Long-interval intracortical inhibition to the biceps brachii is present during arm cycling but is not different than a position-matched tonic contraction

by

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Abstract

Locomotor outputs are controlled by a combination of descending input, sensory feedback and networks of cells in the spinal cord called central pattern generators (CPGs). In the absence of descending input, sensory feedback and CPGs are able to produce rhythmic muscle activation, which create complex patterns of locomotor outputs such as crawling, swimming, walking, cycling. Arm cycling is used as a model of locomotion in order to examine various changes in neural excitability as humans require descending input in order to preform successful locomotion. The human nervous system is complex with many different pathways and tracts; one of which, the corticospinal tract, is involved in the voluntary control of human locomotion. Research investigating corticospinal excitability during arm cycling found that supraspinal excitability was greater during arm cycling than a position- and intensity-matched tonic contraction, yet the mechanism(s) are unclear. Various cortical circuits, such as short-interval intracortical inhibition (SICI) and interhemispheric inhibition (IHI), have been investigated. However, these results do not shed light on possible mechanisms for greater supraspinal excitability during arm cycling. Only one study assessed long-interval intracortical inhibition (LICI) during locomotion, but it was during leg cycling and did not assess task-dependency. To date, there has yet to be a study that has assessed LICI during arm cycling. Therefore, the purpose of the study was to determine if LICI is 1) present during arm cycling and 2) task-dependent. It was hypothesized that 1) LICI would be observed during arm cycling and 2) the amount of LICI would be less during arm cycling compared to a tonic contraction.

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List of Abbreviations

AP- Action Potential

CMEP- cervicomedullary motor evoked potential

CNS- central nervous system

CPGs- central pattern generators

CS- control stimulus

EEG- electroencephalogram

EMG- electromyography

IHI- interhemispheric inhibition

LICI- long-interval intracortical inhibition

M1- primary motor cortex

MEP- motor evoked potential

Mmax- maximum M-wave

MRI- magnetic resonance imaging

ms- milliseconds

MVC- maximal voluntary contraction

M-wave- compound muscle action potential

PET- positron emission tomography

RPM- revolutions per minute

SD- standard deviation

SICI- short-interval intracortical inhibition

TMES- transmastoid electrical stimulation

TMS- transcranial magnetic stimulation

TS- test stimulus

μV- microvolts

Chapter 1 Introduction

Introduction

1.0 Overview

Humans are dynamic beings, even when standing still, fine adjustments are constantly being made to maintain balance. Before a person is able to walk, they crawl, using their arms and legs in a coordinated fashion to explore the environment. Therefore, we are all born quadrupeds as crawling is the first form of movement. Infants are not taught to crawl rather it is an innate ability. New neural connections within the infants brain are created and strengthen during crawling (Xiong et al 2021). Along with neural connections in the brain there are neural connections in the spinal cord known as Central pattern generators (CPGs) which are important for rhythmic movements such as crawling, walking and swimming (MacKay-Lyons 2002).

Within humans there are countless neural pathways and connections. There are connections between various brain regions, between the brain and spinal cord and between the brain and periphery, such as muscles. For years scientists have been trying to better understand the human nervous system and the countless neural pathways, connections, and circuits in relation to motor output. Initial research mainly investigated the nervous system during a resting state. It is now known that neuronal excitability changes when one transitions from rest to motor output and differs between tonic and dynamic motor outputs. The excitability of the brain during rest and tonic contractions have been extensively researched (Rothwell 1997; Rothwell et al. 1991). However, alterations during dynamic motor output are not well-understood, especially when one considers complex motor outputs such as locomotion. Understanding how different neural circuits within the cortex are altered during motor output is critical for understanding locomotor behaviour. Gaining a better understanding of cortical circuits of young healthy individuals during a locomotor output

is of the utmost importance to advance our basic understanding of the human body and how it is altered during movement. Additionally, improving our understanding of the nervous system of healthy adults will allow for comparisons to that of special populations such as the elderly, or people with neurological disorders.

