Cadence vs Power Output: Corticospinal excitability to muscles of the upper arm is modulated differently with increases in arm cycling intensity

By

© Evan James Lockyer

A thesis submitted to the

School of Graduate Studies

in partial fulfillment of the requirements for the degree of

Master of Science in Kinesiology

School of Human Kinetics and Recreation

Memorial University of Newfoundland

August, 2017

St. John’s Newfoundland

Abstract

Indirect evidence suggests that arm cycling, like other forms of human locomotor outputs, is partially produced by a specialized set of neurones within the spinal cord, called a central pattern generator (CPG), although it is known that the brain also plays a role. Most of what is known regarding the neural control of locomotor outputs has come from research using several different neurophysiology techniques. From this research, it appears that the 'type' of locomotor intensity may be controlled differently by the central nervous system. Specifically, research suggests that the neural control of locomotor outputs is different when speed (i.e. cadence) and load (i.e. power output) are manipulated. To date, no study has compared the influence of cadence and power output on corticospinal and spinal excitability using transcranial magnetic stimulation and transmastoid electrical stimulation during arm cycling. Therefore, the purpose of this study was to examine corticospinal and spinal excitability to the biceps and triceps brachii during arm cycling as the cadence and power output were increased.

Acknowledgements

The completion of this thesis would not have been possible without the unwavering support and encouragement from several influential people. First of all, I would like to express my sincere appreciation to my supervisor and friend, Dr. Kevin Power for providing me with the opportunity to complete my master's degree in my home province at Memorial University of Newfoundland. Under his guidance, I believe I have grown as an individual and have discovered my love for research that has facilitated my desire to pursue a Ph.D. I will be forever grateful for his mentorship, camaraderie, and commitment to enhancing my research experience. I look forward to working alongside him for the next four years as I begin my doctoral studies in September 2017.

I would also like to send my astounding appreciation to my co-authors on this project; Audrey Hynes, Lynsey Alcock, and Alyssa-Joy Spence. The kind and generous assistance that you all have provided me during this process has been incredible. Another sincere thank you goes out to Dr. Duane Button. Even though I was not Duane's master's student, I am very appreciative for the constructive criticism and important feedback that he has provided me, not only with respect to this thesis, but in life in general. He has also been like a mentor to me throughout this whole process and I look forward to collaborating on projects with him again in the future!

A special thank you also goes out to my fellow graduate students, with whom I have had the opportunity to share the past two years. This whole process has been made easier with the advice and support that you have given me. I would like to wish you all the best in your future endeavors.

Finally, I would like to express my heartfelt gratitude to my family and friends who have provided me with unconditional love and support throughout not only my master's degree, but my entire life. To my parents, Debbie and Neil, you will never fully understand the admiration and gratefulness I have for you both. Thank you for allowing me to pursue a high level of education and for always being on my side lifting me up when I needed it most. To my sisters, Erika and Emily, thank you for supporting me over the years. I want to wish both of you the greatest success as you finish your schooling, and I want you both to know that I am proud of you and will always be here for you if and when you need me. To the b'ys (Newfoundland slang), the love and support you have shown me over the years is incredible and I only hope to be able to do the things you guys have done for me two-fold in the future. Last but definitely not least, to my amazing girlfriend Natasha Buckle, I don't know where I would be if it were not for you. Your work ethic and your drive to continuously better yourself are inspiring, and fortunately I think some of these qualities have rubbed off on me. I will forever cherish the years we spent alongside one another throughout our undergraduate and graduate degrees and the obstacles we have overcome. Together, I believe we can get through anything, and I am truly blessed to have you. Keep pushing through; you will make a great physician in years to come!

I would also like to acknowledge the Natural Sciences and Engineering Research Council (NSERC) for providing me with financial support for this project.

> *"If one advances confidently in the direction of his dreams and endeavors to live the life which he has imagined, he will meet with a success unexpected in common hours."*
>
> *-Henry David Thoreau*

Table of Contents

List of Tables

List of Figures

Chapter 2 Figures

Chapter 3 Figures

List of Abbreviations

%MSO – percent of maximal stimulator output

μs – microseconds

CMEP – cervicomedullary motor evoked potential

CNS – central nervous system

CPG – central pattern generator

D-wave – direct wave

EMG – electromyography

I-wave – indirect wave

kg – kilogram

mA – milliamp

MEP – motor evoked potential

MLR – mesencephalic locomotor region

M_{max} – maximum M-wave

ms – milliseconds

mV – millivolts

MVC – maximal voluntary contraction

M-wave – compound muscle action potential

RPM – revolutions per minute

SD – standard deviation

SE – standard error

TMES – transmastoid electrical stimulation

TMS – transcranial magnetic stimulation

W - Watts

W_{max} – maximum power output (wattage)

Chapter 1 Introduction

1.0 Overview

Arm cycling is frequently used as a model to examine the neural control of human locomotion (Carroll et al., 2006; Zehr et al., 2004). This is because arm cycling shares many characteristics of other human locomotor outputs, such as walking and running, in that it is a bilateral motor output that involves rhythmic and alternating patterns of muscle activation. Indirect evidence suggests that the basic rhythmic and alternating pattern of locomotor outputs is produced, at least partially, by functional networks of neurones within the spinal cord (Dietz, 2003), though descending inputs from the motor cortex are also required (Christensen et al., 2001; Forman et al., 2014; Petersen et al., 1998). While it is known that both the brain and spinal cord are involved in the production of locomotor outputs, there is still relatively little known about how the central nervous system (CNS; i.e. brain and spinal cord) controls different locomotor outputs. Moreover, there is much less known regarding the neural control of locomotor outputs as the intensity of the motor output is altered. Of the studies that have investigated the influence of locomotor intensity on neural excitability, the majority have focused on the processing of sensory information and/or the modulation of corticospinal excitability when the speed (i.e. cadence) and/or load (i.e. force production) of the task is altered (Forman et al., 2015; Hundza and Zehr, 2009; Pyndt, Laursen, & Nielsen, 2003; Spence et al., 2016). Interestingly, it appears as though the intensity parameter which is manipulated (i.e. cadence vs load) may influence neural excitability differently. Included in this body of work are two studies from our lab, which have assessed separately the influence of cadence and load (i.e. power output) on corticospinal excitability to muscles

of the upper arm during arm cycling. Using transcranial magnetic stimulation (TMS) of the motor cortex, and transmastoid electrical stimulation (TMES) of corticospinal axons, we have reported phase- and cadence-dependent changes in corticospinal and spinal excitability projecting to the biceps brachii during arm cycling (Forman et al., 2015). Similarly, in a subsequent study using the same techniques, we showed phase-, muscle-, and load-dependent changes in corticospinal and spinal excitability to the biceps and triceps brachii during arm cycling (Spence et al., 2016). There has yet to be a study to compare the influence of cadence and power output on corticospinal and spinal excitability to antagonistic muscles of the upper arm during arm cycling.

1.1 Purpose

The purpose of this study was to compare how corticospinal and spinal excitability to the biceps and triceps brachii were altered during the elbow flexion and extension phases of arm cycling as cadence and power output were increased.

1.2 Research Hypotheses

There were three main hypotheses for this study. It was hypothesized that:

1. corticospinal excitability to the biceps and triceps brachii would increase during both the flexion and extension phases of arm cycling as the intensity (cadence or power output) increased.
2. spinal excitability would increase during the most active phase of each muscle with cycling intensity, while supraspinal excitability would increase and spinal excitability would decrease during the relative inactive phase of each muscle as cycling intensity increased.

3. the modulation of corticospinal and spinal excitability would be different for changes in cadence and changes in power output (i.e. cadence vs power output).

1.3 References

Carroll, T. J., Baldwin, E. R., Collins, D. F., & Zehr, E. P. (2006). Corticospinal excitability is lower during rhythmic arm movement than during tonic contraction. *Journal of Neurophysiology, 95*(2), 914-921.

Christensen, L. O., Andersen, J. B., Sinkjær, T., & Nielsen, J. (2001). Transcranial magnetic stimulation and stretch reflexes in the tibialis anterior muscle during human walking. *Journal of Physiology*, *531*(2), 545-557.

Dietz, V. (2003). Spinal cord pattern generators for locomotion. *Clinical Neurophysiology*, *114*(8), 1379-1389.

Forman, D., Raj, A., Button, D. C., & Power, K. E. (2014). Corticospinal excitability of the biceps brachii is higher during arm cycling than an intensity-matched tonic contraction. *Journal of Neurophysiology, 112*(5), 1142-1151.

Forman, D. A., Philpott, D. T., Button, D. C., & Power, K. E. (2015). Cadence-dependent changes in corticospinal excitability of the biceps brachii during arm cycling. *Journal of Neurophysiology*, *114*(4), 2285-2294.

Hundza, S. R., & Zehr, E. P. (2009). Suppression of soleus H-reflex amplitude is graded with frequency of rhythmic arm cycling. *Experimental Brain Research, 193(2),* 297-306.

Petersen, N. T., Butler, J. E., Marchand-Pauvert, V., Fisher, R., Ledebt, A., Pyndt, H. S., ... & Nielsen, J. B. (2001). Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking. *Journal of Physiology*, *537*(2), 651-656.

Pyndt, H. S., Laursen, M., & Nielsen, J. B. (2003). Changes in reciprocal inhibition across the ankle joint with changes in external load and pedaling rate during bicycling. *Journal of Neurophysiology*, *90*(5), 3168-3177.

Spence, A. J., Alcock, L. R., Lockyer, E. J., Button, D. C., & Power, K. E. (2016). Phase- and workload-dependent changes in corticospinal excitability to the biceps and triceps brachii during arm cycling. *Brain Sciences*, *6*(4), 60.

Zehr, E. P., Carroll, T. J., Chua, R., Collins, D. F., Frigon, A., Haridas, C., ... & Thompson, A. K. (2004). Possible contributions of CPG activity to the control of rhythmic human arm movement. *Canadian Journal of Physiology and Pharmacology*, *82*(8-9), 556-568.

Chapter 2 Review of Literature

2.0 Introduction

The production of rhythmic and alternating activation of agonist and antagonist muscles is characteristic for locomotor outputs in animals as well as in humans. This basic rhythmic and alternating pattern of muscle activation across locomotor outputs, such as walking, running, and cycling (leg and arm), is generated, in part, by specialized interneuronal networks within the spinal cord, referred to as central pattern generators (CPGs) (Dietz, 2002; Grillner, 1981; Grillner & Wallen, 1985; Zehr et al., 2004). In quadrupeds, the complex pattern of locomotor outputs can be evoked in the spinal cord without the influence of supraspinal input and/or sensory feedback (Brown, 1911). In contrast, it appears that descending inputs from the motor cortex are required in humans to produce rhythmic locomotion (Petersen et al., 2001). For example, brain imaging studies during leg cycling (Christensen et al., 2000; Mehta et al., 2009), and studies using sub-threshold transcranial magnetic stimulation (TMS) to show a suppression of ongoing EMG in target muscles of the lower limb during walking (Petersen et al., 2001) and leg cycling (Sidhu et al., 2012) indicate that the motor cortex makes a direct contribution to the motor system output.

Despite the understanding that both the brain and spinal cord are involved in locomotor outputs in humans, the intricate roles for each structure during locomotor outputs remains uncertain. However, there is a growing body of research that is attempting to address this uncertainty, the majority of which has suggested that the central nervous system (CNS; i.e. brain and spinal cord) is modulated differently depending on

the task and/or the phase of the locomotor output (Capaday & Stein, 1987; Carroll, Baldwin, Collins & Zehr, 2006; Pyndt, Laursen & Nielsen, 2003; Sidhu et al., 2012; Simonsen & Dhyre-Poulsen, 1999). Studies assessing the modulation of spinal reflex pathways and/or corticospinal excitability during locomotor tasks have contributed to this current understanding. Moreover, multiple studies report that the CNS control of locomotor outputs may be different depending on the muscles examined, as muscle-dependent modulations in corticospinal excitability have been noted (Carroll et al., 2006; Forman et al., 2014; Sidhu et al., 2012; Spence et al., 2016; Weavil et al., 2015). Additionally, there is some evidence to suggest that the CNS may be modulated as a function of locomotor intensity (Capaday & Stein, 1987; Forman et al., 2015; Martin et al., 2006; Pyndt et al., 2003; Shik, Severin, & Orlovski, 1966; Spence et al., 2016), and that this modulation may be different for changes in locomotor speed and resistive load (Hundza, de Ruiter, Klimstra, & Zehr, 2012; Pyndt et al., 2003) or force production (Shik et al., 1966).

The majority of the work that has assessed the influence of locomotor intensity on the CNS control has focused on the processing of sensory information and the modulation of spinal reflexes during locomotor outputs (Hundza et al., 2012; Pyndt et al., 2003; Simonsen & Dhyre-Poulsen, 1999). While these studies provide valuable information regarding the neural control of locomotor outputs, they do not provide information on the output of the motor system. One way to examine the output of the motor system is to indirectly assess the excitability of the corticospinal tract. As one of the main descending pathways in the CNS that is heavily involved in the voluntary control of movement, the corticospinal tract can be assessed indirectly via numerous electrical and magnetic

stimulation techniques that produce a response in targeted muscles (Rothwell et al., 1999). These responses indicate how 'excitable' the corticospinal tract is at a given instant in time, and thus represent "corticospinal excitability".

Since cycling is frequently used as a model of locomotion and shares similar neural control as other forms of locomotion, it represents an effective and useful way to assess the influence of locomotor intensity on the neural control of movement. To date, there have only been three studies to examine the effect of locomotor intensity on corticospinal excitability during cycling (Forman et al., 2015; Spence et al., 2016; Weavil et al., 2015). Two of these studies have come from our lab. In these studies corticospinal excitability to muscles of the upper limb was measured during arm cycling at a constant power output and different cadences (Forman et al., 2015), and with different power outputs at the same cadence (Spence et al., 2016). There has yet to be a study to compare the influence of cadence at various power outputs on corticospinal excitability to muscles of the upper limb during arm cycling.

This review will first discuss some background information on the characterization of walking and cycling, which will provide the reader with a framework to refer to during the sections that follow. Secondly, this review will provide information on the corticospinal tract and the stimulation techniques that are often used to assess corticospinal excitability. Finally, the overarching aim of this review is to discuss the current literature with regards to intensity-dependent modulation of the CNS during human movement.

2.1 Characterizing Walking and Cycling in Humans

Despite some of the striking similarities that exist in the neural control of walking and cycling in humans, there are also obvious biomechanical differences that need to be highlighted for ease of the reader. This section will characterize the specific phases of walking and cycling (both leg and arm) to provide the reader with a reference for the sections that follow. The terminology used herein will also be used throughout the rest of the review.

As described previously, both leg and arm cycling tasks have similarities to other forms of human locomotion. With respect to walking, cycling tasks have similar levels of muscle activity, joint ranges of motion, and have a similar neural control, as they demonstrate similar rhythmic and alternating patterns of bursting in flexor and extensor motoneurones (Klarner, Bars, Sun, Kaupp, & Zehr, 2014; Zehr, 2005). The rhythmic pattern of muscle activity is thought to be controlled by a similar spinal circuitry (i.e. CPG) across the locomotor tasks (Zehr, 2005; Zehr et al., 2007).

But, as highlighted above, there are also obvious differences between walking and cycling tasks. One main difference between walking and cycling is that walking is a weight-bearing exercise that involves forward propulsion of the centre of gravity all while maintaining balance, while cycling tasks are not weight-bearing, and also do not involve a forward propulsion of the centre of gravity. Additionally, walking involves coordinated movement of both the arms and legs whereas cycling tasks involve coordinated movements of either the lower limbs or upper limbs for leg and arm cycling, respectively.

Walking is comprised of two phases: 1) the stance phase and 2) the swing phase, with each phase having multiple sub-phases. Together, these phases constitute the gait

cycle and form what is known as a stride (Gage, Deluca, & Renshaw, 1995; Vaughn, Davis, & O'Conner, 1992). The stance phase makes up approximately 60% of the gait cycle during walking, and begins with initial heel contact of the foot of reference. Following heel contact, the foot transitions to the loading response where the entire foot is on the ground, to mid-stance where the centre of gravity shifts forward over the stable foot as the ankle dorsiflexes and the contralateral limb swings in preparation for initial contact. The stance phase continues with terminal stance, where the heel leaves the ground getting ready to step forward. The toe-off stage represents the termination of the stance phase and the beginning of the swing phase of walking, which can be further classified into initial, middle, and terminal swing. The swing phase makes up approximately 40% of the gait cycle, and occurs when the foot is no longer in contact with the ground. The gait cycle is formed from initial heel contact of one foot, to initial heel contact of the same foot (Gage et al., 1995; Vaughan, Davis, & O'connor, 1992). This cycle occurs with both lower limbs, which means that the majority of the gait cycle involves only one foot on the ground at any given time (i.e. single support). During running, the gait cycle remains mostly the same, with the exception that, there is a period where neither foot is in contact with the ground. Furthermore, the relative duration of each phase as a percentage of the total cycle decreases as the speed of running increases. Figure 1 (top trace) illustrates the different phases of walking.

Similar to walking, leg and arm cycling can be broken down into two main phases. These phases have been given many names but for the purpose of this review will be classified as: 1) flexion, and 2) extension. Typically, specific positions throughout cycling are often represented in degrees, or are made relative to the face of a clock

(Carroll et al., 2005; Forman et al., 2014, 2015; Pyndt et al., 2003; Sidhu et al., 2012). With reference to the knee joint, the flexion phase of leg cycling (also frequently referred to as up-stroke) begins when the knee is in full extension and the foot and pedal move from 6 o'clock to 12 o'clock (i.e. 180 to 0 degrees). This action is performed predominantly by the knee flexors (Gregor, Broker, & Ryan, 1991). In contrast, during the extension phase of leg cycling (also referred to as down-stroke), the knee is originally in a fully flexed position and the foot and pedal move from 12 o'clock to 6 o'clock (i.e. 0 to 180 degrees). In this phase, the knee extensors are most active in performing the movement (Gregor et al., 1991).

Arm cycling can be broken down in a similar manner to those described during leg cycling. With reference to the elbow joint, the flexion phase occurs from 3 o'clock to 9 o'clock (i.e. 90 to 270 degrees), while the extension phase occurs from 9 o'clock to 3 o'clock (i.e. 270 to 90 degrees). With respect to muscle activity, during the flexion phase of arm cycling, the biceps brachii muscles are involved in flexion of the elbow joint, and thus are akin to the knee flexors during the flexion phase of leg cycling. During the extension phase, the triceps brachii muscles are involved to assist in extension of the elbow, and thus are akin to the knee extensors during the extension phase of leg cycling. It is also important to note that cycling tasks are usually performed with an asynchronous cranking pattern, which means that the pedals are locked 180 degrees out-of-phase with one another. This means that when one limb is in one phase of the cycle, the contralateral limb is in the opposing phase. For example, during arm cycling, when the right arm is in mid-flexion (6 o'clock), the left arm is in mid-extension (12 o'clock), and vice-versa.

Figure 1 (bottom two traces) illustrates the different phases for leg and arm cycling, respectively.

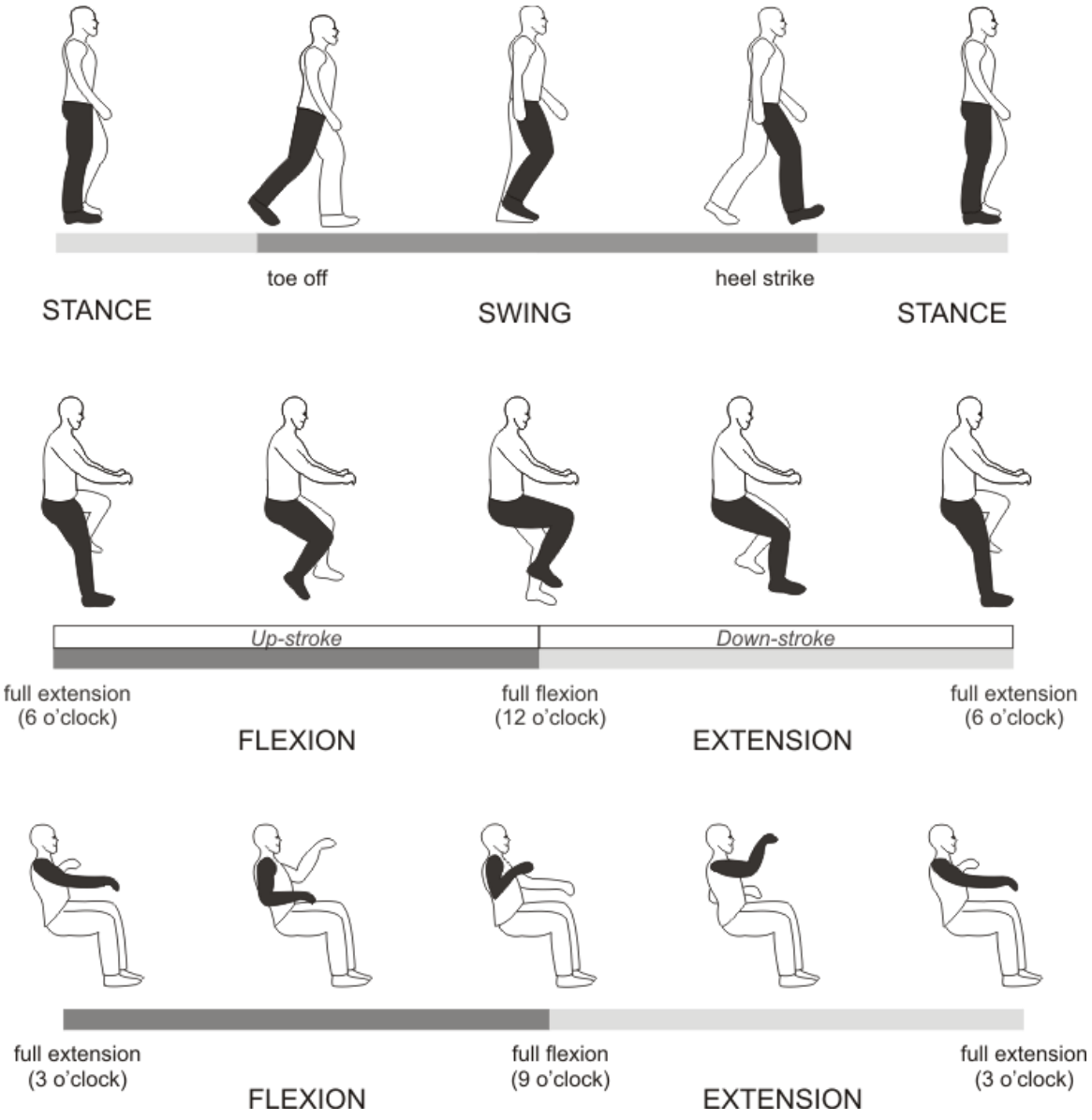

Figure 1: Schematic representation of walking (top trace), leg cycling (middle trace), and arm cycling (bottom trace)

2.2 Relationship between Cadence, Load, and Power Output during Cycle Ergometry

Cycle ergometry provides researchers with the opportunity to manipulate task intensity in a relatively easy and controlled manner (Larson, Voigt, & Grey, 2006; Pyndt et al., 2003). On many ergometers, the intensity or workload can be changed by altering either the cadence and/or the resistive load. Typically, cadence is manipulated by asking the participant to pedal faster or slower, while load may be manipulated in a number of different ways depending on the type of ergometer used. Some ergometers involve the placement of known weights on a weight basket to provide the resistive load during pedalling, while others have a knob that can be turned manually to provide alterations in resistance. Resistance can also be manipulated electronically on some ergometers to control the resistive load applied. Regardless of the type of ergometer used, the combination of cadence and resistive load creates a power output, which is usually measured in Watts (W). Some ergometers, like the one used in the following study (SCIFIT model PRO2 total body ergometer; see Chapter 3), allow the power output to be set at a specified wattage while the cadence and resistance can be inversely altered (Macintosh, Neptune, & Horton, 2000; Price, Collins, Smith, & Gross-Sampson, 2007). This means that at a given cadence, increases in power output are really due to an increase in the resistive load, and at a given power output, increases in cadence result in a decrease in the resistive load. As a result, a resistance-velocity relationship exists during cycle ergometry and it is thought to be similar to the force-velocity relationship of muscle contraction, which states that optimal force production from a muscle is a function of the rate at which the muscle shortens (Wakeling, Blake, & Chan, 2010). In other words, the

force-velocity relationship of a muscle contraction concentric contractions states that at low contraction velocities, higher amounts of force can be produced since more cross-bridges within the muscle are able to form, while at higher contraction velocities, less force can be produced since less cross-bridges are able to form (Wilkie, 1949). During cycle ergometry, this relationship is similar. At a given power output, and at low cycling cadences, the resistive load is high and therefore requires more force to overcome the load, while at higher cycling cadences, the resistive load is low and requires less force to overcome the load. This relationship during cycling also suggests that a given power output can be attained from cycling at a variety of cadences and resistances.

