The Effect of Energy-Matched Exercise Intensity on Brain-Derived Neurotrophic Factor and Motor Learning

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The Effect of Energy-Matched Exercise Intensity on Brain-Derived Neurotrophic Factor and Motor Learning

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Abstract

Brain plasticity is important to motor learning, and is a critical component of motor rehabilitation. Exercise prior to motor training may facilitate plasticity by increasing brain-derived neurotrophic factor (BDNF). However, many studies that have investigated exercise-enhanced plasticity have assessed motor skill performance on tasks involving single finger button presses or small movements of a joystick, results that may not relate to more complex, real-world movements. Additionally, while high-intensity exercise has been shown to benefit motor learning, the effects of low-intensity exercise have yet to be fully investigated. A bout of low-intensity exercise, when completed at an energy expenditure that is equivalent to that of a high-intensity exercise bout, may also benefit learning and might be particularly relevant to individuals with neurological disorders who may only be capable of achieving low-levels of physical activity. Therefore, our first aim was to develop a motor learning task that involved 3-dimensional (3D) reach movements. Our second aim was to investigate the effects of exercise intensity on motor learning of the same task. In Study 1, we developed a motor learning task in a virtual environment that involved 3D reach movements to sequentially presented targets. With this task, we produced results similar to those traditionally observed in the motor learning literature; individuals improved with practice ($p < 0.001$) and performance was maintained at retention ($p = 0.386$). Since our task involved 3D reach movements, results from studies utilizing this
task may be more relatable to real-world movements. In Study 2, we used the 3D reach task to investigate the effects of exercise intensity on motor learning. We compared performance on the 3D reach task and the BDNF response to exercise between a rest group, a high-intensity exercise group, and a low-intensity exercise group. Both exercise groups expended 200 kilocalories of energy. Overall improvement on the motor task, indicated by a reduced response time, did not differ by group. However, exercise at both a high and low-intensity altered the kinematic profile used to improve performance over time. The rest group improved in the spatial domain of performance more than the exercise groups, while both high and low-intensity exercise groups improved more in the temporal domain of performance. Therefore, exercise at a specific energy expenditure, whether at a low or high-intensity, may facilitate the temporal components of motor performance. A significant rise in BDNF was not observed after exercise in either exercise group. Furthermore, the high variability observed in the exercise-related BDNF response was not related to BDNF genotype. However, BDNF genotype did have an effect on performance of the 3D reach task. Individuals with the BDNF polymorphism had faster response times throughout task practice ($p = 0.002$). Future work is needed to fully understand the effects of the polymorphism on motor performance and learning. Our investigation revealed that energy expenditure may be more important than exercise intensity for inducing an exercise-related effect in the kinematics of reach behavior. In addition, exercise may influence motor behavior through neural mechanisms other than BDNF.
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Chapter 1

Introduction

The human brain, while capable of many amazing feats, does not have an infinite amount of resources. The limited capacity of the brain poses an innate constraint, potentially preventing necessary growth and development. Fortunately, the brain is not a static entity, but rather a plastic organization of neural connections capable of adapting to environmental inputs and demands (Pascual-Leone, Amedi, Fregni, & Merabet, 2005). From learning to walk, to throwing a ball, to simply reaching for an object, motor behaviors are accompanied by a restructuring and reorganization of synapses and neural circuits (Pascual-Leone et al., 1995). These neural shifts in organization manifest as behavioral changes observable in everyday life.

The brain’s ability to adapt and learn based on environmental demands is an important aspect of motor development across the lifespan. Additionally, this phenomenon is also an integral aspect of motor rehabilitation. Individuals with brain injuries, such as those induced by a stroke, are often left with debilitating motor impairments. In fact, stroke is a leading cause of disability in the United States (Mozaffarian et al., 2015), and out of approximately seven million stroke survivors, 66% are currently disabled (“Stroke Info: Facts & Statistics,” 2015). Rehabilitation methods, such as physical therapy, utilize the plastic nature of the brain to abate and diminish patients’ motoric impairments. While standard
rehabilitative procedures have shown considerable success in restoring function in many patients, approximately 50-60% of patients still demonstrate some degree of long-term motor deficiency that requires assistance in activities of daily living (Bolognini, Pascual-Leone, & Fregni, 2009). However, research has recently demonstrated therapeutic techniques that can modulate and augment neural plasticity, ultimately leading to better behavioral outcomes. The goal of these techniques is to exploit the existing knowledge of plasticity and brain function to enhance current methodologies, instead of creating completely novel rehabilitative procedures.

Recently, a bout of aerobic exercise is coming to the forefront of research as an effective method of enhancing plasticity and motor learning. Physical exercise does not inherently create plasticity, but rather supports the neural network of motor learning and increases the likelihood of neuroplastic change (Hötting & Röder, 2013). Initial studies on aerobic exercise as an adjunct to motor learning have been promising, but many results are still unclear or conflicting. Specifically, the type, intensity, and duration of exercise, in addition to time of motor training relative to exercise could all affect behavioral outcomes. The ideal combination of the aforementioned variables needs to be established to design and develop the most effective exercise prescription for facilitating neuroplasticity. Furthermore, the underlying mechanisms that support the exercise-related effects on learning and neuroplasticity have yet to be fully identified. Researchers need to deepen their knowledge of how exercise-enhanced plasticity improvements occur to fully maximize their benefits.
Chapter 2
Review of the Literature

2.1 Behavioral Aspects of Motor Learning

The neural processes of motor learning are difficult to directly assess, and therefore researchers must infer changes by observing and quantifying behavior (Kantak & Winstein, 2012). Changes in motor performance, such as reaction time and accuracy, are assessed, and these behavioral outcomes are used to infer motor learning. For example, a greater reduction in reaction time when practice of a task was random as opposed to blocked indicates more robust motor learning occurred in the random group (Shea & Morgan, 1979). Accuracy, indicated as the amount of error in task performance, has also historically been used to assess motor learning. In a paradigm examining delayed knowledge of results versus instantaneous knowledge of results, error was greater in the instantaneous group, suggesting degraded learning (Swinnen, Schmidt, Nicholson, & Shapiro, 1990).

There are two distinct types of learning, explicit and implicit. Originally coined by Reber in 1967 (Reber, 1967), implicit learning is significantly relevant in the acquisition of motor skills as it occurs automatically in response to environmental demands and cues. This automatic process occurs indefinitely, whether it’s making small adjustments to an already learned skill, or performing a completely novel task. In this self-regulatory activity, inputs from the environment
are processed by the brain and the subsequent behavior is automatically altered to meet the demands of the current situation (Wulf, Shea, & Lewthwaite, 2010).

A classic example of implicit motor learning is exhibited with the serial reaction time task (SRTT). Introduced thirty years ago by Nissen and Bullemer (Nissen & Bullemer, 1987), the SRTT, or similar versions which maintain the concept of implicit motor learning, have been utilized in numerous motor learning experimental paradigms (Robertson, 2007). The task involves a series of stimuli presented one at a time which correspond to a particular button press. Unbeknownst to the participant, there is a repeated pattern within the series of stimuli. It is expected that with practice, participants will unwittingly reduce their reaction times at the repeated sequence of stimuli. This is thought to represent a change in implicit motor learning, or learning that occurs without the learner’s awareness (Wulf & Schmidt, 1997).

As previously stated, the SRTT, or tasks following a similar concept, have frequently been used to assess implicit motor learning. However, most of these studies utilize single finger button presses, or minute movements of a computer mouse or joystick (Mang, Snow, Campbell, Ross, & Boyd, 2014; Meehan, Dao, Linsdell, & Boyd, 2011; Nissen & Bullemer, 1987). While capable of assessing the basic principles of implicit motor learning, it is difficult to translate the functional application of these tasks. An implicit motor learning task which uses dynamic whole arm movements could provide a better understanding of implicit motor learning as it relates to functional movement. An implicit motor learning task developed in an immersive 3-dimensional virtual environment would allow
for the control the researcher needs to manipulate the task, while also enabling the learner to make more “real world” movements (Stewart, Gordon, & Winstein, 2013). Knaut et al. (Knaut, Subramanian, McFadyen, Bourbonnais, & Levin, 2009) compared kinematics of a reaching task in a virtual environment to kinematics of a similar reaching task in the real world. The authors concluded that the kinematics were sufficiently similar, indicating that the virtual environment is an appropriate alternative for real world movement. Therefore, development of an immersive virtual environment motor task could provide the opportunity to define the role of functional movement in implicit motor learning.

2.2 Motor Learning and Neuroplasticity

While motor learning ultimately leads to changes in overt behavior, it actually occurs at the neural level as a result of a series of underlying events in the brain. An understanding of the neural processes that support learning can assist researchers in the development of methods that enhance and/or modify these processes, thus optimizing motor learning. There are several suggested mechanisms of brain plasticity involved in learning including: modulation of synaptic strength, unveiling of suppressed neural connections, modulation of neuronal activity in glia, morphological changes, and the reorganization of functional networks (Duffau, 2006). These phenomena have largely been studied in Schaffer collaterals and commissural neurons in the hippocampus, and this research serves as the basis for what is known about learning at the cellular level (Minichiello, 2009). These cellular mechanisms are also present in the primary
motor cortex (M1). Adult rats trained on a skilled motor task demonstrated performance improvements that were accompanied by a strengthening of connections within M1 (Rioultpedotti, Friedman, Hess, & Donoghue, 1998). In humans, paired associative stimulation, a brain stimulation paradigm which closely mimics the neuronal components of learning, demonstrated increased cellular activity in M1 as indicated by an increase in motor evoked potentials (Ziemann, Iliać, Pauli, Meintzschel, & Ruge, 2004). Furthermore, motor training can increase the size of the cortical representation of the trained area in M1 (Bolognini et al., 2009). Taken together, this research indicates synaptic plasticity and the modification of internal neuronal networks as the mechanism underlying motor learning (Sanes, 2003).

Synaptic plasticity is part of a larger process called long-term potentiation (LTP) (Minichiello, 2009). LTP is induced by high-frequency stimulation that is activity dependent. LTP can be broken down into early LTP (E-LTP), which involves changes to existing proteins and regulation of the trafficking of those proteins, and late LTP (L-LTP), which requires new mRNA and protein synthesis (Bramham & Messaoudi, 2005). Most of these modifications occur at the synapse and therefore synaptic plasticity, specifically strengthening of the synapse, is an essential component of LTP.

Within the process of LTP, the N-methyl-D-aspartate receptor (NMDAr), a glutamate receptor, plays a key role. Activation of this receptor requires depolarization of the post-synaptic cell in order to dissociate the Mg^{2+} ion blocking the channel. Once unblocked, glutamate from the presynaptic cell can
activate the receptor, allowing for an influx of Na\(^+\) and Ca\(^{+2}\) (Malenka & Nicoll, 1999). The Ca\(^{+2}\) influx is critical as it activates several necessary enzymes such as Ca\(^{+2}\)/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC), which are crucial for LTP induction (Minichiello, 2009). Once activated, CaMKII and PKC do not require a continuous influx of Ca\(^{+2}\), but rather can run autonomously. This consistent activity initiates several events which support LTP, such as phosphorylation of amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPAR) and increased NMDAR trafficking, both of which are mechanisms that regulate synaptic efficacy (Lau & Zukin, 2007). The increase in neuronal activity also leads to advantageous presynaptic repercussions, such as a rise in neurotransmitter release (Stanton, Winterer, Zhang, & Müller, 2005). With time and continued activation, signaling to the nucleus activates key transcription factors that trigger protein synthesis, which ultimately results in morphological changes at the synapse including new dendritic spines and enlargement of pre-existing spines (Minichiello, 2009).

Further exemplifying the importance of the NMDAR for LTP in M1, synaptic plasticity is dramatically reduced with use of dextromethorphan (an NMDAR blocker), indicating that activation of NMDAR is a critical aspect of neuroplasticity and LTP in this brain region (Bütefisch et al., 2000). Overall, if NMDAR activation is facilitated, LTP and subsequently learning will also be facilitated.

A known prolific modulator of the NMDAR is the protein brain derived neurotrophic factor (BDNF). BDNF is a member of the neurotrophin family, a group of polypeptide growth factors that impact cell differentiation and neuronal
survival (Bath & Lee, 2006). BDNF serves many neuroplastic functions such as regulation of dendritic branching, arborizing axon terminals, potentiation of synaptic transmission, facilitating gene transcription, modifying synaptic efficacy, and enhancing neuronal resilience (Cotman & Berchtold, 2002; Vaynman, Ying, & Gomez-Pinilla, 2004). Also important to motor learning, BDNF modulates NMDAr-dependent LTP by increasing its sensitivity (Antal et al., 2010). To demonstrate the importance of BDNF in LTP, Cotman et al. (Cotman & Berchtold, 2002) showed that mice deficient in BDNF had impaired LTP and learning defects; deficits were reversed with the reintroduction of BDNF. Given the numerous benefits of BDNF, it is crucial to understand its underlying mechanisms in order to further explore these benefits and determine the most advantageous way to utilize the protein.

Release of BDNF at the neuronal level is activity-dependent, and the amount of neurotransmitter release is directly related to the amount of synaptic activity (Schinder & Poo, 2000). Once present in the synapse, BDNF binds to the receptor tyrosine kinase B (TrkB) presynaptically and postsynaptically (Cotman & Berchtold, 2002). Presynaptically, binding of BDNF to TrkB increases vesicle cycling, ultimately resulting in increased release of neurotransmitters including BDNF and glutamate (Murray & Holmes, 2011). Postsynaptically, the binding of BDNF and TrkB activates three prolific pathways: the Ras–mitogen activated protein kinase (MAPK) pathway, which promotes neuronal differentiation and growth; the phosphatidylinositol 3-kinase (PI3K) pathway, which promotes neuronal survival and growth; and the phospholipase Cγ1 (PLCγ1) pathway,
which promotes the release of intracellular Ca^{+2} and key transcription factors (Minichiello, 2009). One such transcription factor is cyclic AMP-responsive element-binding protein (CREB), which initiates the synthesis of crucial proteins required for the maintenance of LTP (Vaynman et al., 2004). Interestingly, one of the CREB-related synthesized proteins is BDNF. Therefore, BDNF promotes learning and, in turn, learning promotes BDNF (Chaieb, Antal, Ambrus, & Paulus, 2014). This cyclic relationship further increases the synaptic efficacy between the pre- and post-synaptic neurons leading to even greater LTP.

Another result of TrkB activation includes increased NMDAr in the membrane, which increases NMDAr sensitivity (Murray & Holmes, 2011; Singh & Staines, 2015). Modulating the efficacy of NMDAr leads to a greater CA^{+2} influx and therefore more activity and increased excitability. Demonstrating the importance of BDNF and TrkB to LTP, blocking the TrkB receptor diminished the synaptic response to high frequency stimulation, and, therefore, the magnitude of LTP at the synapse was reduced (Figurov, Pozzo-Miller, Olafsson, Wang, & Lu, 1996). This result reveals that BDNF modulates LTP by enhancing synaptic efficacy, as all LTP was not prevented, but rather the amount of LTP was lessened.

