Corticospinal excitability and interconnectivity of the limbs during arm cycling

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Abstract

There is evidence of neural interlimb communication between the fore and hind limbs in quadrupeds and the arms and legs in humans. Appropriate coordination between upper and lower limbs is an important characteristic of quadrupedal and human locomotion and part of this coordination involves cortical and spinal control. This communication between the cortex and spinal cord suggests that neural coupling between the upper and lower limbs occurs during rhythmic movement. Most studies aimed at assessing interlimb coordination during locomotor output have utilized H- reflexes and/or surface EMG. However, the effects of rhythmic locomotor output (arm and/or leg cycling) on corticospinal excitability of stationary limbs (arm and/or leg) has yet to be clearly examined. In addition, it seems interlimb pathways probably modulate cortical and spinal pathways during locomotor tasks as a function of movement intensity. The current study demonstrated an intensity-dependent enhancement in corticospinal excitability to the resting biceps brachii and vastus lateralis during leg and arm cycling, respectively. Assessing of interlimb coordination between upper and lower limbs may improve knowledge translation to neurological rehabilitation programs.

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Abbreviations

AMT – Active motor threshold

CMEP – Cervicomedullary evoked motor potentials

CPG – Central pattern generator

CST – Corticospinal tract

EMG – Electromyography

MC – Motor cortex

MEP – Motor evoked potential

RMT – Resting motor threshold

TMES – Transmastoid electrical stimulation

TMS – Transcranial magnetic stimulation

Chapter 1 Introduction

1.1 Overview

Appropriate coordination between the upper and lower limbs is an important characteristic of quadrupedal and human locomotion. This coupling of upper and lower limbs and the well-regulated movement between them involves complex neural processes coordinated by the central nervous system (CNS). Previous studies suggest that neural networks in the cervical and lumbar spinal cord are responsible for regulating the coordination between the fore and hindlimbs during locomotion in quadrupeds (Dietz, 2002; Duysens & Van de Crommert, 1998).

In humans, studies have examined the neural interaction between upper and lower limbs during locomotor output by assessing changes in electromyography activity (EMG) and/or reflexes in the arm and leg muscles (Balter & Zehr, 2007; Haridas & Zehr, 2003). For example, Frigon et al. (2004) demonstrated that soleus H-reflexes were significantly decreased during arm cycling compared to a static arm position. Suppression of H-reflex amplitude in the soleus muscle was likely induced by activation of presynaptic inhibition. Such a suppression indicates neural coupling between arms and legs during locomotor output (Frigon et al., 2004). In another study H-reflex suppression during arm cycling was greater at a cadence of 2 Hz vs 1Hz. Thus, H-reflex suppression is cadence-dependent, indicating that as the intensity of locomotor output increases so too does presynaptic inhibition of the IA afferents (Hundza & Zehr, 2009; Loadman & Zehr, 2007). In summary, the interconnection between the upper and lower limbs is motor-task and intensity-dependent.

Huang and Ferris (2004) demonstrated that EMG activity increased in the lower limbs when participants use their upper limbs to drive their relaxed lower limbs (i.e. self-driven position) compared with when the arms were at rest and another person drove their lower limbs (i.e. externally driven) and externally driven arms and legs (another person drove relaxed upper and lower limbs through the stepping motion). This indicates that connections between the upper and lower limb are capable of facilitating motoneurone firing as locomotor intensity (i.e. resistance) increased. The neural link between the upper- and lower-limbs is mediated by a dense network of neurons known as propriospinal neurons, that relay information between spinal segments (Huang & Ferris, 2004).

Prior work has shown that rhythmic locomotor movement (e.g., leg and arm cycling) does affect corticospinal excitability to the upper and or lower limbs (stationary and/or static conditions (Zehr et al., 2007). However, it is unclear whether there are differences in the relative contributions of cortical and spinal control during rhythmic movement (Petersen et al., 2003). For example, Zehr et.al (2007) investigated the effect of rhythmic leg cycling and a static leg position on corticospinal excitability in the contracted and relaxed flexor carpi radialis (FCR). They showed corticospinal excitability in the FCR was higher during leg cycling compared to the static leg condition when the arms (FCRs) were at rest and isometrically contracting. Higher corticospinal excitability could be due to mechanisms at the cortical and/or spinal level. Again, this intersegmental facilitation of neural excitability is likely mediated by activation of the propriospinal pathways that have excitatory connections projecting directly to motoneurons (Zehr et al., 2007).

Based on prior work, it is clear that there are neural connections between the upper and lower limbs during locomotor tasks. This neural linkage involves complex neural processes coordinated by the CNS. In an attempt to further understand neuronal coordination between the upper and lower limbs in humans, specific non-invasive methods are used to assess the interaction between the upper and lower limbs during locomotor output, including surface EMG (Huang &

Ferris, 2004), reflex modulation (Loadman & Zehr, 2007) and, transcranial magnitude stimulation (TMS) (Zehr et al., 2007), Thus, we hypothesized that corticospinal (brain and spinal cord) excitability in the vastus lateralis and in the biceps brachii will increase during arm cycling and leg cycling, respectively. Further, corticospinal excitability will be higher in the vastus lateralis and in the biceps brachii during arm cycling and leg cycling, respectively as intensity increases.

1.2 Purpose

To examine the effect of arm and leg cycling (at two intensities 25 and 50% of peak power output) on corticospinal excitability to the resting vastus lateralis and biceps brachii, respectively.

1.3 Research Hypotheses

It was hypothesized that:

1. Corticospinal excitability to the vastus lateralis will increase during arm cycling.
2. Corticospinal excitability to the biceps brachii will increase during leg cycling.
3. Corticospinal excitability will be higher to the vastus lateralis during arm cycling as intensity increases.
4. Corticospinal excitability will be higher to the biceps brachii during leg cycling as intensity increases

1.4 References

Balter, J. E., & Zehr, E. P. (2007). Neural coupling between the arms and legs during rhythmic locomotor-like cycling movement. Journal of neurophysiology, 97(2), 1809-1818.

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

Duysens, J., & Van de Crommert, H. W. (1998). Neural control of locomotion; Part 1: The central pattern generator from cats to humans. Gait & posture, 7(2), 131-141.

Frigon, A., Collins, D. F., & Zehr, E. P. (2004). Effect of rhythmic arm movement on reflexes in the legs: modulation of soleus H-reflexes and somatosensory conditioning. Journal of neurophysiology, 91(4), 1516-1523.

Haridas, C., & Zehr, E. P. (2003). Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. Journal of neurophysiology, 90(5), 2850-2861.

Huang, H. J., & Ferris, D. P. (2004). Neural coupling between upper and lower limbs during recumbent stepping. Journal of Applied Physiology, 97(4), 1299-1308.

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

Loadman, P. M., & Zehr, E. P. (2007). Rhythmic arm cycling produces a non-specific signal that suppresses Soleus H-reflex amplitude in stationary legs. Experimental brain research, 179(2), 199-208.

Petersen, N. T., Pyndt, H. S., & Nielsen, J. B. (2003). Investigating human motor control by transcranial magnetic stimulation. Experimental brain research, 152(1), 1-16.

Zehr, E. P., Klimstra, M., Johnson, E. A., & Carroll, T. J. (2007). Rhythmic leg cycling modulates forearm muscle H-reflex amplitude and corticospinal tract excitability. Neuroscience letters, 419(1), 10-14.

2.0 Review of Literature

2.1 Introduction

There is a neural network known as the spinal central pattern generator (CPG) for locomotion in animals (Graham Brown, 1914) and humans (Calancie and others 1994) that when activated can produce rhythmic motor patterns such as walking, running, and swimming in the absence of sensory or descending input. For example, following spinal cord injury when the cortex is unable to communicate below the level of the injury, rhythmic activity of the legs is still possible suggesting the existence of a spinal CPG (Dietz, 2002). However, it is generally accepted that supraspinal commands and sensory feedback are essential for initiating and controlling locomotor movements in humans and animals (Geyer and Herr 2010; Song and Geyer 2015). In addition, according to recent studies using functional magnetic resonance imaging (Debaere et al., 2001), the supplementary motor area might be involved in the supraspinal control of this coupling of upper and lower limb movements. Precise control of interlimb coordination allows humans and animals to produce a variety of coordinated behaviors.

Interlimb coordination in humans shares features with quadrupeds, and it has been suggested that this is due to a similar neural circuitry (i.e. CPGs). The understanding of underlying mechanisms of interlimb coordination in human and quadrupedal locomotion is an interesting topic in neurophysiology research. To date, most studies aimed at examining interlimb coordination have focused on the coordination of left and right limbs produced by commissural circuitry in the spinal cord (Kiehn, 2006). However, much less is known about neural circuitry that coordinates the ipsilateral limbs (Falgairolle et al., 2006).

Arm and leg activity are coordinated during rhythmic motor outputs such as walking, running, and cycling. It has been shown that neural mechanisms between cervical and lumbosacral spinal cord control this interlimb coordination, yet the mechanisms modulating this interaction are unclear. In an attempt to further understand this, specific non-invasive methods are used to assess the interaction between the upper and lower limbs during locomotor output, including surface electromyography activity (EMG) (Huang & Ferris, 2004), reflex modulation (Loadman & Zehr, 2007), as well as transcranial magnetic stimulation (TMS) (Zehr et al., 2007). Thus, the aim of this review is to briefly discuss the neural control of rhythmic human movement and the mechanisms underlying interlimb coordination. I will focus on the effect of rhythmic locomotor movements on corticospinal excitability to the upper and or lower limbs.

2.2 Coordination between Upper and Lower Limbs

One of the main characteristics of humans is their ability to produce a variety of coordinated behaviours that involve the coupling between upper and/or lower limbs during locomotor outputs. Coordinated linkages between the arms and legs begins during infancy. It is part of normal human development and it is similar to quadrupedal locomotion.

Precise control of interlimb coordination allows humans and animals to produce a variety of coordinated behaviors in a continuously changing environment, maintain dynamic stability while changing speed, and/or when transitioning between movement patterns. This ability allows many coordinated movements, such as walking, cycling, and swimming to have synchronized or alternated movements of limb pairs (Frigon, 2017). In addition, previous studies have demonstrated that arm and leg connections are bi-directional.

