortical excitability at 30 minutes post-cTBS in the control session, while no suppression was evident in the exercise session. These results suggest that exercise interferes with, rather than primes, LTD and can modulate excitability changes even when performed after the induction of plasticity.

cTBS and reversal of LTD

CTBS is a rapid, non-invasive method of inducing LTD-like plasticity in M1 and is thought to suppress cortical excitability via the activation of n-methyl d-aspartate (NMDA) receptors [41]. While the effects appear to be maximal at 10-20 minutes post-stimulation, suppression persists for approximately 50 minutes afterwards [443], although effects have been reported to last for up to 60 minutes, including in the original cTBS study conducted by Huang et al. [37]. In the theoretical model of theta-burst stimulation, the rapid rise in calcium triggers binding to the C-lobe of calmodulin and subsequently activates AMPA receptors [444]. While both intermittent and continuous theta-burst stimulation initially

trigger the same response, in cTBS this effect may be reversed by the overstimulation of glutamatergic neurons [443]. While the exact physiology of the response is unknown, the sustained influx of calcium into corticospinal neurons is thought to activate a signalling cascade that results in the dephosphorylation of the cyclic AMP-dependent protein kinase (PKA) binding site of AMPA receptors [19,40,445].

The reversal of cTBS effects has been previously observed as a consequence of homeostatic metaplasticity. The original concept of this phenomenon proposed by Bienenstock et al. [151] proposes that there is a sliding threshold for the induction of synaptic LTP and LTD that is dependent on the prior activity of the synapse. A high level of excitatory activity raises the threshold for further LTP induction and lowers it for LTD, and vice versa. Thus, not only is the response related to prior synaptic activity, but this model suggests that the response to TBS is highly dependent on the parameters and conditions of the stimulation. Indeed, subsequent studies have shown that the effects of cTBS are reversed by doubling the train of stimulation [446], halving the train of stimulation [447], by priming with iTBS [448] or by contraction of the target muscle during or immediately after cTBS [449,450]. Although contractions immediately after cTBS can abolish its effects, this interference is not seen with contractions that occur at later timepoints, suggesting the existence of a critical time window for reversal [449]. Regardless, we ensured that participants remained at rest for 5 minutes following cTBS administration and were carefully monitored during exercise to ensure that the target FDI muscle was at rest. Although exercise has been shown to prime LTD induction, it is important to note that there is no evidence that aerobic exercise alone is sufficient to enhance the excitability of corticospinal neurons in M1. A key characteristic of depotentiation/de-depression is that it

modifies existing LTP/LTD, but does not induce it on its own [451]. Thus, if exercise does not induce LTP in cortical neurons, it is unlikely that our findings are related to homeostatic metaplasticity. Our results suggest that rather than altering the induction of LTD, exercise interfered with the consolidation of LTD. Since we do not know the precise time course of cTBS, we cannot determine whether the lack of LTD observed in the exercise session was due to de-depression (reversal) of existing LTD or whether exercise suppressed the induction of LTD over a prolonged time period. However, the most likely explanation is that exercise induced de-depression of cortical neurons. Since exercise occurred after cTBS, it is unlikely that it altered the threshold for LTD induction. The effects of cTBS, while observable for 60 minutes, are maximal at 5-10 minutes post-stimulation [443]. As such, the maximal suppression would have likely occurred prior to the beginning of exercise. Depotentiation and de-depression are highly time-sensitive, although the window depends on which combination of interventions are used [448,452,453]. Regardless, while most studies have examined LTP rather than LTD, stimulation-induced reversal of LTP is no longer observed after 30 minutes, which is aligned with the end of exercise in the current study.

Potential mechanisms of exercise-induced reversal of cTBS

The influence of exercise in this study appeared to counteract the effects of cTBS. There is limited evidence in this area as, to the best of our knowledge, only one study has previously investigated the interaction of exercise and LTD. McDonnell et al. [299] observed that cTBS after-effects were enhanced following low-intensity exercise, but abolished following moderate-intensity exercise. These results are in line with the current

findings and suggest that moderate exercise interferes with both the induction and consolidation of cTBS effects.