While the power output can remain the same with altered cadence or load, muscle activity does not necessarily remain the same. This is due to the aforementioned force-velocity relationship (Macintosh et al., 2000). For a given power output at high cadences, the resistive load during cycling is low, but there is an increase in muscle activity (recorded via surface EMG) in the working muscles (Wakeling, Blake, & Chan, 2010). This increased muscle activity is likely due to a preferential and selective activation of faster-twitch muscle fibres as the cadence increases along with more frequent, less forceful, cross-bridge formations. Moreover, at low cadences, the resistive load is higher and muscle activity in the working muscles also increase. Recent work from our lab has confirmed this observation as it was shown that EMG from the biceps brachii increased with increased cadence during the flexion phase of arm cycling at a constant power output. This increase in EMG occurred despite a reduction in the amount of torque (i.e. reduction in resistance) required to rotate the crankshaft of the ergometer (Forman et al., 2015). This finding has also been noted in leg cycling (Wakeling et al., 2010). Also from

our lab, Chaytor et al. (2016) have demonstrated a linear increase in EMG from muscles of the upper limb during arm cycling with increased power outputs at a constant cadence. It is currently unknown whether muscle activity is similar during cycling tasks at different cadences and power outputs and how these relationships influence excitability of the corticospinal tract.

2.3 The Corticospinal Tract

The corticospinal tract is one of the most influential descending pathways involved in the voluntary control of motor output (Brouwer & Ashby, 1990; Lemon, Mantel, & Muir, 1986; Nathan & Smith, 1955). As a result, researchers have looked to this tract to gain a better understanding of how motor outputs are controlled. Although most of the original research on the corticospinal tract involved non-human mammals, it is now known that several structures within the brain contain corticospinal projections in humans (Canedo, 1997; Nathan & Smith, 1955, 1990).

In humans, the primary motor cortex (M1) and the premotor and supplementary motor areas are thought to play key roles in controlling movement since approximately 30% of corticospinal projections arise from the M1, and another 30% arise from the premotor and supplementary motor areas (Canedo, 1997). Additionally, the somatosensory areas and the parietal cortex have also been shown to possess corticospinal projections. The cortex, and the M1 in general, is comprised of six layers, each of which that contains neurones that perform different functional tasks. The corticospinal tract arises predominantly in layer V of the M1 via large pyramidal neurones (also known as Betz cells). These neurones have the ability to send both excitatory and inhibitory

information to spinal neurones located contralateral to their origin, to assist in the control of movement (Canedo, 1997; Nudo & Masterton, 1990). These pyramidal neurones have been given many names; however, they will be referred to as upper motoneurones for the remainder of this review. The upper motoneurone classification is based on the fact that the majority of these neurones are located anatomically higher (i.e. within the cerebral cortex) than the neurones that they synapse onto called lower motoneurones, which are located in the brainstem and at specific levels within the spinal cord. Upper motoneurones travelling within the corticospinal tracts can either synapse onto spinal interneurones before synapsing onto a spinal motoneurone (i.e. disynaptic or polysynaptic) or they can synapse directly onto spinal motoneurones (i.e. monosynaptic) (Palmer & Ashby, 1992; Petersen, Taylor, & Gandevia, 2002). The number of polysynaptic and monosynaptic connections varies between motoneurones projecting to different muscles (Palmer & Ashby, 1992). For example, the motoneurones of the biceps brachii receive a large excitatory input from electrical stimulation of corticospinal tract fibres, which has been found to be mostly monosynaptic (Petersen et al., 2002). In contrast, the triceps brachii is thought to have fewer monosynaptic connections, although some monosynaptic connections exist (Brouwer & Ashby, 1990). Regardless of the synaptic connection, it is ultimately the passage of information from the upper motoneurone to specific lower motoneurones that allows for the corticospinal tract to assist in the control of motor outputs.

The actual formation of the corticospinal tract arises from the axons of the upper motoneurones, which project from the precentral gyrus and travel within the white matter of the medulla oblongata where they branch into two distinct tracts: 1) the lateral

corticospinal tract, and 2) the anterior corticospinal tract (Nathan & Smith, 1955). The lateral corticospinal tract is comprised of nerve axons that have decussated at the site of the medulla to the contralateral side of the spinal cord. Although the precise percentage of axons that decussate is not well-known, it is estimated that 80-90% of the descending projections decussate and form the lateral corticospinal tract (Brouwer & Ashby, 1990; Rothwell et al., 1999). Once decussated, the axons travel the length of the spinal cord and synapse onto specific spinal neurones located on the ipsilateral side to which they decussated. The remainder of the axons that do not decussate (approximately 10-20%) pass through the pyramids of the medulla and continue on to form the anterior corticospinal tract. These axons must first decussate to the contralateral side before they synapse onto specific spinal neurones. Thus, both the lateral and anterior corticospinal tracts influence spinal neurones on the contralateral side, although they do not follow the same path. This means that upper motoneurones originating from the left hemisphere of the motor cortex travel on the right side of the spinal cord and innervate muscles of the right limbs, while upper motoneurones that originate from the right hemisphere travel on the left side of the spinal cord and innervate muscles of the left limbs (Nathan & Smith, 1955).

2.4 Assessing the Excitability of the Corticospinal Tract

In brief, the responsiveness of the corticospinal pathway is influenced by several different synaptic inputs that are in constant state of change. Inputs from supraspinal centres, spinal interneurones, primary afferents, as well as the intrinsic properties of the neurones that compose the corticospinal pathway, all influence its responsiveness.

Ultimately, it is the sum of all these various inputs that make the neurons of the corticospinal pathway either more or less likely to produce a motor output (Canedo, 1997; Taylor et al., 2002). The word responsiveness, in neurophysiology, is typically referred to as *excitability*. Thus, the excitability of the corticospinal pathway, termed corticospinal excitability, can be altered by changes in any of the aforementioned inputs, and can change at any given moment or during any different task. For example, corticospinal excitability is lower at rest than it would be during dynamic or isometric contractions of a muscle (McNeil et al., 2013). Researchers are able to assess how corticospinal excitability changes in humans across different conditions by using various indirect electrical and/or magnetic stimulation techniques. While there are many ways to assess corticospinal excitability in humans, transcranial magnetic stimulation (TMS) and transmastoid electrical stimulation (TMES) will be included in this review as they were the techniques utilized in my experiment (see Chapter 3).

2.4.1 Transcranial Magnetic Stimulation

Since its creation by Barker and colleagues in 1985, TMS has been used as a non-invasive and non-painful stimulation technique to examine corticospinal excitability in humans (Barker et al., 1985; Burke et al., 1993; Terao & Ugawa, 2002). TMS is performed using an insulated coil of copper wire that has a high electrical capacitance. Based on Faraday's law of electromagnetic induction, when a high electric current passes rapidly through the coil, it discharges a magnetic field perpendicular to the direction of the electrical current in the coil (Rothwell et al., 1999). When a TMS coil is placed over the scalp, the induced current from the coil is transferred across the scalp and skull to

activate the neural tissue in the cortex (Rothwell, 1991; Rothwell et al., 1999; Di Lazzaro et al., 1998; Terao & Ugawa, 2002).

Single pulse TMS typically does not directly activate the upper motoneurones of the corticospinal tract, but instead activates interneurones in the superficial layers of the motor cortex, which then synapse onto the upper motoneurones of the corticospinal tract located in layer V (Rothwell, 1991). This is known as indirect activation, a because TMS trans-synaptically activates neurones of the corticospinal tract. TMS typically evokes multiple descending volleys, which can be observed at the cervical spinal cord via epidural recordings (Burke et al., 1993; Di Lazzaro et al., 1998). These recordings are referred to as indirect waves (I-waves), and are the result of the trans-synaptic activation of upper motoneurones of the corticospinal tract (Di Lazzaro et al., 1998; Thompson et al., 1991). However, in some individuals TMS can sometimes directly activate the upper motoneurones of the corticospinal tract when the intensity of the magnetic stimulator is high (Di Lazzaro et al., 1998; Edgley et al., 1990). The direct activation of upper motoneurones can also depend on the direction of current flow within the coil, as well as the placement of the coil over the scalp. This direct activation of upper motoneurones results in the production of a direct wave (D-wave) (Day et al., 1989; Nakamura et al., 1996; Thompson et al., 1991) and researchers are able to differentiate between I- and D-waves by examining the latencies of the responses using epidural electromyography (EMG) recordings (Di Lazzaro et al., 1998; Rothwell, 1990). Usually, D-waves have shorter latencies than I-waves by approximately 1-2 ms (Rothwell et al., 1991).

TMS-evoked responses are normally recorded from a target muscle as compound muscle action potentials in the surface EMG trace, and are referred to as motor evoked

potentials (MEPs) (Burke et al., 1990; Taylor et al., 2002). While researchers can assess a variety of MEP properties to examine changes in corticospinal excitability across different experimental conditions, typically, changes in peak-to-peak amplitude of the MEP are used to infer changes in corticospinal excitability (Di Lazzaro et al., 1998). Using this measurement, a corresponding growth or reduction of a MEP represents an increase or a decrease in excitability, respectively. The amplitude of a MEP can be influenced by several factors, including changes in arousal, and the amount of background activity of motoneurones (Hess et al. 1987). In addition, since the MEP pathway involves synapses at the cortical and spinal level as well as the neuromuscular junction, the size of the MEP depends on the excitability of cortical and spinal neurones as well as the muscle fibres involved. Therefore, changes in MEPs across conditions cannot fully disclose where along the motor pathway the change in excitability is occurring (Burke et al, 1993; Taylor et al., 2002). As a result, an independent measure of spinal excitability is often used in conjunction with TMS to help differentiate whether the change in MEP may be due to supraspinal or spinal factors, assuming peripheral excitability is considered (i.e. responses made relative to M_{max}). For the purpose of this review, the only measure of spinal excitability that will be discussed is called transmastoid electrical stimulation (TMES).

2.4.2 Transmastoid Electrical Stimulation

Also referred to as brainstem or cervicomedullary junction stimulation (Ugawa et al., 1991), TMES involves an electrical current being passed through surface electrodes placed over or near the mastoid processes (2 cm above or 4 cm below) (Taylor, 2006).

This electrical stimulation activates the axons of upper motoneurones that run horizontally near the cervicomedullary junction, where the corticospinal tract decussates (Ugawa et al., 1991; Taylor, 2006). This location is thought to be the ideal site for stimulation since the rapid bending of the axons near the decussation provides a larger surface area for stimulation, which may also be more easily excitable (Amassian et al., 1992; Ugawa et al., 1991).

In contrast to TMS of the motor cortex, TMES produces a single descending volley that travels down axons of upper motoneurones and synapses onto spinal motoneurones, the subsequent response recorded from the muscle of interest via surface EMG. The observed response is known as a cervicomedullary motor evoked potential (CMEP), and changes in the peak-to-peak amplitude of these responses (expressed as a percentage of the maximal compound muscle action potential) can be used to help distinguish amongst changes in spinal excitability across experimental conditions. Several researchers have shown that descending volleys produced by TMES and TMS activate at least some of the same axons of the corticospinal tract, as antidromic action potentials from TMES appear to occlude the descending volleys from TMS (Berardelli, Inghilleri, Rothwell, Cruccu, & Manfredi, 1991; Gandevia, Petersen, Butler, & Taylor, 1999; Taylor et al., 2002). This finding confirmed that comparisons between the two methods could be used to discriminate between supraspinal and spinal contributions to corticospinal excitability (Taylor & Gandevia, 2004). TMES-evoked CMEPs reflect a predominantly monosynaptic response of the motoneurones to electrical stimulation of large diameter corticospinal tract axons, and can be performed in awake human participants at rest, during, and/or following voluntary motor outputs (McNeil, Butler, Taylor, & Gandevia,

2013; Taylor & Gandevia, 2004). Unlike other stimulation techniques, CMEPs are not influenced by conventional presynaptic inhibition as descending corticospinal axons are not prone to this type of inhibition (Nielsen & Petersen, 1994; McNeil et al. 2013). This represents an advantage for using TMES in human research. Changes in CMEPs are often looked at as being strictly related to the excitability of the spinal motoneurone; however, they can also provide a measure of the efficacy of transmission across the corticospinal-motoneuronal synapse (Taylor & Gandevia, 2004). Thus, TMES-evoked CMEPs must be interpreted with both spinal motoneurone excitability and corticospinal-motoneuronal excitability in mind.

Like any other stimulation technique, TMES is not without its limitations and pitfalls. The first potential issue to consider when using TMES in experimental research is to recognize that TMES is uncomfortable. The electrical stimulation activates local skin afferents at the stimulation site, and results in transient pain (Taylor & Gandevia, 2004). Unfortunately, there is little that can be done to reduce this short-lasting pain, so it is important for participants to be accustomed to the stimulation before participating in the experiment. A second issue, even with proper electrode placement between the mastoids, is the potential for TMES to activate ventral nerve roots that are exiting the spinal cord in addition to the spinal tracts. These ventral nerve roots have a tendency to bend upon exiting the spinal cord, and this bending provides a larger and more easily activated surface for electrical stimulation (Rossini et al. 1985; Mills and Murray, 1986). If these nerve roots are activated, the onset latency of the resulting CMEP response will decrease by approximately 1-2 ms, which can be observed in the EMG trace (Ugawa et al., 1991; Taylor & Gandevia, 2004). Since some peripheral axons have been activated, this

presents a potential problem for researchers because the CMEP now reflects a mix of both pre- and post-synaptically activated motoneurones, and does not represent the excitability of the motoneurone itself (Taylor & Gandevia, 2004). Thus, when nerve roots are activated, the CMEP cannot be used as a measure of spinal excitability, and as a result, not all participants are able to participate in the TMES portion of experimental protocols.

Similar to the analysis of MEPs from TMS, researchers can analyze CMEPs for peak-to-peak amplitudes, onset latencies, and areas to provide an evaluation of spinal excitability at a given moment. Usually, multiple CMEPs are evoked and averaged, and the peak-to-peak amplitude values are taken from the averaged CMEP trace, as is done with MEPs elicited via TMS.

2.5 Comparing the Neural Control between Locomotor Outputs and Tonic Contractions

As previously mentioned, the basic, rhythmic and alternating patterns of locomotor outputs are thought to be controlled, at least partially, by specialized networks of cells within the spinal cord (Jordan, 1998; Dietz, 2002; Zehr et al., 2004). In contrast, the activation of these networks is absent during non-locomotor outputs, such as tonic or isometric contractions. A common method to examine the neural control of rhythmic motor outputs is to compare a locomotor output to a tonic contraction. This methodology has been used in both animal and human research by several researchers, across several locomotor tasks (Capaday & Stein, 1986; Pyndt et al., 2003; Carroll et al., 2006, Forman et al., 2014; Sidhu et al., 2012). The rationale for why this method is used is that when the

locomotor task and tonic contraction are compared, any observed differences in the measurements used may be due to differences in the neural control of the two tasks.

However, in order for this method to be effective in providing information on the neural control of different tasks, the overall output from the motoneurone pool between the locomotor output and the tonic contraction must be the same. If the output from the motoneurone pool is not the same, then the comparison between the two tasks cannot be made since potential changes in corticospinal excitability may now be due to differences in the amount of drive to the motoneurone pool. One way to match motoneurone pool output between tasks is to match the background EMG, since background EMG provides a crude measure of the intensity of the motor output, and reflects the output of the motoneurone pool (Pyndt & Nielsen, 2003; Sidhu et al., 2012; Zehr, 2002). If the EMG is matched correctly, then any differences in the excitability measurements between the two tasks can more confidently be attributed to differences in the neural control of the tasks.

For example, with matching background EMG, the use of tonic contractions has been used in arm cycling studies whereby it has been shown that corticospinal excitability is increased prior to cycling in a manner that is similar to that of tonic contractions (Copithorne et al., 2014). In addition, it was found that prior to both motor outputs the increase in corticospinal excitability was due to supraspinal, and not spinal mechanisms, suggesting that a similar neural drive may exist to initiate both motor outputs (Copithorne et al., 2014). Interestingly, Forman et al. (2014) found that once cycling reached a steady state, corticospinal excitability to the biceps brachii was significantly greater than during tonic contraction. The authors suggested that an increase in spinal motoneurone excitability could partially account for this finding (Forman et al., 2014). Using this

cycling versus tonic method, the neural control during steady-state arm cycling appeared to be different than the neural control during a tonic contraction.

2.6 Modulation of Spinal Reflexes during Locomotor Outputs

A large part of what is known regarding the neural control of locomotor tasks in humans has come from studies that have assessed the modulation of sensory information and spinal reflexes via the Hoffmann (H-) reflex (Capaday & Stein, 1986, 1987; Brooke et al., 1992; Zehr et al., 2003) and/or the quantification of reciprocal inhibition (Petersen, Morita, & Nielsen., 1999; Pyndt et al., 2003). The main findings from these studies suggest that the processing of sensory information and spinal reflexes during human locomotor tasks are modulated differently depending on the task performed, the phase of the task, and the intensity of the task. The following sections will discuss some of the task-, phase-, and intensity-dependent findings in sensory information during locomotor outputs.

Before focusing on the results from studies that have used the H-reflex and reciprocal inhibition to provide an indication of how sensory information is processed throughout locomotor outputs, it is first important to briefly describe what each of these reflexes are and how they are used in human research. The first reflex that will be discussed is the H-reflex. As the electrical analogue of the predominantly monosynaptic stretch reflex, the H-reflex is one of the most studied reflexes in humans (Knikou, 2008; Magladery & McDougal, 1950). It is evoked by a submaximal electrical stimulation of a peripheral nerve, and results in action potentials propagating along group Ia afferent fibres until they synapse onto motoneurones within the spinal cord (Schieppati, 1987;

Zehr, 2002). The motoneurone then generates action potentials, which travel along efferent fibers until they reach the neuromuscular junction and produce a response (i.e. H-reflex) visible in the surface EMG of a target muscle (Ferris et al., 2001; Magladery & McDougal, 1950; Zehr, 2002). The amplitude of the H-reflex was originally thought to provide a direct measure of alpha-motoneurone excitability, but it has since been shown that this is not the case, since presynaptic inhibition of the Ia afferents modulates the amplitude of the evoked response (Zehr, 2002). Consequently, many researchers highlight the importance of considering presynaptic inhibition when interpreting results from H-reflex studies (Zehr & Stein, 1999; Zehr, 2002). As a result, the H-reflex can provide information on the modulation of presynaptic inhibition across different tasks (Stein, 1995). Presynaptic inhibition is a phenomenon that occurs at an axo-axonic synapse whereby activity in the presynaptic neurone decreases the amount of neurotransmitter released from the postsynaptic neurone (Faist, Mazevet, Dietz, & Pierrot-Deseilligny, 1992; Hultborn, Meunier, Morin, & Pierrot-Deseilligny, 1987). The decrease in neurotransmitter release is mediated by the action of inhibitory interneurones on the Ia afferent terminals, which ultimately results in a reduction in motoneurone depolarization induced by Ia activity (Hultborn et al., 1987).

Reciprocal inhibition is a spinal reflex that is essential for normal flexion-extension movements to occur since it ensures that antagonistic muscles are largely relaxed when the agonist muscle is contracting (Crone et al., 1985, 1987; Shindo et al., 1984; Schiepati, 1987; Tanaka, 1974). This allows the agonist to produce sufficient force to perform the desired motor output. From research conducted in the cat, reciprocal inhibition is thought to be mediated by Ia inhibitory interneurones within the spinal cord

that send inhibitory signals to antagonistic muscles when the agonist muscle is contracting (Crone et al., 1987; Tanaka, 1974**)**. In addition, the excitability of these Ia interneurones is influenced by several factors, including descending motor tracts and peripheral sensory afferents. In humans, the reciprocal inhibition pathway appears to be similar to that in cats, and researchers have been able to examine the influence of reciprocal inhibition during locomotor tasks by measuring a decrease in the amplitude of the H-reflex following stimulation of the antagonist peripheral nerve or by observing a reduction in EMG activity following stimulation to the antagonist peripheral nerve (Knikou, 2008; Zehr, 2002). By measuring the H-reflex and reciprocal inhibition during different phases of locomotor outputs, and comparing them to non-locomotor outputs (e.g. tonic contractions), researchers have been able to develop a better understanding of how spinal reflexes are modulated across a variety of locomotor tasks.

2.6.1 Task- and Phase-dependent Modulation

As mentioned above, there is a growing body of evidence suggesting that the processing of sensory information and spinal reflexes within the spinal cord is modulated in a task- and phase-dependent manner. For example, Capaday and Stein (1986) reported a suppression of the human soleus H-reflex in the stance phase of walking, compared to tonic standing, with similar levels of background EMG. This suppression of the soleus H-reflex could not be simply explained by differences in excitation of the motoneurone pool, since they were matched between tasks via EMG, thus the authors attributed the finding to a task-dependent increase in presynaptic inhibition in the Ia terminals on the soleus motoneurone during walking compared to tonic standing. In addition, the authors

also reported a phase-dependent modulation in the soleus H-reflex during walking, as the H-reflex was measurable during stance, but was absent during swing. These findings have been reported by other walking and running studies as well (Capaday & Stein, 1987; Ferris et al., 2001; Simonsen & Dyhre-Poulsen, 1999). The authors from these studies propose that these phase-dependent findings may be explained by the activation of stretch reflex during stance, whereby increasing muscle activation provides stiffness that may be necessary to help decelerate the body when it makes contact with the ground. However, it was also proposed that central neural mechanisms may be mediating these findings; with presynaptic inhibition and reciprocal inhibition as potential candidates. Examining the reciprocal inhibition pathway between the tibialis anterior and the soleus during walking, Petersen et al. (1999) described an inhibition of the soleus H-reflex following stimulation of the common peroneal nerve, whereby this inhibition was largest during the swing phase of walking, and was nearly absent during stance. The authors suggest that the phase-dependent findings in the suppression of the H-reflex are likely to be explained by the modulation of the reciprocal inhibition pathway, as it may help inactivate antagonistic motoneurones in the appropriate phases of the walking cycle. However, the authors also suggest that other factors, including presynaptic inhibition and supraspinal inputs may also contribute. This pattern of reflex modulation is also not unique to walking and running, since findings from leg and arm cycling studies support a similar modulation in the spinal reflex pathway (Boorman, Becker, Morrice, & Lee, 1992; Pyndt et al., 2003; Zehr et al., 2004; Zehr & Loadman, 2012).

Brooke et al. (1992) compared the modulation of soleus H-reflexes during leg cycling with those during static conditions with matched leg joint angles and soleus

muscle activation, and reported similar task-dependent findings to those found in walking and tonic standing (Capaday & Stein, 1986). They found that the soleus H-reflexes evoked when the subjects performed tonic contractions were significantly larger than those that were evoked during leg cycling, and they attributed the suppression of the soleus H-reflex during cycling to a task-dependent increase in presynaptic inhibition of the Ia afferents. Also during leg cycling, Boorman et al. (1992) reported that the soleus H-reflex progressively increased during down-stroke (when the soleus becomes active), but becomes almost non-existent during up-stroke (when the soleus is relatively inactive). This finding is congruent with the phase-dependent findings in the soleus observed during walking and running (Capaday & Stein, 1986; Ferris et al., 2001). The modulation of the H-reflex during cycling may allow the reflex to enhance ankle stiffness in down-stroke during power generation similar to stance in walking and running. In contrast, during upstroke the inhibition of the soleus H-reflex may function to ensure that muscle activation induced by the stretch reflex does not impede the dorsiflexion of the ankle and the continued propulsion of the crank. The authors postulated that the mechanism underlying the change in H-reflex during cycling may be due to a variety of factors, including presynaptic of Ia terminals and postsynaptic inhibition of Ia inhibitory interneurones. Additionally, the authors suggested that descending commands from supraspinal centres may also play a role into this phasic pattern of control as changes to the soleus H-reflex during passive cycling (i.e. when descending commands are reduced) were suppressed when compared to active cycling.

Carroll et al. (2006) reported that H-reflex amplitudes of the forearm flexors were smaller during arm cycling than intensity- and position-matched tonic contractions.

