Corticospinal and spinal excitability to the biceps and triceps brachii is modulated differently between forward and backward arm cycling

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Abstract

Research on humans and non-human animals has provided indirect evidence that suggests that the fundamental rhythmic motor pattern required for locomotor outputs is partially controlled by neural circuits in the spinal cord, referred to as the central pattern generator (CPG). Specifically, research has shown that the same neural networks operate to control both forward (FWD) and backward (BWD) locomotor outputs. Up until this point researchers have focused on examining reflex modulation patterns during FWD and BWD locomotor outputs to infer activity of the CPG. However, these studies do not provide any insight into how the brain and spinal cord are contributing to the generation of FWD and BWD rhythmic movement. To date, no study has directly compared corticospinal and spinal excitability between FWD and BWD locomotor outputs. Thus, the purpose of this study was to use transcranial magnetic stimulation (TMS) in combination with transmastoid electrical stimulation (TMES) to compare corticospinal and spinal excitability projecting to the biceps and triceps brachii between FWD and BWD arm cycling.

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To honor the strength and optimism instilled within me by my father, I will end with a quote from his favourite song:

“Each day's a gift and not a given right, leave no stone unturned, leave your fears behind and try to take the path less traveled by”

- Nickelback

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List of Abbreviations and Symbols

AHP- Afterhyperpolarization

BWD- Backward

CMEP- Cervicomedullary evoked potential

CPG- Central Pattern Generator

EPSP- Excitatory Post-synaptic Potential

EMG- Electromyography

FWD- Forward

H-reflex- Hoffman reflex

IPSP- Inhibitory Post-synaptic Potential

ISI- Interstimulus interval

KPM- Kilo-pond meters

MEP- Motor Evoked Potential

M_{max}- Maximum compound muscle action potential

MSO- Maximal stimulator output

NMJ- Neuromuscular Junction

PIC- Persistent inward current

TA- Tibialis Anterior

TES- Transcranial Electrical Stimulation

TMS- Transcranial Magnetic Stimulation

TMES- Transmastoid electrical stimulation

V_{th}- Voltage threshold

Chapter 1 Introduction

1.0 Overview

Rhythmic motor outputs, such as walking and cycling, are partially controlled by neural circuits in the spinal cord (Brown, 1911; Grillner, 1981; Zehr, 2005), referred to as the central pattern generator (CPG), however in humans supraspinal input is required (Peterson et al., 2001). As humans our first mode of locomotion was crawling, which requires rhythmic and alternating activation arm and leg muscles. Thus, arm cycling, which is a bilateral motor output that requires rhythmic activation of arm muscles, has been utilized as a paradigm for examining the neural control of rhythmic arm movement (Zehr & Kido, 2001; Zehr & Hundza, 2005; Zehr, Collins, Frigon, & Hoogenboom, 2003). This is because researchers have shown that the muscle activations patterns and reflex modulation patterns, which are used to examine CPG activity, during arm cycling are similar to those obtained during walking and leg cycling (Zehr et al., 2004). When comparing forward (FWD) and backward (BWD) movement a cycling paradigm is useful as it eliminates the differences in postural control, visual input and kinematics that have been observed when comparing FWD and BWD walking (Duysens, Tax, Murrer, Dietz, 1996; Grasso, Bianche, & Lacquanti, 1998). Zehr & Hunda (2005) examined reflex modulation patterns of various upper limb muscles during FWD and BWD arm cycling and found that the reflex modulating patterns were similar for FWD and BWD cycling. This has been supported by a similar study that compared reflex modulation patterns during FWD and BWD leg cycling (Zehr, Hundza, Balter & Loadman, 2009). These findings suggest that FWD and BWD cycling are controlled by the same neural circuits. Although this provides insight into the neural control

of FWD and BWD cycling, little is known about how the brain and spinal cord function to generate BWD cycling.

In humans several non-invasive stimulation techniques can be used to examine corticospinal excitability during voluntary motor outputs. Transcranial magnetic stimulation (TMS) and transmastoid electrical stimulation (TMES) can be used together to provide a measure of suprapsinal and spinal excitability, respectively (Taylor, 2006). Research in our lab has shown that corticospinal excitability projecting to the upper limb muscles is dependent on the phase of the movement cycle (Forman, Raj, Button, & Power, 2014), on the intensity of FWD arm cycling (Forman, Philpott, Button & Power, 2015; Spence et al., 2016), and on the muscle being examined (Spence et al., 2016). To date only one study has examined CSE during BWD locomotor-like movement. Ung et al., (2005) showed that CSE projecting to the soleus muscle is lower during BWD walking when compared to an intensity- and position matched tonic contraction. Conversely, research in our lab has shown that CSE projecting to the biceps brachii was higher during FWD arm cycling when compared to an intensity- and position- matched tonic contraction (Forman et al., 2014). Although corticospinal excitability has been studied during FWD and BWD rhythmic motor outputs, to date there has not been a study that has compared CSE projecting to upper limb muscles between FWD and BWD locomotor-like movements.

1.1 Purpose

The primary purpose of this study is to determine if corticospinal excitability projecting to the biceps and triceps brachii is similarly modulated during FWD and BWD arm cycling.

1.2 Hypothesis

It is hypothesized that corticospinal excitability, as measured by MEP and CMEP amplitudes, projecting to the biceps and triceps brachii will be lower during BWD arm cycling compared to FWD arm cycling.

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Chapter 2 Review of Literature

2.0 Introduction

Both humans and non-human animals have the ability to produce rhythmic and alternating activation of agonist and antagonist muscle groups, which allows rhythmic motor outputs to be generated. The fundamental rhythmic motor pattern for locomotor-like movements, such as walking, swimming and arm and leg cycling, is partially controlled by neural circuits in the spinal cord, referred to as the central pattern generator (CPG) (Brown, 1911; Grillner, 1981; Zehr, 2005). Research on non-human animals, such as quadrupeds, has shown that rhythmic movement can be evoked without supraspinal input or afferent feedback (Brown, 1911). However, supraspinal input is required to initiate rhythmic movement and to modify ongoing movement in humans (Petersen et al., 2001).

Studies on human subjects typically examine reflex modulation patterns during rhythmic motor outputs as a probe of CPG activity. This research has shown that during rhythmic motor outputs reflex amplitudes are not correlated with background EMG activity and are instead related to the phase of the movement cycle that the reflex was evoked (Zehr & Duysens, 2004). When comparing forward (FWD) and backward (BWD) rhythmic motor outputs researchers have shown that reflex modulation patterns are similar for both movement directions and are not correlated with background EMG, suggesting that the same neural circuits are controlling both movement directions (Duysens, Tax, Murrer, & Dietz, 1996; Zehr & Hundza, 2005; Zehr et al., 2009). For example, arm cycling has been used as a paradigm for understanding CPG activity in humans as research has shown that the neural control of walking and arm and leg cycling are similar (Zehr et al., 2004). Zehr and Hundza (2005) have shown that cutaneous reflex responses (ie. amplitude and sign) are similarly modulated during FWD and BWD arm cycling. Based on

these findings researchers have concluded that the same CPG is controlling both cycling directions.

This technique whereby reflex modulation patterns are used to assess the neural control of rhythmic movement does not provide any information regarding the output of the brain and/or spinal cord during movement. Thus, although research examining reflex modulation patterns in humans and non-human animals has supported the claim that FWD and BWD rhythmic motor outputs are controlled by the same CPG, the contributions from suprapsinal and spinal regions to the generation of FWD and BWD rhythmic movement have yet to be studied in detail. The field of research regarding the modulation of corticospinal excitability during rhythmic motor outputs in humans is growing and has shown that the excitability of the corticospinal tract is modulated differently depending on the task being examined, the phase of the movement cycle, and the intensity of the motor output (Forman, Raj, Button & Power, 2014; Forman et al., 2015; Spence et al., 2016). However, to date corticospinal excitability has not been compared between FWD and BWD rhythmic movement.

In human's corticospinal excitability can be assessed using various non-invasive stimulation techniques. The term 'excitability' is used to represent the responsiveness of the corticospinal pathway. Transcranial magnetic stimulation (TMS) can be used to elicit motor evoked potentials (MEPs) in the target muscle, which provide a measure of the overall excitability of the corticospinal tract. However, MEP amplitudes are influenced by changes at the cortical and spinal level, thus in order to make conclusions about changes in supraspinal and spinal excitability a technique to measure spinal excitability is needed. Transmastoid electrical stimulation (TMES) evokes cervicomedullary evoked potentials (CMEPs) in the target muscle and provides a measure of spinal excitability. When used together, TMS and TMES can provide

a measure of supraspinal and spinal excitability, respectively (Forman et al., 2014; Taylor, 2006). Ung et al., (2005) showed that soleus MEP amplitudes were lower during BWD walking when compared to an intensity- and position- matched tonic contraction. However, this study did not compare CSE between FWD and BWD walking. Previous work in our lab has shown that CSE projecting to the biceps brachii, as assessed by MEP amplitudes, was higher during the flexion phase of FWD arm cycling compared to an intensity-matched tonic contraction (Forman et al., 2014). Also, CMEP amplitudes were not different between cycling and tonic contraction, indicating that the increase in MEP amplitude was due to an increase in supraspinal excitability (Forman et al., 2014).

This review of literature will provide an overview of the neural control of FWD and BWD rhythmic movement in humans and quadrupeds and how corticospinal excitability can be assessed in humans. Firstly, the review will provide a brief overview of the CPG and how CPG activity can be studied in humans. Secondly, the review will discuss the existing literature on the neural control of FWD and BWD rhythmic movement. Finally, this review will discuss pertinent information regarding the stimulation techniques that can be used to assess corticospinal excitability in humans. The primary goal of this review is to examine the current literature regarding the neural control of FWD and BWD movement and on the modulation of corticospinal excitability in humans during voluntary rhythmic movement.

2.1 An Overview of Walking, Arm and Leg Cycling

Humans and non-human animals, such as cats, produce a variety of rhythmic motor outputs such as walking and cycling, which have been shown to be controlled by similar neural mechanisms (Zehr, et al., 2007). Although there are similarities between walking and cycling,

there are also major differences between the motor patterns that should be considered when reviewing the literature.

The first mode of locomotion for humans is crawling, which requires coordinated movement of the arms and legs. As humans age and develop bipedal locomotion emerges where the leg musculature is primarily responsible for moving the body forward and backward. Bipedal locomotion is characterized by the gait cycle which can be divided into two phases, stance and swing. The stance phase, also known as the support phase, is the period of the gait cycle where the foot maintains contact with the ground. This phase makes up approximately 60% of the gait cycle (Kharb, Saini, Jain, & Dhiman, 2011). The stance phase of FWD gait begins with heel strike where the knee is extended, and the hip is flexed as the heel makes contact with the ground. After initial heel contact the individuals body weight gets transferred onto the leg that is positioned anterior to the pelvis, which is referred to as the loading response. After the body weight is transferred the ankle of the support leg will dorsiflex, which allows the center of mass to move anteriorly over the support limb, which is referred to as mid-stance. This is followed by ankle plantarflexion as the heel of the support limb rises off the ground, which is referred to as terminal stance. The swing phase is the period of the gait cycle where the foot is not in contact with the ground. This phase makes up approximately 40% of the gait cycle (Kharb et al., 2011). Pre-swing is characterized by double-limb support, where both feet are in contact with the ground, and during this period the support limb is unloaded, and the individuals body weight gets transferred onto the contralateral limb. This transfer of weight allows the foot to leave the ground, which is followed by hip and knee flexion that will move the foot anteriorly to the stance limb, which is referred to as mid-swing. This is followed by knee extension in preparation for heel contact at the beginning of the stance phase.

The mechanics for BWD gait are slightly different than that of FWD gait (Grasso, Bianchi & Lacquaniti, 1998). Notably, while walking BWD the stance phase begins with toe contact as the hip and knee are extended with the limb positioned posteriorly to the pelvis. This is followed by heel contact, as the individual's body weight gets transferred onto the support limb, and hip flexion to propel the body backwards. The stance phase ends with ankle plantarflexion and toe off, which marks the beginning of the swing phase. Unlike FWD walking where the hip flexes during the swing phase to move the limb FWD, BWD swing consists of hip extension to move the limb posteriorly to the pelvis and ends with toe contact.

As human's progress to bipedal locomotion the arm muscles are still rhythmically active as humans naturally swing their arms during gait, which is important for balance control and postural adaptations (Jackson, Joseph, & Wyard, 1983). Notably, similar muscle activation patterns have been reported for the upper and lower limbs during locomotion (Weiss & St. Pierre, 1983). This discovery prompted researchers to examine the neural control of upper limb muscles during arm cycling, which demonstrated that the CPG is partially responsible for controlling rhythmic arm movement (Zehr, Collins, Frigon, & Hoogenboom, 2003). Thus, arm cycling, which requires rhythmic activation of upper limb muscles, has been used as a paradigm for studying CPG activity. Similarly, leg cycling also requires rhythmic and alternating activation of antagonistic muscle groups and has been used to examine CPG activity in the leg muscles (Ting et al., 1999). These cycling paradigms are advantageous compared to walking for studying the neural control of FWD and BWD movement because they remove any differences in postural control, kinematics and visual input that exist between FWD and BWD walking.

While gait is characterized by the stance and swing phase, cycling can be divided into two functional phases: flexion and extension. Leg cycling phases are characterized based on the

movement of the foot relative in the pelvis (Zehr, Hundza, Balter & Loadman, 2009). Thus, flexion is described as the foot moving toward the pelvis and extension involves the foot moving away from the pelvis. Similarly, arm cycling can also be split into flexion and extension phases based on the movement of the hand (Zehr et al., 2004). The movement cycle can be split into 12 positions that correspond with the positions on a clock. Thus, during FWD cycling the extension phase is characterized by elbow extension and shoulder flexion as the arm crank moves the 9 o'clock to the 3 o'clock position, passing the 12 o'clock position. During BWD cycling the extension phase is characterized by elbow extension and shoulder flexion as the arm crank moves from the 9 o'clock to the 3 o'clock position, passing the 6 o'clock position. For both FWD and BWD cycling the extension phase is predominantly produced by the triceps brachii muscle group. The flexion phase of FWD and BWD cycling is characterized by elbow flexion as the arm crank moves from the 3 o'clock to the 9 o'clock position, passing the 6 o'clock position or the 12 o'clock position, for FWD and BWD cycling respectively. The flexion phase is predominantly produced by the biceps brachii (Forman et al., 2014).

2.2 Central Pattern Generator (CPG)

2.2.1 Central Pattern Generator in Quadrupeds

The basic timing of rhythmic alternating muscle activation required for locomotion is produced by the CPG, which is comprised of interneurons that generate the locomotor rhythm and control the pattern of muscle activation (Zehr, 2005). In 1906 Charles Sherrington determined that rhythmic limb movement could be generated in cats with a spinal transection at the level of the brainstem when electrical stimulation was delivered to the skin (Sherrington, 1906). Therefore, Sherrington concluded that rhythmic motor outputs are controlled at the spinal level without input from the brain and resulted from a series of reflexes (Sherrington, 1996).

Following this discovery Graham Brown determined that rhythmic motor outputs could be elicited in decerebrate cats following transection of afferent nerves (Brown, 1911), thus, suggesting that the control of rhythmic movement is occurring at the spinal level and is not dependent on sensory feedback, which led to the development of the 'half-center' model. This model consisted of two half-centers, one for flexors and one for extensors. Activity in one group of neurons (ie. flexor half-center) would activate flexor motoneurons and simultaneously activate inhibitory interneurons, which would inhibit the extensor half-center. Brown also proposed that fatigue of the flexor half-center would release the extensor half-center from inhibition and allow alternating activation of flexor and extensor motoneuron pools (Brown, 1911).

According to the half-center model the locomotor rhythm and pattern of motoneuron activation are controlled by the same neural circuits (Lafreniere-Roula & McCrea, 2005). Thus, a change in the pattern of muscle activation would alter the step cycle timing and shift the phase of the subsequent step cycle (Rybak, Stecina, Shevtsova, & McCrea, 2006a). However, following an interruption in the pattern of motoneuron activity during fictive locomotion in cats the subsequent timing of the step cycle was unchanged (Rybak et al., 2006a). To account for this a two-level CPG model was proposed with two half-centers: (i) a rhythm generator that sets the timing of muscle activation; (ii) a pattern formation network consisting of interneurons that coordinate the pattern of motoneuron activation required for the locomotor task (Rybak, Shevtosova, Lafreniere-Roula & McCrea, 2006b). In cats it was shown that the rhythm generator networks are located in the mid-lumbar segments of the spinal cord (Rossignol et al., 2002; Langlet, Leblond, Rossignol, 2005). Therefore, afferent feedback can independently alter the level of motoneuron activity without altering step cycle timing.

2.2.2 Central Pattern Generator in Humans

The wealth of knowledge on the function of the CPG has come from research on quadrupeds where direct cellular recordings are possible. In humans, this is not possible as it is not realistic or ethical, so research on non-human animals must be used as a guide for researchers to indirectly measure CPG activity in humans. One way to examine CPG activity is to analyze reflex modulation patterns during rhythmic movement (Zehr & Duysens, 2004). This involves stimulating sensory afferents during rhythmic movement and recording the pattern of modulation of motor output. The presence of phase- and task- dependent reflex modulation patterns have been used to infer activity of the CPG.

2.2.2.1 Task- dependent reflex modulation

Task-dependent reflex modulation emerges when comparing patterns of reflex responses during static contractions and rhythmic movement with matched levels of background EMG. During a static contraction, there is a linear relationship between background EMG and reflex amplitude, however during rhythmic motor outputs reflex amplitudes are uncorrelated with background EMG (Brooke et al., 1997; Komiyama, Zehr, & Stein, 2000). For example, an increase in reflex attenuation occurring simultaneously with an increase in EMG activity suggests that the modulation of reflexes is occurring at a premotoneuronal level, which is not affecting the level of activation of the motoneuron pool (Zehr & Duysens, 2004). The mechanism causing this modulation pattern has been attributed to the presynaptic inhibition of afferent feedback by CPG circuits (Duysens & Van de Crommert, 1998), which are not active during tonic contraction. Pre-synaptic inhibition can occur from primary afferent depolarization where there is an increase in chloride ion flow extracellulary from the primary afferent terminal, which results in a smaller amplitude action potential at the axon terminal (Parnas, Rashkovan,

Ravin & Fischer, 2000). This reduces calcium influx into the axon terminal and consequently decreases neurotransmitter release.

2.2.2.2 Phase- dependent reflex modulation

Phase-dependent reflex modulation means that reflex responses (ie. amplitude and sign) vary depending on the phase of the movement cycle that the reflex was evoked. This modulation pattern serves a functional role to allow afferent feedback to contribute to and modify the output of the CPG. Notably, reflexes are facilitated at appropriate times during the step cycle to allow reflexes to contribute to the output of the CPG and are suppressed when not appropriate to prevent reflexes from impairing the movement pattern (Duysens & Van De Crommert, 1998). This modulation pattern has been attributed to activity in CPG circuits (Zehr & Duysens, 2004). This is supported by research examining reflex modulation patterns between passive and active rhythmic movement. The idea behind this research is that the movement related sensory feedback is the same between the two tasks with the primary difference between the tasks being the activity in CPG circuits during active movement. Studies have shown that during passive cycling of the arms or legs cutaneous evoked reflexes are not modulated relative to the phase of the movement cycle (Carroll et al., 2005; Brooke, et al., 1999). Therefore, if peripheral feedback is similar between the tasks the phase-dependent reflex modulation during active rhythmic movement can be attributed to CPG activity.

