This is to certify that the
dissertation entitled

FUNCTIONAL MRI OF CENTRAL MOTOR DRIVE AND CENTRAL MOTOR FATIGUE

presented by

Ryan Michael Francis

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Kinesiology

Major Professor's Signature

8/27/10

Date

MSU is an Affirmative Action/Equal Opportunity Employer

FUNCTIONAL MRI OF CENTRAL MOTOR DRIVE AND CENTRAL MOTOR FATIGUE

By

Ryan Michael Francis

A DISSERTATION

Submitted to
Michigan State University
In partial fulfillment of the degree requirements
for the degree of

DOCTOR OF PHILOSOPHY

Kinesiology

2010

ABSTRACT

FUNCTIONAL MRI OF CENTRAL MOTOR DRIVE AND CENTRAL MOTOR FATIGUE

By

Ryan Michael Francis

Decreased central motor drive from the primary motor cortex is thought to contribute to muscle fatigue. Reductions in central drive can result from either spinal or supraspinal mechanisms. Supraspinal fatigue can be inferred if electrical stimulation of the motor cortex increases the force of a voluntary muscular contraction (MVC). However, stimulation of motor regions that lie deep within the brain is difficult. Therefore there is a need to develop further techniques to assess supraspinal drive during exercise. Functional magnetic resonance imaging (fMRI) is an increasingly popular tool to investigate brain function. If fMRI can be used to supply a measure of supraspinal drive, this technique could be applied to measure alterations in supraspinal drive under conditions during which supraspinal drive may be altered.

Study 1: fMRI of Central Motor drive during Muscle Contraction

Purpose: 1) Determine if fMRI can provide a measure of central motor drive central drive & 2) Determine if muscle fatigue during ischemic submaximal exercise alters central drive. **Methods:** Single-shot echo-planar images (EPI; TR 2s, TE 35 ms, 22 cm FOV, 4 mm axial slices, 0.5 mm gap, 64x64 matrix, 90° pulse, 29-32 slices) were continuously acquired in 12 participants (25.7±3 years) during 5 tasks: 1) 30-s isometric handgrip contractions at 10, 20, 30, and 40% MVC (FV), 2) 3-min occlusion of forearm blood flow (OCL), 3) 3-min isometric contraction at 15% MVC (EX1), 4) 3-min

contraction at 15% MVC with occlusion (EX+OCL), and 5) 3-min contraction at 15% MVC (EX2). **Results:** A strong correlation was observed between changes in muscular force and fMRI signal intensity during FV ($r = 0.70$; $p < 0.001$). Differences were observed between the following comparisons; 1) EX+OCL > EX1 ($p = 0.002$) & 2) EX+OCL > EX2 ($p = 0.008$). A trend was observed where brain activation was greater following OCL+EX vs. OCL ($p = 0.12$). **Conclusion:** The increase in fMRI-measured activity during the ischemic contraction is consistent with the increased central drive needed to maintain the target force as fatigue develops in the peripheral muscles.

Study 2: fMRI of Central Motor Drive during Prolonged Running

Purpose: To assess changes in supraspinal drive following prolonged running using fMRI. **Methods:** Single-shot EPI (64x64 acquisition matrix, coronal plane, TR=2s, TE= 32 ms, 32 slices, 90° pulse) were continuously acquired on 12 participants during 2 exercise tasks before and immediately after 90-min of running at 75% VO_2 max; 1) The forces were four, 8-second MVC plantar flexion contractions & 2) Four 20-sec isometric contractions at 10, 15, 20 and 30% MVC force. **Results:** A trend was observed where supraspinal drive was decreased following 90 minutes of prolonged exercise ($p = 0.12$). **Conclusions:** fMRI can provide a measurement of supraspinal fatigue following prolonged exercise. Furthermore, the results of this study suggest supraspinal drive may be reduced following 90 minutes of running at 75% VO_2 max.

ACKNOWLEDGEMENTS

There are many people who I would like to thank, as without their help this dissertation would not have been able to be completed. First I would like to thank all of my committee members; Drs. Ron Meyer, Karin Pfeiffer, Jill Slade, Joe Carlson and Florian Kagerer. All of you have supplied valuable input throughout the completion of this dissertation that made it the best it could be. I would like to especially thank Drs. Karin Pfeiffer and Ron Meyer. As an orphaned grad student you both took me on and treated me as if I was your own student. Without your support, wisdom and guidance I am certain I would not have been able to complete my degree. I can not say enough about Dr. Jill Slade who during the final weeks was the primary driving force behind the completion of this project. Your willingness to put forth the amount of time that you did to help out with collection and analysis of the data is something that is truly appreciated. Without your help this project would not have been able to be completed. I would also like to thank Dr. Robert Wiseman. Although you were not directly on my committee, with all your help and participation on this project I do consider you to be my 6th committee member. I would also like to thank my former academic advisor Jeffrey Lemmer for initially taking a chance on me as a graduate student. Due to that initial chance I can now leave a much wiser individual with my degree in hand. I also owe thanks to the graduate school for offering me the dissertation completion fellowship. Finally I would like to say thank you to Jo Ann Janes. Being that I am not the type of person who is good with red tape and paperwork which oftentimes leads to a lot of

unnecessary bumps in the road. No matter how rocky I made the road you were always unseemly able to smooth it out.

TABLE OF CONENTS

LIST OF TABLES

LIST OF FIGURES

Images in this dissertation are presented in color

KEY TO SYMBOLS AND ABBREVIATIONS

BOLD	Blood oxygen level dependent
CHO	Carbohydrate
CNS	Central nervous system
EMG	Electromyography
fMRI	Functional magnetic resonance imaging
FOV	Field of view
IT	Interpolated twitch
MRI	Magnetic resonance imaging
MVC	Maximal voluntary contraction
PCr	Phosphocreatine
rCBF	Regional cerebral blood flow
S1+M1	Primary sensorimotor cortex
TE	Echo time
TR	Repetition time
TMS	Transcranial magnetic stimulation

Chapter 1

Introduction

Muscular fatigue is defined as an exercise-induced reduction in the ability of a muscle or muscle group to generate force or power [1]. Developing an understanding of muscular fatigue is important for a variety of reasons. From an athlete's perspective, reductions in muscular force that occur following prolonged activity may not only be crucial for deciding the outcome of a sporting event, but also in reducing the likelihood of injury. In addition, many of the tasks that we perform during our everyday activities, such as walking up a flight of stairs or mowing the lawn, become increasingly difficult as we age as a result of increases in muscular fatigue. Although muscular fatigue has been the subject of thousands of scientific studies, its causes remain poorly understood [2]. At this time most physiological investigations have focused primarily on changes that occur within skeletal muscle following prolonged exercise. However, due to methodological limitations, very little is known regarding neurological limitations to exercise performance, especially with regard to brain function.

Voluntary muscular contractions involve a complex series of events that are initiated in the motor cortex and end with the production of force by the skeletal muscle. The development of muscular fatigue begins almost immediately following the onset of exercise and can originate from both peripheral and central mechanisms [2, 3]. Peripheral fatigue is referred to as the loss of muscular force that occurs at or distal to the neuromuscular junction and can be thought of as fatigue within the muscle itself [3]. The inability of the central nervous system (CNS) to adequately drive the muscle to produce force during a muscular contraction is referred to as central fatigue [3]. Central fatigue

can originate from any site that is proximal to the neuromuscular junction, and can therefore result from either a reduction in central drive from the motor cortex (supraspinal fatigue) or modulations in central drive that occur at the level of the spinal cord (spinal fatigue). Although much of the loss in force during exercise is caused by peripheral mechanisms, muscular fatigue is not merely the result of changes that occur within the muscle alone [3, 4]. In this regard, reductions in central drive contribute significantly to muscular fatigue, and have been attributed for up to 25% of the loss in muscular force during exercise [3].

Until recently, most studies have focused on peripheral mechanisms with little attention given to central fatigue. However, the development of muscular fatigue cannot be fully accounted for by peripheral fatigue alone, as reductions in muscular force are caused by both peripheral and central mechanisms [5]. While it is commonly assumed that fatigue is caused by peripheral mechanisms, such as substrate availability, excitation contraction coupling and the excitability of the peripheral muscle, changes in central drive are often observed well before the work capacity of skeletal muscles is reached [6, 7]. Changes in afferent feedback, motoneuronal discharge, motor cortical output and perceived effort often develop before the exercise capacity of the muscle is reached [7]. Furthermore, during maximal sustained muscular contractions motoneuron discharge rate is slowed, resulting in a reduction of maximal force produced by the muscle, suggesting that part of the development of muscular fatigue is controlled through central mechanisms [7].

Following exercise, the development of central fatigue is evident, as measured through various techniques including twitch interpolation. Twitch interpolation involves

the electrical stimulation of a peripheral nerve during a maximal voluntary contraction. Increases in muscular force observed following the stimulation of the peripheral nerve during an MVC indicate that central drive is insufficient to fully stimulate the muscle [1, 2, 8, 9]. During sustained maximum voluntary contractions (MVC), increases in muscular force are observed following electrical stimulation of the motor nerve in fatigued skeletal muscle, signifying that the loss of muscular force is, in part, caused by a reduction in central drive during muscular contraction [4, 10-12]. Furthermore, using twitch interpolation, reductions in central drive have been observed following a period of prolonged exercise such as running and cycling [13-18]. More specifically, Saldahna et al (2008) observed a 17% reduction in MVC of the plantar flexors and a 19% reduction central drive following 2 hours of running at 75% of VO_2 peak. The reduction in MVC was highly correlated with the reduction in central drive ($r=0.87$), indicating that the reduction in maximal voluntary plantar flexor muscle strength was primarily related to central mechanisms [18]. Although twitch interpolation techniques have shown central drive is reduced during fatiguing exercise, the origination of the site of fatigue can occur anywhere upstream from the site of stimulation. Therefore twitch interpolation cannot be used to differentiate between spinal or supraspinal fatigue during exercise [1].

Although the previous studies using twitch interpolation were unable to assess differences in spinal and supraspinal drive during exercise, some evidence suggests that muscular contractions of a sufficient duration and intensity result in a reduction in supraspinal drive following fatiguing exercise. For example, reductions in both muscular force and EMG activity are observed following a sustained, 70-minute muscular contraction at 5% MVC of the elbow flexors. Following the submaximal

contraction, EMG activity rose by approximately 60-80% and MVC strength was reduced by approximately 72%. Electrical stimulation of the motor cortex using transcranial magnetic stimulation (TMS) elicited an increase in MVC strength and accounted for almost two thirds of the reduction in muscular force, suggesting central drive from the motor cortex is impaired following prolonged exercise[19]. Similarly, Gandevia et al (1996) observed an approximate 10% reduction in muscular force following a sustained, three minute, MVC of the elbow flexors [11]. Cortical stimulation of the motor cortex by TMS resulted in an increase in maximum "voluntary" muscular force, indicating "in part" muscular fatigue is caused by a reduction in cortical output from the motor cortex. Unfortunately, all motor regions of the brain associated with various exercise tasks cannot be stimulated using TMS. In fact, TMS has only been applied to relatively few muscle groups whose associated brain regions lie superficial to the motor cortex, such as the elbow flexors, in addition to the wrist flexors and extensors [1, 3, 12]. Because specific regions of the motor cortex that control other muscle groups (such as the legs) lie deep within the brain, they cannot be easily stimulated using TMS. Therefore, changes in supraspinal drive that occurs during locomotor exercises involving the legs, such as running and cycling, are currently unknown.

Due to these methodological limitations it is presently unknown if alterations in central drive during prolonged locomotor exercise are mediated through spinal or supraspinal mechanisms. Because reductions in central motor drive have been implicated in contributing to muscular fatigue, there is a need to develop further techniques to access brain function during exercise [9]. Functional magnetic resonance imaging (fMRI) is becoming an increasingly popular tool used to investigate brain function, including motor

function, and may provide a non-invasive measure of central motor drive [20-25]. Increases in activated regions of the brain are accompanied by an increase in blood volume and blood oxygenation, which can be used to construct an image of brain activation using fMRI [25]. Because fMRI provides a high degree of spatial and temporal resolution in activated brain regions, it may be ideal to assess changes in supraspinal output during muscular fatigue [20-23, 26]. If fMRI can be used to supply a measure of supraspinal drive, this technique would be able to be applied to measure alterations in supraspinal drive under conditions where supraspinal drive may be altered, but cannot be measured using existing techniques.

The first goal of this dissertation is to determine if fMRI can be used to assess supraspinal drive during exercise. If fMRI does provide a measurement of supraspinal drive during exercise, an additional goal of this dissertation is to apply this technique to measure changes in supraspinal drive during prolonged running exercise, a condition in which central drive is thought to be reduced.

Goal #1: fMRI of Central Motor drive during Muscle Contraction

Previous investigations using electromyography (EMG) observed increases in the EMG signal during sustained, submaximal muscular contractions despite the maintenance of a constant level of force, suggesting increases in central drive are necessary during the development of muscular fatigue so that higher threshold motor units can be recruited to sustain a given level of force output [27-29]. In non-fatigued skeletal muscle motor unit recruitment and activation frequency remain constant during a sustained muscular contraction [28]. However, motor unit recruitment is increased following ischemic exercise that results in the development of muscular fatigue, indicating that supraspinal

drive is increased to maintain muscular force during the development of muscular fatigue [28].

It has been hypothesized that fMRI can be used to provide a measurement of supraspinal drive during exercise. Increases in the fMRI signal intensity are correlated with increases in muscular force, indicating that the output from the motor cortex is increased as the requirements for muscular force increases [30, 31]. Furthermore, following prolonged muscular contractions of a constant force, the fMRI signal increases despite the maintenance of a constant level of muscular force [20, 22, 24]. Therefore it is believed that an increase in supraspinal drive is required to activate higher threshold motor units in order to maintain a constant level of force production during fatiguing exercise. Because ischemic exercise increases motor unit activation due to the development of muscular fatigue, if fMRI does provide a measure of supraspinal drive during a sub-maximal muscular contraction the fMRI signal intensity is likely to be increased following ischemic exercise [28].

If increases in brain activation in the motor cortex are observed following ischemic, submaximal exercise it would provide evidence that fMRI can be used as a tool to measure supraspinal output during exercise. Therefore, the purposes of the first study in this dissertation are to: 1) determine if fMRI can provide a measure of central motor drive central drive and 2) determine if muscle fatigue during ischemic submaximal exercise alters central drive. It was hypothesized that central drive will be greater following ischemic exercise compared to exercise alone. If fMRI can be shown to provide a measurement of supraspinal drive, it would be possible to use this technique to

measure changes in supraspinal drive under conditions where central drive may be impaired.

Goal #2: fMRI of Central Motor Drive during Prolonged Running

Reductions in central drive have been observed following 30-120 minutes of prolonged locomotor exercise [13, 14, 16-18, 32]. Two hours of cycling exercise performed at approximately 65% of VO_2 peak has been shown to elicit reductions in both muscular force and central activation in trained cyclists, suggesting that the development of muscular fatigue is at least partially caused by a reduction of neural input to the muscles [13, 14]. The time course for the development of muscular fatigue appears to be related to the intensity of the work bout. In this regard, following 5 hours of prolonged cycling at 55% VO_2 max, significant reductions in neural drive are not evident until after the 4^{th} hour of exercise [16]. Although the precise relationship between the onset of central fatigue during running in regards to the time and intensity of the work bout has yet to be established, exercising at an intensity greater than 55% VO_2 max results in an earlier onset of central fatigue. In this regard, between 90-120 minutes of running exercise at an intensity of 70-80% VO_2 max elicits significant reductions of central drive in trained runners [17, 18]. However, currently it is unknown whether these reductions in central drive are caused by spinal or supraspinal mechanisms.

Due to methodological limitations discussed previously, earlier studies have been unable to assess supraspinal fatigue following prolonged locomotor exercise such as running and cycling. Although fMRI may provide a valuable tool for measuring supraspinal drive during prolonged locomotor exercise, its use is not met without its own technical challenges. For instance, the subject must lie motionless inside the bore of the

magnet, therefore measuring changes in central drive during locomotor exercises such as running and cycling are difficult [26]. Previous studies using twitch interpolation have indicated that central drive is reduced for up to 4 hours for the involved muscle groups following the end of exercise, with no significant differences observed between 5 and 30 minutes [15-17, 33]. Therefore, although measurements of supraspinal drive would not be able to be performed during the exercise task using fMRI, measurements taken within 30 minutes following exercise using fMRI would most likely reflect the level of central drive at the end of exercise.

Because previous techniques have not been able to differentiate between spinal and supraspinal drive following prolonged locomotor activity, the specific aim of the second study of this dissertation is to use fMRI to assess changes in supraspinal drive following 90 minutes of running at an intensity of 75% VO_2 max. It is hypothesized that the mean % change in fMRI signal intensity (SI) in the motor cortex will be decreased after compared to before 90 minutes of running. If this study is successful it will provide evidence that central fatigue following prolonged activity is "in part" induced by a reduction in cortical output from the motor cortex. This technique could potentially be applied to other conditions where central drive is altered, but where it would be otherwise impossible to differentiate between spinal and supraspinal mechanisms.

Chapter 2

Review of Literature

Muscular Fatigue

During exercise, fatigue is defined as an exercise-induced reduction in the ability of a muscle to maintain force or power [1]. Developing an understanding regarding the mechanisms of muscular fatigue is important to define the limits of physical performance, as well as daily function [2]. Voluntary muscular contractions are a complex series of events that are initiated in the motor cortex. From the motor cortex, action potentials are transmitted through the spinal cord and the α-motor neuron to where they reach the contractile machinery of the muscle fiber resulting in the generation of muscular force [2]. Fatigue can occur from failure at one or more links in this chain and can be objectively measured as a decrease in muscular strength or power [34]. Because the origination of muscular fatigue can occur at one or more points, several definitions are used to define the source of fatigue. Fatigue produced by changes at or distal to the neuromuscular junction is referred to as peripheral fatigue [1]. Any failure in neural drive associated with a progressive reduction in voluntary activation of a muscle during exercise is referred to as central fatigue [1, 35].

Peripheral Fatigue

Peripheral fatigue refers to fatigue-related processes that occur distally to the neuromuscular junction that can be measured as a fall in twitch or titanic force produced by peripheral nerve stimulation [9]. Presently the majority of scientific studies

examining the cause of muscular fatigue have primarily focused on peripheral fatigue. The focus on peripheral fatigue can be attributed to a number of reasons. First it is convenient to assume that exercise performance is limited by the peripheral mechanisms. Therefore, many scientific investigations have focused on the peripheral muscle, without any regard to neural input [6]. Additionally, techniques used to measure peripheral fatigue are more established and accessible compared to those used to study central fatigue. Furthermore, mechanisms of muscular fatigue are similar between human and animal models [2]. Peripheral fatigue results from a variety of factors depending on the frequency, intensity, duration and type of muscular contraction performed. These factors influence a variety of mechanisms leading to the development of peripheral fatigue including substrate availability, excitation contraction coupling and the excitability of the peripheral muscle [2, 6, 36].

Intramuscular Phosphocreatine:

The availability of phosphocreatine (PCr) is likely to limit the force producing capability of a skeletal muscle during brief, high-power muscular contractions lasting between 10-20 seconds [2, 37, 38]. The breakdown of PCr supplies the muscle with the highest rate of ATP resynthesis during maximal exercise [2, 39, 40]. During maximal exercise, intramuscular PCr content begins to decrease as intramuscular PCr stores are metabolized. This results in a reduction of ATP synthesis followed by a reduction in muscular power. Initial signs of fatigue are well correlated with a reduction of intramuscular PCr stores during short-term, high-intensity muscular contractions [40]. The availability of intramuscular PCr may therefore become limited during high-intensity

exercise, reducing muscular power output during the first 10-20 seconds of maximal exercise [40]. Because fast-twitch muscle fibers are able to store approximately 15-20% more PCr compared to slow twitch muscle fibers, fiber type distributions can play an important role in the regulation of peripheral fatigue during high-intensity exercise [41].

Reductions in muscular fatigue have been observed during short-term, high-intensity exercise following oral creatine supplementation [38, 40]. Oral creatine supplementation significantly increases intramuscular creatine and PCr concentrations resulting in an increased work output during repeated bouts of high-intensity exercise [37, 38, 40]. Following creatine supplementation levels of muscular power during high-intensity exercise are able to be sustained due to an attenuation of ATP degradation caused by an increase in intramuscular PCr availability [37]. Conversely, muscular fatigue during high intensity exercise develops sooner in mice lacking the enzyme creatine kinase (CK), which catalyzes the formation of ATP from ADP and phosphocreatine [42, 43]. Furthermore, fatigue related properties of skeletal muscle are restored in CK deficient mice following CK injection [44]. These results indicate that the availability of PCr modulates peripheral fatigue during short-term, high-intensity muscular contractions. However, because creatine supplementation does not improve endurance performance, creatine availability does not contribute to muscular fatigue during prolonged exercise [45].

Muscle Glycogen:

Exercise below the onset of blood lactate accumulation (OBLA) can be maintained over a period of several minutes to several hours [34]. Muscle glycogen is

the primary substrate metabolized to produce energy at exercise above 60% of $VO_{2\ max}$ [2, 40]. During prolonged exercise intramuscular glycogen stores are gradually depleted by reducing available intramuscular glycogen stores. [34]. During continuous exercise between 60-80% of $VO_{2\ max}$ muscular fatigue is correlated with reductions of intramuscular glycogen availability as glycogen depleted muscle fibers are unable generate sufficient amounts of ATP resulting in a reduction of exercise intensity [40, 46]. Muscular fatigue following glycogen depletion is thought to be caused by a reduction in tricarboxylic acid cycle (TCA) activity caused by either reductions in Acetyl CoA or TCA intermediates due to a reduction of glycogenolysis [40].