In contrast to the mechanisms of cTBS, it is unclear how exercise exerts its effects on cortical excitability. Previous studies have primarily investigated the role of aerobic exercise as a primer for the induction of plasticity, but the processes mediating this interaction are unclear. Interneuronal networks, and particularly those responsible for the generation of SICI, are thought to play a critical role in early plasticity. Exercise has previously been shown to suppress SICI and enhance ICF for up to 30 minutes [207,397,398], which could potentially enhance excitatory neurotransmission. In the current study, we observed an increase in ICF following cTBS only in the exercise session. While ICF is not thought to contribute to the effects of cTBS nor the generation of LTD , this finding suggests that an increase in the activity of glutamatergic interneurons may contribute to de-depression. Currently, the role of intracortical networks in the induction of LTD is unclear. While Huang et al. [37] originally observed a reduction in both SICI and ICF following cTBS, other studies have shown no effect of cTBS on SICI [161,396]. Although evidence suggests that increases in GABA contribute to cTBS after-effects [454], and that exercise appears to decrease GABA activity [207,397,398], we did not observe any effect of exercise on levels of SICI, suggesting that the contribution of GABA to the current results was minimal.

In contrast, a prevailing hypothesis suggests that an acute increase in excitatory neurotransmitters and neuromodulators may lead to changes in excitability. Specifically, exercise stimulates the production of the arousal-linked neurotransmitters dopamine (DA), serotonin (5-HT) and norepinephrine (NE), as well as brain-derived neurotrophic factor

(BDNF) [260,263,356–358], all of which can directly enhance M1 excitability [87,98,105,122]. A key component of LTD appears to be the dephosphorylation of AMPA receptors, specifically of the PKA binding site [445]. Both BDNF, acting via the TrkB receptor, and dopamine, via the excitatory D1 receptor, readily phosphorylate this same binding site [124,455–459]. In addition, the primary mechanism of cTBS appears to be calcium-mediated signalling via NMDA receptors, with the rate of calcium entry determining the effect [40]. As well as mediating AMPA receptor activity, DA and BDNF are capable of directly interacting with NMDA receptors and increasing glutamate transmission. In striatal neurons, activation of the D1 receptor increases the surface expression of NMDARs and promotes trafficking to dendritic regions [98], while in pyramidal neurons, D1 activation directly potentiates current flow through NMDARs [99]. Interestingly, the inactivation of D2 receptors appears to abolish cTBS [460].

BDNF also has direct effects on synaptic transmission. The binding of BDNF to TrkB enhances presynaptic glutamate release and increases the open probability of NMDARs postsynaptically, likely via the phosphorylation of NMDAR subunits [125].
Lastly, while similar evidence is lacking for de-depression, activation of D1 and D5 dopamine receptors appears to modulate depotentiation, although these receptors are not required for LTP induction itself [461]. Indeed, Fresnoza et al. [462] report that D1 receptor activation abolishes or reverses the effects of cathodal transcranial direct current stimulation and inhibitory PAS , suggesting that dopamine may have an important role in the reversal of LTD, although it is important to note that this effect is dose-dependent. Thus, we hypothesize that exercise may have altered the physiological state of the neurons stimulated by cTBS. In this model, both cTBS and exercise-associated neurotransmitters

may interact with glutamate receptors, resulting in interference and ultimately decreased effectiveness of cTBS. Taken together with previous evidence, the current results suggest that exercise is effective both when performed prior to or following therapeutic interventions, but that exercise exhibits a bias toward LTP rather than LTD. These results suggest that cTBS is susceptible to de-depression when combined with aerobic exercise and that exercise may limit the effectiveness of cTBS when it is performed afterwards.

While several potential sources of variability in the response to cTBS have been identified, including age, gender, genetics, and time of day [141,407,443], the degree to which these factors affect plasticity is unclear, as Hamada et al. report that none actually influence the response to cTBS [131]. The rates of participants who respond to cTBS in the expected manner range are quite variable, although many studies do not report this information, and rates appear to be at least partially dependent on the stimulation parameters used [463]. In our group of participants, 9 of 10 demonstrated a suppression of MEP amplitude at a minimum of one post-cTBS timepoint in at least one session and thus were classified as responders. We did not eliminate the sole non-responder from the group as the objective of the study was to determine how exercise might modulate the baseline response to cTBS, whatever the response may be. However, we chose to collect the same participants twice rather than using separate experimental groups in order to minimize the variability in the response to cTBS. There is limited evidence of the reproducibility of cTBS measures across sessions, but recent studies suggest good repeatability and low intra-individual variability of cTBS effects over time [463,464]. Finally, is it unclear how fitness levels may affect the response to exercise, although Vallence et al. observed no relationship

between physical activity levels and the response to cTBS in young healthy adults [463]. This remains an important area for future research.