Regarding the connection between the legs, studies demonstrated that both active and passive cycling of the contralateral leg reduces the soleus H-reflex in the ipsilateral leg (Collins et al. 1993; Cheng et al. 1998; Misiaszek et al. 1998). However, studies from arm-to-arm coupling conducted during rhythmic arm movement reported the coordination between two legs are stronger compared to the arms. For instance, Carroll and et.al (2005) measured cutaneous reflexes from the FCR during six arm cycling tasks by stimulating the superficial radial nerve at the wrist. Their results showed that activity of the contralateral arm had no effect on cutaneous reflexes in the ipsilateral arm during cycling movements (Carroll et al. 1998). It seems this weaker coupling between the arms compared to the legs, may be a result of evolution. Bipedal animals gained the ability to move about their environment using the lower limbs, thus allowing the upper limbs to be free for other activities. This freedom in the upper limb would thus not require such a strong interlimb connection for locomotor output (Carroll, Zehr, & Collins, 2005).

Most of the research has focused on understanding how the CNS controls homologous muscle coordination between the limbs (left and right). Commissural neurons project their axons across the midline of the spinal cord to contact neurons on the opposite side and are responsible for coordination of homologous (left and right) limbs (Kiehn, 2006). However much less is known about circuitry coordinating the ipsilateral limbs (upper and lower limbs). It has demonstrated that propriospinal neurons act to coordinate different parts of the body by linking motor circuits that control muscles across the forelimbs, trunk, and hind limbs in non-human animals (Falgairolle et al., 2006). In addition, another possible mechanism controlling coupling between the upper and lower limbs is activation of presynaptic inhibition of Ia afferents. In this regard, Nakajim et.al (2013) demonstrated that performing bilateral leg cycling suppressed FCR H-reflex amplitudes while participants kept a 10% MVC tonic contraction of FCR. Suppression of H-reflex amplitude in the FCR muscle during leg cycling was likely induced by activation of presynaptic inhibition of Ia afferents (Nakajima et al., 2013). Furthermore, it seems this interlimb coordination is intensity-and task-dependent. For instance, it has been shown that as intensities increase in the upper limbs during recumbent stepping, lower limb EMG activity also increases (Ferris, Huang, & Kao, 2006). This indicates that connections between the upper and lower limb are capable of facilitating motoneurone firing as locomotor intensity (i.e. resistance) increased. Regarding task-dependency, Dietz et al., 2001 measured deltoid, triceps and biceps brachii EMG activity during four different walking tasks on a treadmill that involved walking with and without arm swing and standing and sitting. When electrical impulses were applied to one leg, the arm muscle EMG activity were only evoked during walking. These results indicated a task-dependent neuronal coupling between upper and lower limbs (Dietz et al., 2001). Neural structures and mechanism involved in upper and lower limb coordination control of upper and lower limb coordination by propriospinal pathways.

2.3 Neural Structures and Mechanisms Involved in Upper and Lower Limb Coordination

The spinal cord is composed of a dense network of neurons known as propriospinal neurons (Flynn et al., 2011; Jankowska, 1992). These propriospinal neurons have axonal projections that can project over short (1–6 spinal segments) or long distances (>6 spinal segments) (Flynn et al., 2011; Saywell et al., 2011; Skinner et al., 1979). These neurons in the propriospinal system interconnect spinal segments and divide into long descending and ascending pathways that control upper and lower limb coordination. These long pathways involve neurons with cell bodies primarily located in laminae VII and VIII, and they originate from cervical/upper thoracic spinal segments. Cell bodies of the long ascending propriospinal pathways are primarily excitatory with long axonal projections that directly terminate on upper thoracic/cervical neurons (Brockett et al., 2013). Electrophysiological studies have demonstrated that some of the propriospinal pathways have monosynaptic excitatory connections with excitatory and inhibitory interneurons, that project directly to motoneurones (Jankowska et al., 1974; Jankowska et al., 1973). *In vivo* studies using spinal cord transections have demonstrated that crossed propriospinal projections play a key role in the coordination between limbs. In these studies they demonstrated that thoracic spinal cord transection in the adult cat still produced hindlimb stepping and left-right limb alternation on a weight-supported treadmill (Forssberg et al., 1980a, Forssberg et al., 1980b), but the fore- and hind limb coordination was disrupted (Eidelberg et al., 1980).

It has been demonstrated these propriospinal neurons are distributed along the length of the spinal cord, allowing the information transmission to the other side of the spinal cord at different spinal segments. In summary, the spinal cord involves a diverse propriospinal circuitry that can coordinate cervical and lumbar spinal locomotor CPGs through the descending and ascending axonal projections as well as plays an important role in interlimb coordination.

2.4 Control of Upper and Lower Limbs Coordination by Somatosensory Feedback from the Limbs

Sensory feedback plays a role in the activation of the CPG mechanism for controlled rhythmic movements and although it’s not required to generate the basic pattern of locomotor output it does contribute to a smoother movement (Pearcey & Zehr, 2020; Zehr, 2005). The interaction between the position of the body and its environment is informed by the CNS through sensors located in joints, muscles, and skin. During rhythmic movements, sensory information from cutaneous and muscle receptors continuously integrates with the CPG.

Proprioception, a term first introduced by Charles Sherrington (Burke, 2007), refers to the perception of one's own body and movements through information generated inside the body.

Proprioception is mainly provided by muscle receptors, muscle spindles, and Golgi tendon organs (GTOs) but also by some cutaneous receptors that play a role in locomotor control. Locomotor movements can be started or inhibited by some proprioceptive afferent inputs (Frigon et al., 2021). The interaction of the proprioceptive afferent inputs with the spinal locomotor CPG, evoke coordinated motor patterns (C. Sherrington, 1910). However, it has been demonstrated that real locomotor movements (Grillner and Zangger, 1984) and rhythmic patterns called "fictive locomotion" (Grillner & Zangger, 1979) can still be expressed after removing sensory afferents. The role of cutaneous inputs to locomotion as is the case for proprioceptive inputs, also have major effects on initiation and /or blocking of locomotion. For example, stimulation of specific skin areas can evoke rhythmic patterns such as fast paw shake (Langlet et al., 2005) or fictive locomotion (Viala & Buser, 1974; Viala et al., 1978). Langlet et al. (2005) removed cutaneous input (denervation) at L2, L3, as well as, at T13 in the normal cat and measured knee flexors and knee and ankle extensors EMG activity during treadmill locomotion. They reported that denervation at L2 and/or L3 abolishes spinal locomotion, however very few changes in the treadmill locomotor pattern were observed after denervation at T13. They reported that a spinal transection at T13 is not enough to suppress locomotion but that the specific inactivation at L2 and L3 abolishes walking on treadmill.

The most obvious role for cutaneous inputs was demonstrated by electrical and/or mechanical stimulation of the skin of the foot during walking. These experiments showed that cutaneous inputs during locomotion are phase-and stimulation dependent. For instance, during locomotion output, where the limbs constantly change from flexion to extension, the cutaneous stimulations could generate different responses in each position and direction (Rossignol, 1996). However experiments by Sherrington (C. S. Sherrington, 1910) suggested that cutaneous inputs are not essential in the control of locomotion. He reported by removing the cutaneous nerves of the hind and fore limb in quadrupeds, it is unlikely to cause impairment in walking and locomotion-like movements. On the other hand, studies involving locomotion have also shown that cutaneous inputs play a regulatory role (Serge Rossignol, 1996; S Rossignol, Lund, & Drew, 1988). For instance, Stein et al. (2000) indicated stimulation of the foot skin and stretch reflex contributes up to 30% of the ankle extensor activity during normal walking (Stein, Misiaszek, & Pearson, 2000).

In general, even though sensory feedback is not required for the control and generation of rhythmic behavior, performing locomotor movements is the result of a continuous interaction between CPGs and peripheral (sensory) input. In addition, it appears that sensory responses are phase- and muscle-dependent during the step cycle in the cat. For instance a stimulus applied to the foot during walking enhances flexion during the swing phase, but extension during the support phase in cat (Andersson, Forssberg, Grillner, & Lindquist, 1978; Gossard, Cabelguen, & Rossignol, 1989).

2.5 The Corticospinal Pathway

The corticospinal system, the last motor system to develop, is the main descending motor pathway responsible for voluntary limb control and of movements that require high levels of skill (Porter & Lemon, 1993). This pathway has been shown to begin in the cerebral cortex, which receives a range of inputs from primary motor cortex, premotor cortex, and supplementary motor area. It is believed about 30% of the tract originates from the primary motor cortex, 30% arise from premotor and supplementary motor areas while it seems the rest of the tract come from non-motor areas such as the somatosensory areas and the parietal cortex Other parts of the descending motor pathways include the rubrospinal, reticulospinal and vestibulospinal tracts which are all known to originate from the nuclei in the brainstem as well as receive inputs from the corticofugal neurons (Canedo, 1997). The nerve fibres areas from the somatosensory area, play a role in regulating the activity of the ascending tracts (Jang, 2014), then this tract integrate to form bundles, which travel through the internal capsule and cerebral peduncles and finally travel down to the brainstem (Welniarz et al., 2017). As the corticospinal tract continues to travel down into the internal capsule (a white matter pathway, located between the thalamus and the basal ganglia), then pass into the medulla which 75 to 90% of the fibers will decussate to the contralateral side via the pyramidal decussation (Natali et al., 2018). The 5 to 15% of fibers that do not decussate within the pyramidal decussation make up the anterior corticospinal tract. This tract extends into the spinal cord, but only travels down to the levels of the lower thoracic cord (Hong et al., 2009).

After leaving the brainstem and entering the spinal cord, the fibers go down through the anterior and lateral corticospinal tract. The fibers of the anterior corticospinal tract decussate through the anterior white commissure before synapsing with a neuron in the anterior horn of the gray matter. The lateral corticospinal tract fibers decussate at the level of the pyramid and synapse with a neuron on the anterior horn. These neurons, known as motoneurone, then project to the limbs and muscles to control the voluntary motor function (Hong et al., 2009).

