CHANGES IN CORTICOMOTOR PLASTICITY INDUCED BY CARDIOVASCULAR EXERCISE AND STRENGTH TRAINING IN SEDENTARY YOUNG ADULTS

By

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# Table of Contents

Abstract .................................................................................................................................................. 5  
Declaration .................................................................................................................................................. 6  
Acknowledgements ...................................................................................................................................... 7  
List of Abbreviations Used ...................................................................................................................... 8  
Section 1: Literature Review .................................................................................................................. 11  
  1.1: Introduction to Exercise and Neural Plasticity ................................................................................ 12  
  1.2: Inclusion/Exclusion Criteria for Literature Search ...................................................................... 13  
  1.3: Functional Neuroanatomy ............................................................................................................. 15  
    1.3.1: The Basal Ganglia .................................................................................................................. 15  
    1.3.2: The Motor Cortex .................................................................................................................. 16  
    1.3.3: The Cerebellum .................................................................................................................... 18  
    1.3.4: The Corticospinal Tract ....................................................................................................... 19  
    1.3.5: Brain-Derived Neurotrophic Factor ..................................................................................... 21  
  1.4: Neural Plasticity and Exercise ....................................................................................................... 23  
    1.4.1: Mechanisms of Neural Plasticity .......................................................................................... 23  
    1.4.2: Long-Term Potentiation ....................................................................................................... 25  
    1.4.3: Skill Training and Neural Plasticity ....................................................................................... 26  
    1.4.4: Cardiovascular Exercise and Neural Plasticity ................................................................... 27  
    1.4.5: Strength Training and Neural Plasticity ............................................................................... 30  
  1.5: Transcranial Stimulation ................................................................................................................. 33  
    1.5.1: Transcranial Magnetic Stimulation ...................................................................................... 34  
    1.5.2: Motor Evoked Potentials ...................................................................................................... 35  
    1.5.3: Paired Associative Stimulation ............................................................................................. 36  
References .................................................................................................................................................. 41  
Section 2: Manuscript ............................................................................................................................ 49  
  Changes in Corticomotor Plasticity Induced by Cardiovascular Exercise and Strength Training in Sedentary Young Adults .................................................................................................................. 50  
  Abstract .................................................................................................................................................. 51
CHANGES IN CORTICOMOTOR PLASTICITY INDUCED BY CARDIOVASCULAR EXERCISE AND STRENGTH TRAINING IN SEDENTARY YOUNG ADULTS

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Committee Chairperson: Doctor Brenda Gamble

Abstract

Recent studies have further elucidated the properties of cardiovascular and strength training-induced changes in motor cortex (M1) plasticity (i.e. the strengthening and reorganization of neural connections). However, the degree of change in M1 plasticity following the completion of an exercise prescription remains unknown. The purpose of this study was to examine global changes in M1 plasticity of a small hand muscle in sedentary young adults following a 10 week exercise program. Electromyographic recordings were taken from the right abductor pollicis brevis (APB) muscle of 12 young subjects (5 women, 7 men; range 19-23 yrs). Subjects completed a 10 week exercise prescription consisting of two weekly resistance training sessions with a shorter aerobic session at the end as well as a third aerobic session. Exercises were changed to avoid adaptation, and aerobic session workloads were determined by heart rate response. Transcranial magnetic stimulation (TMS) of the left hemisphere was used to assess changes in APB motor-evoked potentials (MEPs) and input-output curve (IO curve). Changes in neural plasticity were induced using paired-associative stimulation (PAS), which consisted of 90 paired stimuli (0.05 Hz for 30 min) of median nerve electrical stimulation at the wrist followed 25 ms later by TMS to the hand area of the left M1. VO₂ increased by 24.9% (P ≤ 0.05; n = 13) and BDNF levels did not significantly change after 10 weeks of exercise (P = 0.623; n = 12). Resting motor threshold (RMT) taken 5 minutes (RMT5) and 30 minutes (RMT30) after PAS did not increase significantly in exercise participants. Control subjects showed a significant decrease in RMT30 MEP amplitude in response to repeating PAS after 10 weeks (P ≤ 0.05; n = 11) and a significant increase in BDNF levels (P ≤ 0.05; n = 8). Post-PAS IO curves in control subjects revealed a significant decrease in excitability of lower threshold motor neurons after 10 weeks. The longitudinal use of PAS appears to yield varying results and our findings indicate that exercise may induce protective benefits against the perhaps diminishing effect of PAS-induced LTP. Findings yielded different excitability levels in RMT5 and RMT30, suggesting that PAS may be used to measure capacity for early-phase and late-phase LTP distinctively in the M1. Future work examining chronic exercise and BDNF levels should aim to control confounding factors shown to influence BDNF production.

Keywords: Neural Plasticity, Motor Cortex, Exercise, BDNF, TMS, PAS
Declarat

I, Hushyar Behbahani, declare that this thesis represents my own work except as acknowledged in the text, and that none of this material has been previously submitted for a degree at the University of Ontario Institute Of Technology, or any other University. The contribution of supervisors and others to this work was consistent with the UOIT regulations and policies. Research for this thesis has been conducted in accordance to UOIT’s Research Ethics Committee.
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List of Abbreviations Used

ACSM: American College of Sports Medicine
AMPA: α-Amino-3-Hydroxy-5-Methyl-4-Isxazolopropionic Acid
ANOVA: Analysis of Variance
APB: Abductor Policis Brevis
BDNF: Brain-Derived Neurotrophic Factor
cAMP: Cyclic Adenosine Monophosphate
CNS: Central Nervous System
CoST: Corticospinal Tract
CREB: Cyclic AMP Response Element-Binding Protein
DA: Dopamine
EDTA: Ethylenediaminetetraacetic acid
EHI: Edinburgh Handedness Inventory
ELISA: Enzyme-Linked Immune-Sorbent Assay
E-LTP: Early-Phase Long-Term Potentiation
EMG: Electromyography
FDI: First Dorsal Interosseous
GABA: Gamma-Aminobutyric Acid
GAP-43: Growth-Associated Protein 43
HR: Heart Rate
H Reflex: Hoffman Reflex
HR Max: Heart Rate Max.
HRR: Heart Rate Reserve
IGF-1: Insulin-Like Growth Factor

IHI: Interhemispheric Inhibition

iM1: Primary Motor Cortex Ipsilateral to Training Limb

INs: Interneurons

IO: Input-Output

IPAQ: International Physical Activity Questionnaire

ISI: Inter-Stimulus Interval

LTD: Long-Term Depression

LTP: Long-Term Potentiation

M1: Primary Motor Cortex

MEP: Motor Evoked Potential

MEP Max: Motor Evoked Potential of Largest Amplitude

MSO: Maximal Stimulator Output

NMDA: N-methyl-D-aspartate

PAR-Q: Physical Activity Readiness Questionnaire

PAS: Paired Associative Stimulation

PAS10: Long-Term Depression Inducing Paired Associative Stimulation Protocol

pCREB: Phosphorylated cAMP Response-Element Binding Protein

PMC: Premotor Cortex

RMT5: Motor Evoked Potential Measured 5 Minutes after Completion of Paired Associative Stimulation Protocol

RMT30: Motor Evoked Potential Measured 30 Minutes after Completion of Paired Associative Stimulation Protocol

RPE: Rated Perceived Exertion

RTh: Rest Threshold
S1: Stimulus 1
S2: Stimulus 2
SM1: Primary Somatosensory Cortex
SMA: Supplementary Motor Area
SICI: Short-Latency Intracortical Inhibition
tDCS: Transcranial Direct Current Stimulation
TMS: Transcranial Magnetic Stimulation
TMC: Transcranial Magnetic Stimulation Checklist
TrkB: Tyrosine Kinase B
VEGF: Vascular Endothelial Growth Factor
VO₂: Maximal Oxygen Consumption
WR: Wheel Running
Section 1: Literature Review
1.1 – Introduction to Exercise and Neural Plasticity

There is a vast body of evidence obtained from both animal models and humans through the use of neurophysiologic and neuroimaging studies which support the paradigm that the central nervous system (CNS) is capable of change and adaptation throughout the duration of one’s lifespan (Cohen et al., 1997; Cramer et al., 2011). This remarkable property of the nervous system has been termed neural plasticity, and can be defined as the ability of the nervous system to respond to intrinsic or extrinsic stimuli through a change in structure, connections and function (Cramer et al., 2011; Fossati, Radtchenko, & Boyer, 2004; Stefan, Kunesch, Cohen, Benecke, & Classen, 2000). This inherent feature of the nervous system can be described in many different levels creating a multitude of different sub-niche topics addressed by various neuroplasticity-specific research. These levels range from molecular to cellular, from systems to behaviour, and can occur during development, in response to one’s environment, in support of learning, in response to disease, and in relation to therapy (Cramer et al., 2011). Along with behavioural and environmental triggers of plasticity, studies have also indicated that elevated levels of stress, clinical depression, and schizophrenia are all associated with a decreased capacity in neural plasticity also (Daskalakis, Christensen, Fitzgerald, & Chen, 2008; Daskalakis, Fitzgerald, & Christensen, 2007; Frantseva et al., 2008). Thus, the paradigm that considered the nervous system as an inflexible structure with little capability for adaptation and modification has become obsolete; in fact, it is evident that the nervous system consists of very dynamic and adaptable tissue structures.

Cardiovascular exercise, a form of physical activity consisting of planned, structured, and repetitive bodily movements has been shown to maintain and/or improve one or more
components of physical fitness (ACSM, 2010). Benefits of regular cardiovascular exercise include improvement in cardiovascular and respiratory function, reduction in coronary artery disease, and decreased morbidity and mortality (ACSM, 2010). With equal importance, musculoskeletal fitness can be improved using various types of strength training exercises including isometric, dynamic, and isokinetic training (Heyward, 2010). Studies have demonstrated that there is a dose-response relationship in the volume of exercise necessary for both health and fitness benefits. The amount of exercise required for the aforementioned benefits to take place can be derived from a combination of frequency, intensity, and duration (time) of the exercises performed (Heyward, 2010). Many epidemiological studies, animals studies, and randomized human clinical interventions have supported the hypothesis that physical activity and exercise serve to protect and also enhance brain and cognitive function (Kramer & Erickson, 2007). While this holds true, the potential effects of an exercise program on the neural plasticity of the corticomotor system is a theme that needs to be further elucidated.

1.2 – Inclusion/Exclusion Criteria for Literature Search

There is a vast and still growing body of literature available which has examined the anatomical organization and physiology of human motor pathways, and helped deepen our understanding of the mechanisms by which changes in plasticity can occur within their functional components. The available literature has examined the effects of exercise on nervous tissue in both animals and humans. Many of these neurocognitive and neural plasticity studies conducted in humans were completed using transcranial magnetic stimulation and paired associative stimulation, the
likes of which are supported by additional research validating the efficacy and reproducibility of these research techniques.

Our inclusion and exclusion criteria were set to include relevant literature that encompassed the aforementioned knowledge gap in order to help us better understand the effects of cardiovascular exercise and strength training on plasticity of the motor pathways, and how this could be measured through transcranial magnetic stimulation, paired associative stimulation, and the collection of serum brain-derived neurotrophic factor. In order to provide an accurate and up-to-date review of pertinent literature the available evidence needed to be extracted, analyzed, and organized into a systematic representation of the current state of knowledge. Various scholarly databases were searched using the following keywords: Neuroplasticity, Exercise, Cardiovascular, Aerobic, Training, Strength, Musculoskeletal, Plasticity, Motor Cortex, Homunculus, Spinal Cord, Corticospinal, Basal Ganglia, Cerebellum, Neurotrophic, Transcranial Magnetic Stimulation, Paired Associative Stimulation, Electromyography, and BDNF. Combinations of these keywords were also searched using the ‘and’ as well as the ‘or’ Boolean operators. Due to the wide scope of the inclusion criteria needed to obtain information regarding all these topics, an exclusion criterion need also be present. Articles must have been written in English for comprehension, and the literature obtained ranged from 1985-2014 in order to capture the introduction and subsequent development of the research techniques of interest. The majority of obtained literature was specific to human participants; however, some animal studies were collected as their findings were both complementary and important in providing a deeper understanding of the underlying mechanisms involved in neural plasticity.
Certain articles obtained were also subject to a hand search in which references that pertained to these studies were collected and analyzed further.

1.3 – Functional Neuroanatomy

The motor pathways arising from the CNS are responsible primarily for controlling movements and involve a mesh of components that enable the organization as well as the execution of voluntary movements (Barker & Barasi, 2008). Within this hierarchy of movement centers, the highest level responsible for translating a desire to move into action includes the basal ganglia, the supplementary motor area (SMA), and the premotor cortex (PMC). Next, the cerebellum has been shown to hold the responsibility of coordinating the planned movement; it does so through comparison of the intended efferent output with the incoming afferents using interneurons (INs) found within the spinal cord. Finally, the supraspinal-derived output descends mainly through the corticospinal/pyramidal tracts conducting through the output neurone of the CNS and ultimately arrives at the effector muscle (Barker & Barasi, 2008).