Altogether, the impact of BDNF on LTP is substantial. Therefore, activities which increase BDNF are likely to enhance learning. As such, activities which increase BDNF could serve as an adjunct to motor learning paradigms in an effort to achieve more robust outcomes. One such activity that has shown to increase serum BDNF levels is acute aerobic exercise.
2.3 Exercise Enhanced Motor Learning: Introduction

The health-related benefits of exercise are well studied with favorable effects on numerous systems of the body. Regular physical activity impacts cardiovascular health, respiratory function, metabolism, and musculoskeletal integrity, and reduces the risk of a variety of disorders including obesity, diabetes, and heart disease (Fletcher et al., 1996). What has not been as clearly elucidated is the effect of exercise on the nervous system and brain health.

Recently, research has indicated the potential of exercise to enhance neuroplasticity as well as prevent cognitive decline with age (Hötting & Röder, 2013). Current investigations examining the effects of chronic exercise on brain health demonstrate a positive relationship between regular exercise across the lifespan and a decrease in age-related cognitive decline (Sofi et al., 2011). However, the effects of an acute bout of aerobic exercise on the brain are less clearly defined. Recent work indicates that a single session of aerobic exercise has the potential to create an optimal environment for neuroplasticity, and ultimately improve motor skill learning (Singh & Staines, 2015). One proposed mechanism underlying the enhancement of neuroplasticity after a single exercise session is through the effect of exercise on BDNF (Mang, Campbell, Ross, & Boyd, 2013).

2.4 The Aerobic Exercise-BDNF Relationship

As a key mediator of neuroplasticity, establishing the relationship between BDNF and a bout of aerobic exercise is integral to determine the effect of acute
exercise on motor learning. Several studies have demonstrated that a single intense bout of aerobic exercise increases serum BDNF two-three fold (Ferris, Williams, & Shen, 2007; Knaepen, Goekint, Heyman, & Meeusen, 2010; Rasmussen et al., 2009; Skriver et al., 2014). When comparing three different exercise sessions, low-intensity cycling (20% below the ventilatory threshold), high-intensity cycling (10% above the ventilatory threshold), and a graded cycle exercise test (exercising with increasing workload until fatigue), a significant increase in BDNF was only observed in the two high-intensity conditions (Ferris et al., 2007). A similar relationship between BDNF and aerobic exercise was evident in another study that compared the effect of a low-intensity warm-up to a high-intensity exercise test to exhaustion. A significant increase in serum BDNF was only present following the high-intensity exercise test whereas there was no change in BDNF concentration following the ten minute warm-up (Vega et al., 2006). Several studies have recently employed a 20 minute, high-intensity interval exercise paradigm, which includes short bursts of high-intensity cycling interspersed with low-intensity “rest” bouts (Roig, Skriver, Lundbye-Jensen, Kiens, & Nielsen, 2012) to further demonstrate the relationship between high-intensity exercise and BDNF. This intense exercise session significantly increases serum BDNF immediately after exercise to levels as high as 3.4 times greater than baseline (Mang et al., 2014; Skriver et al., 2014). Achieving an intense, physically strenuous level of aerobic exercise appears essential to attain a significant increase in BDNF.
Peripheral measurement of serum BDNF (like those utilized by the studies described above) is an indirect method of determining BDNF levels in the CNS. However, BDNF as well as other neurotrophins are capable of crossing the blood brain barrier bi-directionally (Pan, Banks, Fasold, Bluth, & Kastin, 1998; Pan & Kastin, 2004; Poduslo & Curran, 1996). Furthermore, Rasmussen et al. (Rasmussen et al., 2009) recently compared blood samples taken from the radial artery and the internal jugular vein while exercising, and determined that the brain contributes 70-80% of circulating BDNF. By demonstrating a relationship between central and peripheral BDNF levels, peripheral measurements serve as an appropriate marker for BDNF levels in the CNS.

Another important consideration when examining the effects of exercise-dependent BDNF release is the presence of a particular BDNF gene polymorphism. The rs6265 single nucleotide polymorphism (SNP) on the BDNF gene exists in approximately 30% - 50% of the population (Shimizu, Hashimoto, & Iyo, 2004). This SNP results in a change of the amino acid at position 66 from valine to methionine (Val66Met). The effect of the polymorphism on BDNF concentration has not been fully identified. Several studies report no difference of baseline serum BDNF concentration in individuals with and without the polymorphism (Terracciano et al., 2010; Trajkovska et al., 2007; Tramontina et al., 2007). However, Ozan and colleagues (Ozan et al., 2010) indicate a reduction of BDNF concentration in Met carriers, while Lang et al. (Lang, Hellweg, Sander, & Gallinat, 2009) found the opposite to be true.
In addition to the effect on baseline BDNF, the presence of the polymorphism needs to be considered when examining the exercise-dependent BDNF response. Research investigating the polymorphism in relation to activity-dependent BDNF release suggests a differential effect based on the presence of the Met allele. In response to neuronal activation (i.e. depolarization) Met carriers have a diminished BDNF aftereffect (Egan et al., 2003). Work by Chen et al. (Chen et al., 2004) indicates that altered intracellular trafficking of BDNF in response to activation is responsible for this effect. While this evidence implies a discrepant BDNF-related response to activity based on genotype, it is important to distinguish the difference between an ‘activity-dependent’ response, referring to increased electrical activity at the cellular level, and an ‘exercise-dependent’ response, referring to the change post-physical activity. The relationship between the polymorphism and exercise-dependent release of BDNF has yet to be demonstrated. For example, McDonnell and colleagues (McDonnell, Buckley, Opie, Ridding, & Semmler, 2013) did not find a significant difference in BDNF response after a bout of aerobic exercise when comparing a Val66Val group and a Val66Met group. Whether or not a differential BDNF-response exists between the genotypes needs to be further exemplified in research, especially with the continued importance placed on the exercise-dependent release of BDNF, and its subsequent effect on plasticity and learning.

There remains another integral yet unanswered question regarding the intensity of exercise and its effects on BDNF concentration. Is exercise intensity the determining factor in the amount of BDNF release, or is it the amount of total
work (force x distance) that is key? Several studies examining the response of BDNF to varying exercise intensities fail to control for the total amount of work output between the exercise conditions. The high-intensity condition performs a greater amount of overall work compared to the low-intensity condition, but the implications of this have not been considered. Given the complex nature of exercise metabolism (e.g. differences between: anaerobic vs. aerobic, short duration vs. prolonged bouts, continuous exercise vs. interval training), controlling for total work may help identify the underlying mechanisms of exercise-dependent neuroplasticity.

The relationship between exercise, BDNF, and other neural substrates needs to be critically examined as well. Epinephrine, norepinephrine, dopamine, insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), and lactate are all elevated after high-intensity exercise, and their potential role in brain plasticity needs to be investigated (Skriver et al., 2014). Specifically, further examination of the significance of lactate is important. In a study by Winter et al. (Winter et al., 2007), acquisition of novel vocabulary words was 20% faster in an anaerobic sprint condition compared to a low-impact aerobic running condition. In addition to higher levels of BDNF in the sprinting condition, lactate levels were above 10 mmol/l compared to the continuous running condition where lactate remained below 2 mmol/l. While a direct relationship between BDNF and lactate was not established in this study, work by Schiffer et al. (Schiffer et al., 2011) indicates that lactate is involved in the regulation of BDNF blood concentrations. The lactate clamp method, where a sodium lactate solution is infused into the
cubital vein, was performed on eight male subjects at rest to examine the effects of increased blood lactate on BDNF. The experiment revealed a significant increase in blood BDNF concentrations as a result of the lactate infusion. Furthermore, Coco and colleagues (Coco et al., 2013) demonstrated a similar relationship at the cellular level. They examined the effects of lactate on the SH-SY5Y cell line as well as astrocytes, and discovered that lactate increased BDNF in all cell cultures. Continued investigation into the relationship between lactate and BDNF is integral as it could further clarify the underlying mechanisms of neuroplasticity modified by exercise.

2.5 Exercise-facilitated Neuroplasticity

The release of exercise-dependent BDNF primes the neurons, and facilitates mechanisms related to long term potentiation, plasticity, and learning. An increased presence of BDNF can strengthen synapses and facilitate synaptic transmission, which are important neuroplastic processes that promote learning (Cotman & Berchtold, 2002). Exercise alone is not capable of causing neurophysiological change, but rather it creates a neural environment that is optimal for inducing plasticity.

In order to determine the effect of an acute exercise session on plasticity, exercise has been paired with various non-invasive brain stimulation methodologies that have been shown to alter neuronal excitability. Electrophysiological measurements obtained via transcranial magnetic stimulation (TMS) are the outcomes used to assess changes in excitability. In
one example of this approach, Mang et al. (Mang et al., 2014) combined a high-intensity exercise bout with paired associative stimulation (PAS), and compared the resultant change in excitability to an identical stimulation protocol that was not primed with exercise. When examining the slope of the motor evoked potential (MEP) recruitment curve, a 59.8% increase was observed in the combined exercise-PAS condition, whereas the increase was just 14.2% in the stimulation only condition. These results support work by Singh et al. (Singh, Neva, & Staines, 2014) who demonstrated greater area under the MEP recruitment curve in response to PAS when PAS was preceded by exercise. In this study, excitability was also examined in an unstimulated area of M1 where no change was found. This demonstrates that exercise serves as a facilitator for targeted stimulation rather than producing a general increase in excitability across M1. Together these results indicate that a single, high-intensity exercise session can prime neurons for greater LTP-like plasticity.

Another study examined a change in cortical activity using electroencephalogram (EEG) measurements to demonstrate elevated early activation of movement preparation post-exercise (Thacker, Middleton, McIlroy, & Staines, 2014). In this within subject design, EEG data were collected as the subject completed a wrist extension movement. The data were obtained before and after a moderate bout of exercise (20 min cycling at 70% age-predicted max heart rate). The authors suggest that the enhanced cortical activation after exercise is related to an increase of select neurotransmitters in the brain after the exercise bout (although no such neurotransmitters were measured).
Interestingly, an acute bout of exercise can also enhance an inhibitory stimulation protocol. McDonnell and colleagues (McDonnell et al., 2013) demonstrated that a session of low-intensity exercise promoted the inhibitory effect of continuous theta burst stimulation (cTBS). The expected decrease in cortical excitability (MEP amplitude) was more evident in the low-intensity exercise condition (18% reduction) compared to the control resting condition (8% reduction). However, when examining a moderate-intensity exercise bout, the cTBS protocol increased MEP amplitude by 1%. The authors suggest that the higher-intensity exercise caused an increase in cortisol, which may have interfered with BDNF expression. Overall, these results indicate the potential of an acute bout of exercise to modulate M1 plasticity. Furthermore, this study exemplifies the delicate relationship between exercise intensity and its effect on neuroplasticity. Based on the principles of meta-plasticity, which indicates that a synapse’s previous history of activity affects its current likelihood of change (Abraham & Bear, 1996), it is probable that there is a balance point between exercise intensity and the amount of subsequent stimulation (whether via non-invasive brain stimulation or activity-dependent activation such as motor training). Additional investigations examining this relationship are required to establish the optimal intensity (or work) to stimulation ratio.

In addition to enhanced facilitation, a bout of exercise can also reduce inhibition. A decrease of intracortical inhibition is important as it suggests an environment that is more susceptible to activities that promote more permanent synaptic plasticity such as LTP and LTD. This concept was demonstrated by
Singh et al. (Singh, Duncan, Neva, & Staines, 2014) who saw a reduction in short-interval intracortical inhibition (SICI) in response to 20 minutes of moderate-intensity exercise. In a similar study by Smith et al. (Smith, Goldsworthy, Garside, Wood, & Ridding, 2014), a reduction of SICI was evident after both a low-moderate bout of cycling and a moderate-high bout of cycling when compared to baseline measurements. Neither of these experiments observed an increase in MEP amplitude post-exercise indicating that exercise on its own is not capable of altering excitability, but rather it creates a favorable environment for neuroplastic change, and may be effective when combined with plasticity inducing methods such as non-invasive brain stimulation and motor training.

Just as the BDNF polymorphism needs to be considered in the discussion of BDNF production, the effect of the Met allele on synaptic plasticity needs careful examination. No baseline differences of resting and active motor threshold exist between those with the polymorphism and those without (Cárdenas-Morales, Grön, Sim, Stingl, & Kammer, 2014), which supports the idea that differences in those with the Met allele are activity-dependent. Furthermore, Singh et al. (Singh, Duncan, et al., 2014) reported no significant difference in response to exercise-induced plasticity in Met carriers. Of note however, trends indicating a less robust decrease of SICI and a resistance to long-interval intracortical inhibition (LICI) were evident. These findings suggest that Met carriers may have higher thresholds to neuroplastic processes, and as such are less susceptible to stimulation driven changes.
Kleim and colleagues (Kleim et al., 2006) also examined the effect of the BDNF polymorphism on neuroplasticity. Similar to previous work, no baseline differences were observed between Val66Met genotypes. However, when measuring corticospinal output (MEPs) and motor map area after motor training, there was a significant increase in the Val66Val subjects (indicating synaptic plasticity), but no difference in Met carriers. Again, these results suggest that differences between the genotypes are not in the basal state, but rather they manifest as differential responses of activity-dependent plasticity. Results from Cheeran et al. (Cheeran et al., 2008) further support this idea. Three different stimulation protocols, including repetitive TMS (rTMS), a metaplastic paradigm combining tDCS and rTMS, and PAS, all yielded less robust results in Met carriers than homozygous Val66Val subjects.

While the initial neural response to activity is delayed or impaired in Met carriers, this effect may be diminished in longer duration training. McHughen et al (McHughen, Pearson-Fuhrhop, Ngo, & Cramer, 2011) demonstrated that intense motor training over several days can overcome the initial motor performance detriment in subjects with the polymorphism. On day one of motor training (on a marble navigation task), Val66Val subjects experienced the expected motor map enlargement (on M1) associated with plasticity, while the Met carriers did not. After five days of motor task training, this difference was abolished as both groups demonstrated similar short-term cortical plasticity. While this study confirms a genotype-based difference in short-term plasticity, it also indicates that intense training can overcome this initial impairment. Taken together, these
results reveal diminished plasticity in Met carriers, and that this impairment should be considered when devising future research or rehabilitation techniques.

2.6 Acute Exercise and Motor Performance

Neuroplastic changes as indicated by electrophysiological measurements, represent the underpinnings of motor skill learning and acquisition. A bout of acute exercise that increases BDNF and promotes plasticity may also lead to improved motor performance and faster skill acquisition. To date, most research examining the effects of exercise on learning has been focused on cognitive performance. For example, Winter established a relationship between high-intensity exercise and faster acquisition of novel vocabulary words (Winter et al., 2007). In animal models, rats who were exercised prior to practicing the Morris water maze task demonstrated enhanced cognitive function, as they were able to learn and recall the location of the platform better than rats that were kept sedentary (Vaynman et al., 2004). A review by Tomporowski (Tomporowski, 2003) concluded that submaximal aerobic exercise facilitates several aspects of cognitive function and information processing, as long as the exercise does not lead to dehydration and fatigue, which would hinder performance.