2.3 Supraspinal Control of Locomotion

Spinal CPGs produce the fundamental rhythmic motor pattern for walking and other locomotor-like movements in humans and quadrupeds, however supraspinal input is required to initiate, terminate and modulate CPG activity (Jordan et al., 2008; Petersen et al., 2001; Van de Crommert et al., 1998). There are two neuronal structures in the brainstem of vertebrates that are

important for initiating movement; the mesencephalic locomotor region (MLR) and the reticular formation (RF). Supraspinal input from these centers and other areas, such as the primary motor cortex, is transferred through multiple descending pathways, such as the corticospinal, recticulospinal, vestibulospinal and rubrospinal tracts, which link the brain to the spinal cord to control voluntary movement (Jordan et al., 2008).

2.3.1 Mesencephalic Locomotor Region (MLR)

The MLR is an area of the caudal midbrain in the brainstem that contains the cuniform nucleus and the pedunculopontine tegmental nucleus (Takakusaki, 2017). When this area of the brainstem is stimulated it evokes locomotion in quadrupeds (Shik, Severin, & Orlovskii,1966; Le Ray, Juvin, Ryczko, & Dubuc, 2011). The neurons in the MLR will integrate input from higher brain structures and project to and activate reticulospinal neurons in the reticular formation, which relay input from the MLR and will in turn activate the CPG (Le Ray et al., 2011). The MLR is comprised mainly of excitatory neurons and the pathway from the MLR to the CPG is mediated mainly by serotonin (5-HT) and glutamate neurotransmitters. This is supported by research that has shown that glutamate antagonists alter MLR induced locomotion in quadrupeds and glutamate agonists induce locomotor-like activity in the isolated spinal cord of neonatal rats (Cazalets, Sqalli-Houssaini, & Clarac, 1992; Zaporozhets et al., 2006). Also, serotonin receptor antagonists alter MLR-evoked locomotion in neonatal rats (Jordan & Schmidt, 2002), whereas serotonin receptor agonists induce rhythmic activity in the isolated spinal cord of neonatal rats (Feraboli-Lohnherr, Barthe, & Orsal, 1999). It has also been shown that the effect of MLR stimulation on CPG activity is dependent upon the intensity of the stimulation. Notably, as the stimulation intensity was increased the duration of the step- cycle decreased, causing the cat to progress from slow walking to galloping (Shik et al., 1966). Also, when stimulation stopped

locomotion was also stopped, suggesting that the MLR is important for initiating and terminating locomotion (Shik et al., 1999).

2.3.2 Reticulospinal Tract (RST)

The reticulospinal tract (RST) originates in the reticular formation (RF) and has two tracts, the lateral tract arises from the medulla and the medial tract arises from the pons (Siegal & Sapru, 2006). The RF is a group of neurons that are located within the brain stem and are responsible for integrating descending motor commands from higher brain areas, such as the motor cortex and the MLR. The medial RST descends the spinal cord and has both direct and indirect excitatory and inhibitory synaptic contacts with alpha- and gamma- spinal motoneurons to control voluntary movement (Siegal & Sapru, 2006). Specifically, the medial RF reticulospinal neurons are crucial for mediating input from the MLR, cerebellum and subthalamic locomotor region of the lateral hypothalamus (Noga et al., 2003; Sinnamon & Stopford, 1987) and inducing locomotor activity in decerebrate cats (Shik et al., 1966), rats (Skinner & Garcia- Rill, 1984) and other non-human animals. The axons of these neurons descend in the ventrolateral funiculus (Steeves & Jordan, 1980) and are phasically active during locomotion in quadrupeds (Drew, Dubuc, & Rossignol, 1986) and in MLR- evoked fictive locomotion in decerebrate cats (Perreault, Drew, & Rossignol, 1993). This phasic pattern of activation means that reticulospinal neurons modify activity in flexor and extensor motoneuron pools depending on the phase of the step cycle (Drew, Prentice, & Schepens, 2004). Thus, the RST is involved in the activation of the CPG and in adapting the locomotor pattern to the external environment.

2.3.4 Vestibulospinal Tract (VST)

The VST consists of a lateral and medial pathway, one originates from the lateral vestibular nucleus (LVN) and one from the medial vestibular nucleus (MVN), respectively (Siegel & Sapru, 2006). The medial VST innervates spinal motoneurons at the cervical level and is important for rotating and lifting the head (Siegel & Sapru, 2006). The lateral VST projects to all levels of the spinal cord and is important for altering muscle tone in extensor muscles, which is important for maintaining an upright posture (Matsuyama & Drew, 2000a). Notably, stimulation of the LVN during the stance phase of walking in cats enhances activity in extensor muscles, while it has minimal effect on extensor muscles when the stimulation is delivered during the swing phase as flexor muscles are active (Orlovsky, 1972a). Also, the firing frequency of vestibulospinal neurons is modulated relative to the phase of the step cycle, suggesting that the VST is involved in the control of locomotion (Matsuyama & Drew, 2000a). In support of this claim, studies have shown that bilateral lesions to the LVN results in a loss of muscle tone in extensor muscles during locomotion and altered coordination between limbs (Gorska, Bem & Majczynski, 1990; Yu & Eidelberg, 1981). The reticulo- and vestibulospinal neurons have been examined during locomotion in intact cats while walking FWD on an inclined plane (Matsuyama & Drew, 2000b). This research revealed that there is an increase in the firing frequency of VST neurons when walking on an incline compared to level ground, but no changes in the pattern of firing. Thus, suggesting that VST neurons are primarily involved in controlling the overall level of activation in postural muscles that was required to adapt the locomotor pattern to walk on the incline plane (Matsuyama & Drew, 2000b). In contrast, although the RST neurons also showed an increase in activity while walking on an incline plane, this occurred simultaneously with a

change in the pattern of discharge of the RST neurons (Matsuyama & Drew, 2000b). Thus, suggesting that RST neurons are also important for determining the level of activation of different muscles that is required to adapt the locomotor pattern (Matsuyama & Drew, 2000b). Thus, the VST and RST work together to adapt the locomotor pattern and to enable coordinated movement.

2.3.5 Rubrospinal Tract

The rubrospinal tract emerges from the red nucleus of the midbrain and crosses over at the ventral tegmental decussation and descends the spinal cord (Siegal & Sapru, 2006). This tract is well-developed in non-human animals, such as cats, as its fibers descend to both the cervical and lumbar spinal cord (ten Donkelaar, 1988). However, in humans this tract projects mainly to spinal motoneurons in the cervical segments of the spinal cord (Nathan & Smith, 1982). The primary function of this tract is to facilitate activation of flexor muscles as it has been shown that stimulation of the red nucleus increases activity in flexor motoneurons (Rho, Lavoie, & Drew, 1999). Also, during locomotion in decerebrate cats the activity of rubrospinal neurons is modulated relative to the phase of the step cycle and show maximal activation during the swing phase when the flexor muscles are active, suggesting that the rubrospinal tract is involved in the control of locomotion (Orlovsky, 1972b). This is supported by research showing that lesions to the red nucleus in cats leads to deficits in locomotor control during overground walking with greater deficits seen when walking over obstacles (Ingram & Ranson, 1932). Similarly, the firing frequency of rubrospinal neurons increases when cats modify their gait to move over obstacles (Lavoie & Drew, 2002). Thus, in addition to its role in producing the locomotor pattern in cats, it is also important for modifying the locomotor pattern to adapt to the external environment.

2.3.6 Corticospinal Tract (CST)

The corticospinal tract (CST) is one of the major descending pathways that controls voluntary movement in humans, and therefore researchers study the function of this tract to further understand voluntary motor control in humans. This tract originates from several different areas of the cortex. Approximately 60% of the descending fibers arise from the primary motor cortex, supplemental motor area and pre-motor cortex and the remaining 40% arise from primary somatosensory cortex (Siegal & Sapru, 2006). The primary motor cortex, where approximately 30% of the corticospinal projections arise, is made up of six different layers (Barbas & Garcia-Cabezas, 2015). Layer 5 is the most prominent in the primary motor cortex as it gives rise to the CST with originates from large pyramidal neurons (also known as Betz cells or upper motoneurons) (Barbas & Garcia-Cabezas, 2015). These upper motoneurons will project to and activate motoneurons in the spinal cord, referred to as lower motoneurons, which innervate muscles and cause movement. The connections between the upper and lower motoneurons can be direct or indirect. A direct connection is referred to as monosynaptic as there is only one synapse between the upper and lower motoneurons. An indirect connection is referred to a di- or polysynaptic as the upper motoneuron synapses with one or more interneurons which will then synapse onto the lower motoneuron. Regardless of whether the connection is direct or indirect voluntary motor output is generated as a result of the passage of descending commands from the upper to the lower motoneurons.

At the medulla a majority of the descending fibers cross the midline at the pyramidal decussation to form the lateral CST, the remaining fibers do not decussate and form the anterior CST (Snell, 2010). The anterior CST synapses with lower motoneurons in the medial aspect of the ventral horn of the cervical and upper thoracic region, whereas the lateral CST synapses with

lower motoneurons in all spinal cord segments (Snell, 2010). Also, studies have shown that fibers originating from the somatosensory cortex project to the dorsal horn of the spinal cord to modulate the sensory feedback to the cerebral cortex (Siegal & Sapru, 2006). Thus, the lateral CST controls fine movement of the extremities whereas the anterior CST controls muscles in the shoulder, neck and trunk (Siegal & Sapru, 2006). To allow for controlled movement the lateral and anterior CST can relay both excitatory and inhibitory input to lower motoneurons (Welniarz, Dusart & Roze, 2017).

With respect to locomotion, research has shown that CST neurons are active during the gait cycle and their firing frequency is rhythmically modulated based on the phase of the movement cycle (Armstrong & Drew, 1984). Also, the activity of CST neurons in quadrupeds increases when walking over an obstacle and when walking on uneven ground, suggesting that the CST is important for modifying the locomotor pattern to adapt to the external environment (Beloozerova & Sirota, 1993; Drew, 1988). In humans, studies using transcranial magnetic stimulation (TMS) have shown that when TMS is used to inhibit motor cortical neurons during walking, referred to as intracortical inhibition, there is a suppression of ongoing EMG activity (Petersen et al., 2012; Nielsen, 2003). Thus, suggesting that the neurons projecting from the motor cortex through the CST contribute to the level of muscle activation that is required for locomotion.

2.4 Neural Control of Forward and Backward Walking

Humans and animals can naturally change their direction of walking (ie. from FWD to BWD), however the extent to which different directions of locomotion are controlled by the same neural circuits remains unclear. As discussed previously, researchers can examine reflex modulation patterns during rhythmic motor outputs to infer CPG activity. Thus, if the same CPG

circuits were controlling different directions of rhythmic motor outputs (ie. walking or cycling) there would be evidence of similar patterns of reflex modulation and muscle activation patterns between the two tasks (Zehr et al., 2007).

2.4.1 Forward and Backward Walking in Cats

2.4.1.1 Cutaneous reflex modulation patterns

Research on non-human animals has shown that the same neural circuits (CPG) controlling FWD locomotion operate in reverse to control BWD locomotion (Pearson, 1993; Buford & Smith, 1990; Buford, Zernicke, & Smith, 1990; Buford & Smith, 1993). Buford and Smith (1993) examined muscle activation patterns and joint movement in response to perturbations elicited by electrical and mechanical stimulation of cutaneous nerves in the paw of cats. When a dorsal tap was applied to the paw during FWD swing the initial response included knee flexion, hip flexion and plantarflexion, which allowed the paw to move up and over the obstacle (Buford & Smith, 1993). During the swing phase of BWD walking the initial response to a ventral tap included hip and ankle flexion, which drew the paw FWD and away from the ventral obstacle (Buford & Smith, 1993). These responses reflect the "stumble corrective reaction", which results from the transfer of afferent feedback though neural circuits within the CPG and allows the locomotor pattern to be adapted when the limb comes in contact with an unpredicted obstacle (Forssberg, 1979). Thus, due to the role of the CPG in the modulation of motion-related afferent feedback and in the modification of gait, the stumbling corrective reaction can used to examine the neural control of locomotion. Although there were differences in the corrective reaction between FWD and BWD walking in response to the mechanical stimulus, the responses evoked from electrical stimulation delivered at the same location (the foot dorsum) were similar for both forms of walking with only subtle differences (Buford &

Smith, 1993). The subtle differences consisted of a slight shift in the timing of the reflex responses and a slight difference in the amplitude of the evoked responses (Buford and Smith, 1993). The researchers suggested that the differences observed between FWD and BWD walking following mechanical stimulation could be due to the differences in proprioceptive feedback between walking directions and the fact that the stimulus was applied at different locations on the foot (Buford & Smith, 1993). Despite these differences, the researchers concluded that the adaptive responses result from the modulation of afferent feedback by the same CPG for FWD and BWD walking. Thus, these studies support the idea that the same neural control mechanisms for FWD walking are reversed during BWD walking.

2.4.1.2 Muscle activation patterns, hindlimb kinematics and postural adaptations

Muscle activation patterns, hindlimb kinematics and postural adaptations have been compared between FWD and BWD walking in cats. Buford and Smith (1990) examined locomotion in intact cats and determined that cats can readily alter their pattern of locomotion to produce BWD walking. Their research has shown that hindlimb muscle synergies for FWD and BWD walking are similar, with flexor muscles active during swing and extensor muscles active during stance (Buford & Smith, 1990; Perell, Gregor, Buford, & Smith, 1993). However, the timing and recruitment patterns were different for certain muscles, which was attributed to differences in motion-related sensory feedback (ie., input from muscle spindles and Golgi tendon organs) and differences in supraspinal input during BWD walking (Buford & Smith, 1990; Pratt, Buford & Smith, 1996).

Hindlimb kinematics and postural adaptations have been analyzed for FWD and BWD walking in cats. This research revealed slight differences in the postural adaptations required to walk BWD compared to FWD (Buford et al., 1990). Notably, to propel the body BWD the

lumbar spine flexed which allowed the hindlimbs to move posteriorly to the pelvis, this is in contrast to FWD walking as the spine was straight during gait (Buford et al., 1990). During the swing and stance phase of FWD and BWD walking the hip, knee and ankle joints were flexed and extended, but at different times in the step cycle depending the direction of walking (Buford et al., 1990). Notably, during BWD walking the swing phase consisted of hip extension and knee flexion, whereas during FWD walking the hip flexed and the knee extended (Buford et al., 1990). Also, knee extension during the stance phase of BWD walking contributed the most to the BWD propulsion of the body, whereas hip extension during the stance phase of FWD walking was the major contributor to the FWD movement of the body (Buford et al., 1990). A common finding for both FWD and BWD walking was that the movement at the knee and ankle joint were coupled but were not related to the movement at the hip joint. This means that knee extension and flexion occurred simultaneously with ankle extension and flexion, respectively, for FWD and BWD walking (Buford et al, 1990). The researchers concluded that the similar kinematics and the similar muscle activation patterns for FWD and BWD walking suggest that the same neural circuits control both walking directions.

2.4.1.3 Location of spinal networks generating FWD and BWD locomotion

Electrical epidural stimulation (ES) of the spinal cord stimulates sensory fibers in the dorsal roots which results in activation of the spinal networks controlling locomotion, the CPG (Capogrosso et al., 2013; Barthelemy, Leblond, & Rossignol, 2007; Courtine et al., 2009). Thus, ES can be used to identify the segments of the spinal cord that contain the spinal neuronal networks responsible for generating FWD and BWD locomotion. Using this technique in cats it is has been shown that the networks controlling FWD locomotion reside in the lumbosacral spinal cord from L3 to S2 (Merkulyeva et al., 2018). However, BWD locomotion could only be

evoked when ES was applied to the L6 and L7 spinal segments, suggesting that the neuronal networks controlling BWD locomotion are located in these segments (Merkulyeva et al., 2018). To gain further insight into the distribution of neuronal networks controlling FWD and BWD locomotion c-*fos* immunostaining can be used to reveal the location of spinal neurons active during locomotion (Carr et al., 1995; Dai et al., 2005). *C-fos* is an immediate early gene which is activated in response to various stimuli, such as stimulation of the mesencephalic locomotor region (MLR) which evokes fictive locomotion in cats (Huang et al., 2000). C-*fos* labelling occurs in specific locations of the spinal cord during locomotion and does not require sensory feedback from moving limbs as it is evident in fictive preparations (Noga, Douglas, & Jordan, 2005). Thus, c-*fo*s immunohistochemistry can be used to detect the activity of neurons that are part of the CPG during locomotor outputs (Ahn et al., 2006). It has been shown that the number of c-*fos*-positive (FOS +; active neurons) interneurons in the grey area of the L6 and L7 spinal segments of cats during BWD walking was significantly higher than during FWD walking (Merkulyeva et al., 2018). Thus, researchers suggested that the higher number of active neurons during BWD walking reflects the activation of a neuronal network that generates BWD walking that is located within the L5 to L7 spinal segments (Merkulyeva et al., 2018). Also, when ES was applied to the L6 segment both FWD and BWD locomotion could be evoked (Merkulyeva et al., 2018). Therefore, FWD locomotion can be evoked in any segment between L3 and S2, whereas BWD locomotion can only be evoked when ES is applied to L6 and L7, which could suggest that there are separate networks controlling both movement directions.

2.4.1.4 Activity of motor cortex neurons during FWD and BWD walking

As previously mentioned, the basic rhythmic motor pattern required for locomotion is produced by the CPG, however input from supraspinal centers is important for activating the

CPG and modifying ongoing movement. Notably, research in cats has shown that during both FWD and BWD locomotion the activity of motor cortex neurons, also referred to as pyramidal tract neurons, is modulated relative to the phase of the step cycle (Armstrong & Drew, 1984; Beloozerova & Sirota, 1985; Zelenin et al., 2011a; Zelenin et al., 2011b). Thus, suggesting that the motor cortex is involved in the production and control of both directions of walking. Also, when the mean level of activity (f_M) of motor cortex neurons was compared between FWD and BWD walking there was no significant difference between the two movement directions (Zelenin et al., 2011a). Thus, suggesting that BWD walking does not require greater overall cortical activity compared to FWD walking. However, the coefficient of modulation (K_{mod}), which is used to assess the degree of modulation of motor cortex neurons, was compared between FWD and BWD walking in cats and on average the neurons controlling the hindlimbs were modulated to a greater degree during BWD walking compared to FWD walking (Zelenin et al., 2011a). The researchers concluded that there is greater cortical involvement during BWD walking.

The activity of motor cortex neurons can be modulated by motion-related sensory feedback (ie. proprioceptive feedback) (Orlovsky, Deliagina, & Grillner, 1999). Thus, differences in the modulation of motor cortex neurons during BWD walking could be due to changes in afferent feedback during BWD movement. BWD locomotion in cats requires the hindlimbs to lead to propel the body backwards and the forelimbs to trail, which is opposite to that seen during FWD locomotion (Eliam & Shefer, 1992). Research has shown that there are slight differences in hindlimb kinematics during BWD walking and differences in the timing and recruitment of muscles as the hindlimb muscles must function to propel the body backwards (Buford, Zernicke, & Smith, 1990; Buford & Smith, 1990; Eila & Shefer, 1992). These factors could cause differences in motion -related proprioceptive feedback during BWD walking when

compared to FWD walking, which could result in differences in the modulation of motor cortex neurons.