1.3.1 – The Basal Ganglia

Within the highest level of movement centers we have the basal ganglia which consists of the caudate and putamen (dorsal/neostriatum), the internal and external segments of the globus pallidus, the pars reticulate and pars compacta of the substantia nigra, and the subthalamic nucleus. As an aggregate, these structures serve through critical motivation, motor planning, and procedural learning functions (Graybiel, Aosaki, Flaherty, & Kimura, 1994; Hikosaka, Takikawa, & Kawagoe, 2000; Packard & Knowlton, 2002). Furthermore, neural circuitry
involving the basal ganglia has been shown to form a key part of the extrapyramidal motor system; dysfunction of these circuits are associated with an abundance of neurological disorders including Parkinson’s disease, Huntington’s disease, obsessive-compulsive disorder, and others (Kreitzer & Malenka, 2008). Due to the complexity of this circuitry it is important to conceptualize that the primary input nucleus of the basal ganglia is the striatum as it receives excitatory afferents from the cortex and thalamus as well as innervations from midbrain dopaminergic neurones. In contrast, the primary output center of the basal ganglia originates from the internal globus pallidus and the pars reticulata of the substantia nigra (Barker & Barasi, 2008). These outputs are received by the ventroanterior and ventrolateral nuclei of the thalamus which then project to the PMC, SMA, and the prefrontal cortex. Studies have shown that the striatum represents a major site of synaptic plasticity in the basal ganglia (Bolam, Hanley, Booth, & Bevan, 2000; Gerdeman, Partridge, Lupica, & Lovinger, 2003; Gerfen, 2000).

1.3.2 –The Primary Motor Cortex
The primary motor cortex (M1) is an integral component of the motor system and produces a motor response whilst requiring the most minimal amount of electrical stimulation. It lies anterior to the central sulcus, and its efferent projections connect directly and indirectly with the motoneurones of the spinal cord through the corticospinal extrapyramidal tracts respectively, and with the brainstem through the corticobulbar tracts (Barker & Barasi, 2008). Furthermore, M1 receives afferent input from the SMA, PMC, primary somatosensory cortex (SM1) and the cerebellum through the thalamus (Barker & Barasi, 2008). In its entirety, the M1 controls movement through its innervation of populations of motorneurones and functions to control distal musculature and fine skilled movements (Kandel, 2013). M1’s innervation of the
body is organized in a highly topographical manner as the cortical representation of each body part being controlled is proportional to the degree of motor innervation (Barker & Barasi, 2008).

Figure 1. Areas of the M1 responsible for control of the foot, hand, and mouth are much larger than the components controlling other parts of the body (Kandel, 2013).

Anatomical evidence from developmental and neurophysiological studies suggest that the M1 is organized into columnar units of cortical processing and as with the rest of the neocortex has 6 distinct layers which are numbered from the outermost pia matter to its innermost white matter (Barker & Barasi, 2008; Kandel, 2013). These layers are defined mainly by their density,
presence and/or absence of specific cell types, and each individual columnar unit combines motor output with continuous sensory feedback in a somatotopic arrangement (Kandel, 2013). The most superficial layer termed the molecular layer is occupied by the dendrites of cells in deeper layers of the cortex and by axons of cells travelling through this layer that make connections in other areas of the cortex. Layers II and III are termed the external granular cell layer and the external pyramidal cell layer respectively, and contain pyramidal shaped cells which project to other cortical neurons which facilitates intracortical communication. Layer IV is termed the internal granular cell layer and is the main recipient of sensory input from the joint, muscle spindle, and skin. This layer is also the main site of sensory input from the thalamus and is essentially non-existent in the M1. Layer V is the most prominent layer in the M1 termed the internal pyramidal layer and contains larger pyramidal shaped cells that provide major efferent outputs to other cortical areas and to subcortical structures (Barker & Barasi, 2008). Lastly, layer VI is the polymorphic layer and is a heterogeneous blend of cell types its white matter below, thus forming the deepest component of the cortex carrying axons to and from other areas of the cortex.

1.3.3 – The Cerebellum

The cerebellum consists of the cerebellar cortex derived of gray matter, the innermost white matter, and three pairs of deep nuclei: the fastigal nucleus, the interposed nucleus, and the dentate nucleus (Kandel, 2013). Containing more than one-half of the number of neurons found in the entire brain, the cerebellum receives somatosensory information from the spinal cord as well as from descending corticospinal axons. In doing so, the cerebellum is able to compare movement outputs with incoming somatosensory information and adjust commands for
upcoming movements accordingly (Kandel, 2013). While the cerebellum can influence both posture and movement through its connections to the dorsal aspect of the brain stem through the inferior, middle, and superior cerebellar peduncles, its major effects on movement are through the ventrolateral nuclei of the thalamus (cerebrocerebellum) which connect directly to the motor and premotor cortices (Kandel, 2013).

1.3.4 - The Corticospinal Tract

With 30 – 40% of its neurons originating from layer V of the M1 and the rest from the premotor and supplementary motor cortices, the corticospinal tract (CoST) descends through the subcortical white matter, internal capsule, and cerebellar peduncle before carrying motor commands through the ventral portion of the spinal cord (Kandel, 2013). Roughly 90% of corticospinal fibers decussate as they cross the midline in the medulla resulting in a contralateral innervation of this cortically-derived output. However, the remaining 10% of these fibers do not cross until they reach the level in the spinal cord where they terminate. Many cortico-motor neurons connect monosynaptically to the spinal motoneurones of the CoST providing the precision necessary for individuated finger movement, and originate almost exclusively in the posterior part of the M1 (Kandel, 2013). Corticospinal fibers also form synapses with interneurons in the spinal cord as these help coordinate larger groups of muscles during more complex movements.
Figure 2. CoST connections originating from the M1, decussating at the medulla, and terminating at the ventral horn of the spinal cord (Kandel, 2013).

With great importance, just as the various precentral and parietal cortical motor areas are interconnected through a complex network of converging and diverging connections, several cortical areas project to the spinal cord in parallel with projections from M1 as opposed to a serial pathway with M1 as the final output source (Kandel, 2013). Still, despite neurons from
several cortical motor areas sending their axons into the CoST, the M1 has the most direct access through its monosynaptic projections of cortico-motoneurones (Kandel, 2013).

1.3.5 – Brain-Derived Neurotrophic Factor

As postulated by the neurotrophic factor hypothesis - cells at or near the target of a neurone secrete an essential protein called trophic factors - the uptake of which is essential for the survival of surrounding neurons. Alongside nerve growth factor and neorutrophin-3, brain-derived neurotrophic factor (BDNF) is one of three main neurotrophins that are necessary for neuronal survival and the promotion of their growth and differentiation (Kandel, 2013). BDNF is involved in the neuroprotection, neurogenesis, protection of age-induced neurodegeneration, as well as the promotion of cell survival, neurite outgrowth and synaptic plasticity of structures within the CNS (B. L. Jacobs, van Praag, & Gage, 2000; Kramer & Erickson, 2007; Rojas Vega et al., 2006). Interestingly enough, when delivered to the injured spinal cord, BDNF has shown the ability to promote regenerative growth in axons (Bregman, McAtee, Dai, & Kuhn, 1997) as well as stimulate hind-limb stepping (Jakeman, Wei, Guan, & Stokes, 1998) in animals. These observations along with an abundance of other findings suggest that BDNF plays a key component in changing neuronal excitability and synaptic transmission (Causing et al., 1997; Shen, Figurov, & Lu, 1997) as well as in experience-dependent plasticity (Gomez-Pinilla, Ying, Roy, Molteni, & Edgerton, 2002). Furthermore, studies have indicated that the aforementioned benefits induced by BDNF within the nervous system have occurred preferentially in both motor and sensory neurones of the central and peripheral nervous systems (Kishino et al., 2001; Molteni, Zheng, Ying, Gomez-Pinilla, & Twiss, 2004; Skup, 1994).
Similar to the other neurotrophins, BDNF can interact with two major classes of receptors. The first of these receptors is referred to as p75 and is a member of the tumor necrosis factor receptor family. The activation of p75 promotes neuronal survival through a cascade involving the activation of the NF-B enzyme. However, in the absence of exposure to neurotrophins p75 activation promotes neuronal death through the activation of proteases from the caspase family (Kandel, 2013). In contrast to p75’s ability to bind to all neurotrophins, BDNF binds exclusively to the tyrosine kinase B (TrkB) receptor. Alongside the Tyrosine kinase A and Tyrosine kinase C receptors, the TrkB receptor exists as a dimer, which, when bound to BDNF, leads to the phosphorylation of an intracellular domain of the neuron. After its uptake at the axon terminal, the BDNF & TrkB receptor complex becomes internalized and the resulting signal is relayed to the soma of the neuron. This relay occurs through endocytotic vesicles referred to as signaling endosomes, and leads to the activation of signaling pathways and transcriptional programs in various cellular compartments of the neuron. Ultimately, this binding of BDNF to TrkB receptors promotes the survival of neurons through the phosphatidylinositol-3 kinase pathway, Bcl-2 (antiapoptotic protein) expression (Yuan & Yankner, 2000), and the triggering of neuronal differentiation through the mitogen-activated kinase enzymatic pathways (Kandel, 2013). Literature has shown that Synapsin I, growth-associated protein 43, and cyclic AMP response element-binding protein (CREB) may also play an integral role in the mechanisms by which BDNF affects neural and synaptic plasticity (Gomez-Pinilla et al., 2002). The production of BDNF has also been shown to increase in both exercise and estrogen replacement in humans (Berchtold, Kesslak, Pike, Adlard, & Cotman, 2001).
1.4 – Neural Plasticity and Exercise

The paradigm which once considered the human nervous system as an inflexible structure with minimal capacity for adaptation and modification has become obsolete, and has now been shifted to that of a very dynamic and adaptable group of tissues. Neural plasticity has been identified as a key component in memory and learning induced by various behavioural and environmental triggers including elevated levels of stress, skill acquisition, and trauma to the CNS. The mechanisms underlying cortical plasticity have been shown to be dictated by a variety of neurotrophic factors such as BDNF, to involve the unmasking of existent cortico-cortical connections through the removal of GABAergic inhibitory neurotransmission, to involve N-methyl-D-aspartate (NMDA) receptor mediated neurotransmission, and even alterations in dopamine level (Wang et al., 2000). In relation to physical activity, both cardiovascular exercise and strength training stimulate a variety of these mechanisms and subsequently induce changes in neural plasticity (Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002; Adkins, Boychuk, Remple, & Kleim, 2006). These changes have shown to involve various regions of the CNS such as the basal ganglia, cerebellum, red nucleus, and M1.

1.4.1 – Mechanisms of Neural Plasticity

A growing body of evidence has revealed that the adult sensorimotor cortex is capable of reorganizing itself in response to various environmental changes including limb amputation, changes in limb position, nerve stimulation, and focal lesions of the sensorimotor cortex (Brasil-Neto et al., 1992; Donoghue, Suner, & Sanes, 1990; Jenkins, Merzenich, Ochs, Allard, & Guic-Robles, 1990; Nudo, Milliken, Jenkins, & Merzenich, 1996; Sanes, Wang, & Donoghue, 1992). M1 has also shown to reorganize itself in the process of skill acquisition (Le Bihan & Karni, 1995;
Nudo et al., 1996; Pascual-Leone, Grafman, & Hallett, 1994; Pascual-Leone et al., 1995) and repetition of simple movements (Classen, Liepert, Wise, Hallett, & Cohen, 1998). The spinal cord has also demonstrated the capability for adaptive plasticity after traumatic injury (Aagaard, 2003; Bawa, 2002; Cramer, Lastra, Lacourse, & Cohen, 2005; Rosenzweig et al., 2010; Topka, Cohen, Cole, & Hallett, 1991) and following exercise (Aagaard et al., 2002; Adkins et al., 2006; Carroll, Herbert, Munn, Lee, & Gandevia, 2006; Hortobagyi, 2005). Thus, the accumulation of evidence reveals that neural plasticity occurs not only within supraspinal components of the motor pathways but can also take place within their spinal components. It is important to consider however that despite the positive implications of the many forms of adaptive plasticity in the CNS, both maladaptive plasticity during early development as well as plasticity in general can lead to negative consequences. This has been well documented in the induction of seizures caused by traumatic injury and a subsequently maladaptive plasticity (Prince et al., 2009), and in the pathogenesis of disorders such as schizophrenia and depression (Sullivan & Pfefferbaum, 2005). Neural plasticity presents itself at a cellular level through axonal sprouting, synaptic remodeling, modification of dendritic growth and synaptogenesis (Mesulam, 1999). Changes in postsynaptic cell excitability and extensions of specialized areas secondary to neurological disease or learning represent some of the most common results of brain plasticity (Fossati et al., 2004). In addition, neural plasticity has been shown to be mediated by several neurobiological mechanisms such as the unmasking of existent corticocortical connections through the removal of cortical inhibitory Gamma-Aminobutyric acid (GABA) neurotransmission (Daskalakis et al., 2008). NMDA receptor-mediated neurotransmission also plays an integral component in neural plasticity. Lastly, evidence has
shown that striatal plasticity is highly dependent on dopamine (DA) neurotransmission, as DA appears to regulate glutamate release and the function of NMDA and \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on the cell membrane of medium spiny neuron cells (Daskalakis et al., 2008).

1.4.2 – Long-Term Potentiation

Long-term potentiation (LTP) is an important mechanism of learning, memory, and many other forms of experience-dependent plasticity in the mammalian brain (Malenka & Bear, 2004). As postulated by Hebb (1949), this persistent change in synaptic strength due to the coincidental activation of coactive cells can manifest itself as an increase in strength through long-term potentiation (LTP) or a decrease through long-term depression (LTD). Thus, the increase in synaptic efficacy occurring in LTP only occurs in response to time-based correlations between pre-and-post-synaptic activity (Frantseva et al., 2008). A subtype of LTP referred to as associative LTP can also occur through the coincidental activation of presynaptic inputs derived from separate origins that converge onto one common postsynaptic target (Levy & Steward, 1983). This associative LTP provides the conceptual framework to explain how the strength of neural networks with converging inputs such as those found in corticocortical or thalamocortical circuitry can be altered by neural plasticity.