2.6 Transcranial Magnetic Stimulation of the Motor Cortex

The leg area of the human motor cortex is located deeply within the interhemispheric fissure at 3 to 4 cm depth from the scalp surface, while the upper limb motor cortex is located just beneath the scalp (at 2 to 3 cm depth) (Allison, McCarthy, Luby, Puce, & Spencer, 1996; Iles, 1996; Terao et al., 1997). This difference in the leg and arm motor area makes the leg motor area relatively inaccessible for TMS delivered over the scalp. However, applying a high intensity TMS stimulation activates the leg area but the descending volley that are evoked are more complex than those evoked from the hand motor area. For stimulation of the leg motor cortex most use a double-cone as the magnetic pulse is able to penetrate deeper within the cortex. TMS stimulation of the CST will elicit either direct waves (D-waves) or indirect waves (I-waves) (Rossini et al., 2015). Action potentials will descend the pyramidal tract and be recorded as D-waves at higher stimulation intensities. When lower stimulation intensities are used the stimulus will activate cortical interneurons which in turn activate pyramidal neurones resulting in discharge at later intervals, thereby producing I-waves (Rossini et al., 2015). I-waves and D-waves result in the depolarization or hyperpolarization of the postsynaptic cell, resulting in excitatory or inhibitory postsynaptic potentials (EPSPs or IPSPs, respectively) (Barker et al., 2012). If the summation of EPSPs and IPSPs reach threshold for action potential generation, a motor evoked potential (MEP) will be elicited and subsequently recorded from the target muscle. Using the double-cone coil delivers a current at greater depth and in the perpendicular direction to the mesial surface of the cerebral cortex and results in D waves more readily than I waves. It was assumed while using a circle coil to the hand motor area I-waves are volleys predominate, because the TMS intensity activate directly cortical interneurons which in turn activate pyramidal neurones resulting in producing I-waves. While using double-cone coil the intensity of the magnetic stimulator is high, TMS can directly activate the upper motoneurones of the corticospinal tract (Di Lazzaro et al., 1998; Edgley et al., 1990) and, results in the production of a direct wave (D-wave) (Day et al., 1989; Nakamura et al., 1996; Thompson et al., 1991). The direct activation of upper motoneurones can also depend on the direction of current flow within the coil, as well as the placement of the coil over the scalp. Researchers can differentiate between I- and D-waves by measuring the latencies of the responses using epidural EMG recordings (Di Lazzaro et al., 1998; Rothwell, 1990). Usually, D-waves have shorter latencies than I-waves by approximately 1-2 ms (Rothwell et al., 1991).

2.7 Assessment of Corticospinal Excitability

2.7.1 Transcranial Magnetic Stimulation (TMS)

Properties of the human corticospinal pathway can be assessed using electrophysiological techniques like transcranial magnetic stimulation (TMS) (Barker, Jalinous, & Freeston, 1985), and transmastoid electrical stimulation (TMES). In 1980, the first experiments of transcranial stimulation were introduced by Merton and Morton in humans. They succeeded in electrically stimulating the motor cortex through the scalp by using transcranial electrical stimulation (TES) (Merton & Morton, 1980). In these experiments, TES was painful and uncomfortable and it appears only part of the current was passed through the scalp and reached the cortex, whereas the main part of the extended current between the 2 electrodes was measured to evoke contraction of the scalp muscles and cause a local pain. In 1985, TMS was first described by Anthony Barker as a pain free method of stimulating the human cortex using a pulsed magnetic field (Barker et al., 1985). TMS, by placing a coil on the scalp and passing a powerful current through it, produced a magnetic field that penetrates the cortex and it is relatively painless. The magnetic pulse itself creates a magnetic field and acts perpendicular to the current flow. This current penetrates the membranes of the neurons, extracerebral tissues, such as the scalp and the meninges, resulting in action potentials or excitatory (or inhibitory) postsynaptic potentials (Klomjai, Katz, & Lackmy-Vallée, 2015).

Single pulse TMS activates presynaptic corticospinal interneurons. The TMS pulse generates a descending volley of indirect waves (I-waves) that travel down the corticospinal pathway and cause an evoked potential recorded from the muscle target (Day et al., 1989; Di Lazzaro et al., 2004; Klomjai et al., 2015; Pascual-Leone et al., 1995; Pascual-Leone et al., 1998). There are early and late I-waves, denoted as I1, I2, and I3. It was demonstrated the early waves are a result of monosynaptic connections to spinal motoneurons and late waves are from polysynaptic connections (Yuasa et al., 2021) As TMS intensity increases, I3-waves are recruited first, followed by I2, then I1 waves (Klomjai et al., 2015). By increasing the stimulation intensities, TMS directly activate corticospinal neurons causing direct waves (Klomjai et al., 2015; Wagle-Shukla et al., 2009). All these descending volleys activate muscles and can be recorded by using surface EMG.

TMS involves non-invasive magnetic brain stimulation that can be used to quantify corticospinal excitability and inhibition in healthy and neurological patients. TMS preferentially activates neurons that are oriented horizontally in a plane that is parallel to both the coil and the brain surface. TMS applied over the motor cortex induces descending volleys in the pyramidal tract projecting on to spinal motoneurons. Motoneuron activation in response to corticospinal volleys induced by TMS elicits a motor evoked potential (MEP) in muscles of the contralateral limb. Measuring the MEP peak-to-peak amplitude or area from the surface EMG reflects the excitability of the corticospinal pathway (brain and spinal cord) (Klomjai et al., 2015; Tofts, 1990).

Several TMS studies have investigated changes in corticospinal excitability during locomotor and/or tonic task. For example, Forman et.al (2014) measured supraspinal and spinal motoneuron excitability using TMS of the motor cortex and TMES of the corticospinal tract, at three separate positions (3, 6, and 12 o'clock relative to a clock face) during arm cycling and an intensity-matched tonic contraction. They reported an increase in the excitability of corticospinal neurons during arm cycling compared with a tonic contraction at the 3 and 6 o'clock positions. Furthermore, cervicomedullary evoked potentials (CMEPs) during arm cycling were compared with a tonic contraction at the three positions with spinal excitability higher at 3 o'clock during arm cycling. They concluded that supraspinal and spinal motoneurone excitability are phase-and task-dependent (Forman et al., 2014).

Lockyer et.al (2016) examined the effects of cadence and peak power output on the MEP and TMES responses from the biceps and triceps brachii as well as at flexion and extension phases. They reported, at flexion phase, as power output and cadence increased, biceps brachii MEPs increased. Triceps brachii MEPs only increased with cadence, while CMEPs increased with cadence and power output. At the extension phase, biceps brachii MEPs increased as cadence and power output increased, while CMEPs decreased with cadence and did not change with power output. In contrast, triceps brachii MEPs were not different with decadence and /or intensity, while CMEPs increased with cadence and intensity. These data suggested that the supraspinal and spinal excitability are phase- and muscle dependent (Lockyer et al., 2018; Nippard, Lockyer, Button, & Power, 2020).

Aboodarda et.al (2017) measured supraspinal and spinal excitability using TMS and TMES, respectively. MEPs elicited by TMS and CMEPs elicited by TMES were recorded to the non-dominant biceps brachii during isometric contractions at 100%, 50%, and 5% of MVC before and after two sets of 100s unilateral isometric dominant knee extensions (fatigue) and at rest (control). They demonestrated MEP and the MEP/CMEP ratio, in particular at 100% of MVC, was greater after fatigue compared to rest. These data suggest that interlimb coordination between the upper and lower limbs can influence measures of corticospinal excitability (Aboodarda, Šambaher, Millet, & Behm, 2017).

Generally these studies demonstrated changes in corticospinal excitability as task- and intensity-dependent. In addition, previous studies demonstrated interlimb coordination pathway modulate corticospinal excitability during locomotor and tonic tasks. In this study, we are looking for to understanding the neural coordination between upper and lower limbs during rhythmic movements through measuring corticospinal excitability by TMS.

2.8 Conclusion

As it currently stands, it is known that the appropriate coordination between upper and lower limbs is an important characteristic of quadrupedal and human locomotion. This coupling of upper and lower limbs and the well-regulated movement between them involves complex neural processes coordinated by the central nervous system (CNS). Most methodologies for understanding this coordination between upper and lower limbs have used H- reflexes and/or EMG during rhythmic locomotor movement. However the effects of rhythmic locomotor movement (arm and/or leg cycling) on the corticospinal excitability of stationary limbs (arm and/or leg) have yet to be clearly determined. On the other hand, it seems interlimb pathways modulate cortical and spinal pathways during locomotor tasks. However, much less is known regarding the corticospinal excitability during rhythmic movements in the stationary limbs to understand neural coupling mechanisms between the upper and lower limbs.

As such, our lab sought to investigate the effect of arm cycling on corticospinal excitability to the vastus lateralis cortical when the legs were at rest. In addition, effect of leg cycling on corticospinal excitability to the biceps brachii when the arms were at rest. This project aims to explore does arm cycling influence the leg and /or does leg cycling influences the arm, and if so, whether it is intensity-dependent or not. .

2.9 References

Alcock, L., Spence, A., Lockyer, E., Button, D., & Power, K. E. (2019). Short-interval intracortical inhibition to the biceps brachii is present during arm cycling but is not different than a position-and intensity-matched tonic contraction. Experimental brain research, 237(9), 2145-2154.

Allison, T., McCarthy, G., Luby, M., Puce, A., & Spencer, D. D. (1996). Localization of functional regions of human mesial cortex by somatosensory evoked potential recording and by cortical stimulation. Electroencephalography and Clinical Neurophysiology/Evoked Potentials Section, 100(2), 126-140.

Andersson, O., Forssberg, H., Grillner, S., & Lindquist, M. (1978). Phasic gain control of the transmission in cutaneous reflex pathways to motoneurones during ‘fictive’locomotion. Brain research, 149(2), 503-507.

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

Blanc, Y., & Dimanico, U. (2010). Electrode placement in surface electromyography (sEMG)” Minimal Crosstalk Area “(MCA). The Open Rehabilitation Journal, 3(1).

Brockett, E., Seenan, P., Bannatyne, B., & Maxwell, D. (2013). Ascending and descending propriospinal pathways between lumbar and cervical segments in the rat: evidence for a substantial ascending excitatory pathway. Neuroscience, 240, 83-97.

Brown, T. G. (1914). On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. The Journal of physiology, 48(1), 18.

Burke, R. E. (2007). Sir Charles Sherrington's the integrative action of the nervous system: a centenary appreciation. Brain, 130(4), 887-894.

Calancie, B., Needham-Shropshire, B., Jacobs, P., Willer, K., Zych, G., & Green, B. A. (1994). Involuntary stepping after chronic spinal cord injury: evidence for a central rhythm generator for locomotion in man. Brain, 117(5), 1143-1159.

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

Carroll, T. J., Zehr, E. P., & Collins, D. F. (2005). Modulation of cutaneous reflexes in human upper limb muscles during arm cycling is independent of activity in the contralateral arm. Experimental brain research, 161(2), 133-144.

Cheng, J., Brooke, J. D., Misiaszek, J. E., & Staines, W. R. (1998). Crossed inhibition of the soleus H reflex during passive pedalling movement. Brain research, 779(1-2), 280-284.

Cicchetti, F., Barker, R. A., & Robinson, E. S. (2017). Neuroanatomy and Neuroscience at a Glance: John Wiley & Sons.

Day, B., Dressler, D., Maertens de Noordhout, A., Marsden, C., Nakashima, K., Rothwell, J., & Thompson, P. (1989). Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. The Journal of physiology, 412(1), 449-473.