Recently, research examining the effects of acute aerobic exercise on enhanced motor performance has become more prominent. Subjects in which motor skill training is paired with a bout of exercise are demonstrating enhanced immediate performance when compared to subjects who practice the skill without prior exercise. Work by Statton et al. (Statton, Encarnacion, Celnik, & Bastian,
2015) showed improved skill acquisition of a pinch force task when training on the task occurred after moderate-intensity exercise as opposed to after rest. The authors attribute this immediate improvement of performance to elevated neurotransmitters, such as BDNF, that are apparent after a bout of exercise (although BDNF was not specifically measured in the study).

Further demonstrating the effect of exercise on immediate motor performance, those who exercised at a high-intensity prior to motor training, as opposed to quietly resting before practice had quicker skill acquisition of a sequence-specific continuous tracking task (Mang et al., 2014). This study also demonstrated enhanced PAS after an exercise bout which, in conjunction with improved motor performance, suggests that exercise primes plasticity and promotes motor learning that leads to improved performance. However, there was no correlation between PAS response and acquisition of the motor task.

Conversely, other work examining the impact of acute exercise on motor performance has failed to show an immediate effect on skill acquisition. In two similar experimental paradigms, Roig (Roig et al., 2012) and Skriver (Skriver et al., 2014) were unable to elicit an effect of high-intensity exercise on immediate performance of a visuomotor accuracy-tracking task. There was no significant difference in skill acquisition between those who exercised prior to practice and those who rested prior to practice. Furthermore, when tested one hour after training had concluded, there was still no significant difference between the conditions. The authors suggest that the high-intensity exercise may have caused a state of over-arousal, which has the potential to inhibit memory
retrieval. Another possible explanation is that the motor memory was still undergoing consolidation, and thus the beneficial impact of acute exercise had yet to occur.

The effect of the BDNF polymorphism on immediate changes in performance is unclear and under-studied. Experiments examining differences in cognitive performance between BDNF genotypes have indicated that those with the Met allele have impaired performance on hippocampal memory tasks, reduced recall capacity, and diminished episodic memory (Antal et al., 2010; Mang et al., 2013). Results regarding motor performance are less conclusive. The majority of work indicates that there is no genotype-based difference in motor performance tasks at baseline or immediate motor skill acquisition (Cárdenas-Morales et al., 2014; Kleim et al., 2006; McHughen et al., 2011). Alternatively, when comparing performance on a motor learning based driving task, Met carriers showed greater error during short-term learning than Val allele homozygotes (McHughen et al., 2010). It is of great importance to note that none of the studies examining the effect of the polymorphism on immediate motor performance included exercise in their experimental paradigm. How exercise affects motor performance or motor skill acquisition among the varying genotypes has yet to be clearly identified. Answering this key question is significant as it could impact future rehabilitative models that include aerobic exercise.
2.7 Acute Exercise and Motor Learning

Improvements in motor performance and rate of skill acquisition are significant outcomes indicating enhanced short-term plasticity. However, distinguishing between transient motor performance and long-term performance is essential to identify motor skill learning. Examination of a newly acquired motor skill at a delayed retention test is a better indicator of motor learning than testing the skill at the end of an initial practice session (Kantak & Winstein, 2012). Furthermore, motor skill learning is a dynamic process where offline improvements are just as important as online (immediate) gains. The process of memory consolidation is not fully understood, and it is possible that time (and more importantly sleep) is key to solidifying the motor memory (Hotermans, Peigneux, de Noordhout, Moonen, & Maquet, 2006). Therefore, studies that include retention tests provide better indicators of motor learning and long-term synaptic plasticity, whereas those examining immediate gains are demonstrating motor performance and cannot attest to the effects of their interventions on learning.

An example of transient performance improvements without learning is identified by Winter et al. (Winter et al., 2007). As previously described, subjects in a high-impact sprinting condition had quicker acquisition of a novel vocabulary task than those in a low-impact continuous running condition and those at rest. However, when examining immediate performance after acquisition (practice), performance at 1-week post-acquisition, and performance 8-months post-
acquisition, there was no difference between the conditions, indicating that learning of the novel vocabulary had not occurred.

Conversely, Roig (Roig et al., 2012) and Skriver (Skriver et al., 2014), who utilized similar high-intensity exercise paradigms in conjunction with a visuomotor accuracy-tracking task, both failed to show an immediate difference in task performance between the exercise condition and rest condition. However, performance at 24-hours and 7-days post-skill acquisition was better in the exercise condition. These results indicate that more robust motor learning occurred (as demonstrated by improved task performance at retention) when motor training was paired with high-intensity exercise, rather than when it was coupled with rest.

Another example of exercise-enhanced motor learning occurred in the previously discussed Mang et al. study (Mang et al., 2014). Here, those who participated in a high-intensity exercise bout prior to motor training not only had better immediate motor performance than resting controls, but this increase in performance was maintained at a 24-hour retention test. A single bout of aerobic exercise primed the neural environment for LTP induced by the motor training, which modulated synaptic plasticity and improved behavioral outcomes.

In another study combining aerobic exercise and motor training, Statton and colleagues (Statton et al., 2015) were unable to demonstrate retention of the motor skill between consecutive days of the combined exercise/motor training regime. However, on a subsequent day without exercise, individuals who had been training in combination with exercise outperformed those in the control
condition. In other words, individuals who had previously exercised were still able to perform the motor skill better, even without prior exercise on that day. The authors suggest that better performance on the non-exercise day in those who previously trained after aerobic exercise indicates that motor skill performance was encoded and stored (i.e. motor learning occurred) more effectively than in those who did not exercise.

Overall, the limited amount of studies examining the effect of aerobic exercise on motor learning reveals a beneficial relationship. A single session of aerobic exercise serves to prime neurons for subsequent activity-dependent plasticity, which leads to enhanced learning and improved behavioral outcomes. Experiments examining exercise-enhanced motor learning have yet to explore the effects of the BDNF polymorphism, but some studies have analyzed its influence on motor learning separate from exercise.

Based on the limited number of studies examining BDNF genotype-based differences of motor learning, there appears to be an effect of task complexity. When utilizing rather simple motor tasks such as the serial reaction time task or a marble navigation task (requiring movement of just one finger) no discernable genotype-based difference between short-term or long-term motor learning is evident (McHughen et al., 2011; Morin-Moncet, Beaumont, De Beaumont, Lepage, & Théoret, 2014). However, when the task becomes more complex, Met carriers demonstrate poorer retention. For example, on a driving-based motor learning task where subjects were required to turn a steering wheel to guide a vehicle through a winding road, individuals with the polymorphism had impaired
retention (motor learning) measured across four days (McHughen et al., 2010). It is important to continue investigating the effect of the polymorphism on motor learning as impairment would impact future rehabilitative methods.

**2.8 Exercise Enhanced Motor Learning: Conclusions**

The research examining motor learning enhanced by an acute bout of exercise is promising, but more work needs to be done to fill the remaining gaps in the literature. While there are many studies investigating motor learning, few studies have examined the use of an acute bout of exercise as an adjunct. Those that have examined learning and exercise have primarily investigated cognitive learning. Furthermore, establishing the connection between exercise, BDNF, and motor learning is crucial. Several studies have examined key concepts related to exercise-enhanced motor learning such as exercise and BDNF, exercise and plasticity, or BDNF and learning, but the current research is lacking a singular direct examination of the relationship between multiple factors. Instead, researchers rely on the indirect implications from previous studies and are required to strategically finesse these independent results into a logical hypothesis.

Additionally, questions remain regarding the role of exercise intensity. However, the issue is not simply between low-intensity vs high-intensity, but rather how intensity is a factor when overall work (force x distance) is constant. Perhaps the fact that individuals who exercise at a high-intensity perform more work compared to individuals in a low-intensity group is the key rather than the
intensity of the exercise itself. Examination of exercise intensity while controlling for the total amount of work is necessary to determine which is the critical factor. In this way, examining the impact of lactate is also essential. Lactate may be a necessary facilitator of exercise induced BDNF release, but this relationship has not been clearly established. Is high-intensity exercise required because it increases lactate and thereby increases BDNF? Or would a similar amount of work (at a lower intensity) that does not substantially increase lactate also be capable of increasing BDNF and having subsequent effects on motor learning? More clearly defining these relationships is integral to fully understanding the role of acute exercise in enhancing motor learning.

Lastly, there is a need to examine sequence-specific motor skill learning in a virtual environment. Currently, research examining implicit motor learning is primarily limited to button presses, or small movements of a computer mouse or joystick. A motor task designed in 3D space would allow for whole arm reach movements that more closely represent real-world, everyday movements. Establishing that the principles of implicit motor learning are evident in more dynamic, skilled movements is necessary for future research implications of motor learning and rehabilitation.
Chapter 3

Sequence-Specific Implicit Motor Learning Using Whole-Arm 3-Dimensional Reach Movements\(^1\)

3.1 Abstract

Implicit motor learning is essential to the acquisition of motor skills. Examination of implicit motor learning, however, has largely involved single-finger button presses or 2-dimensional movements of a computer mouse or joystick. The purpose of this study was to demonstrate sequence-specific implicit motor learning in individuals that practiced a 3-dimensional (3D) whole-arm reach task. Fifteen young, non-disabled individuals completed two consecutive days of practice of a 3D target task presented in a virtual environment with the dominant, right arm. Stimuli were displayed one at a time and alternated between an 8-target random sequence and an 8-target repeated sequence. Movement of the shoulder and elbow was required to successfully capture a target. Performance was indicated by time to complete a sequence (response time) and analyzed by sequence type (random, repeated). Kinematic data (total distance to complete a sequence, peak velocity, and time to peak velocity) were used to determine how movement changed over time. Results showed significant improvements in performance early in practice, regardless of sequence type. However, individuals completed the repeated sequence faster than the random sequence, indicating sequence-specific implicit motor learning. The difference in response time between the sequence types was driven by the total distance of the hand path; the distance traveled for the repeated sequence was shorter than the distance of the random sequence. Examination of implicit motor learning using 3D reach movements provides the opportunity to study learning using whole-arm movements, an important component of many real-world, functional tasks.
3.2 Introduction

Motor learning principles serve as the conceptual framework for certain aspects of rehabilitation (Krakauer, 2006; Weinstein, Lewthwaite, Blanton, Wolf, & Wishart, 2014). Both motor learning and motor recovery after injury are predicated on neuroplastic adaptations which occur as a result of task practice. Explicit motor learning, which requires higher-order cognitive functions such as working memory, results in a declarative knowledge of the learned skill (Orrell, Eves, & Masters, 2006). Explicit learning of a motor skill is consequently limited by the cognitive functions that govern its underlying processes. When cognitive resources are limited or diminished, such as in individuals post-stroke (Hochstenbach, Mulder, van Limbeek, Donders, & Schoonderwaldt, 1998; Tatemichi et al., 1994), their ability to learn or relearn a motor skill through explicit processes can be impaired. Implicit motor learning occurs when a motor skill is acquired or adapted without explicit awareness of skill performance, and is a fundamental aspect of motor learning and relearning (Maxwell, Masters, & Eves, 2000). Compared to explicit motor learning, motor skills learned implicitly are often more robust (Orrell et al., 2006) and result in greater performance at retention (Maxwell, Masters, Kerr, & Weedon, 2001). Importantly, implicit motor learning processes are preserved in individuals post-stroke (Boyd & Weinstein, 2006). Therefore, a greater understanding of implicit motor learning will further promote the application of these concepts in rehabilitation settings. However, traditional investigations of implicit motor learning, which typically involve button presses (Nissen & Bullemer, 1987; Nitsche, Schauenburg, et al., 2003; E.
Robertson, Tormos, Maeda, & Pascual-Leone, 2001) or 2-dimensional (2D) movements of a computer mouse (Brodie, Borich, & Boyd, 2014; Brodie, Meehan, Borich, & Boyd, 2014; Meehan et al., 2011) or joystick (Mang et al., 2014; Wadden, Brown, Maletsky, & Boyd, 2013), may not translate well to multi-joint, 3-dimensional (3D) movements, which are a large focus of rehabilitation.

Practice of a sequence-specific implicit motor learning task leads to learning of the spatial relationship between the position of the cue and the corresponding movement (Willingham, Wells, Farrell, & Stemwedel, 2000). Completion of a sequence-specific implicit motor learning task in 3D space is not expected to alter the way the task is learned; learning is still presumed to be driven by increased knowledge of the spatial relationship of cues. However, movements of the whole-arm in 3D have increased motor demands compared to 2D tasks as they require greater coordination of muscle recruitment, muscle activation, and kinematic variables such as velocity and force (D’avella & Lacquaniti, 2013). Furthermore, a higher number of degrees of freedom must be controlled when completing 3D reach movements compared to 2D movements (Perrot, Bherer, & Messier, 2012). Additionally, natural, unsupported reach movements require compensation of gravitational forces (Perrot et al., 2012). Research with tasks that require small or 2D movement may minimize or remove these important aspects of functional movement, and may not best represent the whole-arm reach behaviors that are essential to real-world, functional tasks.

Development of a motor learning task that incorporates implicit motor learning concepts with whole-arm reach movements can provide the opportunity
to investigate how increased motor control demands affect known motor learning constructs. A computer-based virtual environment (VE) can be used to replicate the design of traditional sequence-specific implicit motor learning tasks previously used in research. In these tasks, stimuli are presented in patterns of random and repeated sequences, however the performer is not made aware that a repeated sequence of stimuli is present (Meehan et al., 2011; Nissen & Bullemer, 1987; Nitsche, Liebetanz, et al., 2003). Faster reaction times when completing the repeated sequence compared to a random sequence indicates sequence-specific implicit motor learning. Transferring this same task design into a VE would facilitate examination of motor skill learning with whole-arm 3D reach movements. Thus, precise control is maintained over stimuli presentation, while also including more demanding behaviors that incorporate the essential physical components of reach movements.

The purpose of the current study was to examine sequence-specific implicit motor learning for a task that involved whole-arm reach movements within a VE. It was hypothesized that an individual’s overall performance of the task, indicated by a reduction in response time, would improve with practice. Additionally, based on previous research that examined sequence specific implicit motor learning, it was expected that the repeated sequence of stimuli would be completed faster than a sequence of randomly presented stimuli, despite the addition of more demanding 3D reach movements.
3.3 Methods

3.3.1 Participants

Fifteen nondisabled, neurologically-intact adults (23.5 ± 3.7 years, 6 female) were recruited from the university community. In order to be eligible to participate, individuals had to: 1) be right hand dominant as determined by the Edinburgh Handedness Questionnaire (Oldfield, 1971); 2) be between the ages of 18-40; 3) have no current or recent neurological symptoms as determined by a general neurological symptom checklist; and 4) have no pain in the right upper extremity. All participants provided informed consent prior to enrollment in the study. The study was conducted in accordance with the Declaration of Helsinki, and all aspects of the study were approved by the Institutional Review Board at the University of South Carolina.