Muscle glycogen stores are significantly reduced 1-2 hours after the onset of moderate to high intensity exercise resulting in the onset of muscular fatigue [34, 47]. In contrast, increasing intramuscular glycogen stores by the consumption a high carbohydrate diet enhances exercise performance and decreases muscular fatigue [47]. However, muscle fatigue cannot be fully explained by intramuscular glycogen stores alone as substantial quantities of muscle glycogen may still be present in highly trained athletes following exhaustive exercise [48, 49]. Furthermore, in subjects with high pre-exercise muscle glycogen concentrations the consumption of a carbohydrate solution before and during prolonged, intermittent running increases time to exhaustion despite having substantial amounts of muscle glycogen present at the point of fatigue [48]. Upon completion of the exercise, plasma glucose concentrations were higher following the consumption of the carbohydrate solution. Because exercise reduces blood glucose concentration it is possible that the increased plasma glucose levels provided a sustained source of CHO for the central nervous system indicating that fatigue may have been

caused by central rather than peripheral mechanisms [34, 48]. It is believed by some that glycogen depletion in contracting muscle fibers initiates an inhibitory afferent response to the hypothalamus initiating metabolic changes within skeletal muscle resulting in a reduced power output. This indicates that muscle glycogen concentration may act as a signal rather than a determinant of muscular fatigue [36].

Excitation-Contraction (EC) Coupling:

Voluntary muscular contractions originate in the motor cortex. The initialization of an action potential from the motor cortex along the motor neuron, results in the depolarization of the sarcolemma and Ca^{++} release from the sarcoplasmic reticulum (SR). Cytosolic Ca^{++} released from the SR binds with Ca^{++} receptors, exposing the actin/myosin active sites, initiating a muscular contraction. This process is referred to as excitation-contraction (EC) coupling [50]. Metabolic by-products resulting from increases in metabolism during physical activity, including H^+, P_i & lactate in addition to an increased intracellular concentration of K^+ and muscular damage may reduce EC coupling, resulting in the development of muscular fatigue [34].

Following prolonged intensive exercise muscular force recovers slower during low frequency stimulation compared to high frequencies. Muscular force can be maintained during a period of low-frequency fatigue by increasing the frequency or number of recruited motor units [2]. Low frequency fatigue is thought to occur due to an increase in muscular damage resulting in a disruption of the membrane systems involved in Ca^{++} release [5]. Increases in muscle activity result in an increased flux of K^+ across the muscle membrane resulting in fatigue [51]. This results in a reduced intracellular K^+

concentrations, decreasing the chemical gradient across the muscle membrane and therefore decreasing sarcolemmal excitability and Ca^{++} release from the SR [5]. The reduction in cytosolic Ca^{++} would induce muscular fatigue by inhibiting E-C coupling.

Reductions in SR excitability following muscular activity may be dependent on the type of contraction that is being performed. The excitability of the sarcolemma has been shown to decrease following sustained high-intensity muscle contractions but not during low-frequency muscular contractions [51, 52]. Because prolonged physical exercise increases Na^{+}-K^{+} pump activity, it is possible that during low frequency muscular contractions intracellular K^{+} concentration are restored along with the transmembrane potential [5]. Additionally, aerobic training increases the concentration of Na^{+}-K^{+} pumps in skeletal muscle [53]. Therefore the influence of sarcolemmal excitability in response to muscular fatigue may be related to aerobic training status [5]. These effects might not be observed following a sustained, high-intensity muscular contraction where reduced intracellular K^{+} concentrations reduce the excitability of the sarcolemma [51].

The accumulation of intracellular lactic acid during intense physical activity may interfere with EC-coupling is one of the classic theories of muscular fatigue. Lactic acid is a strong acid that disassociates into lactate and H^{+} resulting in a reduction of intracellular pH [54]. Although lactate in itself does not cause of fatigue, the drop in pH caused by the increase in H^{+} has been believed to result in fatigue through a number of potential mechanisms including; 1) inhibiting myosin ATPase, 2) attenuating the frequency and duration of the opening of the Ca^{++} channel of the SR, 3) inhibiting Ca^{+2} binding to troponin C & 4) the inhibition of glycolytic enzymes [2, 39]. Despite the

popularity of this theory, its validity has been questioned [36, 54-57]. For example, in vitro studies have shown reductions in rates of the glycolytic enzymes phosphorylase and phosphofructokinase resulting in a decrease in SR Ca^{++} pumping and cross-bridge formation [54]. However, these results have not been observed in acidified skeletal muscle under normal physiological conditions [34, 40, 58, 59]. Furthermore, oral administration of sodium bicarbonate fails to decrease the prevalence of muscular fatigue during repeated cycling sprints, despite an increase in intracellular pH [60]. In fact, some research has indicated that increased intramuscular lactic acid concentrations counteract the depressing effects of reduced intracellular K^+ during intense exercise, acting as a mechanism to protect against muscular fatigue [61]. These results indicate that reductions of intracellular pH are not as important in peripheral muscular fatigue as previously thought.

Another potential mechanism that disrupts excitation-contraction coupling is the accumulation of intracellular P_i. Intracellular P_i concentrations increase as the result of the hydrolysis of PCr to creatine and P_i. Although creatine has little effect on muscular contractions, increased intramuscular P_i may have an effect on E-C coupling during high intensity muscular contractions [54-56, 62]. Intramuscular P_i concentrations are inversely proportional to muscular force, suggesting that increased intramuscular P_i contribute to the development of peripheral muscular fatigue [62]. Furthermore, increases in intramuscular P_i concentrations reduce the amount of Ca^{++} released from the SR [55, 63]. These results are not observed in skeletal muscle lacking the enzyme creatine kinase and indicate that increases in intramuscular P_i reduce E-C coupling.

resulting in muscular fatigue [55]. It has been proposed that during high intensity exercise P_i enters the sarcoplasmic reticulum combining with Ca^{++} to form calcium phosphate (CaP_i). Increase in CaP_i precipitate in the SR lower calcium release during a sustained contraction resulting in the development of fatigue [34, 39, 54, 56]. However, due to the time course required to elevate intramuscular P_i concentrations during anaerobic metabolism this mechanism is likely only important during high-intensity activities lasting more than 1-2 minutes. Other mechanisms of fatigue are most likely to be more important during lower intensity activities leading to fatigue in > 1 hour [56].

Neuromuscular Junction:

The neuromuscular junction represents another potential site for peripheral fatigue during exercise. The synapse between the motor neuron and a muscle cell is referred to as the neuromuscular junction. The arrival of an action potential from the motor neuron causes acetylcholine (Ach) to be released from the presynaptic nerve terminal. Ach diffuses across the synaptic cleft binding with Ach receptors located on the motor end plate of the muscle cell, initiating a muscular contraction [39]. Following repeated nerve stimulation the release of Ach from the presynaptic nerve terminal is reduced in addition to a decreased sensitivity of the motor end plate [64, 65]. However, measurements with electromyography (EMG) and electrically induced muscle action potentials indicate that the transmission of APs across the neuromuscular junction is not impaired during voluntary contractions [2]. Furthermore, the amplitude of the post synaptic potential largely exceeds the amplitude needed to elicit an end plate potential. This indicates that

the neuromuscular junction is not a likely site of peripheral fatigue in healthy subjects [34].

Central Fatigue

The generation of a voluntary muscular contraction involves a series of events starting in the brain and ending with the development of muscular force. Because muscular fatigue can originate from one or more sites from the motor cortex to the peripheral muscle, the contributions of central and peripheral mechanisms to the loss of muscular force are difficult to determine [3, 5]. Central fatigue is defined as the progressive decrease in neural drive which occurs proximal to the neuromuscular junction leading to a reduction in muscular force [1]. During prolonged exercise, losses of muscular force are partially caused by the failure of the CNS to adequately drive the muscle to contract and can be responsible for as much as 20-25% of the loss of muscular force [3]. Central drive is decreased during prolonged exercise lasting from 30 minutes to several hours [5, 13, 18, 33, 66]. However, the loss of central drive is dependent on a variety of factors including the exercise mode and intensity. For example, both prolonged cycling and running performed at 55% VO_2 max result in the development of central fatigue. However, reductions in central drive were not evident until the 5^{th} hour of cycling, whereas central drive was decreased following 4 hours of running, despite being performed at the same exercise intensity [15, 16]. Furthermore, the development of central fatigue is dependent on the intensity of the exercise. Other investigations observed reductions in central drive during exercise lasting between 30 minutes to 4 hours in duration, performed at exercise intensities >65% VO_2 max [13, 14, 18, 32, 33, 48, 67]. In this regard, 30 minutes of cycling at 80% VO_2 max reduced voluntary activation of the

knee extensors by 13-16%, contributing to a reduction in overall aerobic power output [67]. Therefore, the mode and intensity of exercise should be taken into account when assessing central fatigue.

Spinal vs. Supraspinal fatigue:

Reductions in central drive, resulting in a decrease in muscular force, can occur at either the spinal or supraspinal levels of the CNS. Spinal fatigue results from modulations in neural drive at the level of the spinal cord. During sustained muscular contractions, the firing frequency of the spinal motor neuron decreases as the result of altered input from the muscle spindle, golgi tendon organ, and inhibitory afferents that innervate the fatiguing muscle [1, 68]. Following fatiguing exercise, the excitability of the spinal motoneurons is reduced, making them less responsive to descending cortical drive [69]. Therefore, although a given level of cortical output may be adequate for maximal or near maximal activation of the muscle at the beginning of exercise; the same level of cortical output may be less effective in producing force output as fatigue develops. In this regard, increases in neural drive originating from the motor cortex may be required to maintain the same level of force production during the development of muscular fatigue [3]. In contrast to spinal fatigue, supraspinal fatigue results from the failure of the motor cortex to provide a sufficient level of central activation to maintain a given force output [1]. In fatigued skeletal muscle, increases in muscular force have been observed following direct stimulation of the motor cortex during an MVC [3]. Because the increases in force production are observed during maximum voluntary efforts in fatigued skeletal muscle, the development of fatigue is believed to be of supraspinal, as

opposed to spinal, origin [1]. However, at this time the contributions of each spinal and supraspinal fatigue in relation to central drive during exercise have yet to be determined.

Serogenetic System:

The serogenetic system plays an important role in the development of supraspinal fatigue [2, 34]. During prolonged exercise, serotonin (5-HT) concentrations in the brain are increased, and are associated with the development of central fatigue [70]. The transport of tryptophan, the precursor for 5-HT formation, across the blood brain barrier is the rate limiting step for 5-HT synthesis in the brain [71]. Therefore, increases in plasma tryptophan concentrations during exercise are likely to increase tryptophan transport across the blood brain barrier, contributing to the development of central fatigue [70]. During prolonged exercise, plasma tryptophan concentrations can be increased through a number of ways. First, tryptophan competes with the branch chain amino acids (BCAA) for transport across the blood brain barrier. Because tryptophan and the BCAAs share a common transporter across the blood brain barrier, reductions in plasma BCAA concentrations increase tryptophan's ability to be transported across the blood brain barrier. In this regard, during prolonged exercise BCAAs are metabolized, increasing the tryptophan to BCAA ratio in the plasma, thus increasing tryptophan transport across the blood brain barrier and 5-HT synthesis [72]. Increases in the plasma tryptophan concentrations are also increased due free fatty acid (FFA) release during prolonged exercise. In this regard, both tryptophan and FFA have an affinity for binding with albumin in the plasma. However, because FFAs have a greater affinity for albumin compared to tryptophan, increases in FFA concentrations during prolonged exercise

displaces tryptophan from its binding site and increases free tryptophan concentrations in the plasma. The resulting increase in free tryptophan concentrations in the plasma enhance the transport of tryptophan across the blood brain barrier, leading to the increase of 5-HT in the brain [72, 73].

Several nutritional strategies have been explored to reduce plasma free tryptophan concentrations in hopes of delaying the onset of central fatigue during prolonged exercise. Because tryptophan competes with BCAA for transport across the blood brain barrier, it has been hypothesized that oral BCAA supplementation would inhibit the transportation of free tryptophan across the blood brain barrier by decreasing the free tryptophan to BCAA ratio and attenuate the development of central fatigue [72, 73]. However, BCAA supplementation has not been shown to affect exercise capacity or perceived exertion during prolonged exercise [73, 74]. Although BCAA supplementation has no affect on exercise capacity, carbohydrate supplementation during exercise has been shown to blunt the uptake of tryptophan by the brain, and possibly attenuate the development of supraspinal fatigue [73]. In this regard, carbohydrate supplementation during prolonged exercise decreases plasma FFA release and tryptophan uptake by the brain [48, 73, 75]. Nybo et al (2003) reported 3-hours of cycling resulted in a 20% decrease in muscular force of the knee extensors during a sustained MVC in addition to a decrease in central drive. However, only a 10% decrease in muscular force and no deficits in central drive were observed when the same exercise was performed with glucose supplementation [76]. It should be noted that no differentiations were made between spinal and supraspinal fatigue in this study. It is possible these improvements could be explained by increased levels of blood glucose, resulting in a glycogen sparing

effect in skeletal muscle during the exercise. However, Foskett et al (2008) observed an increase in exercise performance following carbohydrate supplementation independent of blood glucose and muscle glycogen content. Additionally, plasma free fatty acid concentrations were lower following carbohydrate supplementation, likely reducing the transport of tryptophan across the blood brain barrier and serotonin synthesis in the brain [48]. Although previous investigations have found support that carbohydrate supplementation attenuates central fatigue during prolonged exercise, at this time no studies have been conducted assessing the effects of carbohydrate supplementation on supraspinal fatigue during prolonged exercise.

Measurements of Central Fatigue:

Until recently, most studies examining muscular fatigue during exercise focused on peripheral mechanisms, with little attention given to central fatigue. However, reductions in muscular strength cannot be fully explained by peripheral mechanisms alone [5]. In this regard, previous investigations have shown evidence that both peripheral and central fatigue contribute to a reduction in muscular force during prolonged exercise [13-16, 18, 32]. The previous pause in the data regarding central contributions to muscular fatigue can be attributed to several reasons. First, it is convenient to assume that muscular performance is limited by peripheral mechanisms devoid of neural input. There is a common belief held by many physiologists that exercise capacity is limited at the level of the skeletal muscle [6]. However, central changes regarding muscle afferent feedback, neuronal discharge, cortical output and perceived effort develop well before the work capacity of the peripheral muscles is

reached [7]. Furthermore, some of the peripheral mechanisms once thought to induce muscular fatigue, such as lactic acid accumulation and glycogen depletion, may be mediated through central pathways [36, 61].

The study of central fatigue during exercise is also limited by a lack of techniques that are capable of assessing central drive. In the past many different techniques have been used to study the occurrence of central fatigue during exercise, including drug manipulations, twitch interpolation and transcranial magnetic stimulation. Although each of these techniques supplies important information regarding the occurrence of central fatigue, the amount of information gained is often limited in regards to the central mechanism of fatigue and muscle groups that can be measured [1]. Due to these limitations there has been an increased need to develop technologies to further progress the understanding of central fatigue.

Drug Manipulations:

One method commonly used to study central fatigue during prolonged exercise is drug manipulations. Neurons throughout the brain are responsive to a variety of neurotransmitters that influence changes brain function [26]. Physical exercise influences a number of brain neurotransmitters including dopamine, noradrenalin and serotonin [72]. Although it is unlikely that a single neurotransmitter system is responsible for the development of central fatigue, serotonin (5-HT) concentrations are increased during prolonged exercise, contributing to the development of central fatigue [72, 73]. The administration of serotonin reuptake inhibitors (SRI) blocks the reuptake of 5-HT by the presynaptic nerve terminal, increasing 5-HT concentrations in the brain. In

this regard, exercise time to exhaustion is decreased following SRI administration during prolonged moderate intensity (70% VO_2 max) physical activity, suggesting fatigue is, at least in part, mediated through a decrease in central motor drive [77]. However, at higher exercise intensities (>80% VO_2 max) SRI administration has no effect on exercise time to exhaustion and may actually preserve peak power production during repeated Wingate testing [78].

The differences in effects on time to exhaustion during moderate and high-intensity exercise following SRI administration highlights an important limitation of drug manipulations to study central fatigue. Increases in 5-HT concentrations simultaneously affect several functions of the brain. For example, in addition to reductions in central drive, increased 5-HT concentrations also decrease pain perception. Furthermore, the attenuation of ascending afferent feedback following lumbar, epidural anesthesia increases peak power production and muscular activity during a 5-km cycling time trial. It was theorized that the decreased afferent input following anesthesia reduced the perception of pain during exercise, resulting in a reduction of the inhibitory motor response and resultant increase power production [79]. This suggests central fatigue following prolonged exercise can not only be modulated through a reduction in central motor drive, but also through changes afferent input to the CNS. Therefore, drug manipulation studies are limited in the ability to identify the central mechanisms and associated brain areas that are related to the development of fatigue.

Twitch Interpolation:

Another technique commonly used to provide an indirect measurement of central fatigue is twitch interpolation. Twitch interpolation was developed in the 1950s and is now a common tool used to access the development of central fatigue [8]. This technique involves the electrical stimulation of a peripheral nerve during a maximal voluntary contraction (MVC), referred to as an interpolated twitch (IT). Voluntary activation is a measure of how well a subject can drive a muscle to produce force and can be obtained from the following formula: voluntary activation = 100(1 - IT/RT) where IT is the size of the interpolated twitch and RT is the size of a control twitch produced by an identical nerve stimulation of a resting muscle. If an electrically induced superimposed twitch evokes an increase in muscular force during an MVC, central activation is less than 100% resulting in the development of fatigue. Therefore, increases in muscular force following an IT indicate a reduction in the central drive from the CNS to the peripheral muscle [1, 2, 9].

Earlier investigations using twitch interpolation to access muscular fatigue did not report any decrements in central activation and concluded that neural drive was optimal during muscular fatigue. However, the lack of findings was due to the decreased sensitivity of twitch interpolated techniques used in earlier studies. Improvements of this technique over the past 30 years have increased its sensitivity and proven that central drive is submaximal at times of muscular fatigue [1]. Although twitch interpolation has proven central drive is reduced during fatiguing exercise, it is limited in a number of ways. First, stimulation of the motor nerves is restricted to the muscles that can be stimulated to evoke an maximal twitch without stimulating other muscles that either

contribute to or reduce the amount of muscular torque produced [3]. In addition, the origination of the site of fatigue can occur anywhere upstream from the site of stimulation. Therefore twitch interpolation cannot be used to differentiate between spinal or supraspinal fatigue during exercise [1].

Transcranial Magnetic Stimulation (TMS):

Transcranial magnetic stimulation (TMS) is a technique commonly used to study supraspinal fatigue during exercise tasks [1]. TMS works by creating a rapidly changing magnetic field around a circular coil. The rapidly changing magnetic field around the coil induces an electric current in nearby conductive structures. Thus TMS is capable of electrically stimulating the motor cortex by placing the coil over regions of motor cortex associated with a specific task [80]. Increases of a superimposed twitch following TMS during an MVC indicates the level of central drive from the motor cortex is suboptimal [3]. Todd et al (2003) examined voluntary activation of the elbow flexors using both twitch interpolation and TMS. Voluntary activation increased linearly in non-fatigued muscles between 50 and 100% of maximal effort, indicating increases in muscular force are dependent on the level of cortical activation; i.e. increases in muscular force were achieved through an increase in central drive from the motor cortex. Furthermore, all available motoneurons were activated following TMS as both TMS and peripheral nerve stimulation resulted in the same level of muscle activation in non-fatigued muscle [81]. However, TMS increases muscular force output in fatigued skeletal muscle during the performance of an MVC, suggesting central motor drive is suboptimal during muscular fatigue [4, 11, 12]. Additionally, increases in muscular activity, measured by EMG, are

observed following TMS stimulation in fatigued skeletal muscles, further suggesting the excitatory output from the motor cortex is suboptimal during fatigue [29, 82].

The use of TMS as a measure of central fatigue is associated with a number of problems. First, the stimulation of a particular muscle or group of muscles within the motor cortex is very imprecise. Due to this imprecision, magnetic and electrical stimulation of the motor cortex can stimulate cells with divergent projections, activating a muscle or group of muscles that are synergistic or antagonistic to the desired motor task [1]. This is especially problematic when the antagonistic muscle group overpowers the agonistic muscle group especially when the agonists are fatigued, underestimating central fatigue [12]. Due to the potential to increase antagonistic muscle groups, TMS is most reliable when the agonistic muscle group is stronger than the antagonistic muscle group [3, 12]. Therefore, measurements of supraspinal fatigue are limited to a relatively small number of muscle groups including the elbow and wrist flexors using TMS [4, 12, 29, 81, 83]. Because most locomotor activities rely predominantly on the muscles of the leg, TMS has not been used to used to differentiate between spinal and supraspinal contributions to fatigue during running and cycling activities. A second concern with TMS is that a twitch evoked in a relaxed muscle using cortical stimulation cannot be used as a control [12]. Supramaximal stimuli given to a motor neuron result in a maximum contraction that can be used as a standard to judge the size of superimposed twitches or maximal contractions. However, due to the activation of synergistic and antagonistic muscle groups as well as changes in motoneuron excitability during fatigue, a supramaximal cortical stimulus does not yield a control twitch that can be used to quantitatively analyze the degree of supramaximal fatigue [1, 3, 11, 12]. Gandevia et al

(1996) proposed that the increment in torque could be normalized to the voluntary force at the time of stimulation. However, this technique would overestimate the degree of central fatigue [11]. Due to these limitations, there is a need for more precise methods to measure supraspinal fatigue during exercise.