De Luca, C. J. (1997). The use of surface electromyography in biomechanics. Journal of applied biomechanics, 13(2), 135-163.

Debaere, F., Swinnen, S. P., Béatse, E., Sunaert, S., Van Hecke, P., & Duysens, J. (2001). Brain areas involved in interlimb coordination: a distributed network. Neuroimage, 14(5), 947-958.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Mazzone, P., . . . Rothwell, J. (2004). The physiological basis of transcranial motor cortex stimulation in conscious humans. Clinical neurophysiology, 115(2), 255-266.

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

Dietz, V. (2002). Proprioception and locomotor disorders. Nature Reviews Neuroscience, 3(10), 781-790.

Dietz, V., Fouad, K., & Bastiaanse, C. (2001). Neuronal coordination of arm and leg movements during human locomotion. European Journal of Neuroscience, 14(11), 1906-1914.

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

Eidelberg, E., Story, J., Meyer, B., & Nystel, J. (1980). Stepping by chronic spinal cats. Experimental brain research, 40(3), 241-246.

Falgairolle, M., de Seze, M., Juvin, L., Morin, D., & Cazalets, J.-R. (2006). Coordinated network functioning in the spinal cord: an evolutionary perspective. Journal of Physiology-Paris, 100(5-6), 304-316.

Farina, D., Merletti, R., & Enoka, R. M. (2004). The extraction of neural strategies from the surface EMG. Journal of Applied Physiology, 96(4), 1486-1495.

Ferris, D. P., Huang, H. J., & Kao, P.-C. (2006). Moving the arms to activate the legs. Exercise and sport sciences reviews, 34(3), 113-120.

Flynn, J. R., Graham, B. A., Galea, M. P., & Callister, R. J. (2011). The role of propriospinal interneurons in recovery from spinal cord injury. Neuropharmacology, 60(5), 809-822.

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

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

Forssberg, H., Grillner, S., & Halbertsma, J. (1980). The locomotion of the low spinal cat I. Coordination within a hindlimb. Acta Physiologica Scandinavica, 108(3), 269-281.

Forssberg, H., Grillner, S., Halbertsma, J., & Rossignol, S. (1980). The locomotion of the low spinal cat. II. Interlimb coordination. Acta Physiologica Scandinavica, 108(3), 283-295.

Frigon, A. (2017). The neural control of interlimb coordination during mammalian locomotion. Journal of neurophysiology, 117(6), 2224-2241.

Frigon, A., Akay, T., & Prilutsky, B. I. (2021). Control of mammalian locomotion by somatosensory feedback. Comprehensive physiology, 12(1), 2877.

Garcia, M. A., Souza, V. H., & Vargas, C. D. (2017). Can the recording of motor potentials evoked by transcranial magnetic stimulation be optimized? Frontiers in human neuroscience, 11, 413.

Geyer, H., & Herr, H. (2010). A muscle-reflex model that encodes principles of legged mechanics produces human walking dynamics and muscle activities. IEEE Transactions on neural systems and rehabilitation engineering, 18(3), 263-273.

Gossard, J.-P., Cabelguen, J.-M., & Rossignol, S. (1989). Intra-axonal recordings of cutaneous primary afferents during fictive locomotion in the cat. Journal of neurophysiology, 62(5), 1177-1188.

Grillner, S., & Zangger, P. (1979). On the central generation of locomotion in the low spinal cat. Experimental brain research, 34(2), 241-261.

GRILLNER, S., & ZANGGER, P. (1984). The effect of dorsal root transection on the efferent motor pattern in the cat's hindlimb during locomotion. Acta Physiologica Scandinavica, 120(3), 393-405.

Haridas, C., & Zehr, E. P. (2003). Coordinated interlimb compensatory responses to electrical stimulation of cutaneous nerves in the hand and foot during walking. Journal of neurophysiology, 90(5), 2850-2861.

Hong, J. H., Son, S. M., Byun, W. M., Jang, H. W., Ahn, S. H., & Jang, S. H. (2009). Aberrant pyramidal tract in medial lemniscus of brainstem in the human brain. Neuroreport, 20(7), 695-697.

Houlden, D. A., Schwartz, M. L., Tator, C. H., Ashby, P., & MacKay, W. A. (1999). Spinal cord-evoked potentials and muscle responses evoked by transcranial magnetic stimulation in 10 awake human subjects. Journal of Neuroscience, 19(5), 1855-1862.

Huang, H. J., & Ferris, D. P. (2004). Neural coupling between upper and lower limbs during recumbent stepping. Journal of Applied Physiology, 97(4), 1299-1308.

Iles, J. (1996). Evidence for cutaneous and corticospinal modulation of presynaptic inhibition of Ia afferents from the human lower limb. The Journal of physiology, 491(1), 197-207.

Jang, S. H. (2014). The corticospinal tract from the viewpoint of brain rehabilitation. Journal of rehabilitation medicine, 46(3), 193-199.

Jankowska, E. (1992). Interneuronal relay in spinal pathways from proprioceptors. Progress in neurobiology, 38(4), 335-378.

Jankowska, E., Lundberg, A., Roberts, W., & Stuart, D. (1974). A long propriospinal system with direct effect on motoneurones and on interneurones in the cat lumbosacral cord. Experimental brain research, 21(2), 169-194.

Jankowska, E., Lundberg, A., & Stuart, D. (1973). Propriospinal control of last order interneurones of spinal reflex pathways in the cat. Brain research, 53(1), 227-231.

Kiehn, O. (2006). Locomotor circuits in the mammalian spinal cord. Annual review of neuroscience, 29(1), 279-306.

Klomjai, W., Katz, R., & Lackmy-Vallée, A. (2015). Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). Annals of physical and rehabilitation medicine, 58(4), 208-213.

Langlet, C., Leblond, H., & Rossignol, S. (2005). Mid-lumbar segments are needed for the expression of locomotion in chronic spinal cats. Journal of neurophysiology, 93(5), 2474-2488.

Loadman, P. M., & Zehr, E. P. (2007). Rhythmic arm cycling produces a non-specific signal that suppresses Soleus H-reflex amplitude in stationary legs. Experimental brain research, 179(2), 199-208.

Lockyer, E. J., Benson, R. J., Hynes, A. P., Alcock, L. R., Spence, A.-J., Button, D. C., & Power, K. E. (2018). Intensity matters: effects of cadence and power output on corticospinal excitability during arm cycling are phase and muscle dependent. Journal of neurophysiology, 120(6), 2908-2921.

Merton, P., & Morton, H. (1980). Stimulation of the cerebral cortex in the intact human subject. Nature, 285(5762), 227-227.

Misiaszek, J. E., Cheng, J., Brooke, J. D., & Staines, W. R. (1998). Movement-induced modulation of soleus H reflexes with altered length of biarticular muscles. Brain research, 795(1-2), 25-36.

Natali, A. L., Reddy, V., & Bordoni, B. (2018). Neuroanatomy, Corticospinal Cord Tract.

Nikias, C. L., & Raghuveer, M. R. (1987). Bispectrum estimation: A digital signal processing framework. Proceedings of the IEEE, 75(7), 869-891.

Nippard, A. P., Lockyer, E. J., Button, D. C., & Power, K. E. (2020). Corticospinal excitability to the biceps and triceps brachii during forward and backward arm cycling is direction- and phase-dependent. Applied Physiology, Nutrition, and Metabolism, 45(1), 72-80.

Pascual-Leone, A., Nguyet, D., Cohen, L. G., Brasil-Neto, J. P., Cammarota, A., & Hallett, M. (1995). Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. Journal of neurophysiology, 74(3), 1037-1045.

Pascual-Leone, A., Tormos, J. M., Keenan, J., Tarazona, F., Cañete, C., & Catalá, M. D. (1998). Study and modulation of human cortical excitability with transcranial magnetic stimulation. Journal of clinical neurophysiology, 15(4), 333-343.

Pearcey, G. E., & Zehr, E. P. (2020). What lies beneath the brain: Neural circuits involved in human locomotion The Neural Control of Movement (pp. 385-418): Elsevier.

Porter, R., & Lemon, R. (1993). Corticospinal function and voluntary movement: Oxford University Press, USA.

Rossignol, S. (1996). Neural control of stereotypic limb movements. Exercise: Regulation and integration of multiple systems.

Rossignol, S., Lund, J., & Drew, T. (1988). The role of sensory inputs in regulating patterns of rhythmical movements in higher vertebrates. Neural control of rhythmic movements in vertebrates, 201-283.

Rossini, P. M., Burke, D., Chen, R., Cohen, L., Daskalakis, Z., Di Iorio, R., . . . George, M. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an IFCN Committee. Clinical neurophysiology, 126(6), 1071-1107.

Rothwell, J. (1991). Physiological studies of electric and magnetic stimulation of the human brain. Electroencephalography and clinical neurophysiology. Supplement, 43, 29-35.

Sadikoglu, F., Kavalcioglu, C., & Dagman, B. (2017). Electromyogram (EMG) signal detection, classification of EMG signals and diagnosis of neuropathy muscle disease. Procedia computer science, 120, 422-429.

Saywell, S. A., Ford, T. W., Meehan, C. F., Todd, A. J., & Kirkwood, P. A. (2011). Electrophysiological and morphological characterization of propriospinal interneurons in the thoracic spinal cord. Journal of neurophysiology, 105(2), 806-826.

Sherrington, C. (1910). Remarks on the reflex mechanism of the step. Brain, 33(1), 1-25.

Sherrington, C. S. (1910). Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. The Journal of physiology, 40(1-2), 28.

Skinner, R. D., Coulter, J. D., Adams, R. J., & Remmel, R. S. (1979). Cells of origin of long descending propriospinal fibers connecting the spinal enlargements in cat and monkey determined by horseradish peroxidase and electrophysiological techniques. Journal of Comparative Neurology, 188(3), 443-454.

Song, S., & Geyer, H. (2015). A neural circuitry that emphasizes spinal feedback generates diverse behaviours of human locomotion. The Journal of physiology, 593(16), 3493-3511.

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

Stein, R. B., Misiaszek, J. E., & Pearson, K. G. (2000). Functional role of muscle reflexes for force generation in the decerebrate walking cat. The Journal of physiology, 525(3), 781-791.

Terao, Y., & Ugawa, Y. (2002). Basic mechanisms of TMS. Journal of clinical neurophysiology, 19(4), 322-343.

Terao, Y., Ugawa, Y., Hanajima, R., Machii, K., Furubayashi, T., Mochizuki, H., . . . Iwata, N. K. (2000). Predominant activation of I1-waves from the leg motor area by transcranial magnetic stimulation. Brain research, 859(1), 137-146.