LTP depends partially on the activation of NMDA receptors that serve as a coincidence detector. Upon the detection of the coincidental activation of pre-and-post-synaptic neurons, these receptors are able to provide a long-term increase in post-synaptic signal and relieve tonic \( \text{Mg}^{2+} \) inhibition (Bliss & Collingridge, 1993; Rison & Stanton, 1995). In support of this, a
study which involved the administration of Lorazepam, an NMDA disruptor, was shown to disrupt LTP in humans (Butefisch et al., 2000).

1.4.3 – Skill Training and Neural Plasticity

A large body of evidence has demonstrated that motor training can induce neural plasticity within several motor areas including the basal ganglia, cerebellum, red nucleus and M1 (Adkins et al., 2006; Conner, Culberson, Packowski, Chiba, & Tuszyński, 2003; De Zeeuw & Yeo, 2005; Graybiel, 2005; Hermer-Vazquez et al., 2004; Kelley, Andrzejewski, Baldwin, Hernandez, & Pratt, 2003; Kleim, Barbay, & Nudo, 1998; Kleim, Vij, Ballard, & Greenough, 1997). These acquired motor changes have been shown to endure in the absence of continued training demonstrating that there are mechanisms of persistent encoding that can occur within the CNS (Adkins et al., 2006). Skill training, which has been defined as the acquisition and subsequent refinement of novel combinations of movement sequences, has been proposed to induce changes in the connectivity between the neural assemblies that control discrete movement between joints through alterations in synaptic strength, cortical synapse number, and the topography of stimulation-evoked movement representations (Adkins et al., 2006). Studies have shown that the more rapid modulation of motor cortical outputs due to skill acquisition is likely caused by the unmasking of existing but latent cortico-cortical connections through the removal of GABA inhibitory neurotransmission (K. M. Jacobs & Donoghue, 1991) as well as an increase in synaptic efficacy (Pascual-Leone et al., 1999). Animal studies have deduced that the training of a novel skill produces alterations in the neural circuitry of M1 specific to the muscle groups necessary for the execution of the trained task (Adkins et al., 2006; Kleim et al., 1998; Nudo et al., 1996); this however does not occur following simple repetitive use of those same muscle groups.
Furthermore, skilled reach training in rats has shown to increase the complexity and density of forelimb motor cortical dendritic processes (Bury & Jones, 2002; Greenough, Larson, & Withers, 1985; Luke, Allred, & Jones, 2004; Withers & Greenough, 1989) and synapses per neuron (Kleim, Barbay, et al., 2002; Kleim et al., 2004). The observed increase in synapse number was shown to be consistent with enhanced postsynaptic potentials within the hemisphere contralateral to the trained paw in the skill-trained rats (Monfils & Teskey, 2004; Rioult-Pedotti, Friedman, Hess, & Donoghue, 1998). In the context of skill acquisition as a form of activity-dependent neural plasticity, it has been shown that the intensity, specificity, difficulty, and complexity of practice appear to be important parameters for driving neural plasticity and its lasting effect on both brain and behavior (Petzinger et al., 2010).

Bridging the gap between animal studies and evidence obtained directly from humans, TMS and other neuroimaging techniques have been used to demonstrate similar changes in the M1 (Adkins et al., 2006; Classen et al., 1998; Hund-Georgiadis & von Cramon, 1999; Le Bihan & Karni, 1995; Pascual-Leone et al., 1995).

1.4.4 - Cardiovascular Exercise and Neural Plasticity

When evaluating the effectiveness of cardiovascular exercise on the improvement of human health, it is incumbent to understand the recommendations which have been established for the mode, intensity, frequency, duration, and rate of progression of the exercise program; this is to ensure that participants benefit fully from the exercise that they conduct. The American College of Sports Medicine (ACSM) suggests that the mode of rhythmical aerobic activities be
maintained continuously, and involve larger muscle groups and demand little skill to perform (ACSM, 2010). Furthermore, the intensity should be dictated by, and vary depending on the client/patient’s cardiorespiratory fitness classification; moderate-intensity (3.0 – 6.0 METs or 40 – 60% VO₂R), vigorous-intensity (>6.0 METs or >60% VO₂R) or a combination of moderate-and-vigorous-intensity exercise are suggested. Moderate-intensity exercise should be conducted a minimum of 5 days/week, vigorous-intensity exercise should be conducted a minimum of 3 days/week, or a combination of both these intensities can be conducted 3 – 5 days/week. Each exercise session should consist of 20 – 60 minutes of continuous or intermittent activity depending on the exercise intensity prescribed. Finally, ACSM suggests that the exercise prescription for each client should be adjusted in accordance with the conditioning effect, participant characteristics, new exercise test results, or performance during the exercise sessions. The rate of progression should depend on the individual’s age, functional capacity, health status, and goals. Thus, the aerobic exercise prescription should consist of three stages: initial conditioning, improvement, and maintenance (ACSM, 2010).

Endurance training, which refers to exercises that increase the capacity for continued cardiovascular output, has shown to induce its own specific anatomical and neurophysiological changes across the M1 as well as within the spinal cord (Aagaard, 2003; Adkins et al., 2006; Bawa, 2002; Lagerquist, Zehr, & Docherty, 2006). Additionally, endurance training has been shown to increase both the mRNA and protein levels of BDNF in the M1 of rats (Klintsova, Dickson, Yoshida, & Greenough, 2004). Examining the duration of the exercise-induced elevation in BDNF levels it was demonstrated by Oliff et al., (1998) that within the hippocampus BDNF mRNA expression was rapidly influenced by physical activity as soon as 6 hours after
voluntary wheel running (WR) in rats. Repeated WR in rats has been shown to increase the concentration of BDNF not only within the hippocampus, but also in the cerebellum, cortex and the lumbar spinal cord (Rojas Vega et al., 2006). While these changes were sustained after 10 days without exercise in rats, a separate study revealed that serum levels of BDNF in humans were shown to rapidly decline back to baseline only 30 minutes after moderate exercise (Cotman & Engesser-Cesar, 2002; Gold et al., 2003). Another study indicated that a more intense motorized running intervention (60 minutes) of running induced a rapid yet more shortly-lived spike in BDNF levels in rats 2 week after focal ischemia as opposed to voluntary (12 hours) (Ploughman et al., 2007). Furthermore, both forms of exercise not only increased BDNF levels within select neural tissues, but also increased levels of phosphorylated cAMP response-element binding protein (pCREB), insulin-like growth factor (IGF-1) and synapsin-1, each of which has been implicated in neuroplastic processes underlying recovery from ischemia. In addition to synapsin-1, growth-associated protein 43 (GAP-43), and CREB levels were shown to increase in select regions of the brain (Neeper, Gomez-Pinilla, Choi, & Cotman, 1995) as well as the spinal cord (Gomez-Pinilla, Ying, Opazo, Roy, & Edgerton, 2001) in rats who were exposed to voluntary WR for 3 or 7 days. All three of these molecules may play central roles in the mechanisms by which BDNF affects neuronal and synaptic plasticity. Thus, the BDNF response induced by exercise in both rats and humans has been shown to depend on the mode of exercise undergone.

Cardiovascular exercise has been shown to induce increased levels of IGF-1, as well as increased levels of vascular endothelial growth factor (VEGF) in the hippocampus, cerebellum, frontal cortex and M1 (Ding et al., 2004; Klintsova et al., 2004; Kramer & Erickson, 2007; Vaynman &
Gomez-Pinilla, 2005). These molecules are crucial for both exercise-induced angiogenesis and neurogenesis, and promote blood vessel growth across targeted areas through the formation of new capillaries. In accordance with this, the primary effect of endurance training in M1 along with the health-protective and anti-neurodegenerative benefits induced in other supra-spinal regions has shown to be on cerebrovasculature (Adkins et al., 2006). Endurance exercise has proven to induce angiogenesis and increase blood flow in the specific areas of the M1 activated by training in rats that underwent WR for 30 days; however, it did not alter motor map topography (Kleim, Cooper, & VandenBerg, 2002). Further examining the effects of endurance exercise on M1 plasticity it is well known that different exercise types produce experience-specific alterations in cortical maps of movement. However, these changes in M1 function have usually only been examined in the limbs that were involved in the exercise, hence the term activity-dependent neuroplasticity (Adkins et al., 2006). However, TMS studies using paired associative stimulation have deduced that regular physical activity primarily involving lower limb musculature induces a global increase in M1 plasticity (Cirillo, Lavender, Ridding, & Semmler, 2009) similar to the aggregate of neuroprotective benefits that are obtained from exercise in general.

1.4.5 – Strength Training and Neural Plasticity

It is well established that muscular fitness can be improved using various forms of strength training – dynamic (concentric and eccentric), isometric (static), as well as isokinetic (Heyward, 2010). Similar to the principles of cardiovascular exercise prescription, there is a dose-response relationship for the volume (frequency, intensity, and duration) of exercise necessary for desired health & fitness benefits (ACSM, 2010).
Similar, yet different from skill and endurance training through its own distinct forms of anatomical and neurophysiological changes across the M1 and spinal cord, strength training can be defined as training that results in an increase in force capacity (Adkins et al., 2006). While it is apparent that there are changes within skeletal musculature during the development of strength in response to high-intensity strength training (Adkins et al., 2006; D. G. Sale, 1988), the degree to which additional motor units become recruited in response to training must not be overlooked. For example, significant gains in strength have been shown to occur before the occurrence of muscle hypertrophy (Akima et al., 1999; Hickson, Hidaka, Foster, Falduto, & Chatterton, 1994; Jones & Rutherford, 1987; Komi, 1986), and significant loss of strength from either disuse (Duchateau & Hainaut, 1987) or detraining (Hakkinen, Alen, & Komi, 1985; Narici, Roi, Landoni, Minetti, & Cerretelli, 1989) have been shown to precede muscle atrophy. Thus, the aggregate of neural adaptations within the CNS that are induced by strength training also contribute, along with increased skeletal musculature, to the production of a greater net force.

Anatomical and functional adaptations induced by strength training have shown specificity towards the exercised musculature; however, increases in muscle strength on one task do not necessarily transfer over to other tasks that use the same muscle (Enoka, 1997; Higbie, Cureton, Warren, & Prior, 1996; Kraemer, Fleck, & Evans, 1996). A study was conducted by Remple et al., which trained rats on a reaching task with different behavioral demands. The initial task required the acquisition of a skilled reach movement to target and retrieve a food reward while the second task was identical to the first but required an additional increase in forelimb strength to obtain the same reward. Despite the second task requiring an increase in elbow, shoulder, wrist, and digit strength, rats trained on both tasks only showed expansion of wrist
and digit representations (Remple, Bruneau, VandenBerg, Goertzen, & Kleim, 2001). Thus, while skill training has been shown to induce reorganization of movement representations in M1, strength training did not appear to evoke the same change in M1 topography.

Studies have investigated the effects of strength training on cross-education of non-exercised homologous muscles of contralateral limbs where both supraspinal and spinal factors have been proposed to contribute to this neural adaptation (Hortobagyi, 2005; Moritani & deVries, 1979; Zhou, 2000). Recent meta-analyses have shown that on average an 8% increase in strength of the untrained limb is observed following unilateral strength training (Carroll et al., 2006; Munn, Herbert, & Gandevia, 2004). Further studies have demonstrated that unilateral strength training leads to adaptive changes in M1 ipsilateral to the training limb (iMI) in the form of increased corticospinal excitability, reduced short-latency intracortical inhibition (SICI), reduced interhemispheric inhibition (IHI) and increases in voluntary activation (Hortobagyi & Maffiuletti, 2011; Kidgell, Stokes, & Pearce, 2011; Lee, Gandevia, & Carroll, 2009). Additionally, TMS & several imaging studies have shown increased activation of the iMI and increased corticospinal excitability of the contralateral resting limb during unilateral voluntary isometric contractions (Cramer, Finklestein, Schaechter, Bush, & Rosen, 1999; Hortobagyi, Taylor, Petersen, Russell, & Gandevia, 2003; Urbano et al., 1998). In accordance with these findings, a study conducted by Aagaard et al. revealed that the soleus spinal Hoffman reflex (H reflex), a measure of spinal excitability, was facilitated by roughly 20% after 14 weeks of strength training (Aagaard et al., 2002). However, a more recent study measuring the effects of a 5 week unilateral isometric strength-training program through the H reflex in lower limbs revealed that the cross-education effect induced by strength training may be due to a greater amount of
supraspinal as opposed to spinal mechanisms (Lagerquist et al., 2006). Lastly, a recent study conducted by Goodwill et al. 2012 involving 3 weeks of unilateral strength training in right leg dominant participants revealed a 35% increase in strength of their untrained left leg, and a significant increase in the motor-evoked potential amplitude obtained from their left leg. Furthermore, SICI of the iM1 decreased by 21% in the untrained leg which provided evidence of corticomotor adaptation induced by unilateral leg strength training within the iM1 due to modulatory changes in intrahemispheric inhibition (Goodwill, Pearce, & Kidgell, 2012).