Functional Magnetic Resonance Imaging (fMRI)

Magnetic resonance imaging (MRI) relies on the formation of a strong magnetic field to create images of biological tissue. Images are created through of a series of changing magnetic pulses and oscillating electromagnetic fields, referred to as pulse sequences which are absorbed by atomic nuclei. Depending on the pulse sequences being used, MRI can detect differences in tissue properties enabling the construction of a detailed image of biological tissues [26]. Functional magnetic resonance imaging (fMRI) relies on these same principles to construct an image of brain activity. In this regard, blood flow is increased in active regions of the brain and the increase in blood volume can be used to construct an image of brain activation [25]. Since its emergence in 1990, fMRI has been increasingly used to measure central drive during muscular fatigue [20-24]. The primary benefit of using fMRI as a measure of central fatigue is that it is a noninvasive technique that allows for a high degree of both spatial and temporal resolution in relation specific regions of brain activation and central drive during fatigue [26]. Additionally, because fMRI provides a measurement of central output from the brain, it can be used to assess the development of supraspinal fatigue.

Increases in neuronal activity in the brain are accompanied by changes in blood oxygenation and increases in blood volume. Brain activation using fMRI is usually based

on the blood oxygenation level dependent (BOLD) effects. The BOLD response is dependent on the magnetic properties of oxygen and hemoglobin. Oxygenated hemoglobin (Hb) is diamagnetic and therefore has no magnetic moment whereas deoxygenated hemoglobin (dHb) is paramagnetic and thus has a significant magnetic moment. Oxygen consumption increases by approximately 5% following cortical neuron stimulation, thereby increasing dHb concentrations in activated brain regions [25]. The increased magnetic moment caused by the increase in dHb causes dephasing of the magnetic resonance signal (T_2^*) and a resultant decrease in the BOLD response [26]. Initially the BOLD response exhibits a dip lasting for approximately 2-3 seconds due to the increase in dHb in venous blood [84]. However, although the initial BOLD response may be decreased, regional blood flow to activated cortical neurons increases by approximately 50%, consequently decreasing venous dHb concentrations [25, 85]. Following the initial decrease, the BOLD response is ramped for approximately 6-12 seconds before reaching a plateau due to an increase in total blood volume [84]. The increase in total blood volume in activated brain regions lowers the dHb/Hb in the venous blood compared to surrounding regions that remain inactivated. The reduced dHb concentration lowers the rate of dephasing, causing the magnetic resonance signal (T_2^*) to decay at a slower rate compared to the surrounding tissue, thereby increasing the BOLD response [25, 26].

Increases in neuronal activity are associated with an increase in energy metabolism and oxygen consumption which in turn are correlated with an increase in Cerebral blood flow (rCBF) [85]. For example, visual stimulation increases glucose utilization and cerebral blood flow (rCBF) to the visual cortex, whereas these effects are

decreased following visual deprivation [86]. However, despite the correlation between energy metabolism and rCBF, changes in glucose availability or metabolic signals such as decreased O_2, or increases in CO_2 or H^+ cannot fully explain the increase in rCBF evoked by neural activity [87]. Although the exact mechanism is unknown, it is currently thought that increases in rCBF are modulated by neurotransmitter cycling. In this regard, glutamate, the dominant excitatory neurotransmitter in the brain, plays a pivotal role coupling neuronal activity and glucose metabolism [88]. Glutamate injections decouple the relationship between rCBF and glucose metabolism, suggesting the uptake of glutamate by excitatory synapses increases rCBF and thus the fMRI signal [87, 89].

Although both inhibitory and excitatory neurotransmitters increase metabolic demand, inhibition appears to have little to no affect on the BOLD response most likely due to a fewer number of synapses and an increase in metabolic efficiency. Inhibitory synapses are present at only one tenth the density of excitatory synapses in the cortex [87]. In addition, divergence from a single inhibitory interneuron is capable of simultaneously delaying or blocking the generation of action potentials from a large number of projection neurons [90]. Metabolic efficiency may also be increased during inhibition due to the smaller electrochemical gradient of inhibitory synapses compared to excitatory synapses [87]. In this regard, Waldvogel et al (2000) did not observe any measurable changes in the BOLD signal following an inhibitory task, although increases in the BOLD signal were observed following excitatory tasks [91]. Therefore, the increased BOLD response following cortical activation is believed to be mostly affected by excitatory rather than inhibitory effects and would reflect changes in motor output during a voluntary muscular contraction [87, 91-93].

It is important to note that fMRI is not a direct measurement of neuronal activity within the brain, but rather an indirect measurement of blood flow correlated with increased neuronal activity. The fMRI signal is based on the assumption that local energy consumption of the brain is associated with an increase in synaptic activity [85]. This relationship was confirmed by a series of experiments by Logothetis et al (2001) [84]. Simultaneous recordings of fMRI signals were compared with single and multiple spiking activities (neuronal output) and local field potentials (neuronal input) in the visual cortex of monkeys. Following visual stimulation, the BOLD response was highly correlated with local field potentials suggesting fMRI reflects input and intracortical processing rather than spiking output [84]. Although the fMRI data may not directly reflect changes in cortical output, changes in the input response may lead to modifications of output signals in postsynaptic neurons [22].

Early Motor Studies:

The use of fMRI as a tool to study brain function has been cross-validated with previous techniques used to study brain activation patterns during voluntary movement [94-99]. Krings et al (1997) validated the use of fMRI against electrophysiological TMS maps concerning the localization and density of cortical motoneurons [96]. It was concluded that fMRI activation corresponded spatially to the areas of highest motoneuron density. In addition, both Positron emission tomography (PET) and fMRI imaging techniques are based on changes in cerebral blood flow (rCBF) as a measurement of brain activation. In this regard, during simple motor tasks, such as finger tapping and finger opposition, brain activation patterns using PET and fMRI are well correlated [94, 97].

Furthermore, Sadato et al (1997) concluded that due to the higher spatial resolution of fMRI, it is better able to dissociate the area and magnitude of change compared to PET [98].

The increase of the BOLD response during a motor task is dependent on both the force and the rate of muscular contraction. Dettmers et al (1995) reported a direct relationship between muscular force and cerebral blood flow measured by PET. In this regard, increases in index finger flexion force produced a logarithmic increase in cerebral blood flow in motor-related regions of the brain [100]. Because increases in muscular force produced an increase in rCBF, it was later postulated that a similar response would be observed during fMRI analysis [30]. Although the BOLD response is poorly correlated with static finger flexion between 5-10% of MVC, increases in muscular force during a hand gripping exercise between 20 and 80% of the subjects MVC are very well correlated with an increase in BOLD response in the motor-related regions of the brain [24, 30]. Interestingly, a linear increase in the number of activated pixels only occurred between 20 to 65% of MVC and a curvilinear response was observed between 65 to 80% of MVC [30]. It is hypothesized that an increase in the number of pixels is due to an increase in the number of activated neurons that are recruited to produce the required increase in muscular force [25]. Because increases in muscular force above 65% resulted in an increase in signal intensity without any concurrent increase in pixel number, it was believed that all the available cortical neurons were recruited and further increases in fMRI signal intensity are caused by increased firing rate [30]. Similar correlations between muscular force and fMRI signal intensity were observed by van Duinen et al (2008) [31]. Muscular contractions of the right index finger at increasing intensities

between 15 and 70% of MVC produced a linear increase in fMRI signal intensity. Although the curvilinear increase in fMRI signal was not observed in this study as in the previous one, the subjects were not required to perform any muscular contractions above 70% MVC. Therefore it is possible that the exercise intensity was not great enough to recruit all of the available cortical neurons. The correlation between muscular force output and the fMRI signal indicates that neuronal output from the motor cortex is increased as the requirement for muscular force increases. Therefore, fMRI can be used as a measure of central drive during voluntary muscular contractions. However, it should be noted that at this time these relationships have only been performed using handgrip exercise.

fMRI as a Measure of Fatigue:

Over the last decade fMRI has become an increasingly popular tool used to measure cortical drive during fatiguing exercise. The development of central fatigue is dependent on a variety of factors including the intensity of the exercise as well as the type of contraction that is being performed [20-23]. Earlier investigations of central drive during sustained, submaximal contractions noted increases in EMG signal from the muscle despite the maintenance of a constant level of force, suggesting cortical drive increases during muscular fatigue [27, 29]. Similar findings have been reported following fMRI analysis [20, 22, 24]. In this regard, increases in both EMG from the muscle and fMRI signal in the motor-related regions of the brain are observed during both static and intermittent submaximal finger flexion and hand gripping [20, 22]. It is believed that the increase in signal is caused by an increase in cortical activation in

response to fatigue as the muscle attempts to maintain the same level of force output [22].

In contrast to submaximal motor fatigue tasks, muscular fatigue involving a sustained maximal contraction results in the uncoupling of the cortical and muscular signals. Liu et al (2002) observed an almost linear decrease in both muscular force and EMG signal following a maximal, 2-min handgripping exercise. In contrast, fMRI signals from the motor-related regions of the brain increased throughout the first minute of the contraction and later decreased to a level similar to that of the initial period [21]. The uncoupling between muscular activity and brain activation was thought to be caused by an increase in cortical drive required to maintain muscular force. However, because it was a maximal contraction all available motor units should have been recruited and it was unclear if any additional effort could be given. In this regard, two possibilities were given to explain the increase in cortical drive. First, increases in cortical motoneuron excitability could serve to enhance the voluntary drive required to maintain muscular force. However, if motoneuron excitability is increased it would be likely that a similar increase in EMG signal from the muscle would also be observed therefore this is unlikely. Second, the increase in fMRI signal could also be explained by an increase in sensory information coming back to the brain from the muscle during the fatiguing contraction. In this regard, a recent study by Post et al (2009) observed an increase in fMRI signal with concurrent decreases in both voluntary activation and EMG amplitude throughout a 2-minute sustained MVC of the right index finger [101]. Interpolated twitches to the motoneuron increased muscular force, suggesting that central drive was insufficient despite the increase in fMRI signal. It was hypothesized that increased

sensory input from the periphery to the motor cortex resulted in the increase in fMRI signal. Therefore an increase in the BOLD response may not necessarily correlate with an increased cortical output. However, previous studies have shown positive correlations between force output and the BOLD response [30, 31, 100]. Therefore the exact relationship between force output and the BOLD response is yet to be determined.

Differences also exist in cortical activation patterns between maximal sustained, and intermittent muscular contractions. Cortical activation is substantially higher throughout an intermittent handgrip MVCs compared to sustained MVCs despite similarities in the level of muscular fatigue [23]. It is hypothesized that there is a population of neuronal cells that respond to rapid increases in muscular force. This population of neural cells requires a stronger cortical output to recruit high-threshold motor units which may have resulted in the increase in fMRI signal during intermittent compared to sustained contractions. In addition, despite the higher level of cortical activation throughout the intermittent task, a strong trend was observed in which the fMRI signal decreased during the first 2 minutes of the intermittent contractions. In contrast, during the sustained MVC the fMRI signal increased during the first minute and later decreased throughout the second half of the contraction. In this regard, the possibility exists that a greater number of neurons are recruited to strengthen the signal during the first half of the sustained maximal contraction. However, during the intermittent MVCs the fMRI signal all of the motor units had been activated and therefore no increase in signal was observed [23].

fMRI During Dynamic Exercise:

Previous investigations of supraspinal fatigue during dynamic activities predominantly used indirect measurements of cortical drive, such as drug manipulations and TMS. Although useful, these techniques are met with a variety of limitations. For example, drug manipulation studies may influence a variety of neurotransmitter regions across a number of brain region and are therefore not useful for all experimental questions [26]. In addition, TMS does not provide spatial information regarding the precise location of activated brain regions and is limited to muscles where cortical neurons are able to be stimulated transcranially [1, 26]. Although fMRI is a valuable tool that provides a high degree of spatial and temporal resolution, its use to study brain activity in response to fatigue following prolonged dynamic activities is also met with several technical challenges [20-23, 101]. Head movement during gross motor tasks can distort the spatial resolution of the acquired signal data rendering the data useless [26]. Additionally, the subject must remain in a supine position inside of the magnet making central changes in response to dynamic exercise not only difficult to measure but also difficult to perform. Recently a novel technique has been developed to measure brain activity associated with cycling activity using fMRI. In this regard, Mehta et al (2009) observed plausible associations between the BOLD response in the motor-related regions in the brain during a pedaling exercise [102]. Although the pedaling exercise was associated with an increase cortical activation in the motor cortex, as of now this protocol has not been applied to muscular fatigue. Furthermore, the pedaling exercise during this task did not resemble gross locomotor exercises like running and cycling, limiting its

applicability. Due to these limitations, fMRI has not currently been used to study supraspinal fatigue during dynamic activities such as running and cycling.

Time course changes associated with voluntary activation may provide a window of opportunity to measure cortical drive following prolonged exercise using fMRI. Previous studies using electrical nerve stimulation and TMS have observed reductions in voluntary activation immediately following prolonged running and cycling activities at various exercise intensities [13, 18, 32]. Furthermore, reductions in voluntary activation have been observed for up to 30-minutes after moderate intensity exercise [15-17]. Additionally, voluntary activation is not significantly different between 5 and 30-minutes after exercise, indicating that measurements of cortical activity taken within 30 minutes reflect central drive at the end of exercise [17]. However, this window of opportunity is limited as voluntary activation recovers within 4-hours after marathon running [33]. Currently, no studies have assessed changes in central activation between 30-240 minutes following exercise. Voluntary activation is depressed for at least 30 minutes after prolonged activity. Therefore, it is likely that fMRI can be used to measure changes in cortical drive following prolonged exercise.

Chapter 3

Study #1: fMRI of Central Motor Drive during Muscle Contractions

Introduction

The generation of a voluntary muscular contraction involves a complex series of events that is initiated in pre-motor and primary motor cortical areas and ends with the development of a muscular contraction [2, 3]. Muscular fatigue, an exercise-induced reduction in the ability of a muscle to maintain force, begins almost immediately following the onset of exercise and can originate from both peripheral and central mechanisms [2, 3]. At this time, most studies have focused primarily on peripheral mechanisms of fatigue with little attention given to changes that may occur. However, reductions in central drive contribute up to 25% of the decrease in muscular force during exercise and are associated with the development of muscular fatigue [1, 3, 9]. Therefore, central motor drive is an important factor in muscular activation and force production.

Many techniques have been used to study alterations in central drive during exercise. In this regard, peripheral nerve stimulation during a maximal voluntary contraction (MVC) performed following fatiguing exercise increases muscular force production, indicating that central drive is insufficient to maximally contract the muscle following fatiguing exercise [1]. Reductions in central drive can occur at either the spinal or supraspinal levels of the CNS. Spinal fatigue results from modulations in neural drive at the level of the spinal cord, whereas supraspinal fatigue results from the failure of the

motor cortex to provide a sufficient level of central drive to maintain a given force output [1, 68].

Increases in central drive are observed during brief (< 4 min) submaximal muscular contractions in order to maintain a constant level of force during the muscular contraction [22]. Earlier investigations using electromyography (EMG) observed increases in the EMG signal intensity during sustained muscular contractions despite the maintenance of a constant level of force, suggesting that additional motor units are recruited or the rate of activation of recruited motor units is increased in order to maintain a constant level of force during fatiguing exercise [27-29]. Furthermore, increases in motor unit recruitment are dependent on the metabolic state of the muscle. In this regard, following repeated submaximal muscular contractions with unhindered blood circulation motor unit recruitment and activation frequency remain constant [28]. However, motor unit recruitment is increased if blood flow to the muscle is occluded, therefore it is believed that an increase in supraspinal drive required to maintain muscular force during the development of muscular fatigue [28].

Although changes in central drive following fatiguing exercise are well-documented, due to methodological limitations the effects of the spinal and supraspinal mechanisms are difficult to discern [1]. For instance, nerve stimulation that increases force during an MVC is indicative of reduced central drive. However, the origin of the site of fatigue can occur anywhere upstream from the site of stimulation and may be caused by either spinal or supraspinal mechanisms [1]. Some studies have suggested that the origin of fatigue is caused by a reduction in supraspinal drive. In this regard, stimulation of the motor cortex using transcranial magnetic stimulation (TMS) increases

muscular force following fatiguing exercise, suggesting output from the motor cortex is compromised following prolonged muscular activity [11, 12, 29, 81]. However, TMS stimulation is mostly successful in activating the superficial regions of the motor cortex, and therefore has predominantly been used for a relatively small number of muscle groups including the elbow and wrist flexors [4, 12, 51, 81, 83].

Functional magnetic resonance imaging (fMRI) is another method commonly used to study structural and functional relationships in the brain [25]. Functional MRI provides a high degree of both spatial and temporal resolution and therefore offers a noninvasive technique that can be used to assess supraspinal output during muscular activity. Brain activation using fMRI is usually dependent on the blood oxygenation level dependent (BOLD) effects. The BOLD response is reliant on increases in blood volume and blood oxygenation that accompany increases in regional brain activation, resulting in an increased fMRI signal intensity [25, 26]. It has been hypothesized that fMRI can be used to provide a measurement of supraspinal drive during exercise. Increases in the fMRI signal intensity are correlated with increases in muscular force, indicating that the output from the motor cortex is increased as the requirements for muscular force increases [30, 31]. Furthermore, following prolonged muscular contractions of a constant force, the fMRI signal increases despite the maintenance of a constant level of muscular force [20, 22, 24]. Moreover, motor unit recruitment is increased following ischemic exercise that results in the development of muscular fatigue, indicating that supraspinal drive is increased to maintain muscular force during the development of muscular fatigue [28]. Therefore it believed that an increase in supraspinal drive is required to activate higher threshold motor units to maintain a

constant level of force production during fatiguing exercise. In this regard, if fMRI does provide a measure of supraspinal drive during a sub-maximal muscular contraction, the fMRI signal intensity is likely to be increased following ischemic exercise [28].

The purposes of this study were to 1) determine if fMRI can provide a measure of central motor drive and 2) determine if muscle fatigue during ischemic submaximal exercise alters central drive. It was hypothesized that central drive will be greater following ischemic exercise compared to exercise alone. In addition ischemic exercise will result in greater muscle fatigue compared to exercise alone. If these hypotheses are correct this study will show that fMRI can be used to provide a measure of supraspinal drive during fatiguing exercise.

Methods

Participants:

Twelve healthy adult participants (9 males and 3 females) between the ages of 18 and 57 (mean age = 25.7±3 years) were recruited to take part in the study. All participant characteristic data are reported as mean ± standard deviation. All participants gave informed consent prior to their participation in the study. The study protocol was approved by the Institutional Review Board at Michigan State University. Participants were excluded from the study if they had any conditions which would prevent the completion of an MRI exam, including but not limited to, claustrophobia and metallic implants.

MRI data collection:

MRI data was collected using a 3T GE Excite MR system (GE Medical Milwaukee, WI) using an 8 channel head coil. Single-shot echo-planar images for fMRI were constructed using the following pulse sequence; TR= 2000 ms, TE = 35 ms, 22 cm FOV, matrix = 64x64, 90° pulse. Four millimeter axial slices were prescribed with 0.55 mm slice spacing for a total of 29-32 slices. Localizer and shimming sequences were performed prior to the start of the experimental protocol. Following the functional runs a final high resolution T1-weighed anatomy scan of the brain was completed in which the fMRI images were overlayed; Fast SPGR, TR = 2 sec, TE = 1.1 ms, TI = 500 ms, FOV = 22 cm, Matrix = 256 x 192 with 120, 1.5 mm slices, 15° flip.

Study Protocol:

Prior to entering the MRI room, each subject's supine resting systolic blood pressure was recorded using a manual sphygmomanometer. Subjects were then placed in a supine position on the MRI table. Head movement was prevented using padding and a neck brace. Prior to the start of the experimental protocol, each subject performed several 2-3 second handgripping MVCs with the dominant hand, using a rigid handgripping device. The force of each muscular contraction was acquired at 120 Hz using WinDaq data acquisition software (DATAQ Instruments). During the functional scans, target force levels were set between 10 and 40% of the measured MVC. A mirror was affixed to the head coil and force output was displayed using criterion line matching on a computer monitor in real-time so that the target force could be maintained.

The experimental protocol was divided into five separate functional runs. During the initial functional run a series of force varying, submaximal muscular contractions were held for 20 seconds and were used to identify the force-correlated region of the brain. Blood flow to the forearm was occluded using a rapid cuff inflator placed on the arm, proximal to the elbow during the second run to measure the effects of ischemia alone on central drive, without exercise. Runs 3, 4, and 5 included 3-minutes of sustained, isometric exercise at 15% MVC performed with (Run 4) and without (Run 3 & 5) blood occlusion.

Run 1 (FV) was a force varying run for the identification of the force-correlated regions of the motor cortex associated with the generation of grip force. The activation area from Run 1 was used to create a mask that was used to examine brain activation in Runs 2-5. Four separate 20-second isometric contractions performed at a target force of

20, 40, 10 and 30% of the subjects MVC force were interleaved with 40 seconds of rest periods between each contraction. To reduce the effects of muscular fatigue, each subject rested for 5 minutes before the start of the next run.

Run 2 (OCL) examined the effects of blood occlusion alone, without exercise on supraspinal drive. The blood pressure cuff was inflated to 80 mmHg above the subject's resting systolic blood pressure. Following the completion of the second run, each subject rested for 5-minutes to allow for blood redistribution back to the forearm.

Run 3 (EX1) examined the effects of prolonged, submaximal exercise on supraspinal drive. Immediately before EX1, a brief 2-second, handgripping MVC was performed. Following the MVC, a sustained 3-minute isometric contraction was performed at an intensity of 15% of the initial MVC during EPI acquisition. To assess muscular fatigue another MVC was performed immediately following the completion of the 3-minute contraction. To reduce the likelihood of muscular fatigue during subsequent runs, each subject rested for 8 minutes before the next run.

Run 4 (EX+OCL) examined the combined effects of prolonged, submaximal exercise and blood occlusion on supraspinal drive. At the start of EX+OCL, a brief 2 second MVC was performed, followed by a sustained, 3-minute, submaximal contraction at 15% of MVC initial force. Additionally the blood pressure cuff was inflated to a pressure of 80 mmHg above the subject's resting systolic blood pressure for the duration of the run. Immediately following the 3-min contraction, the blood pressure cuff was rapidly deflated and the subject performed an MVC. Each subject rested for 8 minutes before the fifth and final functional run.