Terao, Y., Ugawa, Y., Hanajima, R., Yumoto, M., Kawahara, Y., Yamamoto, T., . . . Kanazawa, I. (1997). Motor cortical reflex myoclonus: a case study with MEG. Electroencephalography and clinical neurophysiology, 102(6), 505-511.

Tofts, P. (1990). The distribution of induced currents in magnetic stimulation of the nervous system. Physics in Medicine & Biology, 35(8), 1119.

Viala, G., & Buser, P. (1974). Inhibition des activités spinales à caractère locomoteur par une modalité particulière de stimulation somatique chez le lapin. Experimental brain research, 21(3), 275-284.

Viala, G., Orsal, D., & Buser, P. (1978). Cutaneous fiber groups involved in the inhibition of fictive locomotion in the rabbit. Experimental brain research, 33(2), 257-267.

Wagle-Shukla, A., Ni, Z., Gunraj, C. A., Bahl, N., & Chen, R. (2009). Effects of short interval intracortical inhibition and intracortical facilitation on short interval intracortical facilitation in human primary motor cortex. The Journal of physiology, 587(23), 5665-5678.

Welniarz, Q., Dusart, I., & Roze, E. (2017). The corticospinal tract: Evolution, development, and human disorders. Developmental neurobiology, 77(7), 810-829.

Yuasa, A., Uehara, S., Ushizawa, K., Toyama, T., Gomez-Tames, J., Hirata, A., & Otaka, Y. (2021). Effects of Cerebellar Transcranial Direct Current Stimulation on Upper Limb Motor Function After Stroke: Study Protocol for the Pilot of a Randomized Controlled Trial.

Zehr, E. P. (2005). Neural control of rhythmic human movement: the common core hypothesis. Exercise and sport sciences reviews, 33(1), 54-60.

Zehr, E. P., & Haridas, C. (2003). Modulation of cutaneous reflexes in arm muscles during walking: further evidence of similar control mechanisms for rhythmic human arm and leg movements. Experimental brain research, 149(2), 260-266.

Zehr, E. P., Klimstra, M., Johnson, E. A., & Carroll, T. J. (2007). Rhythmic leg cycling modulates forearm muscle H-reflex amplitude and corticospinal tract excitability. Neuroscience letters, 419(1), 10-14.

Chapter 3 Corticospinal excitability and interconnectivity of the limbs during arm cycling

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Running head: Examining interlimb coordination during arm and leg Cycling

Key words: Interlimb coordination, MEP, EMG, arm cycling, leg cycling

3.0 Abstract

Aim: The purpose of this study was to investigate the effect of: 1) arm cycling on corticospinal excitability to the resting vastus lateralis, 2) leg cycling on corticospinal excitability to the resting biceps brachii and 3) whether potential effects were intensity-dependent. Main method: Corticospinal excitability was assessed using transcranial magnetic stimulation (TMS) of the motor cortex. Motor-evoked potentials (MEPs) elicited by TMS were recorded at two power outputs25 and 50% peak power output (PPO) from resting biceps brachii and vastus lateralis during leg and arm cycling, respectively. Result: Our results demonstrated that biceps brachii (BB) MEP amplitudes were significantly increased at 25 and 50% PPO during leg cycling compared to rest (0.24 and 0.37mV vs 0.10mV; P=0.03 and P=0.005, respectively), but there was no significant difference in BB MEP amplitudes between 25 and 50% PPO (p=0.13). In addition, MEP amplitudes significantly increased to the vastus lateralis (VL) at 25 and 50% PPO compared to rest (0.3 and 0.5mV vs 0.11mV; P=0.02 and P=0.004, respectively), as well as, increased at 25 compared to the 50% PPO during arm cycling (0.3mV vs 0.5mV; P=0.03). Significance: Our data demonstrate that MEP amplitudes increased in the resting BB and VL during leg and arm cycling, respectively, an effect that was intensity-dependent.

3.1 Introduction

The regulation of human and non-human animals' movement requires the coordination of muscle activation between the upper and lower limbs and the fore- and hind limbs, respectively. This appropriate coordination is an important characteristic of human (Balter & Zehr, 2007; Dietz, 2002; Zehr & Haridas, 2003) and quadrupedal locomotion (Balter & Zehr, 2007; Juvin et al., 2005; Schomburg et al., 1978; Zaporozhets et al., 2006). Evidence shows that effective coordination between limbs during locomotor output is controlled through complex reciprocal connections between cortical and spinal circuits, and through somatosensory feedback that generates the primary pattern of movements and informs the central nervous system of changes within the body and in the environment (Dietz et al., 2001; Zehr et al., 2004; Zehr & Haridas, 2003).

In humans, several studies have examined the neural interaction between upper and lower limbs during locomotor output. Studies have measured changes in electromyography activity (EMG) and/or reflexes modulation in the arm and leg muscles (Balter & Zehr, 2007; Haridas & Zehr, 2003) and demonstrated that observed changes in EMG and reflexes responses were intensity-dependent during rhythmic movements (Frigon et al., 2004; Hundza & Zehr, 2009; Loadman & Zehr, 2007). For example Huang and Ferris (2004) demonstrated that EMG activity increased in the lower limbs while participants used their upper limbs during recumbent stepping, an effect that was intensity-dependent. Frigon and et al (2004) demonstrated that when participants performed arm cycling at 2Hz, the suppression of soleus H-reflex was significantly higher than 1 Hz. Thus, H-reflex suppression is cadence-dependent, indicating that as the intensity of locomotor output increases so too does presynaptic inhibition of the IA afferents (Frigon et al., 2004; Hundza & Zehr, 2009; Loadman & Zehr, 2007).

It appears the interlimb pathways modulates the excitability of the corticospinal pathway during locomotor tasks. It is generally accepted that the coordination of upper and lower limbs during locomotor outputs involves the distribution of cortical and spinal control. However, it is unclear whether there are differences in the relative contributions of cortical and spinal control during rhythmic movement (Petersen et al., 2003). For instance, Zehr et.al (2007) investigated the effect of rhythmic leg cycling and static leg position on corticospinal excitability in the contracted and relaxed flexor carpi radialis (FCR) muscle. They showed corticospinal excitability to FCR increased while the arms were at rest and/or isometrically contractiing during leg cycling. In addition, corticospinal excitability to FCR was higher during leg cycling compared to the isometric contraction. Higher corticospinal excitability could be due to cortical or spinal mechanisms. This intersegmental facilitation of neural excitability is likely mediated by activation of the propriospinal pathways that have excitatory connections projecting directly to motoneurons (Zehr et al., 2007).

Based on prior works, it is clear that there is neural coordination between the upper and lower limbs during locomotor tasks. On the other hand, it seems interlimb pathways modulate corticospinal pathways during locomotor tasks, however, much less is known regarding the corticospinal excitability during rhythmic movements in the stationary limbs. Thus, we hypothesized that corticospinal excitability would increase to the resting vastus lateralis and biceps brachii during arm and leg cycling, respectively, and that this effect would be enhanced as cycling intensity increased.

3.2 METHODS

3.2.1 Ethical Approval

Prior to commencing data collection, all participants were informed of all potential risks and benefits of the study via verbal and written explanation and were given an opportunity to ask questions. All participants then gave written informed consent. This study was conducted in accordance with the Helsinki declaration and all protocols were approved by the Interdisciplinary Committee on Ethics in Human Research at Memorial University of Newfoundland (ICEHR No. 20230113-HK).

3.2.2 Participants

Twelve healthy participants (3 females and 9 males 28.8 ± 3.7 yr of age, height 17.5 ±5.6, and weight 80±7.9, one left hand dominant) with no known neurological impairments participated in our study. The procedure was verbally explained to the participants and written consent was obtained prior to starting the study. All participants completed a magnetic stimulation safety checklist (Rossi et al., 2009), a Physical Activity Readiness Questionnaire [PAR-Q+; Canadian Society for Exercise Physiology (CSEP)] to ensure they could safely perform physical activity, and an Edinburg handedness questionnaire to identify the dominant limb for testing (Veale, 2014).

3.2.3 Experimental Setup

All cycling trials were conducted using an arm-leg cycle ergometer (SCIFIT ergometer, model PRO2 Total Body, Tulsa, UK). Participants were seated upright in a comfortable position at a distance from the arm and leg cranks such that they did not need to lean forward or rotate their torso or hyperextend the knees or elbows while cycling. The seat height was adjusted to fit everyone so that the arm crank was approximately in line with the participant's shoulders. Regarding the leg crank, the seat was positioned so that the knee flexion and extension angle was between 30 and 110 degrees respectively, while the ankle was in the neutral position (Lopes et al., 2014).

During arm cycling participants used wrist braces to limit unwanted flexion or extension of the wrists during arm cycling and to decrease the influence of heteronymous reflex connections that exist between the wrist flexors and the biceps brachii (Manning & Bawa, 2011). Furthermore, the feet were fixed in place on the pedals with a strap during leg cycling. The position of the arm and leg cranks was defined relative to clock face at 6 o'clock. Six o'clock is specified as the "bottom dead center" (Forman et al., 2015; Lockyer et al., 2018). We applied for these positions because, at the 6 o'clock position, the elbow is in mid-elbow flexion, and the knee is in knee-extension. TMS triggered automatically when the dominant arm and leg passed at 6 o'clock.

For all trials, participants arm and leg cycled at two different workloads at 25% and 50% of their maximum peak power output which was determined from an incremental test (see below) during a different session. These intensities were known as low and high intensity and performed at 60 revolutions per minute (RPM).

3.2.4 Time to exhaustion test

All participants performed a time to exhaustion test to determine their maximum aerobic power output. Two tests were performed on the same day, in random order, with a 30-minute rest period between tests. The initial workload for arm cycling was set at 20 watts (W) and increased by 1W every 15 seconds for females, while the initial workload was 40W and increased by 3W every 15 seconds for males. Regarding leg cycling, the initial workload was set at 40W and increased by 1W every 15 seconds for females and it was set at 80W and increased by 3W every 15 seconds for males (Davison et al., 2022).

3.2.5 Electromyography

Electromyography recordings were taken from the target muscles, vastus lateralis and biceps brachii, using pairs of surface electrodes (Ag-AgCl; MediTrace ^{TM} 130 Foam Electrodes with conductive adhesive hydrogel, Covidien IIC, Massachusetts, USA). The electrodes were placed in a bipolar configuration (2-cm interelectrode distance), in line with the muscle fiber direction of the target muscle belly. In addition, a ground electrode was placed over the lateral epicondyle of the elbow and tibia. Prior to electrode placement, the target muscles of the participant were shaved by a razor, then prepared using a gel and alcohol swap to reduce EMG recording impedance and remove all dead dermal cells.. EMG data were collected online at 5 kHz using CED 1401 interface and Signal 5 software program (Cambridge Electronic Design, Cambridge, UK). Signals were amplified and filtered using a three-pole Butterworth with cut-off frequencies of 10–500 Hz.