Arm cycling is used as a model of locomotion during research in order to examine various changes in neural excitability. During cycling, there is rhythmic alterations in flexor and extensor muscle activity which resemble locomotion, but the participant can remain seated meaning they are stationary with minimal trunk movement, thus reducing movement related variability in measures during stimulation protocols. A growing body of literature has examined cortical circuits during locomotor outputs, such as arm and leg cycling (Alcock et al. 2019; Compton et al. 2022; Forman et al. 2014; Sidhu et al. 2013; Sidhu et al. 2018). Utilizing various stimulation techniques such as transcranial magnetic stimulation (TMS), transmastoid electrical stimulation (TMES) and peripheral nerve stimulation, it was determined that spinal excitability was similar during arm cycling compared to a position- and intensity-matched tonic contraction. However, corticospinal excitability was different between the two tasks. Since corticospinal excitability is a combination of supraspinal and spinal components, if spinal excitability did not change but corticospinal excitability increased this change in excitability must be supraspinal. Therefore, supraspinal excitability was greater during arm cycling compared to a position- and intensity-matched tonic contraction yet the mechanisms are unclear. Various cortical circuits such as short-interval intracortical inhibition (SICI) and interhemispheric inhibition (IHI) have been investigated during arm cycling in comparison to a tonic contraction, however, these results do not shed light on possible mechanisms for greater supraspinal excitability during arm cycling. Only one study

assessed long-interval intracortical inhibition (LICI) during locomotion, however, it was during leg cycling and did not assess task-dependency. To date, there has yet to be a study which assesses LICI during arm cycling.

1.1 Purpose

The purpose of this study was to determine if long-interval intracortical inhibition (LICI) is: 1) present during arm cycling and 2) task-dependent.

1.2 Research Hypotheses

It was hypothesized that 1) LICI will be observed during arm cycling and 2) the amount of LICI will be less during arm cycling compared to a tonic contraction.

1.3 References

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Chapter 2 Literature Review

Literature Review

2.1 Introduction

Humans are multicellular organisms with several organs, systems and pathways working together constantly to maintain life. Understanding the anatomy, physiology and biochemistry of humans has been an ongoing task for countless years. Since humans are dynamic beings, understanding how the nervous system controls complex motor outputs is a monumental task. Even with a substantial amount of research constantly being performed, there are countless motor control concepts, neural pathways, and information regarding human motor output that remain unknown. One area in which there are gaps of knowledge is the way in which humans move through the world, specifically how the nervous system helps facilitate successful locomotion. Humans are able to produce complex locomotor patterns which are multilimbed, coordinated movements. However, what remains unclear is what occurs within the central nervous system, specifically neural excitability.

2.2 Locomotion

In order to successfully move throughout the world there are many complex processes which must take place. One of the earliest definitions of locomotion is “the act or power of moving from place to place” (Barnhar et al. 1948). This definition has been altered over the years with the current definition of locomotion focusing on the movement and ability to move from place to place. Nonetheless, locomotion can have many forms, such as walking, swimming, cycling, crawling and more. All of these movements are multijointed and require tight control over muscle and joint timing to result in effective, smooth motion (Inman 1966). The musculoskeletal system is one of the major systems responsible for facilitating locomotion as it provides stability of the

body and forms movement. Walking is the most common form of locomotion, which many humans utilize every day. However, although seemingly easy, walking requires balance control, activation and inhibition of muscles, timing control, to be successful.

2.2.1 Gait

Walking (also known as gait) can be broken into distinct phases. Although there is a specific gait cycle, each individual is able to make small variations based on their age, fitness level, mood, health and personality (Silva & Stergiou 2020). The gait cycle can be broken down into two main phases, stance and swing. For the purposes of this explanation, we will focus on the right foot. The stance phase is when the right foot is in contact with the ground whereas the swing phase is when the right foot is in the air. During regular gait speeds, approximately 60% of the cycle will be stance and 40% swing. The complete gait cycle is the time in which the right foot first contacts the ground up until the right foot contacts the ground again. As gait speed increases, the percentage of stance time decreases (Murray et al. 1964). The stance phase can be further subdivided into various phases, such as the initial contact with the ground (heel contact) and toe-off, directly before the swing phase. Additionally, during the stance phase, there are subphases of double-leg support (both legs in contact with the ground) and single-leg support (one leg in contact with the ground) (Silva & Stergiou 2020). Gait is particularly difficult due to the single-leg stance and thus requires balance control. Even though there can be minor individual differences in gait pattern, overall gait is a cyclical locomotor pattern.