2.4.2 Forward and Backward Walking in Adult Humans

2.4.2.1 Cutaneous reflex modulation patterns

Researchers have suggested that the same neural mechanisms control FWD and BWD locomotion in humans, which is compatible with the claim made in animals (Duysens, Tax, Murrer, & Dietz, 1996; Grasso et al, 1998; Lamb & Yang, 2000; Thorstensson, 1986). To test this theory Duysens et al., (1996) examined cutaneous reflex modulation following stimulation of the sural nerve during FWD and BWD treadmill walking in adult human subjects. During the stance and swing phase of BWD walking there was a slight shift in the timing of reflex suppression and timing of reflex reversal for the tibialis anterior (TA) muscle during the step cycle (Duysens et al., 1996). During FWD walking the largest facilitatory reflex responses in TA were seen during the early swing phase and the largest suppressive responses were seen at the end of the swing phase (Duysens et al., 1996). Notably, large facilitatory reflex responses were seen in several lower limb flexor muscles prior to the beginning of the swing phase, suggesting that there is a facilitation of interneurons that mediate feedback from flexor reflex afferents to assist with knee and ankle flexion (Duysens et al., 1996). The reflex reversal that occurred at the transition from the swing to the stance phase in TA has been widely reported (Yang & Stein, 1990), however it occurs at a different time in the step cycle during BWD walking. The largest facilitatory TA responses during BWD walking occurred during the second half of the swing phase as the ankle was dorsiflexed and suppressive responses occurred during late stance as the soleus was active to push off the ground (Duysens et al., 1996). Although the timing of the facilitatory and suppressive cutaneous reflex responses for all muscles were shifted in the BWD step cycle, the reflex responses (ie. amplitude and sign) were modulated relative to

the phase of step cycle and were uncorrelated with background EMG activity (Duysens et al., 1996). This means that the afferent feedback from stimulation of the cutaneous nerve is being modulated independently from the level of activity in the motoneuron pool, which has been attributed to the presynaptic gating of afferent feedback by the CPG (Duysens & Van de Crommert, 1998). This suggests that the CPG mediating afferent feedback during FWD walking may be operating in reverse to control BWD walking.

2.4.2.2 Muscle activation patterns

A feature of locomotion is the presence of rhythmic and alternating EMG activity characterized by alternating activation of agonist and antagonist muscles. Grasso, et al., (1998) examined muscle activation patterns during overground walking and reported a poor relationship between FWD and BWD walking with respect to EMG patterns and muscle synergies (the temporal sequence of activation of agonist and antagonist muscles). Notably, biceps femoris was reciprocally activated with vastus lateralis and rectus femoris during BWD gait but co-activated with vastus lateralis and rectus femoris during FWD gait (Grasso et al., 1998). However, substantial intersubject variability in EMG patterns during the same movement direction was reported, which could be indicative of error in the EMG measurement, the inherent variability that exists in EMG recordings, and the variability that could exist between subjects. Also, participants were instructed to walk with their arms folded across their chest, which could disrupt balance as the natural arm swing during walking is important for postural adaptations and balance control (Meyns, Bruijn, & Duysens, 2013). Thus, walking with the arms folded could have altered muscle activation patterns. Thorstensson (1986) indicated that muscle activation patterns change during BWD treadmill walking but show similarities to FWD walking when the step cycles are superimposed (ie., heel-strike during FWD matched to heel-off during BWD).

During both directions of walking the hamstring and gluteus maximus muscles were active at the transition from hip flexion to extension (Thorstensson, 1986). Also, at the ankle joint there was reciprocal activation of TA and soleus during both directions of walking (Thorstensson, 1986). However, during FWD walking the level of lateral gastrocnemius activation was higher during stance, whereas the level of TA activation was higher during stance of BWD walking (Thorstensson, 1986). A commonality among these studies is that the magnitude of integrated EMG across a single gait cycle was higher during BWD gait (Grasso et al., 1998; Thorstensson, 1986; Winter, Pluck, & Yang, 1989).

These differences in muscle activation could be due to differences in sensory feedback during BWD walking. For example, during BWD gait, participants cannot see the placement of their foot, which has been shown to result in an "anticipatory H-reflex" in the soleus muscle as participants anticipate foot fall (Capaday & Stein, 1986). This means that during BWD walking, compared to FWD walking, the soleus H-reflex has an earlier onset during the BWD gait cycle (ie. during mid swing phase of BWD walking and during the stance phase of FWD walking) and is higher in amplitude (Capaday & Stein, 1986). Also, during BWD gait the muscles are likely functioning differently as they have to propel the body in the BWD direction, whereas during FWD walking the motion is described as "controlled falling". This could result in differences in feedback from muscle spindles and Golgi tendon organs, which could alter muscle activation patterns and EMG amplitude (Gordan, Ghilardi, & Chez, 1995; Rossingnol, 1996; Bent, McFadyen, & Inglis, 2002).

2.4.3 Forward and Backward Walking in Infants

In adult humans descending supraspinal input can modify the output of the CPG (Nielsen, 2003). To account for this, human infants have been used to study the CPG as indirect

evidence has shown that stepping movements are largely controlled by the brainstem as the connections from the motor cortex to spinal motoneurons are not fully developed (Forssberg, 1985; Muller, Homberg, & Lenard, 1991). Lamb and Yang (2000) examined EMG patterns when human infants (2-11 months) were supported over a treadmill moving at a constant speed which elicited the FWD and BWD stepping reaction. The EMG bursts from lower limb muscles occurred at the same phase of the step cycle regardless of the direction of walking, except for the EMG burst of the hamstring muscle which occurred earlier and with a greater amplitude in the swing phase during BWD walking to extend the hip and flex the knee (Lamb & Yang, 2000). Due to the similarity in muscle activation patterns during both directions of walking they concluded that the same CPG controls both FWD and BWD walking (Lamb & Yang, 2000). Although this observation is compatible with the conclusion from Duysens, et al, (1996) who examined phase-dependent reflex modulation in adult humans, it is unknown if EMG activity alone can indirectly reveal the presence of a CPG. However, it was reported that the infants responded to changes in treadmill speed by altering the duration of the swing and stance phase and step cycle duration in a similar manner during both FWD and BWD walking (Lamb & Yang, 2000). According to the two-level CPG model these findings suggest that the same CPG controls both directions of walking as the pattern formation network controls the pattern of motoneuron activation while the rhythm generator controls step cycle timing.

2.5 Neural Control of Forward and Backward Cycling

To control for the kinematic differences (ie. ankle position) and differences in sensory feedback (ie. postural adjustments and visual input) between FWD and BWD walking, a cycling paradigm was introduced. The pattern of phase-dependent cutaneous reflex modulation during arm and leg cycling is similar to that observed during walking and reflex responses during

cycling are uncorrelated with background EMG activity (Ting, Kautz, Brown & Zajac, 1999; Zehr et al., 2001; Zehr et al., 2004). Therefore, both arm and leg cycling have been used to examine CPG activity.

2.5.1 Cutaneous Reflex Modulation Patterns During Leg Cycling

Zehr et al., (2009), examined cutaneous reflex modulation during FWD and BWD leg cycling and reported that reflex responses (ie. amplitude and sign) were dependent on the functional phase of the movement cycle, rather than being correlated with background EMG activity. The "flexion" phase was described as the foot moving toward the trunk and "extension" phase involved the foot moving away from the trunk. This modulation pattern suggests the presynaptic gating of afferent input to the motoneuron pool by the CPG (Zehr et al., 2009). During the mid-flexion phase of FWD cycling, corresponding to the swing phase of walking, stimulation of the tibial nerve resulted in TA facilitation (Zehr et al., 2009). This corresponds with the reflex responses seen during the swing phase of walking as the flexor reflex afferent pathways are facilitated to assist with keeping the toes off the ground (Zehr et al., 1997; Duysens, 1977). During the same phase of BWD leg cycling stimulation of the superficial peroneal nerve on the dorsum of the foot resulted in a suppressive reflex response in TA (Zehr et al., 2009). This reflex response is functionally useful for adapting to external stimuli as it allows the dorsum of the foot to move away from a simulated obstacle, thus reflecting the role of the stumbling corrective response that is seen during walking (Zehr et al., 1997). These observations are suggestive of CPG activity for FWD and BWD leg cycling and support the hypothesis that the same CPG can produce both directions of locomotor-like movements.

2.5.2 Cutaneous Reflex Modulation Patterns During Arm Cycling

The hypothesis that FWD and BWD rhythmic movement are controlled by the same neural circuits has also been examined during arm cycling. Zehr and Hundza (2005) examined cutaneous reflex modulation following stimulation of the superficial radial nerve during FWD and BWD arm cycling. In contrast to Zehr et al., (2009), similar amplitude excitatory and inhibitory early- (~50- 80 ms) and middle- (~ 80-120 ms) latency reflex responses were reported in the upper limb muscles at the same hand position (relative to the clock face), rather than the functional phase, during the movement cycle for both cycling directions (Zehr & Hundza, 2005). Early-latency reflex responses (~50- 80 ms) have been attributed to activity in the Ia monosynaptic pathway, whereas middle-latency (~ 80-120 ms) reflex responses are mediated by slowly conducting group II afferents (Corna, Grasso, Nardone, & Schieppati, 1995). At the 6 o'clock position during both FWD and BWD arm cycling the reflex responses in the biceps brachii were excitatory as the elbow was in flexion (Zehr & Hundza, 2005). At the 12 and 11 o'clock position during FWD and BWD cycling, respectively, the middle-latency reflex response in the biceps brachii switched to inhibitory as the triceps brachii muscle reached peak activation (Zehr & Hundza, 2005). Also, there was evidence of a reciprocal pattern of reflex responses in the anterior and posterior deltoid (Zehr & Hundza, 2005). This was evidenced by coupling of excitatory reflex responses in posterior deltoid with inhibitory responses in anterior deltoid from the 3 to 9 o'clock position during both directions of cycling (Zehr & Hundza, 2005). Importantly, during arm cycling reflex amplitudes were uncoupled from background EMG suggesting the premotneuronal gating of afferent input to the motoneuron pool by the CPG (Zehr et al., 2009; Zehr & Hundza, 2005). The similar pattern of phase-dependent reflex modulation during both FWD and BWD cycling is indicative of the control of afferent feedback by the same CPG.

2.5.3 Muscle Activation Patterns during Arm and Leg Cycling

The EMG activity of the arm and leg musculature during arm and leg cycling, respectively, is rhythmically modulated during the movement cycle (Brooke et al., 1997; Ting et al., 1999; Zehr et al., 2004). During FWD and BWD leg cycling the pattern of EMG amplitudes for ipsilateral and contralateral TA, biceps femoris, vastus lateralis and medial gastrocnemius was similar for both directions of cycling relative to the functional phase (ie. flexion and extension) of the movement cycle (Zehr et al., 2009; Ting, et al., 1999). Similarly, during FWD and BWD arm cycling the pattern of EMG activity in the biceps brachii, anterior and posterior deltoid and flexor carpi radialis were similarly modulated when analyzed relative to the arm crank position during the movement cycle (Zehr & Hundza, 2005). However, during BWD arm cycling EMG amplitudes were significantly higher in biceps and triceps brachii at the 3 o'clock and 6 o'clock position, respectively (Zehr & Hundza, 2005). A commonality among these studies is that background EMG amplitudes were higher during BWD cycling for a majority of the muscles analyzed, which is the same finding that was reported for BWD walking (Zehr & Hundza, 2005; Zehr et al., 2009; Thorstensson, 1986; Duysens et al., 1996). The increase in muscle activation during BWD leg cycling was attributed to differences in the function of the lower limb muscles with the reversal of cycling direction (Eisner et al., 1999; Ting et al., 1999). Notably, during leg cycling it has been shown that EMG activity of muscles involved in the movement of the foot anteriorly and posteriorly to the pelvis (ie. hamstring and quadriceps muscle groups) is affected by cycling direction (Ting et al., 1999). Specifically, EMG amplitude is significantly higher for biceps femoris during BWD leg cycling and the timing of activation is delayed compared to FWD cycling (Ting et al., 1999). There could also be differences in muscle function, biomechanics and sensory feedback (ie. input from muscle spindles and Golgi tendon

organs) between FWD and BWD arm cycling that could explain the increase in EMG amplitude during BWD arm cycling, however this has not yet been evaluated.

2.6 Techniques Used to Assess Corticospinal Excitability

During tonic and rhythmic motor outputs the excitability of the CST can be modulated. The term *excitability* is used in this context to mean the responsiveness of the CST. The responsiveness of CST, termed corticospinal excitability, can be influenced by changes in the intrinsic properties of spinal motoneurons, interneurons and cortical neurons and it can also be affected by changes in suprasapinal input and sensory feedback. In humans, researchers have examined task-dependent changes in corticospinal excitability using several non-invasive stimulation techniques. The following techniques will be examined in this review: transcranial magnetic stimulation (TMS), transcranial electrical stimulation (TES), and transmastoid electrical stimulation (TMES).

2.6.1 Transcranial Magnetic Stimulation (TMS)

TMS is a non-invasive technique that is used to stimulate corticospinal axons, both directly and indirectly, to assess corticospinal excitability in humans. The TMS device is a capacitor that discharges a large electrical current through the TMS coil. When the TMS coil is placed above the scalp the electrical current will generate a magnetic field perpendicular to the coil and the magnetic field will then evoke a weak electrical current perpendicular to the magnetic field that will stimulate neural tissue in the cortex (Rossini et al., 2015). The direction of the current flow will preferentially activate the left or right motor cortex depending on the orientation of the TMS coil (Martin, Gandevia, & Taylor, 2006).

TMS stimulates corticospinal axons either directly to produce D-waves (direct waves) or transsynaptically to generate I-waves (indirect waves) (Di Lazzaro et al., 2004a). I-waves have a longer latency than D-waves (~ 1- 1.5 ms) and are generated when the electrical current activates

interneurons which will activate pyramidal neurons via a synapse (Terao et al., 2000). The latency of a MEP represents the transmission time from stimulation artifact to the recording of the response in the target muscle. I-waves are generated when the stimulation intensity is around threshold (Rossini et al., 2015). Threshold can be measured during rest (resting motor threshold) or during voluntary contraction (active motor threshold). Resting motor threshold is defined as the lowest stimulation intensity required to produce MEPs with a peak-to-peak amplitude ≥ 50 μV 50% of the time (ie. 4 out of 8 trials) (Rossini, 1990). Active motor threshold is defined as the lowest stimulation intensity required to produce MEPs with an amplitude ≥ 200 μV 50% of the time and are clearly discernible from background EMG (Rossini, 1990). D-waves result from the direct stimulation of pyramidal tract axons in the subcortical white matter or at the initial segment of the neuron and are elicited when a high stimulation intensity is used, which is much higher than threshold (Day et al., 1989; Di Lazzaro et al., 2004b). Both D-waves and I-waves will generate either excitatory (EPSP) or inhibitory (IPSP) post-synaptic potentials in the motoneuron. The EPSP's and IPSP's will summate and if resting membrane potential reaches threshold for action potential generation a response will be evoked in the target muscle, referred to as a motor evoked potential (MEP). The amplitude of the MEP represents the overall excitability of the corticospinal pathway as it is influenced by changes in cortical, spinal and peripheral excitability (Taylor & Gandevia, 2004). Thus, by measuring MEP amplitude it is not possible to distinguish between changes in excitability at the spinal or supraspinal level. Therefore, a technique to measure spinal excitability is often used in combination with TMS to allow researchers to determine if the change in corticospinal excitability is occurring at the supraspinal or spinal level.

2.6.2 Transcranial Electrical Stimulation (TES)

In contrast to TMS, TES is applied using a pair of surface electrodes in a bipolar arrangement with the anode placed at the vertex and the cathode placed on one side, 7 cm lateral to the vertex (Rothwell et al., 1994). When a pulse is delivered the motor cortex in the area of the anode will be stimulated and responses will be recorded on the opposite side of the body (Rossini et al., 2015). TES activates corticospinal axons directly in the subcortical white matter, resulting in descending D-waves (Rossini et al., 2015). However, with increasing stimulation intensity the stimulation delivered by TES can activate interneurons, which will transynaptically activate the same pyramidal neurons to produce I-waves (Rossini et al., 2015). This is evidenced in the recordings of descending volleys in studies using electrodes placed in the epidural space of the spinal cord (Kernell & Chien-Ping, 1967). It was shown that with an increase in stimulation intensity there was an increase in the amplitude of D-waves which were followed by a series of reoccurring smaller amplitude waves with an interval of 1-2 ms, referred to as I-waves (I_1, I_2, I_3) (Kernell & Chien-Ping, 1967). A disadvantage with this technique compared to TMS is the transient discomfort that is felt with high-voltage electrical stimulation (Rossini et al., 2015).

2.6.3 Transmastoid Electrical Stimulation (TMES)

TMES is a non-invasive stimulation technique that is used to assess spinal excitability in humans. This technique causes an electrical stimulation to be passed between a pair of surface electrodes that are placed near the mastoid processes at the level of the cervicomedullary junction near the pyramidal decussation (Taylor, 2006). At this site the axons of upper motoneurons bend, which makes them easier to activate (Maccabee, Amassian, Eberle, & Cracco, 1993). Stimulation at this location results in a single descending volley in corticospinal axons with a latency between 7.5 and 8 msec, which accounts for the synaptic transmission time between the descending axons and the spinal motoneuron. The single descending volley was

confirmed using collision techniques where a supramaximal stimulation to the ulnar nerve at an appropriate interstimulus interval (ISI) produced an antidromic volley that completely occluded the CMEP in the abductor digiti minimi (Berardelli, Inghilleri, Rothwell, Cruccu, & Manfredi, 1991). This is in contrast to the results seen when a peripheral stimulation was delivered after TMS as the peripheral stimulation did not completely occlude the MEP (Hess et al., 1987). This is because TMS results in multiple descending volleys, termed I-waves. Also, occlusion studies have also shown that TMES and TMS produce descending volleys in the same axons. When a TMS stimulus is delivered at an ISI following TMES the antidromic volley almost completely cancels the response to TMS (Taylor, Petersen, Butler, & Gandevia, 2001). Therefore, TMES can be used in conjunction with TMS to determine if changes in MEP amplitude are occurring at the spinal or supraspinal level (Forman et al., 2014). To make conclusions about supraspinal excitability MEP amplitudes can be normalized to CMEP amplitudes to remove the influence of spinal excitability on MEP amplitudes (Forman et al., 2014). For example, if there is an increase in MEP amplitude during or following an intervention but there is no change in CMEP amplitude the change is likely occurring at the supraspinal level.

There are two advantages to this technique that make it a valid and reliable measure of spinal excitability. First of all, it has been shown that CMEPs have a monosynaptic component in the motoneuron pools for upper limb muscles (Petersen, Taylor & Gandevia, 2002). This means that for upper limb muscles, specifically the biceps brachii, there is typically only one synapse between the corticospinal axons and the spinal motoneuron pool. Therefore, CMEPs recorded from the biceps brachii provide a valid measure of the responsiveness of the biceps brachii motoneuron pool to descending input. Also, in comparison to the H-reflex technique for assessing spinal excitability, CMEPs are not affected by pre-synaptic inhibition because

corticospinal axons (Nielsen & Petersen, 1994). Therefore, CMEPs can be used as a valid measure of spinal excitability.

There are limitations to the TMES technique that should be considered when assessing spinal excitability. A common problem with TMES is that the stimulation can be delivered to the ventral roots, rather than to corticospinal axons. This results in a decrease in the latency of the recorded CMEP by ~2 ms as the time for synaptic transmission between the descending axons and the spinal motoneuron is removed (Taylor, 2006). When the stimulation is delivered to the ventral roots (ie. post-synaptic) the response recorded in the muscle is a measure of peripheral excitability and not spinal excitability. Thus, the amplitude of the response will not increase during a voluntary contraction (Taylor, 2006). This is because the increase in CMEP amplitude during a voluntary contraction results from changes in the intrinsic electrical properties of motoneurons and an increase in motoneuron recruitment, which will not be detected if the stimulation is delivered distal to the motoneuron cell body. It is important to monitor the latency of CMEPs during experiments (~8 msec) to ensure the recorded responses result from stimulation of the descending axons and provide a measure of spinal excitability. Also, electrical stimulation at the cervicomedullary junction can be quite painful, thus it is important to familiarize participants with the technique prior to the experimental session. Another disadvantage is that valid CMEP responses without ventral root stimulation and sufficient amplitude cannot be recorded in some subjects (McNeil, Butler, Taylor & Gandevia, 2013).