3.2.6 Transcranial Magnetic Stimulation

Motor-evoked potentials (MEPs) from the target muscles (vastus lateralis and biceps brachii) were elicited during arm and leg cycling using a double cone coil (2*12.8cm outside diameter) and circular coil (13.5 cm outside diameter) using TMS (Magstim, Whitland, Dyfed, UK).. The coil was held on the vertex, parallel to the floor with the direction of current flow preferentially activating the dominant motor cortex. The vertex was located by measuring nasion to inion and tragus to tragus; marking the location on the scalp halfway between them; defining the vertex as the intersection of the halfway marks. This method has previously been used in our lab to measure MEPs (Forman et al., 2015). Stimulation was delivered every 8 seconds.

3.2.7 Resting Motor Threshold

The resting motor threshold (RMT) was defined as the minimum stimulation intensity at which a clearly discernable MEP was found in the target muscles (defined as having an amplitude ≥50µV in five out of ten trials). Once RMT was determined, an average of ten MEPs was recorded at this stimulation intensity during the resting condition (arms and legs completely at rest). Stimulation intensity started at ~30% maximal stimulator output (MSO) and at ~40% MSO, while arms and legs were at rest, respectively. The intensity was increased gradually until RMT was detected. This percent intensity of MSO was then used for the entire experiment (Forman et al., 2015).

3.2.8 Experimental Protocol

Participants were asked to visit to the lab on two separate sessions:

The first session was used to familiarize the participants with the stimulation techniques. In addition, they completed an incremental arm and leg cycling test on the ergometer to determine their maximum aerobic power output. On the second session, we determined RMT for arm and legs. Participants were asked to sit at the arm-leg cycling ergometer and TMS beginning at 30% MSO for arm and at 40% of the (MSO) for leg, while they were at rest, without cycling, and increasing in intensity until clearly identifying discernable MEPs from the arm and leg were recorded.

Following the determination of RMT for the arm and leg muscles, participants were required to perform the second session which consisted of performing arm and leg arm and/or leg cycling that were randomized, while their legs and arms were completely at rest respectively, at two different power outputs (25 and 50% peak power output (PPO) that was determined from the incremental test. Each cycling bout involved 10 stimulation, and 2 blank by random. Once the stimulation intensities for TMS were determined, the 10 stimulations were performed to make sure we found MEP. See Figure.1 for experimental set-up.

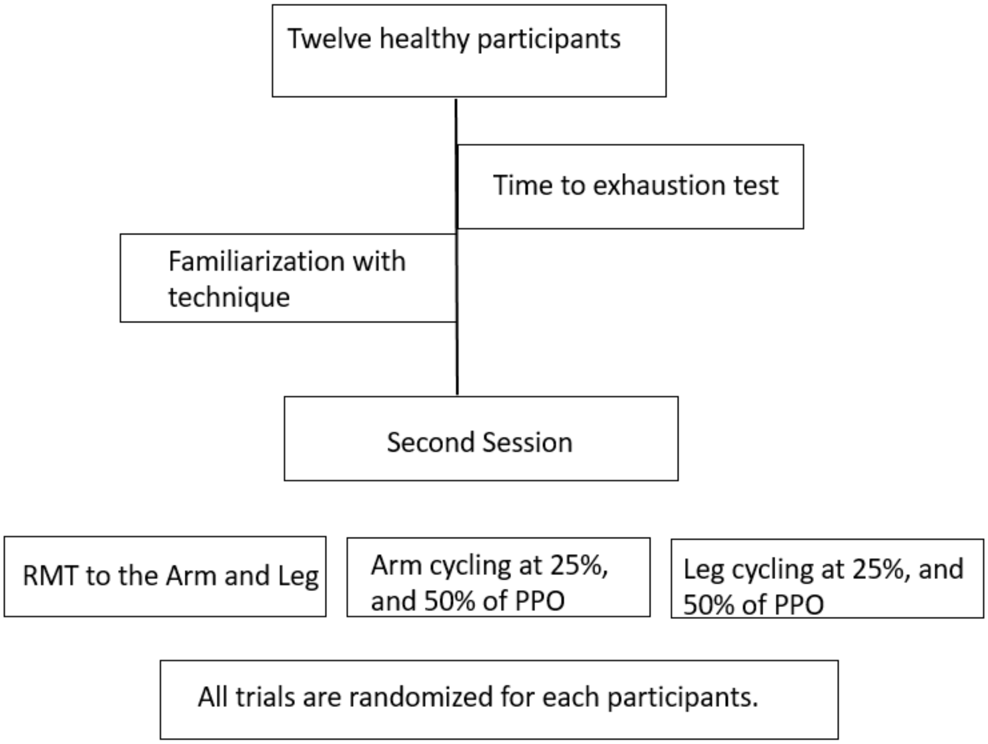


Figure 1. Experimental Protocol

3.2.9 Data Analysis

All data were analyzed using Signal 5.08 data collection software (Cambridge Electronic Design). The averaged peak-to-peak amplitudes of MEPs for each muscle were determined at rest and during 25 and 50% PPO cycling of the distal musculature. Additionally, background EMG, defined as the mean rectified EMG 50ms preceding the stimulation artifact, was assessed to ensure the muscle of interest was at rest.

3.2.10 Statistical Analysis

All statistics were analyzed using IBM's SPSS Statistics version 22 and were expressed as mean ± SD. To compare the differences in MEP amplitude and pre-stimulus EMG in biceps brachii and vastus lateralis muscles between rest, 25% and 50% of peak power output during leg and arm cycling respectively, a One Way Repeated Measures ANOVA was used. In addition, to compare resting motor threshold and maximum stimulator output between arm and leg, paired t-test were employed. The significance level was accepted at $P \leq .05$.

3.3 RESULTS

3.3.1 Stimulation intensities

TMS intensities used for arm and leg cycling at the 6 o'clock position ranged from 40 to 55% and 50 to 70% of MSO, respectively. On average, the TMS intensity used at vastus lateralis was higher than that used at biceps brachii. There was significant difference between %MSO to the arm and leg ($t_{(11)} = -3.43$ $p = 0.006$) (Table 2).

Condition	Arm	Leg
%MSO	51.5±8.3 (n=12)	62.8±.14.17 (n=12)

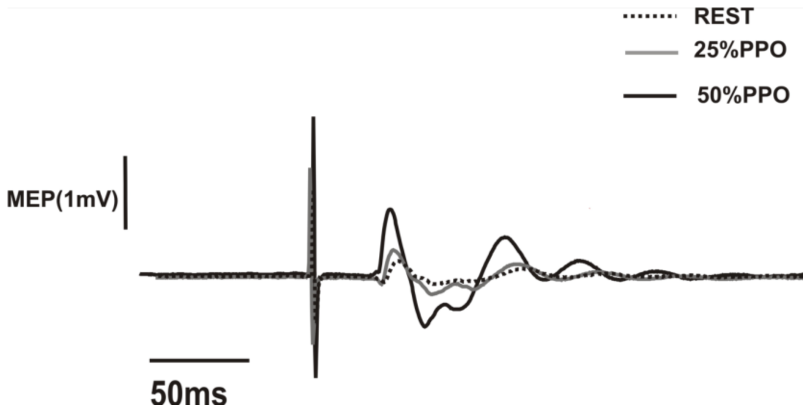
Table2: Maximum stimulation outputs using stimulation intensity is shown.

3.3.2 Peak power output

Each participant completed a continuous, incremental arm and leg cycling test on the SCIFIT ergometer to determine their PPO. The relative power outputs at 25 and 50% PPO during arm cycling (26.6 ± 6.9 and 53.3 ± 13.8 watts, respectively) and leg cycling (46.3 ± 11.7 and 92.5 ± 23.7 watts, respectively) were determined.

3.3.3 Corticospinal excitability to the biceps brachii during leg cycling

Figure 2, shows data for the MEP amplitudes to the biceps brachii at rest (Mean=0.10, SD=0.05), 25 (Mean=0.24, SD=0.17), and 50% PPO (Mean=0.37, SD=0.23) during leg cycling. There was a significant increase in MEP amplitudes at 25 and 50% PPO compared to rest (P=0.03, P=0.005, respectively), but MEP amplitudes were not significantly different between 25 and 50% PPO (P=0.13) (Figure 2).



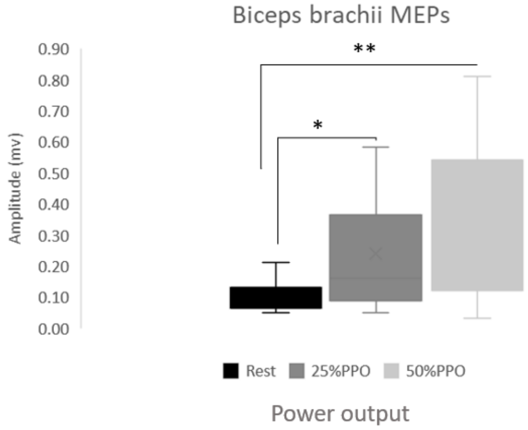


Figure 2: Biceps brachii MEP amplitude (mV) at rest, 25 and, 50% PPO. MEP amplitudes was increased at 25 and 50% PPO during leg cycling compared to rest (0.24mV vs 0.10 mV; P=0.03) and (0.37mV vs 0.10 mV; P=0.005) (n=12). * denotes a significant difference between rest and 25% PPO (P < 0 .05), ** denotes a significant difference between rest and 50% PPO (P < 0 .01).

3. 3.4 Pre-stimulus EMG for biceps brachii

As expected, there was no significant difference in background EMG of the biceps brachii at rest (9.2μv), 25% (9.6 μv) and 50% (10 μv) PPO during leg cycling (P=0.23; Figure.3).

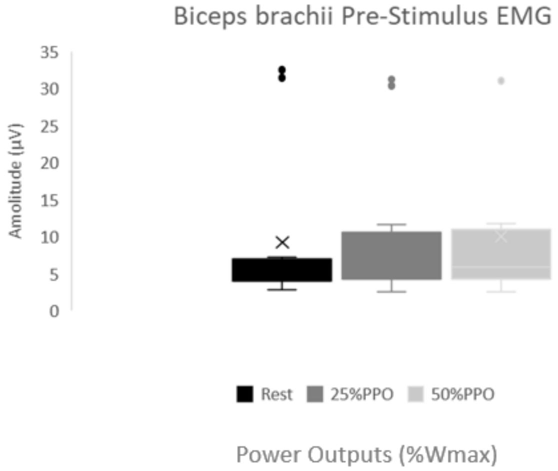


Figure 3: Background EMG for the biceps brachii at rest, 25% and 50% PPO during leg cycling (n=12).