3.3.2 Experimental Task

Participants sat facing a virtual display (Innovative Sport Training Inc., Chicago, IL), and the task was projected down into the workspace directly in front of them (Figure 3.1a). Stereoscopic glasses were worn to provide 3D visualization of the targets. An electromagnetic marker was secured to the right index finger, and provided position data during reaching. The marker was displayed as a white sphere (25 mm diameter) on a simple black background, which provided visual feedback to the participant on finger position throughout the task; visual feedback of arm position was not provided.
Task parameters for the current study were adapted from a previous implicit motor learning serial target task that required 2D movements (Brodie, Borich, et al., 2014; Brodie, Meehan, et al., 2014; Meehan et al., 2011). Targets were displayed as red spheres (28 mm diameter) and were presented one at a time. Participants were instructed to reach towards each target as quickly and accurately as possible. Once the center of the cursor was within 5 mm of the center of the target for 500 msec, that target was considered “hit” and would disappear as the next target was displayed. All targets were presented at one of nine pre-determined target locations (Figure 3.1b). Eight target locations were placed equidistant in a circular array (96 mm radius), with the remaining target location positioned directly in the center. The tangent distance between any adjacent target locations was 75 mm. The array of targets was positioned to the right of the midline of the trunk, permitting the participant to reach all targets without any trunk flexion or rotation. All targets were in the same Z-plane (up/down direction) but required unsupported 3D movement of the arm for successful capture.

Individuals reached to targets under two sequence conditions: repeated and random. Each sequence consisted of eight targets and was controlled for overall difficulty by keeping the total distance traveled constant (93.8 cm). Individual movements between any two targets were assigned an index of difficulty (ID) based on Fitts’ Law (Fitts & Peterson, 1964; Meehan et al., 2011). Calculated values of each ID were 2.42, 2.78, 3.28, 3.66, and 3.78. To simplify, each calculated value was assigned an ID value 1-5, with 1 being the shortest
movement (calculated ID 2.42) and 5 being the longest movement (calculated ID 3.78). Each sequence was then assigned targets consisting of the same ID levels such that every eight-target sequence was comprised of one movement at ID levels 1 and 4, and two movements at ID levels 2, 3, and 5 (8 total movements). The repeated sequence (targets: 1, 8, 6, 5, 9, 4, 8, 2) was the same across all trials. For all random sequences, target position and ID level were randomly presented but overall difficulty level for the sequence remained constant.

3.3.3 Experimental Procedure

Participants completed the 3D reach task over two consecutive days. On Day 1, individuals practiced 144 sequences in an alternating random-repeated sequence order, such that every other sequence of eight targets was the repeated sequence. Participants were not made aware of the presence of the repeated sequence. A 10 second rest was provided after every third sequence to prevent fatigue. All participants returned on Day 2 (24 ± 2 hours) for a retention test, and completed 72 alternating random-repeated sequences. All other task procedures were identical to Day 1.

After completing the retention test on Day 2, explicit awareness of the repeated sequence was assessed. Participants were asked if they noticed the presence of a repeated sequence. If the individual answered ‘Yes’, he or she was asked to recall the sequence. All participants then completed six explicit awareness tests. For each test, the participant viewed three eight-target sequences presented in the VE. After each explicit test, the participant was
asked if the repeated sequence was present. Three of the six explicit tests contained the repeated sequence.

3.3.4 Data Analysis

The position of the electromagnetic marker was sampled at a rate of 120 Hz throughout the task and data were analyzed with a custom MATLAB script (Mathworks, Inc., Natick, MA). Total time to complete an eight-target sequence (response time) was the primary measure of task performance consistent with previous studies that used a similar task (Brodie, Borich, et al., 2014; Brodie, Meehan, et al., 2014; Mang, Snow, Wadden, Campbell, & Boyd, 2016). To determine how performance changed over time, kinematic variables of both spatial and temporal components of performance were evaluated. Spatial aspects of performance were indicated by total length of the hand path (sum of total distance moved) when completing a sequence. A shorter distance moved indicates a straighter hand path between the targets. Temporal aspects of performance were assessed using peak velocity and time to peak velocity; both values were extracted for each reach movement and averaged across each eight-target sequence. A higher peak velocity indicates faster reach speed, and an earlier time to peak velocity suggests heavier reliance on feedforward control (Sainburg & Schaefer, 2004; Schmidt, 1975).

SPSS 22.0 (IBM Corp., Armonk, NY) was used for all statistical analyses (α = 0.05). Data from each sequence type (random and repeated) were combined and averaged into blocks of nine sequences for analysis (Day 1 = 8
blocks of 9 sequences, Day 2 = 4 blocks of 9 sequences). Changes across the eight blocks of Day 1 were assessed to examine motor skill acquisition. A within-subject 2x8 repeated-measures analysis of variance (ANOVA) with factors for sequence type (repeated, random) and block (Day 1 blocks 1-8) was run for response time and each kinematic variable. Retention was examined as the amount of forgetting between the end of Day 1 (block 8) and the start of Day 2 (block 9) with a within-subject 2x2 repeated-measures ANOVA with factors for sequence (random, repeated) and time (block 8, block 9). Post-hoc pairwise comparisons with Bonferroni corrections were used to further assess any significant effects.

3.4 Results

3.4.1 Acquisition

Figure 3.2 shows response time for the random (solid line) and repeated (dashed line) sequences over practice on Day 1. As expected, response time was significantly reduced by the end of task practice, regardless of sequence (main effect of time $F(7, 8) = 12.66, p = 0.001$). Pairwise comparisons indicated that by the second block, participants were already moving significantly faster than the first block (mean difference = 1.89 sec, $p = 0.04$). A subsequent 2x9 repeated-measures ANOVA on the first block only (first nine sequences of each sequence type) was performed to investigate how quickly a significant change in response time occurred. A main effect of time ($F(1,14) = 5.32, p = 0.02$) was evident and revealed that, compared to the first sequence, response time was
significantly faster by the sixth sequence of practice (mean difference = 1.79 sec, 
$p = 0.034$). In addition to changes over time, a difference in response time by 
sequence type was found (main effect of sequence $F_{(1, 14)} = 57.76, p < 0.001$), 
and revealed that the repeated sequence was completed significantly faster than 
the random sequence throughout the acquisition period. When examining the 
first block only, the repeated sequence was completed significantly faster than 
the random sequence by the eighth trial (mean difference at sequence 8 = 1.15 
sec, $p = 0.001$). Performance up to that point (through the first seven trials) was 
similar for both sequences types.

Total distance moved, as determined by the length of the hand path, was 
examined to represent spatial aspects of task performance. Figure 3.3a 
demonstrates that, irrespective of sequence, there was a significant decrease in 
total distance over practice (main effect of time $F_{(7, 8)} = 5.67, p = 0.013$), 
suggesting a straighter, more efficient hand path was used while traveling 
between the targets. Pairwise comparisons indicated that total distance 
significantly decreased as early as block 2 (mean difference = 7.08 cm; $p = 
0.029$). A 2x9 repeated-measures ANOVA was completed on the first block of 
task practice and revealed that, when compared to the first sequence, the 
distance of the hand path was significantly reduced by the seventh sequence 
(main effect of time $F_{(1, 14)} = 4.863, p = 0.025$; mean difference 11.07 cm, $p = 
0.019$). Like response time, total distance of the hand path also differed by 
sequence type (main effect of sequence $F_{(1, 14)} = 44.72, p < 0.001$). The
distance travelled for the repeated sequence type was shorter than the random sequence type.

Neither peak velocity (Figure 3.3b) nor time to peak velocity (Figure 3.3c), both temporal components of performance, differed by sequence type (no main effect of sequence: peak velocity, \( p = 0.72 \); time to peak velocity, \( p = 0.075 \)). Peak velocity did not significantly change during practice (no main effect of time, \( p = 0.368 \)), however time to peak velocity was significantly shortened over practice regardless of sequence type (main effect of time \( F(7,8) = 7.44, p = 0.006 \)), indicating participants adopted more feedforward control as practice progressed. Pairwise comparisons indicated that, when compared to the first block, a significant temporal shift occurred as early as block 2 (mean difference = 0.03 sec, \( p = 0.001 \)). Closer examination of the first practice block revealed that, unlike response time and distance of the hand path, no significant change was evident during the first nine trials (no main effect of time, \( p = 0.184 \)).

### 3.4.2 Retention

Performance, indicated by response time, was maintained on Day 2 (no main effect of time, \( p = 0.386 \)), regardless of sequence. While overall performance was retained for both sequences, the repeated sequence was completed significantly faster than the random sequence at both time points (main effect of sequence \( F(1,14) = 24.999, p < .01 \), mean difference = 0.358 seconds).
Like acquisition, differences in response time between the repeated and random sequences on retention appeared to be driven by differences in the spatial component of task performance. Total distance moved was significantly less for the repeated sequence than for the random sequence (main effect of sequence $F_{(1, 14)} = 17.831, p < .01$) at both the end of Day 1 and the start of Day 2. However, regardless of sequence, total distance was not significantly different at retention (no main effect of time, $p = .301$).

Temporal aspects of performance were also maintained at retention, regardless of sequence (no main effect of time for: peak velocity, $p = 0.491$; time to peak velocity, $p = 0.382$). No differences between the sequences for either temporal component were present (no main effect of sequence for: peak velocity, $p = 0.714$; time to peak velocity, $p = 0.073$).

3.4.3 Explicit Awareness

Five participants stated they recognized some repetition, but none were able to recall the repeated sequence from memory when provided a template of target position. Recognition of the repeated sequence was assessed as a measure of sensitivity and specificity to the explicit awareness tests. Individuals who correctly identified two out of the three positive tests, while correctly rejecting two out of three negative tests were considered to have recognition of the repeated sequence ($n = 6$). A Group X Time repeated measures ANOVA was performed for each sequence type to examine differences in response time across task practice between participants who recognized the sequence and
participants who did not. Results indicated that individuals who recognized the sequence did not improve response time differently than individuals who did not recognize the sequence (no main effect of group: random, $p = 0.655$; repeated, $p = 0.702$). The results suggest that recognizing the repeated sequence did not influence task performance.

3.5 Discussion

This study examined sequence-specific implicit motor learning with a whole-arm 3D reach task. Improvements in performance, indicated by faster response times, were evident regardless of sequence type. However, the repeated sequence was completed faster throughout the acquisition and retention phases, suggesting implicit motor learning of the sequence occurred. Examination of temporal and spatial kinematic variables revealed that the faster response times during the repeated sequence were driven by a shorter, more direct hand path. The current 3D reach task demonstrates sequence-specific implicit motor learning with whole-arm functional movements. Results from studies using this task may inform rehabilitation methods, which often include the practice of functional tasks that require 3D, whole-arm movements.

Results of the current study completed in 3D space are comparable to experiments where a similar 2D task was used to examine implicit motor learning (Boyd & Linsdell, 2009; Brodie, Borich, et al., 2014; Mang et al., 2016; E. Vidoni, Acerra, Dao, Meehan, & Boyd, 2010). Regardless of sequence type, generalized improvements of motor performance were observed during acquisition (Day1)
with changes in performance evident early in practice (within Block 1). The rapid improvement in performance was supported by quick changes in both spatial (distance of the hand path) and temporal (time to peak velocity) kinematic variables. Increased trajectory accuracy, indicated by a shorter hand path, is an integral aspect of movement optimization and sequence learning (Moisello et al., 2009), and signifies greater coordination of muscle activity (Diedrichsen, Shadmehr, & Ivry, 2010). Earlier time to peak velocity indicates increased reliance of feedforward control of movement, which facilitates faster and more accurate movements (Adams, 1971; Sainburg & Schaefer, 2004; Seidler-Dobrin & Stelmach, 1998; Seidler, Noll, & Thiers, 2004). The changes in hand path distance and time to peak velocity occurred in parallel with response time, which suggests that improvements in response time were driven by these kinematic variables.

The rapid decrease in response time early in practice was not unexpected. This is likely supported by three factors: the level of task complexity, visuospatial adaptation to the VE, and redundant sensory feedback. While the motor demands for the current task were greater than 2D tasks, the relative simplicity of the task allowed for large gains in performance to occur after only minutes of practice (Dayan & Cohen, 2011). In addition, while not strictly a motor adaptation task, the need to transfer reach movements from the real-world into the VE necessitates adaptation of the visuospatial aspects of the reach behavior (Levin, Knaut, Magdalon, & Subramanian, 2009) which may have occurred early in practice. Given that the current task provided multimodal sensory feedback and
information about motor accuracy, quick adjustments could be made to meet the
demands of the novel environment (Krakauer, Ghilardi, & Ghez, 1999; Wolpert,
Diedrichsen, & Flanagan, 2011). Further promoting quick improvements in
performance, the current 3D reach task places a higher demand on
proprioceptive feedback compared to 2D laboratory tasks, as the arm is
unsupported and the performers needed to control more degrees of freedom
(Mongeon, Blanchet, & Messier, 2013). Proprioceptive feedback is thought to be
especially important in the execution of sequential movements (E. D. Vidoni &
Boyd, 2008), and therefore may have provided additional feedback that
supported fast motor learning.

Regardless of sequence, the observed improvement in performance across
Day 1 was maintained at retention on Day 2. In addition, none of the measured
kinematic variables were significantly different between the end of Day 1 and the
start of Day 2. The lack of forgetting between days is evidence of motor learning,
rather than a transient change in motor performance (Kantak & Winstein, 2012).
Motor learning is evident in many 2D motor tasks (Boyd & Winstein, 2004; Mang
et al., 2014; Roig et al., 2012), and results from such studies have been used to
support conclusions concerning complex, 3D movements. The current task,
which demonstrates motor learning with whole-arm reach movements, may be
more ecologically valid, and results may be more directly transferable to real-
world settings.

In addition to generalized motor learning, individuals demonstrated sequence-
specific implicit motor learning. Throughout practice (Day 1) and at retention (Day
2), participants completed the repeated sequence faster than the random sequence. The difference in response time between the two sequence types was evident as early as the first block of task practice. Further examination of the kinematic variables identified a shorter hand path as the driver of this difference. It is unclear why sequence-specific differences were only present for hand path distance, a spatial component of performance, and not for either of the temporal components examined (peak velocity, time to peak velocity). Given that task performance was limited by spatial accuracy (cursor required to be within 5 mm of the center of the target), and not by any temporal constraints, participants likely adopted a movement strategy that prioritized spatial aspects of performance. In addition, similar to other implicit motor learning tasks, improved performance of the repeated sequence is likely improved as a spatial relationship between the targets and the reach movement is developed (Willingham et al., 2000). The development if this spatial relationship supports straighter, more efficient movement to the targets.

Previous research that utilized a continuous tracking task to examine implicit motor learning demonstrated that changes in temporal, rather than spatial, components of performance facilitated improved tracking of a repeated sequence compared to randomly presented sequences (Mang et al., 2014). Contrasting results in the current study and this previous work are likely driven by differences in the demands of the task. A continuous tracking task requires the performer to meet both spatial and temporal demands to successfully follow the target. Improvement in either the temporal or spatial domains could enhance task
performance. However, in the serial target task there is no temporal restriction that limits performance. The performer’s ability to navigate 3D space is the major requirement in this task, and therefore changes in the spatial domain are necessary for performance to improve. Continued investigation of both serial discrete motor tasks and continuous motor tasks are necessary as they not only present different behavioral demands, but the underlying neuroanatomical processes associated with each type of task may differ (Doya, 2000; Mang et al., 2016; Vakil, Kahan, Huberman, & Osimani, 2000).