To account for any changes in supraspinal drive that may have occurred due to muscular fatigue, EX1 was again repeated in Run 5 (EX2). Anatomical Fast gradient recalled echo (GRE) scans were taken immediately following the fifth and final run. To ensure changes in the BOLD response were not associated with changes in heart rate during the function runs, heart rate was recorded during each of the runs using an infrared pulse sensor that was placed on the subject's left index finger and interfaced with the MRI scanner.

Data Analysis:

The following was used to determine statistical power. Previously collected data from 8 participants of 4 repeated fMRI exams yielded a mean BOLD increase of 1.97% signal intensity with a standard deviation of 0.34%. Calculated statistical power from 12 participants using an effect size of 15% and an alpha of 0.05 would result in a statistical power of 0.7.

The Talairach-averaged location of the most highly force correlated contiguous clusters ($r > 0.50$) in the motor cortex during the handgripping exercise were identified during FV and this map was used for analysis of subsequent functional runs (EX1, EX+OCL & EX2). Analysis of functional neuroimaging (AFNI) software was used to extract the percent signal change from the map during each of the five runs [103]. BOLD response (calculated as the change in slope over the final 90 seconds of each run), muscular force and heart rate were compared using repeated measures ANOVA. Within the repeated measures ANOVA the following selected multiple comparisons were made using a Bonferroni-adjusted level of significance of $p= 0.017$; 1) OCL vs. EX+OCL, 2)

EX1 vs. EX+OCL & 3) EX2 vs. EX+OCL. Paired t-tests were performed to determine individual differences between selected variables as the study was underpowered for all possible comparisons. Pearson correlations were used to determine if any relationships existed between the BOLD response for each of the functional runs and heart rate. All data are represented as mean ± SE unless stated otherwise.

Results

Participant characteristics:

The average age and weight of the participants was 25.7±3 years and 72.4±13.6 kg, respectively. Average recorded systolic and diastolic blood pressures were 119.2±10.5 and 73.9±6.9. All participants in the study were right handed. Participant characteristic data are presented as mean ± standard deviation.

Force Varying Contractions:

Force-correlated regions of the motor cortex were defined as the Talairach-averaged location of the most highly force-correlated voxel cluster ($r>0.5$) during FV. A strong correlation was observed between changes in muscular force and fMRI signal intensity during FV ($r = 0.70$; $p < 0.001$). The location of the most highly force-correlated cluster was in the left precentral gyrus centered at location S55, P23, L37 (Figure 1). In every subject the most highly force-correlated cluster was centered in this region, although typically the cluster included both pre-central (motor) and post-central (somatosensory) voxels and therefore is identified as S1+M1. Muscular force produced and percent changes in fMRI signal intensity are presented in Figure 2. Muscular force produced during FV was standardized as the percentage of force relative to the initial MVC. Measurements of muscular force during the force varying contractions were 18.2±2.6, 35.3±4.9, 12.3±0.7 and 27.9±4.1 % for the respective target forces of 20, 40, 10 and 30% MVC. Average changes in fMRI signal intensity during FV are presented as percent changes from baseline. The increase in the BOLD response above baseline for the force varying contraction were 0.92±0.35, 1.66±0.57, 0.55±0.22, & 1.25±0.41%, for the respective target forces of 20, 40, 10 and 30% MVC.

Blood occlusion and exercise:

The percent change in BOLD signal intensity per minute during the final 90 seconds of exercise during OCL, EX1, EX+OCL & EX2 was 0.27±0.13, 0.10±0.05, 0.78±0.14 & 0.17±0.12 %, respectively (Figures 3-5). Following a repeated measures ANOVA with a Bonferroni adjustment, significant differences were observed during the final 90-seconds of exercise between the following comparisons; 1) EX+OCL > EX1 ($p = 0.002$) & 2) EX+OCL > EX2 ($p = 0.008$). Although no significant differences were observed between OCL+EX vs. OCL, a trend was observed where brain activation was greater following OCL+EX vs. OCL ($p = 0.123$). In this regard, an independent paired t-test revealed brain activation to be greater following OCL+EX vs. OCL ($p = 0.021$). No significant differences were observed between EX1 and EX2 ($p = 0.62$). Mean recorded heart rates during OCL, EX1, EX+OCL & EX2 were 69±2, 72±3 & 69±3 bpm respectively. Following a repeated measures ANOVA no differences in heart rate were observed between EX+OCL compared to either the EX1 or EX2 conditions ($p = 0.13$ & 0.38 respectively). A significant difference in heart rate was observed between OCL vs. EX+OCL ($p= 0.005$), however no correlations were observed between heart rate and fMRI signal intensity ($r = -0.45$; $p = 0.15$ & $r = -0.25$; $p = 0.44$, respectively).

Submaximal forces, represented as a percentage of MVC, during the 3 minute sustained muscular contraction for each of the condition (EX1, EX+OCL & EX2) were 14.31±0.08, 14.69±0.05, & 14.43±0.09 %, respectively. No significant differences were observed in submaximal force between any of the functional runs or between the beginning and end of exercise. Following multiple paired t-tests with a Bonferroni adjustment, significant reductions in MVC ($n = 8$) were observed after EX1, EX+OCL &

EX2 ($p = 0.015$, 0.008 & 0.002 respectively; Figure 6). Although reductions in MVC were not significantly different between any of the conditions, EX+OCL showed the greatest percent reduction in MVC force compared to EX1 and EX2 (12.2±9.6% vs. 7.4±7.2% and 8.1±4.5%, respectively). The MVC measurements were not recorded for two subjects prior to Runs EX1 & EX2 and for one additional subject in EX+OCL; therefore these cases have been excluded from all comparisons related to changes in MVC force before and after each run.

Discussion

The purposes of this study were to 1) establish if fMRI can provide a measure of central motor drive and 2) determine if altered central motor output contributes to muscular fatigue during submaximal exercise. The observed increase in the BOLD response during ischemic exercise versus exercise alone suggests that increases in fMRI signal intensity are reflective of an increased supraspinal drive during fatiguing exercise. In addition, the development of muscular fatigue during a sustained, submaximal contraction was not caused by a reduction in central output, rather central output increased to match demand. These findings suggest that fMRI can be used as a measure of supraspinal drive during exercise.

The known correlation between muscular force and brain activity allowed for the identification of active regions of the brain. A high correlation was observed during FV between muscular force and fMRI signal intensity in the S1+M1 during the handgripping exercise. These results are similar to earlier investigations examining the relationships between rCBF and central motor output. Both Positron Emission Topography (PET) and fMRI techniques rely on changes in cerebral blood flow to measure changes in brain activity. During simple motor tasks, such as finger tapping and finger opposition, there is a high correlation of brain activation patterns measured by fMRI and PET indicating that fMRI can be used as a measure of central motor output [95, 97]. Furthermore, increases of brain activation measured by fMRI are linearly related to the degree of muscular force that is produced. In this regard, increases in muscular force during a handgripping exercise between 10-80% of MVC strength results in a linear increase in the BOLD response in the motor-related regions of the brain [24, 30, 31]. Although the

relationships observed between the BOLD response and muscular force are only correlational, perturbing force generation and observing related signal changes in the associated region of the motor cortex supplies strong evidence that fMRI signal intensity is representative of central motor output during exercise.

Greater increases in fMRI signal intensity were observed in the S1+M1 during exercise plus occlusion vs. exercise alone. Although no differences were observed during the first 90 seconds of exercise between each of the runs, the BOLD response during the final 90 seconds of exercise was approximately 3-fold greater during ischemic vs. non-ischemic exercise. The increase in fMRI signal intensity observed during the final 90 seconds of exercise during the ischemic condition occurred despite the maintenance of a constant level of muscular force. Previously, it has been hypothesized that during fatiguing exercise an increase in central drive is required to recruit higher threshold motor units in order to maintain a constant level of muscular force as lower threshold motor units begin to fatigue [20, 22, 24, 27, 29]. In this regard, studies using EMG analysis have shown that during submaximal contractions of a constant force the EMG signal is amplified, indicating the development of muscular fatigue [10, 27, 29, 104]. This suggests that voluntary effort is increased during prolonged, fatiguing contractions in order to recruit higher threshold motor units so a constant level of muscular force can be maintained. Furthermore, Liu et al (2003) observed similar increases in both EMG activity and BOLD signal intensity during sustained handgripping contractions performed at 30% MVC strength of approximately 4 minutes [22]. In addition, during intermittent handgripping contractions performed at 30% of MVC force, the BOLD response from the contralateral sensorimotor is increased during the later stages of the exercise task

suggesting central motor drive increases when muscular fatigue develops [20, 22]. The results from the present study further support that corticomotor drive is increased during fatiguing exercise. The increase in supraspinal drive likely results in the recruitment of higher threshold motor units to preserve force output during exercise.

The design of the present study was unique in that central drive was measured during exercise under both ischemic and non-ischemic conditions. Increases in motor unit recruitment are dependent on the metabolic state of the muscle. EMG activity remains constant following submaximal handgripping contractions if blood flow to the muscle is not occluded. However, ischemic exercise under the same conditions increases the EMG activity of the contracting muscles, suggesting that the metabolic state of the active muscle plays a role in the regulation of motor unit recruitment [28]. Therefore, the occlusion of blood flow to the working muscle is likely to induce a greater amount of muscular fatigue, resulting in an increase in central motor drive in order to recruit higher threshold motor units so that the same level of force output can be maintained. If the ischemic condition did result in a greater increase in muscular fatigue, it would be expected that MVC loss would be greater following EX+OCL compared to exercise alone. Although no significant differences in MVC were observed between any of the functional runs, a trend was observed in which the greatest amount of force loss occurred following the occlusion of blood flow. This trend is supported by previous data of a similar experiment in our lab where the pH of the forearm, measured by ^{31}P MRS, was significantly reduced following a 3-minute sustained contraction at 15% MVC when blood flow is occluded compared to exercise alone [105]. Interestingly the pH of the forearm muscle was not significantly reduced until after approximately 90 seconds of

exercise. In the current study, the slope of the fMRI signal intensity remained constant for the first 90 seconds of the exercise task and did not increase until the final 90 seconds of exercise. Therefore it is likely that muscular function did not become compromised until after approximately 90 seconds of exercise, at which time central motor drive was increased so that higher threshold motor units could be recruited in order to maintain the target level of muscular force.

An increase in the BOLD response was observed following blood occlusion without exercise, suggesting that some of the increase in central drive may have been caused by an increase of afferent feedback from the muscle in response to ischemia. In this regard, an increase in afferent activity to the sensorimotor cortex may have led to an increase in cerebral blood flow (rCBF), increasing fMRI signal intensity. However, Williamson et al (1996) reported that ischemia alone does not increase rCBF [106]. Because the BOLD response is dependent on changes in regional blood volume and oxygenation in active regions of the brain, it would be doubtful that increases in fMRI signal intensity would be observed following the inflation of the BP cuff alone. One potential reason for the differences observed in central activation in the present study and the previous study is the ischemic protocol that was used. Williamson et al (1996) induced muscle ischemia during the final 15 seconds after a 2 minute muscular contraction, whereas in the present study ischemia was induced during 3 minutes of rest. It is possible that the present protocol induced a greater amount of muscle ischemia, leading to an increase in afferent feedback resulting in an increase of the BOLD response. However, this was not observed during 3 minutes of ischemia alone without exercise. Regardless, the increase in fMRI signal intensity following ischemic exercise was still

significantly greater than the additive effects of exercise alone plus those observed following occlusion alone. This indicates that the increase in central motor output following ischemia was most likely due to an increase in motor unit recruitment as opposed to an increase in afferent activity to the sensorimotor cortex.

It is also unlikely that the increase in fMRI signal intensity observed during ischemic exercise was caused by an order effect throughout the exercise protocol. Although it would be possible that the participants could experience a greater amount of muscular fatigue in the later Runs of the exercise protocol, a second "exercise only" Run was performed following exercise with blood occlusion. In this regard, no significant differences were observed between Runs 3 and 5. Furthermore, Run 4 was significantly greater than both of the exercise only runs, indicating that there was no effect of order on increasing cortical activity over time. Differences in heart rate during the individual Runs may have the potential to influence cortical drive throughout the protocol. However, although significant differences in heart rate were observed between each of the Runs, heart rate was not correlated with fMRI activity.

In conclusion, ischemic exercise compromises peripheral muscle function resulting in an increase in central motor drive in order to maintain the same target force. This suggests that central motor drive is not compromised during sustained, sub-maximal, fatiguing exercise in young healthy adults. Overall these results show that fMRI can be used to study central motor drive and may be a useful tool to study conditions in which central motor drive may be impaired. For example, while this study shows that central drive increases to maintain force during submaximal exercise, it is unclear if maximal cortical drive is affected following fatiguing exercise. Therefore

future studies are needed to measure changes in maximal cortical drive following fatiguing exercise, such as prolonged endurance exercise. This may be accomplished by measuring brain activation during maximal contractions before/after strenuous exercise that elicits muscular fatigue.

Chapter 4

Study#2: fMRI of Central Motor Drive during Prolonged Running

Introduction

Muscular fatigue is defined as an exercise-induced reduction in the ability of a muscle or muscle group to generate force or power [1]. Voluntary muscular contractions involve a complex series of events that are initiated in the motor cortex and end with the production of force by the skeletal muscle. The development of muscular fatigue begins almost immediately following the onset of exercise and can originate from both peripheral and central mechanisms [2, 3]. Peripheral fatigue is referred to as the loss of muscular force that occurs at or distal to the neuromuscular junction and can be thought of as fatigue within the muscle itself [3]. The inability of the central nervous system (CNS) to adequately drive the muscle to produce force during a muscular contraction is referred to as central fatigue [3]. Central fatigue can originate from any site that is proximal to the neuromuscular junction and can therefore result from either a reduction in cortical drive from the motor cortex (supraspinal fatigue) or modulations in central drive that occur at the level of the spinal cord (spinal fatigue).

Although muscular fatigue has been the subject of numerous scientific investigations, its causes remain poorly understood [2]. Until recently, most studies have focused on peripheral mechanisms of fatigue, such as substrate availability, excitation contraction coupling and the excitability of the peripheral muscle; however, little attention has been given to the central mechanisms of fatigue. For example, increasing intramuscular glycogen stores by the consumption of a high carbohydrate diet enhances

exercise performance and decreases muscular fatigue [47]. Popular belief is that substrate depletion within the muscle itself is the direct cause of fatigue. However, the highest work rates are often observed at the end of exercise, when muscle glycogen would not be available to fuel intense exercise [107]. It is believed by some that glycogen depletion in contracting muscle fibers initiates an inhibitory afferent response to the hypothalamus, resulting in a reduction in muscular power [36]. This suggests that dietary factors, such as carbohydrate intake, may reflect central, rather than peripheral, mechanisms of fatigue.

The development of central fatigue during exercise has been measured through various techniques, including twitch interpolation. Twitch interpolation involves the electrical stimulation of a peripheral nerve during a maximal voluntary contraction (MVC). Increases in muscular force observed following the stimulation of the peripheral nerve during an MVC indicate that central drive is insufficient to fully contract the muscle [1, 2, 8, 9]. Using twitch interpolation, reductions in central drive have been observed following a period of prolonged exercise such as running and cycling [13-18]. Saldahna et al (2008) observed a 17% reduction in MVC of the plantar flexors and a 19% reduction central drive following 2 hours of running at 75% of VO_2 peak. The reduction in MVC was highly correlated with the reduction in central drive ($r=0.87$), indicating that the reduction in maximal voluntary plantar flexor muscle strength was primarily related to central mechanisms [18]. Additionally, two hours of cycling exercise performed at 65% of VO_2 peak elicits reductions in both muscular force and central activation in trained cyclists, suggesting that the development of muscular fatigue was at least partially caused by a reduction of neural input to the muscles [13, 14]. The time course for the

development of muscular fatigue appears to be related to the intensity of the work bout. Following 5 hours of prolonged cycling at 55% VO_2 max, significant reductions in neural drive are not evident until after the 4^{th} hour of exercise [16]. Although the precise relationship between the onset of central fatigue during running in regards to the time and intensity of the work bout has yet to be established, exercising at an intensity greater than 55% VO_2 max results in an earlier onset of central fatigue. In this regard, between 90-120 minutes of running exercise at an intensity of 70-80% VO_2 max elicits significant reductions of central drive in trained runners [17, 18]. However, currently it is unknown whether these reductions in central drive are caused by spinal or supraspinal mechanisms.

Some evidence suggests that muscular contractions of a sufficient duration and intensity result in a reduction in supraspinal drive following fatiguing exercise. For example, reductions in both muscular force and EMG activity are observed following a sustained, 70-minute muscular contraction at 5% MVC of the elbow flexors. Following the submaximal contraction, EMG activity rose by approximately 60-80% and MVC strength was reduced by approximately 72%. Electrical stimulation of the motor cortex using transcranial magnetic stimulation (TMS) elicited an increase in MVC strength and accounted for almost two thirds of the reduction in muscular force [19]. Similarly, Gandevia et al (1996) observed an approximate 10% reduction in muscular force following a sustained, three minute, MVC of the elbow flexors [11]. Cortical stimulation of the motor cortex by TMS resulted in an increase in maximum "voluntary" muscular force, indicating "in part" muscular fatigue is caused by a reduction in cortical output from the motor cortex. Unfortunately, all motor regions of the brain associated with various exercise tasks cannot be stimulated using TMS. At this time, TMS has mostly

been applied to relatively few muscle groups whose associated brain regions are located superficially to the brain, such as the elbow flexors as well as the wrist flexors and extensors [1, 3, 12]. Because specific regions of the motor cortex that control other muscle groups (such as the legs) lie deep within the brain, they cannot be easily stimulated using TMS. Therefore, changes in supraspinal drive that occurs during locomotor exercises involving the legs, such as running and cycling, are currently unknown.

Because reductions in central motor drive have been implicated in contributing to muscular fatigue and current techniques cannot always differentiate between supraspinal vs. spinal fatigue, there is a need to develop further techniques to access brain function during exercise [9]. In this regard, functional magnetic resonance imaging (fMRI) is becoming an increasingly popular tool to investigate brain function, including motor function during muscular fatigue, and may provide a non-invasive measure of central motor drive [20-25]. Increases in activated regions of the brain are accompanied by an increase in blood volume and blood oxygenation, which can be used to construct an image of brain activation using fMRI [25]. Because fMRI provides a high degree of spatial and temporal resolution in activated brain regions, it may be ideal to assess changes in supraspinal output during prolonged locomotor activity [20-23, 26].

Although fMRI may provide a valuable tool for measuring supraspinal drive during prolonged locomotor exercise, the use of fMRI is not met without technical challenges. For instance, the subject must lie motionless inside the bore of the magnet, therefore changes in central drive during locomotor exercises such as running and cycling cannot be measured during the exercise task [26]. Previous studies using twitch

interpolation have indicated that central drive is reduced for up to 4 hours for the involved muscle groups following the end of exercise, with no significant differences observed between 5 and 30 minutes [15-17, 33]. Therefore, although measurements of supraspinal drive would not be able to be performed during the exercise task, fMRI, measurements taken within 30 minutes following exercise would most likely reflect the level of central drive at the end of exercise.

The specific aim of this study is to use fMRI to assess changes in supraspinal drive to the legs following 90 minutes of running at an intensity of 75% VO_2 max. It is hypothesized that the mean percent change in fMRI signal intensity (SI) in the motor cortex will be decreased following 90 minutes of running compared to before exercise. Additionally, because dietary factors may be related to the development of central fatigue, dietary intake was assessed and correlations between nutritional intake (carbohydrate, protein, fat & total energy intake) and fatigue were determined.

Methods

Participants:

Twelve healthy adult males between the ages of 18 and 35 years were recruited through word of mouth and various sports clubs from the University community to participate in this study. Previous studies measuring the affects of central fatigue following prolonged running have only included male participants [13-15, 17, 18, 32, 67]. Because gender effects are unknown at this time, only males were included in this study. Participants were excluded from the study if they have any condition preventing an MRI exam, including but not limited to, metallic implants or prostheses and claustrophobia. Additional exclusion criteria included any chronic condition such as heart disease, joint disorders or neurological disorder which would prevent performance of a 90 minute run or leg exercise. Prior to the study each participant was informed of all of the risks associated with the study and signed a document of informed consent. The experimental protocol was approved by the institutional review board at Michigan State University.

Outline of study design:

During the course of the study the participants performed two separate testing sessions. During the initial testing session, aerobic fitness was assessed using a graded treadmill test to volitional fatigue. During the second session the participant was instructed to run on a treadmill at 75% of his maximal aerobic capacity for 90-minutes. fMRI measurements were taken immediately before and after the 90-min run during a series of isometric plantar flexion contractions to assess the effects of prolonged running

on central motor drive. Previous studies using twitch interpolation have observed reductions in central drive following prolonged running of a similar intensity and duration [16-18]. Participants were instructed not to engage in any intense physical activity for a period of 24 hours prior to each testing session.

Initial testing session:

After a detailed explanation of the study and signing of the consent form, each participant's height and weight was recorded and maximum oxygen consumption was measured using a graded maximal treadmill test. Prior to the maximal treadmill exercise test each subject performed a brief warm-up at a speed of 3.5 mph for 3 minutes. Immediately following the warm-up period, the treadmill speed was increased to a self-selected speed (equivalent to a speed the participant would expect to run at for 30 minutes) and remained constant throughout the remainder of the test. After running for 2 minutes at the participant's self-selected pace, the grade of the treadmill increased by 1% every minute thereafter until volitional exhaustion was achieved. Heart rate (HR) was recorded using a Polar HR telemetry system (Gays Mills, WI) and maximal oxygen consumption (VO_2 max) was measured using a Parvo Medics True One 2400 metabolic measurement cart (East Sandy, UT). VO_2 max was defined as the highest 20-sec average recorded VO_2 during the test. To ensure a maximal effort was achieved, subjects had to meet two of the three criteria: 1) 95% of age-predicted maximal heart rate; 2) a respiratory exchange ratio (RER) greater than or equal to 1.15; or 3) a plateau in VO_2 max.