Additionally, they found that forearm flexor H-reflexes were largest during the extension phase of arm cycling, and were lowest during flexion. These results are directly in opposition with studies that have examined the soleus H-reflex during walking and leg cycling (Capaday & Stein, 1986; Brooke et al., 1992; Boorman et al., 1992). The authors concluded that the discrepancy between their results and those of the lower limbs might be due to the function of the muscle examined. The soleus has distinct bursting patterns of muscle activity during the down-stroke of leg cycling, whereas the flexor carpi radialis is active throughout arm cycling without any distinct bursting of muscle activity. Regardless, the similar task- and phase-dependent modulation of sensory information across locomotor tasks provides indirect evidence of the likelihood that spinal CPGs are involved in the control of these movements, although it is likely that increases in presynaptic inhibition along with descending inputs may also be mediating these findings.

2.6.2 Intensity-dependent Modulation

In addition to the task- and phase-dependent spinal reflex modulation during locomotor tasks, intensity-dependent modulations have been shown to exist as well (Petersen et al., 1999; Simonsen & Dyhre-Poulsen, 1999; Ferris et al., 2001; Pyndt et al., 2003). Several researchers have reported a suppression of the soleus H-reflex gain during running compared to walking (Capaday & Stein, 1987; Edamura et al., 1991; Ferris et al., 2001). Capaday & Stein (1987) were amongst the first to report this effect, as they found that the soleus H-reflex was larger during the stance phase of walking than during the stance phase of running, even though the background EMG was on average 2.4 times greater during running. The authors suggested that a reduced H-reflex during running

must not simply be a passive consequence of the excitation of the motoneurone pool, but must be influenced by other central neural mechanisms. It was suggested that modulation of presynaptic or postsynaptic inhibition was a likely candidate to explain these results, since these types of inhibition strongly modulate the reflex pathway (Brooke et al. 1997). In contrast to these findings, Simonsen & Poulsen (1999) evaluated soleus H-reflex amplitudes during walking and running at faster speeds than those reported by Capaday and Stein (1987). They showed that soleus H-reflex amplitudes were similar during walking at 4.5 km/h and during running at 8 km/h, but were larger at faster running speeds. The authors suggested that the discrepancy between the findings might be due the methodology employed by the other studies as they did not correct for fluctuations in M_{max} throughout the gait cycle, which would have influenced the stimulation intensities used to elicit the H-reflexes. Thus, they argued that stretch reflexes (as measured through H-reflexes) play more of a prominent role in the control of running than presynaptic or postsynaptic inhibition in the spinal cord. However, regardless of the discrepancy, the CNS is clearly modulated as the intensity of the motor output is increased.

Researchers examining cycling tasks have also reported intensity-dependent findings. Staines et al. (1997) reported that somatosensory evoked potential amplitudes and soleus H-reflex amplitudes were suppressed as leg cycling cadence and rate of stretch on the knee extensors increased. The authors suggested that these findings were likely due to an increased proprioceptive discharge from the muscle spindles at higher cycling cadences, which would reduce the ascending transmission in the somatosensory evoked potential and H-reflex pathways by synapsing onto Ia inhibitory interneurones in the spinal cord. Thus, the authors suggested that afferent feedback from sensory receptors

have at least some role in the central control of locomotion when the intensity of the movement is increased. Work done by Pyndt et al. (2003) examining the effect of increased external load and cadence during leg cycling has demonstrated that reciprocal inhibition from the tibialis anterior to the soleus, as measured via a decrease in soleus background EMG and the soleus H-reflex, decreases as external load increases, and tends to decrease as cycling rate increases. Similar to Staines et al. (1997), these authors also suggested that modulation in the group Ia afferents leading onto opposing inhibitory interneurones is the likely mechanism underlying this increase in reciprocal inhibition.

The effects of cycling frequency (i.e. cadence) and external load have also been examined during arm cycling (Hundza et al., 2009; 2012). In two separate papers, the authors investigated the effect of arm cycling frequency (Hundza et al., 2009) and arm cycling load (Hundza et al., 2012) on H-reflex amplitudes from the soleus of the lower limbs. The authors found that arm cycling at higher frequencies significantly suppressed the soleus H-reflex, with the largest suppression occurring at the highest frequency. In contrast, cycling load did not alter the amplitudes of the soleus H-reflex. Although these studies examined the influence of arm cycling on the processing of sensory information in a muscle of the lower limb, it still suggests that locomotor outputs may be controlled differently by the CNS as the cadence and load are altered. The authors suggested that supraspinal and/or spinal mechanisms are the dominant sources of this modulation, and that afferent feedback plays a lesser role.

While it is clear from the aforementioned studies that the processing of sensory information is altered when the intensity of the locomotor output is changed, it remains unclear what effect increasing the intensity of the task has on the output of the motor

system. One way to examine the effects of intensity on motor system output is to assess the excitability of the corticospinal tract.

2.7 Modulation of Corticospinal Excitability during Tonic Contractions

Most of what is known regarding the excitability of the corticospinal tract in humans has come from research examining tonic contractions (Martin et al., 2006; Oya et al., 2008; Taylor et al., 1997). When a person performs a voluntary muscle contraction, there is an increase in excitability at the motor cortex and the motoneurone pool, as motor unit firing frequency and recruitment are increased (Bawa & Lemon, 1993; Brouwer & Ashby, 1990; Martin et al., 2006). As the force of a muscle contraction increases, the recruitment of motor units increase until a certain limit is reached, at which point the only way to increase the force output is to increase the firing frequency (Martin et al., 2006). Additionally, this 'limit' at which a muscle reaches its maximum motor unit recruitment varies from muscle to muscle (Kischka et al., 1993). For example, relatively large muscles that have the potential to produce large amounts of force, such as the biceps brachii, can recruit new motor units up until ~90% of MVC, while smaller muscles, such as the adductor pollicis, have all of its motor units recruited at ~50% of MVC (De Luca et al., 1982; Kukulka & Clamann, 1981).

In addition, with an increase in motor unit firing frequency, the likelihood of obtaining a response to a stimulus from the motor cortex or motoneurone pool is decreased, irrespective of the recruitment threshold of the motor unit and the type of input (Oya et al., 2008). For low force contractions of the upper limbs, there is a linear increase between voluntary effort (frequently measured via EMG) and the size of MEPs evoked by

TMS and CMEPs evoked by TMES (Taylor et al., 1997). However, this increase is not necessarily continuous, as several studies, especially in the upper limbs, have observed a plateau and subsequent decrease in MEP and CMEP responses at particularly high contraction intensities (Pearcey, Power, & Button, 2014; Martin et al., 2006; Søgaard et al., 2006; Taylor et al., 1997; Todd et al., 2003).

For the purposes of this review, only studies that investigated corticospinal excitability during upper limb tonic contractions will be discussed, although similar findings have been reported in the lower limbs (e.g., Goodall et al., 2009). Todd and colleagues (2003) examined TMS-evoked MEPs of the biceps brachii during four different submaximal tonic contractions that were made relative to each participant's MVC torque. The authors found that MEPs increased up until ~50% MVC, where the MEP was ~90% of M_{max}, and from there decreased to a low of 77% M_{max} as the force increased towards 100% MVC. The authors suggested that this decrease in corticospinal excitability was likely due to the inability of some motoneurones to fire in response to the excitatory input from the magnetic stimulus. Martin et al. (2006) reported similar findings, and used TMS and TMES to examine responses from the biceps brachii and brachioradialis during three different submaximal tonic contractions intensities of 50, 75, and 90% MVC. The researchers found that MEPs elicited in the biceps brachii reached a maximum at ~50% MVC, where they were equal to ~90% M_{max}, and decreased to ~75% M_{max} as the contraction intensity increased to 100% MVC. Interestingly, they found that CMEPs followed a similar pattern, suggesting that this modulation of corticospinal excitability (as indicated via MEP area) was likely due to mechanisms acting at the spinal motoneurone pool (Martin et al., 2006). Responses from the brachioradialis followed the

same trend as well, with the MEPs and CMEPs reaching maxima at ~50% MVC, at which point they progressively decreased as contraction intensity increased to 100% MVC. This plateauing and subsequent decrease in corticospinal excitability during tonic contractions has been reported by several researchers from different muscles, which suggests that these motor outputs are controlled similarly as the intensity of the contraction is increased. Additionally, since MEPs and CMEPs appear to follow the same trend during tonic contractions, the change in excitability is likely due to spinal mechanisms.

2.8 Modulation of Corticospinal Excitability during Locomotor Outputs

The modulation of corticospinal excitability during locomotor outputs has been previously reported across several walking and cycling tasks (Capaday et al., 1999; Forman et al., 2014; Petersen et al., 1998; Sidhu et al., 2012; Schubert et al., 1997). Schubert and colleagues (1997) were the first to use TMS to investigate the corticospinal control during a locomotor output. Examining MEPs from the tibialis anterior and the gastrocnemius during walking, the authors reported phase-dependent increases in corticospinal excitability to both muscles. MEPs from the tibialis anterior were largest during the swing phase of walking, while MEPs from the gastrocnemius were largest during the stance phase. The authors suggested that this modulation in MEPs was likely due to increases in EMG from each muscle during the corresponding phase of walking; the tibialis anterior EMG was largest during swing, while the gastrocnemius EMG was largest during stance. However, they noted that facilitation of the MEP was larger in the tibialis anterior (i.e. ankle flexor) than the gastrocnemius (i.e. ankle extensor). Although it

was not suggested by the authors, this muscle-dependent finding in corticospinal excitability coincides with the idea that the corticospinal tract makes more monosynaptic connections to flexor motoneurones than extensor motoneurones, specifically more to the tibialis anterior than to the gastrocnemius (Brouwer & Ashby, 1992). In addition, the authors reported a task-dependent change in corticospinal excitability as MEPs were larger during the corresponding phases of walking than when compared to an intensity-matched tonic dorsiflexion contraction. The authors suggested that the facilitation of the MEPs between tasks cannot simply reflect changes in motoneurone activity since EMG was matched, and therefore may be due to supraspinal and/or spinal mechanisms. Similar phase-dependent modulation of corticospinal excitability to the tibialis anterior has been noted by Christensen et al. (2001), who found that MEPs were larger during the swing phase of walking (i.e. when the tibialis anterior is most active) than the stance phase (i.e. when the tibialis anterior is less active). While this was not a main finding of this study, it does provide further evidence that corticospinal excitability is modulated in task- and phase-dependent manners during locomotor outputs.

Capaday et al. (1999) performed a similar study in which they looked at the corticospinal contribution to the tibialis anterior and soleus muscles during the stance phase of walking and a tonic plantar flexion task. In opposition to the findings from Schubert et al. (1997), the authors reported a suppression of MEPs from the soleus (i.e. an ankle extensor) during the stance phase of walking, while MEPs from the tibialis anterior were enhanced when both were compared to an intensity-matched tonic plantar flexion contraction. It was suggested that during stance, corticospinal excitability to the tibialis anterior was enhanced due to increased cortical activity, which consequently would bring

the tibialis anterior motoneurones closer to threshold. As for the reduction in corticospinal excitability to the soleus, the opposite was proposed. They suggested that during stance, motor cortical activity to the ankle extensor (i.e. soleus) was reduced, which would account for the suppression of the MEP. Although not discussed in the paper, a potential reason for the observed discrepancy between the two studies could represent a muscle-dependent modulation in corticospinal excitability likely associated to the number of monosynaptic projections made to each muscle or the muscle fibre type composition. The gastrocnemius and the soleus are both ankle extensor muscles; however the amount of monosynaptic connections to each of these muscles varies (Brouwer & Ashby, 1992), as does the muscle fibre type composition (Edgerton, Smith, & Simpson, 1975). These factors could potentially explain the discrepancy found between studies. However, despite the discrepancy between the two studies, they both provide evidence for task- and phase-dependent modulation in corticospinal excitability during locomotor tasks. Similar task-, phase-, and muscle-dependent findings have been replicated in cycling studies (Carroll et al., 2006; Forman et al., 2014; Pyndt & Nielsen, 2003; Sidhu et al., 2012).

During leg cycling, Pyndt and Nielsen (2003) investigated the transmission in the corticospinal and Ia afferent pathways, using TMS and H-reflexes respectively, to soleus and tibialis anterior motoneurones throughout the full crank cycle. They reported that soleus H-reflexes and MEPs were modulated similarly throughout the crank cycle, whereby responses were largest during down-stroke and smallest during up-stroke. In contrast, tibialis anterior MEPs were large during up-stroke and much smaller during down-stroke. Thus, the responses were directly related to the EMG from each muscle during each phase, since during down-stroke the soleus is most active and during up-

stroke the tibialis anterior is most active. Additionally, at matching ankle angles and levels of background EMG, the authors found that soleus MEPs were larger during early down-stroke during bicycling compared to a tonic plantarflexion contraction, while soleus H-reflexes were depressed during late down-stroke compared to tonic contraction. These findings are similar to the modulation observed during walking (Capaday et al., 1999; Schubert et al., 1997), which suggests that a common neural control may exist across locomotor tasks. The authors suggested that task-dependent facilitation in soleus MEPs during early down-stroke may be due to changes in the transmission of the corticospinal pathway, while the suppression of the H-reflex during late down-stroke of leg cycling was likely due to changes in the Ia afferent pathway.

Sidhu et al. (2012) found that corticospinal and spinal excitability to upper leg muscles during leg cycling were modulated in a similar manner throughout the phase of a full cycle. Using TMS and TMES, the researchers activated the descending corticospinal pathway projecting to the vastus lateralis muscle throughout one full revolution of leg cycling. Responses from these stimulation techniques were grouped into 12 ‘equal time bins’, and each of the 12 ‘bins’ represented one of the 12 pedal positions that were examined. These positions were made relative to a clock face. The results showed that MEP amplitudes of the vastus lateralis were largest just prior to the most active phase of the muscle, but significantly decreased for the remainder of the cycle. The modulation of CMEP amplitudes followed a similar pattern, suggesting that the changes in corticospinal excitability were due to spinal mechanisms. This study also provides evidence for phase-dependent modulation in corticospinal excitability during a locomotor output.

During arm cycling, Carroll et al. (2006) have reported similar modulations in corticospinal excitability. Similar to the technique used by Pyndt and Nielsen (2003), Carroll and colleagues (2006) used TMS and H-reflexes to evoke responses in the flexor carpi radialis at four different positions during arm cycling. These positions were 3, 6, 9, and 12 o'clock, which were made relative to a clock face. Additionally, stimulations were also delivered during position- and intensity-matched tonic contractions. During the mid-flexion phase of arm cycling (6 o'clock position), MEP and H-reflex amplitudes were significantly smaller than when compared to position- and intensity-matched tonic contractions. In addition, at the onset of flexion (3 o'clock position), MEPs remained unchanged, while H-reflexes were significantly larger during tonic contraction than during cycling. When the researchers conditioned the H-reflex with TMS during tonic contractions, there was a facilitation of the H-reflex but this facilitation did not exist once subjects cycled. Thus, the authors concluded that their findings reflect a decrease in the contribution of the motor cortex to the generation of motor output during arm cycling when compared to tonic contractions. They suggested that this modulation is likely due to the contribution of the spinal CPG during rhythmic arm cycling.

Recently, findings from Forman et al. (2014) suggest that the modulation of corticospinal excitability during arm cycling is not only task and phase-dependent, but it is also muscle-dependent. Examining corticospinal excitability of the biceps brachii via TMS and TMES, the authors performed a similar experiment to Carroll et al. (2006) and reported that both MEPs and CMEPs were modulated throughout the full cycle. In contrast to Carroll et al. (2006), they found that MEPs were significantly larger during cycling at both the 3 and 6 o'clock position than during position- and intensity-matched

tonic contractions. CMEPs were significantly larger only at the 3 o'clock position, as they remained unchanged at the 6 o'clock position. The authors suggested that the discrepancy between their results and those by Carroll et al. (2006) is likely due to the role that the target muscle from each experiment plays throughout arm cycling. The biceps brachii is a prime mover, while the flexor carpi radialis is more of a stabilizing muscle, and thus is active throughout the entire locomotor output. Nonetheless, the results from all of these studies suggest that corticospinal excitability is modulated in a task- and phase-dependent manner. While there is increasing evidence available for the modulation of corticospinal excitability throughout locomotor tasks, there is very little known regarding how this system is modulated as the intensity of the motor output is increased.

2.9 Modulation of Corticospinal Excitability as Cycling Intensity is Altered

To date, there are only three studies that have examined the modulation of corticospinal excitability as the intensity of a cycling task is altered (Forman et al., 2015; Spence et al., 2016; Weavil et al., 2015). Using leg cycling as their model of locomotion, Weavil and colleagues (2015) used TMS and TMES to investigate the effects of increased cycling workload on the modulation of corticospinal excitability. Recording from vastus lateralis, participants performed brief constant-load bouts on a cycle ergometer at a constant cadence of 80 rpm, and at various power outputs (100, 200, 300, and 400 W). Stimulations were delivered in a randomized order throughout the cycling bouts at 45 degrees after top-dead-centre, which was chosen because it represented the peak EMG in the vastus lateralis during cycling. Tonic contractions were also performed at matching levels of background EMG, and stimulations were delivered in a randomized order. The

authors found that increases in workload, via increases in power output, resulted in an increase in corticospinal excitability during cycling, with both MEPs and CMEPs following a similar pattern. MEPs and CMEPs increased with increasing power output until a plateau was reached, in which further increases in power output did not cause additional changes in MEP and CMEP amplitude. The similar pattern of modulation between MEPs and CMEPs suggests that the observed increase in corticospinal excitability during leg cycling with increasing power output was mainly driven by spinal mechanisms, although the exact mechanism remains unknown. Additionally, MEPs and CMEPs from the rectus femoris increased with increased power output but did not demonstrate a plateauing effect. Thus, there may also be muscle-dependent modulation in corticospinal excitability as intensity increases.

Work from our lab by Forman et al. (2015) examined corticospinal excitability (via TMS and TMES) projecting to the biceps brachii during both the flexion (6 o'clock) and extension (12 o'clock) phases of arm cycling as the cadence was increased. The authors reported cadence-dependent changes in overall corticospinal excitability, as both MEPs and CMEPs increased in a similar manner as the cadence increased from 30 to 60 to 90 rpm at the 6 o'clock position. Thus, it was concluded that enhanced spinal excitability during the flexion phase of arm cycling must be partially responsible for the increase in overall corticospinal excitability. However, at the 12 o'clock position, overall corticospinal excitability (MEPs) increased with increased cadence, but spinal excitability (CMEPs) decreased in a manner that was exactly opposite to MEPs. This modulation suggests that at the 12 o'clock position, supraspinal mechanisms must account for the

increase in overall corticospinal excitability as the cadence of the motor output is increased.

A recent study from our laboratory (Spence et al., 2016) has examined the effect that arm cycling at different relative power outputs has on corticospinal excitability projecting to muscles of the upper limb during arm cycling. In this study, MEPs and CMEPs were recorded from the dominant arm biceps and triceps brachii during cycling trials at 5 and 15% of each participant's peak power output (PPO), which was calculated from a maximal arm cycling sprint test performed on a cycle ergometer. While cycling at a constant cadence of 60 rpm, participants received stimulations at the 6 and 12 o'clock position in a randomized order at each of the two power outputs (i.e. 5 and 15% PPO). We reported both phase- and workload-dependent changes in corticospinal excitability projecting to the biceps brachii, as both MEPs and CMEPs increased in amplitude with power output, with MEPs and CMEPs being larger at the 6 o'clock position than the 12 o'clock. Corticospinal excitability to the triceps brachii did show a workload-dependent change in corticospinal excitability (i.e. higher at the highest power output), but did not show a phase-dependent change. The most interesting finding was that spinal excitability projecting to the triceps brachii, indicated via CMEP amplitude, was larger at the 6 o'clock position during than at the 12 o'clock position. This was unexpected since the triceps brachii is most active during the extension phase of arm cycling, as demonstrated by the higher EMG. We suggested that this finding might be related to a potential dissociation between corticospinal excitability and EMG, and that differences in central motor command instead of central drive may be mediating the increase in triceps brachii EMG at that position. The findings from this study suggest that corticospinal excitability

projecting to antagonistic muscles during arm cycling is modulated differently as the intensity, via an increase in power output, is altered. A critique to this study is that the power outputs that were chosen, although relative to each participant, were calculated from an anaerobic maximal arm cycling sprint while the remainder of the session was predominantly aerobic exercise. While this enabled the authors to look at the influence of different relative power outputs on corticospinal excitability, it remains unknown if corticospinal excitability would be modulated differently to muscles of the upper limb when the power outputs were calculated during an aerobic arm cycling test.

Currently, the effect of cadence at one power output, and the effect of power output at one cadence on corticospinal excitability have been examined during arm cycling. It remains unknown what effect increasing the cadence will have on corticospinal excitability projecting to the muscles of the upper limb at different relative power outputs during arm cycling. This represents a gap in the literature that is important to investigate as it will contribute to our current understanding of how corticospinal excitability is altered as the intensity of a rhythmic motor output is increased.

2.10 Conclusion

The present understanding of human locomotor outputs suggests that the CNS is modulated through a complex combination of descending inputs from the motor cortex, spinal influences, and sensory feedback. In addition, this modulation appears to be task-, phase-, and muscle-dependent. While these modulations are becoming more well-known during locomotor outputs, it remains unclear how corticospinal excitability is modulated as the intensity of the motor output increases. The following project will explore this idea,

and examine how corticospinal excitability to muscles of the dominant upper limb is modulated at different relative power outputs during arm cycling when cadence is altered. The findings from this research may have functional application for clinical settings, where designing appropriate training plans for individuals with central motor control impairments is of practical value.

2.11 References

Amassian, V. E., Eberle, L., Maccabee, P. J., & Cracco, R. Q. (1992). Modelling magnetic coil excitation of human cerebral cortex with a peripheral nerve immersed in a brain-shaped volume conductor: The significance of fiber bending in excitation. *Electroencephalography and Clinical Neurophysiology, 85*(5), 291-301.

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *The Lancet, 325*(8437), 1106-1107.

Berardelli, A., Inghilleri, M., Rothwell, J. C., Cruccu, G., & Manfredi, M. (1991). Multiple firing of motoneurones is produced by cortical stimulation but not by direct activation of descending motor tracts. *Electroencephalography and Clinical Neurophysiology, 81*(3), 240-242.

Brown, T. G. (1911). The intrinsic factors in the act of progression in the mammal. *Proceedings of the Royal Society of London. Series B, containing papers of a biological character, 84*(572), 308-319.

Boorman, G., Becker, W. J., Morrice, B. L., & Lee, R. G. (1992). Modulation of the soleus H-reflex during pedalling in normal humans and in patients with spinal spasticity. *Journal of Neurology, Neurosurgery & Psychiatry, 55*(12), 1150-1156.

Brooke, J. D., McIlroy, W. E., & Collins, D. F. (1992). Movement features and H-reflex modulation. I. Pedalling versus matched controls. *Brain Research, 582*(1), 78-84.

Brouwer, B., & Ashby, P. (1990). Corticospinal projections to upper and lower limb spinal motoneurones in man. *Electroencephalography and Clinical Neurophysiology, 76*(6), 509-519.

Burke, D., Hicks, R., Gandevia, S. C., Stephen, J., Woodforth, I., & Crawford, M. (1993). Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. *Journal of Physiology*, *470*(1), 383-393.

Canedo, A. (1997). Primary motor cortex influences on the descending and ascending systems. *Progress in Neurobiology*, *51*(3), 287-335.

Capaday, C., & Stein, R. B. (1986). Amplitude modulation of the soleus H-reflex in the human during walking and standing. *Journal of Neuroscience*, *6*(5), 1308-1313.

Capaday, C., & Stein, R. B. (1987). Difference in the amplitude of the human soleus H reflex during walking and running. *Journal of Physiology*, *392*(1), 513-522.

Capaday, C., Lavoie, B. A., Barbeau, H., Schneider, C., & Bonnard, M. (1999). Studies on the corticospinal control of human walking. I. Responses to focal transcranial magnetic stimulation of the motor cortex. *Journal of Neurophysiology*, *81*(1), 129-139.

Carroll, T. J., Baldwin, E. R., Collins, D. F., & Zehr, E. P. (2006). Corticospinal excitability is lower during rhythmic arm movement than during tonic contraction. *Journal of Neurophysiology*, *95*(2), 914-921.