The stance phase can be compared to the extension phases of other locomotor outputs, as the leg must be extended to be on the ground and support the body weight. In addition, the swing phase can be compared to the flexion phase as the hip and knee flex to create foot clearance. All

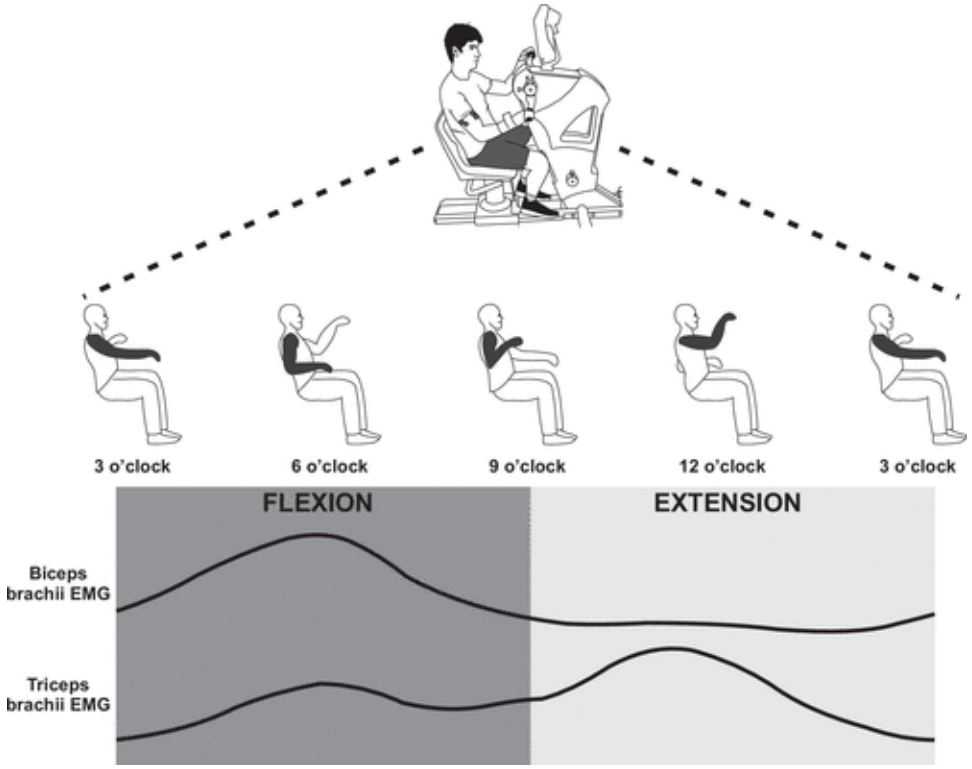
forms of locomotion have joint specific extension and flexion phases as it is rhythmic with specific joint angles.

2.2.2 Arm Cycling

Arm cycling is used in research as a model of locomotion. Arm cycling shares many similarities with locomotion, specifically gait. Similarities between arm cycling and gait include, similar joint range of motion, neural control and muscle activity (flexion and extension as described earlier) (Klarner et al. 2014; Zehr 2005). Therefore, arm cycling is an effective model used to assess the neural control of locomotion. There are many benefits to using arm cycling as a model of locomotion, one of which is the fact that it is a non-weight bearing activity. Gait requires balance and tight timing control of muscle activation to effectively propel oneself forward. Since arm cycling is a seated activity no balance control is required. Thus, research can be completed on individuals who struggle with balance control. In addition, variability can be reduced when stimulations such as transcranial magnetic stimulation are given during arm cycling where a participant remains in a seated position as opposed to other locomotor outputs such as gait which are not static positions. Additionally, arm cycling is a model of locomotion as this rhythmic movement allows for investigation of the upper limb neural pathway. Since cycling can either be arm or leg cycling, individualized analyses can be made of the upper or lower limbs. In comparison to gait which the upper and lower limbs work in unison, the upper and lower limbs cannot be easily isolated. In addition, humans swing their arms rhythmically during walking. Therefore, arm cycling can be used as a model to only assess the upper limbs but can also compare connections between arms and legs during locomotion (Pearcey & Zehr 2020; Zehr 2005).

Similar to walking, arm cycling involves a rhythmic movement where muscles facilitate joint flexion and extension. As seen in Figure 1, during arm cycling there are distinct phases of elbow flexion and extension (Lockyer et al. 2021). The biceps brachii is one of the major muscles that produces elbow flexion, and the triceps brachii muscle produce elbow extension. When discussing arm cycling the movement is compared to a clock face to define positions. 12 o'clock is dead centre at the top of the movement, and 6 o'clock is dead centre at the bottom of the circular movement. Therefore, elbow flexion occurs from 3 o'clock to 9 o'clock, and elbow extension occurs from 9 o'clock to 3 o'clock. It is the combination of these two muscles, along with many others, that are able to produce movement alternation. The process of muscle activation is complex as many factors are involved such as muscle fibres, ions, neurotransmitters and neurons.