2.6.4 Setting Stimulation Intensity for TMS and TMES

Prior to conducting an experiment using TMS and/or TMES it is important to set stimulation intensities in such a way that will allow the amplitude of responses to both increase and decrease during the protocol and in a way that will allow conclusions to be drawn about the

collected data. For example, to make conclusions about supraspinal excitability MEP amplitudes must be normalized to CMEP amplitudes. In order to do so stimulation intensities for TMS and TMES should be set to produce MEPs and CMEPs with similar amplitudes to ensure that the same portion of the motoneuron pool is being activated (Gandevia et al., 1999; Gruber et al., 2009). One approach that has been done in previous research is to first find the participants Mmax, as described previously, and then set TMES stimulation intensity to produce CMEPs (average of 8) with an amplitude equal to 15-20% of the participants Mmax. After the stimulation intensity for TMES is set the stimulation intensity for TMS is set to produce MEPs (average of 8) with an amplitude equal to the amplitude of the average of 8 CMEPs (Forman et al., 2014; Forman et al., 2016; Spence et al., 2016).

2.6.5 Brachial Plexus Stimulation

Although MEPs and CMEPs provide a measure of overall CSE and spinal excitability, respectively, when the responses are recorded from the muscle using surface EMG they are influenced by changes in peripheral excitability. After an action potential is generated in the spinal motoneuron it travels down the axon, located in the peripheral nerve, and across the neuromuscular junction (NMJ) and along the sarcolemma. Thus, changes in the amplitude of MEPs and CMEPs could result from changes at the NMJ or in the propagation of the action potential along the muscle fiber membrane, which can be affected by fatigue (Gruet et al., 2013). Therefore, in order to make conclusions about changes in supraspinal and spinal excitability it is important to account for changes in peripheral excitability. This can be done by normalizing MEPs and CMEPs to a maximum compound muscle action potential (Mmax). To determine a participants Mmax a stimulation is delivered to a peripheral nerve (ie. brachial plexus) and the intensity of the stimulation is increased until the recorded response (M-wave) reaches a plateau

or the amplitude starts to decrease with an increase in stimulation intensity (Forman et al., 2014). Therefore, by normalizing MEPs and CMEPs to Mmax researchers can remove the influence of peripheral excitability on MEP and CMEP amplitudes and can determine if changes in excitability are occurring within the corticospinal tract.

2.7 Changes in Intrinsic Motorneuron Properties During Rhythmic Motor Outputs in Quadrupeds

Locomotion is initiated by descending commands from supraspinal regions, such as the MLR and primary motor cortex, which increases the excitability of spinal motoneurons and allows the CPG to recruit the motoneurons required to generate rhythmic motor outputs. Thus, the intrinsic electrical properties of spinal motoneurons change during rhythmic motor outputs, such as scratch and fictive locomotion. There are several intrinsic motoneuron properties that can be examined to determine the excitability of the cell in non-human animals. Some of these properties include: afterhyperpolarization amplitude (AHP) and voltage threshold (V_{th}).

2.7.1 Reduction in Afterhyperpolarization (AHP) Amplitude

AHP is a temporary hyperpolarization of membrane potential that occurs after an action potential. This results from the prolonged opening of potassium (K+) channels, which results in an outward flow of K+ ions. This outward flow of ions causes a brief hyperpolarization of membrane potential. The AHP amplitude influences the firing rate of motoneurons, with a reduction in AHP amplitude leading to an increase in firing frequency. A study on decerebrate cats has shown that during fictive locomotion AHP amplitude is significantly lower that the AHP amplitude observed during rest (Brownstone et al., 1992). This reduction in AHP amplitude allows for the high motoneuron firing rates that are required for locomotion. This was supported by a more recent study that showed a reduction in AHP amplitude during both the approach and

rhythmic phases of fictive scratch in spinal intact cats and in cats with a spinal transection (Power et al., 2010).

2.7.2 Hyperpolarization of Voltage Threshold (V_{th})

Voltage threshold (V_{th}) is the level of depolarization of membrane potential that is required to reach threshold for action potential generation (Gardiner, 2011). Thus, it is the membrane potential that causes the voltage gated sodium (Na+) channels to open, resulting in a rapid influx of Na+ ions into the cell, which causes an action potential. During fictive locomotion in adult decerebrate cats, induced by stimulation of the MLR, there is a hyperpolarization of V_{th} for action potential initiation in lumbar motoneurons (Brownstone et al., 1992). Thus, with a hyperpolarization of voltage threshold there is a reduction in the amount of inward current that is required to produce an action potential and as a result the motoneuron is in a facilitated/ excited state (Krawitz et al., 2001). This finding was supported by a study that assessed changes in V_{th} during fictive scratch in adult decerebrate cats. It was found that during fictive scratch there was a hyperpolarization of V_{th} causing the spinal motoneurons to be in a facilitated state (Power et al., 2010). Thus, during rhythmic motor outputs motoneuron excitability is increased in adult decerebrate cats, which reduces the input needed from the spinal CPG to recruit motoneurons (Power et al., 2010).

2.8 Modulation of Supraspinal and Spinal Excitability During Rhythmic Motor Outputs in Humans

2.8.1 Task-dependent Modulation of CSE

As discussed previously, TMS and TMES can be used to assess CSE in humans during voluntary movement. Researchers have revealed that there is a task-dependent neural control of arm musculature during arm cycling. Forman et al., (2014) examined CSE projecting to the biceps brachii during FWD arm cycling and compared responses to an intensity- and position-

matched tonic contraction. A tonic contraction was chosen to produce the same level of central motor drive, measured by EMG, as arm cycling but without the input from CPG circuits (Forman et al., 2014). TMS evoked MEPs were higher in amplitude during arm cycling when elicited at the 6 and 3 o'clock position, relative to the clock face, compared to an intensity- (ie. same level of background EMG) and position- matched tonic contraction (Forman et al., 2014). Also, CMEP amplitudes were significantly higher at the 3 o'clock position during arm cycling but were not different at the 6 o'clock position, suggesting that the increase in MEP amplitude during mid- elbow flexion was due to an increase in supraspinal excitability (Forman et al., 2014). The increase in excitability of the spinal motoneuron pool at the 3 o'clock position could have resulted from changes in the intrinsic electrical properties (ie. AHP, V_{th}) of the motoneurons or an increase in excitatory input to the motoneuron pool, as can occur in the presence of PICs. PIC is an intrinsic motoneuron property that is capable of producing a prolonged inward flow of sodium or calcium ions and is facilitated in the presence of serotonin and norepinephrine (Heckman, 2003). These results are in contrast with a study by Carroll, Baldwin, Collins & Zehr (2006) which showed a decrease in MEP amplitude of the flexor carpi radialis during the flexion phase of arm cycling compared to an intensity- and position- matched tonic contraction. These researchers suggested that there was greater input from CPG circuits during arm cycling, thus reducing the reliance on cortical input.

CSE has also been examined during FWD and BWD walking where MEP amplitudes were assessed during the stance and swing phase of walking and compared to MEPs evoked during an intensity- and position- matched tonic contraction. Soleus MEPs elicited during the stance phase of FWD walking were smaller in amplitude than MEPs evoked during a plantar flexion tonic contraction with matched levels of background EMG (Capaday et al., 1999).

However, during the same phase of the FWD gait cycle the TA had larger MEPs relative to MEPs measured during voluntary ankle plantar flexion (Capaday et al., 1999). The researchers concluded that during the stance phase of FWD walking there is greater input from the corticospinal tract to the flexor muscle, TA, than the extensor muscle, soleus (Capaday et al., 1999). Ung et al., (2005) examined MEPs in TA and soleus elicited during the BWD gait cycle and compared the amplitudes to MEPs elicited during voluntary dorsiflexion or plantar flexion, respectively, with matched levels of background EMG. Linear regression analysis of MEP amplitude versus background EMG for the soleus muscle was completed and revealed that the slope of the linear regression was steeper for voluntary contraction than for BWD walking. Thus, suggesting that for a given level of background EMG MEP amplitudes of the soleus muscle are smaller during BWD walking than a voluntary contraction (Capaday et al., 1999). This task-dependent modulation of CSE could reflect a decrease in the contribution of the motor cortex to the generation of BWD walking in comparison to a tonic contraction. Therefore, suggesting that there may be greater input from subcortical regions (CPG) to the control of BWD walking. However, this was not seen for the TA muscle as there was no difference in the slope of the regression line between BWD walking and a voluntary contraction (Capaday et al., 1999). This supports the observations from FWD walking where input from the corticospinal tract is higher for the TA than the soleus due to the fine motor control required to keep the toes from touching the ground.

2.8.2 Intensity-dependent Modulation of CSE

The intensity of a cycling task can be altered by modifying the cycling cadence, the power output or both. Recent studies have examined the influence of arm cycling cadence and workload on the excitability of the nervous system in humans by using TMS and TMES.

Forman, Philpott, Button and Power (2015) examined the influence of arm cycling cadence on CSE projecting to the biceps brachii. The researchers reported cadence-dependent changes in CSE as MEPs and CMEPs increased with an increase cadence during elbow flexion (Forman et al., 2015). However, during elbow extension although MEP amplitudes progressively increased with an increase in cadence spinal excitability, as measured by CMEP amplitude, decreased with an increase in cadence (Forman et al., 2015). Thus, suggesting that changes in spinal excitability are not only cadence-dependent but also phase-dependent. Therefore, during elbow extension the increase in MEP amplitude that occurred with an increase in cadence was due to an increase in supraspinal excitability. A potential mechanism that was discussed to explain the decrease in spinal excitability was the reciprocal inhibition from the triceps brachii, which is active during elbow extension (Forman et al., 2015). Reciprocal inhibition is a neuromuscular reflex whereby the agonist muscle, in this case the triceps brachii, inhibits the activity of the antagonist muscle, the biceps brachii, through the activation of Ia inhibitory interneurons (Latash, 1998).

The influence of workload on CSE has also been assessed in humans during arm and leg cycling. A study by Weavil et al., (2015), examined the effects of leg cycling intensity on CSE projecting to the quadriceps. Participants performed eight FWD leg cycling trials at a constant cadence (80 rpm) at four different workloads (100 W, 200 W, 300 W & 400W) (Weavil et al., 2015). The researchers found that MEP and CMEP amplitudes of the vastus lateralis increased by 65% with an increase in power output and then reached a plateau. Similarly, rectus femoris MEPs and CMEPs increased by 110% with an increase in workload but the amplitudes did not plateau. Therefore, CSE is likely muscle dependent, as discussed in the following section. Interestingly, the MEP-to-CMEP ratio did not change with increases in workload, suggesting that the increase in MEP amplitude was due to an increase in spinal excitability (Weavil, et al., 2015).

A similar study examined workload-dependent changes in CSE projecting to the biceps and triceps brachii during arm cycling (Spence et al., 2016). Participants completed four arm cycling trials at a constant cadence (60 rpm) at 5% and 15% of their peak power output (PPO), which was measured during an arm cycling sprint test, with stimulations delivered at either the 6 or 12 o'clock position (Spence et al., 2016). CSE projecting to the biceps and triceps brachii, as measured by MEP amplitude, increased with an increase in workload during elbow flexion and extension (Spence et al., 2016). Also, significantly higher CMEP amplitudes were reported at 15% PPO for the triceps brachii. but there was no significant difference in CMEP amplitudes recorded from the biceps brachii between the two power outputs (Spence, et al., 2016). Therefore, CSE projecting to the biceps and triceps brachii is workload-dependent, but CSE is modulated differently for each muscle.

2.8.3 Muscle-dependent Modulation of CSE

The research that has been done thus far regarding the modulation of CSE during rhythmic motor outputs in humans has revealed that CSE is muscle dependent. As mentioned previously, this can be seen when comparing the results from Forman et al. (2014) and Carroll et al. (2006). The study by Forman et al., (2014) showed an increase in CSE projecting to the biceps brachii during FWD arm cycling compared to a tonic contraction. This is opposite to the results from the study by Carrol et al. (2006) that reported lower CSE projecting to the flexor carpi radialis during FWD arm cycling compared to a tonic contraction. A potential explanation for the differences between these studies is the muscle examined. The biceps brachii, which was used in the study by Forman et al., (2014), is a prime mover that contributes to the arm cycling movement, whereas the flexor carpi radialis is important for wrist stabilization during the arm cycling task. Intermuscle differences in CSE were previously reported for the biceps and triceps

brachii in a study that examined changes in CSE during FWD arm cycling with changes in workload (Spence et al., 2016). Both supraspinal and spinal excitability projecting to the biceps brachii were phase-dependently modulated as MEPs and CMEPs were significantly higher during elbow flexion than elbow extension with increase in power output (Spence et al., 2016). However, this pattern of modulation was not reported for the triceps brachii. In fact, although background EMG was higher during elbow extension than elbow flexion, CSE projecting the triceps brachii was not dependent on the phase of the movement cycle (Spence et al., 2016). Thus, the presence of phase-dependent modulation of CSE in the biceps brachii suggests that the two antagonistic muscles may be under different neural control and that cortical input may be more important for the control of the biceps brachii muscle.

In fact, greater cortical control of flexor motoneuron pools has been reported previously for the tibialis anterior muscle during locomotion. Capaday et al., (1999) showed that tibialis anterior MEPs are higher during the stance phase of FWD walking compared to MEPs evoked during a voluntary plantarflexion contraction, however soleus MEPs were lower during the stance phase compared to a voluntary contraction. The researchers concluded that the CST is more involved in the control of the neural circuits controlling flexor motorneuron pools than extensors. This seems likely as it has been shown that there is a greater contribution of PICs to the intrinsic excitability of extensor motor units than flexors, as was reported in a study that compared PICs between the biceps and lateral head of the triceps brachii during an isometric contraction in humans (Wilson et al., 2015). PICs are characterized by an inward sodium and calcium current that increase neuron excitability due to the presence of a plateau potential, which is a sustained depolarization of membrane potential (Heckman, 2003). As a result, PICs amplify synaptic input to the motoneuron pool and can cause self-sustained firing, thus reducing the

reliance on descending input to recruit motorneurons (Heckman, 2003). Therefore, during rhythmic motor outputs extensor motoneuron pools may require less input from the CST than flexors, which can partially explain the muscle-dependent differences in CSE that exist in humans.

To further understand the muscle-dependent differences in CSE to the biceps and triceps brachii the properties of the muscles should be assessed. Notably, a majority of the synapses between the CST and the biceps brachii motoneuron pool are monosynaptic (ie., one synapse), whereas the triceps brachii motoneuron pool has a larger portion of polysynaptic (ie., multiple synapses) connections (Palmer & Ashby, 1992). Therefore, both MEP and CMEP amplitudes of the triceps brachii are more likely to be influenced by changes in the excitability of interneurons rather than representing changes in the excitability of the motoneuron pool controlling the triceps brachii (Spence et al., 2016). This is unlikely for the biceps brachii as a majority of the connections are monosynaptic. There are also differences in the intrinsic electrical properties of motoneurons within a muscle group. The triceps brachii is a 3-headed muscle consisting of the medial, long and lateral head. Research has shown that during an isometric contraction with the elbow flexed and extended the motoneurons controlling the lateral head of the triceps brachii have a lower recruitment threshold than the long head (Davidson & Rice, 2010). Recruitment threshold is the level of depolarization of membrane potential that is required to recruit a motoneuon (Gardiner, 2011). Thus, with a lower recruitment threshold motoneurons are more easily recruited as less synaptic input is required to recruit the motoneurons.

2.9 Conclusion

Current research on FWD and BWD locomotor outputs suggests that both movement directions are controlled by the same neural networks. However, this does not provide any

insight into how CSE is modulated during FWD compared to BWD locomotor outputs. In fact, present research has shown that during locomotor-like movements the modulation of CSE is dependent on the phase of the movement cycle, the task being examined, and the muscle being studied. Currently, it is unknown how CSE is modulated during FWD and BWD locomotor outputs. The following study will explore how the modulation of CSE and spinal excitability to the upper limb is different between FWD and BWD arm cycling. The findings from this research will further the understanding of how CSE is modulated during arm cycling and has potential clinical applications for designing rehabilitation programs for individuals with neural impairments.

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Chapter 3 Corticospinal and spinal excitability to the biceps and triceps brachii is modulated differently between forward and backward arm cycling

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

Key words: Forward, Backward, transmastoid, transcranial, corticospinal, CMEP, MEP,

3.0 ABSTRACT

The purpose of this study was to evaluate supraspinal and spinal excitability to the biceps and triceps brachii when comparing forward (FWD) and backward (BWD) arm cycling. Supraspinal and spinal excitability were assessed non-invasively using transcranial magnetic stimulation (TMS) to elicit motor evoked potentials (MEPs) and transmastoid electrical stimulation (TMES) to elicit cervicomedullary evoked potentials (CMEPs), respectively. MEPs and CMEPs were recorded from the biceps and triceps brachii during FWD and BWD arm cycling at two positions, the 6 and 12 o'clock position. The 6 o'clock position corresponded to mid elbow flexion and extension during FWD and BWD cycling, respectively, while the 12 o'clock position corresponded to mid elbow extension and flexion during FWD and BWD cycling, respectively. Participants completed four arm cycling trials, two FWD and two BWD, at 60 rpm and 25 W. During the flexion phase MEP (p = .001) and CMEP (p = .001) amplitudes of the biceps brachii were higher during FWD cycling. However, during the extension phase MEP (p = .006) and CMEP (p = .003) amplitudes were higher during BWD cycling. For the triceps brachii MEP amplitudes were higher during FWD cycling compared to BWD (p = .027) regardless of the functional phase (flexion vs. extension) of the movement cycle. However, spinal excitability to the triceps brachii was dependent on the functional phase of the movement cycle. During the flexion phase CMEPs of the triceps brachii were higher during FWD cycling compared to BWD (p = .032), but during the extension phase CMEPs were higher during BWD cycling compared to FWD (p = .001). This data suggests that the modulation of CSE and spinal excitability to the biceps brachii is dependent on the functional phase of the movement cycle and on the cycling direction. Also, spinal excitability but not CSE to the triceps brachii is dependent on the functional phase of the movement cycle when comparing FWD and BWD cycling.

3.1 INTRODUCTION

Humans and non-human animals, such as cats, can naturally change their direction of locomotion from forward (FWD) to backward (BWD). Locomotion and other locomotor-like movements, such as cycling, require rhythmic and alternating activation of antagonistic muscle groups within a limb and coordinated movement between limbs. In non-human animals the basic motor pattern required for locomotion can be produced by neural circuits in the spinal cord, referred to as the central pattern generator (CPG), however input from supraspinal centers is required to initiate and terminate movement (Jordan et al., 2008). Similarly, research has revealed that the CPG is also active during rhythmic motor outputs in humans, however humans rely on descending drive from cortical centers to a greater degree than non-human animals to initiate and control the motor output (Petersen et al., 2001). Thus, in humans and non-human animals the CPG is activated and modulated by descending drive from supraspinal centers.