5.5 Conclusion

In conclusion, we have demonstrated that an acute session of moderate-intensity exercise suppresses the response to cTBS in young healthy individuals. Interventions aimed at inducing LTD may not benefit from the use of exercise as a primer, while exercise remains a suitable adjunct for techniques and therapies that promote LTP.

Chapter 6: General discussion

6.1 Summary of main findings

In this thesis, we sought to investigate the influence of acute aerobic exercise on excitability changes and the induction of experience-dependent plasticity in M1. In chapter 2, we reported that 20 minutes of moderate stationary biking was sufficient to suppress SICI and enhance ICF in a non-exercised upper limb muscle for at least 30 minutes following exercise completion. These findings suggest that a post-exercise window exists in which the neural environment might be more receptive to experience-dependent plasticity. Thus, in chapter 3, we administered PAS, a technique known to induce LTP, during the 30 minutes immediately following exercise and observed an upregulation of excitability in M1 when PAS was paired with exercise compared to PAS alone. While the degree of facilitation was the same between groups, exercise resulted in a more rapid response. In Chapter 4, we extended these findings to investigate whether exercise could enhance the behavioural response to a motor training task, and whether these changes were evident throughout the entire cortical representation of the trained muscle. We employed a grid mapping technique and observed that exercise enhanced the cortical excitability of the target muscle when compared to training alone, but did not result in greater performance improvements. Finally, we investigated whether the benefits of exercise apply to interventions that induce inhibition rather than excitation. The findings from Chapter 4 suggest that in contrast to LTP, exercise does not enhance the induction of LTD in M1.

Exercise prevented the suppression of cortical excitability due to cTBS when it was performed immediately post-stimulation, demonstrating that exercise interferes with cTBS after-effects.

6.2 Implications of current results and generalization of findings

a) Priming the brain for plasticity

Taken together, these four studies suggest that exercise may create an ideal environment for the induction of plasticity, and also that the effects of exercise may be biased towards LTP-like processes rather than LTD. The state of M1 neurons reflects the sum of excitatory and inhibitory inputs upon them, and exercise appears to shift the balance towards excitation. This suggests that exercise is a suitable adjunct to therapies and interventions that aim to increase M1 excitability, which includes motor learning.

The motor cortex has a remarkable capacity for reorganization and adaptation, even after an acute session of training or rehabilitation. However, the ability of a given intervention to induce plasticity is influenced by a number of both internal and external factors, and the effectiveness of a given intervention will depend on the responsiveness of the cortex. The concept of priming the brain for learning is not a new one, and in the past, several attempts have been made to alter the state of M1 to render it more receptive to plasticity, including pharmacological treatments such as amphetamines [463], nicotine [464], 5-HT [465], L-dopa [89], and non-invasive stimulation techniques such as TBS [466] or TDCS [467]. The findings in this thesis suggest that exercise can modify the brain in a similar fashion. The ideal priming technique should comply with principles of homeostatic

metaplasticity, and thus should not induce LTP on its own. Currently, there is no evidence to suggest that exercise alone is sufficient to trigger LTP, which supports its use an adjunct therapy. While these changes may be quite subtle compared to pharmacological interventions, exercise is safe, cost-effective, non-invasive, and can be performed virtually anywhere. Importantly, exercise does not represent an additional intervention, but instead takes advantage of an activity with myriad health benefits in which people are already encouraged to participate.

The studies that comprise this thesis also connect to a much larger field of research into the benefits of exercise on brain function. While a focus on the motor cortex is quite recent, it is generally accepted that both acute and chronic exercise can enhance cognitive function through their effects on regions such as the prefrontal cortex. Our research suggests that such benefits are not limited to frontal regions, but that exercise might induce an increase in excitability in multiple cortical areas. If exercise is capable of producing a generalized increase in cortical excitability, the potential applications are extensive. Even if such effects are localized to those regions involved with the performance and maintenance of exercise, this may include not only M1, but the premotor and supplementary motor areas, the basal ganglia, somatosensory cortices, the visual and auditory cortices, prefrontal areas, and the limbic system. Indeed, the unique potential of exercise may lie in its ability to engage a vast network of brain regions and trigger system-wide changes in excitability.