Sequential motor skill learning may require both explicit and implicit processes working in parallel to learn both the sequence of elements which comprise a task, and the sequence of movements required to complete the task (Ghilardi, Moisello, Silvestri, Ghez, & Krakauer, 2009). However, the current task was designed to limit the explicit processes associated with sequential motor skill learning. Participants exhibited faster performance of the repeated sequence compared to the random sequence without explicit awareness, which indicates that implicit processes alone may be enough to facilitate some sequence learning tasks (Willingham, Salidis, & Gabrieli, 2002). Therefore, results of the current study may be especially relevant in clinical populations, such as individuals post-stroke, where implicit processes are often preserved and explicit processes may be limited (Boyd & Weinstein, 2006).

A variety of tasks have been used to examine implicit motor learning, such as sequential button presses (Nissen & Bullemer, 1987), computer based continuous tracking tasks (Boyd & Weinstein, 2001), and 2D serial target tasks
(Mang et al., 2016). However, it is important to understand how implicit motor learning translates to tasks requiring whole-arm, 3D movements. An increased understanding of implicit motor learning may better inform learning, or relearning, of real-world functional tasks. Examination of implicit motor learning is specifically important as it is a fundamental aspect of motor skill learning (Maxwell et al., 2000), and often leads to motor skills that are more durable and less prone to forgetting (Baars, Newman, & Taylor, 1998; Kahneman, 1973). Our finding that implicit motor learning is evident in a whole-arm reach task may better translate to future work in older adults or individuals with clinical diagnoses such as stroke, who often practice functional tasks that require whole-arm movement in rehabilitation.

While the virtual environment allows 3D reach movements that are closer to real-world movements than many previously studied laboratory tasks, the current task was not performed in an actual “real-world” environment. However, reach kinematics have been found to be similar when comparing movements made in a virtual reality system and a real-world setting (Stewart et al., 2013; Viau, Feldman, McFadyen, & Levin, 2004). Furthermore, while the random and repeated sequences were matched for difficulty based on the distance between the targets, the resultant spatial configuration produced by reaching to the targets in a specific order was not controlled for between sequence types. It is possible that the participants implicitly learned the spatial configuration of the targets rather than the sequence of targets. In addition, the current work examined implicit motor learning as a series of discrete movements. A continuous motor
task designed to examine implicit motor learning in a 3D virtual environment may yield differing results (Mang et al., 2016) and warrants future investigation. Results may also differ when examining the non-dominant arm. Previous work examining the scaling of reach movements has demonstrated different control mechanisms for the dominant vs non-dominant arm (Sainburg & Schaefer, 2004). Future work examining 3D reach movements could investigate interlimb differences in implicit learning using a whole-arm reach task.

3.6 Conclusion

Results from the current study indicate that a motor task requiring whole-arm 3D reach movements demonstrates sequence-specific implicit motor learning. Compared to previously researched 2D laboratory tasks, results from the current task may be more applicable to the learning of functional tasks that often require whole-arm movement. Furthermore, the current task enables researchers to examine specific kinematic variables that may be important in understanding how reach movements are learned over time. Future research utilizing this novel task may better inform rehabilitation practice, where similar functional movements are often an important component of motor practice.
Figure 3.1 Experimental setup. a Side view of a participant sitting at the virtual display. Stereoscopic glasses provided a 3-dimensional view of the virtual environment. Virtual objects were sent from the projector, reflected off the mirror, and presented in the area below the glass. b Representation of the nine possible target locations. Each target was 28 mm in diameter. Targets were presented in a circular array with a radius of 96 mm and a tangent distance between any adjacent targets of 75 mm. The repeated sequence consisted of targets 1, 8, 6, 5, 9, 4, 8, 2.
Figure 3.2 Average time (sec) to complete a sequence across acquisition on Day 1 and at retention on Day 2. Each block (1-8 on Day 1 and 9-12 on Day 2) consists of nine sequences. The solid line represents the sequences of randomly presented stimuli and the dashed line represents the repeated sequence. Error bars represent standard error.
Figure 3.3 Distance of the hand path (a), peak velocity (b), and time to peak velocity (c) across acquisition on Day 1 and at retention on Day 2. Each block (1-8 on Day 1 and 9-12 on Day 2) consists of nine sequences. The solid line represents the sequences of randomly presented stimuli and the dashed line represents the repeated sequence. Error bars represent standard error.
Chapter 4

The Effect of Energy-Matched Exercise Intensity on Brain-Derived Neurotrophic Factor and Motor Learning

4.1 Abstract

High-intensity exercise induces an increase in brain-derived neurotrophic factor (BDNF), a neurotrophin that facilitates synaptic plasticity, suggesting that an exercise-induced rise in BDNF prior to practice of a motor task may enhance learning of the practiced motor skill. However, previous work that has compared high and low-intensity exercise has failed to control for overall energy expenditure. Therefore, it is unclear if results were related to the intensity of the exercise or the overall amount of work. The purpose of the current study was to examine the effect of different exercise intensities on BDNF levels and motor learning while controlling for exercise-related energy expenditure. Forty-eight non-disabled participants (23.3 ± 3.2 years) were assigned to one of three groups: high-intensity exercise [High], low-intensity exercise [Low], or quiet rest [Rest]. The duration of the exercise bouts was individually adjusted so that each participant expended 200 kilocalories regardless of exercise intensity. Blood samples were collected immediately before and after each intervention to assess change in BDNF concentration. After exercise or rest, all participants practiced a 3-dimensional motor learning task, which involved reach movements made to sequentially presented targets. Task retention was assessed 24 hours after initial task practice. Saliva DNA samples were obtained from each participant to determine BDNF genotype. All participants equally improved performance, indicated by a reduction in time to complete the task ($p < 0.001$). However, the kinematic profile used to control the reach movement and augment response time differed by group. The Rest group improved by reducing the distance
travelled between the targets, the High group had higher reach speed (peak velocity), and the Low group had earlier peak velocities ($p < 0.001$ for all group differences). The rise in BDNF post-exercise was not significant, regardless of exercise intensity, and the change in BDNF was not associated with motor learning. The BDNF polymorphism did not affect the BDNF response to exercise, however, performance differed between those with the polymorphism (Met carriers) and those without (Val/Val). Compared to the Val/Val genotype, Met carriers had faster response times throughout task practice ($p = 0.002$), which was supported by higher reach speeds ($p < 0.001$). Conversely, Val/Val homozygotes executed the task with a significantly shorter distance travelled between the targets ($p < 0.001$). The effects of the BDNF polymorphism need further investigation. Results indicate that both low and high-intensity exercise can alter the kinematic approach used to complete a reach task, and these changes are not related to a change in BDNF, which suggests other exercise-related neural mechanisms may affect motor behavior.

4.2 Introduction

When an individual learns a novel motor skill, changes in behavior are accompanied by a reorganization of underlying neural circuits (Pascual-Leone et al., 1995). Neuroplasticity is regulated by changes in synaptic efficacy through the process of long term potentiation (LTP) (Duffau, 2006; Kleim et al., 2002; Pascual-Leone, Amedi, Fregni, & Merabet, 2005). Brain-derived neurotrophic factor (BDNF), a protein that influences neuronal growth and function, can modify
synaptic efficacy, which facilitates LTP and promotes plasticity (Bath & Lee, 2006). BDNF plays an important role in both the initiation and maintenance of LTP (Bramham & Messaoudi, 2005). Moreover, LTP is diminished when BDNF is absent (Fritsch et al., 2010), which further identifies BDNF as a mediator of neuroplasticity. Therefore, activities that increase BDNF may facilitate LTP and enhance motor learning.

Recently, a single session of aerobic exercise has been investigated as a potential mechanism to increase BDNF. When compared to a rest group, individuals who completed an acute session of high-intensity interval exercise demonstrated a significant increase in BDNF (Mang, Snow, Campbell, Ross, & Boyd, 2014; Skriver et al., 2014). Importantly, there is also evidence that supports the relationship between high-intensity exercise and enhanced motor learning. Performance of a novel motor skill was better when task practice was preceded by a bout of high-intensity exercise compared to when task practice was preceded by rest (Mang et al., 2014). In addition, retention of motor task performance, assessed a minimum of 24 hours after initial practice, was greater when task practice was paired with high-intensity exercise, compared to a no-exercise control group (Mang, Snow, Wadden, Campbell, & Boyd, 2016; Roig, Skriver, Lundbye-Jensen, Kiens, & Nielsen, 2012; Skriver et al., 2014). Taken together, results from studies that examined the BDNF response to exercise and exercise-enhanced motor learning indicate that high-intensity exercise has the potential to create a favorable environment for neuroplastic change, and this may
be supported by an exercise-dependent increase in BDNF (Hötting & Röder, 2013).

Notably, intensity appears to be a critical factor in the effects of exercise on BDNF. Studies that compared bouts of high and low-intensity exercise observed a significant increase of BDNF levels in the high-intensity exercise condition only (Ferris, Williams, & Shen, 2007; Vega et al., 2006). However, the current literature has failed to consider the importance of overall energy expenditure. When high and low-intensity exercise bouts were compared, the difference in energy expenditure (work) was overlooked, and conclusions were based on differences in intensity only (Etnier et al., 2016; Ferris et al., 2007; Thomas, Beck, et al., 2016; Vega et al., 2006). This can be misleading, especially since modest effects of low-intensity exercise are often evident. For example, a moderate rise of BDNF has been shown after submaximal or low-intensity exercise (Etnier et al., 2016; Ferris et al., 2007; Gustafsson et al., 2009). However, it is unknown whether low-intensity exercise produces a significant BDNF response when the duration of exercise is extended and more energy is expended. Further investigation of energy-matched exercise bouts is needed to determine if intensity or energy expenditure is the critical component of exercise-enhanced motor learning.

In addition, many studies that have investigated exercise-enhanced motor learning have assessed motor skill performance on tasks that involve single-finger button presses or small movements of a joystick (Mang et al., 2014; Roig et al., 2012; Skriver et al., 2014; Thomas, Beck, et al., 2016). Results from these
studies may not relate well to more complex, real-world movements. Examination of exercise-enhanced motor learning where the motor demands of the task more closely resemble the motor demands of everyday movements may yield results that are more applicable to a real-world setting (Baird & Stewart, 2017).

Therefore, the purpose of this study was to determine the effect of a single bout of high and low-intensity exercise on the BDNF response and learning of a 3-dimensional (3D) serial target task, while controlling for overall energy expenditure of the exercise. We hypothesized that individuals in both exercise groups (low and high-intensity) would demonstrate a greater increase in BDNF compared to a no-exercise rest group. We suspected that the change in BDNF concentration would be associated with the change in performance on the motor task, and therefore individuals in the exercise groups would improve more than the rest group. Furthermore, we expected low and high-intensity to effect BDNF and task performance similarly, indicating the importance of energy expenditure over exercise intensity.

4.3 Methods

4.3.1 Participants

Forty-eight healthy, young adults between the ages of 20 and 29 years (23.35 ± 3.2 years) were recruited to participate from the local university community. To participate, individuals had to: 1) be right hand dominant as determined by the Edinburgh Handedness Questionnaire (Oldfield, 1971); 2) have no current or recent neurological symptoms; 3) have no pain in the right
upper extremity; and 4) have no contraindications to strenuous exercise. All participants gave written informed consent prior to enrollment in the study. The Institutional Review Board at the University of South Carolina approved all study procedures, and the study was conducted in accordance with the Declaration of Helsinki. Participants received a total of $30 for their involvement in the study.

4.3.2 Experimental Design
All participants completed three experimental sessions. Experimental session one was a graded exercise test (GXT) on a cycle ergometer to estimate peak aerobic capacity (VO$_{2\text{peak}}$). Results from session one were used to define block randomization of participants into one of three experimental conditions (High-intensity, Low-intensity, or Rest), and in the prescription of the exercise bout completed during session two. Experimental session two, which occurred at least 48 hours after session one, consisted of a bout of exercise or quiet rest followed by practice of a 3D serial target task (STT) (Baird & Stewart, 2017). Twenty-four hours after experimental session two, participants returned to the lab for experimental session three which included additional practice of the STT to assess retention.

4.3.3 Maximal Exercise Test Procedure
All exercise procedures were performed on a cycle ergometer (Monark 828 E; Monark Exercise, Vansbro, Sweden). Participants were asked to refrain from strenuous physical activity for at least 24 hours before session one.
Individuals were instructed to maintain a set pedaling cadence (60 revolutions per minute [rpm]), which was indicated by a metronome. After the participant was comfortably seated on the bike, the test began with a two-minute warm-up at a resistance of 0 kiloponds (kp). Following the warm-up, the resistance of the cycle ergometer was increased to 2 kp. From this point, resistance was incrementally increased 0.5 kp every two-minutes until the individual was not able to maintain the cadence, the individual reached volitional exhaustion, or age-predicted maximal heart rate (220 – age) was met. At the end of each stage, heart rate (Polar Electro, Kempele, Finland) and rating of perceived exertion (RPE) (Borg, 1982) were recorded.

After the GXT, estimated VO$_{2peak}$ was calculated using the following ACSM metabolic equation:

$$VO2_{ml/kg/min} = \left(\frac{1.8 \times work\ rate\ (kp/min/m)}{body\ mass\ (kg)}\right) + 3.5\ ml/kg/min + 3.5\ ml/kg/min$$

Work rate was determined as: resistance at task completion (kp) × 60 rpm × 6 m (representing the distance covered with one full revolution of the wheel). Each participant was classified by fitness level (very poor, poor, fair, good, excellent, or superior) based on their age, sex, and estimated VO$_{2peak}$ results (Brodowicz, 1998). Individuals were then assigned into one of three conditions (high-intensity exercise [High], low-intensity exercise [Low], or quiet rest [Rest]) via block randomization to ensure that fitness level was evenly distributed between the groups. Group characteristics are presented in Table 4.1.
4.3.4 Acute Exercise Intervention

After a minimum of 48-hours after the GXT, participants returned to the lab for experimental session two, which included either rest or a bout of exercise followed by practice of the STT. All participants were asked to refrain from exercise within 24-hours of session two. For the exercise groups, the maximal resistance achieved during the GXT was used to individually determine the exercise prescription for each participant. For the High group, resistance was initially set at 80% of maximal resistance. This resistance was maintained until the individual expended 100 kilocalories (kcals) of energy. The resistance was then decreased 0.5 kp, and cycling continued until another 100 kcals were expended. Individuals in the Low group cycled at a resistance set at 40% of their maximal resistance. This resistance was maintained throughout exercise until each participant expended 200 kcals of energy. The following equation was used to determine how many kcals per minute each individual expended during their respective exercise interventions:

\[
\text{cal/min} = \left( \frac{V_{O2}(ml/kg/min)}{\text{body mass (kg)}} \right) \div 1000 \text{ L/min} \times 5 \text{ kcal/min}
\]

The duration of exercise was based on the predicted length of time it took each participant to expend 200 kcals (Medicine, 2013), thereby keeping each participant’s total energy expenditure constant (high-intensity average duration = 16.75 min, low-intensity average duration = 28.67 min). For both exercise groups, the pedaling cadence was maintained at 60 rpm, and HR and RPE were recorded every two-minutes (see Table 4.2 for exercise characteristics). Individuals in the Rest group were required to sit quietly for 20 minutes. Use of
electronic devices and sleeping were prohibited. For all groups, blood lactate was assessed with a portable lactate analyzer (Lactate Plus; Nova Biomedical, Waltham, MA) immediately before and after their respective interventions.