Christensen, L. O., Andersen, J. B., Sinkjær, T., & Nielsen, J. (2001). Transcranial magnetic stimulation and stretch reflexes in the tibialis anterior muscle during human walking. *Journal of Physiology*, *531*(2), 545-557.

Copithorne, D. B., Forman, D. A., & Power, K. E. (2015). Premovement changes in corticospinal excitability of the biceps brachii are not different between arm cycling and an intensity-matched tonic contraction. *Motor Control*, *19*, 223-241.

Crone, C., Hultborn, H., & Jespersen, B. (1985). Reciprocal Ia inhibition from the peroneal nerve to soleus motoneurones with special reference to the size of the test reflex. *Experimental Brain Research*, *59*(2), 418-422.

Crone, C., Hultborn, H., Jespersen, B., & Nielsen, J. (1987). Reciprocal Ia inhibition between ankle flexors and extensors in man. *The Journal of Physiology*, *389*, 163.

Dietz, V. (2002). Do human bipeds use quadrupedal coordination? *Trends in Neurosciences*, 25(9), 462-467.

Di Lazzaro, V., Oliviero, A., Profice, P., Saturno, E., Pilato, F., Insola, A., ... & Rothwell, J. C. (1998). Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control*, *109*(5), 397-401.

Edgerton, V. R., Smith, J. L., & Simpson, D. R. (1975). Muscle fibre type populations of human leg muscles. *The Histochemical Journal*, *7*(3), 259-266.

Faist, M., Mazevet, D., Dietz, V., & Pierrot-Deseilligny, E. (1994). A quantitative assessment of presynaptic inhibition of la afferents in spastics. *Brain*, *117*(6), 1449-1455.

Ferris, D. P., Aagaard, P., Simonsen, E. B., Farley, C. T., & Dyhre-Poulsen, P. (2001). Soleus H-reflex gain in humans walking and running under simulated reduced gravity. *Journal of Physiology*, *530*(1), 167-180.

Forman, D. A., Philpott, D. T., Button, D. C., & Power, K. E. (2015). Cadence-dependent changes in corticospinal excitability of the biceps brachii during arm cycling. *Journal of Neurophysiology*, *114*(4), 2285-2294.

Forman, D., Raj, A., Button, D. C., & Power, K. E. (2014). Corticospinal excitability of the biceps brachii is higher during arm cycling than an intensity-matched tonic contraction. *Journal of Neurophysiology*, *112*(5), 1142-1151.

Gage, J. R., Deluca, P. A., & Renshaw, T. S. (1995). Gait analysis: Principles and applications. *Journal of Bone & Joint Surgery Am*, *77*(10), 1607-1623.

Gandevia, S. C., Petersen, N., Butler, J. E., & Taylor, J. L. (1999). Impaired response of human motoneurones to corticospinal stimulation after voluntary exercise. *The Journal of Physiology*, *521*(3), 749-759.

Goodall, S., Romer, L. M., & Ross, E. Z. (2009). Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. *Experimental Physiology*, *94*(9), 995-1004.

Grillner, S. (1981). Control of locomotion in bipeds, tetrapods, and fish. In: *Handbook of Physiology*. *The Nervous System*. *Motor Control*. Bethesda, MD: Am. Physiol. Soc., sect. 1, vol. II, 1179–1236.

Grillner, S., & Wallen, P. (1985) Central pattern generators for locomotion, with special reference to vertebrates. *Annual Reviews of Neuroscience*, *8*, 233-261.

Gregor, R. J., Broker, J. P., & Ryan, M. M. (1991). The Biomechanics of Cycling. *Exercise and Sport Sciences Reviews*, *19*(1), 127-170.

Hultborn, H., Meunier, S., Morin, C., & Pierrot-Deseilligny, E. (1987). Assessing changes in presynaptic inhibition of Ia fibres: A study in man and the cat. *The Journal of Physiology*, *389*, 729.

Hundza, S.R., de Ruiter, G.C., Klimstra, M., & Zehr, E.P. (2012). Effect of afferent feedback and central motor commands on soleus H-reflex suppression during arm cycling. *Journal of Neurophysiology*, *108(11)*, 3049-3058.

Hundza, S. R., & Zehr, E. P. (2009). Suppression of soleus H-reflex amplitude is graded with frequency of rhythmic arm cycling. *Experimental Brain Research, 193(2),* 297-306.

Jordan, L. M. (1998). Initiation of locomotion in mammals. *Annals of the New York Academy of Sciences*, *860*(1), 83-93.

Kischka, U., Fajfr, R., Fellenberg, T., & Hess, C. W. (1993). Facilitation of motor evoked potentials from magnetic brain stimulation in man: A comparative study of different target muscles. *Journal of Clinical Neurophysiology*, *10*(4), 505-512.

Knikou, M. (2008). The H-reflex as a probe: Pathways and pitfalls. *Journal of Neuroscience Methods*, *171*(1), 1-12.

Klarner, T., Barss, T. S., Sun, Y., Kaupp, C., & Zehr, E. P. (2014). Preservation of common rhythmic locomotor control despite weakened supraspinal regulation after stroke. *Frontiers in Integrative Neuroscience*, *8*(1), 1-9.

Larsen, B., Voigt, M., & Grey, M. J. (2006). Changes in the soleus stretch reflex at different pedaling frequencies and crank loads during pedaling. *Motor Control*, *10*(3), 265-279.

Lemon, R. N., Mantel, G. W., & Muir, R. B. (1986). Corticospinal facilitation of hand muscles during voluntary movement in the conscious monkey. *Journal of Physiology*, *381*(1), 497-527.

Macintosh, B. R., Neptune, R. R., & Horton, J. F. (2000). Cadence, power, and muscle activation in cycle ergometry. *Medicine and Science in Sports and Exercise*, *32*(7), 1281-1287.

Magladery, J. W., & McDougal Jr, D. B. (1950). Electrophysiological studies of nerve and reflex activity in normal man. I. Identification of certain reflexes in the electromyogram and the conduction velocity of peripheral nerve fibers. *Bulletin of the Johns Hopkins Hospital*, *86*(5), 265.

Martin, P. G., Gandevia, S. C., & Taylor, J. L. (2006). Output of human motoneurone pools to corticospinal inputs during voluntary contractions. *Journal of Neurophysiology*, *95*(6), 3512-3518.

McNeil, C. J., Butler, J. E., Taylor, J. L., & Gandevia, S. C. (2013). Testing the excitability of human motoneurons. *Frontiers in Human Neuroscience*, *152*(7), 1-9.

Mehta, J. P., Verber, M. D., Wieser, J. A., Schmit, B. D., & Schindler-Ivens, S. M. (2009). A novel technique for examining human brain activity associated with pedaling using fMRI. *Journal of Neuroscience Methods*, *179*(2), 230-239.

Mills, K. R., & Murray, N. M. F. (1986). Electrical stimulation over the human vertebral column: Which neural elements are excited? *Electroencephalography and Clinical Neurophysiology*, *63*(6), 582-589.

Nathan, P. W., & Smith, M. C. (1955). Long descending tracts in man. *Brain*, *78*(2), 248-303.

Nielsen, J., & Petersen, N. (1994). Is presynaptic inhibition distributed to corticospinal fibres in man? *Journal of Physiology*, *477*(1), 47-58.

Nudo, R. J., & Masterton, R. B. (1990). Descending pathways to the spinal cord, III: Sites of origin of the corticospinal tract. *Journal of Comparative Neurology*, *296*(4), 559-583.

Petersen, N. T., Butler, J. E., Marchand-Pauvert, V., Fisher, R., Ledebt, A., Pyndt, H. S., ... & Nielsen, J. B. (2001). Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking. *Journal of Physiology*, *537*(2), 651-656.

Petersen, N., Christensen, L. O., & Nielsen, J. (1998). The effect of transcranial magnetic stimulation on the soleus H reflex during human walking. *Journal of Physiology*, *513*(2), 599-610.

Petersen, N. T., Taylor, J. L., & Gandevia, S. C. (2002). The effect of electrical stimulation of the corticospinal tract on motor units of the human biceps brachii. *Journal of Physiology*, *544*(1), 277-284.

Power, K. E., & Copithorne, D. B. (2013). Increased corticospinal excitability prior to arm cycling is due to enhanced supraspinal but not spinal motoneurone excitability. *Applied Physiology, Nutrition, and Metabolism*, *38*(11), 1154-1161.

Price, M. J., Collins, L., Smith, P. M., & Goss-Sampson, M. (2007). The effects of cadence and power output upon physiological and biomechanical responses to

incremental arm-crank ergometry. *Applied Physiology, Nutrition, and Metabolism*, *32*(4), 686-692.

Pyndt, H. S., Laursen, M., & Nielsen, J. B. (2003). Changes in reciprocal inhibition across the ankle joint with changes in external load and pedaling rate during bicycling. *Journal of Neurophysiology*, *90*(5), 3168-3177.

Pyndt, H. S., & Nielsen, J. B. (2003). Modulation of transmission in the corticospinal and group Ia afferent pathways to soleus motoneurones during bicycling. *Journal of Neurophysiology*, *89*(1), 304-314.

Rossini, P. M., Di Stefano, E., & Stanzione, P. (1985). Nerve impulse propagation along central and peripheral fast conducting motor and sensory pathways in man. *Electroencephalography and Clinical Neurophysiology*, *60*(4), 320-334.

Rothwell, J. C. (1990). Physiological studies of electric and magnetic stimulation of the human brain. *Electroencephalography and Clinical Neurophysiology. Supplement*, *43*, 29-35.

Schieppati, M. (1987). The Hoffmann reflex: A means of assessing spinal reflex excitability and its descending control in man. *Progress in Neurobiology*, *28*(4), 345-376.

Shik M., Severin R., & Orlovsky, G. (1966). Control of walking and running by means of electrical stimulation of the mid-brain. *Biophysics*, 11, 756-765.

Shindo, M., Harayama, H., Kondo, K., Yanagisawa, N., & Tanaka, R. (1984). Changes in reciprocal Ia inhibition during voluntary contraction in man. *Experimental Brain Research*, *53*(2), 400-408.

Simonsen, E. B., & Dyhre-Poulsen, P. (1999). Amplitude of the human soleus H reflex during walking and running. *Journal of Physiology*, *515*(3), 929-939.

Sidhu, S. K., Hoffman, B. W., Cresswell, A. G., & Carroll, T. J. (2012). Corticospinal contributions to lower limb muscle activity during cycling in humans. *Journal of Neurophysiology*, *107*(1), 306-314.

Spence, A. J., Alcock, L. R., Lockyer, E. J., Button, D. C., & Power, K. E. (2016). Phase- and workload-dependent changes in corticospinal excitability to the biceps and triceps brachii during arm cycling. *Brain Sciences*, *6*(4), 60.

Staines, W. R., Brooke, J. D., Misiaszek, J. E., & McIlroy, W. E. (1997). Movement-induced gain modulation of somatosensory potentials and soleus H-reflexes evoked from the leg II. Correlation with rate of stretch of extensor muscles of the leg. *Experimental Brain Research, 115*(1), 156-164.

Tanaka, R. (1974). Reciprocal Ia inhibition during voluntary movements in man. *Experimental Brain Research, 21*(5), 529-540.

Taylor, J. L., Allen, G. M., Butler, J. E., & Gandevia, S. C. (1997). Effect of contraction strength on responses in biceps brachii and adductor pollicis to transcranial magnetic stimulation. *Experimental Brain Research*, *117*(3), 472-478.

Taylor, J. L., Petersen, N. T., Butler, J. E., & Gandevia, S. C. (2002). Interaction of transcranial magnetic stimulation and electrical transmastoid stimulation in human subjects. *Journal of Physiology*, *541*(3), 949-958.

Taylor, J. L. (2006). Stimulation at the cervicomedullary junction in human subjects. *Journal of Electromyography and Kinesiology*, *16*(3), 215-223.

Terao, Y., & Ugawa, Y. (2002). Basic mechanisms of TMS. *Journal of Clinical Neurophysiology*, 19(4), 322-343.

Todd, G., Taylor, J. L., & Gandevia, S. C. (2003). Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *Journal of Physiology*, *551*(2), 661-671.

Ugawa, Y., Rothwell, J. C., Day, B. L., Thompson, P. D., & Marsden, C. D. (1991). Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. *Annals of Neurology*, *29*(4), 418-427.

Vaughan, C. L., Davis, B. L., & O'connor, J. C. (1992). *Dynamics of human gait* (pp. 88-92). Champaign, Illinois: Human Kinetics Publishers.

Wakeling, J. M., Blake, O. M., & Chan, H. K. (2010). Muscle coordination is key to the power output and mechanical efficiency of limb movements. *Journal of Experimental Biology*, *213*(3), 487-492.

Weavil, J. C., Sidhu, S. K., Mangum, T. S., Richardson, R. S., & Amann, M. (2015). Intensity-dependent alterations in the excitability of cortical and spinal projections to the knee extensors during isometric and locomotor exercise. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *308*(12), R998-R1007.

Wilkie, D. R. (1949). The relation between force and velocity in human muscle. *The Journal of Physiology*, *110*(3-4), 249-280.

Zehr, P. E. (2002). Considerations for use of the Hoffmann reflex in exercise studies. *European Journal of Applied Physiology*, *86*(6), 455-468.

Zehr, E. P. (2005). Neural control of rhythmic human movement: The common core hypothesis. *Exercise and Sport Sciences Reviews*, *33*(1), 54-60.

Zehr, E. P., Balter, J. E., Ferris, D. P., Hundza, S. R., Loadman, P. M., & Stoloff, R. H. (2007). Neural regulation of rhythmic arm and leg movement is conserved across human locomotor tasks. *Journal of Physiology*, *582*(1), 209-227.

Zehr, E. P., Carroll, T. J., Chua, R., Collins, D. F., Frigon, A., Haridas, C., ... & Thompson, A. K. (2004). Possible contributions of CPG activity to the control of rhythmic human arm movement. *Canadian Journal of Physiology and Pharmacology*, *82*(8-9), 556-568.

Zehr, E. P., Collins, D. F., Frigon, A., & Hoogenboom, N. (2003). Neural control of rhythmic human arm movement: phase dependence and task modulation of Hoffmann reflexes in forearm muscles. *Journal of Neurophysiology*, *89*(1), 12-21.

Chapter 3 Cadence vs Power Output: Corticospinal excitability to muscles of the upper arm is modulated differently with increases in arm cycling intensity

Evan J. Lockyer

School of Human Kinetics and Recreation, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

Running Head: Intensity-dependent changes in CSE during arm cycling

Key words: intensity, cadence, power output, transcranial, transmastoid, MEP, CMEP

3.0 ABSTRACT

This is the first study to compare the influences of cadence and power output on the modulation of corticospinal excitability (CSE) to the biceps and triceps brachii during arm cycling. Supraspinal and spinal excitability were assessed using transcranial magnetic stimulation (TMS) of the motor cortex and transmastoid electrical stimulation (TMES) of the corticospinal tract, respectively. Motor evoked potentials (MEPs) elicited by TMS and cervicomedullary motor evoked potentials (CMEPs) elicited by TMES were recorded from the biceps and triceps brachii at two different positions during arm cycling corresponding to mid-elbow flexion and mid-elbow extension (i.e. 6 and 12 o'clock made relative to a clock face, respectively). Arm cycling was performed at two cadences (60, 90 rpm) and three power outputs (20, 40, 60% W_{max}). At the 6 o'clock position, MEP amplitudes from the biceps brachii increased with cadence (p = .005) and power output (p = .001), while CMEP amplitudes increased with power output (p < .001) and tended to increase with cadence (p = .076). At the 12 o'clock position, MEP amplitudes from the biceps brachii increased with power output (p = .012), and tended to increase with cadence (p = .069), while the pattern of CMEP amplitude was noticeably different. CMEPs did not change with power output (p = .257), and decreased with cadence (p = .012). In the triceps brachii, MEP amplitudes at the 6 o'clock position increased with cadence (p = .047) but were not affected by power output, while CMEP amplitudes increased significantly with power output (p = .030) and tended to increase with cadence (p = .053). At 12 o'clock, MEP amplitudes increased with power output (p = .040) but were not different with changes in cadence, while CMEP amplitudes increased with both cadence (p = .030) and power output (p = .018). Collectively, the data suggest that the

‘type’ of cycling intensity affects CSE and spinal excitability differently and that these findings are muscle- and phase-dependent.

3.1 INTRODUCTION

The basic activation patterns of locomotor outputs (e.g., walking, running, or cycling) are generated in part, by a set of specialized interneurones within the spinal cord, referred to as a central pattern generator (CPG) (Grillner, 1975; Zehr et al., 2004). In animal models, CPG-mediated motor outputs can be produced in the absence of cortical and/or sensory input. Though spinal CPGs are thought to also contribute to rhythmic motor outputs in humans (Duysens and Van de Crommert, 1998; Zehr et al., 2004), it appears as though humans rely on descending input from cortical centres to a greater extent than animals (Christensen et al., 2000; Petersen et al., 2001; Petersen et al., 2012).

Most of what is known with regards to the neural control of locomotor outputs in humans has come from studies that have assessed reflex modulation (Brooke et al., 1992; Brooke et al., 1997; Capaday and Stein, 1987; de Ruiter et al., 2010; Palomino et al., 2011; Zehr and Chua, 2000), with the overarching findings indicating that reflexes, and thus the processing of afferent feedback, are modulated in a phase-, task-, and muscle-dependent manner. Additionally, the gain of spinal reflex pathways and ascending sensory pathways have been shown to be differentially modulated by changes in locomotor intensity (i.e. intensity-dependent modulation) (Hundza and Zehr, 2009; Hundza et al., 2012; Larsen et al., 2006; Pyndt et al., 2003; Sakamoto et al., 2004). Most of these studies report velocity- or cadence-dependent modulation, though load-dependent modulation in supraspinal and spinal reflex excitability have also been reported. For example, the suppression of somatosensory evoked potentials (SEPs), short latency stretch reflexes (SLRs), and H-reflexes have all been reported during leg cycling with increased cadence, while changes in cycling load did not affect SEP or SLR amplitudes,

and increased H-reflex amplitudes (Hundza et al., 2012; Larsen and Voigt, 2004; Larsen et al., 2006; Sakamoto et al., 2004). Thus, the results from these studies suggest that supraspinal and spinal reflex excitability are modulated differently during locomotor outputs in a manner that is also dependent on the manner in which the intensity is altered.

Recently, researchers have started to understand how the motor system is modulated during locomotor outputs by assessing the excitability of the corticospinal pathway during both leg (Sidhu et al., 2012; Sidhu et al., 2013; Weavil et al., 2015) and arm cycling (Carroll et al., 2006; Copithorne et al., 2015; Forman et al., 2014; Forman et al., 2015; Forman et al., 2016; Power and Copithorne, 2013; Spence et al., 2016). To examine corticospinal excitability (CSE), researchers frequently examine amplitudes of motor evoked potentials (MEPs) in target muscles elicited by transcranial magnetic stimulation (TMS) of the motor cortex. This provides an instantaneous evaluation of the excitability of the corticospinal pathway, from the motor cortex to the muscle fibres. Since changes in MEPs can be influenced by changes at the supraspinal, spinal and/or peripheral level, an independent technique called transmastoid electrical stimulation (TMES) is often employed to provide an indication of spinal excitability. When normalized to the M_{max}, using both TMS and TMES together can provide an indication of the relative contribution of supraspinal and spinal factors to overall changes in CSE (McNeil et al., 2013; Pearcey et al., 2014; Taylor, 2006).

The current understanding of CSE during locomotor outputs is that, similar to spinal reflex modulation studies, CSE and spinal excitability are modulated in a phase-, and task-dependent manner (Carroll et al., 2006; Copithorne et al., 2015; Forman et al., 2014; Sidhu et al., 2012; Sidhu et al., 2013). For example, work from our lab has

demonstrated that CSE to the biceps brachii during arm cycling at a constant cadence and power output is higher than during an intensity-matched tonic contraction (*task-dependence*), a finding that was only different at the 3 o'clock and 6 o'clock position (*phase-dependence*) made relative to the face of a clock (Forman et al., 2014).

To date, only three studies have examined the effect of intensity on CSE during locomotor outputs; one during leg cycling and two during arm cycling. Weavil and colleagues (2015) showed that MEP and CMEP amplitudes elicited during the most active phase of the vastus lateralis during leg cycling increased with cycling workload up to a certain point (approximately 300 W), after which there was a subsequent suppression in MEP and CMEP amplitudes. In two separate studies, we have recently shown cadence- (Forman et al., 2015) and load-dependent (Spence et al., 2016) changes in supraspinal and spinal excitability to muscles of the upper limb during arm cycling. More specifically, we reported that CSE to the biceps brachii was enhanced throughout arm cycling with increased cadence, while spinal excitability increased during the elbow flexion phase of cycling and decreased during elbow extension (Forman et al., 2015). In the study where cycling load was manipulated (Spence et al., 2016), overall CSE to the biceps and triceps brachii increased during both elbow flexion and extension, whereas the pattern of spinal excitability to the biceps and triceps tended to increase with load. Interestingly, these findings suggest that the manner in which the intensity is modulated (i.e. cadence vs power output) may be important in determining CSE during arm cycling, and that the findings are muscle-dependent. Since in our previous studies we looked at the effect of cadence and load separately, there has yet to be a study to compare the influence of

cadence and load (which will be referred to in the present study as power outputs) on CSE to the biceps and triceps brachii during arm cycling.

The purpose of the present study was to compare the influence of cadence and power output on CSE projecting to the biceps and triceps brachii during arm cycling. We hypothesized that: (1) CSE to both the biceps and triceps brachii would increase in each position examined (flexion and extension; see Methods) as cycling intensity (both cadence and power output) increased, (2) supraspinal excitability would account for increases in CSE during elbow extension for the biceps and elbow flexion for the triceps brachii, while spinal excitability would be largely responsible for the increase in excitability during elbow flexion for the biceps and elbow extension for the triceps brachii, and (3) supraspinal and spinal excitability would be modulated differently with changes in cadence versus changes in power output.

3.2 METHODS

3.2.0 Ethical approval

Prior to data collection, all participants received verbal explanation of the experimental protocol. Once all questions were answered, written informed consent was obtained. This study was conducted in accordance to the Helsinki declaration and all protocols were approved by the Interdisciplinary Committee on Ethics in Human Research at Memorial University of Newfoundland (ICEHR no. 20170217-HK). Additionally, the protocols were carried out in accordance with the Tri-Council Guidelines in Canada, with the potential risks being fully disclosed to all participants.

3.2.1 Participants

Eleven healthy, recreationally active (> 10 hours of physical activity per week), male volunteers (24.7 ± 4.4 years of age, height = 179 ± 7.2 cm, weight = 86 ± 9.5 kg, 9 right-hand dominant, 2 left-hand dominant), with no known neurological impairments participated in *part 1* of the study. Eight of those participants also participated in *part 2* (see below). Following consent, all participants completed a safety checklist to screen for contraindications to magnetic stimulation (Rossi et al., 2009), and a Physical Activity Readiness Questionnaire to screen for any contraindications to physical activity (Canadian Society for Exercise Physiology, 2002). Hand dominance was determined using the Edinburg handedness inventory (Veale, 2014). This was done to ensure that the evoked potentials (see stimulation conditions Section 2.5) were measured from the dominant arm, given that differences in the neural control may be different between dominant and non-dominant limbs (Daligadu et al., 2013).

3.2.2 General setup

This study was conducted over three separate days and consisted of two parts: *part 1* assessed corticospinal excitability and *part 2* assessed spinal excitability during arm cycling at different relative cadences and power outputs. All arm cycling was performed using an arm cycle ergometer (SCIFIT ergometer, model PRO2 Total Body, Tulsa, OK, USA) with the forearms fixed in a pronated position and the pedals locked 180 degrees out of phase (i.e. asynchronous cranking pattern; see Figure 1A). Participants were seated in an upright position at a comfortable distance from the hand pedals to ensure there was no reaching or trunk variation during cycling. The height of the

ergometer seat was adjusted so that participants' shoulders were approximately the same height as the axis of rotation of the arm cranks. Participants wore wrist braces to limit the amount of wrist flexion and extension during cycling in order to reduce the influence of heteronymous reflex connections that exist between the wrist flexors and the biceps brachii (Manning and Bawa, 2011).