The significance of this generalized response likely has its roots in evolutionary biology. An emerging theory put forth by Raichlen et al. [479] postulates that the increase in human brain size over an evolutionary timescale is linked to an increase in aerobic capacity, which was driven by the emergence of the hunter-gatherer lifestyle. As hunting

skills evolved, the neurochemical response to exercise stimulated brain growth, along with conferring a survival advantage. If true, the response that is observed to acute exercise today likely reflects and utilizes a framework that has been in place for millions of years. Thus, if exercise is linked to neurogenesis and increased synaptic complexity, it seems unlikely that the response would be localized to one or two regions. Indeed, research into the cognitive and motor benefits of acute exercise may be two pieces in a puzzle that will eventually demonstrate similar responses in a number of cortical areas.

b) Synthesis of the neurochemical hypothesis model

The proposed model for the findings observed here link to the neurochemical hypothesis. Support for this hypothesis requires positive findings in three related fields: a) observing increased neurotransmitter/neuromodulator release with exercise; b) establishing that these transmitters can affect M1 excitability; and c) demonstrating that M1 excitability is influenced by exercise. Thus, the findings in the current thesis contribute to one component of this model. In addition, these results suggest that as well as increasing the release of arousal-linked neurotransmitters, exercise may suppress GABA activity. Synthesizing a variety of findings from across human and animal studies, it is clear that the exercise state is associated with the release of a number of potential neurochemical modulators. As this research is still in the early stages, it may be useful to examine them in isolation. Future studies might examine which component of the response, if any, is driving the effects of exercise. This could be investigated by comparing the effects of exercise vs. the administration of L-dopa, an infusion of lactate, or an increase in generalized arousal.

However, it is unlikely that a single factor or response is mediating the effects of exercise. Exercise increases catecholamine levels, but also increases lactate, BDNF, and cortisol and suppresses GABA activity, suggesting a unified response from multiple brain and body systems. This is supported by a number of studies demonstrating that these factors can interact at the neuronal level. Dopamine receptor activation appears to suppress both GABA receptor currents and the probability of GABA release [431,468]. Dopamine enhances BDNF expression [469], and activation of the D1 dopamine receptor induces the phosphorylation and surface expression of TrkB [470]. Activation of 5-HT receptors triggers transcription of the BDNF gene and increases BDNF expression [471,472]. Both BDNF and NE upregulate the neuronal lactate transporter MCT2 [473,474], and BDNF suppresses synaptic $GABA_A$ receptor activity [129]. Taken together, these findings indicate that the multifactorial response to exercise may underlie its ability to enhance plasticity.

Given the inherent difficulties in measuring neurotransmitter levels in humans, this model remains largely theoretical and limited to animal studies. Exceptions include lactic acid, which is produced in working muscles, and BDNF, which is able to cross the BBB and can be measured in the peripheral circulation. Although BDNF has traditionally been associated with structural changes such as neurogenesis and synaptic restructuring [188,475–477], there is strong evidence to suggest that it can acutely enhance cortical excitability and induce early plasticity [120,121,123,129,478]. Given its established roles in both rapid excitability changes and long-term potentiation, BDNF has emerged as a potential link between the acute benefits of exercise and the adaptive changes that occur in M1 with chronic physical activity. BDNF not only interacts with the arousal-linked

neurotransmitters, but its activity-dependent release may distinguish exercise from other states in which arousal is increased.

c) Chronic exercise and M1

While the study of acute exercise remains an important field in its own right, it is also useful in order to shed light on mechanisms that mediate the chronic response to exercise. Physical activity throughout the lifespan is the best path to neurological health, and examining the acute and incremental changes induced during a bout of exercise may inform how the accumulation of these changes leads to more permanent alterations in brain structure and function. The documented benefits of long-term physical activity include increased brain volume, neurogenesis and angiogenesis, as well as altered biochemistry [185,186,191,465,466]. Although few studies have examined the effects of chronic exercise specifically in M1, preliminary evidence indicates that both structural and functional changes occur over time. Swain et al. [467] report increased capillary perfusion and capillary reserve in M1 after 30 days of running exercise, while the measurement of cytochrome oxidase activity, a marker of ATP production, was significantly higher in the motor cortex following 6 months of voluntary exercise [344]. Correspondingly, 30 days of voluntary exercise induces angiogenesis within M1 [345]. Garcia et al. [468] report an increased expression of the synaptic proteins synapsin I (SYS) and synaptophysin (SYP) in M1 following four weeks of treadmill training, indicating enhanced synaptic transmission. Whether such changes occur incrementally with each bout of exercise or whether a critical threshold is eventually reached that triggers changes in gene expression is yet to be

determined. While growth factors such as BDNF may play a key role, at present the precise links between acute and chronic adaptations to exercise remain largely unknown.