Researchers that study the neural control of FWD and BWD rhythmic movement have relied primarily on examining reflex modulation patterns during locomotion and other locomotor-like movements as an indirect measure of CPG activity. This technique is based on the discovery that neural pathways relaying sensory feedback from the periphery to the spinal cord are integrated with CPG circuits, thus reflex responses are modulated by the CPG (Zehr. 2005). Thus, by stimulating sensory afferents and recording the pattern of motor output this technique indirectly reveals the characteristics of neural control (Zehr, 2005). Buford & Smith (1990;1993) conducted a sequence of experiments on cats to examine the neural control of the hindlimb muscles during FWD and BWD walking. The researchers examined muscle activation patterns, joint movements and reflexes in response to perturbations elicited by electrical and mechanical stimulation of cutaneous nerves in the paw of cats (Buford & Smith, 1993). The

amplitude and sign of the reflex output in response to electrical stimulation was dependent on the phase of the step cycle that the stimulation was delivered for FWD and BWD walking (Buford & Smith, 1993). Thus, it was concluded that the adaptive responses result from the modulation of afferent feedback by the same CPG for FWD and BWD walking. Duysens, Tax, Murrer, & Dietz (1996) conducted a similar study in adult humans and found that the modulation of cutaneous reflexes was similar for FWD and BWD walking.

More recently researchers have utilized arm and leg cycling paradigms to examine the neural control of rhythmic motor outputs in humans (Zehr & Hundza, 2005). Notably, similarities in muscle activation patterns and reflex modulation have been found for locomotion during arm and leg cycling suggesting that arm and leg movement during these tasks are controlled by similar neural mechanisms (Zehr. Hundza, Balter & Loadman, 2009; Zehr, 2005). Zehr & Hundza (2005) examined cutaneous reflex modulation patterns of the upper limb muscles during FWD and BWD arm cycling. This research revealed similar phase-dependent reflex modulation patterns for FWD and BWD cycling, meaning that at similar phases of the movement cycle the reflex responses were the same sign and were similar in amplitude for both FWD and BWD cycling (Zehr & Hundza, 2005). These results correspond with the reflex modulation patterns seen in the lower limb muscles during FWD and BWD walking and leg cycling (Duysens et al., 1996; Zehr et al., 2009). Thus, providing support to the claim that FWD and BWD rhythmic motor outputs are controlled by similar neural mechanisms.

Although it appears that FWD and BWD rhythmic motor outputs are controlled by the same CPG, little is known about how the brain and spinal cord contribute to the generation of BWD rhythmic movement compared to FWD. In humans, the motor cortex is a major center involved in the control of voluntary motor outputs. During FWD and BWD locomotion motor

cortex neurons, also known as pyramidal tract neurons, are rhythmically active during the step cycle (Armstrong & Drew, 1984; Beloozerova & Sirota, 1985; Zelenin et al., 2011a; Zelenin et al., 2011b). Thus, suggesting that the motor cortex is involved in the production and control of both directions of walking. Zelenin et al., (2011a) examined the activity of pyramidal tract neurons during FWD and BWD walking in cats. This research revealed no significant difference in the mean level of activity (f_M) of motor cortex neurons between FWD and BWD walking (Zelenin et al., 2011a). Thus, suggesting that BWD walking does not require greater overall cortical activity compared to FWD walking. However, the coefficient of modulation (K_{mod}), which is used to assess the degree of modulation of motor cortex neurons, was on average higher during BWD walking (Zelenin et al., 2011a). This indicates that the neurons controlling the hindlimbs were modulated to a greater degree during BWD walking compared to FWD walking (Zelenin et al., 2011a). This could suggest that there is greater cortical involvement during BWD walking in cats.

Up until this point, the activity of the central nervous system (brain and spinal cord) has not been compared between FWD and BWD rhythmic motor outputs in humans. This can be assessed non-invasively by examining the responsiveness of the corticospinal tract to stimulation, referred to as corticospinal excitability (CSE), during arm (Carroll, Baldwin, Collins & Zehr, 2006; Copithorne, Forman & Power, 2015; Forman, Raj, Button & Power, 2014; Forman, Philpott, Button & Power, 2015; Forman et al., 2016; Spence et al., 2016) and leg cycling (Weavil et al., 2016). CSE can be assessed using transcranial magnetic stimulation (TMS) of the motor cortex to elicit motor evoked potentials (MEPs) in the target muscle. MEP amplitudes provide a measure of the overall excitability of the corticospinal tract, but do not allow researchers to make conclusions about changes in supraspinal and spinal excitability. To

do so transmastoid electrical stimulation (TMES) at the level of the pyramidal decussation, which elicits cervicomedullary evoked potentials (CMEPs) in the target muscle, can be used in conjunction with TMS (Taylor, 2006). Using these techniques previous work in our lab has shown that CSE and spinal excitability projecting to the biceps brachii muscle are phase-dependent during FWD arm cycling such that it is higher during the flexion phase of arm cycling compared to the extension phase (Forman et al., 2014; Spence et al., 2016). However, previous research has shown that CSE to the triceps brachii was not phase-dependent during FWD arm cycling, however spinal excitability was higher during the flexion phase compared to the extension phase (Spence et al., 2016). Also, Forman et al., (2014) showed that CSE projecting to the biceps brachii is higher during the elbow flexion phase of FWD arm cycling compared to an intensity- and position- matched tonic contraction (Forman et al., 2014). To date only one study has examined CSE during BWD rhythmic motor outputs. Ung et al., (2005) showed that soleus MEP amplitudes were lower during BWD walking when compared to an intensity- and position-matched tonic contraction. Although it appears that CSE is different between FWD and BWD rhythmic motor outputs, no study has directly compared CSE between FWD and BWD locomotor outputs. However, based on the research done in our lab and the study by Ung et al., (2005) we have hypothesized that CSE projecting to the biceps and triceps brachii will be lower during BWD cycling compared to FWD cycling.

The primary purpose of this study was to determine if CSE projecting to the biceps and triceps brachii was similarly modulated during FWD and BWD arm cycling. We hypothesized that: (1) CSE and spinal excitability projecting to the biceps and triceps brachii would be lower during BWD arm cycling and (2) CSE and spinal excitability projecting to the biceps brachii and

spinal excitability projecting to the triceps brachii would be phase-dependent during FWD and BWD arm cycling.

3.2 METHODS

3.2.0 Ethics Approval

All procedures were verbally explained to the participants and written consent was obtained prior to the start of the session. The research was conducted in accordance with the Helsinki Declaration and approved by the Interdisciplinary Committee on Ethics in Human Research at Memorial University of Newfoundland (ICEHR#: 20181250-HK). Procedures were in accordance with the Tri-Council guideline in Canada and potential risks were fully disclosed to participants.

3.2.1 Participants

Twelve healthy male volunteers (ten right hand dominant, two left hand dominant) with no known neurological deficits participated in the study. All 12 participants received TMS and 11 of those also received TMES. One participant did not receive TMES as the required stimulation intensity was not tolerable. Prior to testing each participant completed a magnetic stimulation safety-checklist to screen for existing contraindications to magnetic stimulation (Rossi et al, 2009). To determine hand dominance participants completed an Edinburg handedness inventory questionnaire to ensure that all evoked responses were recorded from the dominant arm (Oldfield, 1971). Responses were recorded from the dominant arm for all participants because differences in neural control have been reported between the dominant and non-dominant limb (Davidson & Tremblay, 2013). Additionally, to screen for existing contraindications to physical activity each participant completed a Physical Activity Readiness Questionnaire (PAR- Q+) (Warburton et al., 2011).

3.2.2 Experimental Set-up

A one-group within subjects design was used. Participants attended one familiarization session (~ 30 min) and one testing session (~ 1 hour).

3.2.2.0 Familiarization session

During the familiarization session the hand dominance of each participant was determined using the Edinburgh handedness questionnaire (Oldfield, 1971). Participants then performed FWD and BWD arm cycling at 25 W while maintaining 60 rpm to ensure they were able to complete the task. Participants then received TMS, TMES and Erb's point stimulations to ensure they were comfortable with the stimulation paradigm that was used during the testing session.

3.2.2.1 Testing session

During the testing session each participant completed both FWD and BWD arm cycling on an arm cycle ergometer (SCIFIT ergometer, Berkshire, UK). All participants were advised to maintain an upright posture throughout each cycling protocol to limit forward and backward bending of the trunk. The arm cranks were set at 180 degrees out of phase and seat height was adjusted to ensure the participants shoulders were in line with the center of the arm shaft. Each participant was informed to lightly grip the arm cranks with the forearms in pronation. All participants wore wrist braces to restrict wrist joint movement during cycling due to the reflex connections that exist between the wrist flexors and extensors and the biceps brachii (Manning & Bawa, 2011).

Measurements were taken at the 6 and 12 o'clock position, relative to the clock face. The 6 o'clock position was defined as the "bottom dead center" and the 12 o'clock position was

defined as the “top dead center” of the movement cycle (see Figure 1; Forman et al., 2014; Forman et al., 2015; Spence et al., 2016). These positions were relative the participants dominant hand such that the 12 o’clock position for a right-handed individual occurred when their right hand was at the “top dead center”. These positions were selected because they represent maximum and minimum biceps brachii activation (Forman et al., 2014). During FWD cycling the movement of the arm crank from the 3 o’clock (elbow extension) to 6 o’clock (mid elbow flexion) to the 9 o’clock position (elbow flexion) was defined as elbow flexion. During this phase the biceps brachii is most active and peak EMG occurs at the midway point, at the 6 o’clock position (Forman et al., 2014). In contrast, movement of the arm crank from 9 o’clock (elbow flexion) to 12 o’clock (mid elbow extension) to 3 o’clock position (elbow extension) was defined as elbow extension for FWD cycling. During BWD arm cycling the arm cranks were moving in the reverse direction (ie. counterclockwise). Thus, movement of the arm crank from the 3 o’clock position to 12 o’clock to the 9 o’clock position was defined as elbow flexion. Elbow extension was defined as movement of the arm crank from the 9 o’clock to the 6 o’clock to the 3 o’clock position.

All stimulations were triggered automatically as the arm crank of the dominant arm passed each position (6 and 12 o’clock). Measurements at each position were obtained during FWD and BWD arm cycling during separate trials, for a total of four cycling trials. Participants were instructed to cycle at a constant power output of 25 W and a cadence of 60 rpm (Forman et al., 2014). This power output was chosen based on previous research completed in our lab (Forman et al., 2014) as it did not induce fatigue in the participants and it produced sufficient EMG in the muscles of interest allowing MEP and CMEP responses to be recorded. The order of

the four cycling trials (FWD and BWD with responses elicited at 6 and 12 o'clock) were randomized for each participant.

3.2.2 Electromyography (EMG)

Electromyography (EMG) was recorded from anterior and posterior deltoid, biceps brachii, triceps brachii and brachioradialis of the dominant arm using pairs of surface electrodes (Kendall^{TM} 130 conductive adhesive electrodes, Covidien IIC, Massachusetts, USA). EMG was recorded using a bipolar configuration (Ag-AgCl) with an interelectrode distance of 2 cm. A ground electrode was placed on the lateral epicondyle of the dominant arm. Prior to electrode placement the skin at the recording site was shaved to remove hair, abraded using an abrasive pad to remove dead epithelial cells and cleaned with an isopropyl alcohol swab to reduce impedance for EMG recordings. The EMG signals were sampled online at 5 kHz using CED 1401 interface and Signal 5.11 software (Cambridge Electronic Design (CED) Ltd., Cambridge, UK). EMG signals were amplified (x300) and filtered using a 3-pole Butterworth band-pass filter, with cut-off frequencies from 10-1000 Hz, using a CED 1902 amplifier.

3.2.3 Stimulation Conditions

Responses from the biceps and triceps brachii were elicited using TMS, TMES, and electrical stimulation at Erb's point. All stimulation intensities were set while the participant was cycling forward at 25 W and 60 rpm and stimulations were triggered automatically when the arm crank passed the 6 o'clock position. At this position the shoulder was at ~ 0 degrees of shoulder flexion and the elbow was flexed to ~ 90 degrees. The stimulation intensities for Erb's point, TMS and TMES were set relative to the biceps brachii muscle. Responses were also recorded from the triceps brachii muscle, which has been done previously (Spence et al., 2016).

3.2.3.0 Brachial Plexus Stimulation

Electrical stimulation of the brachial plexus at Erb's point was used to elicit maximal compound muscle action potentials (M_{max}) (DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK). M_{max} is a measure of maximal electrical activity that can be produced in the muscle (Wee, 2006). The anode was placed over the acromion process and the cathode was placed over the skin in the supraclavicular fossa. A pulse duration of 200 μs was utilized and the stimulation intensity was gradually increased from 25 mA until the M-wave amplitude of the biceps brachii reached a plateau or started to decrease, referred to as M_{max}. This stimulation intensity was increased by 10% and used for the remainder of the experiment to ensure maximal M-waves were elicited during each trial (Forman et al., 2014; Copithorne, Forman & Power, 2015).

3.2.3.1 Transmastoid Electrical Stimulation (TMES)

TMES was applied using adhesive Ag-AgCl electrodes placed inferior to the mastoid processes (200 μs duration; Digitimer; model DS7AH). The stimulation intensity was increased until the peak-to-peak amplitude of the average of 8 CMEPs was equal to approximately 15-20% of the peak-to-peak amplitude of the participants M_{max}. This stimulation intensity was used for the remainder of the experiment. The latency of the CMEP responses was monitored throughout the experiment to ensure the latency was between 7.5-8 ms to be confident that the corticospinal tract was being stimulated and not the ventral roots (Taylor, 2006).

3.2.3.2 Transcranial Magnetic Stimulation (TMS)

The stimulation intensity for TMS was always set following TMES as it easier to match MEPs to CMEPs (Forman et al., 2014). TMS was applied over the participants vertex using a circular coil (13.5- cm outside diameter) attached to a Magstim 200 (Magstim, Dyfed, United Kingdom). The vertex was located by measuring the distance between nasion and inion and

between the participants tragi and placing marks halfway directly on the scalp. The intersection of the halfway marks was defined as the vertex (Power & Copithorne, 2013). Stimulation intensity was measured as a percentage of maximum stimulator output (MSO) and the stimulation intensity was increased until the peak-to-peak amplitude of the average of 8 MEPs was matched to that of the CMEP, approximately 15-20% of the participants $M_{max.}$ This stimulation intensity was used for the remainder of the experiment.

3.2.4 Experimental protocol

After stimulation intensities for Erb's point stimulation, TMES and TMS were set, participants completed 4 arm cycling trials in a randomized order; 2 FWD and 2 BWD with stimulations delivered at the 6 or 12 o'clock position. For each cycling trial participants cycled at 60 rpm and 25W. During each cycling trial participants received a total of 10 MEPs, 10 CMEPs and 2 M_{max}. Stimulations were triggered automatically as the arm crank passed the predetermined crank position. The order of the stimulations was randomized throughout the trial and were evoked every 7-8 s. To prevent anticipation of the stimulation 2 frames without stimulation were added.

3.2.5 Data Analysis

Data was analyzed off-line using Signal 5.11 software (Cambridge Electronic Design Ltd., Cambridge, UK). To determine if central motor drive projecting to the biceps and triceps brachii was similar between the 4 arm cycling trials the mean rectified 50 ms pre-stimulus EMG prior to TMS and TMES stimulus artifact was measured (Forman et al., 2014). The peak-to-peak amplitude of all evoked responses (MEP, CMEP and M_{max}) of the biceps and triceps brachii were measured from the initial deflection of the voltage trace from background EMG to the return of the trace to the baseline level. MEP and CMEP amplitudes can change as a result of changes to

M_{max}, thus MEPs and CMEPs were normalized to M_{max} evoked during the same trial to account for changes in the peripheral muscle (Forman et al., 2014). Also, MEP amplitudes were normalized to CMEP amplitudes evoked during the same trial to provide a measure of supraspinal excitability. All measurements were taken from the averaged files of all 10 MEPs, 10 CMEPs and 2 M_{max}.

3.2.6 Statistics

All statistical analysis was completed using IBM® SPSS® Statistics Version 23 (IBM, Markham, Ontario, Canada). A two-way repeated-measures ANOVA with factors 'direction' and 'phase' were used to determine if statistically significant differences in MEP or CMEP amplitudes (normalized to M_{max}), or pre-stimulus EMG occurred as a main effect of arm cycling direction or phase. All data was normally distributed as determined using Kolomogorov-Smirnov normality test. Sphericity is not an issue for this data set as there are less than three conditions (Field, 2013). If a significant main effect was found paired t-tests were used to examine changes in MEP and CMEP amplitudes and pre-stimulus EMG between cycling directions at the two arm crank positions. All statistical analysis was performed on group data with a significance level of $p < .05$. All data is reported in text as mean ± standard deviation (*SD*) and in figures as mean ± standard error (*SE)*.

3.3 RESULTS

Refer to Figure 1 for a schematic of the functional phases (flexion vs. extension) of the movement cycle and corresponding arm crank positions for both FWD and BWD cycling.

3.3.0 Biceps brachii

3.3.0.1 Corticospinal excitability

MEP amplitude. Figure 2A and Figure 3A show representative and grouped data for MEP amplitudes (normalized to M_{max}), respectively, from the biceps brachii during the elbow flexion phase (6 o'clock for FWD cycling and 12 o'clock for BWD cycling) and extension phase (12 o'clock for FWD cycling and 6 o'clock for BWD cycling) of arm cycling (refer to Figure 1). Group data demonstrated a significant main effect of arm cycling direction (FWD > BWD, $F_{(1,11)} = 82.257$, $p = .001$) and arm crank position (6 > 12, $F_{(1,11)} = 7.498$, $p = .019$) and a significant interaction effect between cycling direction and arm crank position ($F_{(1, 11)} = 49.52$, $p = .001$). Paired t-tests revealed that during the elbow flexion phase MEP amplitudes of the biceps brachii were significantly higher while arm cycling FWD compared with BWD (FWD: 19. 67 ± 5.03% of M_{max}, BWD: 8.47 ± 6.27% of M_{max}, $p = .001$). In contrast, during the elbow extension phase MEP amplitudes of the biceps brachii were significantly higher while arm cycling BWD compared with FWD (FWD: 1.28 ± 0.69 % of M_{max}, BWD: 5.86% ± 4.92% of $M_{max,}$ $p = .006$). During FWD cycling MEP amplitudes were significantly higher at the 6 o'clock position compared to the 12 o'clock position ($p = .001$). However, during BWD cycling there was no significant difference in MEP amplitudes between the 6 and 12 o'clock position ($p > .20$). MEP amplitudes at the 6 o'clock position ($p = .001$) and the 12 o'clock position ($p = .002$) were significantly different between FWD and BWD cycling for the biceps brachii.

Pre-stimulus EMG for MEPs. Figure 3B shows grouped data for pre-stimulus EMG for MEPs for the biceps brachii, there were no significant main effects of cycling direction ($F_{(1, 11)} = .153$, $p > .20$) or arm crank position ($F_{(1, 11)} = .934$, $p > .20$). There was a significant interaction effect ($F_{(1, 11)} = 12.24$, $p = .005$). As a group, pre-stimulus EMG for FWD (6 and 12 o'clock) and BWD (6 and 12 o'clock) cycling was 30.98, 19.43, 27.78 and 20.91 μV, respectively. Paired t-

tests revealed that pre-stimulus EMG for the biceps brachii was significantly higher at the 6 o'clock position compared to the 12 o'clock position during FWD cycling (p = .001). However, there was no significant difference between pre-stimulus EMG at the 6 and 12 o'clock position during BWD cycling (p = .150) for the biceps brachii.