Responses were evoked at two positions during arm cycling: 6 and 12 o'clock, defined relative to a clock face. Similar to previous arm cycling studies, 6 o'clock was specified as "bottom dead centre" and 12 o'clock was specified as "top dead centre" (Balter and Zehr, 2007; Copithorne et al., 2015; Forman et al., 2014; Forman et al., 2015; Forman et al., 2016). These two positions were examined because they occur during mid-elbow flexion (6 o'clock) and extension (12 o'clock) during arm cycling (Figure 1B), which is important given our interest in both the biceps and triceps brachii. Stimuli were triggered automatically when the right hand passed a magnetic sensor at one of the predetermined positions (6 and 12 o'clock). For a left-handed participant, stimulations at the 6 o'clock position occurred when the right hand passed the sensor at the 12 o'clock position, while stimulations at the 12 o'clock position occurred when the right hand passed the sensor at the 6 o'clock position. The movement from 3 o'clock (full elbow extension) to 9 o'clock (full elbow flexion) was defined as elbow flexion, and the movement from 9 o'clock to 3 o'clock was defined as elbow extension.

The study required participants to cycle at combinations of two different cadences and three different relative power outputs. Measurements were taken separately at 6 and 12 o'clock for a total of 12 trials. The order of the two positions was randomized across all participants.

3.2.3 Electromyography recordings

Electromyograph (EMG) signals were recorded from the biceps and triceps brachii of the dominant arm using pairs of disposable Ag-AgCl surface electrodes (MediTrace^{TM} 130 Foam Electrodes with conductive adhesive hydrogel, Covidien IIC, Massachusetts, USA). Using a bipolar configuration, electrodes were positioned approximately 2 cm apart (centre to centre) over the midline of the biceps brachii and on the lateral head of the triceps brachii. A ground electrode was positioned on the lateral epicondyle of the dominant arm. Preceding electrode placement, the skin was thoroughly prepared by removing hair (via a handheld razor) and dead epithelial cells (via abrasive paper), followed by sanitization using isopropyl alcohol swabs. This was done to reduce the impedance for EMG recordings. The EMG was sampled at a rate of 5 KHz using CED 1401 interface and the associated Signal 5 (Cambridge Electronic Design Ltd., Cambridge, UK) software. All signals were amplified (x300) and bandpass filtered using a 3-Pole Butterworth filter with cut-off frequencies ranging from 10-1000 Hz.

3.2.4 Stimulation conditions

Evoked potentials were elicited via 1) electrical stimulation at Erb's point, 2) TMS, and 3) TMES. All participants had prior experience with these stimulation procedures before participating. Initially, stimulation intensities were determined with participants seated comfortably in the chair of the SCIFIT ergometer with their hands in their lap. This position was defined as "rest". Each stimulation technique is described in more detail below. Stimulation intensities were set at rest to coincide with our previous work upon which this study was based (Forman et al., 2015).

3.2.5 Brachial plexus stimulation

For *parts 1* and *2*, electrical stimulation of the brachial plexus at Erb's point was delivered to elicit maximal compound muscle action potentials (M-waves). The cathode was placed in the supraclavicular fossa, and the anode was placed on the acromion process. A constant current stimulator (model DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) with a pulse width of 200 μs was used for all participants. The stimulator intensity was initially set at 25 mA and was gradually increased until the size of the M-wave plateaued (i.e. maximal M-wave, M_{max}). At this point, the stimulation intensity was then increased by 20% to ensure that M_{max} was elicited throughout the study since the M_{max} can change throughout the course of an experiment (Crone et al., 1999). The stimulation intensity used to elicit M_{max} was 186 ± 55.8 mA (mean ± SD) and ranged from 100 mA – 300 mA.

3.2.6 Transcranial magnetic stimulation

TMS to the motor cortex was applied using a Magstim 200 stimulator (Magstim, Whitland, Dyfed, UK). A circular coil (13.5 cm outside diameter) was positioned over the vertex of each participant's skull, with the direction of current flow in the coil preferentially activating the left or right motor cortex, depending on hand dominance. The vertex was determined by measuring the mid-point between the participant's nasion and inion, and the mid-point between the participant's tragi (Copithorne et al., 2015; Forman et al., 2014; Forman et al., 2015; Pearcey et al., 2014). The location at which these two points intersected was marked with a marker and defined as the vertex. The coil was held tangentially and firmly against the participant's skull by an investigator who ensured

careful and consistent placement of the coil over the vertex throughout the entire experiment. Stimulation intensity started at approximately 30% maximal stimulator output (MSO) and was increased gradually until resting motor threshold (RMT) was found. RMT was defined as a clearly discernable MEP in the biceps brachii with an amplitude ≥50 μV in four out of eight trials. Once RMT was determined, the %MSO was increased by 20% (i.e., 120% RMT), and an average of eight MEPs were recorded at this new intensity in the rest position. The stimulator was increased by 20% to ensure that MEPs could be measured during both flexion and extension phases of arm cycling, as was done by Forman et al. (2015). This intensity of MSO was then used for the remainder of the experiment (Forman et al., 2015).

3.2.7 Transmastoid electrical stimulation

TMES was delivered using Ag-AgCl surface electrodes placed slightly inferior to the mastoid processes (Taylor and Gandevia, 2004; Taylor, 2006). A second Digitimer constant current stimulator (model DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) with a pulse width of 200 μs was used to pass the current between the electrodes. Stimulation intensity began at 25 mA and was gradually increased until a clearly discernable cervicomedullary motor evoked potential (CMEP) was found. This was defined as CMEP threshold ($CMEP_{Thres}$). This intensity was then increased by 20% to ensure that a CMEP could be recorded and could either increase or decrease in amplitude to avoid potential 'flooring' or ceiling effects of the CMEP (Taylor and Gandevia, 2004). This new intensity was then used for the remainder of the experiment. The stimulation intensity that was used to evoke CMEPs throughout the experiment was

137 ± 37.0 (mean ± SD) and ranged from 75 mA – 198 mA. The latency of the CMEP was monitored to ensure that the corticospinal tract, and not ventral roots was being stimulated (Taylor, 2006). Latencies were visually monitored by recording the time between two vertical cursors, one of which placed at the stimulation artefact and the other placed at the initial deflection of the CMEP.

3.2.8 Experimental protocol

On the initial visit, participants were familiarized with the various stimulation techniques and were required to perform an incremental aerobic arm cycling test (described in *Incremental test*). *Part 1* was conducted on a separate day and required participants to cycle at two different cadences (60 and 90 rpm) at three different power outputs (20, 40, and 60% of their maximum power output determined from the *Incremental test*) while receiving TMS and brachial plexus stimulation. *Part 2* was also conducted on a separate day and involved the same protocol as *part 1* except participants received TMES and brachial plexus stimulation. Within each part of the study, the order of the different conditions was randomized for each participant. Additionally, whether participants participated in *part 1* or *part 2* first was randomized for each participant.

3.2.9 Incremental test

Each participant completed a continuous, incremental test on the SCIFIT ergometer to determine their maximum aerobic power (W_{max}). The initial work rate was set at 40 W and increased by 20 W every two minutes, as per the guidelines for untrained participants (Price et al., 2007). Participants were asked to maintain a constant cadence of 60 rpm throughout the entire incremental test. The W_{max} was considered to be the power

recorded at the last completed stage. Participant mean power outputs at 20%, 40%, and 60% W_{max} were 25.5 ± 3.54 W, 51.0 ± 7.08 W, and 76.5 ± 10.62 W, respectively. All participants exercised until volitional exhaustion or until the cadence dropped below 60 rpm for a period of 5 seconds. Following the test, participants completed a two-minute self-selected pace cool-down at 25 W. This test was completed to set the power outputs used during *part 1* and *part 2* of the study relative to each participant's W_{max}.

3.2.10 Part 1: CSE during arm cycling at different cadences and power outputs

Once the stimulation intensities for brachial plexus stimulation, and TMS were determined, the 12 different trials were performed (20, 40, 60% W_{max} at 60 and 90 rpm at the 6 and 12 o'clock position) in a randomized order. The arm cycle ergometer was set at the predetermined power output and participants were required to maintain the specified cadence. While cycling, a trial consisting of 10 MEP frames, 4 blank frames, and 2 M_{max} frames was completed at one of the two predetermined positions. Blank frames were given to reduce the effect of participants anticipating the stimulations. The order of the stimulations throughout each trial was randomized, and stimulations were separated by approximately 7-8 s. The total length of each cycling trial was approximately 2.5 minutes. To reduce the potential influence of fatigue, 30 s rest periods were given half-way through the 20 and 40% W_{max} trials, and a 60 s rest period was given half-way through the 60% W_{max} trials. Additionally, following the completion of all six trials at one position, a 5-minute rest period was provided. Following this rest period, these steps were completed for the remaining position.

3.2.11 Part 2: Spinal excitability during arm cycling at different cadences and power outputs

Part 2 of the study was conducted the exact same way as *part 1* with the exception that participants (n = 8) now received TMES instead of TMS while cycling. These trials consisted of 8 CMEP frames, 2 blank frames, and 2 M_{max} frames, which were randomized for each trial, as in *part 1*. Fewer TMES were given than TMS since TMES is more transiently painful than TMS. Rest periods were given in the same manner as *part 1*, and trials were completed in a randomized order.

3.2.12 Data analysis

All data were stored and analyzed off-line using Signal 5.08 data collection software (CED, UK). The averaged peak-to-peak amplitudes of MEPs, CMEPs, and M_{max} were measured from the biceps and triceps brachii of the dominant arm, from the initial deflection of the voltage trace to the return of the trace to baseline background EMG levels. Additionally, latencies of all evoked responses were examined and monitored. To account for changes in peripheral neuromuscular propagation during exercise (Taylor, 2006), averaged MEP and CMEP amplitudes were normalized to the averaged M_{max} evoked during the same trial. Pre-stimulus EMG, defined as the mean rectified EMG immediately prior to the stimulation artifact, was measured from the rectified virtual channel created for each muscle. The window used for mean EMG calculation was determined by the cadence at which the participant was cycling. For trials at 60 rpm, the mean was calculated by taking the mean of a 50 ms window, while at 90 rpm, it was

calculated by taking the mean of a 33.3 ms window. The timeframes were chosen to represent 5% of one complete revolution (Forman et al., 2015).

3.2.13 Statistical analysis

All statistics were performed using IBM's SPSS Statistics version 23. Assumptions of sphericity were tested using Mauchly's test and if violated, the appropriate correction was made to the degrees of freedom. To assess whether there were statistical differences in MEP and CMEP amplitudes (normalized to M_{max}), and average pre-stimulus EMG between the two cadences and three power outputs, separate two-way repeated-measures ANOVAs (cadence x power output) were used for each position. Where significant results were found, repeated pairwise comparisons using Sidak post-hoc tests were used to determine where the significance existed within conditions. All statistics were performed on group data and a significance level of $p < .05$ was used.

3.3 RESULTS

All data are provided in Tables 1 and 2. Table 1 shows the raw and normalized data during the 'active' phase of arm cycling. This means that for the biceps brachii the data in Table 1 is during mid-elbow flexion (6 o'clock) while the data for the triceps brachii is during mid-elbow extension (12 o'clock). Table 2 shows the raw and normalized data for the relative 'inactive' phase of each muscle during arm cycling, which is opposite to that above for each muscle (i.e. 12 o'clock for biceps brachii and 6 o'clock for triceps brachii). All data are reported in text as means ± standard deviation (*SD*), but are illustrated in figures as means ± standard error (*SE*).

3.3.0 Biceps brachii

3.3.01 CSE to the biceps brachii during elbow flexion

MEP amplitude. Figure 2 (top row) and Figure 3A show representative and grouped data for MEP amplitudes from the biceps brachii at the 6 o'clock position, respectively. There were significant main effects for cadence ($F_{(1,10)}$= 13.06, p = .005), and power output ($F_{(1.199,\ 11.989)}$= 41.31, p = .001), as well as the interaction between cadence and power output ($F_{(2,20)}$= 4.00, p = .035). Pairwise comparisons revealed that MEPs increased with cadence (90 rpm > 60 rpm, p = .005) and power output (60% > 40% > 20% W_{max}, p < .05 for all comparisons) at this position. To determine where the significant interaction effect was between cadence and power output, independent paired t-tests were conducted. MEPs increased at 20% (p = .004) and 40% (p = .003) W_{max} with increased cadence (90 > 60 rpm). There was no significant change in MEP amplitudes with increased cadence at 60% W_{max} (p = .265).

Pre-stimulus EMG for MEPs. As a group, pre-stimulus EMG at 60 rpm at the 6 o'clock position were 58.02, 110.54, and 160.49 μV at 20%, 40%, and 60% W_{max}, respectively; at 90 rpm, pre-stimulus EMG were 92.59, 171.66, and 219.75 μV at 20%, 40%, and 60% W_{max}, respectively (Figure 3C). Significant main effects for cadence ($F_{(1,10)}$ = 14.32, p = .004) and power output ($F_{(2,\ 20)}$= 47.76, p < .001) were observed, however no significant interaction effects were observed ($F_{(2,20)}$= 1.33, p = .286). Pairwise comparisons indicated that pre-stimulus EMG increased prior to MEPs as cadence (90 rpm > 60 rpm, p = .004) and power output (60% > 40% > 20% W_{max}, p < .001 for all comparisons) increased.

3.3.02 Spinal excitability to the biceps brachii during elbow flexion

CMEP amplitude. Figure 2 (bottom row) and Figure 3B show representative and grouped data for CMEP amplitudes from the biceps brachii at the 6 o'clock position, respectively. There was a significant no main effect for cadence ($F_{(1,7)}$= 4.32, p = .076), but there was a main effect of power output ($F_{(2, 14)}$= 36.28, p < .001), and there was no interaction between cadence and power output ($F_{(2, 14)}$= 2.41, p = .126). Pairwise comparisons indicated that CMEP amplitudes increased as power output increased (60% > 40% > 20% W_{max}, p < .05 for all comparisons).

Pre-stimulus EMG for CMEPs. As a group, pre-stimulus EMG at the 6 o'clock position were 59.97, 80.04, and 124.47 μV at 20%, 40%, and 60% W_{max}, respectively for the 60 rpm condition and 69.10, 128.97, and 189.44 μV at 20%, 40%, and 60% W_{max}, respectively for the 90 rpm condition (Figure 3D). Significant main effects for cadence ($F_{(1,7)}$ = 5.93, p = .045) and power output ($F_{(1.222, 8.551)}$= 27.83, p < .001) were observed. However, no significant interaction effects were observed ($F_{(1.163, 8.144)}$= 4.38, p = .065). Pairwise comparisons indicated that pre-stimulus EMG increased prior to CMEPs as cadence (90 rpm > 60 rpm, p = .045) and power output (60% > 40% > 20% W_{max}, p < .001 for all comparisons) increased.

3.3.03 CSE to the biceps brachii during elbow extension

MEP amplitude. Figure 4 (top row) and Figure 5A show representative and grouped data for MEP amplitudes from the biceps brachii at the 12 o'clock position, respectively. There was no effect of cadence ($F_{(1, 10)}$ = .28, p = .069), but there was a significant main effect for power output ($F_{(2, 20)}$= 5.18, p = .012). There was no significant

interaction effect between cadence and power output ($F_{(2, 20)}$= .75, p = .361). Pairwise comparisons revealed that MEP amplitudes increased with power output, with 60% W_{max} being greater than both 40% (p = .047) and 20% W_{max} (p = .039). There was no significant difference in MEP amplitude during the 40% and 20% W_{max} condition, although there was a trend towards significance (p = .069).

Pre-stimulus EMG for MEPs. As a group, pre-stimulus EMG at 60 rpm were 33.23, 36.25, and 36.92 μV at 20%, 40%, and 60% W_{max}, respectively, while pre-stimulus EMG at 90 rpm were 39.37, 36.87, and 38.01 μV at 20%, 40%, and 60% W_{max}, respectively (Figure 5C). There were no significant main effects for cadence ($F_{(1, 10)}$= .95, p =.352), power output ($F_{(1.179, 11.788)}$= .48, p = .533), or interaction effects ($F_{(1.071, 10.712)}$= .99, p = .348) were observed.

3.3.04 Spinal excitability to the biceps brachii during elbow extension

CMEP amplitude. Figure 4 (bottom row) and Figure 5B show representative and grouped data for CMEP amplitudes from the biceps brachii at the 12 o'clock position, respectively. There was a significant main effect for cadence ($F_{(1, 7)}$ = 11.50, p = .012), but no significant effects were found for power output ($F_{(2, 14)}$= 1.50, p = .257), or the interaction between cadence and power output ($F_{(2, 14)}$= .66, p = .535). Pairwise comparisons revealed that CMEP amplitudes decreased as cadence increased (60 rpm > 90 rpm, p = .012).

Pre-stimulus EMG for CMEPs. As a group, pre-stimulus EMG at 60 rpm were 30.89, 33.27, and 36.69 μV at 20%, 40%, and 60% W_{max}, respectively, while pre-stimulus EMG at 90 rpm were 31.21, 31.34, and 38.39 μV at 20%, 40%, and 60% W_{max},

respectively (Figure 5D). There was no significant main effect for cadence ($F_{(1, 7)}$= .00, p = .987), but there was a significant main effect for power output ($F_{(2, 14)}$= 6.00, p = .013).There was no interaction effect between cadence and power output ($F_{(2, 14)}$= 1.19, p = .333). Pairwise comparisons revealed that pre-stimulus EMG increased only when power output increased from 40% to 60% W_{max} (p = .009).

3.3.1 Triceps Brachii

3.3.1.1 CSE to the triceps brachii during elbow flexion

MEP amplitude. Figure 6 (top row) and Figure 7A show representative and grouped data for MEP amplitudes from the triceps brachii at the 6 o'clock position, respectively. There was a significant main effect of cadence ($F_{(1, 10)}$ = 13.57, p = .047) but no effect of power output ($F_{(2, 20)}$ = 2.35, p = .121), and no significant interaction effect ($F_{(2, 20)}$ = .636, p = .540). Pairwise comparisons revealed that MEPs at 90 rpm were higher than MEPs at 60 rpm (p = .047).

Pre-stimulus EMG for MEPs. As a group, pre-stimulus EMG were 24.61, 34.87, and 40.80 μV at 20%, 40%, and 60% W_{max}, respectively for the 60 rpm condition and 31.19, 48.88, and 52.94 μV at 20%, 40%, and 60% W_{max}, respectively for the 90 rpm condition (Figure 7C). There were significant main effects for cadence ($F_{(1, 10)}$ = 5.13, p = .004) and power output ($F_{(2, 20)}$ = 22.51, p < .001), but there was no significant interaction effect ($F_{(2, 20)}$ = 1.72, p = .204). Pairwise comparisons revealed that pre-stimulus EMG at 90 rpm was higher than pre-stimulus EMG at 60 rpm (p = .004) and that pre-stimulus EMG at 20% W_{max} was smaller than pre-stimulus EMG at 40% W_{max} (p < .001) and 60% W_{max} (p = .001), but 40% W_{max} was not different from 60% W_{max} (p = .331).

3.3.1.2 Spinal excitability to the triceps brachii during elbow flexion

CMEP amplitude. Figure 6 (bottom row) and Figure 7B show representative and grouped data for CMEP amplitudes from the triceps brachii at the 6 o'clock position, respectively. There was a significant main effect of power output ($F_{(2, 14)} = 4.53$, $p = .030$), but there was no main effect of cadence ($F_{(1, 7)} = 5.39$, $p = .053$), nor was there an interaction effect ($F_{(2, 14)} = 1.85$, $p = .194$). Despite the significant main effect of power output, pairwise comparisons revealed that no significant differences in CMEP amplitudes existed between any of the power outputs ($p > .05$ for all comparisons). There was a trend towards significance for cadence with the higher cadence resulting in a larger CMEP (90 > 60 rpm, $p = .053$).

Pre-stimulus EMG for CMEPs. As a group, pre-stimulus EMG were 19.42, 26.35, and 31.93 μV at 20%, 40%, and 60% W_{max}, respectively for the 60 rpm condition and 23.38, 34.25, and 42.29 μV at 20%, 40%, and 60% W_{max}, respectively for the 90 rpm condition (Figure 7D). There were significant main effects for cadence ($F_{(1, 7)} = 9.52$, $p = .018$), and power output ($F_{(2, 14)} = 21.54$, $p < .001$), but there was no significant interaction effect ($F_{(2, 14)} = 1.28$, $p = .315$). Pairwise comparisons revealed pre-stimulus EMG at 90 rpm was larger than pre-stimulus EMG at 60 rpm ($p = .018$), and that with increased power output, pre-stimulus EMG also increased (60% > 40% > 20% W_{max}, $p < .05$ for all comparisons).

3.3.1.3 CSE to the triceps brachii during elbow extension

MEP amplitude. Figure 8 (top row) and Figure 9A show representative and grouped data for MEP amplitudes from the triceps brachii at the 12 o'clock position,

respectively. There was a significant effect of power output ($F_{(2, 20)} = 3.78$, $p = .040$), but there was no significant main effect of cadence ($F_{(1, 10)} = .022$, $p = .884$), nor was there a significant interaction effect ($F_{(2, 20)} = .01$, $p = .993$). Despite the significant main effect of power output, pairwise comparisons revealed that no significant differences between MEPs existed among the power outputs ($p > .05$ for all comparisons).

Pre-stimulus EMG for MEPs. As a group, pre-stimulus EMG during elbow extension at 60 rpm was 51.75, 105.29, and 149.22 μV at 20%, 40%, and 60% W_{max}, respectively, while pre-stimulus EMG at 90 rpm was 58.20, 94.97, and 133.88 μV at 20%, 40%, and 60% W_{max}, respectively (Figure 9C). There was a significant main effect of power output ($F_{(1.096, 10.955)} = 10.01$ $p = .008$), but there was no significant main effect of cadence ($F_{(1, 10)} = .24$, $p = .634$), nor was there a significant interaction effect ($F_{(1.269, 12.689)} = 1.12$, $p = .328$). Pairwise comparisons revealed that pre-stimulus EMG increased as power output increased (60% > 40% > 20%, all comparisons $p < .05$).

3.3.1.4 Spinal excitability to the triceps brachii during elbow extension

CMEP amplitude. Figure 8 (bottom row) and Figure 9B show representative and grouped data for CMEP amplitudes from the triceps brachii at the 12 o'clock position, respectively. There were significant main effects of cadence ($F_{(1, 7)} = 10.38$, $p = .018$), and power output ($F_{(2, 14)} = 4.53$, $p = .030$), but there was no significant interaction effect ($F_{(2, 14)} = 1.85$, $p = .194$). Pairwise comparisons revealed that CMEPs at 90 rpm were larger than CMEPs at 60 rpm ($p = .018$), and that CMEPs were greater at 40% W_{max} than at 20% W_{max} ($p = .049$).

Pre-stimulus EMG for CMEPs. As a group, pre-stimulus EMG at 60 rpm was 53.08, 105.94, and 162.95 μV at 20%, 40%, and 60% W_{max}, respectively, while pre-stimulus EMG at 90 rpm was 66.10, 118.50, and 178.12 μV at 20%, 40%, and 60% W_{max}, respectively (Figure 9D). There was a significant main effect of power output ($F_{(1.060, 6.360)}$ = 8.89, p =.022), but no main effect of cadence ($F_{(1, 6)}$ = 1.14, p = .327), nor an interaction effect ($F_{(2, 12)}$ = 0.16, p = .985). Pairwise comparisons revealed that only pre-stimulus EMG at 40% and 60% W_{max} were significantly different from one another (60% > 40%, p = .020).

3.4 DISCUSSION

There are three main novel observations in the current study. First, during the inactive phase of the biceps brachii during arm cycling, spinal excitability is modulated differently with alterations in cadence and power output. Second, we have demonstrated the influence of cadence on CSE and spinal excitability to the triceps brachii during arm cycling and have added information to our previous study which examined the influence of power output on CSE and spinal excitability to the triceps brachii during arm cycling (Spence et al., 2016). Lastly and perhaps the most interesting finding in this study, is that there appears to be muscle-dependent (biceps vs. triceps brachii) changes in CSE and spinal excitability that are specific to the relative active and inactive phases during arm cycling.

3.4.0 Intensity-dependent changes in CSE and spinal excitability to the biceps brachii

During the most active phase of the biceps brachii (i.e. 6 o'clock position; see Figure 1B), CSE (as indicated by MEP amplitudes; Figure 3A) and spinal excitability (as

indicated by CMEP amplitudes (p = .053); Figure 3B) increased with increases in cycling cadence and power output, a finding that we have previously shown in two separate reports (Forman et al., 2015; Spence et al., 2016). In these studies, we suggested that the increase in MEP and CMEP amplitudes reflected an increase in the descending motor drive to the spinal cord necessary to increase the recruitment and/or firing frequency of spinal motoneurones, thus providing adequate muscle activation for movement. In the present study, the same rationale can be applied.