1.5 – Transcranial Stimulation

Transcranial magnetic stimulation (TMS) is now established as an investigative tool in the field of cognitive neurosciences. Introduced in 1985 by Barker and associates, it proved to be a non-invasive and painless method of stimulating both brain tissue and peripheral nerves (Barker, Jalinous, & Freeston, 1985). This research technique provided the means to deepen our understanding of motor conduction in human development, human motor control, movement disorders, swallowing, vision, attention, memory, speech and language, epilepsy, depression, stroke, pain, as well as neural plasticity. Prior to its introduction, the first major advancement in this soon-to-be research technique occurred when Merton and Morton discovered that a high voltage electrical pulse applied to the head could sufficiently penetrate the skull and excite the motor cortex (Merton & Morton, 1980). This method however was noted as quite painful (Amassian & Maccabee, 2006; Hallett, 2000). Thus, when TMS was first introduced it was praised due to its absence of pain when compared to its predecessor.
1.5.1 – Transcranial Magnetic Stimulation

TMS operates through Faraday’s principle of electromagnetic induction, and operates using a stimulating coil that produces a magnetic field perpendicular to its underside. This can be used to stimulate the M1 both safely and non-invasively. The design of TMS proved effective as the skull provides little impedance to the passage of the evoked magnetic field; thus, it passes readily through the skull and into the cortical structures of the brain (Barker et al., 1985; Hallett, 2000; Siebner & Rothwell, 2003). The desired effect was to stimulate neural tissue and change the excitation and organisations of neuronal firings in the target region of stimulation (Hallett, 2000). Initially, all TMS coils consisted of a single round coil of wire which would be centered on the head, and thus would generate a magnetic field that would penetrate the skull.

In today’s practice however, the most commonly used TMS coil is the figure-of-eight coil which allows researchers to target focal regions of cortical tissue with approximately twice as strong a current as its single-coiled predecessor (Amassian & Maccabee, 2006).

TMS over the M1 has been used in a plethora of studies to evoke motor evoked potentials in the fingers, hand, arm, face, trunk and legs in a pattern that matches the organisation of the motor homunculus. Thus, studies have demonstrated the specificity in TMS as the positioning of the coil on the scalp at locations spaced between 0.5 and 1 cm apart was sufficient in selectively activating these different muscles (Barker et al., 1985). Furthermore, TMS techniques have been further validated through as well as used in conjunction with electroencephalography, functional magnetic resonance imaging and other neuroimaging techniques (Hallett, 2000). TMS has also proven to be an effective measure of plasticity within the human motor cortex through the creation of virtual lesions and subsequent cortical
reorganization, and in the measurement of changes in excitability of cortico-cortical and cortico-subcortical connections (Siebner & Rothwell, 2003). The majority of these studies have been performed on the motor cortex in human models following skill acquisition, stroke, and immobilization.

1.5.2 – Motor Evoked Potentials

When the current induced by TMS provides sufficient and appropriate amplitude, duration, and direction, it will induce the depolarization of cortical neurons and subsequently generate action potentials that travel down the CoST (Siebner & Rothwell, 2003). This can be measured as a motor evoked potential (MEP) in the contralateral hand as it is fact that in a normal motor system each cerebral hemisphere controls movements of the contralateral effector muscles. These MEPs represent the net facilitatory effect of TMS stimuli, and provide an easily quantifiable measurement of cortical & non-cortical neuronal properties (Siebner & Rothwell, 2003). It is important to note however that due to the physiological variance in neuroanatomy and excitability levels of nervous tissue amongst individuals, the amount of electrical current needed exogenously from said TMS coil has shown to vary from person to person, as well as from one brain region to the next (George et al., 2003; Stewart, Battelli, Walsh, & Cowey, 1999).
Figure 3. Depiction of focal TMS applied over the left M1 producing stimulus artifact (1). This MEP will conduct down the CoST and reach the target effector muscle and be recorded through the use of surface electromyography (2).

1.5.3 – Paired Associative Stimulation

As previously alluded too, Hebb (1949) was largely concerned with neural assemblies and the phenomenon of neural plasticity. Through the use of TMS researchers have been able to measure various parameters in the motor cortex and evaluate different aspects of cortical excitability. Taking such measures has proven useful in understanding the changes in brain physiology that occur during changes in cortical plasticity as well as in brain disorders (Hallet, 2000). Somewhat recently, a research protocol was established based on Hebb’s law of coincidental summation. Hebb postulated that ‘When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased’ (Hebb, 1949). Established upon the principle where a presynaptic neuron is depolarized at the same time as its connecting postsynaptic neuron is the fairly recent research
protocol termed paired associative stimulation (PAS) (Stefan, Kunesch, Benecke, Cohen, & Classen, 2002; Stefan et al., 2000; Wolters et al., 2003).

During this protocol, a subthreshold stimulus delivered as a peripheral nerve volley is timed to arrive at the postsynaptic cell before a second suprathreshold stimulus (induced by TMS) (Di Lazzaro et al., 2009; Mrachacz-Kersting, Fong, Murphy, & Sinkjaer, 2007; Stefan et al., 2002; Stefan et al., 2000). If the TMS pulse is applied at an interstimulus interval (ISI) slightly longer than the time needed for the afferent input (peripheral nerve stimulation) to reach the cortex, and if a sufficient number of pairs of stimuli are delivered, then the excitability of the sensorimotor cortex increases (Di Lazzaro et al., 2009; Ridding & Uy, 2003; Stefan et al., 2000) (McKay, Brooker, Giacomin, Ridding, & Miles, 2002; Ridding & Taylor, 2001). This induced plasticity has shown to be rapidly evolving (30 minutes to show changes), long-lasting (>60 minutes in duration), reversible, timing-dependent, and topographically specific in its changes (Stefan et al., 2002). However, it was also observed that if the ISI is shorter than the time needed for the afferent inputs to reach the cerebral cortex (10ms) then the excitability of the sensorimotor cortex would decrease (PAS10) (Wolters et al., 2003). Elaborating on the aforementioned dichotomy of changes in plasticity induced by different ISI’s, it has been noted that in order for PAS to be effective in producing LTP the interval between the preceding conditioning stimulus and the following test stimulus must not exceed 40-60 ms (Baranyi & Feher, 1981). Thus, exact synchronicity of this pair of events is not necessary as plasticity was also induced when pairing stimuli at 35 ms ISI as opposed to 25 ms ISI (Stefan et al., 2000). Furthermore, Mrachacz-Kersting et al. observed that at a set ISI across a cohort of subjects PAS was more effective when it was applied to an active muscle as opposed to one at rest.
(Mrachacz-Kersting et al., 2007). Lastly, Godde et al. observed that there may be an importance to presenting stimuli with a randomised timing between successive pairs as failure to do so could result in habituation and adaptive effects (Godde, Spengler, & Dinse, 1996).

The mechanism underlying this change in plasticity has been proposed to be similar to that of LTP which has been observed in the hippocampus of animal preparations (Bi & Poo, 1998). Pharmacological studies have shown that the effects of PAS were influenced by drugs that acted upon the NMDA receptor, supporting the hypothesis that the after effects of PAS involve LTP-like changes in cortical synapses (Stefan et al., 2002). Experiments have aimed to locate the level of the CNS in which the changes in plasticity took place. By measuring F-waves and electrical brainstem stimulation in a resting muscle of the hand, both of which are sensitive to spinal excitability but not supraspinal changes, Stefan et al. were able to deduce that PAS most likely induced plasticity changes that were cortical in nature (Stefan et al., 2000). It has also been thought that the organisational changes induced during this LTP are similar in nature to those seen during motor learning (Ridding & Uy, 2003).

The PAS protocol has been implemented to alter the excitability of the cortical projections to a variety of hand muscles in various plasticity studies (Kujirai, Kujirai, Sinkjaer, & Rothwell, 2006; Ridding & Flavel, 2006; Stefan et al., 2002; Stefan et al., 2000; Stefan et al., 2006; Weise et al., 2006) as well as the tibialis anterior muscle of the lower limb (Prior & Stinear, 2006; Stinear & Hornby, 2005). A study conducted by Ridding et al. revealed that PAS causes reorganisations within the sensorimotor cortex which manifested itself as an increase in excitability of the corticospinal projection to the stimulated muscles (Ridding et al., 2000) and an increase in the
cortical representation of the stimulated muscles (McKay et al., 2002a; Ridding et al., 2001). These studies demonstrate the dynamic nature of the motor cortex’s organisation, as well as the large role that afferent input has in modulating this organisation (Ridding & Uy, 2003).

Studies have determined details about optimal ways of performing PAS as well as the variability that may be observed amongst different subjects. Ridding & Uy (2003) determined that in the Abductor Pollicis Brevis (APB) and first dorsal interosseous (FDI) muscles the greatest MEP facilitation was induced 1 hour following the PAS intervention. This time proponent was in accordance with a previously observed finding by Fraser et al. in 2002 where an increase in excitability induced on the pharynx peaked at 60 – 90 minutes following the period of afferent stimulation. While the aforementioned findings applied to median nerve afferent stimulation, there seems to be different time values for ulnar nerve stimulation. Ridding & Uy thus observed that following a 2 hour period of ulnar nerve stimulation, cortical excitability to ulnar nerve-innervated muscles peaked at 15 minutes post-intervention (Ridding & Uy, 2003). Thus, the nerve stimulated appears to influence the peak time for increased excitability. A study conducted by Sale, Ridding & Nordstrom sought to determine if there were any differences in excitability induced by a short PAS protocol involving 132 paired stimuli at 0.2 Hz vs. a long PAS protocol involving 90 paired stimuli at 0.05 Hz. The short PAS protocol produced greater APB MEP facilitation (51%) than the long protocol (11%) (M. V. Sale, Ridding, & Nordstrom, 2007). The same study sought to determine if there were differences in the PAS induced MEP facilitation in the morning, afternoon or in the evening, and there proved to be a significantly higher facilitation induced in the afternoon. Thus, studies involving PAS should be conducted at a fixed time of the day (M. V. Sale et al., 2007). The same study alluded to various internal
variability factors that should be considered such as attention, hormonal fluctuations & other anatomical or physiological variables that may influence a participant’s response to TMS as there was variability amongst subjects in the level of MEP facilitation that was induced by PAS. Finally, a recent study revealed that the magnitude of MEP increase induced by PAS in young and middle-aged individuals was much more than that found in the elderly (>60 years old), re-enforcing that the capacity for changes in plasticity are correlated with age (Fathi et al., 2010).
References


Section 2: Manuscript
Changes in Corticomotor Plasticity Induced by Cardiovascular Exercise and Strength Training in Sedentary Young Adults

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Abstract

Recent studies have further elucidated the properties of cardiovascular and strength training-induced changes in motor cortex (M1) plasticity (i.e. the strengthening and reorganization of neural connections). However, the degree of change in M1 plasticity following the completion of an exercise prescription remains unknown. The purpose of this study was to examine global changes in M1 plasticity of a small hand muscle in sedentary young adults following a 10 week exercise program. Electromyographic recordings were taken from the right abductor pollicis brevis (APB) muscle of 12 young subjects (5 women, 7 men; range 19-23 yrs). Subjects completed a 10 week exercise prescription consisting of two weekly resistance training sessions with a shorter aerobic session at the end as well as a third aerobic session. Exercises were changed to avoid adaptation, and aerobic session workloads were determined by heart rate response. Transcranial magnetic stimulation (TMS) of the left hemisphere was used to assess changes in APB motor-evoked potentials (MEPs) and input-output curve (IO curve). Changes in neural plasticity were induced using paired-associative stimulation (PAS), which consisted of 90 paired stimuli (0.05 Hz for 30 min) of median nerve electrical stimulation at the wrist followed 25 ms later by TMS to the hand area of the left M1. VO$_2$ increased by 24.9% ($P \leq 0.05; n = 13$) and BDNF levels did not significantly change after 10 weeks of exercise ($P = 0.623; n = 12$). Resting motor threshold (RMT) taken 5 minutes (RMT5) and 30 minutes (RMT30) after PAS did not increase significantly in exercise participants. Control subjects showed a significant decrease in RMT30 MEP amplitude in response to repeating PAS after 10 weeks ($P \leq 0.05; n = 11$) and a significant increase in BDNF levels ($P \leq 0.05; n = 8$). Post-PAS IO curves in control subjects revealed a significant decrease in excitability of lower threshold motor neurons after
10 weeks. The longitudinal use of PAS appears to yield varying results and our findings indicate that exercise may induce protective benefits against the perhaps diminishing effect of PAS-induced LTP. Findings yielded different excitability levels in RMT5 and RMT30, suggesting that PAS may be used to measure capacity for early-phase and late-phase LTP distinctively in the M1. Future work examining chronic exercise and BDNF levels should aim to control confounding factors shown to influence BDNF production.
Introduction

There is a vast body of evidence derived from animal models and humans through the use of both neurophysiologic and neuroimaging techniques supporting the paradigm that the central nervous system (CNS) is capable of change and adaptation throughout the duration of one’s lifespan (Cohen et al., 1997; Cramer et al., 2011). This remarkable property has been termed neural plasticity, and can be defined as the inherent ability of the nervous system to respond to intrinsic or extrinsic stimuli through a change in structure, connections and function (Cramer et al., 2011; Fossati et al., 2004; Stefan et al., 2000). Responsible for neuronal survival and the promotion of neurite growth and differentiation, brain-derived neurotrophic factor (BDNF) is involved in the neuroprotection, neurogenesis, protection of age-induced neurodegeneration, as well as the promotion of cell survival, neurite outgrowth and synaptic plasticity of structures within the CNS (B. L. Jacobs et al., 2000; Kramer & Erickson, 2007; Rojas Vega et al., 2006). Studies have elucidated BDNF’s fundamental role in changing neuronal excitability and synaptic transmission (Causing et al., 1997; Shen et al., 1997) as well as in experience-dependent plasticity (Gomez-Pinilla et al., 2002). Synapsin I, growth-associated protein 43, and cyclic AMP response element-binding protein (CREB) have also been shown to play an integral role in the mechanisms by which BDNF promotes neural and synaptic plasticity (Gomez-Pinilla et al., 2002). BDNF has shown to regulate plasticity most significantly in both motor and sensory neurones of the central and peripheral nervous systems (Kishino et al., 2001; Molteni et al., 2004; Skup, 1994). In particular, the motor cortex (M1) has shown the capability of adapting in response to various environmental changes including limb amputation (Brasil-Neto et al., 1992), changes in limb position (Sanes et al., 1992), nerve stimulation (Jenkins et al., 1990), focal
lesions of the sensorimotor cortex (Donoghue et al., 1990) and skill acquisition (Nudo et al., 1996; Pascual-Leone et al., 1994; Pascual-Leone et al., 1995). Plasticity in M1 has shown to be dictated by several neurobiological mechanisms including the unmasking of existent corticocortical connections through the removal of cortical inhibitory Gamma-Aminobutyric acid (GABA) neurotransmission (Daskalakis et al., 2008), and the involvement of a variety of neurotrophic factors such as BDNF.