3.3.0.1 Spinal excitability

CMEP amplitude. Figure 2B and Figure 4A show representative and grouped data for CMEP amplitudes (normalized to M_{max}), respectively, from the biceps brachii during the elbow flexion phase and extension phase of FWD and BWD arm cycling. Grouped data demonstrated a significant main effect of cycling direction (FWD>BWD, $F_{(1, 10)}$ = 15.13, p = .003) and arm crank position (6 > 12, $F_{(1, 10)}$ = 34.07, p = .001) and a significant interaction effect between cycling direction and arm crank position ($F_{(1, 10)}$ = 60.71, p = .001). During the elbow flexion phase CMEP amplitudes were significantly higher while arm cycling FWD compared with BWD for the biceps brachii (FWD: 16.9 ± 4.89% of M_{max}, BWD: 7.49 ± 4.36% of M_{max}, p = .001). In contrast, during the elbow extension phase CMEP amplitudes were significantly higher while arm cycling BWD compared with FWD (FWD: 1.78 ± 1.63 % of M_{max}, BWD: 5.61% ± 3.28% of $M_{max,}$ p = .003). During FWD cycling CMEP amplitudes were significantly different between the 6 and 12 o'clock position (p = .001), however during BWD cycling there was no significant difference in CMEP amplitudes between the 6 and 12 o'clock position (p > .20) for the biceps brachii. CMEP amplitudes at the 6 o'clock position (p = .001) and the 12 o'clock position (p = .001) were significantly different between FWD and BWD cycling.

Pre-stimulus EMG for CMEPs. Figure 4B shows grouped data for pre-stimulus EMG for CMEPs for the biceps brachii. There was a significant main effect of cycling direction (FWD > BWD, $F_{(1, 10)}$ = 5.11, p = .047) and arm crank position (6 > 12, $F_{(1, 10)}$ = 14.03, p = .004) and a

significant interaction effect between cycling direction and arm crank position ($F_{(1, 10)} = 13.25$, $p = .005$). During the elbow flexion phase pre-stimulus EMG prior to CMEPs for the biceps brachii was significantly higher while arm cycling FWD compared to BWD (FWD: 36.19 ± 20.18 μV, BWD: 26.77 ± 15.74 μV, $p = .008$). During the elbow extension phase there was no significant difference in pre-stimulus EMG between FWD and BWD arm cycling (FWD: 18.06 ± 14.66 μV, BWD: 20.67 ± 15.34 μV, $p = .071$). During FWD cycling pre-stimulus EMG was significantly higher at the 6 o'clock position compared to the 12 o'clock position ($p = .001$). However, during BWD cycling there was no significant difference in pre-stimulus EMG between the 6 and 12 o'clock position ($p = .087$) for the biceps brachii.

MEP/CMEP. Figure 5 shows group data of MEP amplitudes (normalized to CMEP recorded at the same arm crank position and cycling direction) during flexion and extension phases of FWD and BWD cycling. There was no significant main effect of cycling direction ($F_{(1, 9)} = 0.397$, $p > .20$) or arm crank position ($F_{(1, 9)} = 0.017$, $p > .20$) and no significant interaction effect ($F_{(1, 9)} = 1.95$, $p > .20$).

3.3.1 Triceps brachii (TB)

3.3.1.0 Corticospinal excitability

MEP amplitude. Figure 6A and Figure 7A show representative and grouped data for MEP amplitudes (normalized to M_{max}), respectively, from the triceps brachii during the elbow flexion and extension phases of arm cycling. Group data demonstrated a significant main effect of arm cycling direction (FWD > BWD, $F_{(1,11)} = 6.54$, $p = .027$). There was no significant main effect of arm crank position ($F_{(1,11)} = 2.45$, $p = .145$) and no significant interaction effect ($F_{(1, 11)} = 2.68$, $p = .130$). During the elbow flexion phase MEP amplitudes were 18.01± 18.81% and 8.84 ± 4.94% of M_{max} for FWD and BWD cycling for the triceps brachii, respectively. During the elbow

extension phase MEP amplitudes were 9.87 ± 6.61% and 8.31 ± 5.56% of M_{max} for FWD and BWD cycling, respectively. Paired t-tests revealed no significant difference in MEP amplitudes when FWD and BWD cycling were compared relative to the functional phase of the movement cycle (Flexion: $p = .058$, Extension: $p > .20$). However, MEP amplitudes were significantly higher at the 6 o'clock position during FWD cycling compared to the 6 o'clock position during BWD cycling ($p = .048$) for the triceps brachii.

Pre-stimulus EMG for MEPs. Group data for pre-stimulus EMG of the triceps brachii prior to MEPs can be seen in Figure 7B. There was no main effect of arm crank position ($F_{(1, 11)} = 0.440$, $p > .20$) or arm cycling direction ($F_{(1, 11)} = 1.86$, $p = .20$) on pre-stimulus EMG for the triceps brachii. Also, there was no significant interaction effect observed ($F_{(1, 11)} = 2.81$, $p = .122$). As a group, pre-stimulus EMG during the elbow flexion phase of FWD and BWD cycling for the triceps brachii was 21.98 ± 10.86 μV and 21.60 ± 9.34 μV, respectively. During the elbow extension phase pre-stimulus EMG of the triceps brachii was 24.29 ± 9.14 μV and 22.54 ± 10.73 μV for FWD and BWD arm cycling, respectively.

3.3.1.1 Spinal excitability

CMEP amplitude. Representative and group data for CMEP amplitudes (normalized to M_{max}) can be seen in Figure 6B and Figure 8A, respectively, for the triceps brachii. Significant main effect of arm crank position (6 > 12, $F_{(1, 10)} = 16.66$, $p = .002$) and significant interaction effect ($F_{(1, 10)} = 9.13$, $p = .013$) of arm cycling direction and arm crank position were observed. There was no significant main effect of arm cycling direction ($F_{(1, 12)} = 0.361$, $p > .20$). While arm cycling FWD MEP amplitudes were significantly higher at the 6 o'clock position compared to the 12 o'clock position (6 o'clock: 20.09 ± 14.84%, 12 o'clock: 5.56 ± 4.98%, $p = .003$). While arm cycling BWD there was no significant difference in MEP amplitudes between the 6

and 12 o'clock position (6 o'clock: 10.02 ± 6.15%, 12 o'clock: 13.99 ± 10.03%, $p = .196$). Paired t-tests revealed that during the flexion phase CMEPs were significantly higher during FWD cycling compared to BWD cycling ($p = .032$) for the triceps brachii. During the extension phase CMEPs were significantly higher during BWD cycling compared to FWD cycling ($p = .001$) for the triceps brachii.

Pre-stimulus EMG for CMEPs. Figure 8B shows group data of pre-stimulus EMG of the triceps brachii prior to CMEPs. There was a significant main effect of cycling direction ($F_{(1, 10)} = 10.31$, $p = .009$). There was no significant main effect of arm crank position ($F_{(1, 10)} = 2.91$, $p = .119$) and no significant interaction effect ($F_{(1, 10)} = 2.94$, $p = .117$). As a group, pre-stimulus EMG during the elbow flexion phase was 21.95± 10.34 μV and 21.17 ± 10.21 μV for FWD and BWD arm cycling, respectively. During the elbow extension phase pre-stimulus EMG of the triceps brachii was 25.97 ± 11.58 μV and 21.83 ± 10.68 μV for FWD and BWD cycling, respectively. Pre-stimulus EMG was significantly higher at the 12 o'clock position during FWD cycling compared to the 12 o'clock position during BWD cycling ($p = .02$) for the triceps brachii.

MEP/CMEP. Figure 9 shows group data of MEP amplitudes (normalized to CMEP recorded at the same arm crank position and cycling direction) during flexion and extension phases of FWD and BWD cycling. There was a significant main effect of cycling direction (FWD > BWD, $F_{(1, 9)} = 7.28$, $p = .027$) and a significant interaction effect ($F_{(1, 9)} = 7.56$, $p = .025$). There was no significant main effect of arm crank position ($F_{(1, 9)} = 2.86$, $p = .130$). MEP amplitudes, normalized to CMEPs, were significantly higher at the 12 o'clock position during FWD cycling (elbow extension) compared to the 12 o'clock position during BWD cycling (elbow flexion) (FWD: 187.62 ± 141.3%, BWD: 62.38 ± 25.45%, $p = .023$).

3.4 DISCUSSION

To our knowledge this is this first study to examine CSE and spinal excitability projecting to upper limb antagonistic muscles during FWD and BWD arm cycling. As hypothesized, during the elbow flexion phase of arm cycling CSE and spinal excitability projecting to the biceps brachii was higher during FWD cycling compared to BWD. However, surprisingly the opposite was found for the extension phase such that CSE and spinal excitability projecting to the biceps brachii was higher during BWD cycling. For the triceps brachii, spinal excitability was higher at the 6 o'clock position (the flexion phase of FWD cycling and the extension phase of BWD cycling) compared to the 12 o'clock position. Also, overall CSE to the triceps brachii was higher during FWD cycling compared to BWD cycling. BWD cycling provided interesting results as there was no phase-dependent differences in CSE projecting to the biceps or triceps brachii. This is in contrast with FWD cycling as CSE projecting to the biceps brachii was phase-dependent, which has been shown previously (Forman et al., 2014; Spence et al., 2016).

3.3.0 Direction-dependent modulation of corticospinal and spinal excitability to the biceps brachii

During the elbow flexion phase of arm cycling CSE (as indicated by MEP amplitudes; Figure 3) to the biceps brachii was higher during FWD cycling compared to BWD. Changes in spinal excitability (as indicated by CMEP amplitudes; Figure 4) followed the same pattern as MEPs, suggesting that increased spinal excitability can partially account for the higher MEP amplitudes during the flexion phase of FWD cycling. However, during the inactive phase for the biceps brachii (ie. extension) MEP and CMEP amplitudes were significantly lower during FWD cycling compared to BWD. The differences in MEP amplitudes between FWD and BWD cycling occurred despite no significant differences in pre-stimulus EMG (Figure 3). However, pre-

stimulus EMG prior to CMEPs was significantly higher during the elbow flexion phase of FWD cycling compared to BWD cycling (Figure 4). Despite the higher EMG during FWD cycling a correlational analysis revealed no correlation between CMEP amplitudes and pre-stimulus EMG ($p > .20$).

One putative mechanism that could be contributing to the observed differences in CSE and spinal excitability between FWD and BWD cycling is the position of the shoulder during the flexion and extension phases. During the flexion phase of the FWD cycling (ie. arm crank at the 6 o'clock position) the shoulder is at ~ 0° flexion. However, during the flexion phase of BWD cycling (ie. arm crank at the 12 o'clock position) the shoulder is flexed to ~ 90°. The biceps brachii is a biarticular muscle that crosses the shoulder joint, so the length of the muscle changes with alterations in shoulder position (Landenderfer et al., 2004). Collins et al., (2017) examined MEPs and CMEPs at 0 and 90° of shoulder flexion and showed that CSE projecting to the biceps brachii is shoulder position-dependent. In fact, during a 10% MVC both MEPs and CMEPs decreased when the shoulder was flexed to 90° compared to 0° (Collins et al., 2017). It was concluded that the position-dependent change in CSE was mainly of spinal origin as CMEPs decreased in amplitude (Collins et al., 2017). This supports our current finding that MEPs and CMEPs were lower during the elbow flexion phase of BWD cycling, where the shoulder was flexed to 90°, compared to FWD cycling. Similarly, during the elbow extension phase MEP and CMEP amplitudes of the biceps brachii were lower during FWD cycling compared to BWD cycling. This coincides with the previous findings as the shoulder was flexed to ~ 90° during the extension phase of FWD cycling but was at ~ 0° of shoulder flexion during the extension phase of BWD cycling.

Although it appears that the changes are occurring at the spinal level it is possible that the posture dependent changes in MEP and CMEP amplitudes resulted from peripheral factors. For example, the biceps brachii tendon has a proximal attachment on the scapula and therefore flexion of the shoulder shortens the biceps brachii muscle. This alters the muscle fiber length and diameter and can change the orientation of the muscle relative to the electrode, which can influence the recorded MEP and CMEP responses (Fortune & Lowery, 2012). However, if peripheral factors were the primary mechanism for explaining the posture related changes in CSE, an increase in CSE would be expected when muscle length was decreased (Fortune & Lowery, 2012). Yet, in this study CSE was lower when the shoulder was flexed to 90°, suggesting that the changes in MEP and CMEP amplitudes are likely occurring at the central level rather than the peripheral level. This is supported by Mogk et al., (2014) who showed a reduction in biceps brachii MEP amplitudes during an overhead reach (ie. shoulder flexion) compared to a horizontal reach, despite a shorter muscle length in the overhead reach position.

Changes in afferent feedback to the brain and spinal cord is another mechanism that could contribute to the differences in CSE and spinal excitability between FWD and BWD cycling. Notably, it has been shown that changes in joint angle can influence the degree of Ia reciprocal inhibition from synergist muscles, which alters the synaptic input into the motoneuron pool (Hyngstrom, Johnson, Miller & Heckman, 2007). Thus, in the current study it is possible that the decrease in spinal excitability during the flexion phase of BWD cycling resulted from increased inhibition from the brachioradialis (Naito et al., 1996), pronator teres (Naito et al., 1998), or triceps brachii, to the biceps brachii motoneuron pool. This would reduce the discharge rate of the biceps brachii motoneuron pool and consequently decrease MEP and CMEP responses. In fact, previous research has shown that an inhibitory reflex pathway exists between

brachioradialis group I afferents and inhibitory interneurons in humans which synapse onto the biceps brachii motoneuron pool (Barry, Riley, Pascoe & Enoka, 2008). Also, Naito et al., (1998) demonstrated that an oligosynaptic inhibitory pathway exists between the pronator teres nerve and the biceps brachii motoneuron pool in humans. However, more research is needed regarding the influence of these muscles on the excitability of the biceps brachii motoneuron pool.

While CMEPs represent changes occurring at the spinal level it is possible that the recorded MEPs were influenced by changes occurring at the supraspinal level. Zelenin et al., (2011) examined the activity of motor cortex neurons in cats during BWD locomotion and compared it with that during FWD locomotion. There was no significant difference between FWD and BWD walking with respect to the mean level of activity of motor cortex neurons, concluding that BWD walking does not require higher or lower cortical activity compared to FWD walking (Zelenin et al., 2011). In the current study the MEP/CMEP ratio, which provides a measure of supraspinal excitability (Gandevia, Petersen, Butler & Taylor, 1999), calculated for the biceps brachii was not significantly different between FWD and BWD cycling. Thus, the changes in MEP amplitudes between FWD and BWD cycling were mainly of spinal origin. In fact, Ugawa et al., (1995) has shown that the excitability of the biceps brachii is altered mainly by spinal mechanisms at low contraction intensities. In the current study participants cycled at a power output of 25 W, thus it is likely the contraction intensity of the biceps brachii was low. However, whether the decrease in spinal excitability during the flexion phase of BWD cycling and the extension phase of FWD cycling was due to greater inhibition from the triceps brachii, brachioradialis or pronator teres, greater inhibition from descending sources or from changes in the intrinsic properties of the biceps brachii motoneurons remains unknown.

3.4.1 Direction-dependent modulation of corticospinal and spinal excitability to the triceps brachii

In this study we recorded the activity of the lateral head of the triceps brachii, which is a monoarticular muscle that extends the elbow. We hypothesized that for both the triceps and biceps brachii CSE would be lower during BWD cycling compared to FWD. Although this was shown for the biceps brachii during the flexion phase, the triceps brachii provided some interesting results. In support of our hypothesis there was a main effect of cycling direction such that CSE to the triceps brachii was higher during FWD cycling compared to BWD cycling. However, unlike for the biceps brachii, paired t-tests revealed no significant difference in MEP amplitudes between FWD and BWD cycling when flexion and extension phases were compared. In contrast, the modulation of spinal excitability did depend on the phase of the movement cycle such that during the flexion phase spinal excitability was higher during FWD cycling compared to BWD cycling and during the extension phase spinal excitability was higher during BWD cycling compared to FWD cycling.

The results for the modulation of CSE to the triceps brachii contrast with the findings for the biceps brachii as overall CSE to the biceps brachii was modulated relative to the functional phase of the movement cycle. Unlike for the biceps brachii, there was no significant difference in pre-stimulus EMG prior to MEPs or CMEPs during the flexion and extension phases between FWD and BWD cycling or between the 6 and 12 o'clock position for FWD or BWD cycling for the triceps brachii. Therefore, the output of the motoneuron pool prior to MEPs and CMEPs was not different between the two phases of the movement cycle or between the two cycling directions. The dissociation between changes in spinal excitability and pre-stimulus EMG suggests that the differences in CMEP amplitudes are not resulting from differences in central motor drive but rather are due to changes in spinal excitability.

The results from the current study show that when FWD and BWD cycling are compared spinal excitability to the triceps brachii was higher at the 6 o'clock position (flexion during FWD and extension during BWD) compared to the 12 o'clock position (extension during FWD and flexion during BWD), despite no differences in pre-stimulus EMG. A previous study in our lab showed that during FWD cycling spinal excitability to the triceps brachii was higher during elbow flexion (6 o'clock position) than elbow extension (12 o'clock position) (Spence et al., 2016). This was an unexpected outcome as pre-stimulus EMG of the triceps brachii was higher at the 12 o'clock position (Spence et al., 2016). This coincides with the current finding that spinal excitability was higher during the flexion phase of FWD cycling (6 o'clock position) compared to BWD cycling (12 o'clock position). Spence et al., (2016) explained several mechanisms that could account for this finding, one mechanism being input from Ia afferents. For example, during the flexion phase of FWD cycling the triceps brachii muscle is stretched as the elbow is flexed which could result in an increased activation of Ia afferents (Spence et al., 2016). This would increase the excitatory input into the triceps brachii motoneuron pool which could increase the recruitment of spinal motoneurons and the activation of persistent inward currents (PICs), which amplify synaptic input, resulting in higher amplitude responses (Spence et al., 2016). However, in the current study at the 12 o'clock position during BWD cycling the triceps brachii was also stretched as the elbow was flexed yet spinal excitability was higher during the flexion phase of FWD cycling at the 6 o'clock position. This suggests that there is likely another mechanism aside from the stretch of the triceps brachii during the elbow flexion phase that is contributing to the increase in spinal excitability at the 6 o'clock position.

Another mechanism that could partially account for the differences in spinal excitability between FWD and BWD cycling is differences in excitatory and inhibitory afferent

feedback and the amount of synaptic input to the motoneuron pool. For example, flexion of the shoulder at the 12 o'clock position may result in an increase in inhibitory synaptic input to the triceps brachii motoneuron pool from the biceps brachii, brachioradialis or other ascending or descending sources, which decreases the intrinsic excitability of the motoneurons and decreases CMEP amplitudes. Notably, Sato et al., (2018) reported that inhibitory oligosynaptic connections mediated by Ia afferents exist between the brachioradialis and the triceps brachii motoneuron pool. Also, it is possible that during the flexion phase of BWD cycling there is greater pre-synaptic inhibition of Ia afferents to the triceps brachii motoneuron pool compared to the flexion phase of FWD cycling. This could also explain the decrease in CMEP amplitudes during the flexion phase of BWD cycling.

Unlike for the biceps brachii, the reduction in spinal excitability to the triceps brachii during BWD cycling in the current study is unlikely to result solely from peripheral factors arising from differences in shoulder position. This is because in the current study we recorded the activity of the lateral head of the triceps brachii, which is a monoarticular muscle that does not cross the shoulder joint and is primarily responsible for extending the elbow (Ali et al., 2014). Thus, it is unlikely that shoulder flexion would alter the muscle membrane properties (ie. muscle fiber length and diameter) to an extent that would influence the recorded responses. Thus, it is plausible that the changes in spinal excitability resulted from changes in the intrinsic properties of the spinal motoneurons or from differences in afferent feedback to the triceps brachii motoneuron pool.