During the inactive phase for the biceps brachii (i.e. 12 o'clock; see Figure 1B), the pattern of MEP amplitudes significantly increased with power output, and tended to increase with cadence (p =.069; Figure 5A). In contrast, CMEP amplitudes significantly decreased as cadence increased and did not change with increased power output (Figure 5B). These changes occurred despite no change in pre-stimulus EMG (Figure 5C, 5D). We have recently shown similar findings and we attributed the increase in overall CSE at this position to supraspinal factors (since CMEP amplitudes decreased or did not change). In these studies, however, we were not able to compare the influence of cadence and power output since we examined the two intensity paradigms separately (Forman et al., 2015; Spence et al., 2016). The present study, however, permitted this comparison. The observed differences in spinal excitability with changes in cadence versus changes in power output during arm cycling, likely reflects differences in synaptic input to the spinal motoneurone pool between the two intensity paradigms.

One form of input that could be underlying the differences in excitability at the spinal motoneurone pool of the biceps brachii during elbow extension is reciprocal inhibition. Reciprocal inhibition has been shown to be modulated in an intensity- and

phase-dependent manner during locomotor outputs in the leg muscles (Petersen et al., 1999; Pyndt et al., 2003). During leg cycling, Pyndt and colleagues (2003) showed that the amount of disynaptic Ia reciprocal inhibition between the soleus and the tibialis anterior was phase-dependent, with inhibition being largest during upstroke and smallest during downstroke. Additionally, they showed that during downstroke reciprocal inhibition decreased significantly with cycling load and tended to decrease with cycling cadence (Pyndt et al., 2003). This suggests that reciprocal inhibition may be modulated differently depending on the manner in which the 'intensity' of a locomotor output is altered. This study, however, did not examine reciprocal inhibition during upstroke as cycling intensity increased, and thus it remains to be determined if reciprocal inhibition is modulated in a similar manner with changes in load and cadence during upstroke as was found during downstroke. In the present study, it is thus possible that during elbow extension, there is increased disynaptic Ia reciprocal inhibition from the triceps brachii to the relatively inactive biceps brachii spinal motoneurone pool (Katz et al., 1991). Perhaps at this position (i.e. 12 o'clock), reciprocal inhibition is amplified when cadence is increased, which could help to explain the observed decrease in spinal excitability as the cadence increased from 60 to 90 rpm in the present study. Whether the lack of change in the CMEP with increased power output is due to less reciprocal inhibition or more concomitant excitation from other ascending or descending inputs remains unknown.

Differences in the processing of afferent feedback to the brain and spinal cord is another putative mechanism that could be underlying the observed differences in spinal excitability with cadence and power output. During leg cycling, soleus H-reflexes as well as cerebral somatosensory evoked potentials (SEPs) are significantly suppressed with

increases in cadence, while increases in cycling load have been shown to increase H-reflex amplitudes with no change in SEP amplitudes (Sakamoto et al., 2004; Staines et al., 1997). Similarly, during arm cycling, soleus H-reflex amplitudes in the stationary legs are unaffected by changes in cycling load, but are significantly suppressed as cadence increases (Hundza et al., 2012), suggesting that the amount of presynaptic inhibition may be different between changes in cadence and load. In the present study, it is possible that differences in presynaptic inhibition to the spinal motoneurone and/or supraspinal centres may help to explain the difference in the TMES-evoked CMEPs (i.e. suppression of CMEP with cadence vs no change in CMEP with power output). Perhaps as cadence increases, presynaptic inhibition of group Ia afferents to the biceps motoneurone pool at the 12 o'clock position is enhanced more than when cycling at an increased power output, therefore explaining the reduction in CMEP amplitude with increased cadence.

3.4.1 Intensity-dependent changes in CSE and spinal excitability to the triceps brachii

During arm cycling, the triceps brachii is least active at the 6 o'clock position and is most active at the 12 o'clock position (Figure 1B). In the current study, it was hypothesized that increases in cycling intensity, both cadence and power output, would result in an increase in CSE and spinal excitability to the triceps brachii regardless of the position, since we previously showed that MEPs and CMEPs increased at the 6 and 12 o'clock position during arm cycling as power output increased (Spence et al., 2016). However, the results from the current study suggest otherwise. In the least active phase (i.e. 6 o'clock), CSE to the triceps brachii was enhanced only when the cadence of arm cycling was increased from 60 to 90 rpm (Figure 7A), while spinal excitability

significantly increased with power output (Figure 7B), and there was a trend for spinal excitability to increase with cadence (p = .053; Figure 7B). Thus, as arm cycling cadence increases, the increase in MEP amplitude is likely mediated by both supraspinal and spinal factors. In contrast, the lack of change in MEP amplitude along with a concomitant increase in CMEP amplitude with increased power output suggests that supraspinal excitability may have decreased, though the precise mechanisms are currently unknown. It is likely that the enhanced spinal excitability with cycling intensity was influenced by similar mechanisms as discussed above, namely decreased reciprocal inhibition from the biceps brachii.

Surprisingly, contrary to our hypothesis, CSE to the triceps brachii in the most active phase (i.e. 12 o'clock) was not significantly different as cadence or power output increased (Figure 9A), although there was a significant main effect of power output, there was no significant differences between power outputs when post-hoc analyses were conducted. In contrast, spinal excitability significantly increased with both cadence and power output (Figure 9B). The lack of increase in overall CSE with increased power output at either position is in direct opposition to the findings by Spence et al. (2016). Although we cannot be certain, it is likely that this observation can be at least partially explained by differences in the methodology employed by each study. In our previous study (Spence et al., 2016), the two power outputs (5 and 15% of each participant's peak power output) were obtained from a 10-s maximal anaerobic sprint test and were significantly different from one another in terms of wattage and muscle contraction intensity (as indicated by the large difference in pre-stimulus EMG). In the current study, however, the power outputs (20, 40, and 60% W_{max}) were obtained from an incremental

aerobic test, and thus were much smaller than those obtained from the maximal 10-s sprint test. This also means that the difference in wattage between the power outputs was smaller. Additionally, unlike our previous study, pre-stimulus EMG at the 6 o'clock position was not different between the two highest power outputs (40 and 60% W_{max}; Figure 7C). Thus, the output of the motoneurone pool at these power outputs was not different, which may help to explain the lack of change in the TMS-evoked MEP at this position. At the 12 o'clock position, however, pre-stimulus EMG significantly increased as power output increased (Figure 9C), yet there was still no change in CSE (Figure 9A). While we do not have a satisfactory explanation for this finding, dissociations between CSE and EMG have been reported before (Matthews, 1999; Olivier et al., 1995), suggesting that differences in central motor commands (i.e. upstream of the primary motor cortex (M1)) as opposed to differences in central drive (i.e. output of the M1) to increase the ongoing EMG levels may be underlying this finding.

3.4.2 Muscle-dependent changes in CSE and spinal excitability

Perhaps the most interesting finding in the current study is that the intensity-dependent modulation of corticospinal and spinal excitability during arm cycling appears to be different between the biceps and triceps brachii when the relative active and inactive phases of the respective muscles are taken into consideration. For example, when considering the relative inactive phases of each muscle (12 o'clock for biceps brachii and 6 o'clock for triceps brachii), overall CSE to the biceps brachii increased with increased cycling intensity, while spinal excitability either decreased with cadence or did not change with power output (see above). Overall CSE to the triceps brachii increased with

cadence, but did not change with power output, while the pattern of spinal excitability increased with both cadence and power output. Thus, the pattern of modulation, specifically in regards to spinal excitability, during the inactive phase of each muscle are different. These findings add to the argument that there are inter-muscle differences in phase- (Carroll et al., 2006; Sidhu et al., 2012) and intensity-dependent (Spence et al., 2016; Weavil et al., 2015) modulation of corticospinal and spinal excitability during locomotor outputs. Several factors likely mediate these inter-muscle differences in corticospinal and spinal excitability, such as the amount of monosynaptic projections to the respective motoneurone pools arising from the cortex, differences in the intrinsic properties of the spinal motoneurones (flexor vs extensor) or functional characteristics of the muscles examined (mono- vs bi-articular).

The differential arrangement of monosynaptic corticospinal projections to each muscle may help to explain the muscle-dependent changes in corticospinal and spinal excitability. Although monosynaptic corticospinal excitation of motoneurone pools to both the biceps and triceps brachii occur, there are considerably more monosynaptic connections to the biceps brachii (Brouwer and Ashby, 1990; Palmer and Ashby, 1992). Thus, the direct influence of cortical centres on the spinal motoneurones to the triceps brachii would presumably be less than that of the biceps brachii given that the triceps brachii motoneurone pool would be more heavily influenced by spinal interneurones.

Differences in the levels of intrinsic excitability of the motoneurone pools related to persistent inward currents (PICs) for the biceps and triceps brachii may also play a role. Previous work has indeed shown differences in the degree of PIC activation between flexor and extensor motoneurone pools in animals (Cotel et al., 2009; Hounsgaard et al.,

1988) and more recently in humans (Wilson et al., 2015). Wilson and colleagues (2015) showed that the contribution of PICs to motoneurone excitability is larger to the motoneurones of the lateral head of the triceps brachii than to the long head of the biceps brachii. In the present study, differences in intrinsic excitability of the motoneurone pools for the biceps and triceps brachii may help to explain the different pattern of MEP and CMEP modulation during the inactive phase of each muscle. At the 6 o'clock position during arm cycling, the triceps brachii is assisting in elbow stabilization and is in a stretched position, therefore ascending input from Ia afferents is likely increased. This afferent information would also presumably be increased with arm cycling intensity and could potentially increase spinal motoneurone excitability. If the intrinsic properties of the triceps brachii motoneurone pool are already enhanced (i.e. increased PICs), then this increase in synaptic input with increased cycling intensity could help to explain the observed increase in TMES-evoked CMEP amplitudes. The observed decrease and lack of change in CMEP amplitudes from the biceps brachii with increases in cadence and power output, respectively, suggests that during this phase, the intrinsic excitability of the biceps brachii motoneurone pool is lowered, likely as a result of reciprocal inhibition from the triceps brachii (see above) as it becomes active to produce adequate force to overcome the inertial load on the crankshaft of the ergometer.

Some of the differences between the biceps and triceps brachii in the current study may also be explained by the fact that the lateral head of the triceps brachii has a monoarticular function (elbow extension) compared to the biarticular function of the biceps brachii (elbow and shoulder flexion). Previous studies reporting inter-muscle differences in corticospinal and spinal excitability have related their findings to the

specific articular function of the muscles examined (Carroll et al., 2006; Sidhu et al., 2012; Spence et al., 2016; Weavil et al., 2015). Differences in the amount and/or degree of synaptic input resulting from the biarticular function of the biceps brachii may be of importance. For example, at the 12 o'clock position synaptic inputs may cause a lowering of the excitability of the biceps brachii motoneurone pool, resulting in a suppression in CMEP amplitude. Although Katz et al. (1991) reported similar amounts of reciprocal inhibition between the biceps and triceps brachii, the biceps brachii receives additional inhibitory information from the brachioradialis (Naito et al., 1996) and the pronator teres (Naito et al., 1998).

3.4.3 Methodological considerations

In the current study, there are several factors that need to be considered in the interpretation of the results. One of our main objectives in this study was to examine the effects of increasing the "intensity" of cycling, by comparing the effect of increases in cadence and power output. We originally sought to match the output of the spinal motoneurone pools (i.e. pre-stimulus EMG) between the two intensity paradigms so that we could directly compare the two types of intensity, however, due to non-linear relationships between EMG and muscle output during cycling tasks (Wakeling et al., 2010), we were not able to do so. For example, increases in pre-stimulus EMG as cadence increased were not the same as increases in pre-stimulus EMG as power output increased. Thus, in our results, we compared the patterns of MEP and CMEP modulation between changes in cadence and power output, rather than compare the two types of intensity directly. Regarding MEPs and CMEPs, it is also noted that the amplitudes of these

potentials were not matched, and thus represent the activation of different portions of the motoneurone pool. MEPs were much larger than CMEPs.

An interesting finding in the current study was that during the active phase of the biceps brachii, increases in cadence had no effect on the MEP amplitudes at the highest power output (i.e. 60% W_{max}) (Figure 3A), despite large increases in ongoing EMG at 90 rpm (Figure 3C). CMEP amplitudes, however, at this power output increased with increased cadence (Figure 3B), suggesting that supraspinal excitability at this intensity may have decreased to account for the lack of change in MEP amplitude. Previous work during isometric contractions have demonstrated that CSE to the biceps brachii increases as contraction intensity increases, up to a limit of approximately 60% of MVC force output (Martin et al., 2006; Pearcey et al., 2014), after which there is a subsequent suppression in CSE and spinal excitability. It is currently unknown if a similar plateau response exists in the biceps brachii during a locomotor output as the contraction intensity is increased. However, recently, Weavil et al. (2015) have reported a plateauing of MEP and CMEP amplitudes as leg cycling workload is increased, suggesting that the plateau or 'ceiling effect' in CSE may be common across different motor outputs. We are also currently unsure if stimulus intensity from TMS was too low at the highest power output and cadence to recruit additional motoneurones, therefore yielding no change in MEP amplitude. Future work should consider assessing the effect of higher relative power outputs and cadences on CSE during arm cycling. Additionally, this work could be conducted at different intensities of TMS to determine the mechanism related to this finding.

3.5 CONCLUSION

The present study demonstrates inter-muscle differences in CSE and spinal excitability during rhythmic arm cycling that are dependent on the relative active and inactive phase of the muscle as well as the type of intensity (i.e. cadence vs power output). Whether the inter-muscle differences in CSE and spinal excitability are related to differences in synaptic input or intrinsic excitability between biceps and triceps brachii spinal motoneurones remains unknown, although it is likely that differences in reciprocal inhibition and/or afferent feedback may play a role. Future studies should evaluate the influence of locomotor intensity on different muscles to provide insight into the potential mechanisms underlying the observed changes in CSE reported in this study. This work would contribute to the existing literature surrounding the neural control of locomotor outputs as the intensity of the output is altered.

3.6 FIGURE LEGEND

Figure 1. (A) Example of the experimental setup. Participants were seated with their shoulders at approximately the same height as the axis of the crank shaft on the cycle ergometer while cycling at either 60 or 90 rpm at three different power outputs (20, 40, and 60% maximum power (W_{max}) attained from an incremental aerobic test (see Methods)). Black arrows label each of the stimulation techniques utilized and the location of EMG electrodes. Measurements were taken at the 6 o'clock (shown here) and 12 o'clock positions from the dominant arm. **(B)** Raw electromyography (EMG) trace for the biceps (black, solid trace) and triceps brachii (gray, solid trace) of a single participant while arm cycling at 60 rpm and 20% W_{max}. No stimulations were given in this example, and the black arrows denote the 12 and 6 o'clock positions, accordingly.

Figure 2. Representative example for the biceps brachii at the 6 o'clock position (n = 1). Average motor evoked potentials (MEPs; top row) and cervicomedullary motor evoked potentials (CMEPs; bottom row) traces during arm cycling at 20%, 40%, and 60% W_{max} at 60 rpm (dashed black line), and 90 rpm (solid black line). In this example, MEP amplitudes at 60 rpm were 37.7%, 58.1%, and 71.6% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively. At 90 rpm, MEP amplitudes were 68.7%, 69.4%, and 77.8% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively. CMEP amplitudes at 60 rpm were 31.7%, 35.6%, and 40.4% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively. At 90 rpm, CMEP amplitudes were 43.8%, 45.9%, and 53.2% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively. Abbreviation is: W_{max}, max wattage attained from incremental aerobic test.

Figure 3. (A) Group data (mean ± *SE*, n = 11) for biceps brachii MEP amplitudes at the 6 o'clock position. At 60 rpm, MEP amplitudes were 40.5%, 67.1%, and 79.5% M_{max} at 20%, 40%, and 60% W_{max}, respectively, while at 90 rpm, MEP amplitudes were 53.4%, 75.1%, and 81.3% M_{max} at 20%, 40%, and 60% W_{max}, respectively. **(B)** Group data (mean ± *SE*, n = 8) for CMEP amplitudes at the 6 o'clock position. At 60 rpm, CMEP amplitudes were 22.1%, 29.1%, and 32.8% M_{max} at 20%, 40%, and 60% W_{max} trials, respectively, while at 90 rpm, CMEP amplitudes were 24.3%, 33.0%, and 41.3% M_{max} at 20%, 40%, and 60% W_{max} trials, respectively. **(C,D)** Average pre-stimulus EMG prior to TMS (shown in **C**), and TMES (shown in **D**). * denotes a significant difference for power output, † denotes significant difference for cadence (p < .05).

Figure 4. Representative example for the biceps brachii at the 12 o'clock position (n = 1). Average MEPs (top row) and CMEPs (bottom row) traces during arm cycling at 20%, 40%, and 60% W_{max} at 60 rpm (dashed black line), and 90 rpm (solid black line). In this example, MEP amplitudes at 60 rpm were 7.5%, 7.8%, and 7.7% M_{max} during the 20%,

40%, and 60% W_{max} trials, respectively. At 90 rpm, MEP amplitudes were 6.9%, 10.3%, and 14.2% M_{meax} during the 20%, 40%, and 60% W_{max} trials, respectively. CMEP amplitudes, at 60 rpm were 2.1%, 4.5%, and 5.4% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively, while at 90 rpm, CMEP amplitudes were 2.1%, 2.9%, and 2.1% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively.

Figure 5. (A) Group data (mean ± *SE*, *n* = 11) for biceps brachii MEP amplitudes at the 12 o'clock position. At 60 rpm, MEP amplitudes were 5.0%, 7.3%, and 11.5% M_{max} at 20%, 40%, and 60% W_{max}, respectively, while at 90 rpm, MEP amplitudes were 6.5%, 10.2%, and 14.9% M_{max} at 20%, 40%, and 60% W_{max}, respectively. **(B)** Group data (mean ± *SE*, *n* = 8) for biceps brachii CMEP amplitudes at 12 o'clock. At 60 rpm, CMEP amplitudes were 5.4%, 7.1%, and 6.3% M_{max} at 20%, 40%, and 60% W_{max} trials, respectively, while at 90 rpm, CMEP amplitudes were 2.3%, 4.9%, and 4.8% M_{max} at 20%, 40%, and 60% W_{max} trials, respectively. **(C,D)** Average pre-stimulus EMG prior to TMS (shown in **C**), and TMES (shown in **D**). * denotes a significant difference for power output, † denotes significant difference for cadence ($p < .05$). # denotes trend for significance ($p = .069$).

Figure 6. Representative example for the triceps brachii at the 6 o'clock position (*n* = 1). Average MEPs (top row) and CMEPs (bottom row) traces during arm cycling at 20%, 40%, and 60% W_{max} at 60 rpm (dashed black line), and 90 rpm (solid black line). In this example, MEP amplitudes at 60 rpm were 14.2%, 18.4%, and 19.5% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively. At 90 rpm, MEP amplitudes were 20.4%, 18.5%, and 22.9% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively. CMEP amplitudes, at 60 rpm were 18.3%, 19.9%, and 24.5% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively, while at 90 rpm, CMEP amplitudes were 28.4%, 32.5%, and 34.8% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively.

Figure 7. (A) Group data (mean ± *SE*, *n* = 11) for triceps brachii MEP amplitudes at the 6 o'clock position. At 60 rpm, MEP amplitudes at this position were 21.3%, 25.3%, and 25.4% M_{max} at 20%, 40%, and 60% W_{max}, respectively, while at 90 rpm, MEP amplitudes were 25.1%, 29.5%, and 26.8% M_{max} at 20%, 40%, and 60% W_{max}, respectively. **(B)** Group data (mean ± *SE*, *n* = 8) for triceps brachii CMEP amplitudes at 6 o'clock. At 60 rpm, CMEP amplitudes were 23.6%, 26.0%, and 26.0% M_{max} at 20%, 40%, and 60% W_{max} trials, respectively, while at 90 rpm, CMEP amplitudes were 26.2%, 28.7%, and 31.9% M_{max} at 20%, 40%, and 60% W_{max} trials, respectively. **(C,D)** Average pre-stimulus EMG prior to TMS (shown in **C**), and TMES (shown in **D**). * denotes a significant difference for power output, † denotes significant difference for cadence ($p < .05$).

Figure 8. Triceps brachii representative example at the 12 o'clock position (n = 1). Average MEPs (top row) and CMEPs (bottom row) traces during arm cycling at 20%, 40%, and 60% W_{max} at 60 rpm (dashed black line), and 90 rpm (solid black line). In this example, MEP amplitudes at 60 rpm were 26.5%, 27.2%, and 40.3% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively. At 90 rpm, MEP amplitudes were 35.2%, 41.9%, and 40.9% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively. In CMEP amplitudes, at 60 rpm were 21.5%, 21.7%, and 19.8% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively, while at 90 rpm, CMEP amplitudes were 18.9%, 19.6%, and 22.6% M_{max} during the 20%, 40%, and 60% W_{max} trials, respectively.

Figure 9. (A) Group data (mean ± *SE*, n = 11) for triceps brachii MEP amplitudes at the 12 o'clock position. At 60 rpm, MEP amplitudes were 32.1%, 42.8%, and 46.2% M_{max} at 20%, 40%, and 60% W_{max}, respectively, while at 90 rpm, MEP amplitudes were 31.2%, 42.5%, and 46.0% M_{max} at 20%, 40%, and 60% W_{max}, respectively. **(B)** Group data (mean ± *SE*, n = 8) for triceps brachii CMEP amplitudes also at 12 o'clock. At 60 rpm, CMEP amplitudes were 19.0%, 21.2%, and 24.0% M_{max} at 20%, 40%, and 60% W_{max} trials, respectively, while at 90 rpm, CMEP amplitudes were 20.2%, 23.4%, and 24.5% M_{max} at 20%, 40%, and 60% W_{max} trials, respectively. **(C,D)** Average pre-stimulus EMG prior to TMS (shown in **C**), and TMES (shown in **D**). * denotes a significant difference for power output, † denotes significant difference for cadence (p < .05).

3.7 LIST OF TABLES

Table 1. Raw and normalized MEP, CMEP and M_{max} amplitudes during the active phase of arm cycling at different cadences and relative power outputs.

Active phase

	Cycling Workload					
	60 rpm			90 rpm		
	20% W_{max}	40% W_{max}	60% W_{max}	20% W_{max}	40% W_{max}	60% W_{max}
	Biceps brachii (6 o'clock)					
M_{max}						
-Peak-to-peak, mv	14.6 ± 1.19	14.4 ± 1.27	13.9 ± 1.26	14.6 ± 1.22	14.1 ± 1.26	14. 0 ± 1.19
-Latency, ms	4.8 ± 0.10	4.8 ± 0.13	4.9 ± 0.11	4.9 ± 0.12	4.8 ± 0.10	4.9 ± 0.13
MEP						
-Peak-to-peak, mv	5.5 ± 0.55	9.4 ± 0.61	10.7 ± 0.82	7.3 ± 0.99	10.2 ± 0.77	11.2 ± 0.98
-%M_{max} Peak-to-peak	40.5 ± 5.21	67.1 ± 4.38	79.5 ± 4.72	53.4 ± 7.46	75.1 ± 5.85	81.3 ± 4.72
-Latency, ms	11.8 ± 0.13	11.8 ± 0.17	11.8 ± 0.17	11.7 ± 0.19	11.8 ± 0.19	11.9 ± 0.18
CMEP						
-Peak-to-peak, mv	3.0 ± 0.56	3.8 ± 0.69	4.1 ± 0.65	3.4 ± 0.79	4.3 ± 0.87	5.4 ± 1.08
-%M_{max} Peak-to-peak	22.1 ± 3.68	29.1 ± 4.15	32.8 ± 4.91	24.3 ± 4.41	33.0 ± 4.52	41.3 ± 5.92
-Latency, ms	8.0 ± 0.19	8.1 ± 0.17	7.9 ± 0.21	8.1 ± 0.10	8.1 ± 0.21	8.0 ± 0.21
	Triceps brachii (12 o'clock)					
M_{max}						
-Peak-to-peak, mv	4.5 ± 0.44	4.3 ± 0.41	4.4 ± 0.43	4.2 ± 0.38	4.2 ± 0.40	4.3 ± 0.45
-Latency, ms	5.4 ± 0.07	5.5 ± 0.09	5.4 ± 0.09	5.4 ± 0. 60	5.6 ± 0.10	5.5 ± 0.09
MEP						
-Peak-to-peak, mv	1.5 ± 0.49	1.9 ± 0.59	2.1 ± 0.68	1.4 ± 0.51	1.9 ± 0.49	2.0 ± 0.55
-%M_{max} Peak-to-peak	32.1 ± 8.17	42.8 ± 10.55	46.2 ± 11.44	31.2 ± 7.00	42.4 ± 9.00	46.0 ± 10.13

-Latency, ms	12.5 ± 0.37	12.5 ± 0.38	12.3 ± 0.47	12.8 ± 0.61	12.5 ± 0.53	12.4 ± 0.61
CMEP						
-Peak-to-peak, mv	0.9 ± 0.3	1.1 ± 0.2	1.3 ± 0.3	1.0 ± 0.2	1.1 ± 0.3	1.2 ± 0.3
-%M_{max} Peak-to-peak	19.0 ± 4.33	21.2 ± 3.65	24.0 ± 4.93	20.2 ± 4.02	23.4 ± 5.24	24.5 ± 4.10
-Latency, ms	7.0 ± 0.53	6.9 ± 0.42	6.8 ± 0.33	7.4 ± 0.43	7.2 ± 0.44	7.0 ± 0.32

Data are reported as means ± *SE*.