4.3.5 Serial Target Task

Task Setup and Design: Participants sat facing a virtual display (Innovative Sports Training Inc., Chicago, IL), and the task was projected down into the workspace in front of them (Figure 4.1A). Specialized glasses were worn to provide 3D visualization of the targets. Eight targets, represented as red spheres (28 mm in diameter), were positioned equidistant in a circle (96 mm radius) with an additional target in the center (nine total target placements, Figure 4.1B). An electromagnetic marker was secured to the right index finger. Its position was indicated by a white sphere (25 mm diameter) providing a visual representation of the movement and position data during reaching. One target was presented at a time, and participants were required to move the white sphere (cursor) through 3D space to capture the target. For a target to be considered “hit”, the center of the cursor was required to be within 5 mm of the center of the target for 500 msec. Targets were presented in eight-target sequences under two sequence conditions: repeated and random. The two sequence types provide a distinction between improvements in generalized motor control (random sequences) and changes associated with implicit motor learning (repeated sequences). Users were not made aware of the repeated
sequence, and were instructed to move to all targets as quickly and accurately as possible.

Throughout the task, the position of the electromagnetic marker was sampled at a rate of 120Hz, and data were analyzed with a custom MATLAB script (Mathworks, Inc.; Natick, MA). Response time, the total time to complete an eight-target sequence, was the primary measure of task performance. Other kinematic variables (total distance travelled to complete a sequence, mean peak velocity during a sequence, and mean time to obtain peak velocity) were assessed to define the kinematic profile of the reach, and assess how the kinematics changed over time by group.

**Baseline:** Prior to exercise or rest, all participants completed one trial of the random sequence, which served as a baseline measurement of task performance. **Acquisition:** The task acquisition period immediately followed the second blood draw after exercise or rest. Task procedures were the same for all groups. Sequence presentation alternated between the two sequence types throughout task practice. A total of 144 sequences (72 random alternating with 72 repeated) were completed. **Retention:** All participants returned 24 hours (± 2 hours) after experimental session two for a retention test, where an additional 72 sequences (36 random alternating with 36 repeated) were completed. All other STT procedures were identical to task practice the previous day. **Explicit Awareness Testing:** After completion of the retention period, explicit awareness of the repeated sequence was assessed. All participants completed six explicit awareness tests. The participants viewed three different sequences during each
test. The participants were then asked if they recognized the presence of the repeated sequence. Three of the six tests contained the repeated sequence.

4.3.6 BDNF Sample Collection and Analysis

Immediately before and after exercise or rest, 10 ml of blood was obtained from an antecubital vein into Vacutainer tubes containing EDTA. All samples were centrifuged, and plasma was aliquotted and stored at -80°C for further analysis. Plasma BDNF concentrations were later analyzed in duplicate using a sandwich ELISA kit (PromoCell, Heidelberg, Germany) per the manufacturer’s instructions. A coefficient of variation (CV) was calculated between duplicate samples according to the formula \[
\text{CV} = \left( \frac{\text{SD}}{\text{Mean}} \right) \times 100
\] to assess the relative variability between the two measurements. The average intra-assay CV across all BDNF assays was 8.36%.

Approximately thirty percent of humans possess a single nucleotide polymorphism (SNP) on the BDNF gene (rs6265), which results in a substitution of the amino acid methionine for valine at position 66 (Val/Met) (Shimizu, Hashimoto, & Iyo, 2004). Effects of the polymorphism are largely unknown, but individuals with the polymorphism (Val/Met or Met/Met) have demonstrated altered cortical plasticity (Cheeran et al., 2008) and motor skill learning (McHughen et al., 2010). Studies that have examined the impact of the polymorphism on the BDNF response to exercise have found varying results. Some studies indicate that the BDNF response may be diminished in individuals with the polymorphism (Leech & Hornby, 2017), while others report no effect of
the polymorphism on the BDNF response (Helm et al., 2017). BDNF genotype data were collected in the current study population to further examine the impact of the polymorphism on the BDNF response to exercise, and to potentially inform unexpected results. To determine each participant’s BDNF genotype, 2 ml of saliva was collected with an Oragene Kit (DNA Genotek, Ottawa, Ontario, Canada). Genetic analysis was carried out at AKESOgen genomics lab (Norcross, GA) with a TaqMan genotype assay (c__11592758_10) per manufacturer’s instructions.

4.3.7 Data Analysis

Statistical analyses were completed using SPSS software (SPSS 24.0; IBM Corporation, Armonk, NY). Significance level was set at \( p < 0.05 \) for all statistical tests. Assumptions of normality of the distribution of all STT variables were explored through histograms and assessed with the Shapiro-Wilk’s test for normality. A reciprocal transformation was applied to any non-normal data.

Data from each sequence type (random or repeated) were combined and averaged into blocks of nine sequence trials for analysis (acquisition = eight blocks of nine sequences, retention = four blocks of nine sequences). A Univariate Generalized Linear Model Analysis (GLM) with fixed factors for group (High, Low, Rest), sequence type (Random, Repeated), and time (Blocks 1 – 8), and dependent variable response time, compared the effects of exercise intensity on motor task performance during acquisition. To assess how group kinematic profiles changed over time, similar GLMs with fixed factors for group, sequence
type, and time were conducted for each kinematic variable. Furthermore, the first block of practice (first nine trials) was separately investigated to determine the immediate effect of exercise on task performance. A GLM compared group differences for response time and all kinematic variables at the start of task practice. Data from two individuals were not included in this analysis. One participant in the High group was identified as an outlier (response times on the first three trials were more than three standard deviations from the group mean), and one participant in the Low group had missing data on five of the first nine sequences due to an error during data collection. Retention was defined as the degree of forgetting between the end of the acquisition phase (Block 8) and the beginning of retention phase (Block 9), and was examined with an additional GLM with fixed factors for group, sequence type, and time (Blocks 8–9), and dependent variable response time. Fisher’s least significant difference was used to further investigate any significant differences.

To account for varying BDNF concentrations at baseline, BDNF levels were examined as percent change from pre-intervention (before exercise or rest) to post-intervention (after exercise or rest). The following equation was used to assess percent change:

\[
\text{Percent change} = \left( \frac{\text{BDNF concentration Post} - \text{BDNF concentration Pre}}{\text{BDNF concentration Pre}} \right) \times 100
\]

A one-way ANOVA for BDNF percent change with factor Group (High, Low, Rest) was conducted to determine the effect of exercise intensity on BDNF concentration. To determine if the presence of the Met allele affected the BDNF response to exercise, an independent samples T-test was carried out for BDNF
percent change between individuals with the Met allele (Val/Met or Met/Met) and individuals without the Met allele (Val/Val). We also investigated whether BDNF genotype influenced motor behavior. A GLM with fixed factors for exercise group, time, sequence type, and genotype were conducted for response time and all kinematic variables.

A series of bivariate comparisons (Pearson’s correlations) were conducted to compare the change in BDNF to the change in motor task performance. The percent change in BDNF was compared to the percent change (of response time) from the baseline trial to the first trial of acquisition, from the baseline trial to the last trial of acquisition, and the baseline trial to the first trial of retention.

4.4 Results

4.4.1 Participant Randomization and Exercise Intervention

Fitness level was evenly distributed between the groups (Table 4.1). Groups did not differ by VO_{2peak} \( (F_{(2,45)} = 0.099, p = 0.91) \) or age \( (F_{(2,45)} = 0.647, p = 0.53) \). During the exercise intervention, individuals in the High group achieved a significantly higher exercise HR and RPE compared to the Low group (HR: \( t = 7.40, p < 0.001 \); RPE: \( t = 8.59, p < 0.001 \) [Table 4.2]). Blood lactate concentration increased in the High group, but remained stable in the Low and Rest groups \( (F_{(2,44)} = 46.18, p < 0.001 \) [Table 4.2]). Taken together, the data indicate that a high level of exercise intensity was achieved in the High group, a low level of exercise intensity was maintained in the Low group, and no change in physical activity level was observed in the Rest group.
4.4.2 STT Acquisition

At baseline, prior to rest or exercise, response time did not significantly differ by group ($F_{(2,45)} = 1.78, p = 0.18$). Following the experimental intervention, there was an immediate effect of high-intensity exercise on task performance (Figure 4.2). Individuals in the High group completed sequences significantly faster than those in the Rest group during the first block of task practice ($F_{(2,808)} = 3.179, p = 0.042$, mean difference = 0.93 sec). However, the effect of high-intensity exercise was not maintained throughout acquisition as there were no group differences in performance by the end of the acquisition period ($F_{(2,734)} = 2.287, p = 0.10$; Figure 4.3). All groups significantly reduced response time during the acquisition period ($F_{(7,734)} = 32.158, p < 0.001$). Changes in performance occurred quickly; significantly faster times (across all groups) were evident as early as the second block of practice (mean difference = 2.35 sec). Furthermore, all groups completed the repeated sequence faster than the random sequence throughout acquisition ($F_{(1, 734)} = 6.68, p = 0.01$). There was no interaction between group and sequence type ($F_{(2,734)} = 0.15, p = 0.86$), which indicated that neither high nor low-intensity exercise had a positive impact on implicit motor learning specifically (performance of the repeated sequence).

While overall performance (response time) was not different between the groups, the kinematic profiles of the reach movement differed significantly (Figure 4.4). Individuals in the Rest group completed the sequences with a shorter distance travelled (greater spatial accuracy) than the High (mean
difference = 7.45 cm) and Low (mean difference = 6.9 cm) groups (F(2,734) = 7.99, p < 0.001). In contrast, individuals in the High group reached with higher peak velocities. A significant group effect (F(2,734) = 8.85, p < 0.001) showed that the High group had faster reaches than both the Low (mean difference = 2.55 cm/s) and Rest (mean difference = 3.23 cm/s) groups. Time to peak velocity occurred earlier for the Low group (F(2,734) = 22.78, p < 0.001) compared to the High (mean difference = 0.02 sec) and the Rest (mean difference = 0.02 sec) groups. An earlier time to peak velocity suggests a greater reliance on feedforward control, an important characteristic of sequence-specific motor learning (Sainburg & Schaefer, 2004; Schmidt, 1975).

4.4.3 STT Retention

Retention was assessed as a lack of forgetting between the end of acquisition (Block 8) and the start of retention (Block 9). It is important to demonstrate maintenance of task performance to distinguish between motor learning and a transient change in motor performance (Kantak & Weinstein, 2012). Time to complete a sequence was maintained at retention (F(1,180) = 0.65, p = 0.80), and did not change differently by group from the end of acquisition to the start of retention (F(2,180) = 0.15, p = 0.86). Similar results were found for all kinematic variables. For each kinematic variable, performance was maintained at retention (distance: F(1,180) = 0.05, p = 0.82; peak velocity: F(1,180) = 0.16, p = 0.69; time to peak velocity: F(1,180) = 0.02, p = 0.90), and there were no group by time interactions (distance: F(2,180) = 0.58, p = 0.56; peak velocity: F(2,180) = 0.02, p =
0.98; time to peak velocity: \( F_{(2,180)} = 0.33, \ p = 0.72 \), which indicated that exercise did not affect how performance changed between the end of acquisition and the start of retention.

4.4.4 STT Explicit Awareness

No participant was able to recall the repeated sequence from memory. Recognition of the repeated sequence was assessed as a measure of sensitivity and specificity. Participants who correctly identified two out of three positive tests (when the repeated sequence was present), while also correctly rejecting two out of three negative tests (when the repeated sequence was not present), were considered to have recognition of the repeated sequence. A total of seven participants met these criteria and were deemed to have recognition of the repeated sequence. A subsequent repeated measures ANOVA that compared response time for individuals with recognition and those without revealed there was no significant difference in performance between the groups (\( F_{(1,46)} = 2.587, \ p = 0.12 \)).

4.4.5 BDNF Response

We were unable to obtain blood samples for three participants (two in the Low group and one in the Rest group), and therefore BDNF data was not available for those individuals. Although both exercise groups had a relatively large increase in BDNF concentration compared to the Rest group (Table 4.2), there was no significant difference between the groups (\( F_{(2,42)} = 0.60, \ p = 0.55 \)).
Lack of significance is likely due to high variability within each group. Individuals in the High group had a BDNF response that ranged from -87.04% to 1740.25%. In the Low group, the BDNF response ranged from -84.57% to 986.77%. Similarly, a range of -95.51% to 685.29% was found in the Rest group.

The distribution of BDNF genotype did not deviate from Hardy-Weinberg equilibrium ($X^2 = 1.92, p = 0.17$), as results from genetic testing revealed the Met allele was present in 33% of the study population (Val/Met $n = 16$, Val/Val $n = 32$, no participants had genotype Met/Met; see Table 4.1 for group distribution). There was no difference in BDNF concentration at baseline between the two genotype groups (Val/Val = 419.43 ± 381.06 pg/ml, Val/Met = 334.29 ± 244.05 pg/ml, $t = 0.75, p = 0.46$). To examine the association of the polymorphism on the BDNF response to exercise, the Val/Val and Val/Met participants from both exercise groups (High and Low) were combined (Val/Val = 22, Val/Met = 8). Although a large mean difference was evident in the percent change of BDNF between groups (Val/Val = 203.98% ± 444.94, Val/Met = 15.65% ± 99.83), this difference was not statistically significant ($t = 1.18, p = 0.25$). The BDNF response to exercise was highly variable regardless of genotype or exercise group (Figure 5).

**4.4.6 BDNF and Motor Learning**

No relationship was found between the percent change of BDNF concentration and motor learning, measured as a percent change of response time from baseline (baseline to first trial of acquisition: $r = -0.091, p = 0.56$;
baseline to last trial of acquisition: \( r = -0.198, p = 0.20 \); baseline to first trial of retention: \( r = -0.213, p = 0.17 \).