Long-term potentiation (LTP) has shown to be one of the most important underlying mechanisms of learning and other forms of experience-dependent plasticity in M1 (Malenka & Bear, 2004). Partially dependent on N-methyl-D-aspartate (NMDA) receptor-mediated activity, LTP responds to time-based correlations between pre-and-post-synaptic activity thus leading to a long-term increase in post-synaptic signal and efficacy through the relieving of tonic Mg$^{2+}$ inhibition (Bliss & Collingridge, 1993; Rison & Stanton, 1995). Through the use of transcranial magnetic stimulation (TMS), studies have been able to non-invasively induce motor-evoked potentials (MEP) in a variety of distal muscles and in doing so measure changes in excitability of cortico-cortical and cortico-subcortical connections (Siebner & Rothwell, 2003). Established upon the same time-based parameters of LTP is the TMS-based protocol of paired associative stimulation (PAS). During this protocol, a subthreshold stimulus delivered as a peripheral nerve volley is timed to arrive at the postsynaptic cell before a second suprathreshold stimulus induced by TMS (Di Lazzaro et al., 2009; Mrachacz-Kersting et al., 2007; Stefan et al., 2002; Stefan et al., 2000). If the TMS pulse is applied at an interstimulus interval (ISI) slightly longer than the time needed for the afferent input to reach the cortex and a sufficient number of pairs of stimuli are delivered, then the excitability of the sensorimotor cortex has shown to increase
(Di Lazzaro et al., 2009; McKay et al., 2002; Ridding & Taylor, 2001; Ridding & Uy, 2003; Stefan et al., 2000). This induced plasticity has shown to be rapidly evolving (30 minutes to show changes), long-lasting (>60 minutes in duration), reversible, timing-dependent, and topographically specific in its changes (Stefan et al., 2002). In support of the hypothesis that the after effects of PAS involve LTP-like changes studies have deduced that its effects are most likely cortical in nature (Stefan et al., 2000), NMDA receptor-dependent (Stefan et al., 2002), and similar in mechanism to motor learning (Ridding & Uy, 2003).

Randomized human clinical interventions, epidemiological and prospective observational studies, and animal studies have demonstrated that exercise enhances both brain function and cognition, and protects against the development of neurodegenerative disease (Kramer & Erickson, 2007). Similar to this aggregate of benefits induced by exercise, more recent information has emerged linking frequent exercise to a global increase in M1 plasticity (Cirillo et al., 2009). Although the mechanisms by which plasticity occurring in M1 have been elucidated to great detail, much less is known about the effects of exercise on its structure and function. Furthermore, the amount of exercise necessary to increase BDNF levels in humans remains unknown. While studies have demonstrated varying exercise prescriptions leading to an increase in serum BDNF levels (Erickson et al., 2011; Seifert et al., 2010; Zoladz et al., 2008), studies with similar parameters of exercise yielded non-significant changes also (Goekint et al., 2008; Levinger et al., 2008; Schiffer, Schulte, Hollmann, Bloch, & Struder, 2009; Yarrow, White, McCoy, & Borst, 2010). Thus, the question of how much exercise is necessary to induce an increase in baseline levels of circulating BDNF and M1 plasticity requires further elucidation.
The purpose of this study was to examine circulating BDNF levels and M1 plasticity in a cohort of sedentary young adults at baseline and after a 10 week cardiovascular and strength-training prescription. Our aim was to prescribe an exercise regime that adhered to previously established recommendations in mode, intensity, frequency, duration, and rate of progression of exercise in order to meet a baseline level of physical activity that any sedentary individual could partake in; we chose the recommendations set forth by the American College of Sports Medicine (ACSM). We hypothesize that through the completion of 10 week exercise prescription sedentary young adults will exhibit an increase in circulating BDNF levels and global M1 plasticity in the motor cortical projection to a small hand muscle.
Methodology

Neurophysiological measurements of corticospinal excitability taken through TMS, serum BDNF levels, and maximal oxygen consumption (VO$_2$) were measured prior to the start of the 10 week exercise prescription and immediately following its completion. Using TMS, motor-evoked potentials (MEP) were recorded from the relaxed right abductor pollicis brevis (APB) muscle of participants. Each TMS session involved measures of excitability followed by plasticity evoked using the PAS protocol through the synchronized pairing of TMS with suprathreshold percutaneous stimulation of the right median nerve. This was followed by repeating pre-PAS measures of excitability in order to determine the capacity for LTP-like plasticity of the M1. Control group underwent the same TMS and BDNF testing as the exercise group and were excluded from the exercise prescription and asked to refrain from exercise throughout the 10 week period to maintain their sedentary status. All our participants were matched closely for age as MEP increases induced by PAS in young and middle-aged individuals was found to be much greater than the elderly (Fathi et al., 2010), and both RMT5 and RMT30 measurements were taken at exactly the same time between all subjects as the induction of plasticity through PAS has been shown to be temporal in its synaptic potentiation (Ridding & Uy, 2003). Furthermore, we attempted to undergo experimentation during the afternoon in both pre-and-post sessions as the time of day has been shown to influence PAS-induced plasticity also (M. V. Sale et al., 2007). Subjects in both our exercise and control groups were well matched for gender, handedness, and all participants were deemed healthy and free of neurological disease as part of the screening process. All participants were deemed sedentary as evaluated by the International Physical Activity Questionnaire (IPAQ).
Participant Eligibility

Experiments were performed on 12 participants (mean age, 21.3; range, 19-24; 7 men and 5 women). Average weight of participants was 71.7 kg; range 54.5-97.7 kg. Average height was 67.5”; range 65-73”. 10 of the 12 participants were right-handed as assessed by the Edinburgh Handedness Inventory whilst 2 were ambidextrous, and no participants had any history of neurological disease. Participants were screened for contraindications to TMS including the consumption of neuroactive medications, metal fragments or implants in the upper body or head, epilepsy, heart disease, severe headaches, skull fracture or serious head injury, and pregnancy. Participants also were screened using the Physical Activity Readiness Questionnaire (PAR-Q) to ensure adequate health status and absence of contraindications to exercise prior to VO$_2$ testing. An additional 11 healthy participants were recruited as a control group (mean age, 19.81; range, 18-22; 5 men and 6 women). Eligibility for this group included the same criteria as our exercise group and participants were not allowed to partake in a volume of cardiovascular exercise or strength training that would cause them to no longer be considered sedentary as according to IPAQ. This study was approved by the University Of Ontario Institute Of Technology Research Ethics Board and conducted in accordance with regulations set by the Declaration of Helsinki.

Exercise Regime

Our exercise group underwent a 10 week exercise prescription consisting of both cardiovascular exercise and strength training. The mode, intensity, frequency, duration, and rate of progression of our exercise prescription adhered to the criteria set by ACSM. All exercise sessions were supervised by a certified personal trainer. Strength training took place twice a
week and consisted of a 9 exercise circuit providing a full-body workout per session. Each exercise was performed with 12 repetitions using a weight that evoked sufficient muscular fatigue as opposed to failure. Participants were given 30 seconds to rest in between each exercise and upon completion of the circuit were given 2-3 minutes to rest before repeating the circuit a second time. To prevent plateau and ensure proper progression all participants completed the circuit twice between weeks 1 and 3 and three times during weeks 4 and 10. Weights were increased in response to adaptation; however, the number of repetitions did not decrease to facilitate heavier weights as we wanted to maintain a high heart rate (HR) throughout. Weeks 1-3 consisted primarily of machine exercises, weeks 4-6 consisted primarily of free weights (barbells and dumbbells) and weeks 7-10 introduced body weight exercises i.e. push-ups and pull-ups for even greater difficulty. Despite the varying exercises over the 10 weeks, the same muscle groups were targeted by the 9 exercise circuit per session in order to ensure a full body prescription.

Cardiovascular exercise was performed three times a week and consisted of two short aerobic sessions following strength training sessions and one longer aerobic-only session. The duration of shorter aerobic sessions was 20 minutes at week 0 and progressed to 30 minutes by week 10. Radial pulse was taken frequently during exercise to ensure that the minimum intensity of 60% heart rate reserve (HRR) was met and that the maximum intensity of 80% heart rate max. (HRmax) was not exceeded. Some participants preferred using the seated cycle, however all participants were using the treadmill by week 3. The longer aerobic session maintained the same minimum intensity of 60% HRR and maximum of 80% HRmax. The initial duration was 30 minutes at week 0 and increased to 60 minutes by week 10.
**Electromyographic Recordings**

Surface electromyography (EMG) was recorded from the abductor pollicis brevis (APB) muscle of the right hand using Ag-AgCl electrodes placed 2 cm apart in a belly-tendon arrangement. An electrode impedance meter was used to assess the quality of our electrode setup (Grass Medical, Quincy Mass, USA). Experimentation did not begin unless the impedance fell under 50 kilohms. The ground electrode was placed around the wrist of the right arm between the stimulating coil and the surface electrodes. EMG signals were amplified (x 1000), filtered (13Hz-1000 Hz) with a Cambridge Electronic Design 1902 isolated amplifier (Cambridge Electronic Design, Cambridge, UK), digitized online (1 kHz/channel) via a CED 1401 interface, and stored on a laboratory computer for offline analysis. Data were analyzed using SIGNAL© software version 4.08 (Cambridge Electronic Design). Participants were seated in a padded chair with a pillow on their lap and their right hand rested on this pillow, palm up, and relaxed throughout the entire experiment. Participants were asked to maintain an upright posture throughout the experiment with a pillow placed behind their lower back for lumbar support. Background EMG activity was assessed visually prior to experimentation and subsequent EMG activity was monitored to ensure that the muscle was at rest throughout experimentation.

**VO₂ Max Testing**

Submaximal VO₂ was estimated using the YMCA cycle ergometer test. Participants were instructed to pedal at a rate of 50 rpm with an initial workload of 25W and HR was taken via radial pulse every minute. Dependent on the 3rd HR reading taken from this initial workload subsequent workloads were set according to the pre-determined progression of the YMCA test protocol. The test was terminated once the steady-state HR of participants fell within 10 beats
of 85% of their HRmax or if they felt discomfort or too high of difficulty as determined according to the rated perceived exertion (RPE) scale.

**BDNF Acquisition**

Peripheral venous blood was drawn mid-day in the UOIT laboratory by a medical lab technician. Collection times and other information that could affect biomarker levels such as illness or infection were logged by the technician. A total of 5 mL of blood was collected from each participant by venipuncture into ethylenediaminetetraacetic acid (EDTA) tubes. Blood samples were prepared within 30 minutes by centrifugation (3000g for 5 minutes), and the fibrinogen containing plasma supernatant was aliquot into thirty 0.5 mL labeled tubes and stored at -85°C until assayed.

Plasma BDNF was quantified using enzyme-linked immune-sorbent assay (ELISA) according to the manufacturer’s protocol (R&D Systems, Minneapolis, MN, USA). The ELISA plates were read at a wavelength of 450 nm using a Synergy HTTR microplate reader (BioTek, Winooski, VT, USA). All assays were performed in triplicate and the average was recorded.

**Transcranial Magnetic Stimulation**

Cortical stimulation was performed using a custom figure-of-8 coil (outer diameter, 10 cm) and was applied over the M1 on the left hemisphere of the brain responsible for evoking a MEP in the relaxed right APB muscle. The optimal location of coil placement was marked with a felt-tip pen onto a cap that the participant was asked to wear throughout the entire procedure and the positioning of the cap and coil was continually checked throughout the experiment to ensure there was no displacement. Magnetic stimulation was given via the use of 2 Magstim
200 stimulator units (Magstim Co, Whitland, Dyfed, UK) connected together with a BiStim unit. The coil was held with its handle pointed backwards and rotated approximately 45° away from the midsagittal line, with the current flowing posteriorly for optimal stimulation.

**Paired Associative Stimulation**

PAS was performed as previously described by Stefan *et al.* (2000) consisting of percutaneous electrical stimulation of the median nerve at the right wrist (300% of perceptual threshold) followed by TMS (100% resting motor threshold) 25 ms later over the left M1. The intervention consisted of 90 paired stimuli that were non-randomized in timing between successive pairs and delivered at 0.05 Hz with the entire procedure lasting 30 minutes. Electrical stimuli were applied to the median nerve at the wrist using a constant current stimulator (DS7A stimulator, Digitimer Ltd, Welwyn Garden City, UK) with bipolar surface electrodes separated by 20 mm, and with the cathode proximal. Stimuli were square wave pulses with a pulse width of 200 µs. Subjects were asked to focus on their right hand and keep it relaxed throughout the procedure and count the peripheral stimuli they perceived during the PAS intervention as this has been shown to be an important factor influencing the effectiveness of PAS (Stefan *et al.* 2004).