3.4.2 Phase-Dependent modulation of corticospinal and spinal Excitability during FWD and BWD cycling

During FWD cycling CSE and spinal excitability to the biceps brachii was significantly higher during the elbow flexion phase (6 o'clock) compared to the elbow extension phase (12 o'clock). In contrast, during BWD cycling there was no phase-dependent modulation of CSE or spinal excitability, such that MEPs and CMEPs were not different between the 6 and 12 o' clock phases of BWD cycling. This corresponds with the finding that during FWD cycling pre-stimulus EMG of the biceps brachii was modulated relative to the phase of the movement cycle but during BWD cycling there was no phase-dependent modulation of pre-stimulus EMG observed. This could suggest that during FWD cycling the biceps brachii has a monophasic activation pattern such that there is a high level of activation at the 6 o'clock position and a low level of activation at the 12 o'clock position, which has been shown previously (Spence et al., 2016). In contrast, during BWD cycling the biceps brachii could have a biphasic activation pattern such that there isn't distinct "on" and "off" periods of muscle activation but rather the level of activation is similar at both the 6 and 12 o'clock positions.

Zehr & Hundza (2005) showed that during FWD and BWD arm cycling background EMG for the biceps and triceps brachii was significantly modulated by phase in the movement cycle and was significantly higher during BWD cycling for the biceps brachii. This was not found in the current study as the only reported difference in pre-stimulus EMG between FWD and BWD cycling was that pre-stimulus EMG prior to CMEPs for the biceps brachii was significantly higher during FWD cycling. The differences between the current study and the study by Zehr and Hundza (2005) could be due to differences in methodology as participants in their study were cycling against 0.5-1.0 kpm (kilopond-metres), whereas in the current study a 25 W resistance was used. Also, in the study by Zehr and Hundza (2005) EMG and reflex

responses were compared based on the position of the arm whereas in the current study EMG and evoked responses were compared based on the functional phase of the movement cycle (flexion and extension).

3.4.3 Muscle-dependent modulation of corticospinal excitability

Past research has shown that the modulation of CSE is muscle-dependent. In the current study we showed that the modulation of CSE to the biceps brachii is phase-dependent for FWD cycling and was dependent on the phase of the movement cycle when FWD and BWD cycling were compared. However, for the triceps brachii CSE was not phase-dependent for FWD or BWD cycling and did not depend on the phase of the movement cycle when the two cycling directions were compared. Interestingly, Spence et al., (2016) also reported no phase-dependent modulation of CSE to the triceps brachii. This suggests that flexor muscles (ie. biceps brachii) may be under greater cortical control than extensor muscles (Power, Lockyer, Forman & Button, 2018). This is supported by research that has shown that there are a larger number of monosynaptic connections between cortical neurons and flexor motoneuron pools than extensor motoneuron pools (Brouwer & Ashby, 1990). Also, researchers have reported that flexor and extensor motoneurons have different intrinsic properties. In fact, studies using animal models have shown that there is a greater incidence of PICs in extensor motoneurons compared to flexor motoneurons (Cotel, Antri, Barthe & Orsal, 2009). Also, Wilson, Thompson, Miller & Heckman (2015) showed that in humans there is a greater incidence of PICs in the triceps brachii motor units compared to the biceps brachii motor units. A PIC is a persistent inward flow of calcium and sodium ions that amplifies synaptic input and causes self-sustained firing of the motoneuron (Heckman, 2003). Thus, with the activation of PICs less synaptic input from the motor cortex

and other descending sources is required (Power et al., 2018). Thus, extensor motoneurons require less input from supraspinal centers in comparison to flexor motoneurons.

3.5 Methodological considerations

In the current study there are several factors that should be considered when interpreting the results. To compare FWD and BWD cycling we chose to compare MEP and CMEP responses relative to the functional phase (elbow flexion and extension) of the movement cycle, as was done in the study by Zehr et al., (2009). To compare MEP and CMEP responses during the flexion phase of FWD and BWD arm cycling responses were examined at the 6 o'clock position during FWD cycling and were compared with responses at the 12 o'clock position during BWD cycling. To compare MEP and CMEP responses during the extension phase of FWD and BWD cycling responses were examined at the 12 o'clock position during FWD cycling and compared with responses at the 6 o'clock position during BWD cycling. The 6 and 12 o'clock positions differ with respect to the position of the shoulder, such that at the 12 o'clock position the shoulder is flexed to ~ 90° whereas the shoulder is at ~ 0° flexion at the 6 o'clock position. Thus, comparing changes in CSE at 0° and 90° may be problematic for several reasons. For example, at the 12 o'clock position the muscle length changes (mainly the biceps brachii as it crosses the shoulder joint) which can alter the orientation of the recording electrodes relative to the muscle and can result in the recording of a different area of the motor units compared to the 6 o'clock position. Also, in the current study stimulation intensities for TMS, TMES and Erb's point were set during FWD cycling at the 6 o'clock position. Collins et al., (2017) showed that M_{max} amplitude is shoulder position-dependent and significantly increased when the shoulder position was changed from 0° to 90° flexion. Thus, when comparing MEP and CMEP amplitudes, that are normalized to $M_{max,}$ between the 6 and 12 o'clock position, as was

done in the current study, it is possible that the excitability of the corticospinal tract was under- or overestimated. Another factor that should be considered when comparing MEP and CMEP amplitudes between FWD and BWD arm cycling is pre-stimulus EMG. In this study pre-stimulus EMG was not matched between FWD and BWD arm cycling and therefore the differences found between FWD and BWD arm cycling could have been due to differences in background EMG. However, there were no significant correlations between MEP and CMEP amplitudes and background EMG for FWD or BWD arm cycling.

3.6 CONCLUSION

The current study demonstrates that CSE and spinal excitability to the biceps and triceps brachii during arm cycling is direction-dependent. Spinal excitability to the biceps and triceps brachii was higher during the flexion phase of FWD cycling compared to BWD cycling but higher during the extension phase of BWD cycling compared to FWD. Overall CSE to the biceps brachii showed the same modulation pattern as spinal excitability, however for the triceps brachii CSE was higher during FWD cycling compared to BWD but did not depend on the phase of the movement cycle. As expected CSE and spinal excitability to the biceps brachii and spinal excitability to the triceps brachii was phase-dependent during FWD cycling, however it was not phase-dependent during BWD cycling. These findings suggest that the neural control of the biceps and triceps brachii may be different between FWD versus BWD cycling. Whether the decrease in spinal excitability to the biceps and triceps brachii motoneuron pools during the extension phase of FWD cycling and the flexion phase of BWD cycling is related to inhibitory input from heteronymous muscles or from changes in the intrinsic motoneuron properties remains unknown. Further investigation is needed to determine the underlying mechanisms contributing to the observed differences in CSE between FWD and BWD cycling.

3.7 FIGURE LEGEND

Figure 1. Schematic representing the cycling directions, arm crank positions and corresponding functional phases of the movement cycle (flexion and extension). Arm crank positions are shown with 6 o'clock representing "bottom dead center" and 12 o'clock representing "top dead center". The functional phases of the movement cycle are labeled on the diagram with the black arrows representing elbow extension and the white arrows representing elbow flexion. The arrows on the outside of the circle indicate FWD cycling and the arrows on the inside of the circle indicate BWD cycling. The table shows the functional phases of the movement cycle and corresponding arm crank positions for both FWD and BWD cycling.

Figure 2. (A) Biceps brachii representative examples (n = 1) of MEPs at the 6 and 12 o'clock position during FWD and BWD cycling. Average MEP traces during the flexion phase (6 o'clock FWD and 12 o'clock BWD) and during the extension phase (12 o'clock FWD and 6 o'clock BWD). In this example MEP amplitudes were 19.4 % and 8.6 % for FWD at 6 o'clock and BWD at 12 o'clock, respectively, and 1.5 % and 4.5 % for FWD at 12 o'clock and BWD at 6 o'clock, respectively. **(B)** Biceps brachii representative example (n = 1) of CMEPs at the 6 o'clock and 12 o'clock position during FWD and BWD cycling. In this examples CMEP amplitudes were 15.3 % and 6.7 % for FWD at 6 o'clock and BWD at 12 o'clock, respectively, and 1.2 % and 5.8 % for FWD at 12 o'clock and BWD at 6 o'clock, respectively.

Figure 3. (A) Group data (mean ± SE, n = 12) MEP amplitudes as a percentage of M_{max} of the biceps brachii during FWD and BWD cycling. During FWD cycling at the 6 o'clock and 12 o'clock positions average MEP amplitudes were 19.67% and 1.28% of M_{max}, respectively. During BWD cycling at the 6 o'clock and 12 o'clock position average MEP amplitudes were 5.86% and 8.48% of M_{max}, respectively. **(B)** Average biceps brachii pre-stimulus EMG prior to TMS. Black bars represent BWD cycling and white bars represent FWD cycling. * denote a significant difference ($p < .05$) for cycling direction, while the # denotes a significant difference for position (6 vs 12 o'clock; $p < .05$).

Figure 4. (A) Group data (mean ± SE, $n = 11$) for CMEP amplitudes as a percentage of M_{max} of the biceps brachii during FWD and BWD cycling. During FWD cycling at the 6 o'clock and 12 o'clock positions average CMEP amplitudes were 16.90% and 1.78% of M_{max}, respectively. During BWD cycling at the 6 o'clock and 12 o'clock positions average CMEP amplitudes were 5.62% and 7.49%, respectively. (B) Average biceps brachii pre-stimulus EMG prior to TMES. Black bars represent BWD cycling and white bars represent FWD cycling. * denotes a significant difference ($p < .05$) for cycling direction, while # denotes a significant difference for position (6 vs 12 o'clock; $p < .05$).

Figure 5. Group data (mean ± SE, $n = 11$) for MEP amplitudes, normalized to CMEPs evoked during the same trial, for the biceps brachii during FWD and BWD cycling. Black bars represent BWD cycling and white bars represent FWD cycling

Figure 6. **(A)** Triceps brachii representative examples ($n = 1$) of MEPs at the 6 and 12 o'clock position during FWD and BWD cycling. Average MEP traces during the flexion phase (6 o'clock FWD and 12 o'clock BWD) and during the extension phase (12 o'clock FWD and 6 o'clock BWD). In this example MEP amplitudes were 19.9 % and 8.1 % for FWD at 6 o'clock and BWD at 12 o'clock, respectively, and 9.7 % and 8.1 % for FWD at 12 o'clock and BWD at 6 o'clock, respectively. **(B)** Triceps brachii representative example ($n = 1$) of CMEPs at the 6 o'clock and 12 o'clock position during FWD and BWD cycling. In this examples CMEP amplitudes were 21.4% and 13.0 % for FWD at 6 o'clock and BWD at 12 o'clock, respectively, and 3.9 % and 11.3 % for FWD at 12 o'clock and BWD at 6 o'clock, respectively.

Figure 7. (A) Group data (mean ± SE, $n = 12$) for MEP amplitudes as a percentage of M_{max} of the triceps brachii during FWD and BWD cycling. During FWD cycling at the 6 o'clock and 12 o'clock positions average MEP amplitudes were 18.01% and 9.87% of M_{max}, respectively. During BWD cycling at the 6 o'clock and 12 o'clock position average MEP amplitudes were 8.31% and 8.84% of M_{max}, respectively. **(B)** Average triceps brachii pre-stimulus EMG prior to TMS. Black bars represent BWD cycling and white bars represent FWD cycling. * denote a

significant difference ($p < .05$) for cycling direction, while the # denotes a significant difference for position (6 vs 12 o'clock; $p < .05$).

Figure 8. (A) Group data (mean ± SE, $n = 11$) for CMEP amplitudes as a percentage of M_{max} of the triceps brachii during FWD and BWD cycling. During FWD cycling at the 6 o'clock and 12 o'clock positions average CMEP amplitudes were 20.09% and 5.56% of M_{max}, respectively. During BWD cycling at the 6 o'clock and 12 o'clock positions average CMEP amplitudes were 10.02 % and 13.99 %, respectively. (B) Average triceps brachii pre-stimulus EMG prior to TMES. Black bars represent BWD cycling and white bars represent FWD cycling. * denotes a significant difference ($p < .05$) for cycling direction, while # denotes a significant difference for position (6 vs 12 o'clock; $p < .05$).

Figure 9. Group data (mean ± SE, $n = 11$) for MEP amplitudes, normalized to CMEPs evoked during the same trial, for the triceps brachii during FWD and BWD cycling. Black bars represent BWD cycling and white bars represent FWD cycling * denotes a significant difference ($p < .05$) for cycling direction.

3.8 LIST OF TABLES

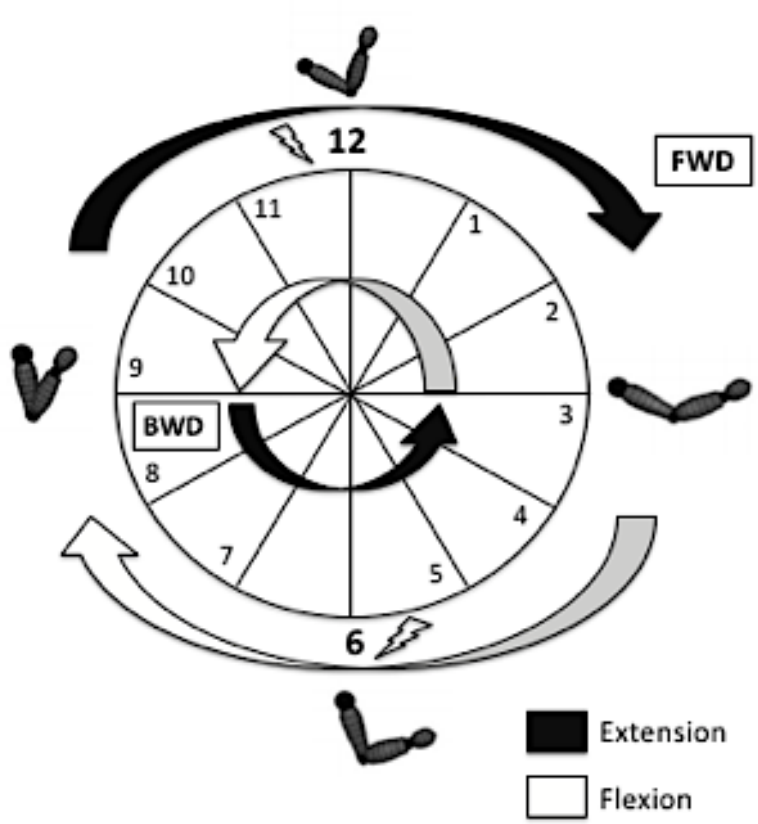
Table 1 Raw and normalized data for the biceps brachii. MEP, CMEP, M_{max} and pre-stimulus EMG values during the flexion and extension phases of FWD and BWD cycling.

			Forward		**Backward**	
			6 o'clock	*12 o'clock*	*6 o'clock*	*12 o'clock*
MEPs						
	Pre-EMG (μV)		30.9 ± 4.25	19.4 ± 4.06	20.9 ± 4.05	27.8 ± 3.87
	Latency, (ms)		11.7 ± 0.18	12.6 ± 0.32	12.3 ± 0.21	12.2 ± 0.17
	Amplitude	*mv*	2.4 ± 0.31	0.2 ± 0.03	0.7 ± 0.14	1.15 ± 0.25
		% M_{max}	19.6 ± 1.45	1.3 ± 0.20	5.9 ± 1.31	8.5 ± 1.67
CMEPs						
	Pre-EMG		36.2 ± 6.08	18.1 ± 4.42	20.7 ± 4.62	26.8 ± 4.75
	Latency		8.0 ± 0.29	7.7 ± 0.38	7.9 ± 0.3	7.9 ± 0.29
	Amplitude	*mv*	2.0 ± 0.24	0.2 ± 0.07	0.7 ± 0.15	1.0 ± 0.17
		% M_{max}	16.9 ± 1.47	1.8 ± 0.49	5.6 ± 0.90	7.5 ± 1.20
M_{max}						
	Amplitude (mv)		12.3 ± 1.11	13.4 ± 1.18	13.1 ± 1.01	14.2 ± 1.07

Table 2 Raw and normalized data for the triceps brachii. MEP, CMEP, M_{max} and pre-stimulus EMG values during the flexion and extension phases of FWD and BWD cycling.

			Forward		**Backward**	
			6 o'clock	*12 o'clock*	*6 o'clock*	*12 o'clock*
MEPs						
	Pre-EMG (μV)		21.9 ± 3.14	24.3 ± 2.63	22.5 ± 3.09	21.6 ± 2.69
	Latency (ms)		11.9 ± 0.23	11.9 ± 0.26	11.6 ± 0.28	12.4 ± 0.19
	Amplitude	*mv*	0.6 ± 0.13	0.4 ± 0.06	0.4 ± 0.05	0.4 ± 0.04
		% M_{max}	18.0 ± 5.43	9.9 ± 1.91	8.3 ± 1.60	8.8 ± 1.43
CMEPs						
	Pre-EMG(μV)		21.9 ± 3.12	25.9 ± 3.49	21.8 ± 3.21	21.2 ± 3.08
	Latency (ms)		7.9 ± 0.20	7.5 ± 0.23	7.2 ± 0.29	7.8 ± 0.17
	Amplitude	*mv*	0.8 ± 0.09	0.2 ± 0.04	0.5 ± 0.06	0.6 ± 0.08
		% M_{max}	20.1 ± 4.47	5.6 ± 1.50	10.0 ± 1.85	13.9 ± 3.02
M_{max}						
	Amplitude (mv)		4.3 ± 0.36	4.4 ± 0.5	4.8 ± 0.4	4.5 ± 0.47

3.9 LIST OF FIGURES



FWD	BWD	Phase
9	3	Extension
10	4	
11	5	
12	**6**	
1	7	
2	8	
5	9	Flexion
4	10	
5	11	
6	**12**	
7	1	
8	2	

Figure 1 Schematic representing the arm cycling directions, arm crank positions and corresponding functional phases (flexion vs. extension) of the movement cycle.