Table 2. Raw and normalized MEP, CMEP and M_{max} amplitudes during the inactive phase of arm cycling at different cadences and relative power outputs.

Inactive phase

	Cycling Workload					
	60 rpm			90 rpm		
	20% W_{max}	40% W_{max}	60% W_{max}	20% W_{max}	40% W_{max}	60% W_{max}
	Biceps brachii (12 o'clock)					
M_{max}						
-Peak-to-peak, mv	15.5 ± 1.32	15.0 ± 1.27	15.4 ± 1.32	15.9 ± 1.36	15.1 ± 1.37	15.3 ± 1.37
-Latency, ms	4.8 ± 0.13	4.8 ± 0.11	4.8 ± 0.11	4.9 ± 0.11	4.9 ± 0.12	4.9 ± 0.14
MEP						
-Peak-to-peak, mv	0.8 ± 0.17	1.0 ± 0.26	1.7 ± 0.39	1.0 ± 0.23	1.4 ± 0.33	2.1 ± 0.52
-%M_{max} Peak-to-peak	5.0 ± 1.17	7.3 ± 2.04	11.6 ± 2.99	6.5 ± 1.69	10.2 ± 2.70	14.9 ± 4.34
-Latency, ms	14.9 ± 0.61	14.8 ± 0.49	14.5 ± 0.37	14.7 ± 0.43	14.8 ± 0.25	14.8 ± 0.48
CMEP						
-Peak-to-peak, mv	0.8 ± 0.41	1.1 ± 0.54	0.9 ± 0.48	0.7 ± 0.39	0.7 ± 0.50	0.7 ± 0.44
-%M_{max} Peak-to-peak	5.4 ± 2.53	7.1 ± 3.10	6.2 ± 2.92	4.4 ± 2.33	4.9 ± 2.94	4.7 ± 2.64
-Latency, ms	7.8 ± 0.24	8.1 ± 0.33	8.1 ± 0.45	7.8 ± 0.19	7.8 ± 0.44	8.0 ± 0.41

			Triceps brachii (6 o'clock)			
M_{max}						
-Peak-to-peak, mv	4.9 ± 0.44	4.8 ± 0.43	4.8 ± 0.41	4.6 ± 0.43	4.7 ± 0.47	4.9 ± 0.44
-Latency, ms	5.5 ± 0.10	5.5 ± 0.14	5.6 ± 0.14	5.6 ± 0.10	5.5 ± 0.11	5.5 ± 0.12
MEP						
-Peak-to-peak, mv	0.9 ± 0.09	1.1 ± 0.05	1.1 ± 0.06	1.0 ± 0.10	1.1 ± 0.08	1.2 ± 0.09
-%M_{max} Peak-to-peak	21.3 ± 5.15	25.3 ± 4.29	25.4 ± 3.56	25.1 ± 5.80	29.5 ± 7.51	26.8 ± 4.21
-Latency, ms	15.5 ± 0.90	14.1 ± 0.87	15.1 ± 0.85	15.1 ± 0.91	14.7 ± 0.87	14.3 ± 0.80
CMEP						
-Peak-to-peak, mv	1.3 ± 0.24	1.4 ± 0.25	1.4 ± 0.22	1.4 ± 0.27	1.5 ± 0.22	1.6 ± 0.20
-%M_{max} Peak-to-peak	23.6 ± 4.48	26.0 ± 4.42	26.0 ± 4.29	26.2 ± 4.92	28.7 ± 3.93	31.9 ± 3.94
-Latency, ms	7.4 ± 0.58	7.6 ± 0.54	7.8 ± 0.53	7.6 ± 0.60	7.8 ± 0.48	7.7 ± 0.54

Data are reported as means ± *SE*.

3.8 LIST OF FIGURES

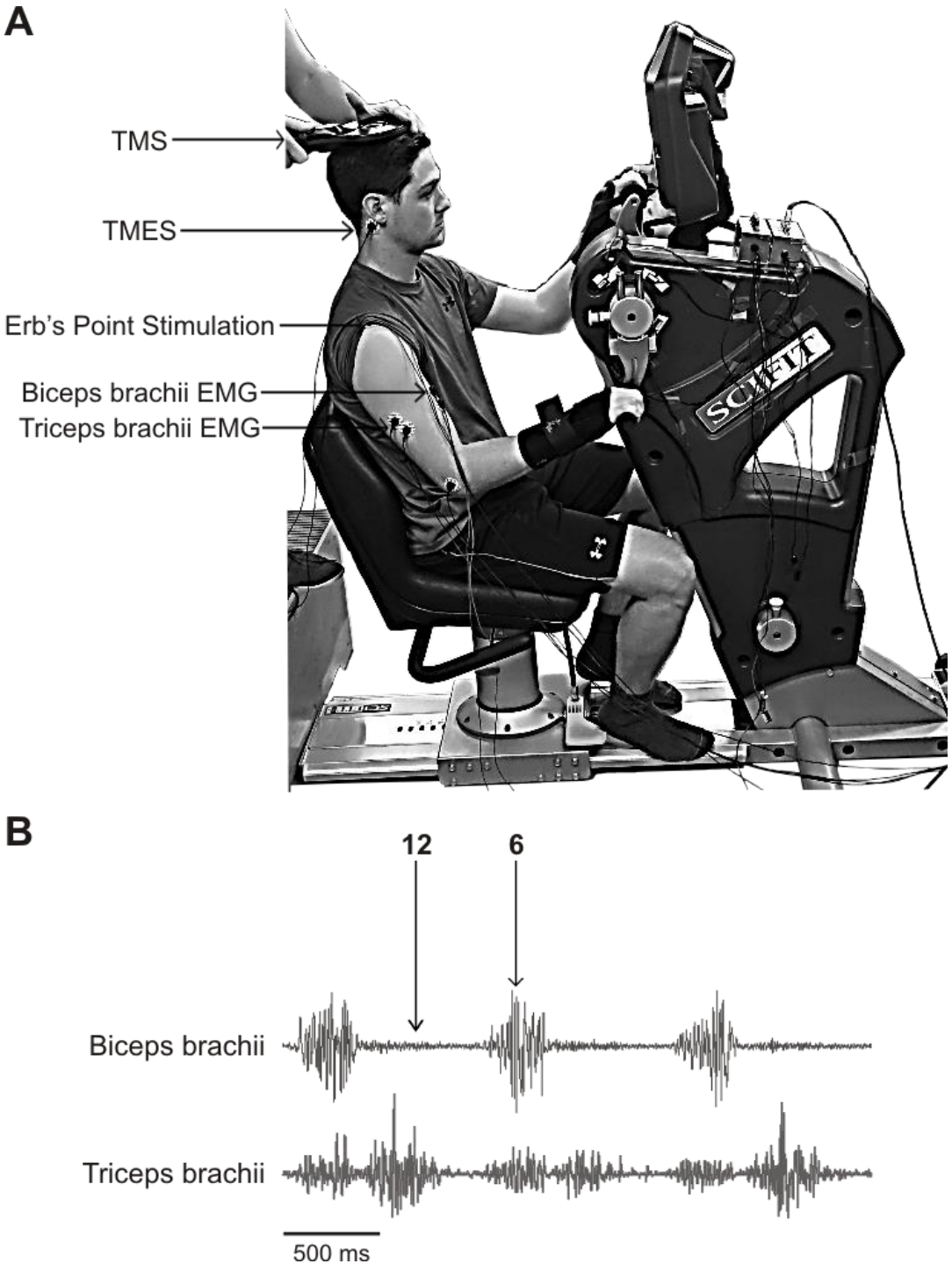

Figure 1 Experimental setup and EMG patterns during arm cycling at 60 rpm and 20% W_{max}

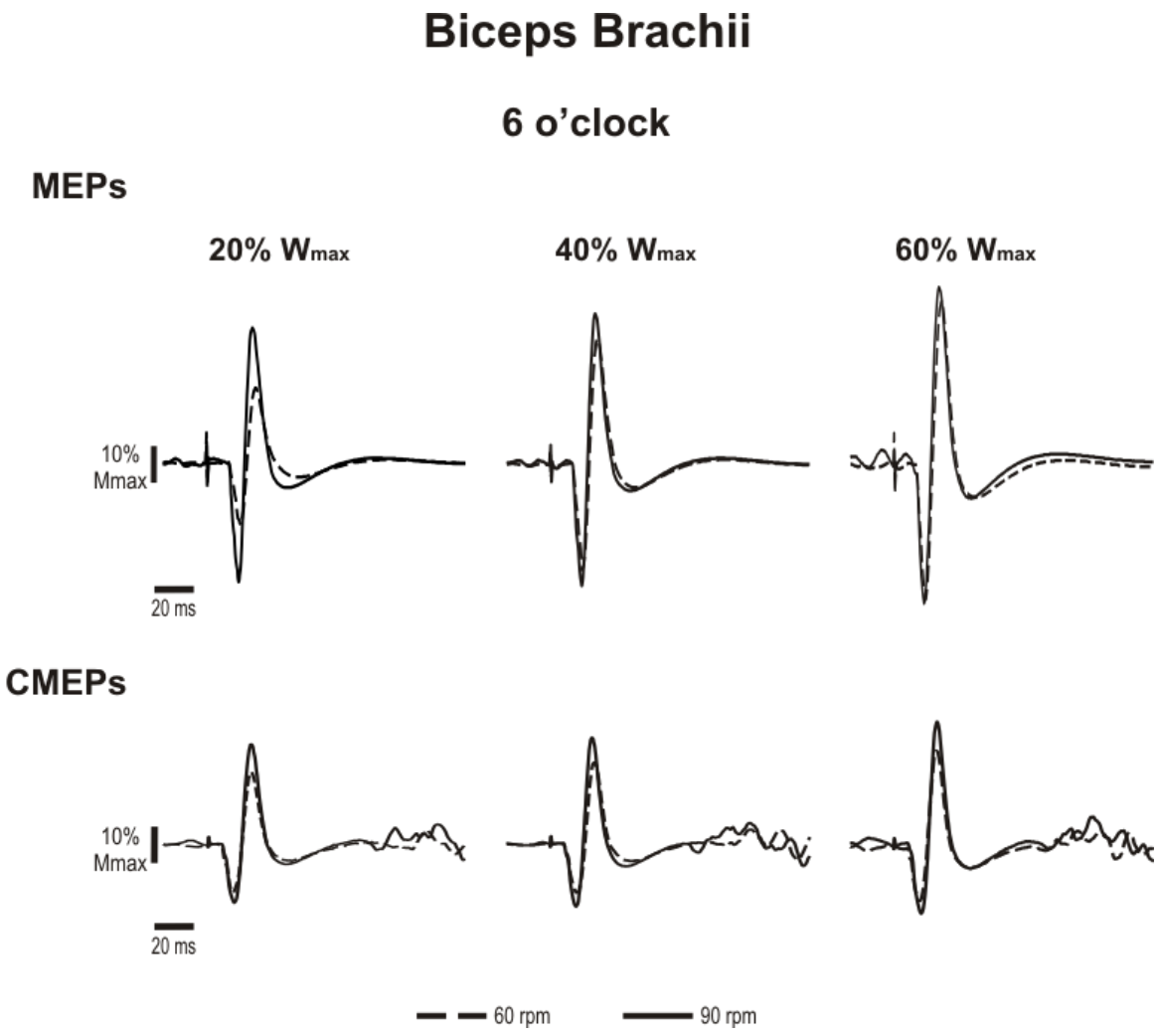

Figure 2 Representative MEP and CMEP amplitudes from biceps brachii at 6 o'clock

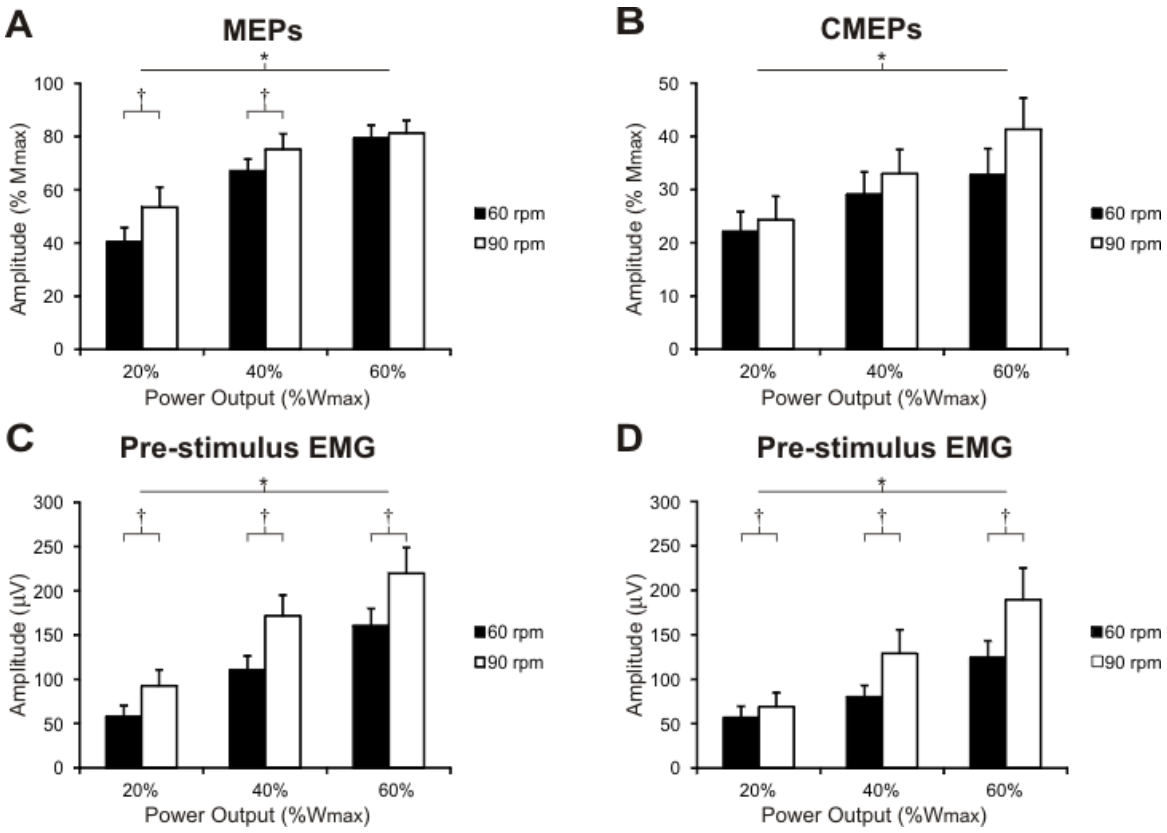

Figure 3 Group MEP, CMEP, and pre-stimulus EMG from biceps brachii at 6 o'clock

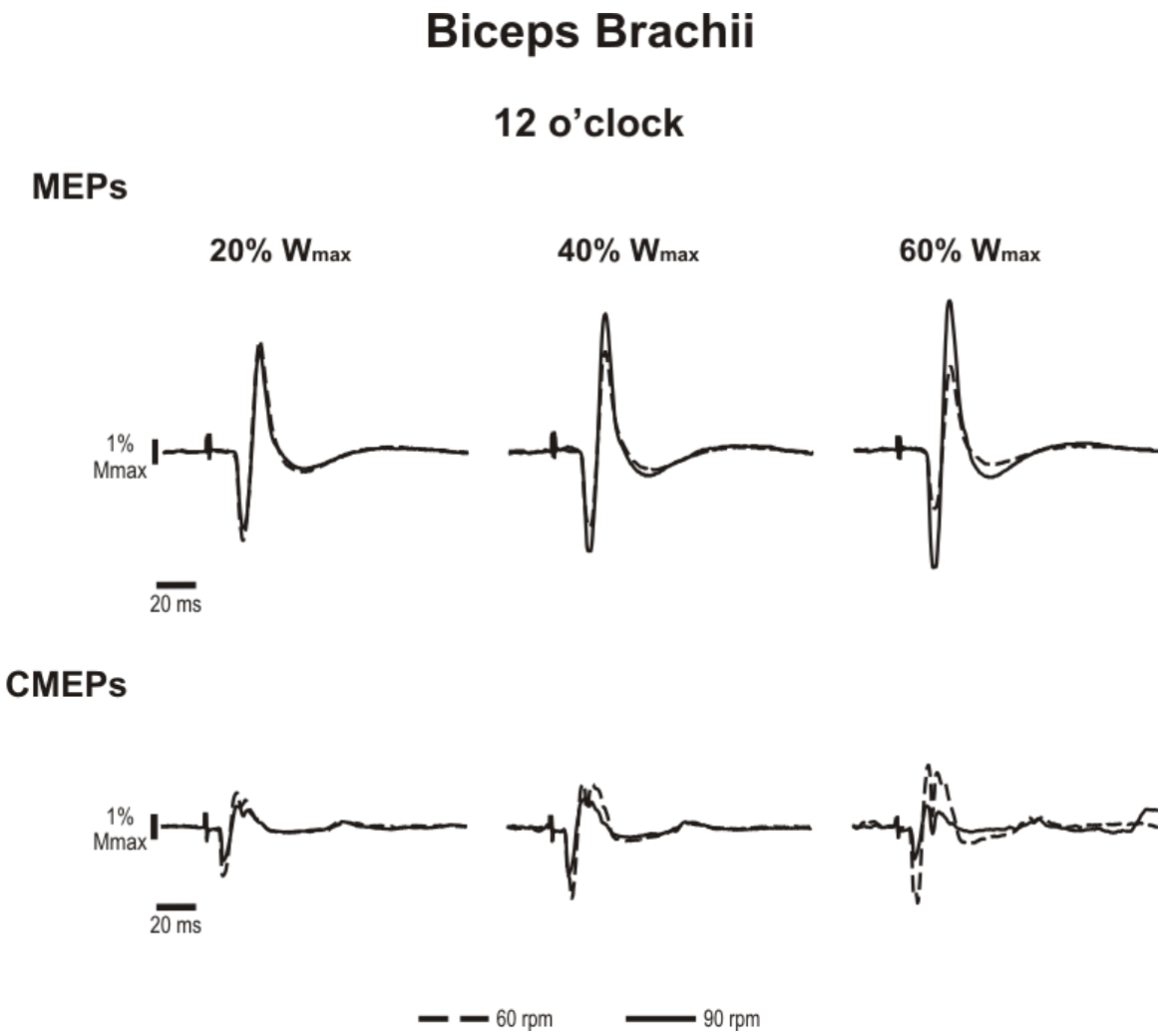

Figure 4 Representative MEP and CMEP amplitudes from biceps brachii at 12 o'clock

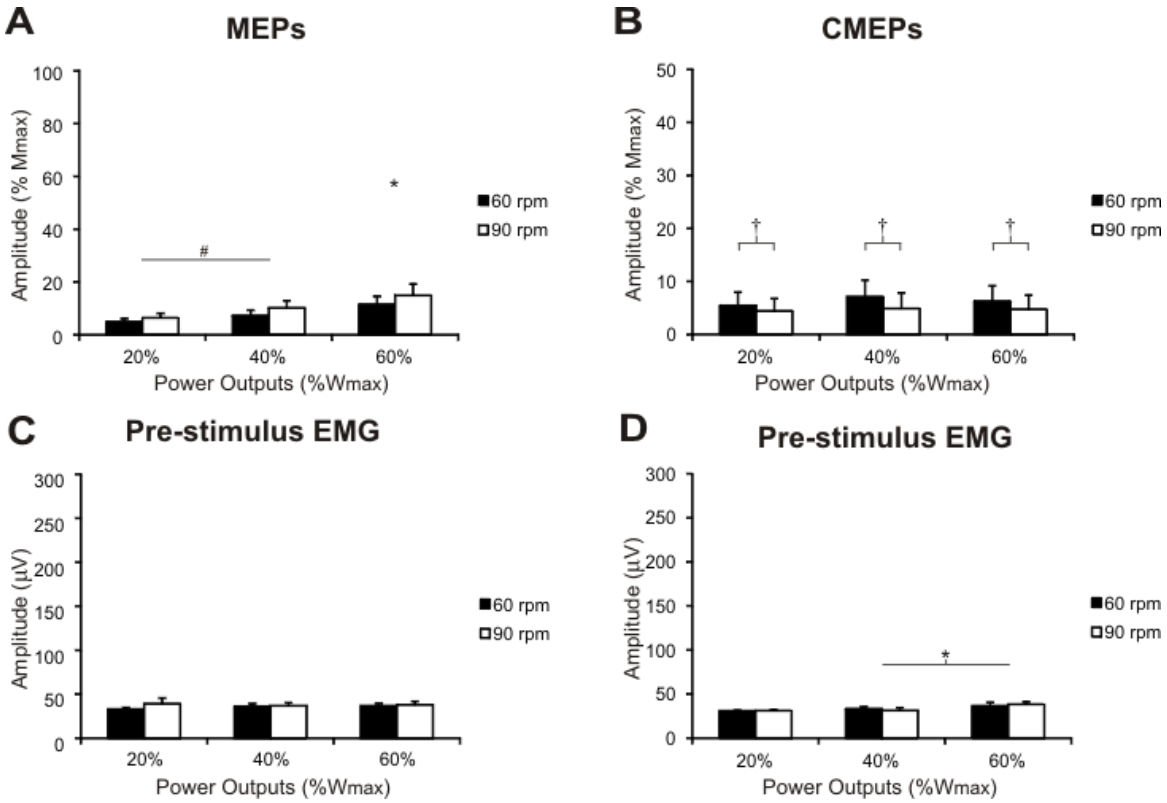

Figure 5 Group MEP, CMEP, and pre-stimulus EMG from biceps brachii at 12 o'clock

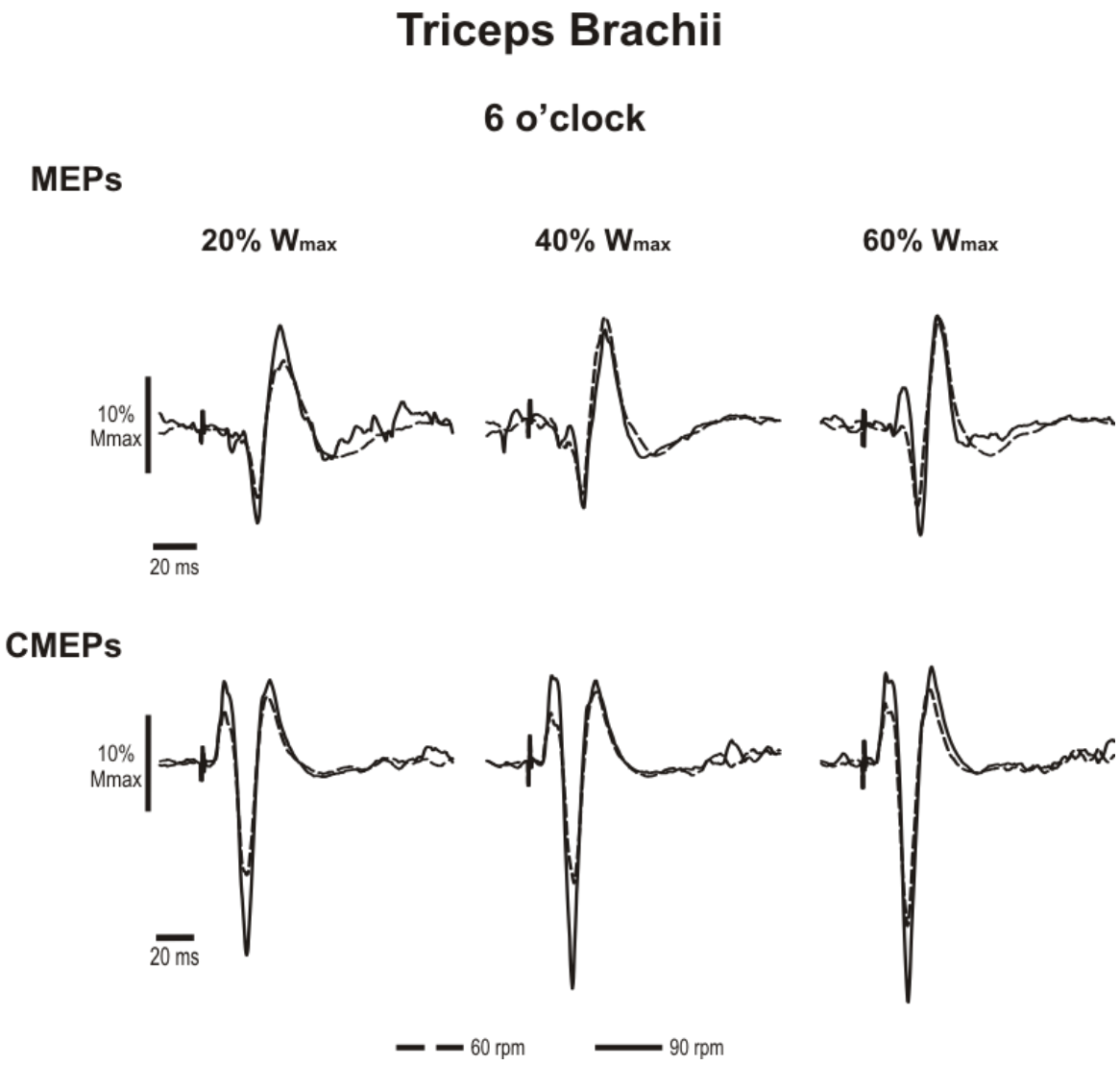

Figure 6 Representative MEPs and CMEPs amplitudes from triceps brachii at 6 o'clock