Following the maximal aerobic fitness test, each participant was positioned prone on a mock scanning table and was familiarized with the MRI protocol to be performed during the experimental session. All force measurements were taken with the leg slightly elevated (approximately 25 cm above the table) with the ankle rigidly fixed at a 90° angle. Inelastic Velcro straps were used to maintain foot position. In order to maximally recruit the gastrocenemius muscle, force measurements were performed with the legs in a straightened position. The participant performed several 1-2 second maximum isometric plantar flexion contractions using the right leg. The force of the isometric contractions was measured using a custom-made force measuring device (the "force boot"). Additionally, the participant performed several sustained, 8-second MVCs. To ensure that the target intensity was achieved during each muscular contraction visual feedback was provided to the participant using an LCD display. These familiarization procedures were necessary to reduce the possibility of head movement that may occur during force measurements taken during the experimental session. However, no fMRI data was collected at this time.

Experimental Session:

During the experimental session the participant performed 90-minutes of running on a motor-driven treadmill at an intensity corresponding to 75% of the participant's VO_2 max. Due to the possibility of VO_2 drift during exercise, participants were allowed to adjust the speed of the treadmill within ±10% of their predicted pace during the run. To ensure that adequate exercise intensity was maintained during the run, VO_2 measurements were collected during the final 5-minutes of each 30-minute interval

during the exercise task. Speed and heart rate were recorded continuously throughout the run. To reduce the affects of dehydration during exercise water was provided ad libitum, however, sports drinks were not allowed before, during or after the run.

fMRI measurements:

fMRI measurements were recorded prior to and immediately following 90-minutes of treadmill running. Each participant was positioned on the MRI table with the same force boot and leg positioning as during the initial testing session. To minimize head movement, the participant's head was firmly fixed within the MRI coil using inelastic tape and foam padding, in addition to a neck brace that was fitted to the participant. The head coil was fitted with a mirror such that the subject could see and respond to instructions presented on a computer display screen, and the subject was positioned in the magnet bore. To assess muscular strength the participant performed several, one-two second plantar flexion MVCs. The participant's MVC was defined as the average of the two strongest muscular contractions. All subsequent muscular contractions were defined as a percentage of the pre-exercise MVC strength. Two separate functional exercise tasks were performed during the fMRI exam. The first exercise task that was performed was a series of MVCs. Each participant performed a series of four, 8-second MVC plantar flexion contractions of the right leg interspersed with 52-seconds of rest. A computer monitor was positioned behind the participants, instructing them to rest and when to contract. No feedback of force was provided during the MVC runs. A mask was made from the MVC run for the regions with a force correlation or $r \geq 0.35$.

The second exercise task consisted of a series of force varying contractions. Four separate 20-second isometric contractions performed at a target forces of 10, 30, 15 and 20% of MVC, respectively, were interleaved with 40 seconds of rest. Visual feedback was displayed using criterion line matching on a computer monitor in real-time so that the target force could be maintained. Following the second functional run, the participant was instructed to lie motionless in the bore of the magnet for 8-minutes, so that a final high resolution, T1-weighed, anatomy scan of the brain could be performed in which the fMRI images were overlayed. The same protocol was followed immediately after the completion of the 90-minute running task with the exception that the force varying contractions were not performed.

The following pulse sequences were performed during the fMRI exam: 1) Fast 3-Plane gradient echo localizer sequence (Minimum TR/TE, 20 degree pulse, 256x128 acquisition matrix, 1 NEX, five 5 mm thick slices in each plane). Approx. 1 min scan time); 2) A coronal T1-weighted spin-echo sequence (Thirty-two 4.5 mm slices, 0.5mm gap, minimum TR=100 ms, minimum TE, 20 degree pulse, 256x128) was used to confirm anatomical positioning for the subsequent functional scans (approx. 1.5 min scan time); 3) Higher Order Shim (i.e., a fast low resolution volumetric phase mapping scan, similar to #1 above (approx. 1 min scan time); 4) Single-shot echo-planar functional scanning (EPI, 64x64 acquisition matrix, coronal plane, TR=2s, TE= 32 ms, same slices as scan 2, 90° degree pulse). This scan was continuously acquired during each of the exercise tasks; & 5) High resolution T1-weighted anatomical scan (spoiled gradient recalled echo, pulse flip = 8°, TR 4.4 ms, TE=1.1 ms, TI=500 ms, 1.5 mm axial slices, 256x192 matrix).

Nutrition Assessment:

To account for how nutritional status may impact the development of fatigue during prolonged exercise, a three-day dietary assessment was performed prior to the 90-minute run. Following the initial testing session participants were given a dietary log and asked to record their nutritional intake for the 72 hours prior to the experimental testing session. Participants were provided with instructions and a handout regarding how to record their nutritional intake as well as quantifying portion sizes. The three-day dietary assessment was analyzed using ESHA food processor software (ESHA Research, Salem, Oregon) for the following variables; 1) Total Calories, 2) carbohydrate (g/kg), 3) protein (g/kg) and 4) fat (g/kg).

Data Analysis:

Previously collected data from 8 participants of 4 repeated fMRI exams yielded a mean increase of 1.97% signal intensity with a standard deviation of 0.34%. Calculated statistical power from 12 subjects using an effect size of 15% and an alpha of 0.05 would result in a statistical power of 0.7.

Following motion correction, the Talairach-averaged location of the most highly force correlated contiguous clusters ($r > 0.35$) in the motor cortex were identified during MVC and this map was used for analysis. Analysis of functional neuroimaging (AFNI) software was used to extract the percent signal change of the activated regions of the motor cortex before and after the running protocol [103]. The percent signal change was extracted during each 8-sec contraction. The signal intensity immediately before each contraction was established as the baseline. It was expected that running 90-minutes

would induce muscular fatigue of the plantar flexors. Therefore, it was hypothesized that running 90-min at 75% VO_2 max decreases plantar flexor force in trained runners. Additionally, it was hypothesized that the mean percent change in signal intensity (SI) in the force-correlated region of the motor cortex during the 8-second MVC's would be decreased after compared to before the run. This hypotheses was tested using paired t-tests with the level of significance set at $p = 0.05$ (SPSS; version 17). Muscular and central fatigue indexes were calculated using the following formula: [1-(post/pre*100)]. A Pearson correlation was used to identify associations between muscular and central fatigue and the following dietary variables; 1) protein intake (g/kg), 2) carbohydrate intake (g/kg), 3) fat intake (g/kg) and 4) total energy intake (Kcals).

Results

Participant Characteristics:

Participant characteristic data are presented in Table 1. Twelve participants between the ages of 21 and 34 years (mean age = 28.0±4.4 years) were recruited to take part in the study. Due to movement artifact three subjects were excluded from the final data set, and all analyses were performed on the remaining nine participants. The average weight of the participants was 69.6±9.1 kg. The average VO_2 max was 62.3±7.4 ml/kg*min^{-1}. The mean VO_2 during the 90-minute run was 45.08±5.52 ml/kg*min^{-1} with a VO_2 of 45.4±5.3, 44.9±5.7 and 44.9±5.9 ml/kg*min^{-1} observed at 30, 60 and 85 min during the exercise protocol. Mean heart rate and RER responses during the run were 159.3±14.3 bpm and 0.87±0.04, respectively.

Force varying contractions:

Brain activation regions were defined as the location of the most highly force-correlated voxel clusters for each individual participant (r= 0.35-0.65; mean = 0.48±0.01) during the force varying contractions. Three force-correlated voxel clusters were identified during the force varying run. The first force-correlated cluster (Region 1) was located in the left SMA (BA6) and left medial frontal gyrus; centered at location L1, P7, S54 (Figure 7). The second force-correlated cluster (Region 2) was located in the left precentral gyrus and BA6 (supplementary motor area); centered at location L0, P6, S57. The third force-correlated cluster (Region 3) was located in the left paracentral lobule, including the BA4 and BA6; centered at location L8, P33, S64. Additionally, the inferior parietal lobe (including the BA7) was activated during the force varying contractions.

However, this region is involved in motor tasks that involve sensory feedback and visual-motor coordination, and was therefore excluded from further analyses. During the force varying run, the inferior parietal lobe was also activated. However, activation in this region was not observed during either MVC run.

The average MVC force obtained prior to the subsequent fMRI testing was 442.8±99.6 N. Muscular force produced during the force varying contractions was standardized as the percentage of muscular force relative to the initial MVC. Measurements of muscular force and brain activation of the motor-related brain regions are shown in Figure 8. The average muscular forces during the force varying contractions were 8.1±0.7, 26.3±3.1, 12.7±0.9, & 17.4±1.3% for the respective target forces of 10, 30, 15, & 20% MVC. Average changes in BOLD signal intensity (SI) from Regions 1 and 3 are standardized as percent changes from baseline from the Pre MVC run. The percent changes in SI for Region 1 during the force varying contractions at 10, 30, 15 and 20% MVC were 100.87±0.08, 101.22±0.08, 101.01±0.10, and 100.98±0.13%, respectively. No significant differences in SI were observed between any of the force varying contractions in Region 1. In Region 3 the percent changes in SI during the force varying contractions of 10, 30, 15 and 20% MVC force were 100.67±0.07, 101.00±0.05, 100.79±0.07 and 100.67±0.07%, respectively. A trend was observed where the 30% MVC was higher than MVCs of 10% ($p = 0.12$), 15% ($p= 0.11$) and 20% ($p= 0.11$). No significant differences were observed in SI between any of the other force varying contractions.

MVCs Pre and Post exercise:

MVC force and brain activation before and after 90 minutes of running are shown in Figure 9. Brain activation maps for the MVC runs are shown in Figure 10. The averages of the 8-sec MVC forces before and after the 90-min run were 342.67±61.59 and 288.63±42.64 N, respectively. Following the 90-min run, approximately a 16% reduction in MVC force was observed ($p = 0.04$). Brain activation during the maximal contractions in Regions 1 and 2 are presented in three ways; 1) average of the full 8-sec MVC, 2) average of the four highest continuous seconds and 3) average of the highest two continuous seconds (Tables 4 and 5). The functional MRI exam was completed in less than 30 minutes following the run for all participants with a mean time of 22.7±2.5 min. No significant differences were observed in SI in either Region 1 or Region 3 immediately following exercise. However, during the 8-sec MVC, two participants showed no signs of muscular fatigue. When these participants were excluded from the analysis, a trend was observed where the BOLD response was reduced following exercise in region 3 ($p = 0.12$), but no differences were observed in Region 1. No correlation was observed between muscular and central fatigue ($r= 0.21$; $p = 0.46$).

Dietary Analysis:

Dietary analysis data are presented in Table 3. One participant did not complete the dietary assessment prior to the 90-min run and was excluded from the analysis (n=6). The average energy intake for the eight remaining participants was 2801.3±193.7 Kcal. The average percentage consumption of carbohydrate, fat, and protein were 56.9±3.0, 27.4±2.9, and 15.3±1.3%, respectively. The total number of grams of carbohydrate, fat,

and protein were standardized to the participant's body weight in kilograms and were 5.6±0.5, 1.1±0.2 and 1.5±0.2 g/kg, respectively. A Pearson correlation did not reveal associations between muscular or supraspinal fatigue and any of the dietary variables. However, when excluding the subjects who did not have muscle fatigue, a negative correlation was observed between fat intake and central fatigue ($r = -0.84$; $p = 0.04$). A trend was observed where total caloric intake was negatively correlated with both muscular and central fatigue ($r = -0.74$; $p = 0.06$ & $r = -0.67$; $p = 0.15$, respectively). Protein intake was correlated with muscular ($r = -0.76$; $p = 0.08$) but not central fatigue ($r = -0.33$; $p = 0.53$). No correlations were observed between carbohydrate intakes with either muscular or central fatigue.

Discussion

The purpose of this investigation was to assess changes in supraspinal drive to the legs following 90-minutes of prolonged running exercise. Previous investigations have observed a reduction in central drive to the legs following prolonged running [16-18]. However, due to methodological limitations it was uncertain whether reductions in central drive were mediated through spinal or supraspinal processes. The results from this study demonstrate that supraspinal drive may be reduced following prolonged running activity, contributing to a reduction in muscular force.

Activated brain regions:

Correlations between muscular force production and increases in SI were observed in three distinct regions of the brain. Region 1 included the left SMA (BA6) and left medial frontal gyrus. Region 2 was located in the left paracentral gyrus and BA6. Although region 2 was associated with the production of muscular force during the force varying contractions, it was not associated with the generation of muscular force during the 8-sec MVCs. This may be because Region 2 is closely mapped to the motor cortex for muscular contractions involving the hands and fingers [108, 109]. It is possible that the participants flexed their hands and fingers in conjunction with the 20-sec, force varying, plantar flexion contractions, resulting in an increased activation in this region of the brain and was not directly associated with the plantar flexion contractions. Region 3 was located in the left paracentral lobule, including the BA4, BA6 and the post central gyrus. During the force varying run, the inferior parietal lobe was also activated. However, this region is involved in motor tasks that involve sensory feedback and visual-

motor coordination. Because visual feedback was displayed during the force varying run using criterion line matching, in which the participant was instructed to maintain a given level of force output, the criterion line matching task may have activated this region of the brain independent of the plantar flexion contractions. Previous studies have observed activation regions similar to Regions 1 and 3 in the present study, and therefore only Regions 1 and 3 were included in the analysis [110-113].

Force varying contractions:

Changes in muscular force were correlated with changes in brain activation during the force varying run. Interestingly, brain activation was similar between the varying levels of muscular force between 10-30% of MVC strength in both regions 1 and 3. However, in Region 3, a trend was observed in which 30% MVC force was higher than the target forces of 10, 15 and 20% MVC strength. Similar trends were not observed in Region 1 in response to the varying degrees of muscular force. This is likely because this region does not include the primary motor cortex, but rather areas that assist motor output. This is in contrast to previous studies which have observed a linear increase in muscular force and the BOLD response during maximal handgripping contractions [24, 30, 31]. Additionally, contractions of the right index finger between 15 and 70% of MVC force are positively correlated with an increase in the BOLD response, further suggesting that increases in the BOLD response are correlated with muscular force [31]. In contrast, static finger flexion between 5 and 10% of MVC strength is poorly correlated with the BOLD response, suggesting a minimal threshold of force must be reached before a linear increase in brain activation is observed [24]. It is important to note that previous

studies have measured associations between muscular force output and the BOLD response in muscle groups involving the hands and fingers, whereas the current study used the plantar flexors. It is possible that levels of force used during the force varying contractions (<30% of MVC strength) were insufficient to induce significant differences in the BOLD response in activated brain regions during plantar flexion tasks. However, the trend observed in brain activation was greater following an MVC of 30% compared to MVCs between 10-20%, indicating that changes in brain activation may be poorly correlated with plantar flexion forces of < 30% MVC strength. In a separate experiment the force varying contractions were performed at higher exercise intensities of 15, 65, 30 and 90% MVC of the plantar flexors. In this experiment a distinction was observed between the level of brain action in Region 3 between 65 and 90% MVC force compared to all other force outputs (Figure 11). However, this experiment was only performed on a single participant, so no decisive conclusions can be made regarding correlations between muscular force and the BOLD response during plantar flexion. Because this is the only known study to have examined the relationship between the plantar flexion force and the BOLD response, future studies are needed to assess these relationships.

Muscular and supraspinal fatigue following exercise:

Approximately a 16% reduction in muscular force was observed following the 90-min run compared to before. Similar reductions in muscular fatigue have been reported by Saldhana et al, who observed an approximately 18% reduction in plantar flexion force following 2 hours of running at 75% $VO_{2\ peak}$ [18]. Despite the reduction in muscular force, no changes in brain activation were observed in either Region 1 or 3. It is possible

that central drive recovered between the end of the 90-min run and the time it took to complete the second fMRI exam. The second fMRI exam was completed in less than 30 minutes for all the participants with an average time of < 23 minutes. However, reductions in central drive are not significantly different between 5 and 30-minutes after exercise, making this unlikely [17]. It is also possible that the results of the study were influenced by a single participant whose MVC was approximately 10% greater following exercise compared to before. Due to the increase in muscular force observed following exercise, it is likely that this participant had given a submaximal effort during the pre condition. If this subject was excluded from the analysis, a reduction in supraspinal drive was observed in Region 3. However, no differences in brain activation were observed in Region 1 even after the exclusion of this single participant. A strong correlation was observed between muscular and central fatigue indexes, indicating the loss of muscular force was associated with a reduction in supraspinal drive. These results suggest that supraspinal drive is reduced following 90 minutes of prolonged running at 75% VO_2 max.

Previous studies have reported reductions in central drive following prolonged exercise, including running and cycling [13-18, 32, 67]. Reductions in central drive have been attributed to account for up to 25% of the reduction in muscular force during exercise [3]. However, due to methodological limitations, it is difficult to discern whether the reductions in central drive were mediated through spinal or supraspinal mechanisms. For example, many of the previous studies have examined the issue of central fatigue using twitch interpolation, in which a peripheral nerve is electrically stimulated following a MVC. During an MVC, increases in muscular force observed

following the electrical stimulation of the motor nerve signifies that central motor drive to the muscle is insufficient to fully contract the muscle. However, the origination of the site of fatigue can occur anywhere upstream from the site of stimulation and therefore cannot be used to differentiate between spinal or supraspinal fatigue [1]. Cortical stimulation of the motor cortex during fatiguing handgripping contractions using TMS results in an increase in force output during an MVC, suggesting that central fatigue is at least "in part" mediated through supraspinal mechanisms [11, 19]. However, motor regions associated with leg movements are difficult to stimulate using TMS, therefore changes in supraspinal drive during locomotor tasks such as running and cycling are difficult to determine. The results of the present study show that fMRI may be a valuable tool to investigate the origin of central fatigue during prolonged physical activity. The reductions in the brain BOLD response following prolonged exercise further supports that the development of central fatigue during prolonged exercise is mediated through supraspinal mechanisms.

The use of fMRI may also provide insight regarding specific regions of the brain that are associated with the development of muscular force output. Both Regions 1 and 3 in the current study have been previously associated with the development of muscular force during plantar flexion contractions [110-113]. However, at this time no studies have examined the relationship between muscular force output and activated regions of the brain. In this regard, although both Regions 1 and 3 were activated during the MVCs, a reduction in supraspinal drive was only observed in Region 3 following exercise. Additionally, as stated previously, a trend was only observed in Region 3 where increased levels of force output may be associated with an increase in brain activation

during the force varying contractions. Because similar trends were not observed in Region 1, it appears as though the activated brain areas associated with Region 3 are more closely correlated with muscular force output during plantar flexion.

Carbohydrate intake and fatigue:

High carbohydrate diets have been shown to result in a reduction in muscular fatigue during prolonged exercise [47]. It is commonly believed that high carbohydrate diets reduce muscular fatigue by increasing muscle glycogen stores, and therefore providing a greater source of carbohydrate needed to fuel high intensity exercise for a longer period of time. However, it has been suggested that reductions in muscular fatigue associated with high carbohydrate diets may be mediated through central mechanisms [36, 107]. Because dietary factors, such as carbohydrate intake, may influence the development of central fatigue, dietary assessments were performed during the 3 days prior to the 90-min run. Interestingly, the results of this study did not show any association between, carbohydrate intakes with either muscular or central fatigue following the run. Carbohydrate intakes between 6-10 g/kg per day have been associated with improvements in exercise performance in endurance athletes [114-116]. Although several of the participants had carbohydrate intakes less than 6 g/kg per day, carbohydrate intake was similar between all the participants and therefore these associations would not be expected to be observed. Due to the small sample size and the small amount of variability between the participant's diets, this study was not adequately designed to examine these relationships.

Conclusions:

In conclusion, this study shows that reductions in supraspinal drive are likely to be responsible for at least "portion" of the reduction in muscular force observed following prolonged running. Although several regions of the brain showed activation during plantar flexion before and after exercise, the brain regions that were most associated with changes in force output were the left paracentral lobule, including BA4 and BA6 and the post central gyrus. Therefore, these regions may represent target areas of the brain for future studies examining relationships between force output and supraspinal spinal drive. Therefore, fMRI may provide a valuable tool for assessing relationships between nutrition and supraspinal fatigue.

Chapter 5

Limitations and Future Research

The specific aims of this dissertation were to 1) determine if fMRI can be used to assess supraspinal drive during exercise and 2) to assess changes in supraspinal drive following 90 minutes of running using fMRI.

The first study in this dissertation demonstrates that fMRI provides a measurement of supraspinal drive during exercise. Previous fMRI studies have shown that the BOLD response is positively correlated with muscular force production, suggesting that the BOLD response is indicative of supraspinal drive [30, 31]. Furthermore, the brain BOLD response was increased during fatiguing muscular contractions of a constant force; a condition where an increase in supraspinal drive is required to activate higher threshold motor units so that a constant force can be maintained [20, 22, 24, 28]. Therefore, if muscular fatigue was induced by occluding the blood flow to the working muscle during a contraction of a constant force, it would be expected that supraspinal drive would be increased [28]. The observed increase in the BOLD response during ischemic exercise vs. non-ischemic exercise further supports that fMRI can be used to provide a measurement of supraspinal drive during exercise.