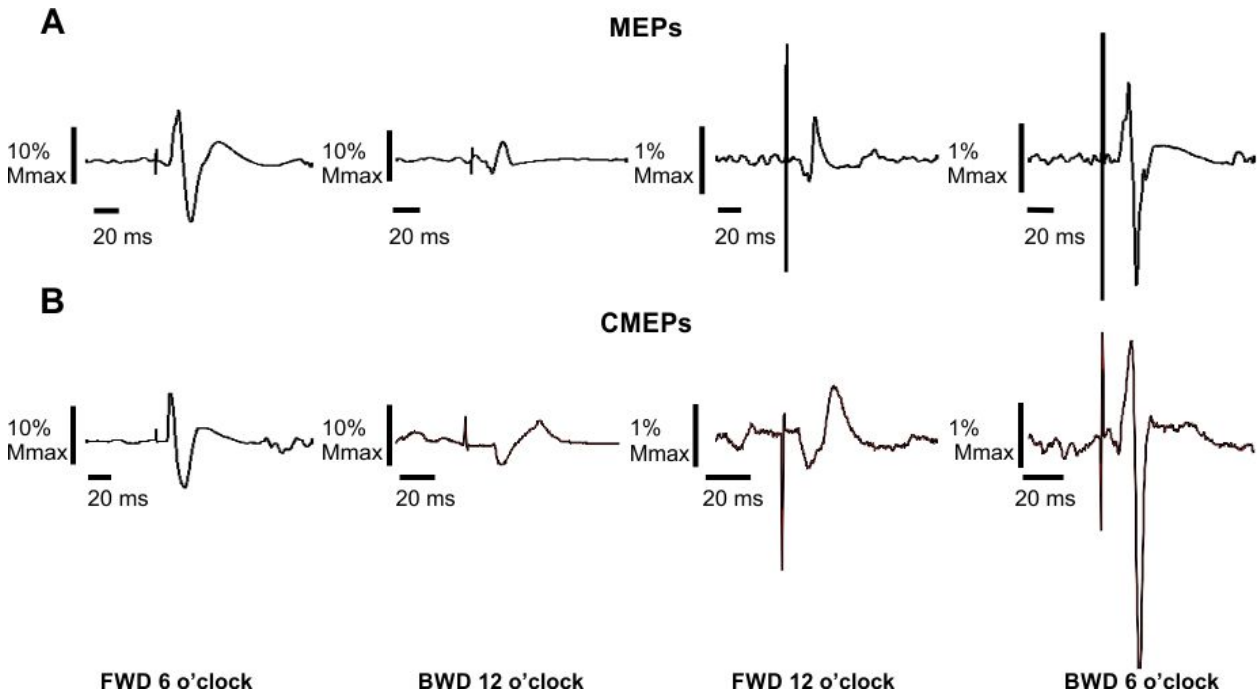


Figure 2 Representative MEP and CMEP amplitudes from the biceps brachii during FWD and BWD cycling at the 6 and 12 o'clock positions.

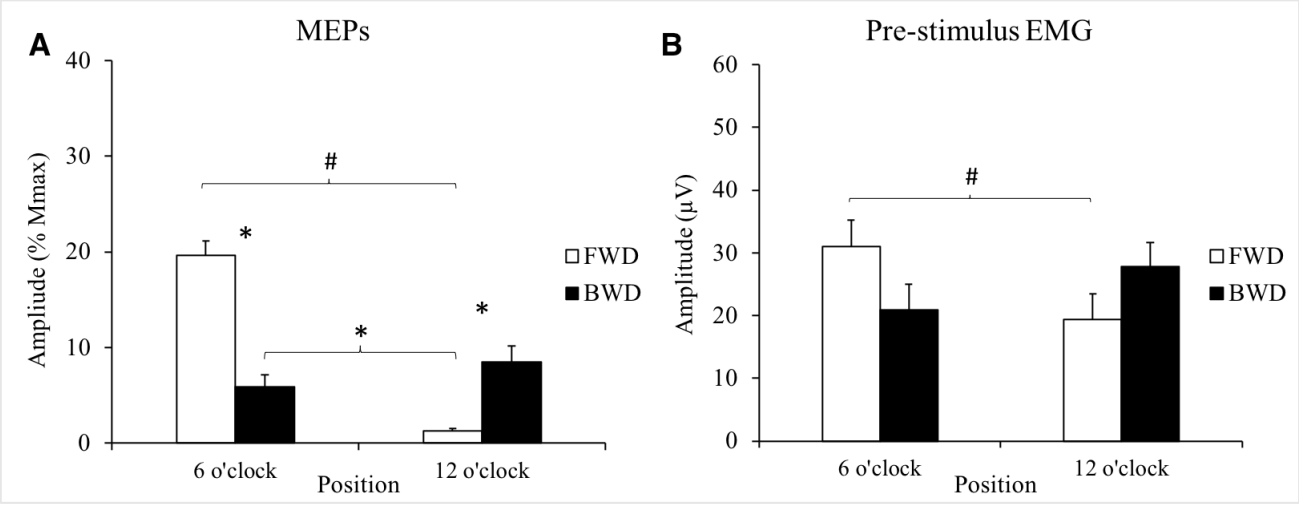


Figure 3. Group MEP amplitudes and pre-stimulus EMG prior to TMS from the biceps brachii during FWD and BWD cycling.

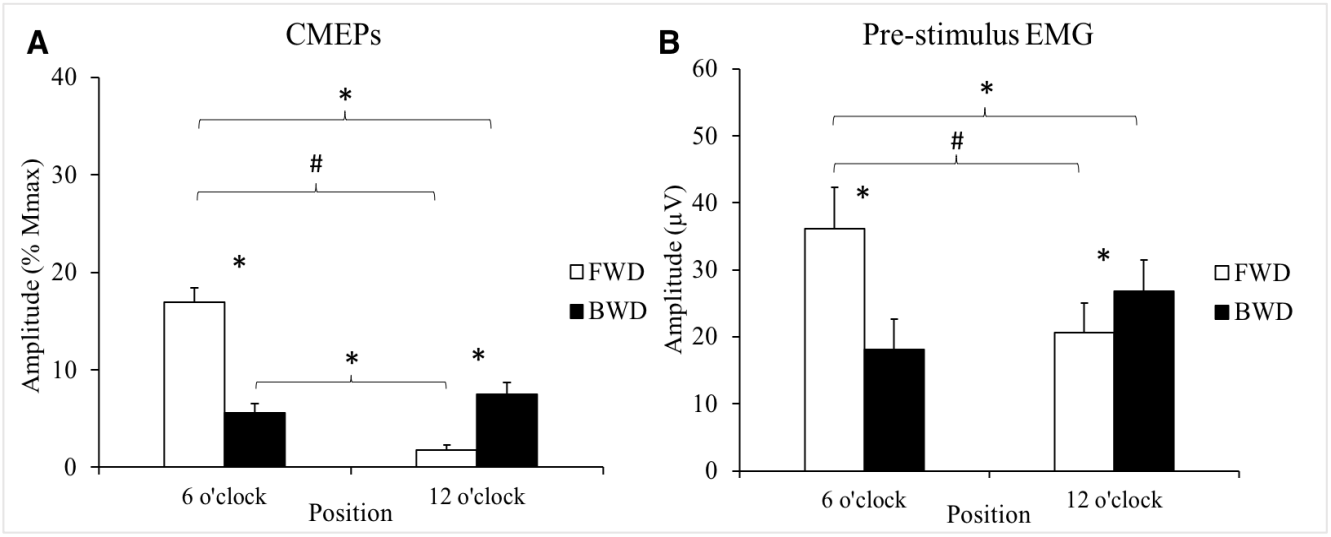


Figure 4 Group CMEP amplitudes and pre-stimulus EMG prior to TMES from the biceps brachii during FWD and BWD cycling at the 6 and 12 o'clock positions.

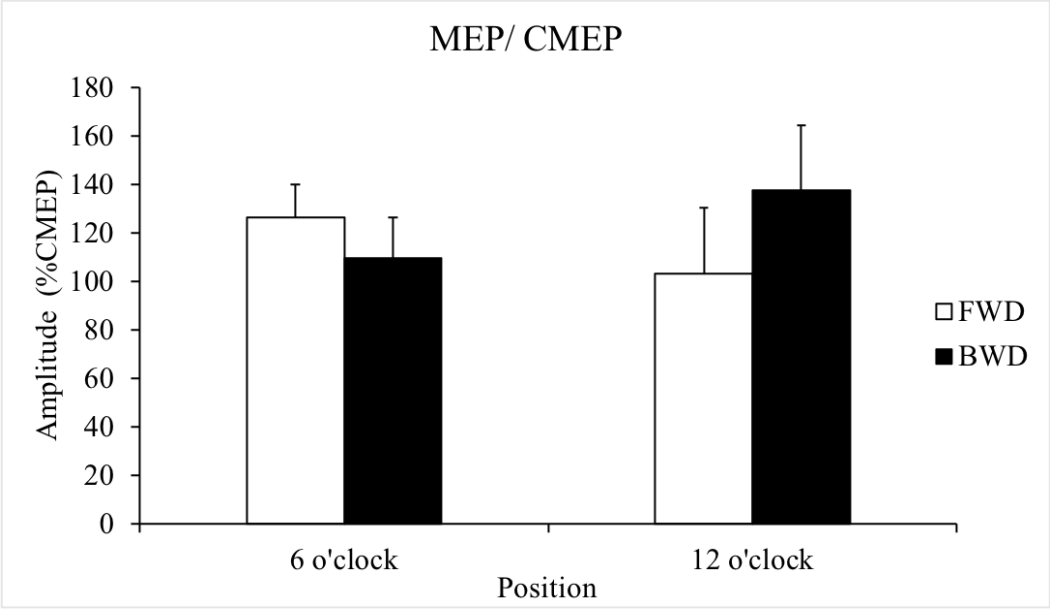


Figure 5 Group MEP amplitudes as a percentage of CMEP for the biceps brachii during FWD and BWD cycling at the 6 and 12 o'clock positions.

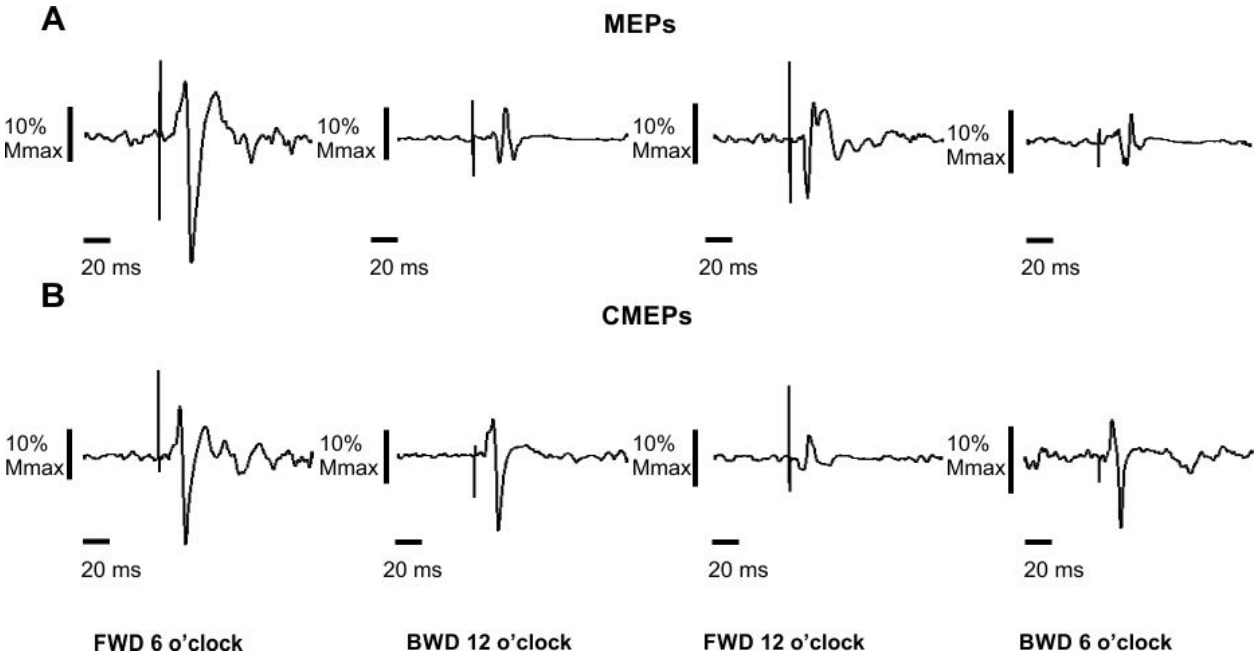


Figure 6 Representative MEP and CMEP amplitudes from the triceps brachii during FWD and BWD cycling at the 6 and 12 o'clock positions.

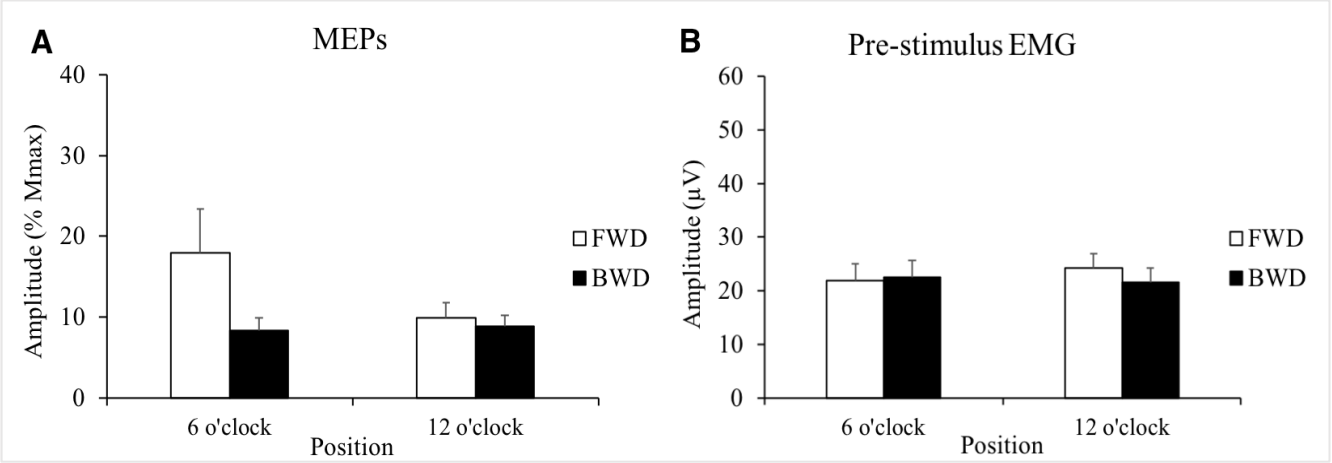


Figure 7 Group MEP amplitudes and pre-stimulus EMG prior to TMS from the triceps brachii for FWD and BWD cycling at the 6 and 12 o'clock positions.

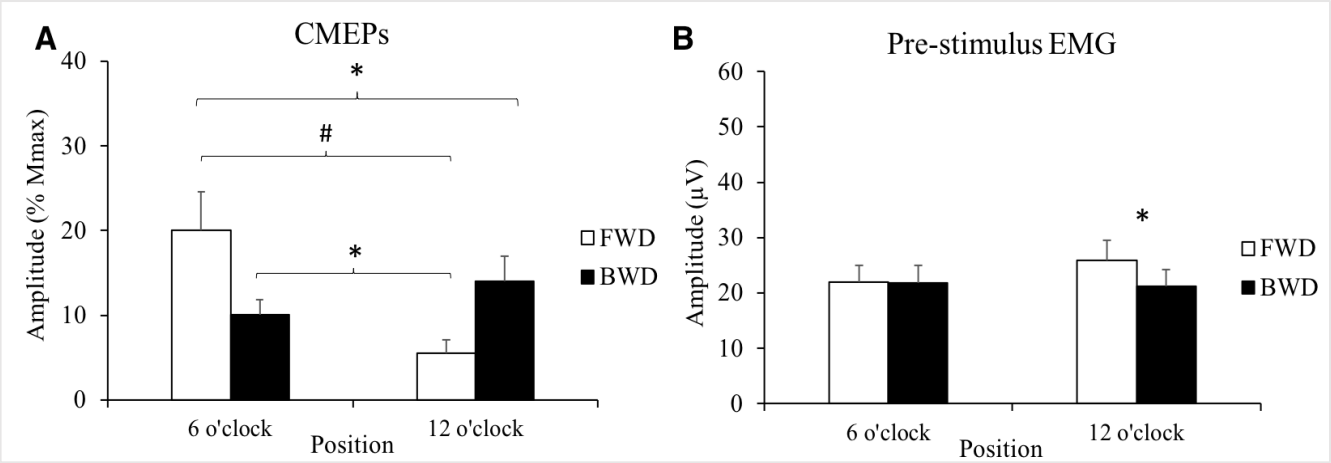


Figure 8 Group CMEP amplitudes and pre-stimulus EMG prior to TMES from the triceps brachii for FWD and BWD cycling at the 6 and 12 o'clock positions.

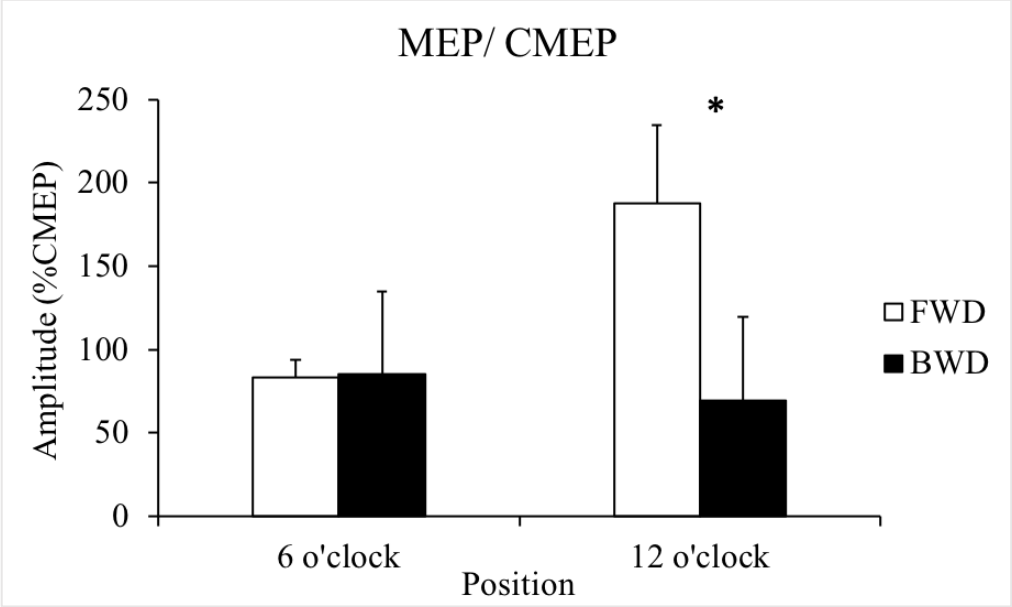


Figure 9 Group MEP amplitudes as a percentage of CMEP for the triceps brachii during FWD and BWD cycling at 6 and 12 o'clock positions.

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Chapter 4 Future Directions

The aim of this study was to further the understanding of how the brain and spinal cord contribute to the neural control of FWD and BWD locomotor outputs. In this study, we examined the modulation of CSE and spinal excitability to the biceps and triceps brachii muscles during FWD and BWD arm cycling. The results from this study suggest that the modulation of CSE and spinal excitability during arm cycling is dependent on cycling direction and dependent on the muscle being examined. Since this was the first study to compare CSE and spinal excitability between FWD and BWD cycling we chose to only examine the responses at one cadence (60 rpm) and one workload (25 W). Thus, further research should examine how CSE and spinal excitability are modulated during FWD and BWD cycling with an increase in cadence and workload. Also, researchers should attempt to characterize how CSE and spinal excitability to other upper limb muscles are modulated during FWD and BWD cycling. One issue with the current study is that we only recorded evoked responses from the dominant arm. Arm cycling, like locomotion, is a bilateral motor output and therefore the activity of the non-dominant arm should also be examined in future studies. Examining CSE and spinal excitability of different muscles and at different cadences and workloads will provide a more extensive understanding of the neural control of FWD and BWD rhythmic movement. It will also provide further insight into the potential mechanisms leading to the differences in CSE and spinal excitability between FWD and BWD rhythmic movement.

This study used TMS and TMES to examine corticospinal and spinal excitability, respectively. Although these techniques allow researchers to determine if changes are occurring at the suprapsinal or spinal level, they do not allow researchers to make conclusions about the

exact mechanisms leading to the modulation of CSE and spinal excitability. Rather, researchers must make assumptions and speculation about the potential mechanisms involved. Thus, future research should use other techniques that provide a more thorough understanding of the mechanisms contributing to the modulation of CSE. One technique that could be used is paired pulse TMS which provides researchers with more information on the modulation of supraspinal excitability.