e) Potential clinical applications

In neurological patient populations, such as those living with the effects of a stroke, one aim of therapy is often to restore or improve motor function. This is generally achieved by targeted interventions such as constraint-induced therapy, bimanual motor training, or traditional physiotherapy. All of these strategies aim to promote long-term cortical reorganization. As reorganization requires increases in excitatory neurotransmission, exercise may enhance the ability of these therapies to stimulate structural changes within M1. Priming techniques have previously been shown to enhance recovery in stroke patients [469–471], and if exercise acts as a general primer for excitatory changes in synaptic transmission, it may have promising applications in clinical populations where voluntary movements are impaired. Aerobic exercise facilitates motor recovery after stroke [376] and interestingly, levels of BDNF appear to be a determining factor in the degree of recovery [472]. In addition to facilitating plasticity, if exercise targets the GABA system, it may be useful in reducing the excessive inhibition of M1 that is often observed in stroke patients [368,473,474]. Indeed, a reduction in GABA activity has been shown to promote motor recovery post-stroke [371]. As well as its importance for overall health and well-being, exercise also decreases the risk of developing diseases such as Alzheimer's [475,476], alleviates depressive symptoms [477,478], and reduces the risk of stroke [479–481]. Thus, any treatment plan that incorporates exercise will have multifactorial benefits

for primary and secondary prevention of neurological diseases, as well as improving quality of life for those living with motor impairments.

6.3 Future considerations

a) Role of fitness and recommendations for exercise prescription

The role of fitness-related adaptations in the neural response to exercise remains an open question. Of particular relevance to this thesis is the question of how chronic participation in aerobic exercise influences the response to an acute bout of exercise. In the motor cortex, chronic activity may modulate resting excitability levels, as Cirillo et al. [388] demonstrate greater short-term plasticity in physically active individuals. However, activity levels in this study were compared between two extreme populations (ie. very sedentary vs. very active), and when a more moderate group is studied, no differences in plasticity are observed [461]. In addition to aiding exercise prescription, there are two reasons why fitness may be relevant if the neurochemical hypothesis is correct: the first is if resting levels of neurotransmitters are correlated with fitness, and the second is if the threshold for the release of neurotransmitters is dependent on fitness. Currently, these questions are the source of much debate. Zoladz et al. [115] observed that 5 weeks of endurance training in young healthy men increased resting BDNF levels, while others report that aerobically trained men have lower levels of serum BDNF than sedentary men [493,494]. This may also be dependent on activity state, as three months of endurance training appears to increase BDNF release at rest but not during exercise [495]. Indeed, it

has been reported that exercise-induced increases in serum BDNF do not differ between low and high fit individuals [496].

Adding to the confusion is a lack of clarity surrounding the definition of fitness and how it is determined. What, if any, is the relationship between cardiovascular fitness, physical activity levels, nutritional status, metabolism, and body composition, and how do these affect the brain's response to exercise? Furthermore, does physical fitness reflect a lifetime of activity, or can it be attained through a single training program? Future studies might investigate this cross-sectionally using participants of varying fitness and physical activity levels, or longitudinally by designing a training program to observe changes in M1 excitability before and after the intervention.

Another area for future research concerns not just the cortical but the behavioural and performance outcomes related to exercise. In Chapter 4 of this thesis, we addressed this question and did not observe any additional benefits to motor performance when training was preceded by exercise, despite enhanced cortical adaptations. Changes in motor function are particularly relevant in clinical populations, where far greater emphasis is placed on behavioural outcomes. Thus, it is important to investigate whether particular types of motor learning are more likely to be enhanced by exercise, or whether the "dose" of exercise needs to be greater in order to observe benefits at the behavioural level. This relates to a larger and important question surrounding the optimal exercise prescription for neurological benefits. While the prescription used in this thesis was sufficient to modulate excitability in M1, it is unknown whether altering the parameters of the exercise bout would have optimized the response. It is possible, and indeed likely, that the ideal exercise bout varies according to physical and psychological status, which may pave the

way for a more individualized approach to exercise prescription. Thus, a final role for fitness status relates to the psychological response to exercise. In unfit individuals, the perceived stress of exercise may override the beneficial neurochemical response, and thus exercise should be prescribed at a lower relative intensity. The goal seems to be to challenge the cardiovascular system while avoiding placing extreme stress upon this system and consequently the brain. The difficulty lies is applying existing principles that are true in the periphery to the brain. The role of fitness is confounded by a lack of understanding of how peripheral markers of fatigue and intensity are reflected in the brain. For example, the accumulation of lactic acid in working muscles is one of the key indicators of fatigue, yet lactate is metabolized quite readily by the brain and in fact enhances excitability in M1 [255,256]. How to prescribe exercise for neurological benefits rather than cardiovascular benefits remains an important question for future study.