If the first study of this dissertation was successful, and fMRI could provide a measurement of supraspinal drive, the second aim was to assess changes in supraspinal drive during prolonged running, a condition in which central drive is thought to be impaired. Previous studies have reported reductions in central drive following prolonged exercise, including running and cycling [13-18, 32, 67]. However, due to methodological

limitations, it was unclear whether reductions in central drive were mediated through spinal or supraspinal mechanisms [1]. Although reductions in supraspinal drive were not statistically significant following 90-minutes of running, this may have been influenced by the small sample size in the study. In this regard, a single subject's force increased during the 8-sec MVC following the run as compared to before, indicating that a submaximal effort may have been given during the pre condition. If this subject was excluded from the analysis, a significant reduction in supraspinal drive was observed in the following brain regions; 1) the left paracentral lobule, including BA4 and BA6 and 2) the post central gyrus. Although other brain regions were plantar flexion during the MVCs, these brain regions were not associated with the muscular force output. Additionally, a 3-day dietary analysis indicated that dietary variables such as protein and total Caloric intake may be associated with the development of both muscular and supraspinal fatigue following prolonged running. The results of this study suggest that not only is supraspinal drive reduced following prolonged running, but also that changes in supraspinal drive may be influenced through nutritional intake.

Limitations:

While fMRI appears well suited to noninvasively investigate central motor drive, there are some limitations associated with this method. One critical limitation is head motion during the acquisition. While the analysis software can make corrections for small changes, corrections greater than 2 mm cannot be appropriately accounted for within the software and the data cannot be analyzed with any confidence. The analysis of the brain activation data can also be difficult as there are many approaches to consider. For

example a group analysis can be done comparing overall brain activity between conditions or selecting a region of interest in a target area of brain when the task region has been well investigated, as was done in the current studies. Other considerations within a region of interest analysis include how the region is defined. One may choose to extract activation during the target task or one may choose to select the activation that corresponds to the highest brain activation during or after a task. This may occur if there are temporal differences between subjects or between different conditions. A final challenge is controlling for baseline drifts between blocks of the target task.

Areas of further investigation:

1) Associations between muscular force and supraspinal drive: Previous studies have shown a linear relationship between handgripping force and the BOLD response at exercise intensities greater than 10-15% MVC force [24, 30, 31]. However, during the plantar flexion contractions, differences in supraspinal drive were not observed between 10, 15, 20 or 30% MVC force. However, a trend did exist where 30% MVC was higher compared to 10, 15, and 20% MVC force. Additionally, in a separate experiment consisting of a single subject, there appeared to be a distinction between supraspinal drive during plantar flexion contractions performed at higher exercise intensity. This data suggests that a minimum threshold force of greater than 30% MVC is required to observe relationships between muscular force and the BOLD response during plantar flexion. Therefore future studies are needed in order to examine relationships between muscular force and changes in supraspinal drive in other muscle groups using fMRI.

2) Exercise mode, intensity and duration: In the current study reductions in supraspinal drive were assessed following 90 minutes of running at an intensity of 75% of the participant's VO_2 max. However, previous studies have also observed reductions in central drive during other modes of exercise such as cycling [13-16, 32, 67]. At this time it is unclear whether these reductions in central drive in other modes of exercise are due to spinal or supraspinal fatigue. Additionally, differences between exercise mode, intensity, and duration also lead to different responses in regards to the development of central fatigue. For example, the onset of central fatigue has been shown to occur during the 4^{th} hour of running compared at 55% VO_2 max whereas it does not occur until the 5^{th} hour of cycling exercise performed at similar exercise intensity [15, 16]. Furthermore, the results of this dissertation, as well as other studies, have observed differences in the rate of development of central fatigue depending on not only the mode of exercise, but intensity and duration as well [13, 14, 18, 67]. Future studies examining the relationships between exercise mode, intensity and duration may be beneficial in designing and implementing exercise programs where central fatigue may limit performance.

3) Nutrition and supraspinal fatigue: Previously it has been suggested that dietary variables such as carbohydrate intake may be associated with the development of central fatigue [36, 107]. Although carbohydrate intake was not associated with either muscular or supraspinal fatigue, other studies have suggested that increased carbohydrate intake may reduce the development of central fatigue during prolonged activity, possibly through supraspinal mechanisms [48, 73, 75, 76]. The results in this study were limited in that most participants were consuming a diet that was adequate (based on

recommendations for endurance performance) in carbohydrate, and therefore sufficient muscle glycogen stores may have been available following the end of the 90-min run. Additionally, carbohydrate supplementation during exercise, irrespective of muscle glycogen content, has been shown to reduce muscular fatigue during prolonged running [48]. Therefore it is possible that carbohydrate consumption during the exercise protocol would have resulted in a reduction in fatigue. Although the participants in the present study were allowed to drink water ad libitum, sports drinks containing carbohydrate were forbidden.

Relationships between both muscular and central fatigue were observed between fat, protein and total kcal intake. In this regard, a negative correlation was observed between fat intake and central fatigue, but not muscular, fatigue. Additionally, a trend was observed where protein intake was negatively correlated with muscular, but not central fatigue. A trend was observed in which total energy intake was negatively correlated with both muscular and central fatigue, however, these relationships were not significant. Although these relationships were observed, caution must be taken when interpreting these results. First, due to the small sample size and the small amount of variability between the participant's diets, this study was not adequately designed to fully examine these relationships. Secondly, correlations do not imply causation and therefore these dietary factors may only be associated with and are not the cause of fatigue. Additionally, other nutrients, including vitamins and minerals, may also impact the development of central fatigue were not assessed in this study. Therefore at this time these results remain highly speculative and future studies are required to validate these findings and explore possible mechanistic links.

APPENDIX A

Tables

Table 1: Participant characteristics

Age (years; n= 9)	28.0±4.4
Weight (kg)	69.6±9.1
VO_2 max (ml/kg*min^{-1})	62.3±7.4
Heart rate max (bpm)	189.6±9.6
RER	1.14±0.05

Data displayed are ± SD

Table 2: 90-min run results

Average VO_2 (ml/kg*min^{-1})	45.1±5.5
30-min VO_2 (ml/kg*min^{-1})	45.4±5.3
60-min VO_2 (ml/kg*min^{-1})	44.9±5.7
85-min VO_2 (ml/kg*min^{-1})	44.9±5.9
RER (n=7)	0.87±0.04
Heart Rate (bpm; n=7)	159.3±14.3
Rating of Perceived Exertion (RPE)	7.2±1.4
fMRI completion time (min)	22.7±2.5

Data displayed are ± SE

Table 3: Dietary intake and muscular & central fatigue

	(n=6)	Muscular fatigue correlations	Central fatigue correlations
Calories (Kcal)	2801.3±193.7	r = -0.74 p = 0.06	r = -0.67 p = 0.15
Pro (g/kg)	1.5±0.2	r = -0.76 p = 0.08	r = -0.33 p = 0.53
CHO (g/kg)	5.6±0.5	r = -0.41 p = 0.42	r = -0.31 p = 0.55
Fat (g/kg)*	1.1±0.2	r = -0.29 p = 0.57	r = -0.84 p = 0.04*

* A correlation was observed between dietary fat intake and central fatigue. Data displayed are mean ± SE

Table 4: Brain activation Region 1

(n=7)	Pre (% change)	Post (% change)	p-value
8 sec MVC	2.88±0.29	2.85±0.30	0.94
Highest 4 sec	3.49±0.30	3.42±0.36	0.88
Highest 2 sec	3.71±0.32	3.70±0.41	0.99

Region 1 includes the left SMA (BA6) and left medial frontal gyrus. Data displayed are ± SE.

Table 5: Brain activation Region 3

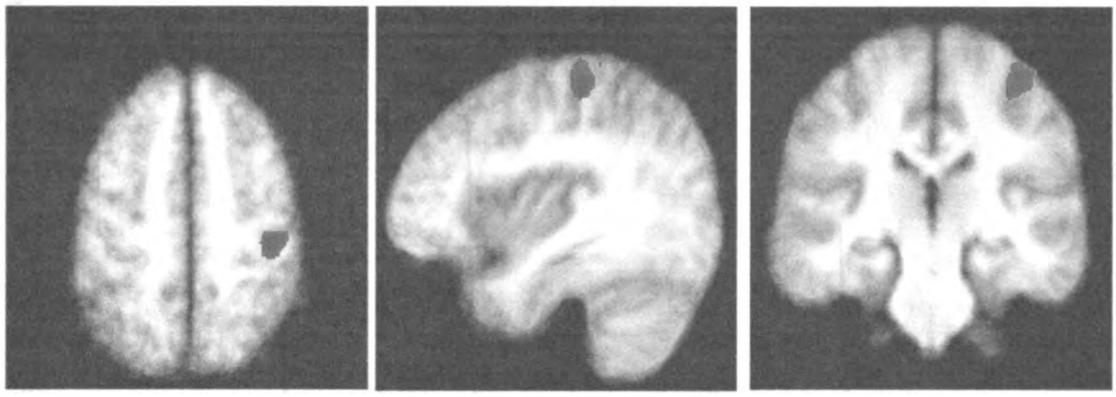
(n=7)	Pre (% change)	Post (% change)	p-value
8 sec MVC	2.01±0.18	1.46±0.23	0.12
Highest 4 sec	2.73±0.27	2.14±0.28	0.18
Highest 2 sec	2.87±0.28	2.29±0.27	0.18

Region 3 was located in the left paracentral lobule, including the BA4, BA6 and the post central gyrus. Data displayed are ± SE.

APPENDIX B

Figures

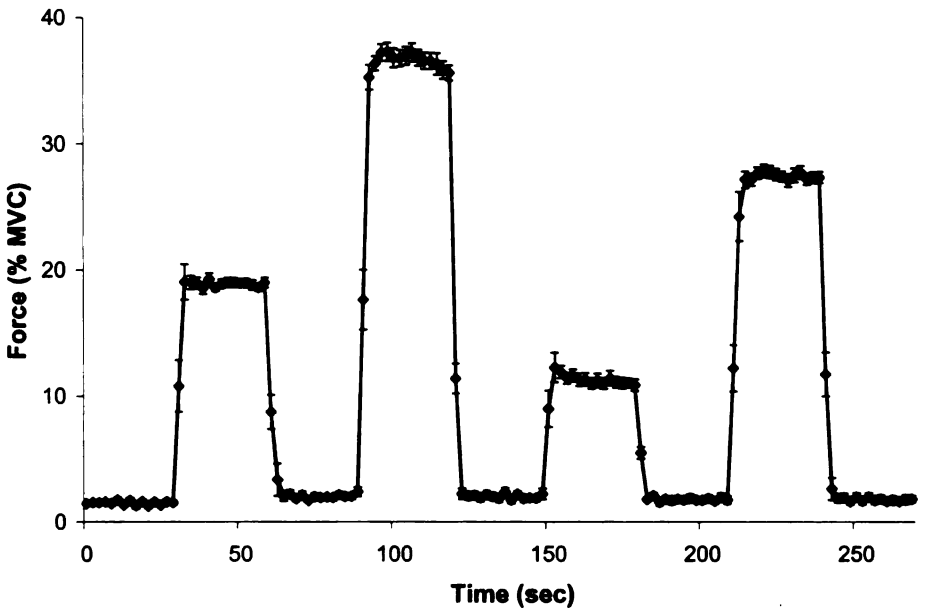
Figure 1: Force correlated regions of the brain



The location of the force-correlated clusters during FV was in the contralateral, primary sensorimotor cortex (S1+M1) centered at location L37, P23, S55.

Figure 2: Muscular force and brain activation during force varying contractions

A.



B.

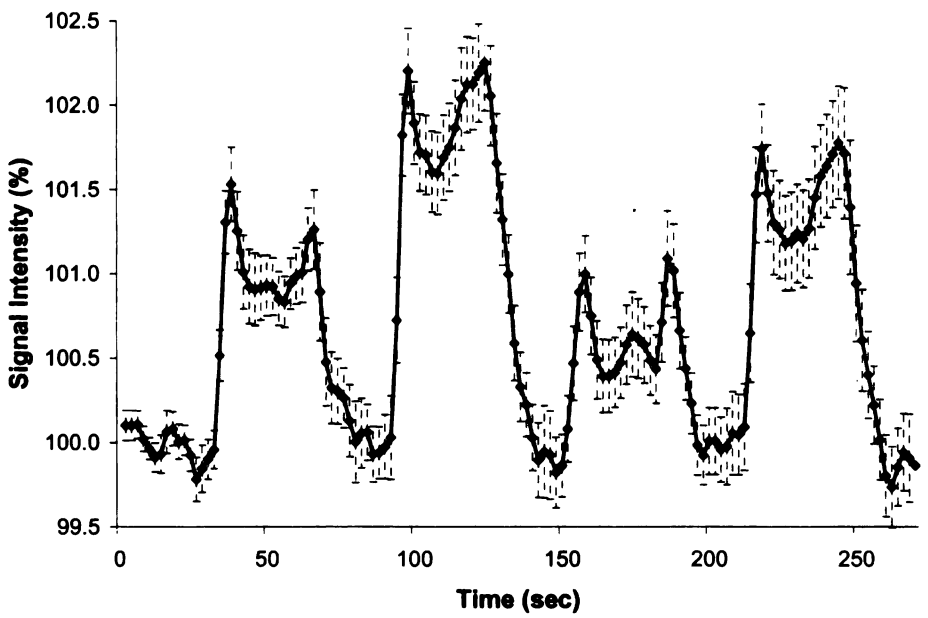
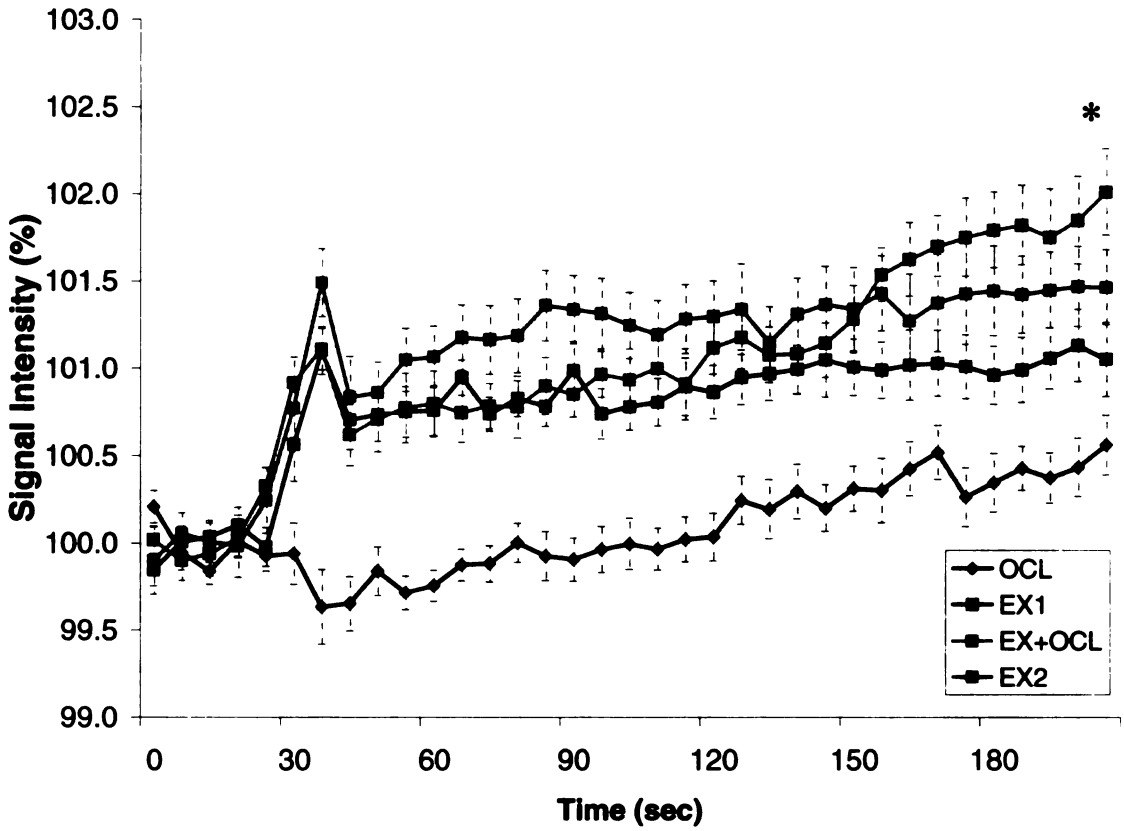


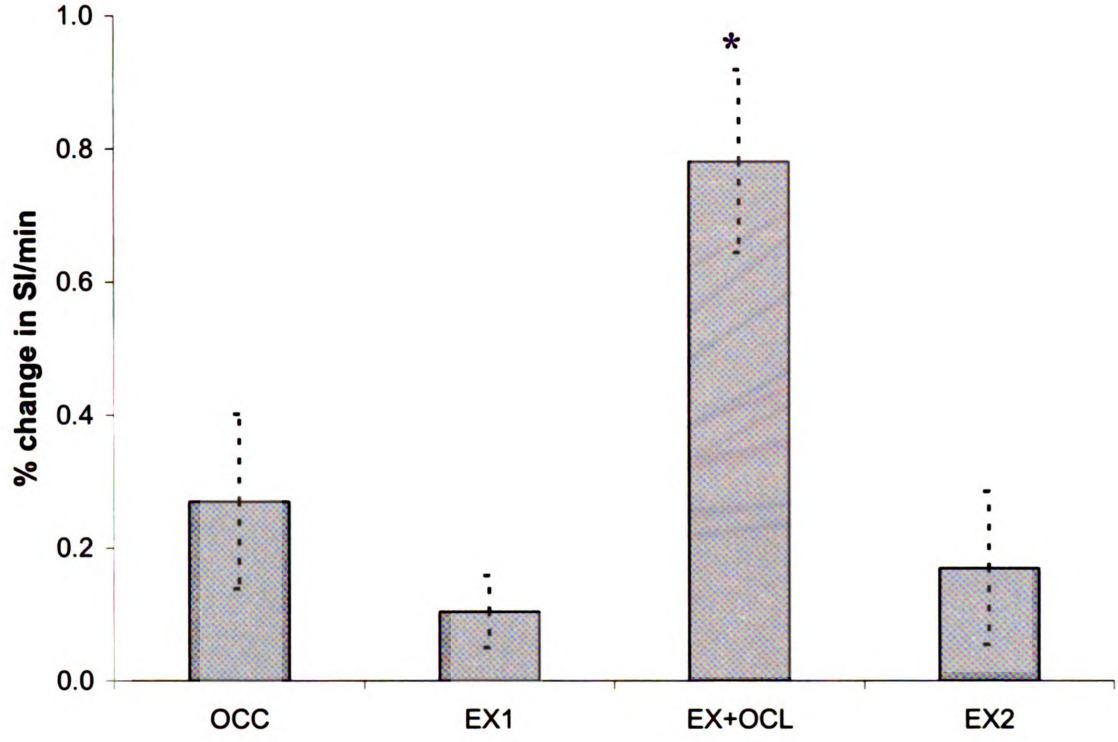
Figure 2-A: Measured percentage of MVC force during FV at target forces 20, 40, 10 & 30% MVC held for 30 seconds. Figure 2-B: % fMRI signal change following force varying contractions of approximately 20, 40, 10 & 30 % of MVC force. A strong correlation was observed between changes in muscular force and fMRI signal intensity during FV ($r = 0.70$; $p < 0.001$).

Figure 3: fMRI percent signal intensity over time



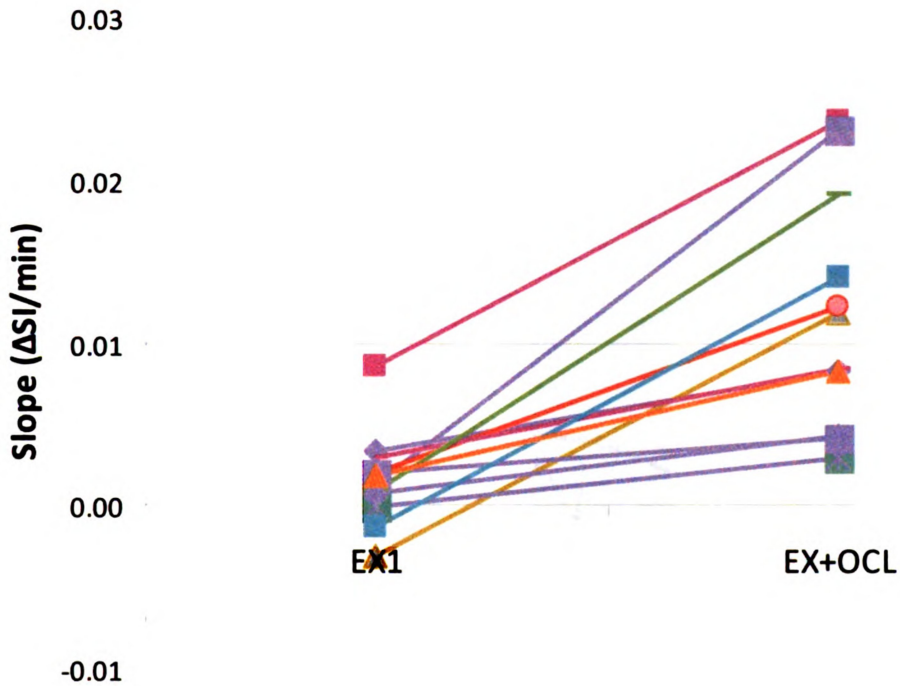
During the initial 30 sec of rest there were no differences in brain activation between any of the groups. Brain activation increased across all conditions following the initiation of the handgripping contraction at 30 seconds. * SI in the left somatosensory cortex showed a greater increase in central drive during the final 90 sec of exercise during exercise plus occlusion vs. exercise alone.