**Experimental Parameters**

Measures of corticospinal excitability using TMS included resting motor threshold (RMT) and input-output (IO) curves. RMT was determined as the minimum stimulus intensity required to produce a MEP in the relaxed APB of approximately ≤1 mV in 3 out of 5 consecutive trials and was determined before PAS. Using this stimulator intensity, 10 trials were recorded to
investigate resting MEP amplitude before PAS, 5 minutes after PAS (RMT5), and 30 minutes after PAS (RMT30). PAS has shown to be most effective during the afternoon as observed by M. V. Sale et al., 2007. To account for this variance in PAS efficacy, experiments were conducted around mid-day at similar times for each participant between week 0 and 10.

The intensities used to construct the IO curves were determined for each participant according to their RMT before PAS. Ten trials at 90, 100, 110, 120, 130 and 140% of RMT were recorded for each subject at rest. The order of presentation of the six intensities was randomized throughout the trials and stimuli were given every 5 seconds. The IO curve was recorded before PAS as well as 15 minutes after PAS in all participants. The mean amplitudes were then calculated for each TMS intensity.

**Statistical Analysis**

Once data was acquired the peak-to-peak amplitude for each sweep was measured offline and averaged using Signal© (Cambridge Electronic Design) and input into Microsoft Excel (Redmond, WA). A paired t-test was used to analyse changes in estimated VO\textsubscript{2} max after 10 weeks. Repeated-measures analysis of variance (ANOVA) was used to evaluate the effects of exercise on RMT5 and RMT30 values after 10 weeks. Two-way repeated measures ANOVA was used to detect between group significance in RMT, RMT5 and RMT30 values between exercise and control groups as well as within group significance after 10 weeks. IO curve values were normalized relative to the MEP of highest amplitude (MEP Max.) obtained from each participant’s IO curve during each session, and points between 110 and 140% RMT were used in the Boltzmann Sigmoidal Function to determine the slope of IO curves as this portion has
shown to represent the linear segment of the sigmoidal recruitment curve (Devanne, Lavoie, & Capaday, 1997). IO curves were then tested for difference in slope using a two-way repeated measures ANOVA examining between group significance as well as within group significance after 10 weeks. The purpose of this analysis was twofold and consisted of the comparison of two sets of data. First, analysis was performed to detect changes in excitability through the comparison of IO curve slope taken pre-PAS before and after 10 weeks and between groups. Second, this was also performed to detect changes in plasticity through the comparison of IO curve slope taken post-PAS before and after 10 weeks of exercise and between groups. All data was reported as mean ± standard error.

RESULTS

A total of 12 out of 13 subjects from the exercise group completed TMS measurements and reported no side effects or discomfort; one participant was excluded due to contraindications however all 13 subjects from the exercise group successfully completed submaximal VO\(_2\) testing. A total of 12 subjects were recruited for TMS as part of the control group but only 11 completed the 10 week pre-and-post measurements as one participant dropped out. Thus, results obtained reflect the aggregate of 12 exercise and 11 control group participants.

Weight and VO\(_2\) Max

Both weight and VO\(_2\) max measurements taken at weeks 0 and 10 were analyzed through a paired sample t-test for significance. Exercise group had a mean weight of 71.67 ± 3.92 kg at week 0 and 67.46 ± 3.63 kg at week 10 indicating a mean decrease in weight of 0.66 ± 0.66 kg after 10 weeks of exercise (P = 0.34; n = 13). Weight loss was observed in 7 participants, weight
gain in 5, and no change in 1. Exercise group had a mean VO₂ max of 17.84 ± 1.80 at week 0 and 26.0 ± 3.0 at week 10 indicating a mean increase in maximal oxygen consumption of 24.9 ± 6.99% as depicted in Fig. 1 (P ≤ 0.05; n = 13). Increase in VO₂ max was observed in 10 participants, no change was observed in 1, and decrease in 2.

![INCREASE IN ESTIMATED VO₂ MAX AFTER 10 WEEKS OF EXERCISE](image)

**Figure 1.** Estimated VO₂ max measured using the YMCA submaximal cycle ergometer test.

**BDNF**

Fig. 2 depicts serum BDNF levels from our exercise group measuring at 899.31 ± 92.80 pg at week 0 and 868.0 ± 109.16 pg at week 10. This indicated a non-significant mean decrease of 13.35 ± 10.7% (P = 0.623; n = 12). Fig. 3 depicts serum BDNF levels from our control group measuring at 955.30 ± 117.19 pg at week 0 and 1215.63 ± 89.66 pg at week 10. This indicated a significant mean increase of 21.37 ± 7.21% after the 10 week period (P ≤ 0.05; n = 8).
**Figure 2.** Plasma BDNF concentrations in healthy, sedentary young adults (n = 12) determined before the first exercise session at week 0 and immediately after completion of the 10 week exercise prescription.

**Figure 3.** Plasma BDNF concentrations in healthy, sedentary young adults (n = 8) determined before the first exercise session at week 0 and after 10 weeks of remaining sedentary.
Two-way repeated measures ANOVA was used to test the difference in RMT, RMT5 and RMT30 between exercise and control groups at both time periods. Week 0 comparison showed no significant time interaction $F(2, 22) = 2.950, P = 0.069$, however a significant group x time interaction was observed $F(2, 22) = 3.823, P \leq 0.05$. Statistical analysis between groups at week 10 also showed no significant time interaction $F(2, 22) = 1.155, P = 0.848$ or significant group x time interaction $F(2, 22) = 0.001, P = 1.000$. The main effect of baseline capacity for PAS-induced plasticity between exercise and control groups at week 0 was found to be significant $F(1, 22) = 4.62, P \leq 0.05$ however the main effect of exercise on RMT values was not $F(1, 22) = 205.18, P = 0.980$.

Our exercise group yielded mean MEP amplitudes of $0.64 \pm 0.08$ mV in RMT, $0.47 \pm 0.05$ mV in RMT5, and $0.50 \pm 0.06$ mV in RMT30 at week 0. MEP amplitudes following week 10 in our exercise group were $0.66 \pm 0.06$ mV in RMT, $0.6 \pm 0.06$ mV in RMT5, and $0.65 \pm 0.09$ mV in RMT30. As depicted in Fig 4, RMT5 and RMT30 data were normalized and expressed as a percentage of mean RMT taken pre-PAS. Repeated-measures ANOVA was used to compare pre- and post means between values. Mean RMT5 amplitude increased non-significantly by 10.1% $F(1, 22) = 0.702, P = 0.420$ and mean RMT30 amplitude increased non-significantly by 8.23% $F(1, 22) = 0.241, P = 0.633$ after 10 weeks. The main effect of time on capacity for PAS-induced plasticity after 10 weeks was non-significant for RMT5 $F(1, 22) = 0.702, P = 0.420$ and for RMT30 $F(1, 22) = 0.241, P = 0.633$ in our exercise group.
In our control group, analysis of RMT, RMT5 and RMT30 yielded mean MEP amplitudes of 0.64 ± 0.1 mV in RMT, 0.60 ± 0.07 mV in RMT5, and 0.86 ± 0.16 mV in RMT30 at week 0. MEP amplitudes following week 10 were 0.60 ± 0.08 mV in RMT, 0.49 ± 0.1 mV in RMT5, and 0.5 ± 0.13 mV in RMT30. Fig. 5 depicts RMT5 and RMT30 data normalized and expressed as a percentage of mean RMT taken pre-PAS. Mean RMT30 amplitude decreased significantly by 33.8% after the 10 week period of inactivity $F(1, 22) = 10.74, \ P \leq 0.05$. The main effect of time on capacity for PAS-induced plasticity after the 10 weeks of inactivity was non-significant for RMT5 $F(1, 22) = 0.349, \ P = 0.568$ but significant for RMT30 $F(1, 22) = 10.736, \ P \leq 0.05$ in our control group.

![Graph](image)

**Figure 4.** RMT5 and RMT30 MEPs were normalized and expressed as a percentage of each individual’s mean RMT taken prior to the PAS protocol from our exercise group. Depicted are RMT5 and RMT30 mean’s relative to the RMT measured during either week 0 or 10. A value of 100% thus represents the amplitude of the response before PAS. RMT5 increased by 10.1% but this was not significant $F(1, 22) = 0.702, \ P = 0.425, \ n = 12$ and RMT30 increased by 8.23% after 10 weeks which was also not significant $F(1, 22) = 0.241, \ P = 0.633; \ n = 12$. 
Figure 5. RMT5 and RMT30 MEPs normalized and expressed as a percentage of each individual’s mean RMT taken prior to the PAS protocol during both week 0 and 10 in our control group. RMT30 decreased significantly by 33.8\% \( F (1, 22) = 10.74, P \leq 0.05; n = 12 \) and RMT5 decreased by 13.43\% although non-significantly \( F (1, 22) = 0.349, P = 0.57; n = 11 \).

**IO Curves**

IO curve values were normalized relative to the largest obtained MEP (MEP Max) and slopes were derived from these values using the Boltzmann sigmoidal function. In our control group, pre-and-post-PAS slopes were \( 0.0129 \pm 0.0013 \%\text{MEP Max} \cdot \%\text{RMT}^{-1} \) and \( 0.013 \pm 0.0015 \%\text{MEP Max} \cdot \%\text{RMT}^{-1} \) at week 0, respectively, and pre-and-post-PAS slopes were \( 0.0266 \pm 0.0035 \%\text{MEP Max} \cdot \%\text{RMT}^{-1} \) and \( 0.0278 \pm 0.0053 \%\text{MEP Max} \cdot \%\text{RMT}^{-1} \) at week 10, respectively. Thus, both pre-and-post-PAS slopes indicated an increase after 10 weeks. In our exercise group, pre-and-post-PAS slopes were \( 0.0119 \pm 0.0004 \%\text{MEP Max} \cdot \%\text{RMT}^{-1} \) and \( 0.0108 \pm 0.0008 \%\text{MEP Max} \cdot \%\text{RMT}^{-1} \) at week 0, respectively, and pre-and-post-PAS slopes were \( 0.0114 \pm 0.0010 \%\text{MEP Max} \cdot \%\text{RMT}^{-1} \) and \( 0.0125 \pm 0.0007 \%\text{MEP Max} \cdot \%\text{RMT}^{-1} \) at week 10, respectively. Thus, pre-PAS slope indicated a decrease and post-PAS slope indicated an increase after 10 weeks of exercise. Two-way repeated measures ANOVA was used to test for changes in IO curve slope.
This was done to compare IO curve slopes measured pre-PAS in weeks 0 and 10 between exercise and control groups to detect changes in corticospinal excitability. This was also done to compare IO curve slopes measured post-PAS in weeks 0 and 10 between exercise and control groups as a means of detecting changes in plasticity. Analysis of pre-PAS IO curve slopes revealed a significant group x time interaction after 10 weeks $F(1, 22) = 14.783, P \leq 0.05$ and analysis of post-PAS IO curve slope also revealed a significant group x time interaction $F(1, 22) = 5.455, P \leq 0.05$. The main effect of time on baseline corticospinal recruitment efficacy measured through pre-PAS IO curve slope after 10 weeks of exercise was significant $F(1, 22) = 17.05, P \leq 0.05$ as was the main effect of time on PAS-induced changes in corticospinal recruitment efficacy measured through post-PAS IO curve slope $F(1, 22) = 10.879, P \leq 0.05$. The aforementioned properties are shown in Fig. 6 and Fig. 7.

**Figure 6.** MEPs were normalized and are depicted as a percentage of MEP Max obtained from each IO curve trial taken either pre-or-post-PAS. Values were then used to calculate slope using the Boltzmann sigmoidal function.
Figure 7. MEPs were normalized and are depicted as a percentage of MEP Max obtained from each IO curve trial taken either pre-or-post-PAS. Values were then used to calculate slope using the Boltzmann sigmoidal function.

Figure 8. Between-group comparison of normalized IO curve measurements taken pre-PAS before and after 10 weeks.
**Figure 9.** Between-group comparison of normalized IO curve measurements taken post-PAS before and after 10 weeks. Significant decrease at 90% RMT observed after 10 weeks in control group ($P \leq 0.05$; $n = 11$).

Further analysis of IO curves included paired sample t-tests comparing both pre-PAS and post-PAS stimulator intensity responses after 10 weeks. Our results indicated that there was a significant difference between MEPs produced at 90% RMT post-PAS after 10 weeks in our control group; mean MEP amplitude post-PAS measured $27.86 \pm 19.22 \%$ MEP Max at week 0 and $11.79 \pm 13.56$ MEP Max after 10 weeks ($P \leq 0.05$; $n = 11$). All other comparisons were non-significant in difference.
Discussion

This study sought to determine the effects of a 10 week exercise prescription on serum BDNF levels and the excitability and plasticity of M1 in sedentary young adults. Data yielded three findings. First, the control group demonstrated a significant increase in serum BDNF levels after the 10 week period of further inactivity. Second, there was a significant decrease in synaptic plasticity in the control group in response to repeating PAS after the 10 week period as measured in RMT30. Lastly, there was a decrease in excitability of lower threshold motor neurons at 90% RMT measured following PAS in control group in response to repeating PAS after the 10 week period; this was absent in the exercise group. We suggest that the capacity for early-phase LTP (E-LTP) and late-phase LTP (L-LTP) may be measured separately using PAS, and is dependent on the time in which M1 excitability is measured after the PAS protocol as both of these subtypes of LTP are timing-dependant. The control group indicated a significant decrease of 33.8% in RMT30 measurements following 10 weeks of further inactivity while RMT5 did not decrease by a significant amount. Furthermore, we suggest that there is an unknown effect when repeating PAS over a greater period of time that may confound results obtained. To date, there are no longitudinal studies which have used PAS in evoking plasticity in the same subject group in order to test its reliability. Since the main difference between both groups after the 10 week period was the introduction of regular exercise, we suggest that these differential changes in the capacity for plasticity are mediated by exercise or the lack thereof.