b) Genetic variability

The concept of an individualized approach to exercise may become more relevant as research continues to demonstrate that the ability of exercise to prime plasticity is ultimately influenced by a number of different factors, including genetic variability that mediates the neurochemical response to exercise. While there is strong evidence that variation in the BDNF gene modulates the induction of plasticity, genes rarely work in isolation and a number of other candidate genes are being identified. One gene of interest in the modulation of cortical excitability is catechol-O-methyl transferase, or COMT, an enzyme that catalyzes the degradation of DA, E and NE. Three polymorphisms of COMT

have been identified: rs4680 (Val158Met), rs737865 and rs165599. In particular, the Val158Met is associated with decreased enzymatic activity, thereby slowing the degradation of DA with a resultant increase in prefrontal DA levels [482]. A third candidate is known as kidney and brain associated protein, or KIBRA. KIBRA is expressed in multiple brain regions, including the cerebral cortex, and codes for a cytoplasmic protein that interacts with Dendrin, a cytoskeleton-associated protein thought to be involved in cellular signalling between the synapse and the nucleus [483]. A common polymorphism, rs17070145, results from a T→C substitution in the ninth intron of KIBRA [484]. To date, the influence of KIBRA in the motor cortex has not been studied; however, carriers of the KIBRA-C polymorphism have increased hippocampal volume [485] and enhanced performance on episodic memory tasks [484]. Furthermore, C-carriers display increased synchronization in multiple regions of the default mode network, although not in sensorimotor regions [486].

It is also critical to identify how these genes may interact. Li Voti et al. [338] observed no difference between BDNF Val/Val and Met individuals in MEP amplitude or performance on a motor learning task following iTBS, indicating that cortical excitability changes are not determined by a single genotype, but that multiple genes likely interact to determine the susceptibility to plasticity induction. In support of this, Witte et al. [339] report that PAS-induced plasticity is not influenced solely by BDNF genotype, but rather by the interaction of BDNF and COMT. In this study, BDNF Met carriers displayed smaller increases in post-PAS excitability than BDNF Val/Val individuals, but only when COMT genotype was taken into account. This is expected to be an area of great interest in the future, as the effects of variation in genes critical for plasticity begin to emerge, such as

NMDARs and DA receptors. In addition to elucidating a key determinant of rapid plasticity responses, developing a genetic profile of those patients and individuals most likely to respond to a particular technique will assist in the development of targeted therapies and result in more effective clinical interventions.

c) Time course of exercise effects

On a smaller scale, some unanswered questions from this work remain, which can be addressed in individual studies. We have not yet tested the time course and time limitations of these effects. In subsequent work, it will be useful to extend the measurements until excitability levels have returned to baseline, and consequently to identify the period of maximal facilitation. In addition, altering the timing of stimulation will shed light on how exercise interacts with the processes that induce LTP and LTD. It will also be important to assess the response to exercise using different techniques, such as TDCS, or possibly with pharmacological interventions, to add to the knowledge base in this area.

6.4 Limitations

In addition to the specific limitations outlined in each chapter, there are some general limitations across studies. As identified above, we did not control for differences in fitness and physical activity levels between participants, which may have increased the variability. While it is currently unclear how fitness levels may affect the acute response to exercise, it

may be useful to have a measure of physical activity levels or cardiovascular fitness to examine the influence of such factors on the exercise response. In addition, other variables that may have affected M1 excitability were not assessed or controlled for, such as gender differences and hormonal levels. While choosing more restrictive inclusion criteria can decrease variability, it can also limit the applicability of the findings to the larger population.