Figure 4: Rate of brain activation across all conditions



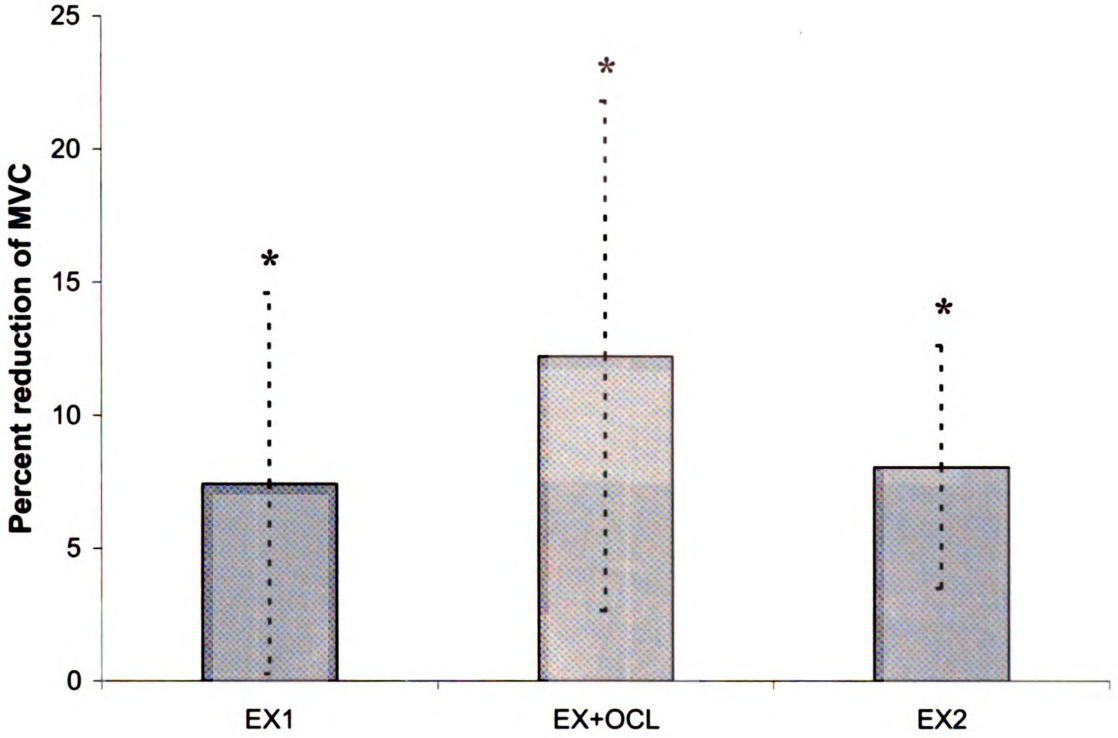
*The slope of the last 90 seconds of exercise was 3-fold greater ($p<0.02$) during EX+OCL compared to EX1 and EX2.

Figure 5: Individual comparisons between changes in SI following exercise alone vs. exercise plus occlusion



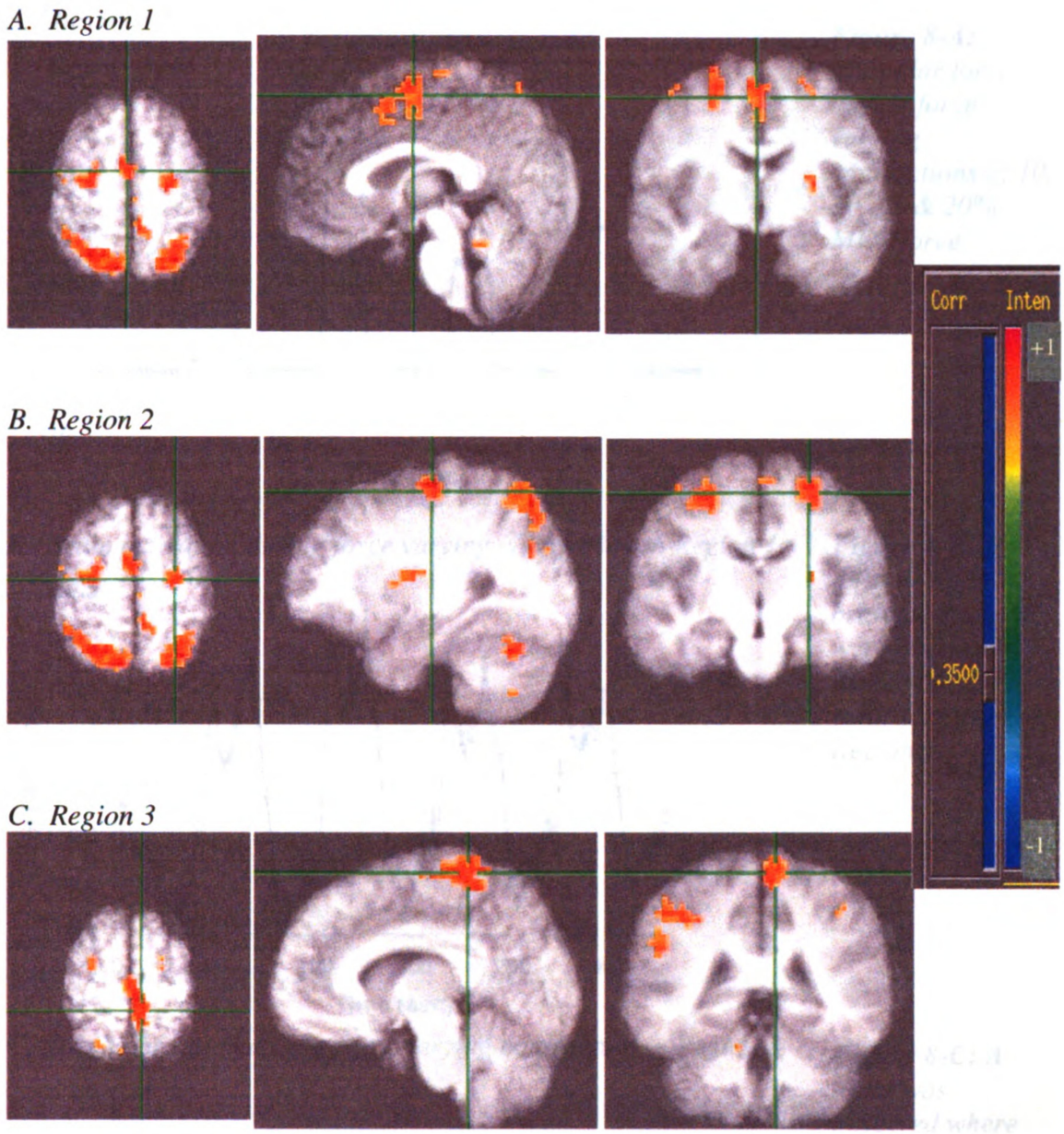
All individual brain plots trended towards a greater increase in supraspinal drive during exercise plus occlusion compared to exercise alone.

Figure 6: Reduction in MVC force following each condition



* MVC force was reduced following each of the exercise conditions ($p < 0.05$). No significant differences in MVC force were observed between any of the exercise conditions. However, a greater trend towards fatigue was observed following exercise plus ischemia compared to exercise alone.

Figure 7: Activated brain regions during force varying contractions



The three activated brain regions that were correlated with muscular force during the force varying contractions are depicted above. **Figure 7-A:** Region 1 included the left SMA (BA6) and left medial frontal gyrus (L1, P7, S54). **Figure 7-B:** Region 2 included the left precentral gyrus and BA6 (L0, P6, S57). **Figure 7-C:** Region 3 included the left paracentral lobule, including BA4 and BA6 and the post central gyrus (L8, P33, S64). Region 2 was not activated during the 8-sec MVCs and was excluded from further analyses.

Figure 8: Muscular force and brain activation during force varying contractions

A. Muscular force

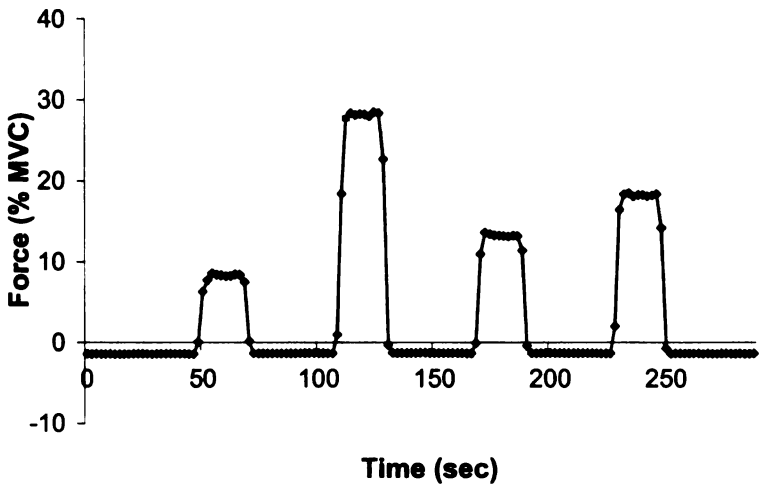


Figure 8-A: *Muscular force during force varying contractions of 10, 30, 15 & 20% MVC force.*

B. Brain activation during force varying contraction in Region 1

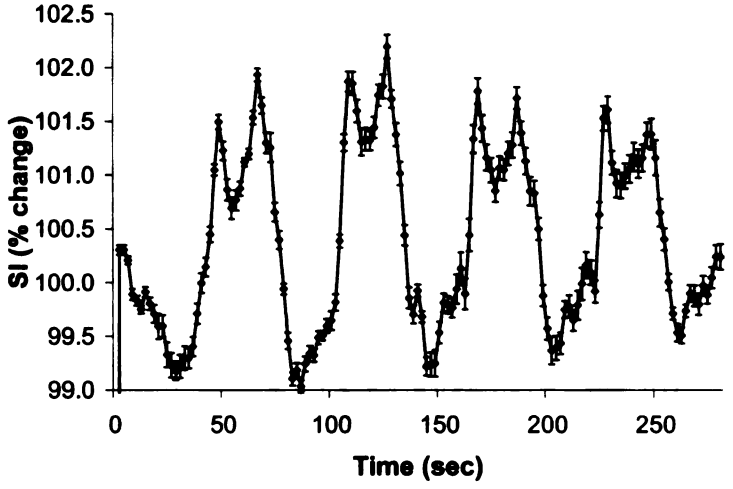


Figure 8-B: *No differences in brain activation were observed during the FV contractions in Region 1.*

C. Brain activation during force varying contraction in Region 3

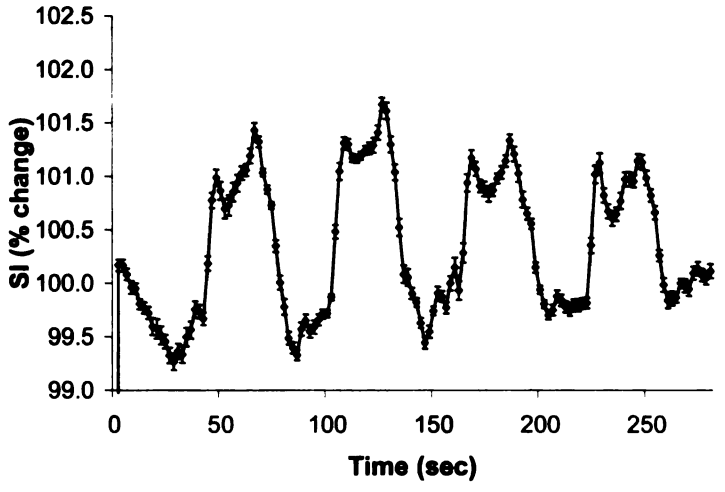
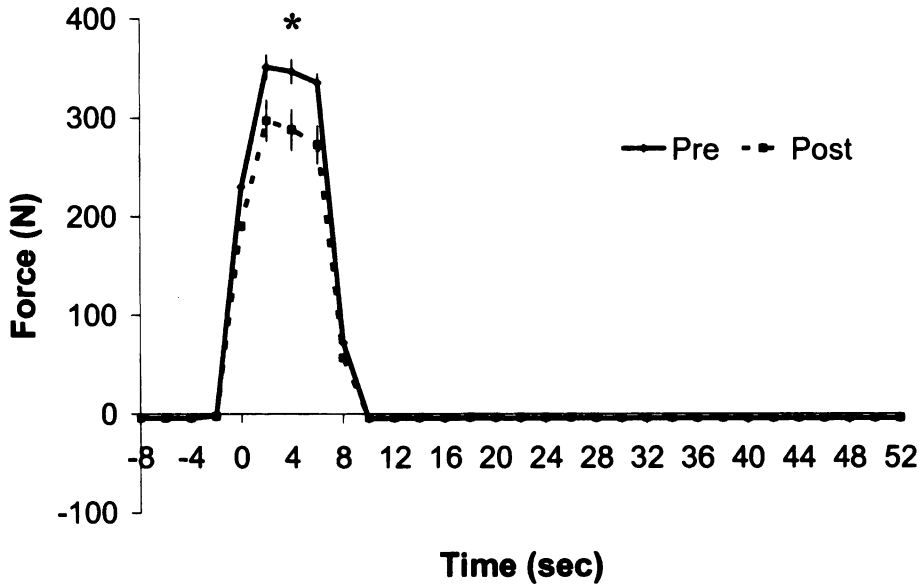


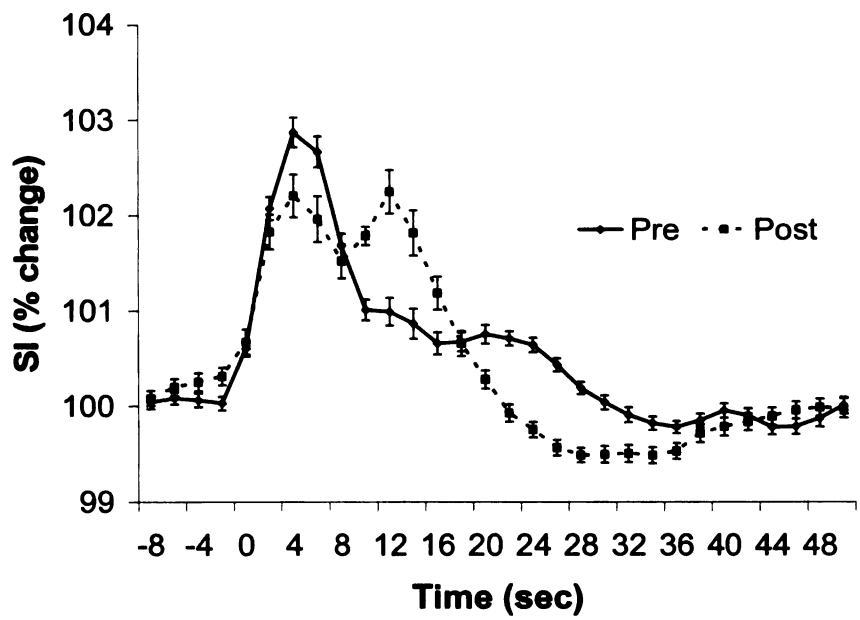
Figure 8-C: *A trend was observed where brain activation was > during 30% MVC compared to 10, 15 & 20% in Region 3 ($p \leq 0.12$)*

Figure 9: Reductions in muscular force and brain activation following exercise

A. Average of muscular force during 8-sec MVCs



B. Average of brain activation in Region 3 during 8-sec MVCs



* signifies a reduction in muscular force following 90 minutes of running. *Figure 9-A:*The average of the 8-sec MVCs following 90 minutes of running resulted in a 16% reduction in muscular force ($p = 0.04$). *Figure 9-B:* A reduction in brain activation in the left paracentral lobule (BA4 & BA6) and the post central gyrus were observed following 90 minutes of running ($p=0.05$).

Figure 10: Activated brain region during MVC contractions

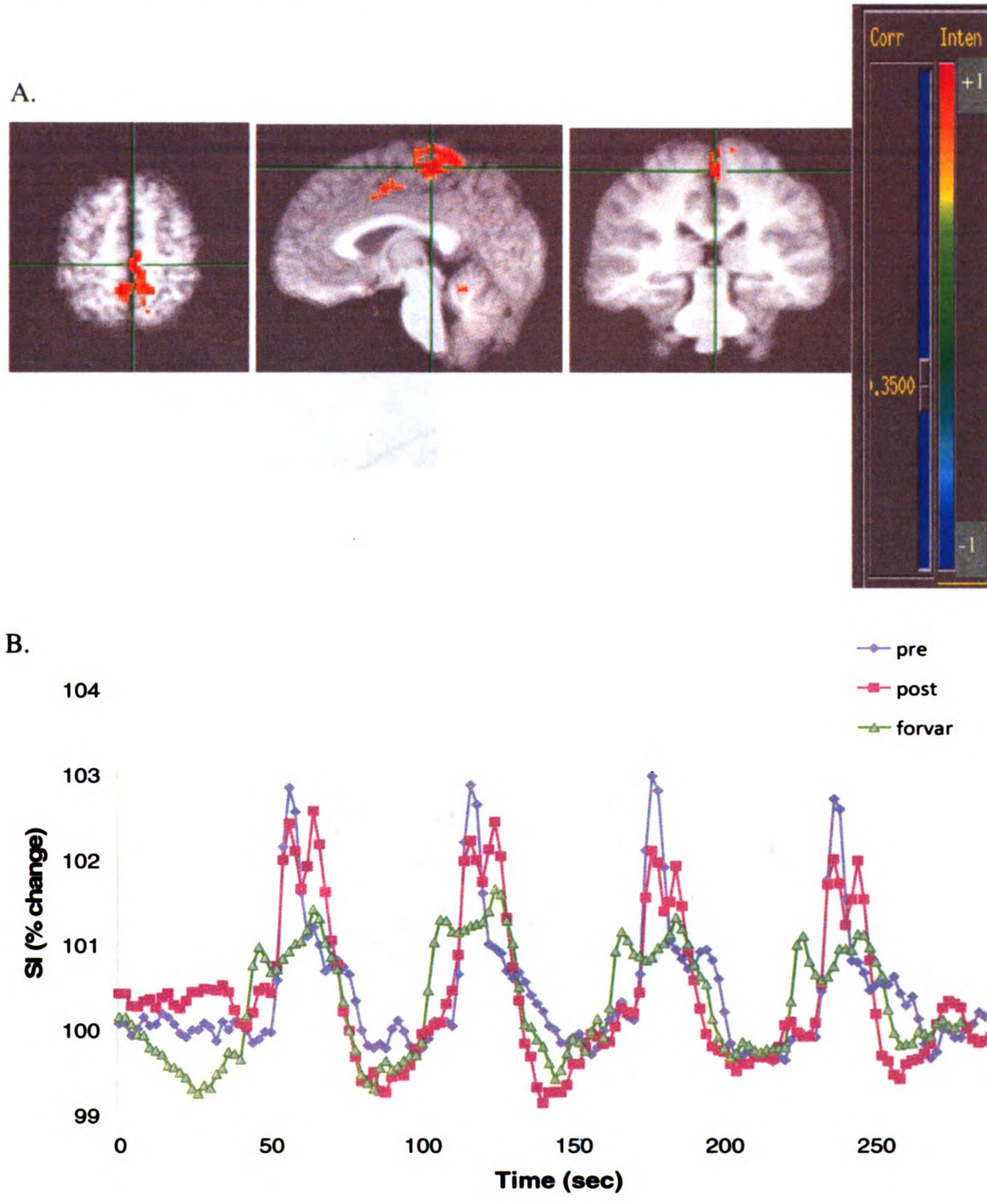
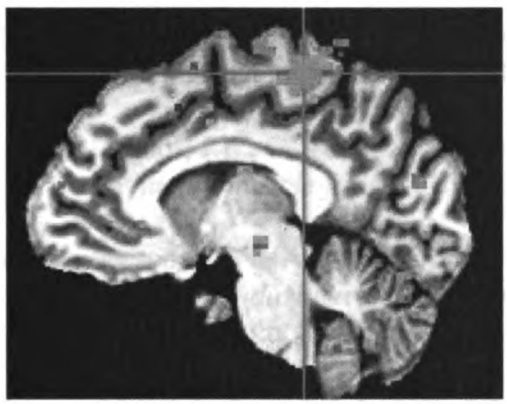


Figure 10-A: Activated brain areas during the MVC contractions included the left paracentral lobule, including BA4 and BA6 and the post central gyrus (L4, P26, S59; n = 7). **Figure 10-B:** Time course changes in brain activation in Region 3 during all conditions (Pre, Post & FV).

Figure 11: Brain activation during force varying contractions at 15, 65, 30 and 90% MVC

A.



B.

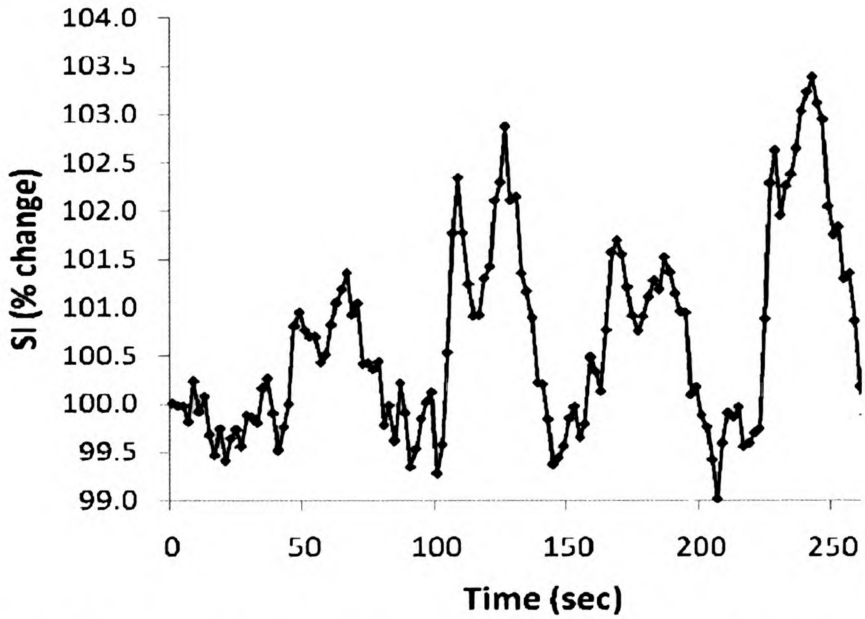


Figure 11-A: Brain activation region for a single participant performing the force varying at a higher %MVC force (15, 65, 30 & 90% MVC, respectively) in a separate experiment. **Figure 11-B:** A clear distinction was observed between forces of 65 and 90% MVC between all other contractions in Region 3. This suggests brain activation may be correlated to muscular force during plantar flexion contractions > 30% MVC.