Increase in maximal oxygen consumption after 10 weeks of exercise

VO₂ max was measured at week 0 before the start of the exercise prescription and immediately upon its completion. VO₂ increased by an average of 24.9 ± 6.99% as depicted in
Fig. 1 (P ≤ 0.05; n = 13) in our exercise group after the 10 week period. This increase in maximal oxygen consumption can be expected to have happened in a linear fashion over the 10 weeks (Hickson, Bomze, & Holloszy, 1977). In comparison, the same study by Hickson et al. found an average increase of 44% in VO$_2$ max after a 10 week period of purely cardiovascular training. Considering our exercise program was introduced to completely sedentary individuals and involved a divided emphasis of musculoskeletal and cardiovascular training, the average increase that we observed in our exercise group is within reason.

**Increase in serum BDNF levels in control group**

Serum BDNF levels in control group increased by a mean of 21.37 ± 7.21% after the 10 week period of further inactivity. In humans, there have been numerous chronic exercise studies that have tested exercise’s ability in raising circulating BDNF levels. However there appears to be great variability amongst these findings as some studies have demonstrated an increase in circulating BDNF levels from chronic aerobic exercise (Erickson et al., 2011; Seifert et al., 2010; Zoladz et al., 2008) while the majority of studies with similar exercise prescriptions have revealed no observed increase (Goekint et al., 2010; Levinger et al., 2008; Schiffer et al., 2009; Yarrow et al., 2010). Thus, a clearly defined dose-response relationship between chronic exercise and circulating BDNF levels has yet to be determined. It is partially due to this discrepancy that our exercise group was prescribed an exercise program that adhered to ACSM’s baseline requirements for physical activity. Although literature has not put great emphasis on strength training increasing circulating BDNF levels, the aerobic component of our exercise prescription was comparable to the aforementioned studies that did in fact observe an
increase in circulating BDNF levels. Interestingly enough, a study conducted by Currie et al. observed an inverse relationship between $VO_2$ and circulating BDNF levels (Currie, Ramsbottom, Ludlow, Nevill, & Gilder, 2009). This suggests that reduced levels of serum BDNF in more physically active individuals could be indicative of a more efficient uptake mechanism of serum BDNF into the CNS, and adds to the body of literature affirming that BDNF can be affected by cardiovascular fitness (Chan, Tong, & Yip, 2008; Laurin, Verreault, Lindsay, MacPherson, & Rockwood, 2001; Nofuji et al., 2008). Although non-significant, our exercise group had a mean decrease of $13.35 \pm 10.7\%$ in circulating BDNF levels which could relate to their significant increase of $24.9 \pm 6.99\%$ $VO_2$ max after 10 weeks of exercise. Furthermore, other studies have observed that peripheral BDNF levels were positively related to cardiovascular risk factors such as body mass index (BMI), abdominal fat, and fasting plasma glucose (Babaei, Azali Alamdari, Soltani Tehrani, & Damirchi, 2013; Golden et al., 2010; Jung, Kim, Davis, Blair, & Cho, 2011; Laurin et al., 2001; Levinger et al., 2008; Meyer et al., 2008). The aggregate of findings thus suggest that decreased peripheral BDNF levels may demonstrate improved cardiovascular conditions in physically active individuals.

Another important factor to consider is the source of the up and/or down regulation of BDNF in the body in response to chronic exercise. It is known that BDNF is primarily produced by the CNS as well as a range of peripheral tissues (Donovan et al., 2000). Platelets have been shown to be the largest non-neuronal contributor of BDNF (Yamamoto & Gurney, 1990) and serum BDNF levels have been shown to correlate with platelet number (Lommatzsch et al., 2005). A study conducted by Seifert et al. consisting of 3 months of endurance training in healthy participants observed no increase in BDNF from peripheral blood, however found a significant
increase in serum BDNF levels in venous blood taken from the internal jugular vein (Seifert et al., 2010). This alludes to the importance of identifying which sources of BDNF are up and/or down regulated by chronic exercise as these findings most likely reflect an increased output of BDNF from the brain in particular. Our experimental design involved blood samples measured through peripheral venipuncture as have most previously conducted studies. Thus, we would not have noticed this potential difference in central vs. peripherally-derived circulating BDNF.

The role of the Val66Met single nucleotide polymorphism (SNP) in BDNF expression has been associated with variation in brain volume (Nemoto et al., 2006) and cognitive function (Erickson et al., 2008). The BDNF SNP has shown to moderate the effect of physical activity on cognitive function and dementia (Kim et al., 2011) as well as depression (Mata, Thompson, & Gotlib, 2010). Thus, indicating that genetic makeup is important in understanding the extent to which exercise could influence BDNF output. As a confounding factor, this is something that should be identified in participants of future studies which seek to observe the influence of exercise on circulating BDNF levels also.

**Decrease in synaptic plasticity of control group in response to repeating PAS after 10 weeks**

Our results showed an average decrease of 33.8% in RMT30 MEP amplitude in our control group after the 10 week period. Although not statistically significant, RMT5 in the same group showed a 13.43% decrease after 10 weeks. PAS can be used to induce both LTP and LTD. The dichotomy of effects however is programmed experimentally through the timing of the ISI of the synchronized stimuli. If the TMS pulse is applied at an ISI slightly longer than the time needed for the afferent input to reach the cortex, and if a sufficient number of pairs of stimuli
are delivered, then the excitability of the sensorimotor cortex increases (Stefan et al., 2000). However, it has also been observed that if the ISI is shorter than the time needed for the afferent input to reach the cerebral cortex (10ms) then the excitability of the sensorimotor cortex will decrease (Wolters et al., 2003). Although exact synchronicity is not necessary to induce plasticity through PAS (Stefan et al., 2000), our protocol was designed to evoke LTP exclusively. Thus, the observed decrease in RMT30 and RMT5 MEP amplitudes in control group after repeating PAS could not have been induced by a different PAS protocol during week 10 measurements as the same PAS protocol was executed in all experiments. A vast body of existing literature has examined synaptic plasticity of the M1. However, the focus in human studies using PAS has been on the induction and early phase of LTP or LTD whilst the question of its maintenance has not received as great of attention. It has been established that the plasticity induced by PAS is rapidly evolving (<30 minutes to show changes), long-lasting (>60 minutes in duration) yet reversible, and timing-dependent in its changes (Stefan et al., 2002). However, due to the difficulty in elucidating the exact mechanism(s) behind this non-invasive technique it remains unclear as to exactly when and to what extent L-LTP contributes to this induction of plasticity vs. that of E-LTP. In the hippocampus, studies have examined E-LTP and L-LTP in Schaffer collateral and mossy fiber pathways and have helped further our understanding of how E-LTP and its extension of L-LTP contribute to plasticity in hippocampal tissue. Evidently, LTP induced by PAS depends partially on the activation of NMDA receptors providing a persistent increase in post-synaptic signal through the relieving of tonic Mg²⁺ inhibition (Bliss & Collingridge, 1993; Rison & Stanton, 1995). Furthermore, pharmacological studies have shown that the effects of PAS were negated by drugs that acted upon the NMDA receptor (Butefisch et
In addition to NMDA receptors, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors of post-synaptic cells affected by LTP have shown to play an integral role in this process. As an aggregate, E-LTP occurs almost entirely through postsynaptic mechanisms in its induction, maintenance, and expression. In contrast to E-LTP’s independence of protein synthesis, L-LTP requires both gene transcription and protein synthesis in the postsynaptic cell and takes place both during and continues after E-LTP ceases. In the CA1 region of the hippocampus, E-LTP has shown to persist for only 1-2 hours post-stimuli while L-LTP has shown to persist for more than 8 hours (Huang et al., 1994). Due to the non-significant change in RMT5 taken only 5 minutes post-PAS vs. the significant decrease in RMT30 taken 30 minutes post-PAS in our control group, we suggest the possibility of a distinction in the effects of PAS-induced E-LTP vs. that of PAS-induced L-LTP, and the ability for their capacity to change in a perhaps mutually exclusively fashion over time. L-LTP has been evoked in the human M1 through conventional anodal transcranial direct current stimulation (tDCS) protocols in the past (Monte-Silva et al., 2013). Thus, the activation of dual and temporally-different LTP subtypes induced by PAS in the M1 is a possibility.

We observed MEP amplitude increases of 10.1% and 8.23% in RMT5 and RMT30, respectively after 10 weeks in our exercise group. Studies have shown that cardiovascular exercise in the form of wheel running in rats not only increases BDNF levels within select neural tissues, but also increases levels of phosphorylated cAMP-response-element binding protein (pCREB) in the brain (Neeper et al., 1995) as well as the spinal cord (Gomez-Pinilla et al., 2001). With relevance to L-LTP, cAMP-response element-binding protein (CREB) contributes to the process of sensing the coincidence of calcium and cAMP signals in neurons. Subsequently,
converting it into a transcriptional response that ultimately leads to the synthesis of factors required for enhanced synaptic transmission – the process of which takes place in L-LTP, and not in E-LTP (Kovacs et al., 2007). Although non-significant, the distinction between the two subtypes of LTP could explain the slight increase in RMT30 in our exercise group after 10 weeks of exercise in comparison to the significant decrease in our control group after the 10 week period of inactivity, suggesting that these changes in L-LTP are mediated by exercise or the lack thereof.

**Decrease in excitability of lower threshold motor neurons of control group in response to repeating PAS after 10 weeks**

The input (% of maximum stimulator output) and output (MEP amplitude) relationship has been shown to have a sigmoidal shape with three distinct traits; each of which relates to specific neurophysiological properties of the corticospinal pathway under investigation (Ginanneschi, Dominici, Biasella, Gelli, & Rossi, 2006). First, the threshold of the response reflects the stimulus intensity required to activate the most excitable corticospinal cells and motor neurons. Second, the slope indicates the recruitment efficiency of the corticospinal pathway. Lastly, the plateau value reflects the magnitude of the excitatory component that determines the number of spinal motoneurones which are ultimately recruited (Carroll, Riek, & Carson, 2001; Devanne et al., 1997; Rothwell, Thompson, Day, Boyd, & Marsden, 1991). It has been deduced that the threshold and slope of the IO curve are dictated by different neural mechanisms (Devanne et al., 1997). In the context of cortical motor neuron recruitment, multiple discharges of single units due to increasing stimulus intensity occur very rarely (Day et al., 1989). As a result, it is well known that stimuli of increasing strength recruit motoneurons
with increasing motor unit potentials (Henneman, 1957), and the sigmoidal shape alludes to the wide distribution of spike amplitudes. Our IO curves taken pre-PAS in both exercise and control groups revealed no changes in the aforementioned properties of their curves. In support of these results, TMS has shown great capacity in distinguishing changes in cortical excitability (Carroll et al., 2001). This is due partially to the fact that at near-threshold intensities TMS activates almost all cortico-spinal neurons trans-synaptically, and eventually greater activation at higher stimulation intensities through direct connections with neurons located deeper in the cortex (Di Lazzaro et al., 1998). Thus, changes in near-threshold excitability can be distinguished between cortical and sub-cortical changes much more accurately through TMS than other conventional techniques. Furthermore, it has been shown that properties of the corticospinal pathway can be measured longitudinally and reliably in experimental sessions on different days when measuring IO curves (Carroll et al., 2001).

In contrast of our pre-PAS measurements, the IO curves taken post-PAS revealed a significant decrease in MEPs produced whilst stimulated with 90% RMT in our control group. The circuitry involved in a TMS-evoked MEP is proven quite complex and remains to be fully elucidated. TMS is assumed to activate neurons superficial to the output layer V (Sanes & Donoghue, 2000) and probably activates intracortical fibres that travel horizontally relative to the surface of the cortex which in turn activate postsynaptic pyramidal output cells either directly (Porter, 1996) or trans-synaptically after synapsing through cortical layer II/III (Kaneko, Caria, & Asanuma, 1994), and eventually influencing the activity of muscles through mono-synaptic connections with spinal motoneurons (Berardelli, 1991; Di Lazzaro et al., 1998; Edgley, Eyre, Lemon, & Miller, 1997; Rothwell et al., 1991). Intracortical connections have been studied
further in rats revealing that horizontal axons in layer II & III provide strong monosynaptic input to dendrites of layer II & III pyramidal cells in a distant column (Aroniadou & Keller, 1993). Thus, columnar units of M1 are interconnected and interact with one another through both strong and weak connections. Evidence derived from many sources suggest that the contacts made by superficially travelling horizontal cortical pathways underlie much of the experience-dependent plasticity in the neocortex (Diamond, Armstrong-James, & Ebner, 1993; Hess, Aizenman, & Donoghue, 1996; Rioult-Pedotti, Friedman, & Donoghue, 2000) and in the acquisition of motor skills (Rioult-Pedotti et al., 1998). These horizontal connections have shown the capability to have their strength increased or decreased through LTP and LTD respectively (Aroniadou & Keller, 1995; Hess & Donoghue, 1994). Direct evidence obtained from slice preparations in rats revealed larger field potentials across layer II – III in the trained M1 after learning a skilled reaching task. This was supported by a decreased capability in further LTP induction, linking M1 horizontal connections to LTP processes that occur during skill learning (Rioult-Pedotti et al., 1998). Furthermore, complex motor maps in M1 have been revealed when focally blocking GABAergic inhibition which resulted in the unmasking of existing horizontal connections likely blocked by feed-forward inhibition (K. M. Jacobs & Donoghue, 1991). Thus, LTP in the intrinsic horizontal circuitry of M1 within its most superficial layers appears to be a candidate mechanism in its plasticity.