A further limitation is that we only tested one type of exercise, using a standard exercise prescription. It is possible that the optimal mode, duration or intensity of the exercise bout differs from the parameters used in this thesis. Indeed, there is emerging evidence that high-intensity interval training might be a more effective intervention than steady-state exercise [221,222]. In addition, providing more mental engagement during exercise, through visual, sensory or auditory stimulation might have led to greater increases in excitability. Finally, we did not assess the arousal level of our participants during and following exercise. Such measures, whether via skin conductance or anxiety questionnaires would lend support to an arousal-based model.

All of the studies that comprise this thesis employed TMS either as an intervention or an assessment tool. While it is a safe and effective form of brain stimulation, a number of drawbacks can limit the interpretation of TMS results. The utility of TMS can be limited by the inability to prevent the spread of excitability from the site of stimulation, and also the inability to identify which neuronal pools are contributing to the generation of MEPs. Locating the identical site of stimulation across multiple sessions remains a challenge, as small differences in electrode placement or coil position can potentially affect the reliability and repeatability of TMS measures. The low spatial resolution of TMS increases the

likelihood that additional brain regions are contributing to the response in the target muscle. With regard to the parameters of cTBS, it has been argued that the muscle contractions required for the determination of the AMT influence the after-effects of cTBS [447]. While some researchers have chosen to use a percentage of RMT for this reason, the majority of studies employing TBS still use AMT, which makes comparisons more difficult when using RMT.

Finally, as MEPs reflect the excitability of the entire corticospinal tract, the contribution of changes at the level of the spinal cord cannot be excluded. While interventions such as PAS have been shown to be cortically mediated, motor learning paradigms such as those used in Chapter 4 likely influence both cortical and spinal circuits, and we did not distinguish between these changes.

A final limitation of this thesis, which is reflective of this field of research in general, is the inability to connect in vitro and in vivo results. That is, the putative mechanisms developed from findings in animal research cannot directly be tested in humans. For example, while we observed decreases in SICI in Chapters 2 and 3, which are presumed to reflect a suppression of GABA activity, we did not assess GABA levels or receptor activity. Similarly, our models assume that LTP and LTD-like changes are mediated by NMDAR activity, which cannot be observed in vivo. There is evidence that metabotropic glutamate receptors may also contribute to the induction of LTP [487], which may alter the model of the exercise response. The difficulties in measuring changes in neurochemical factors in human participants are a limitation not only to the development of a model, but also to exercise prescription. It is likely that the optimal exercise prescription differs based on which neurotransmitter system is targeted. The parameters of exercise that maximize

BDNF release, for example, may not be the same ones that maximize catecholamine release. While these are likely to remain limitations in the future, they are not a barrier to exercise prescription. In addition, more advanced neuroimaging techniques, such as positron emission tomography, may be able to bridge this gap by examining the release, activity and distribution of specific neurotransmitters within the brain.

6.5 Conclusion

In this thesis, we have demonstrated that a single bout of aerobic exercise has beneficial effects on cortical excitability in M1 and enhances the induction of LTP-like plasticity. Exercise suppresses intracortical inhibition, increases intracortical facilitation and alters the state of M1 to create a more receptive environment for motor learning and short-term adaptations. The mechanisms by which this occurs are not clear but likely include an increase in excitatory neuromodulators such as DA, 5-HT, NE, BDNF and lactate, in conjunction with a suppression of GABA activity. These findings suggest that exercise may be a useful adjunct to techniques and therapies that aim to induce plasticity in motor areas in healthy populations and may also be beneficial in neurological patient populations.
The findings in this thesis continue to support the promotion of physical activity for overall brain health and also demonstrate that a single session of exercise is sufficient to enhance the ability of the motor cortex to undergo experience-dependent plasticity.

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Appendix: Exercise and heart rate (HR) data

Chapter 2

Resting heart rate range = 56-88 beats per minute (bpm)
Range for 65-70% age-predicted maximal HR = 125-135 bpm
Average HR during exercise: 124.8 bpm
Average Borg rating: 3-4

Chapter 3

Resting heart rate range = 53-76 bpm
Range for 65-70% age-predicted maximal HR = 125-135 bpm
Average HR during exercise: 127.8 bpm
Average Borg rating: 3-4

Chapter 4

Resting heart rate range = 66-88 bpm
Range for 65-70% age-predicted maximal HR = 120-130 bpm
Average HR during exercise: 127.0 bpm
Average Borg rating: 3-4

Chapter 5

Resting heart rate range = 72-93 bpm
Range for 65-70% age-predicted maximal HR = 120-130 bpm
Average HR during exercise: 115.2 bpm
Average Borg rating: 3-4