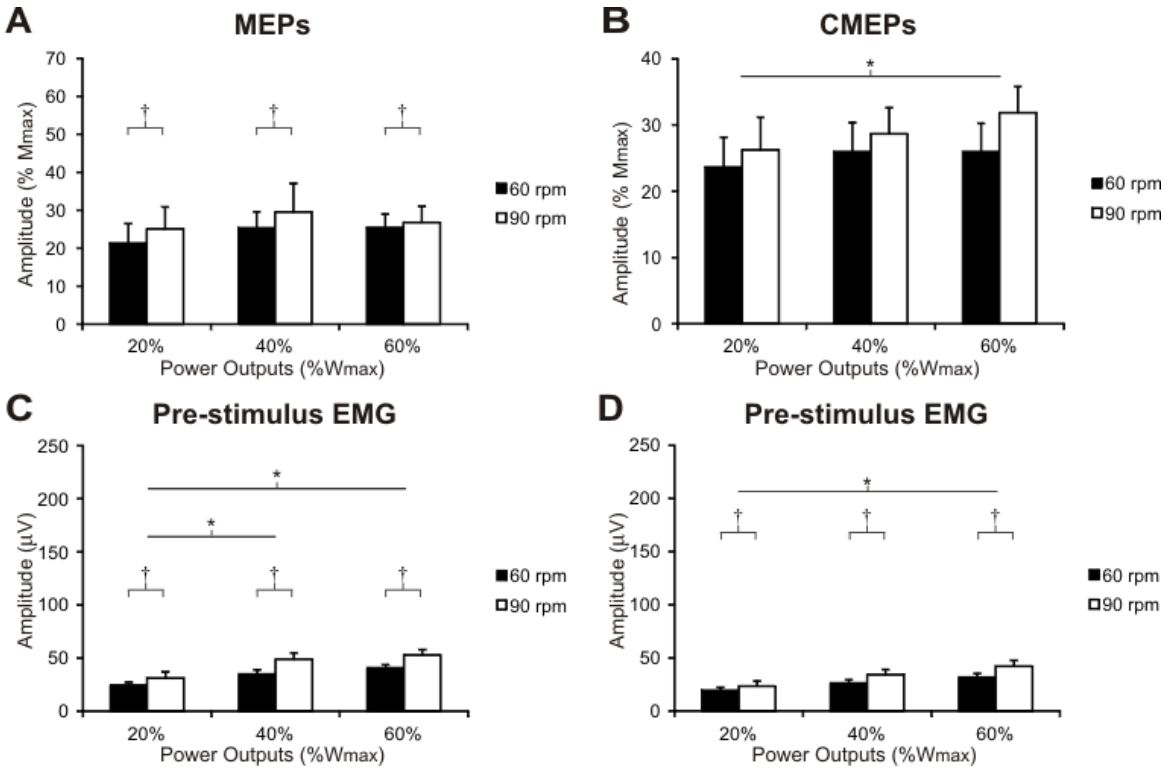

Figure 7 Group MEP, CMEP, and pre-stimulus EMG from triceps brachii at 6 o'clock

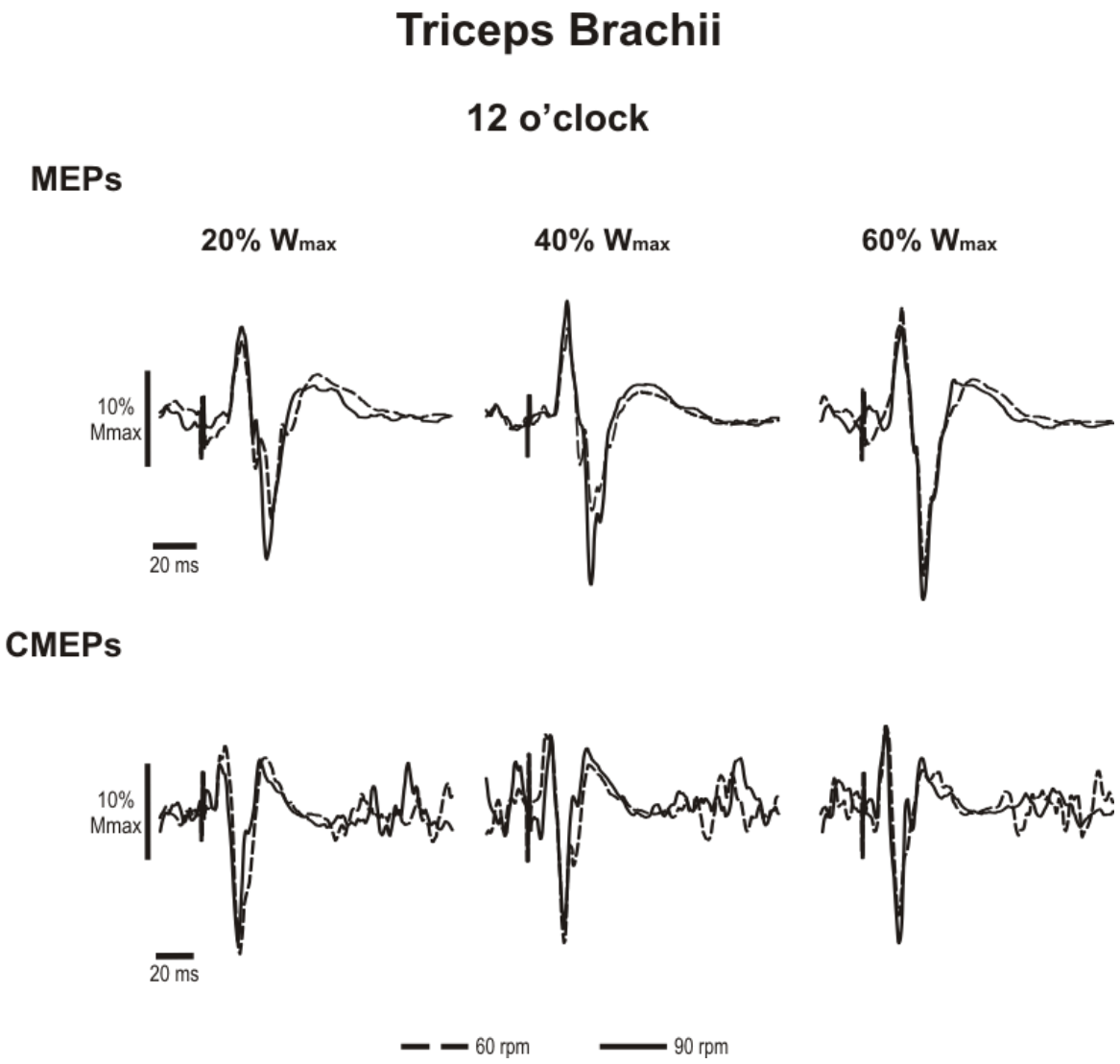

Figure 8 Representative MEP and CMEP amplitudes from triceps brachii at 12 o'clock

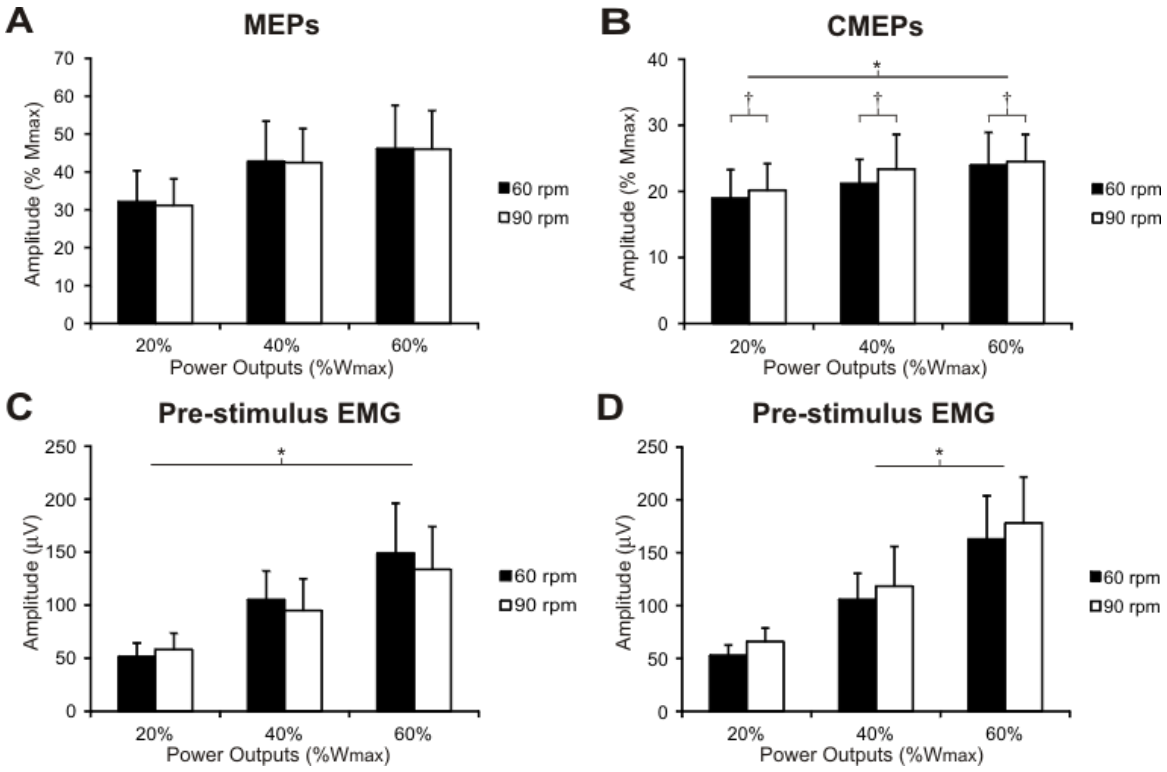

Figure 9 Group MEP, CMEP, and pre-stimulus EMG from triceps brachii at 12 o'clock

3.9 REFERENCES

Balter, J.E., Zehr, E.P., 2007. Neural coupling between the arms and legs during rhythmic locomotor-like cycling movement. J Neurophysiol. 97**,** 1809-18.

Brooke, J.D., McIlroy, W.E., Collins, D.F., 1992. Movement features and H-reflex modulation. I. Pedalling versus matched controls. Brain Res. 582**,** 78-84.

Brooke, J.D., et al., 1997. Modulation of cerebral somatosensory evoked potentials arising from tibial and sural nerve stimulation during rhythmic active and passive movements of the human lower limb. Electromyogr Clin Neurophysiol. 37**,** 451-61.

Brouwer, B., Ashby, P., 1990. Corticospinal projections to upper and lower limb spinal motoneurons in man. Electroencephalogr Clin Neurophysiol. 76**,** 509-19.

Capaday, C., Stein, R.B., 1987. Difference in the amplitude of the human soleus H reflex during walking and running. J Physiol. 392**,** 513-22.

Carroll, T.J., et al., 2006. Corticospinal excitability is lower during rhythmic arm movement than during tonic contraction. J Neurophysiol. 95**,** 914-21.

Christensen, L.O., et al., 2000. Cerebral activation during bicycle movements in man. Exp Brain Res. 135**,** 66-72.

Copithorne, D.B., Forman, D.A., Power, K.E., 2015. Premovement Changes in Corticospinal Excitability of the Biceps Brachii are Not Different Between Arm Cycling and an Intensity-Matched Tonic Contraction. Motor Control. 19**,** 223-41.

Cotel, F., et al., 2009. Identified ankle extensor and flexor motoneurons display different firing profiles in the neonatal rat. J Neurosci. 29**,** 2748-53.

Daligadu, J., et al., 2013. TMS stimulus-response asymmetry in left- and right-handed individuals. Exp Brain Res. 224**,** 411-6.

de Ruiter, G.C., Hundza, S.R., Zehr, E.P., 2010. Phase-dependent modulation of soleus H-reflex amplitude induced by rhythmic arm cycling. Neurosci Lett. 475**,** 7-11.

Duysens, J., Van de Crommert, H.W., 1998. Neural control of locomotion; The central pattern generator from cats to humans. Gait Posture. 7**,** 131-141.

Forman, D., et al., 2014. Corticospinal excitability of the biceps brachii is higher during arm cycling than an intensity-matched tonic contraction. J Neurophysiol. 112**,** 1142-51.

Forman, D.A., et al., 2015. Cadence-dependent changes in corticospinal excitability of the biceps brachii during arm cycling. J Neurophysiol. 114**,** 2285-94.

Forman, D.A., et al., 2016. Changes in Corticospinal and Spinal Excitability to the Biceps Brachii with a Neutral vs. Pronated Handgrip Position Differ between Arm Cycling and Tonic Elbow Flexion. Front Hum Neurosci. 10**,** 543.

Grillner, S., 1975. Locomotion in vertebrates: central mechanisms and reflex interaction. Physiol Rev. 55**,** 247-304.

Hounsgaard, J., et al., 1988. Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. J Physiol. 405**,** 345-67.

Hundza, S.R., Zehr, E.P., 2009. Suppression of soleus H-reflex amplitude is graded with frequency of rhythmic arm cycling. Exp Brain Res. 193**,** 297-306.

Hundza, S.R., et al., 2012. Effect of afferent feedback and central motor commands on soleus H-reflex suppression during arm cycling. J Neurophysiol. 108**,** 3049-58.

Katz, R., Penicaud, A., Rossi, A., 1991. Reciprocal Ia inhibition between elbow flexors and extensors in the human. J Physiol. 437**,** 269-86.

Larsen, B., Voigt, M., 2004. Changes in the gain of the soleus H-reflex with changes in the motor recruitment level and/or movement speed. Eur J Appl Physiol. 93**,** 19-29.

Larsen, B., Voight, M., Grey, M.J., 2006. Changes in the soleus stretch reflex at different pedaling frequencies and crank loads during pedaling. Motor Control. 10**,** 265-79.

Manning, C.D., Bawa, P., 2011. Heteronymous reflex connections in human upper limb muscles in response to stretch of forearm muscles. J Neurophysiol. 106**,** 1489-99.

Martin, P.G., Gandevia, S.C., Taylor, J.L., 2006. Output of human motoneuron pools to corticospinal inputs during voluntary contractions. J Neurophysiol. 95**,** 3512-8.

Matthews, P.B., 1999. The effect of firing on the excitability of a model motoneurone and its implications for cortical stimulation. J Physiol. 518 (Pt 3)**,** 867-82.

McNeil, C.J., et al., 2013. Testing the excitability of human motoneurons. Front Hum Neurosci. 7**,** 152.

Naito, A., et al., 1996. Inhibitory projection from brachioradialis to biceps brachii motoneurones in human. Exp Brain Res. 111**,** 483-6.

Naito, A., et al., 1998. Inhibitory projections from pronator teres to biceps brachii motoneurones in human. Exp Brain Res. 121**,** 99-102.

Olivier, E., Bawa, P., Lemon, R.N., 1995. Excitability of human upper limb motoneurones during rhythmic discharge tested with transcranial magnetic stimulation. J Physiol. 485 (Pt 1)**,** 257-69.

Palmer, E., Ashby, P., 1992. Corticospinal projections to upper limb motoneurones in humans. J Physiol. 448**,** 397-412.

Palomino, A.F., Hundza, S.R., Zehr, E.P., 2011. Rhythmic arm cycling differentially modulates stretch and H-reflex amplitudes in soleus muscle. Exp Brain Res. 214**,** 529-37.

Pearcey, G.E., Power, K.E., Button, D.C., 2014. Differences in supraspinal and spinal excitability during various force outputs of the biceps brachii in chronic- and non-resistance trained individuals. PLoS One. 9**,** e98468.

Petersen, N., Morita, H., Nielsen, J., 1999. Modulation of reciprocal inhibition between ankle extensors and flexors during walking in man. J Physiol. 520 Pt 2**,** 605-19.

Petersen, N.T., et al., 2001. Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking. J Physiol. 537**,** 651-6.

Petersen, T.H., et al., 2012. The motor cortex drives the muscles during walking in human subjects. J Physiol. 590**,** 2443-52.

Power, K.E., Copithorne, D.B., 2013. Increased corticospinal excitability prior to arm cycling is due to enhanced supraspinal but not spinal motoneurone excitability. Appl Physiol Nutr Metab. 38**,** 1154-61.

Price, M.J., et al., 2007. The effects of cadence and power output upon physiological and biomechanical responses to incremental arm-crank ergometry. Appl Physiol Nutr Metab. 32**,** 686-92.

Pyndt, H.S., Laursen, M., Nielsen, J.B., 2003. Changes in reciprocal inhibition across the ankle joint with changes in external load and pedaling rate during bicycling. J Neurophysiol. 90**,** 3168-77.

Rossi, S., et al., 2009. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 120**,** 2008-39.

Sakamoto, M., et al., 2004. Load- and cadence-dependent modulation of somatosensory evoked potentials and Soleus H-reflexes during active leg pedaling in humans. Brain Res. 1029**,** 272-85.

Sidhu, S.K., et al., 2012. Corticospinal contributions to lower limb muscle activity during cycling in humans. J Neurophysiol. 107**,** 306-14.

Sidhu, S.K., Cresswell, A.G., Carroll, T.J., 2013. Short-interval intracortical inhibition in knee extensors during locomotor cycling. Acta Physiol (Oxf). 207**,** 194-201.

Spence, A.J., et al., 2016. Phase- and Workload-Dependent Changes in Corticospinal Excitability to the Biceps and Triceps Brachii during Arm Cycling. Brain Sci. 6.

Staines, W.R., et al., 1997. Movement-induced gain modulation of somatosensory potentials and soleus H-reflexes evoked from the leg. II. Correlation with rate of stretch of extensor muscles of the leg. Exp Brain Res. 115**,** 156-64.

Taylor, J.L., Gandevia, S.C., 2004. Noninvasive stimulation of the human corticospinal tract. J Appl Physiol (1985). 96**,** 1496-503.

Taylor, J.L., 2006. Stimulation at the cervicomedullary junction in human subjects. J Electromyogr Kinesiol. 16**,** 215-23.

Veale, J.F., 2014. Edinburgh Handedness Inventory - Short Form: a revised version based on confirmatory factor analysis. Laterality. 19**,** 164-77.

Wakeling, J.M., Blake, O.M., Chan, H.K., 2010. Muscle coordination is key to the power output and mechanical efficiency of limb movements. J Exp Biol. 213**,** 487-92.

Weavil, J.C., et al., 2015. Intensity-dependent alterations in the excitability of cortical and spinal projections to the knee extensors during isometric and locomotor exercise. Am J Physiol Regul Integr Comp Physiol. 308**,** R998-1007.

Wilson, J.M., et al., 2015. Intrinsic excitability of human motoneurons in biceps brachii versus triceps brachii. J Neurophysiol. 113**,** 3692-9.

Zehr, E.P., Chua, R., 2000. Modulation of human cutaneous reflexes during rhythmic cyclical arm movement. Exp Brain Res. 135**,** 241-50.

Zehr, E.P., et al., 2004. Possible contributions of CPG activity to the control of rhythmic human arm movement. Can J Physiol Pharmacol. 82**,** 556-68.

Chapter 4 Summary and Future Directions

Obtaining a better understanding of how the brain and spinal cord function to produce locomotor outputs, such as rhythmic arm cycling, is of great importance for the basic scientific advancement of human locomotion-related knowledge. In this study, we examined the modulation of corticospinal and spinal excitability to the biceps and triceps brachii during arm cycling as the intensity of the output was altered. The findings from this study add to the literature suggesting that the neural control of locomotor outputs is dependent on the type of locomotor intensity (i.e. cadence vs power output). Moreover, the findings suggest that the observed intensity-dependent modulation is also dependent on the muscle examined (i.e. biceps vs triceps brachii). Future studies should attempt to characterize how corticospinal and spinal excitability are modulated with intensity across several different muscles of the upper body during arm cycling. Doing this would not only gain insight into potential mechanisms and muscle-dependent effects on corticospinal and spinal excitability during arm cycling, but it would also provide a more thorough understanding of how the central nervous system controls rhythmic locomotor outputs. This type of research, although not applied in a clinical setting in the current study, could help guide potential 'best-practice' rehabilitation treatments to reduce spasticity for individuals with central nervous system disorders who may use arm cycling for exercise/training.

By using both TMS and TMES together in the present study, we were able to differentiate where along the corticospinal pathway that differences in excitability may have occurred as arm cycling intensity increased. However, we were still limited in terms of understanding the mechanisms that may be underlying the observed findings. Although

we can speculate on potential mechanisms, future research should be devoted to obtaining more information on the factors and mechanisms that may contribute to the production of human locomotor outputs, including arm cycling. For example, examining reciprocal inhibition to the upper arms while arm cycling could provide additional information in terms of mechanism to the current study, as it is currently unknown how reciprocal inhibition is modulated between the biceps and triceps brachii during a locomotor output. Additionally, paired-pulse TMS could be used to assess changes in supraspinal excitability, which would provide a better understanding of potential cortical mechanisms that may be involved during arm cycling as the locomotor intensity changes.

Looking back, there are a number of things that could have been done differently in terms of methodology that could have provided us with more information. First, as discussed briefly in the literature review (section 2.2), the cycle ergometer used in this study allowed us to increase the cadence and power output. However, at a set power output, increases in cadence results in a decrease in resistance to the crankshaft. Therefore, the amount of voluntary effort would presumably be less at a faster cadence and a constant power output (i.e. reduced resistance). Unfortunately, with the equipment used, we were not able to monitor the resistance or load applied to the crankshaft. If this study were to be repeated, perhaps the use of a constant-load ergometer, like ones used in previous arm and leg cycling studies would allow the load to be kept constant, which would be unaffected by cadence and may provide different results. Second, we hoped to be able to match pre-stimulus EMG between the two intensity parameters but were unsuccessful in our attempts. Future work, albeit easier said than done, should try to do this again but use a method of matching that would allow for the direct comparison

between changes in cadence and changes in power output/load. Lastly, arm cycling is a bilateral motor output, meaning that both arms contribute to the desired movement. In the current study, we have only reported on the corticospinal and spinal control of the dominant limb. Thus, future work (already in progress in our lab) should examine the bilateral modulation of corticospinal and spinal excitability during arm cycling in order to understand whether the findings are similar between limbs. To date, inter-limb differences in corticospinal excitability during arm cycling have yet to be reported.