3.3.5 Corticospinal excitability to the vastus lateralis during arm cycling

Figure 4, shows data for the MEP amplitudes in vastus lateralis at rest (Mean=0.11, SD=0.10), 25 (Mean=0.30, SD=0.19) and 50% (Mean=0.50, SD=0.26) PPO during arm cycling. All participants demonstrated significant increase in MEP amplitude at 25 and 50% PPO compared to rest (P=0.02 and P=0.004, respectively), as well as an increase at 25 compared to 50% PPO (P=0.03) (Figure 4).

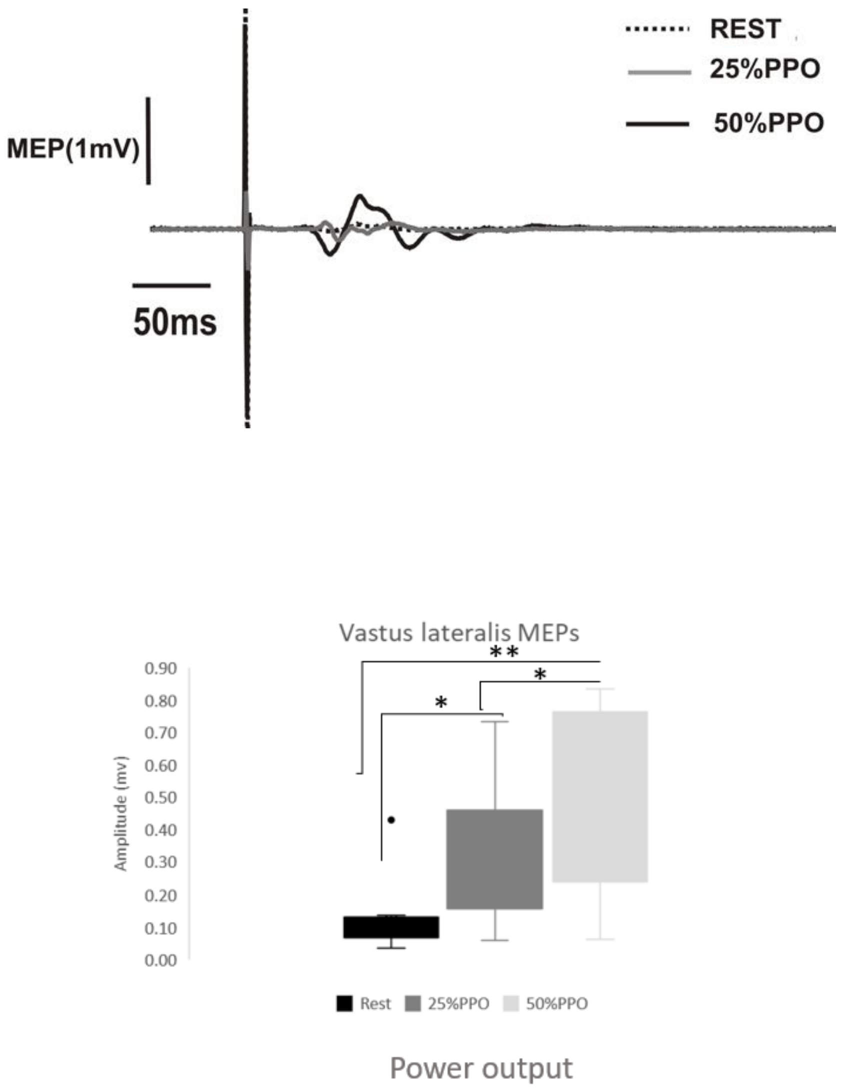


Figure 4: Vastus lateralis MEP amplitude (mV) at rest, 25 and, 50% PPO. MEP amplitudes was increased at 25 and 50% PPO during arm cycling compared to rest (0.3mV vs 0.11 mV; P=0.02) and (0.5mV vs 0.11 mV; P=0.004) as well as at 25 compared to the 50% PPO during arm cycling (0.3mV vs 0.5mV; P=0.03) (n=12). * denotes a significant difference between rest and 25% PPO, as well as between 25 and 50% PPO. (P < 0 .05) and ** denotes a significant difference between rest and 50% PPO. (P < 0 .01).

3.3.6 Pre-stimulus EMG for vastus lateralis

As expected, there was no significant difference in background EMG of the vastus lateralis between rest (9.5µv), 25% (15 µv) and, 50% (18.6 µv) of peak power output during arm cycling (P=0.09), (Figure.5).

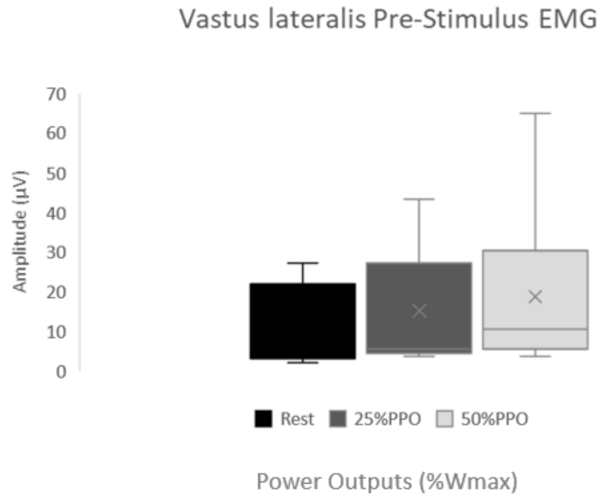


Figure 5: Background EMG for the vastus lateralis at rest, 25% and 50% of peak power output during arm cycling (n=12).

3.4 Discussion

Our study, for the first time, investigated the effect of arm and leg cycling on corticospinal excitability to the resting limbs. In the present study we demonstrated that corticospinal excitability increased to biceps brachii and vastus lateralis during leg and arm cycling, respectively, an effect that was enhanced as cycling intensity increased.

3.4.1 Corticospinal excitability to the resting biceps brachii and vastus lateralis muscles is increased during leg and arm cycling, respectively.

Previous evidence reported that rhythmic leg cycling increased corticospinal excitability in contracted and relaxed flexor carpi radialis (FCR) muscle (Sakamoto, Tazoe, Shiozawa, & Komiyama, 2008; Zehr, Klimstra, Johnson, & Carroll, 2007). In line with this research, our data demonstrated an increase in corticospinal excitability to the biceps brachii and vastus lateralis during leg and arm cycling, respectively. A possible mechanism for increased corticospinal excitability to the biceps brachii and vastus lateralis during leg and arm cycling respectively could result from mechanisms at the cortical and/or spinal level (Sakamoto et al., 2008; Zehr et al., 2007). Previous work has shown that the coordination between arm and leg was modulated through spinal intersegmental linkages that included propriospinal pathways, connecting cervical and lumbar locomotor networks (Dietz, 2002; Duysens & Van de Crommert, 1998).

In our study, we observed that corticospinal excitability to biceps brachii and vastus lateralis increase during leg and arm cycling respectively. One of the possible reasons may be due attributed to the excitation of cervical and lumbar networks, which may enhanced the corticospinal excitability to the arm and leg. This result may suggest a potential propriospinal neurons effect in interlimb coordination between upper and lower limbs.

Another possible mechanism for increases in corticospinal excitability to the biceps brachii and vastus lateralis during leg and arm cycling, respectively, can be cortically mediated. Debaere et al. (2001) reported the supplementary motor area is involved in the coordination of limb movements and play a leading role in the coordination of the homologous limbs. Therefore, it is possible that increased activity in various cortical areas could have led to 'spread' to other areas, such as those contributing to the corticospinal pathway.

3.4.2 Corticospinal excitability to the resting biceps brachii and vastus lateralis is intensity-dependent during leg and arm cycling, respectively

Our data showed significant differences in the MEP amplitude for the biceps brachii and vastus lateralis at 25 and 50% PPO during leg and arm cycling, respectively, compared to rest (Figures. 4 and 5). In addition, MEP amplitude in vastus lateralis was significantly higher at 25 than 50% PPO during arm cycling (Figure. 5). In line with the results of the present study, Sakamoto et.al (2008) reported that corticospinal excitability to the active (isometric contraction) and relaxed FCR was increased as cadence increased during leg cycling. In the present study we expected corticospinal excitability to increase in both biceps brachii and vastus lateralis during leg and arm cycling, respectively as workload increased. One of the possible mechanisms is that increases in intensity (workload), resulted in a decrease in presynaptic inhibition of the IA afferents (Forman, Philpott, Button, & Power, 2015; Spence, Alcock, Lockyer, Button, & Power, 2016).

3.5 Methodological considerations

There are factors that should be considered when interpreting this study's results. First, we only used TMS to measure corticospinal excitability in biceps brachii and vastus lateralis during leg and arm cycling, respectively. Therefore, our results may not necessarily reflect the spinal excitability. As previous studies has been shown, the main neural mechanism that control of upper and lower limb coordination is propriospinal pathways (Flynn et al., 2011; Jankowska, 1992). Furthermore, it seems using TMES can be improve our understanding about neural connection mechanism during rhythmic movements.

3.6 Conclusion

The present study was the first that demonstrated that corticospinal excitability increased to the resting biceps brachii and vastus lateralis during leg and arm cycling, respectively. It has been suggested that that neural networks in the cervical and lumbar spinal cord are responsible for regulating the coordination between the upper and lower limbs during rhythmic movements. Therefore, our study strongly supports this hypothesis that rhythmic activity of one limb pair affects corticospinal excitability of the other limb pair. In addition, our results demonstrates that changes in corticospinal excitability during rhythmic movements (arm and/or leg cycling) is intensity-dependent.

3.7 References

Debaere, F., Swinnen, S. P., Béatse, E., Sunaert, S., Van Hecke, P., & Duysens, J. (2001). Brain areas involved in interlimb coordination: a distributed network. Neuroimage, 14(5), 947-958.

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

Duysens, J., & Van de Crommert, H. W. (1998). Neural control of locomotion; Part 1: The central pattern generator from cats to humans. Gait & posture, 7(2), 131-141.

Flynn, J. R., Graham, B. A., Galea, M. P., & Callister, R. J. (2011). The role of propriospinal interneurons in recovery from spinal cord injury. Neuropharmacology, 60(5), 809-822.