As our IO curves revealed a decrease in excitability of lower threshold motor neurons caused by the PAS protocol and only in the non-exercising group, we suggest that the decreased capacity in LTP after 10 weeks arose from transient changes in the horizontal intracortical circuitry connecting lower threshold motor units located in the more superficial layer of the
cortex. This would potentially explain why only connections involving threshold motor units were influenced by PAS while the slope and peak amplitude remained unchanged. Furthermore, as with our RMT5 & RMT30 results taken between groups there appears to be an exercise-mediated interaction that prevented the observed decrease in lower threshold motor neuron excitability in our exercise group after 10 weeks.

Limitations

To take part in our study participants in both the exercise and control group had to be deemed sedentary as according to IPAQ. Participants in our exercise group were supervised during workouts to ensure adequate prescription and completion. However, our control group could only be asked to maintain their current level of physical activity for the duration of the 10 weeks. Beyond such, there is no way to determine exactly how much or how little exercise or prolonged physical activity they may have taken part in during the 10 week period.

Due to the inherent nature of submaximal testing, VO2 measurements obtained are limited in their reliability. Our study design chose to incorporate submaximal testing however as we were targeting sedentary individuals who have limited experience exercising. Submaximal testing is more comfortable and less rigorous than the gold standard method of direct VO2 max testing, and we wanted to ensure that participants were not overly-intimidated as a potential bad experience prior to the commencement of our 10 week program could have negatively impacted further compliance. Whilst some members of our exercise group demonstrated significant improvements in VO2 max after 10 weeks this could not be said for all participants. 2 participants showed only a slight improvement (less than 5%) and 2 participants showed a
decrease after 10 weeks. In addition to the previously mentioned limitations in submaximal VO₂ testing motivation during testing can play a significant factor in performance in the test. We suggest that there is in fact a range of fitness levels that need to be considered for future studies as the dichotomized view of sedentary vs. active may prove too simple-minded for longitudinal plasticity studies.

Conclusion

As our study is the first to report an unknown inconsistency when testing PAS longitudinally, future studies using PAS should test this effect and determine what the cause may be. Furthermore, the capacity for E-LTP and L-LTP in the M1 should be analyzed distinctively, as both subtypes of LTP appear to be regulated exclusively from one another and perhaps modulated by exercise or the lack thereof. Further research needs to be conducted in order to better understand the intracortical circuitry affected by PAS, and the role in which exercise affects LTP in the M1 in healthy individuals. Future studies attempting to bring us closer to a dose-response relationship between chronic exercise and circulating BDNF levels should take into account as many of the previously established variables affecting BDNF levels as possible. This includes, however is not limited to: changes in VO₂ levels, central vs. peripheral sources of BDNF, BMI and the Val66Met SNP.
References


Section 3: Appendices
Data Collection Spreadsheet – Pre-PAS

Subject Name: ______________________ Date: ________________

1) Resting Motor Threshold (RMT)
   1) Produce a MEP of at least 50 µV in relaxed APB; at least 5/10 consecutive trials.
   2) Then, find the % MSO required to get an amplitude a little less than ±1mV.
   3) Finally, take 10 trials of the RMT; set at 5 second interval, with 20% variance.

   % MSO used for RMT: ______

2) Input Output (IO) Curve
   ❖ Intensities based off of RMT before PAS.
   ❖ 10 trials at 90, 100, 110, 120, 130, and 140% RMT – Randomized, and given every 5 seconds with 20% variance.

   %RMT #1 ______
   %RMT #2 ______
   %RMT #3 ______
   %RMT #4 ______
   %RMT #5 ______
   %RMT #6 ______

PAS: Threshold Value _______  PAS: 300% Value _______
Data Collection Spreadsheet – Post-PAS

Subject Name: ___________________________  Date: ______________

1) Resting Motor Threshold (RMT)
   1) Use the same %MSO as used in pre-PAS RMT.
   2) Take 10 trials
      a. 5 minutes post-PAS.
      b. 30 minutes post-PAS.

2) Input Output (IO) Curve
   ❖ Intensities based off of RMT before PAS.
   ❖ Do 15 minutes post-PAS.
   ❖ 10 trials at 90, 100, 110, 120, 130 & 140 % RMT – Randomized, and given every 5 seconds.

   %RMT #1 _____
   %RMT #2 _____
   %RMT #3 _____
   %RMT #4 _____
   %RMT #5 _____
   %RMT #6 _____
TMS safety checklist:
The following questions are to ensure it is safe for you to have TMS applied. If you answer yes to any of the questions below, we may need to exclude you from TMS experiments.

<table>
<thead>
<tr>
<th>QUESTION</th>
<th>ANSWER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Do you suffer from epilepsy, or have you ever had an epileptic seizure?</td>
<td>Yes</td>
</tr>
<tr>
<td>2. Does anyone in your family suffer from epilepsy?</td>
<td>Yes</td>
</tr>
<tr>
<td>3. Do you have any metal implant(s) in any part of your body or head? (Excluding tooth fillings)</td>
<td>Yes</td>
</tr>
<tr>
<td>4. Do you have an implanted medication pump?</td>
<td>Yes</td>
</tr>
<tr>
<td>5. Do you wear a pacemaker?</td>
<td>Yes</td>
</tr>
<tr>
<td>6. Do you suffer any form of heart disease?</td>
<td>Yes</td>
</tr>
<tr>
<td>7. Do you suffer from reoccurring headaches*?</td>
<td>Yes</td>
</tr>
<tr>
<td>8. Have you ever had a skull fracture or serious head injury?</td>
<td>Yes</td>
</tr>
<tr>
<td>9. Have you ever had any head surgery?</td>
<td>Yes</td>
</tr>
<tr>
<td>10. Are you pregnant?</td>
<td>Yes</td>
</tr>
<tr>
<td>11. Do you take any medication or use recreational drugs (including marijuana)*?</td>
<td>Yes</td>
</tr>
<tr>
<td>12. Do you suffer from any known neurological or medical conditions?</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Comments

Name
Signature
Date

*Note if taking medication or using recreational drugs please read through the medication list on the next page to see if you use contraindicated drugs or medications. You do not need to tell the researcher which medications or drugs you use, unless you wish to. However, all researchers have signed confidentiality agreements and this information will not recorded in writing, if you do wish to discuss this issue.

**Dr. Murphy will meet with participants who answer yes to this question to seek further information.
Medications contraindicated with magnetic stimulation:

1) Tricyclic antidepressants

<table>
<thead>
<tr>
<th>Name</th>
<th>Brand</th>
</tr>
</thead>
<tbody>
<tr>
<td>amitriptyline (&amp; butriptyline)</td>
<td>Elavil, Endep, Tryptanol, Trapeutic</td>
</tr>
<tr>
<td>desipramine</td>
<td>Norpramin, Pertofrime</td>
</tr>
<tr>
<td>dothiepin hydrochloride</td>
<td>Prothiaden, Thadon</td>
</tr>
<tr>
<td>imipramine (&amp; dibenamin)</td>
<td>Tofranil</td>
</tr>
<tr>
<td>ipindole</td>
<td></td>
</tr>
<tr>
<td>noripryline</td>
<td>Pamelor</td>
</tr>
<tr>
<td>opipramol</td>
<td>Opipramol-neuraxpharm, Insidon</td>
</tr>
<tr>
<td>proipryline</td>
<td>Vivactil</td>
</tr>
<tr>
<td>trimipramine</td>
<td>Suernontil</td>
</tr>
<tr>
<td>amoxapine</td>
<td>Aserdol, Aserdol, Defamyl, Dexamoax, Monadil</td>
</tr>
<tr>
<td>doxepin</td>
<td>Adapin, Sinequian</td>
</tr>
<tr>
<td>clomipramine</td>
<td>Anafranil</td>
</tr>
</tbody>
</table>

2) Neuroleptic or Antipsychotic drugs

A) Typical antipsychotics

<table>
<thead>
<tr>
<th>Phenothiazines:</th>
<th>Thiothixene:</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Chlorpromazine (Thorazine)</td>
<td>* Chlorpromazine</td>
</tr>
<tr>
<td>* Fluphenazine (Prolixin)</td>
<td>* Fluphenazine (Depixol and Fluoxol)</td>
</tr>
<tr>
<td>* Perphenazine (Trilafon)</td>
<td>* Perphenazine (Navane)</td>
</tr>
<tr>
<td>* Prochlorperazine (Compazine)</td>
<td>* Prochlorperazine (Clopixol and Acuphase)</td>
</tr>
<tr>
<td>* Thioridazine (Mellaril)</td>
<td>* Thioridazine (Navane)</td>
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<tr>
<td>* Trifluoperazine (Stelazine)</td>
<td>* Trifluoperazine (Stelazine)</td>
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<tr>
<td>* Mesoridazine</td>
<td>* Mesoridazine</td>
</tr>
<tr>
<td>* Promazine</td>
<td>* Promazine</td>
</tr>
<tr>
<td>* Trifluoperazine (Vaspin)</td>
<td>* Trifluoperazine (Vaspin)</td>
</tr>
<tr>
<td>Levomepromazine (Nordim)</td>
<td>Levomepromazine (Nordim)</td>
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</tbody>
</table>

B) Atypical antipsychotics

<table>
<thead>
<tr>
<th>Clozapine (Clozani)</th>
<th>Quetiapine (Seroquel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Olanzapine (Zyprane)</td>
<td>* Olanzapine (Geodon)</td>
</tr>
<tr>
<td>Palipradone (Invoga)</td>
<td>* Palipradone (Sotom)</td>
</tr>
<tr>
<td>* Risperidone (Risperdal)</td>
<td>* Risperidone (Risperdal)</td>
</tr>
</tbody>
</table>

C) Dopamine partial agonists: Aripiprazole (Abilify)

D) Others

Symbytom: A combination of olanzapine and fluoxetine is used in the treatment of bipolar depression.

Tetrahydrocannabinol (THC) is a natural component in cannabis and is found in many of Europe's main psychoactive components. Some parts of Europe use cannabis more often than once per week, and/or cannabis use is in the past 4 days. Regular use of recreational drugs, or single episode within the past three weeks.
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Resistance (kg or watts)</th>
<th>Cadence (rpm)</th>
<th>HR (bpm)</th>
<th>KPE</th>
<th>BP (mmHg)</th>
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<tbody>
<tr>
<td>1</td>
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<td>Workload One</td>
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<td>4+</td>
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<td>Workload Two</td>
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<td>Workload Three</td>
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<td>Workload Four</td>
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<td>Recovery** (reduce resistance)</td>
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<td>4</td>
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</tbody>
</table>

**HR at end of 3rd minute**

<table>
<thead>
<tr>
<th>&lt;80 bpm</th>
<th>80 - 89 bpm</th>
<th>90 - 100 bpm</th>
<th>&gt;100 bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd workload</td>
<td>2.5 kp</td>
<td>2.0 kp</td>
<td>1.5 kp</td>
</tr>
<tr>
<td></td>
<td>750 kpm·min⁻¹</td>
<td>600 kpm·min⁻¹</td>
<td>450 kpm·min⁻¹</td>
</tr>
<tr>
<td></td>
<td>125 W</td>
<td>100 W</td>
<td>75 W</td>
</tr>
<tr>
<td>3rd workload</td>
<td>3.0 kp</td>
<td>2.5 kp</td>
<td>2.0 kp</td>
</tr>
<tr>
<td></td>
<td>900 kpm·min⁻¹</td>
<td>750 kpm·min⁻¹</td>
<td>600 kpm·min⁻¹</td>
</tr>
<tr>
<td></td>
<td>150 W</td>
<td>125 W</td>
<td>100 W</td>
</tr>
<tr>
<td>4th workload</td>
<td>3.5 kp</td>
<td>3.0 kp</td>
<td>2.5 kp</td>
</tr>
<tr>
<td></td>
<td>1050 kpm·min⁻¹</td>
<td>900 kpm·min⁻¹</td>
<td>750 kpm·min⁻¹</td>
</tr>
<tr>
<td></td>
<td>175 W</td>
<td>150 W</td>
<td>125 W</td>
</tr>
</tbody>
</table>

**subsequent workloads**

Keep adding 0.5 kp until calculated target zone for test is reached. Most clients will not require this extra time.
Edinburgh Handedness Inventory

Your Initials: ______________

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

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

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

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

<table>
<thead>
<tr>
<th>Task / Object</th>
<th>Left Hand</th>
<th>Right Hand</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Writing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Drawing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Throwing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Scissors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Toothbrush</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Knife (without fork)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Spoon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Broom (upper hand)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Striking a Match (match)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Opening a Box (lid)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total checks: LH = ____________ RH = ____________

Cumulative Total
CT = LH + RH = ____________

Difference
D = RH - LH = ____________

Result
R = (D / CT) × 100 = ____________

Interpretation:
(Left Handed: R < -40)
(Ambidextrous: -40 ≤ R ≤ +40)
(Right Handed: R > +40)