4.4.7 BDNF Genotype and Motor Learning

When examining the effect of BDNF genotype on motor learning, data for Val/Val individuals and data for Val/Met individuals were combined across all conditions (high-intensity, low-intensity, and rest). BDNF genotype had a significant effect on task performance. Throughout acquisition, individuals with the polymorphism (Val/Met) had faster response times compared to individuals without the polymorphism (Val/Val) (Figure 4.6A; \( F_{(1,746)} = 9.51, p = 0.002 \), Val/Val mean response time = 16.43 ± 3.13 sec, Val/Met mean response time = 15.58 ± 2.21 sec). Group differences remained statistically significant \( (p < 0.001) \) after an additional GLM analysis with adjustments for sex, fitness level, and baseline BDNF level (Val/Val adjusted mean response time = 16.43 ± 1.84 sec, Val/Met adjusted mean response time = 15.42 ± 1.58 sec). The kinematic profile of reach performance also differed by genotype. Val/Val participants had a significantly shorter hand path when reaching to the targets compared to the Val/Met participants (Figure 4.6B; \( F_{(1,746)} = 46.46, p < 0.001 \)). Conversely, Val/Met participants had higher peak velocities compared to Val/Val participants (Figure 4.6C; \( F_{(1,746)} = 69.58, p < 0.001 \)). Time to peak velocity did not differ by genotype (Figure 4.6D; \( F_{(1,746)} = 3.85, p = 0.05 \)). Furthermore, a comparison between genotype groups of the percent change for response time from the start of acquisition to the end of acquisition revealed that the groups improved the
same amount over time for both sequence types (random sequence type: $t = -0.20$, $p = 0.844$; repeated sequence type: $t = -0.67$, $p = 0.504$).

4.5 Discussion

The purpose of the current study was to examine the effects of high and low-intensity acute aerobic exercise on BDNF concentration and motor learning when exercise bouts were matched for overall energy expenditure. To our knowledge, this is the first investigation into the effects of exercise intensity on motor learning where total work was considered. While an acute bout of exercise prior to practice did not lead to overall improvements in STT performance or retention, exercise appeared to have an effect on the kinematic variables that control reaching. Similar to previous work utilizing the 3D STT (Baird & Stewart, 2017), the Rest group increased spatial accuracy to improve performance. Conversely, individuals in the exercise groups altered temporal components of performance to improve response time. The High group completed reaches with higher peak velocities, while the Low group had earlier peak velocities. While exercise did not affect response time, the differences between the kinematic profiles that control reach movements provide insight into the relationship between exercise intensity, energy expenditure, and motor learning.

Our results indicated that an acute bout of exercise, whether at a high or low-intensity, did not enhance motor learning of a 3D sequential target task. This result conflicts with results from other studies that have demonstrated a relationship between high-intensity exercise and improved motor learning (Mang
et al., 2014; Roig et al., 2012; Skriver et al., 2014). It is possible that the effects of exercise on motor learning are task dependent. The previously observed effects of exercise on learning were evident when the examined motor task involved relatively simple movements of either the thumb or wrist (Mang et al., 2014; Roig et al., 2012; Skriver et al., 2014). Exercise may impact motor learning differently when the task involves more complex movement. For example, an exercise-related learning effect was not evident when participants were asked to learn a novel locomotor pattern on a split-belt treadmill (Helm et al., 2017). The lack of effect for tasks that involve relatively more complex movements may be due to an inability to capture changes in motor behavior with practice in the particular task, a difference in the dose-response relationship between exercise and motor learning when the motor task requires a higher degree of movement difficulty, or lack of facilitation of the neuroplastic processes associated with learning of more motorically demanding tasks via acute exercise.

Task dependent differences that dictate how performance is defined may also explain contradicting results between our study and previous work. In the current study, motor learning was indicated by the change of a single STT performance measurement (response time), while the kinematic components of performance (spatial and temporal) were examined to describe how performance changed over time. In comparison, other studies have primarily defined motor learning by a change in either the spatial or temporal components of performance (Mang et al., 2014; Roig et al., 2012; Skriver et al., 2014; Thomas, Johnsen, et al., 2016). Therefore, it is difficult to compare the results of the
current study, which assessed an overall change in performance, to previous studies that have specifically examined the individual kinematic components of performance.

While there were no group differences in STT performance, our examination of the individual kinematic variables that comprise reach performance provided information about how exercise intensity may differentially affect motor behavior. Compared to the Low and High groups, individuals in the Rest group had the shortest hand path distance when completing a sequence (Figure 4.4A). Consistent with previous work from our lab (Baird & Stewart, 2017) the change in hand path distance occurred in parallel with response time, which suggests that, for the Rest group, improvements in response time were supported by this particular kinematic variable. As a spatial relationship is developed between the targets and the reach movement, straighter movements can be made to the targets, which supports a faster response time (Willingham, Wells, Farrell, & Stemwedel, 2000). Together with our previous work, results reveal that augmenting the spatial component of performance is the natural approach to reducing response time on the 3D STT.

Both high and low-intensity exercise appear to differentially modify the kinematic profile that controls reaching, with an overall shift in the temporal components of performance compared to the spatial component. Throughout acquisition and retention, individuals in the High group had higher peak velocities compared to the Rest and Low groups (Figure 4.4B). During a reach movement, velocity of the hand is encoded by the motor cortex, and greater cortical activity
is associated with a higher velocity (Moran & Schwartz, 1999; Wang, Chan, Heldman, & Moran, 2007). The faster velocity observed in the High group may therefore be related to an increase in motor cortical excitability. Studies that utilize non-invasive brain stimulation have shown that an acute bout of exercise leads to an increase in motor cortical excitability (Mang et al., 2014; Singh, Neva, & Staines, 2014). The mechanisms that support a change in motor cortex excitability following exercise are unknown, but a change in cerebral metabolism, an increase in specific neurotransmitters such as norepinephrine and serotonin, and an increase in BDNF are all potentially involved (Singh & Staines, 2015; Smith, Goldsworthy, Garside, Wood, & Ridding, 2014).

Individuals in the Low group had a significantly earlier peak velocities during the reach movement compared to the High and Rest groups (Figure 4.4C). An earlier time to peak velocity indicates increased reliance on feedforward motor control, which is characterized by movements that are less dependent on sensory feedback and instead rely more on preplanning of the action (Adams, 1971; Sainburg & Schaefer, 2004; Seidler-Dobrin & Stelmach, 1998). Brain regions associated with feedforward motor control include the motor cortex, premotor cortex, and basal ganglia (Seidler, Noll, & Thiers, 2004). This network of regions is involved in motor planning and programming of sequential motor patterns (Halsband, Ito, Tanji, & Freund, 1993; Hikosaka, Nakamura, Sakai, & Nakahara, 2002; Houk & Wise, 1995; Mitz, Godschalk, & Wise, 1991). Previous work using non-invasive brain stimulation to characterize changes in neuroplasticity post-exercise have shown a decrease in intra-cortical inhibition in
the motor cortex following low to moderate-intensity exercise (McDonnell, Buckley, Opie, Riddin, & Semmler, 2013; Smith et al., 2014), suggesting a decrease in the inhibitory influences of other brain regions on motor cortex. This disinhibition within the motor network may promote greater network communication between regions responsible for the planning and execution of movement, which may facilitate behavioral changes in the temporal components of sequential motor learning.

With regard to the changes in BDNF, our results highlight four important concepts: 1) baseline levels of BDNF and the BDNF response to exercise are highly variable; 2) there may be an energy expenditure threshold that must be met to induce a BDNF response, and the value of that threshold may vary by person; 3) other mechanisms besides BDNF may be important for exercise-related neuroplasticity; and 4) peripheral measurement of BDNF concentration may not accurately indicate central levels of BDNF. The lack of a significant effect of exercise on BDNF concentration is possibly because of the high variability present at baseline and in response to exercise between the groups. As the presence of the Met allele has been shown to limit the BDNF response to exercise (Leech & Hornby, 2017), we speculated that BDNF genotype may be the source of the observed variability. However, there was no significant difference in BDNF concentration between individuals with and without the Met allele at baseline or in response to exercise. A high amount of variability was also observed for the BDNF response to exercise within each genotype group, which indicates other factors may be influencing the BDNF response. We also did not
find a relationship between the BDNF response and fitness level ($r = -0.02$, $p = 0.90$), body mass index ($r = -0.005$, $p = 0.97$), or the percent change in blood lactate ($r = -0.097$, $p = 0.53$). It is unclear what could be driving the variability observed in the BDNF response to exercise. Further investigation is necessary to determine what factors may be influencing BDNF concentration at baseline and in response to exercise.

Another variable that may limit the BDNF response to exercise is the possibility of an energy expenditure threshold which must be met for a BDNF response to occur. This concept is supported by previous work that demonstrates a modest rise in BDNF following low to moderate-intensity exercise (Etnier et al., 2016; Ferris et al., 2007; Gustafsson et al., 2009). If exercise, even at a low-intensity, meets the energy expenditure threshold, a significant increase in BDNF may occur. Our results support the concept of an energy expenditure threshold, as the rise in BDNF concentration was equivalent between the exercise groups when total work was kept constant. The lack of a universal effect of exercise on the BDNF response in our study population may indicate that the minimum energy expenditure required for an exercise-related effect on BDNF varies by person. Determining if a dose-response relationship exists between energy expenditure and BDNF, and exploring what individualized factors may be influencing this BDNF threshold, is necessary to further the investigation of exercise-enhanced motor learning.

Although response time was not influenced by exercise, differences in kinematic profiles that control reaching were evident between the groups. An
increase in BDNF has been suggested to facilitate the neuroplasticity associated with changes in motor behavior following exercise (Cotman & Berchtold, 2002). However, in the current study, a significant increase in BDNF was not present, and no relationship between the change in BDNF and the change in motor performance exists. Other studies have also shown no association between BDNF levels and learning (Etnier et al., 2016; Helm et al., 2017; Mang et al., 2014). These results suggest that exercise, specifically different exercise intensities, may influence neuroplasticity through alternate mechanisms. Neurotransmitters such as norepinephrine, epinephrine, and dopamine increase after high-intensity exercise (Skriver et al., 2014), but the possible effects of these neurotransmitters on neuroplasticity and motor learning are largely unknown. Furthermore, changes in cortical excitability are evident post-exercise (Mang et al., 2014; McDonnell et al., 2013; Singh, Duncan, Neva, & Staines, 2014; Smith et al., 2014), but the neural mechanisms that support these changes are undetermined. While BDNF should continue to be investigated, there are other potential exercise-related mechanisms that may influence motor learning and these need to be carefully considered.

Another potential source of variability in the BDNF response to exercise is the peripheral measurement of BDNF concentration. Peripheral measurement of systemic BDNF is currently the most feasible way to assess BDNF concentration in humans. However, as an indirect measurement, it possesses an innate level of uncertainty and variability. Animal research has shown that exercise induces an increase of BDNF in the brain (Rasmussen et al., 2009; Vaynman, Ying, &
Gomez-Pinilla, 2004), and that BDNF bidirectionally crosses the blood brain barrier (Pan, Banks, Fasold, Bluth, & Kastin, 1998; Pan & Kastin, 2004). This indicates that the central nervous system is likely the primary source of the systemic rise in BDNF observed with exercise, but whether peripheral measurements provide an accurate indication of BDNF levels in the brain is undetermined. For example, when BDNF concentrations were simultaneously measured from the internal jugular vein and the radial artery during exercise, there was a difference of 84% between the two locations (Rasmussen et al., 2009). These results indicate that peripheral measurements of BDNF may not accurately capture the exercise-related increase in central BDNF, and the potential facilitatory effects of BDNF should not automatically be discounted if a change in peripheral concentration is not found.

We found an immediate effect of high-intensity exercise on motor performance, as individuals in the High group had significantly faster response times compared to the Rest group throughout the first block of task practice (Figure 4.2). It is unclear why there was an initial boost in performance for the High group, and why the advantage was not maintained after the first block. The transient improvement in performance may be related to an initial boost in BDNF following high-intensity exercise (Skriver et al., 2014; Vega et al., 2006). However, given the lack of a significant BDNF response following high-intensity exercise, other mechanisms likely influenced initial performance. One possible mechanism is an exercise-induced increase in arousal, which has been shown to enhance cognitive task performance immediately following high-intensity
exercise (Lambourne & Tomporowski, 2010). Further examination into the immediate effects of high-intensity exercise may provide insight about possible exercise-induced neuroplastic mechanisms that support motor learning, and how to best take advantage of those effects to maximize their influence on performance.

Differences in task performance were found based on the presence of the BDNF polymorphism. When comparing response times between genotype groups, individuals with the Met allele completed the task faster than Val/Val homozygotes. This was an unexpected finding as previous research has either shown no effect of the Met allele on motor learning (Helm et al., 2017; McHughen, Pearson-Fuhrhop, Ngo, & Cramer, 2011), or individuals with the Met allele have demonstrated impaired motor learning compared to those without the Met allele (Fritsch et al., 2010; McHughen et al., 2010). It is important to note that while Val/Met individuals completed the task faster, the overall change in performance was similar between the genotype groups, which indicated group differences in motor performance rather than motor learning. The kinematic profiles that define reach control also differed by genotype. The Val/Val genotype had a significantly shorter hand path than the Val/Met genotype, while the Val/Met genotype had higher peak velocities than the Val/Val genotype. An advantage in the spatial domain of performance for Val/Val individuals has previously been reported by McHughen et al. (2010). However, an important distinction between the current study and the previous work from McHughen and colleagues (2010) is the measurement used to indicate performance. The
advantage in the spatial domain of performance for the Val/Val genotype in the previously indicated study (McHughen et al., 2010) was in fact the same measurement used to determine overall performance. As such, an advantage in the spatial domain of performance for the Val/Val genotype was also determined to be an advantage in overall motor performance. In the current study, aspects of both spatial (distance of the hand path) and temporal (peak velocity and time to peak velocity) components of performance were considered to examine the effect on overall motor performance (response time). It is therefore possible that Val/Val individuals used a spatially driven kinematic approach to control reach performance, while Val/Met individuals used a temporally driven kinematic approach to control reach performance. This dichotomous effect of the BDNF polymorphism on reach control kinematics has not been previously identified because the tasks used to investigate it were unable to distinguish between the spatial and temporal aspects of task performance. Future work is needed to fully understand the effects of the polymorphism on motor performance and learning, and these studies should consider both spatial and temporal aspects of motor tasks.

The between-subjects design of the current study presents an inherent amount of variability that may have been prevented with a within-subjects design. Particularly, the variability that surrounds the baseline levels of BDNF and the BDNF response to exercise may have been limited if all participants completed each of the experimental conditions. However, a within-subjects design would introduce a practice effect on the motor task which would have prevented an
accurate measure of motor learning. The chosen time course for the retention test may have also limited our ability to find an effect of exercise on learning. Other studies have shown that the effects of exercise on motor learning may not be apparent until at least seven days after the initial learning phase (Roig et al., 2012; Skriver et al., 2014; Thomas, Beck, et al., 2016). It is possible that a retention test at a later time point may have shown an exercise-induced effect on learning. Also, an effect of exercise on motor learning may have been masked by a floor effect present in the current task. Participants were able to learn quickly and performance plateaued by the middle of the acquisition phase. Differences in performance between groups may have been evident if the task examined was more difficult and took longer to learn. Furthermore, while we attempted to keep energy expenditure constant between the exercise groups, work levels were estimated rather than directly measured through calorimetry, and thus small differences in energy expenditure may exist. Lastly, results relating to the effects of the BDNF polymorphism on the BDNF response and motor performance should be considered with some caution as the current study was not powered to find differences between the genotype groups, and therefore the sample size is relatively small.