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

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

Jankowska, E. (1992). Interneuronal relay in spinal pathways from proprioceptors. Progress in neurobiology, 38(4), 335-378.

Jankowska, E., Lundberg, A., Roberts, W., & Stuart, D. (1974). A long propriospinal system with direct effect on motoneurones and on interneurones in the cat lumbosacral cord. Experimental brain research, 21(2), 169-194.

Lockyer, E. J., Benson, R. J., Hynes, A. P., Alcock, L. R., Spence, A.-J., Button, D. C., & Power, K. E. (2018). Intensity matters: effects of cadence and power output on corticospinal excitability during arm cycling are phase and muscle dependent. Journal of neurophysiology, 120(6), 2908-2921.

Sakamoto, M., Tazoe, T., Shiozawa, S., & Komiyama, T. (2008). Effect of arm or leg cycling on motor-evoked potentials and H-reflexes in static limbs. Japanese Journal of Physical Fitness and Sports Medicine, 271-284.

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

Zehr, E. P., Klimstra, M., Johnson, E. A., & Carroll, T. J. (2007). Rhythmic leg cycling modulates forearm muscle H-reflex amplitude and corticospinal tract excitability. Neuroscience letters, 419(1), 10-14.

Zehr, E. P., Frigon, A., Hoogenboom, N., & Collins, D. F. (2004). Facilitation of soleus H-reflex amplitude evoked by cutaneous nerve stimulation at the wrist is not suppressed by rhythmic arm movement. *Experimental brain research*, *159*(3), 382-388.

Zehr, E. P., & Haridas, C. (2003). Modulation of cutaneous reflexes in arm muscles during walking: further evidence of similar control mechanisms for rhythmic human arm and leg movements. *Experimental brain research*, *149*(2), 260-266.

Zehr, E. P., Klimstra, M., Johnson, E. A., & Carroll, T. J. (2007). Rhythmic leg cycling modulates forearm muscle H-reflex amplitude and corticospinal tract excitability. *Neuroscience letters*, *419*(1), 10-14.

Chapter 4 Summary and Future Directions

The result of the study was conducted by the Human Neurophysiology Lab (HNL) at Memorial University of Newfoundland and Labrador. The HNL aims to understand how the brain and spinal cord produce complex movements and controls locomotor outputs in humans. The HNL lab has conducted several investigations to understand the modulation of corticospinal excitability during rhythmic movement by using TMS and TMES. As mentioned through this thesis, this project was the first study in the HNL lab that focused in the interlimb connection between upper and lower body during arm and/or leg cycling. Coordinated sequence of rhythmic interactions between the upper and lower limbs is essential to activities such as walking, running, and cycling that these smooth and well-regulated movement between the arms and legs, involves complex neural processes and control by central nervous system. In general, in this thesis had two purposes:

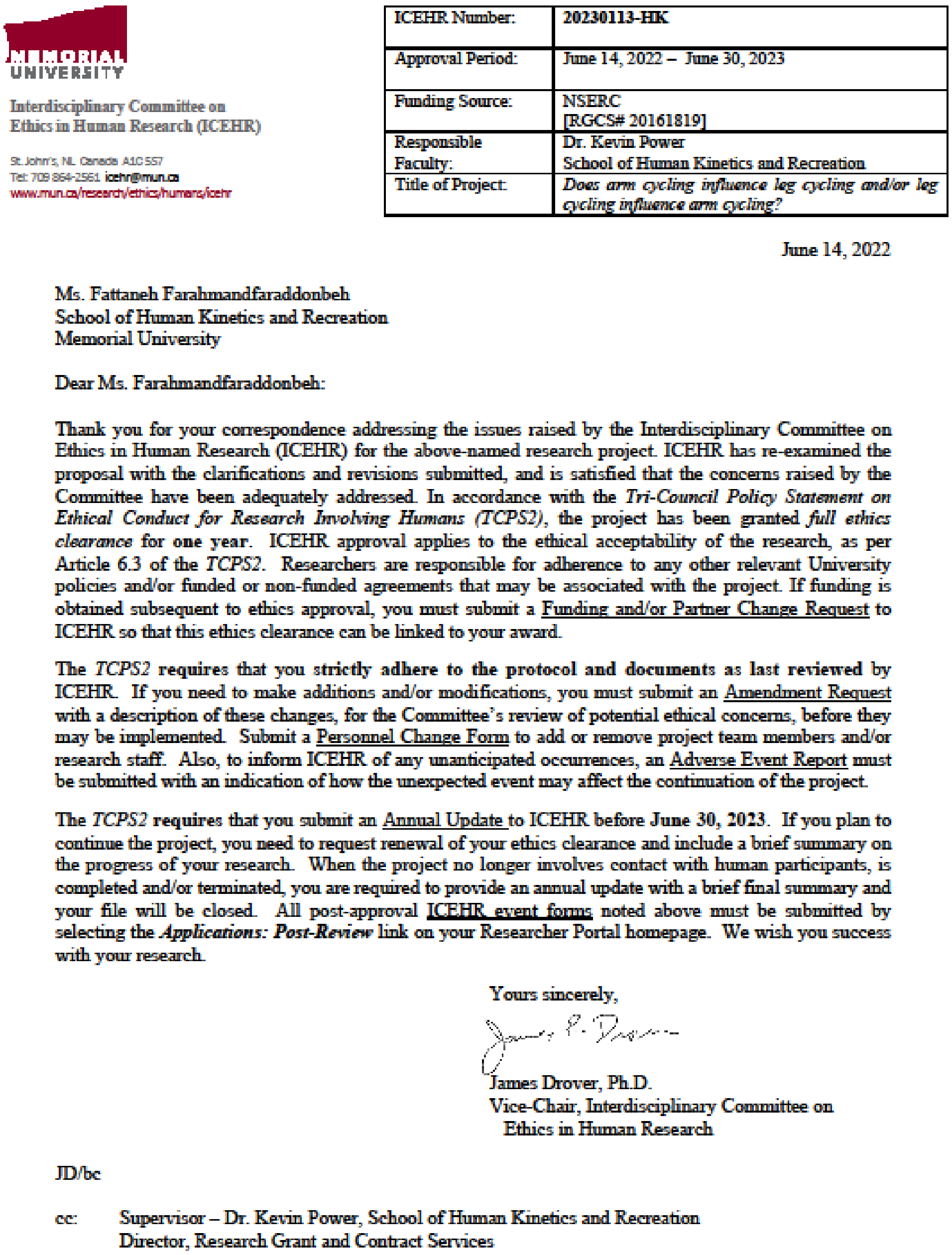
First to determine if corticospinal (brain and spinal cord) excitability to the biceps brachii and vastus laterlis is modulated by leg and arm cycling respectively and second if the relationship between arm and leg cycling and corticospinal excitability of the vastus laterlis and biceps brachii is dependent on cycling intensity or not.

Using the transcranial magnetic stimulation (TMS), MEPs were elicited during rhythmic cycling at stationary limbs. The results of this thesis showed that MEP in the vastus lateralis and biceps brachii was increased during arm and leg cycling respectively. In addition, for the second purpose, this study found there were a significant intensity-dependent modulation of MEP to the biceps brachii and vastus laterlis during leg and arm cycling respectively. It seems in addition of

MEP, future studies should assess cervicomedullary motor evoked potential (CMEP) to the biceps brachii and vastus laterlis during leg and arm cycling to examine the interlim connection. As well, MEP and/or CMEP should be examined at different intensities and/or cadences of arm and leg cycling.

The results from this study may be used for guiding rehabilitation strategies and exercise interventions for clinical and non-clinical populations. For example, in persons living with stroke and/or multiple sclerosis the motor control of the upper and/or lower limbs are affected. It may be possible to increase the ability of the nervous system to regain some motor control to the affected limb(s) by using arm and/or leg cycling to increase excitability to the region of the brain that controls the affected limb(s). Increasing brain excitability in that region could make that region more susceptible to rehabilitation induced neuroplasticity.

Appendix A: Ethical Approval



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Interdisciplinary Committee on
Ethics in Human Research (ICEHR)

St. John's, NL Canada A1C 5S7
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www.mun.ca/research/ethics/humans/icehr

ICEHR Number:	**20230113-HK**
Approval Period:	June 14, 2022 – June 30, 2023
Funding Source:	NSERC [RGCS# 20161819]
Responsible Faculty:	Dr. Kevin Power School of Human Kinetics and Recreation
Title of Project:	*Does arm cycling influence leg cycling and/or leg cycling influence arm cycling?*

June 14, 2022

Ms. Fattaneh Farahmandfaraddonbeh
School of Human Kinetics and Recreation
Memorial University

Dear Ms. Farahmandfaraddonbeh:

Thank you for your correspondence addressing the issues raised by the Interdisciplinary Committee on Ethics in Human Research (ICEHR) for the above-named research project. ICEHR has re-examined the proposal with the clarifications and revisions submitted, and is satisfied that the concerns raised by the Committee have been adequately addressed. In accordance with the *Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans (TCPS2)*, the project has been granted *full ethics clearance* for **one year**. ICEHR approval applies to the ethical acceptability of the research, as per Article 6.3 of the *TCPS2*. Researchers are responsible for adherence to any other relevant University policies and/or funded or non-funded agreements that may be associated with the project. If funding is obtained subsequent to ethics approval, you must submit a Funding and/or Partner Change Request to ICEHR so that this ethics clearance can be linked to your award.

The *TCPS2* **requires that you strictly adhere to the protocol and documents as last reviewed by ICEHR.** If you need to make additions and/or modifications, you must submit an Amendment Request with a description of these changes, for the Committee's review of potential ethical concerns, before they may be implemented. Submit a Personnel Change Form to add or remove project team members and/or research staff. Also, to inform ICEHR of any unanticipated occurrences, an Adverse Event Report must be submitted with an indication of how the unexpected event may affect the continuation of the project.

The *TCPS2* **requires** that you submit an Annual Update to ICEHR before **June 30, 2023**. If you plan to continue the project, you need to request renewal of your ethics clearance and include a brief summary on the progress of your research. When the project no longer involves contact with human participants, is completed and/or terminated, you are required to provide an annual update with a brief final summary and your file will be closed. All post-approval ICEHR event forms noted above must be submitted by selecting the ***Applications: Post-Review*** link on your Researcher Portal homepage. We wish you success with your research.

Yours sincerely,

James Drover, Ph.D.
Vice-Chair, Interdisciplinary Committee on
Ethics in Human Research

JD/bc

cc: Supervisor – Dr. Kevin Power, School of Human Kinetics and Recreation
Director, Research Grant and Contract Services