References

1. Gandevia, S.C., *Spinal and supraspinal factors in human muscle fatigue.* Physiol Rev, 2001. **81**(4): p. 1725-89.

2. Sahlin, K., *Metabolic Factors in Fatigue*, in *Exercise Metabolism.* 2006, Human Kinetics: Champaign. p. 163-186.

3. Taylor, J.L., G. Todd, and S.C. Gandevia, *Evidence for a supraspinal contribution to human muscle fatigue.* Clin Exp Pharmacol Physiol, 2006. **33**(4): p. 400-5.

4. Loscher, W.N. and M.M. Nordlund, *Central fatigue and motor cortical excitability during repeated shortening and lengthening actions.* Muscle Nerve, 2002. **25**(6): p. 864-72.

5. Millet, G.Y. and R. Lepers, *Alterations of neuromuscular function after prolonged running, cycling and skiing exercises.* Sports Med, 2004. **34**(2): p. 105-16.

6. Conley, K.E., W.F. Kemper, and G.J. Crowther, *Limits to sustainable muscle performance: interaction between glycolysis and oxidative phosphorylation.* J Exp Biol, 2001. **204**(Pt 18): p. 3189-94.

7. Gandevia, S.C., *Neural control in human muscle fatigue: changes in muscle afferents, motoneurones and motor cortical drive [corrected].* Acta Physiol Scand, 1998. **162**(3): p. 275-83.

8. Merton, P.A., *Voluntary strength and fatigue.* J Physiol, 1954. **123**(3): p. 553-64.

9. Taylor, J.L. and S.C. Gandevia, *A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions.* J Appl Physiol, 2008. **104**(2): p. 542-50.

10. Loscher, W.N., A.G. Cresswell, and A. Thorstensson, *Central fatigue during a long-lasting submaximal contraction of the triceps surae.* Exp Brain Res, 1996. **108**(2): p. 305-14.

11. Gandevia, S.C., et al., *Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex.* J Physiol, 1996. **490 (Pt 2)**: p. 529-36.

12. Taylor, J.L., et al., *Supraspinal fatigue during intermittent maximal voluntary contractions of the human elbow flexors.* J Appl Physiol, 2000. **89**(1): p. 305-13.

13. Kremenic, I.J., et al., *Central fatigue after cycling evaluated using peripheral magnetic stimulation.* Med Sci Sports Exerc, 2009. **41**(7): p. 1461-6.

14. Lepers, R., et al., *Evidence of neuromuscular fatigue after prolonged cycling exercise.* Med Sci Sports Exerc, 2000. **32**(11): p. 1880-6.

15. Lepers, R., et al., *Neuromuscular fatigue during a long-duration cycling exercise.* J Appl Physiol, 2002. **92**(4): p. 1487-93.

16. Place, N., et al., *Time course of neuromuscular alterations during a prolonged running exercise.* Med Sci Sports Exerc, 2004. **36**(8): p. 1347-56.

17. Racinais, S., et al., *Failed excitability of spinal motoneurons induced by prolonged running exercise.* J Neurophysiol, 2007. **97**(1): p. 596-603.

18. Saldanha, A., M.M. Nordlund Ekblom, and A. Thorstensson, *Central fatigue affects plantar flexor strength after prolonged running.* Scand J Med Sci Sports, 2008. **18**(3): p. 383-8.

19. Smith, J.L., et al., *Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles.* J Appl Physiol, 2007. **103**(2): p. 560-8.

20. Benwell, N.M., F.L. Mastaglia, and G.W. Thickbroom, *Changes in the functional MR signal in motor and non-motor areas during intermittent fatiguing hand exercise.* Exp Brain Res, 2007. **182**(1): p. 93-7.

21. Liu, J.Z., et al., *Nonlinear cortical modulation of muscle fatigue: a functional MRI study.* Brain Res, 2002. **957**(2): p. 320-9.

22. Liu, J.Z., et al., *Human brain activation during sustained and intermittent submaximal fatigue muscle contractions: an FMRI study.* J Neurophysiol, 2003. **90**(1): p. 300-12.

23. Liu, J.Z., et al., *Fatigue induced by intermittent maximal voluntary contractions is associated with significant losses in muscle output but limited reductions in functional MRI-measured brain activation level.* Brain Res, 2005. **1040**(1-2): p. 44-54.

24. Thickbroom, G.W., et al., *Differences in functional magnetic resonance imaging of sensorimotor cortex during static and dynamic finger flexion.* Exp Brain Res, 1999. **126**(3): p. 431-8.

25. Toma, K. and T. Nakai, *Functional MRI in human motor control studies and clinical applications.* Magn Reson Med Sci, 2002. **1**(2): p. 109-20.

26. Huettel, S.A., Song, Allen W., & McCarthy, Gregory, *Functional Magnetic Resonance Imaging*. 2004, Sunderland: Sinauer Associates, Inc.

27. Bigland-Ritchie, B., F. Furbush, and J.J. Woods, *Fatigue of intermittent submaximal voluntary contractions: central and peripheral factors.* J Appl Physiol, 1986. **61**(2): p. 421-9.

28. Moritani, T., et al., *Oxygen availability and motor unit activity in humans.* Eur J Appl Physiol Occup Physiol, 1992. **64**(6): p. 552-6.

29. Sogaard, K., et al., *The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles.* J Physiol, 2006. **573**(Pt 2): p. 511-23.

30. Dai, T.H., et al., *Relationship between muscle output and functional MRI-measured brain activation.* Exp Brain Res, 2001. **140**(3): p. 290-300.

31. van Duinen, H., et al., *Relation between muscle and brain activity during isometric contractions of the first dorsal interosseus muscle.* Hum Brain Mapp, 2008. **29**(3): p. 281-99.

32. Lepers, R., et al., *Neuromuscular fatigue following constant versus variable-intensity endurance cycling in triathletes.* J Sci Med Sport, 2008. **11**(4): p. 381-9.

33. Ross, E.Z., et al., *Corticomotor excitability contributes to neuromuscular fatigue following marathon running in man.* Exp Physiol, 2007. **92**(2): p. 417-26.

34. Ament, W. and G.J. Verkerke, *Exercise and fatigue.* Sports Med, 2009. **39**(5): p. 389-422.

35. Maclaren, D.P., et al., *A review of metabolic and physiological factors in fatigue.* Exerc Sport Sci Rev, 1989. **17**: p. 29-66.

36. Lambert, E.V., A. St Clair Gibson, and T.D. Noakes, *Complex systems model of fatigue: integrative homoeostatic control of peripheral physiological systems during exercise in humans.* Br J Sports Med, 2005. **39**(1): p. 52-62.

37. Greenhaff, P.L., *Creatine and its application as an ergogenic aid.* Int J Sport Nutr, 1995. **5 Suppl**: p. S100-10.

38. Lambert, C.P. and M.G. Flynn, *Fatigue during high-intensity intermittent exercise: application to bodybuilding.* Sports Med, 2002. **32**(8): p. 511-22.

39. Mougios, V., *Exercise Biochemistry.* 2006, Champaign: Human Kinetics.

40. Sahlin, K., M. Tonkonogi, and K. Soderlund, *Energy supply and muscle fatigue in humans.* Acta Physiol Scand, 1998. **162**(3): p. 261-6.

41. Soderlund, K. and E. Hultman, *ATP and phosphocreatine changes in single human muscle fibers after intense electrical stimulation.* Am J Physiol, 1991. **261**(6 Pt 1): p. E737-41.

42. Dahlstedt, A.J., et al., *Is creatine kinase responsible for fatigue? Studies of isolated skeletal muscle deficient in creatine kinase.* Faseb J, 2000. **14**(7): p. 982-90.

43. Steeghs, K., et al., *Altered Ca2+ responses in muscles with combined mitochondrial and cytosolic creatine kinase deficiencies.* Cell, 1997. **89**(1): p. 93-103.

44. Dahlstedt, A.J., et al., *Creatine kinase injection restores contractile function in creatine-kinase-deficient mouse skeletal muscle fibres.* J Physiol, 2003. **547**(Pt 2): p. 395-403.

45. Demant, T.W. and E.C. Rhodes, *Effects of creatine supplementation on exercise performance.* Sports Med, 1999. **28**(1): p. 49-60.

46. Noakes, T.D., *The central governor model of exercise regulation applied to the marathon.* Sports Med, 2007. **37**(4-5): p. 374-7.

47. Bangsbo, J., F.M. Iaia, and P. Krustrup, *Metabolic response and fatigue in soccer.* Int J Sports Physiol Perform, 2007. **2**(2): p. 111-27.

48. Foskett, A., et al., *Carbohydrate availability and muscle energy metabolism during intermittent running.* Med Sci Sports Exerc, 2008. **40**(1): p. 96-103.

49. Noakes, T.D., et al., *Carbohydrate ingestion and muscle glycogen depletion during marathon and ultramarathon racing.* Eur J Appl Physiol Occup Physiol, 1988. **57**(4): p. 482-9.

50. Brooks, G., Fahey, T., White, T., & Baldwin, K., *Exercise Physiology: Human Bioenergetics and its Applications.* 3rd ed. 2000, Boston: McGraw Hill.

51. Sjogaard, G., *Potassium and fatigue: the pros and cons.* Acta Physiol Scand, 1996. **156**(3): p. 257-64.

52. Green, H.J., *Mechanisms of muscle fatigue in intense exercise.* J Sports Sci, 1997. **15**(3): p. 247-56.

53. Evertsen, F., et al., *Hard training for 5 mo increases Na(+)-K+ pump concentration in skeletal muscle of cross-country skiers.* Am J Physiol, 1997. **272**(5 Pt 2): p. R1417-24.

54. Westerblad, H., D.G. Allen, and J. Lannergren, *Muscle fatigue: lactic acid or inorganic phosphate the major cause?* News Physiol Sci, 2002. **17**: p. 17-21.

55. Allen, D.G., A.A. Kabbara, and H. Westerblad, *Muscle fatigue: the role of intracellular calcium stores.* Can J Appl Physiol, 2002. **27**(1): p. 83-96.

56. Allen, D.G. and H. Westerblad, *Role of phosphate and calcium stores in muscle fatigue.* J Physiol, 2001. **536**(Pt 3): p. 657-65.

57. Adams, G.R., M.J. Fisher, and R.A. Meyer, *Hypercapnic acidosis and increased H2PO4- concentration do not decrease force in cat skeletal muscle.* Am J Physiol, 1991. **260**(4 Pt 1): p. C805-12.

58. Bangsbo, J., et al., *Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man.* J Physiol, 1996. **495 (Pt 2)**: p. 587-96.

59. Pate, E., et al., *Reduced effect of pH on skinned rabbit psoas muscle mechanics at high temperatures: implications for fatigue.* J Physiol, 1995. **486 (Pt 3)**: p. 689-94.

60. Matsuura, R., et al., *Effect of oral administration of sodium bicarbonate on surface EMG activity during repeated cycling sprints.* Eur J Appl Physiol, 2007. **101**(4): p. 409-17.

61. Nielsen, O.B., F. de Paoli, and K. Overgaard, *Protective effects of lactic acid on force production in rat skeletal muscle.* J Physiol, 2001. **536**(Pt 1): p. 161-6.

62. Wilkie, D.R., *Muscular fatigue: effects of hydrogen ions and inorganic phosphate.* Fed Proc, 1986. **45**(13): p. 2921-3.

63. Westerblad, H. and D.G. Allen, *The effects of intracellular injections of phosphate on intracellular calcium and force in single fibres of mouse skeletal muscle.* Pflugers Arch, 1996. **431**(6): p. 964-70.

64. Smith, D.O., *Acetylcholine storage, release and leakage at the neuromuscular junction of mature adult and aged rats.* J Physiol, 1984. **347**: p. 161-76.

65. Feltz, A. and A. Trautmann, *Desensitization at the frog neuromuscular junction: a biphasic process.* J Physiol, 1982. **322**: p. 257-72.

66. Petersen, K., et al., *Muscle mechanical characteristics in fatigue and recovery from a marathon race in highly trained runners.* Eur J Appl Physiol, 2007. **101**(3): p. 385-96.

67. Lepers, R., G.Y. Millet, and N.A. Maffiuletti, *Effect of cycling cadence on contractile and neural properties of knee extensors.* Med Sci Sports Exerc, 2001. **33**(11): p. 1882-8.

68. Kandel, E., Schwartz, J., Jessell, T., *Principles of Neural Science.* 4th ed. 2000, New York: McGraw Hill.

69. Andersen, B., B. Westlund, and C. Krarup, *Failure of activation of spinal motoneurones after muscle fatigue in healthy subjects studied by transcranial magnetic stimulation.* J Physiol, 2003. **551**(Pt 1): p. 345-56.

70. Newsholme, E.A. and J.C. Stanley, *Substrate cycles: their role in control of metabolism with specific references to the liver.* Diabetes Metab Rev, 1987. **3**(1): p. 295-305.

71. Pardridge, W.M., *Blood-brain barrier carrier-mediated transport and brain metabolism of amino acids.* Neurochem Res, 1998. **23**(5): p. 635-44.

72. Meeusen, R., et al., *Brain neurotransmitters in fatigue and overtraining.* Appl Physiol Nutr Metab, 2007. **32**(5): p. 857-64.

73. Blomstrand, E., et al., *Effect of carbohydrate ingestion on brain exchange of amino acids during sustained exercise in human subjects.* Acta Physiol Scand, 2005. **185**(3): p. 203-9.

74. Cheuvront, S.N., et al., *Branched-chain amino acid supplementation and human performance when hypohydrated in the heat.* J Appl Physiol, 2004. **97**(4): p. 1275-82.

75. Davis, J.M., et al., *Effects of carbohydrate feedings on plasma free tryptophan and branched-chain amino acids during prolonged cycling.* Eur J Appl Physiol Occup Physiol, 1992. **65**(6): p. 513-9.

76. Nybo, L., *CNS fatigue and prolonged exercise: effect of glucose supplementation.* Med Sci Sports Exerc, 2003. **35**(4): p. 589-94.

77. Wilson, W.M. and R.J. Maughan, *Evidence for a possible role of 5-hydroxytryptamine in the genesis of fatigue in man: administration of paroxetine, a 5-HT re-uptake inhibitor, reduces the capacity to perform prolonged exercise.* Exp Physiol, 1992. **77**(6): p. 921-4.

78. Parise, G., et al., *Selective serotonin reuptake inhibitors: Their effect on high-intensity exercise performance.* Arch Phys Med Rehabil, 2001. **82**(7): p. 867-71.

79. Amann, M., et al., *Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans.* J Physiol, 2009. **587**(Pt 1): p. 271-83.

80. Rothwell, J.C., et al., *Stimulation of the human motor cortex through the scalp.* Exp Physiol, 1991. **76**(2): p. 159-200.

81. Todd, G., J.L. Taylor, and S.C. Gandevia, *Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation.* J Physiol, 2003. **551**(Pt 2): p. 661-71.

82. Taylor, J.L., et al., *Changes in motor cortical excitability during human muscle fatigue.* J Physiol, 1996. **490 (Pt 2)**: p. 519-28.

83. Lee, M., S.C. Gandevia, and T.J. Carroll, *Cortical voluntary activation can be reliably measured in human wrist extensors using transcranial magnetic stimulation.* Clin Neurophysiol, 2008. **119**(5): p. 1130-8.

84. Logothetis, N.K., et al., *Neurophysiological investigation of the basis of the fMRI signal.* Nature, 2001. **412**(6843): p. 150-7.

85. Jueptner, M. and C. Weiller, *Review: does measurement of regional cerebral blood flow reflect synaptic activity? Implications for PET and fMRI.* Neuroimage, 1995. **2**(2): p. 148-56.

86. Phelps, M.E., et al., *Tomographic mapping of human cerebral metabolism visual stimulation and deprivation.* Neurology, 1981. **31**(5): p. 517-29.

87. Attwell, D. and C. Iadecola, *The neural basis of functional brain imaging signals.* Trends Neurosci, 2002. **25**(12): p. 621-5.

88. Magistretti, P.J., et al., *Energy on demand.* Science, 1999. **283**(5401): p. 496-7.

89. Hirose, S., et al., *De-coupling of blood flow and metabolism in the rat brain induced by glutamate.* Ann Nucl Med, 2009. **23**(3): p. 293-300.

90. Koos, T. and J.M. Tepper, *Inhibitory control of neostriatal projection neurons by GABAergic interneurons.* Nat Neurosci, 1999. **2**(5): p. 467-72.

91. Waldvogel, D., et al., *The relative metabolic demand of inhibition and excitation.* Nature, 2000. **406**(6799): p. 995-8.

92. Chatton, J.Y., L. Pellerin, and P.J. Magistretti, *GABA uptake into astrocytes is not associated with significant metabolic cost: implications for brain imaging of inhibitory transmission.* Proc Natl Acad Sci U S A, 2003. **100**(21): p. 12456-61.

93. Lauritzen, M. and L. Gold, *Brain function and neurophysiological correlates of signals used in functional neuroimaging.* J Neurosci, 2003. **23**(10): p. 3972-80.

94. Dettmers, C., et al., *Quantitative comparison of functional magnetic resonance imaging with positron emission tomography using a force-related paradigm.* Neuroimage, 1996. **4**(3 Pt 1): p. 201-9.

95. Dettmers, C., et al., *Comparison of regional cerebral blood flow with transcranial magnetic stimulation at different forces.* J Appl Physiol, 1996. **81**(2): p. 596-603.

96. Krings, T., et al., *Functional magnetic resonance imaging and transcranial magnetic stimulation: complementary approaches in the evaluation of cortical motor function.* Neurology, 1997. **48**(5): p. 1406-16.

97. Ramsey, N.F., et al., *Functional mapping of human sensorimotor cortex with 3D BOLD fMRI correlates highly with H2(15)O PET rCBF.* J Cereb Blood Flow Metab, 1996. **16**(5): p. 755-64.

98. Sadato, N., et al., *Frequency-dependent changes of regional cerebral blood flow during finger movements: functional MRI compared to PET.* J Cereb Blood Flow Metab, 1997. **17**(6): p. 670-9.

99. Stippich, C., et al., *Motor, somatosensory and auditory cortex localization by fMRI and MEG.* Neuroreport, 1998. **9**(9): p. 1953-7.

100. Dettmers, C., et al., *Relation between cerebral activity and force in the motor areas of the human brain.* J Neurophysiol, 1995. **74**(2): p. 802-15.

101. Post, M., et al., *Voluntary activation and cortical activity during a sustained maximal contraction: an fMRI study.* Hum Brain Mapp, 2009. **30**(3): p. 1014-27.

102. Mehta, J.P., et al., *A novel technique for examining human brain activity associated with pedaling using fMRI.* J Neurosci Methods, 2009. **179**(2): p. 230-9.

103. Cox, R.W. and J.S. Hyde, *Software tools for analysis and visualization of fMRI data.* NMR Biomed, 1997. **10**(4-5): p. 171-8.

104. Fuglevand, A.J., et al., *Impairment of neuromuscular propagation during human fatiguing contractions at submaximal forces.* J Physiol, 1993. **460**: p. 549-72.

105. Forbes SC, S.J., Carlson JJ, Wiseman RW, Meyer RA., *Ischemia increases central drive from the motor cortex during submaximal exercise in healthy adults.* Med. Sci. Sport Exerc, 2009. **41**(S5): p. S521.

106. Williamson, J.W., et al., *Mechanisms regulating regional cerebral activation during dynamic handgrip in humans.* J Appl Physiol, 1996. **81**(5): p. 1884-90.

107. Rauch, L.H., et al., *The effects of carbohydrate loading on muscle glycogen content and cycling performance.* Int J Sport Nutr, 1995. **5**(1): p. 25-36.

108. Mori, E., A. Yamadori, and M. Furumoto, *Left precentral gyrus and Broca's aphasia: a clinicopathologic study.* Neurology, 1989. **39**(1): p. 51-4.

109. Yousry, T.A., et al., *Localization of the motor hand area to a knob on the precentral gyrus. A new landmark.* Brain, 1997. **120 (Pt 1)**: p. 141-57.

110. Christensen, L.O., et al., *Cerebral activation during bicycle movements in man.* Exp Brain Res, 2000. **135**(1): p. 66-72.

111. Ciccarelli, O., et al., *Identifying brain regions for integrative sensorimotor processing with ankle movements.* Exp Brain Res, 2005. **166**(1): p. 31-42.

112. Johannsen, P., et al., *Cerebral functional anatomy of voluntary contractions of ankle muscles in man.* J Physiol, 2001. **535**(Pt 2): p. 397-406.

113. Newton, J.M., et al., *Reliable assessment of lower limb motor representations with fMRI: use of a novel MR compatible device for real-time monitoring of ankle, knee and hip torques.* Neuroimage, 2008. **43**(1): p. 136-46.

114. *Nutrition and Athletic Performance.* Med Sci Sports Exerc, 2009.

115. Rodriguez, N.R., N.M. Di Marco, and S. Langley, *American College of Sports Medicine position stand. Nutrition and athletic performance.* Med Sci Sports Exerc, 2009. **41**(3): p. 709-31.

116. Rodriguez, N.R., N.M. DiMarco, and S. Langley, *Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance.* J Am Diet Assoc, 2009. **109**(3): p. 509-27.