4.6 Conclusion

The current study indicated that a session of acute aerobic exercise at a specific energy expenditure does not influence peripheral BDNF concentration or motor learning. However, exercise at both a high and low-intensity modified the
kinematic approach that controls reach movements and augments motor performance. High-intensity exercise was associated with higher peak velocities of reach movements, and low-intensity exercise facilitated earlier peak velocities. Given the high inter-individual variability of the BDNF response, other mechanisms are suspected to support the underlying neural processes related to the changes in behavior. Further investigation of exercise-enhanced motor learning is necessary to identify other facilitatory mechanisms, and to better understand the role of energy expenditure and exercise intensity.
Table 4.1 *Group Demographics*

<table>
<thead>
<tr>
<th></th>
<th>High</th>
<th>Low</th>
<th>Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.19 ± 2.9</td>
<td>22.81 ± 3.3</td>
<td>24.06 ± 3.4</td>
</tr>
<tr>
<td>Sex</td>
<td>7m/9f</td>
<td>7m/9f</td>
<td>3m/13f</td>
</tr>
<tr>
<td>O$_{2}$peak (ml/kg/min)</td>
<td>40.33 ± 6.2</td>
<td>41.27 ± 4.2</td>
<td>41.12 ± 8.2</td>
</tr>
</tbody>
</table>

**Fitness Level (n)**

<table>
<thead>
<tr>
<th>Level</th>
<th>High</th>
<th>Low</th>
<th>Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Poor</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Poor</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Fair</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Good</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Excellent</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Superior</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

**BDNF Genotype (n)**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>High</th>
<th>Low</th>
<th>Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val/Val</td>
<td>14</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Val/Met</td>
<td>2</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

Values represent group mean ± standard deviation.
Table 4.2 *Exercise Response Characteristics*

<table>
<thead>
<tr>
<th></th>
<th>High</th>
<th>Low</th>
<th>Rest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max Exercise HR</td>
<td>168.14 ± 15.1*</td>
<td>132.27 ± 10.7</td>
<td>-</td>
</tr>
<tr>
<td>Max Exercise RPE</td>
<td>17.25 ± 1.7*</td>
<td>11.19 ± 2.2</td>
<td>-</td>
</tr>
<tr>
<td>Change in Lactate (mmol/l)</td>
<td>5.09 ± 0.6*</td>
<td>0.63 ± 0.3</td>
<td>-0.06 ± .3</td>
</tr>
<tr>
<td>Percent Change BDNF</td>
<td>164.53 ± 465.6</td>
<td>152.76 ± 324.8</td>
<td>37.8 ± 195.7</td>
</tr>
</tbody>
</table>

Values represent group mean ± standard deviation. * indicates \( p < 0.05 \) for difference between groups.
Figure 4.1 Sequential target task (STT) setup. A. Side view of a participant sitting at the virtual display. Stereoscopic glasses provided a 3-dimensional view of the virtual environment. Virtual targets were sent from the projector, reflected off the mirror, and presented in the area below the glass. B. Representation of the nine possible target locations. Each target was 28 mm in diameter. Targets were presented in a circular array with a radius of 96 mm and a tangent distance between any adjacent targets of 75 mm. The repeated sequence consisted of targets 1, 8, 6, 5, 9, 4, 8, 2.
Figure 4.2 Response time (sec) to complete a sequence during the first block of task practice (first nine trials for each sequence type). The High-intensity exercise group completed the sequences, regardless of sequence type, significantly faster than the Rest group ($p = 0.042$). Error bars represent standard error. Error bars ascend from the marker for the random sequences and descend from the marker for the repeated sequences.
Figure 4.3 Response Time. A. Response time (sec) to complete a sequence across the acquisition phase and the retention phase for all groups. Each data point consists of an average of nine sequences. Error bars represent standard error. Error bars ascend from the marker for the random sequences and descend from the marker for the repeated sequences. No group differences in response time were evident. B. Response time for the High-intensity group. C. Response for the Low-intensity group. D. Response time for the Rest group.
Figure 4.4 Kinematic Variables. Distance of the hand path (A), peak velocity (B), and time to peak velocity (C) across the acquisition phase and the retention phase for all groups. Each data point consists of an average of nine sequences. Error bars represent standard error. Error bars ascend from the marker for the random sequences and descend from the marker for the repeated sequences. A. The Rest group travelled the shortest distance when completing a sequence compared to the High and Low groups ($p < 0.001$). B. The High group had the highest peak velocity compared to the Rest and Low groups ($p < 0.001$). C. The Low group had the earliest time to peak velocity compared to the Rest and High groups ($p < 0.001$).
Figure 4.5 BDNF exercise response by BDNF genotype. The Low-intensity group is represented by the gray bars and the High-intensity group is represented by the black bars. Each bar represents an individual participant. The presence of the polymorphism did not affect the BDNF response to exercise.
Figure 4.6 Response time and kinematic variables of performance by BDNF genotype. Response time (A), distance of the hand path (B), peak velocity (C), and time to peak velocity (D) across the acquisition phase and the retention phase for both genotype groups. Error bars represent standard error. Error bars ascend from the marker for the random sequences and descend from the marker for the repeated sequences. A. The Val/Met genotype had significantly lower response times for both sequence types compared to the Val/Val genotype (p = 0.002). B. The Val/Val genotype has a significantly shorter distance when completing a sequence compared to the Val/Met genotype (p < 0.001). C. The Val/Met genotype had higher peak velocities when reaching to the targets compared to the Val/Val genotype (p < 0.001). D. No difference in time to peak velocity was present between the genotypes (p = 0.05).
Chapter 5

Conclusion

A single session of aerobic exercise may be a beneficial adjunct to motor training and rehabilitation. However, more evidence based knowledge is needed to establish the effectiveness of exercise-enhanced motor learning and the neural mechanisms that support such an effect before the concept can be applied in the real-world. Therefore, the purpose of the current research was to expand the knowledge of exercise-enhanced motor learning through two distinct studies. First, we developed a 3-dimensional (3D) serial target task (STT) that involves whole-arm reach movements to sequentially presented targets. This task enabled the investigation of sequence-specific implicit motor learning with movements that have similar motoric demands as movements in the real-world. Second, we used the 3D STT to investigate the effects of energy-matched exercise bouts at different intensities on motor learning. While no effect of exercise was found on overall motor performance, exercise at both a high and low-intensity modified the kinematic approach that controlled reach movements and augmented motor performance over time. Together, the results of these studies help elucidate the principles defining exercise-enhanced motor learning, and expand the current evidence base.

With the development of the 3D STT, we can investigate motor learning with movements that more closely resemble real-world movements.
(Baird & Stewart, 2017). Therefore, results from studies that utilize the 3D STT may be more applicable to the learning of functional tasks that often require whole-arm movements. Furthermore, the STT enables researchers to examine specific kinematic variables that control reaching, which may be important in understanding how reach movements are learned over time. In addition, examination of the kinematic profile that controls reaching enables researchers to understand how specific kinematic variables change compared to others (spatial vs temporal), and how changes in those variables impact overall motor performance. The 3D STT will be a useful tool in future motor learning investigations.

In our second study, we used the 3D STT to examine the effects of energy-matched exercise bouts at high and low-intensities on motor learning. Although no effect of exercise on overall motor performance was found, high and low-intensity exercise differentially affected the kinematic variables that controlled reach performance. Compared to the rest and low-intensity groups, the high-intensity group had higher reach speeds (peak velocity); compared to the rest and high-intensity groups, the low-intensity group had earlier time to peak velocity. Therefore, regardless of intensity, exercise at a specific energy expenditure facilitated a temporally driven approach to improving reach performance within the 3D STT.

The fact that both high and low-intensity exercise had an effect on the control of reach movements indicated that energy-expenditure, not exercise intensity, was the critical component in inducing an exercise-related change in
motor behavior. This is an important finding, as high-intensity exercise was previously thought to be necessary to affect motor behavior (Mang, Snow, Campbell, Ross, & Boyd, 2014; Roig, Skriver, Lundbye-Jensen, Kiens, & Nielsen, 2012; Skriver et al., 2014). In addition, this finding may be particularly relevant to individuals with neurological disorders who may only be capable of achieving low-levels of physical activity.

Brain-derived neurotrophic disorder (BDNF) is often considered the neural mechanism through which exercise facilitates neuroplasticity and motor learning (Cotman & Berchtold, 2002; Cotman, Berchtold, & Christie, 2007). BDNF levels have been shown to significantly increase following high-intensity exercise (Ferris, Williams, & Shen, 2007; Mang et al., 2014), and an increase in BDNF has been associated with enhanced motor learning (Fritsch et al., 2010; Vaynman, Ying, & Gomez-Pinilla, 2004). Our investigation revealed two predominant findings regarding BDNF, exercise, and motor learning. First, the BDNF response to exercise was equivalent between the high and low-intensity exercise groups. This indicates that exercise at a low-intensity can induce a rise in BDNF if the bout of exercise requires a specific amount of energy. Second, the change in BDNF concentration was not associated with the change in motor learning. This finding has several possible implications. First, the BDNF response to exercise appears to be highly variable and future research needs to identify the characteristics of “responders” versus “non-responders”. Additionally, a peripheral measurement of BDNF, while currently the most feasible measurement technique, may not accurately indicate central levels of BDNF.
Lastly, mechanisms other than BDNF may influence exercise-induced neuroplasticity and motor learning.

Our findings also highlight the need for the continued investigation of the effects of the BDNF polymorphism on the BDNF response to exercise and motor learning. We did not find an effect of BDNF genotype on the BDNF response to exercise, which is consistent with some studies (Helm et al., 2017), but contradicts others (Leech & Hornby, 2017). Interestingly, we found an effect of BDNF genotype on motor performance that is in contrast to what has been previously reported (McHughen et al., 2010). Compared to individuals without the polymorphism, individuals with the polymorphism had better task performance, indicated by a shorter response time, throughout task practice. Furthermore, the BDNF genotype groups each used a different kinematic approach to control reach movements. Individuals with the polymorphism had higher reach speeds (peak velocity), while individuals without the polymorphism travelled a shorter distance when reaching to the targets. The effects of the polymorphism on motor performance were unexpected, and the implication of these results is unclear. Future work is needed to fully understand the effects of the polymorphism on motor performance and learning.

In conclusion, results from our first study indicate that a motor task requiring whole-arm 3D reach movements demonstrates sequence-specific implicit motor learning. Use of the 3D STT in future motor learning research may yield results that are more applicable to a real-world setting. Additionally, our second study indicated that a session of acute aerobic exercise at a specific
energy expenditure does not influence BDNF concentration or motor learning. However, exercise at both a high and low-intensity modified the kinematic approach that controls reach movements and augments performance. Other mechanisms than BDNF are suspected to support the underlying neural processes related to the kinematic changes in motor behavior. While this work constructively adds to the evidence based knowledge of exercise-enhanced motor learning, further investigation is necessary to identify other facilitatory mechanisms, and to better understand the role of energy expenditure and exercise intensity.
References


Medicine, A. C. o. S. (2013). *ACSM's guidelines for exercise testing and prescription*: Lippincott Williams & Wilkins.


Appendix A: Edinburgh Handedness Inventory

For each of the activities listed below, please indicate your hand preference by circling the most appropriate response. Some of the activities require the use of both hands. In these cases, the part of the task or object for which hand preference is wanted is indicated in brackets. Also, please indicate whether you ever use the other hand for each activity.

<table>
<thead>
<tr>
<th>Which hand do you prefer when:</th>
<th>Do you ever use the other hand?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Writing: No Preference</td>
<td>Right Yes No</td>
</tr>
<tr>
<td>2. Drawing: No Preference</td>
<td>Right Yes No</td>
</tr>
<tr>
<td>3. Throwing: No Preference</td>
<td>Right Yes No</td>
</tr>
<tr>
<td>4. Using scissors: No Preference</td>
<td>Right Yes No</td>
</tr>
<tr>
<td>5. Using a toothbrush: No Preference</td>
<td>Right Yes No</td>
</tr>
<tr>
<td>6. Using a knife: No Preference</td>
<td>Right Yes No</td>
</tr>
<tr>
<td>(without a fork)</td>
<td></td>
</tr>
<tr>
<td>7. Using a spoon: No Preference</td>
<td>Right Yes No</td>
</tr>
<tr>
<td>8. Using a broom: No Preference</td>
<td>Right Yes No</td>
</tr>
<tr>
<td>(upper hand)</td>
<td></td>
</tr>
<tr>
<td>9. Striking a match: No Preference</td>
<td>Right Yes No</td>
</tr>
<tr>
<td>10. Opening a box: No Preference</td>
<td>Right Yes No</td>
</tr>
</tbody>
</table>
Appendix B: Neurologic Symptom Checklist

Study Subject ID#________

For safety reasons, it is important that you answer all the following questions carefully. Please ask if you have any questions.

<table>
<thead>
<tr>
<th>Check All That Apply</th>
<th>Yes</th>
<th>No</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you experience frequent dizziness or vertigo?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you experience frequent headaches?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you experience tremors?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Are you prone to strange movements or bizarre behavior?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you experience memory loss or problems?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you recently experienced double vision change or loss of vision?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you experience abnormal muscle weakness?</td>
<td></td>
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<tr>
<td>Do you experience burning, tingling or numbness?</td>
<td></td>
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<tr>
<td>Have you noticed any sudden change in your sleep patterns?</td>
<td></td>
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<tr>
<td>Do you experience extreme fatigue or become fatigued easily?</td>
<td></td>
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<tr>
<td>Do you experience staring or twitching spells?</td>
<td></td>
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<tr>
<td>Do you experience difficulty or slowness understanding what others say to you?</td>
<td></td>
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<tr>
<td>Do you experience any unexplained pain in your hands, feet or face?</td>
<td></td>
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</tbody>
</table>
Appendix C: Health History Questionnaire

Assess your health status by marking all true statements

History
You have had:
- A heart attack
- Heart surgery
- Cardiac catheterization
- Coronary angioplasty (PTCA)
- Pacemaker/implantable cardiac defibrillator/rhythm disturbance
- Heart valve disease
- Heart failure
- Heart transplantation
- Congenital heart disease

Symptoms
- You experience chest discomfort with exertion
- You experience unreasonable breathlessness
- You experience dizziness, fainting, or blackouts
- You take heart medications

Other health issues
- You have diabetes
- You have asthma or other lung disease
- You have burning or cramping sensation in your lower legs when walking short distances
- You have musculoskeletal problems that limit your physical activity
- You have concerns about the safety of exercise
- You take prescription medications
- You are pregnant

Cardiovascular risk factors
- You are a man older than 45 years
- You are a woman older than 55 years,
have had a hysterectomy, or are postmenopausal

You smoke, or quit smoking within the previous 6 months

Your blood pressure is >140/90mmHG

You do not know your blood pressure

You take blood pressure medication

Your blood cholesterol level is >200 mg/dL

You do not know your cholesterol level

You have a close blood relative who had a heart attack
or heart surgery before age 55 (father or brother) or
age 65 (mother or sister)

You are physically inactive (i.e., you get <30 minutes
of physical activity on at least 3 days per week)

You are >20 pounds overweight

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None of the above

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