Figure 1: Arm cycling position and EMG comparison during flexion and extension phases (Lockyer et al. 2021).



2.3 Nervous System

The nervous system is responsible for transmitting electrical and chemical signals to communicate with regions of the body and also for sending signals that create movement. The nervous system can be broken down into two sections, central and peripheral**.** The central nervous system is comprised of two parts, supraspinal (brain and brainstem) and spinal. The peripheral nervous system is comprised of nerves that exit the spinal cord, innervating all parts of the body in addition to the nerves that have yet to enter the spinal cord (Brodal 2016). Within the human body there are billions of neurons, in the brain alone there are approximately 100 billion neurons (Herculano-Houzel 2009).

2.3.1 Neurons

Neurons are composed of a cell body, dendrites and axon (Brodal 2016; Sivadas & Broadie 2020)**.** Briefly, dendrites receive information from other neurons, and if the cell is excited, an action potential (AP) is generated at the axon hillock and travels down the axon to the terminal end. At the terminal end, the electrical signal will cause the release of neurotransmitters into the synapse, thus chemically communicating to another neuron (Sivadas & Broadie 2020)**.** The brain is able to send descending information to the periphery for the voluntary control of movement, through spinal motoneurones. Spinal motoneurones synapse onto muscle fibres releasing neurotransmitters to initiate muscle contraction (Purves et al. 2001). In addition, information can be transmitted from the periphery to the central nervous system, through sensory neurons. Spinal nerves are known as ‘mixed nerves’ as they contain both motor and sensory nerve fibres (Kaiser & Lugo-Pico 2022; Purves et al. 2001). However, when the spinal nerves exit the spinal cord, they separate into motor and sensory components (Kaiser & Lugo-Pico 2022). There are several

peripheral sensory neurons, one of which is the 1a afferent which provide sensory information on the rate of change and length of the intrafusal muscle fibres (Kaiser & Lugo-Pico 2022). The sensory afferent axons carry information to the spinal cord via the dorsal root (Kaiser & Lugo-Pico 2022). On the other hand, the motoneurone cell bodies are found within the spinal cord and are referred to as alpha motoneurones. These axons exit the spinal cord via the ventral roots which send information from the central nervous system to the muscles (Purves et al. 2001).

2.3.2 Spinal Cord

As mentioned above, the central nervous system is comprised of two parts, supraspinal (brain and brainstem) and spinal. Supraspinal components are vital for the voluntary control of locomotion. However, the importance of the spinal cord during locomotion must not be overlooked. In humans, there are two spinal cord enlargements, one located within the cervical region and one in the lumbar region. The purpose of these enlargements is to accommodate the large number of nerve cells as well as neural connections in relation to the arms and legs (Purves et al. 2001). The cervical enlargement includes spinal segments C5-T1 whereas the lumbar enlargement includes spinal segments L2-S3 (Purves et al. 2001). The spinal cord is very important to send information to and from the periphery, but it is more than simply an ‘information highway’. The spinal cord has many important neural connections to enable spinal reflexes and even house central pattern generators (CPGs).

2.3.3 Animal Models Used to Assess Spinal Pathways

In the initial stages of neural research, scientists analyzed the nervous system of animals or assessed rare human cases such as illnesses or following accidents. For example, an accident occurred in 1848 where a tamping iron exploded through the skull of Phineas Gage (O'Driscoll &

Leach 1998). Phineas miraculously survived the incident and maintained his cognitive ability, ability to move and working memory (O'Driscoll & Leach 1998). However, his personality changed. Leading doctors at the time to begin to understand how different areas of the brain have different functions (O'Driscoll & Leach 1998). However, these special cases were rare and were unable to be controlled. Therefore, small animal research was popular as specific research questions could be investigated due to researcher having full control over the experimental conditions.

Over 100 years ago, animal models displayed the first evidence that the control of locomotion was not dependent on the cortex but instead on the spinal locomotor CPG networks (Brown 1911; Klarner & Zehr 2018; Sherrington 1910). A very famous chicken, named Mike, was a headless chicken who stayed alive for 18 months after decapitation and continued to walk (Lockyer et al. 2022). Mike displayed clear evidence that a brain is not required to facilitate movement. Animal models are still used to analyze various spinal pathways, as invasive surgeries can be done to analyze various neural connections housed within the spinal cord (Pearcey and Zehr 2020).

2.3.4 Central Pattern Generators (CPGs)

Within the spinal cord, there are CPGs that control basic rhythmic movement. CPGs are specialized networks of neurons located within the spinal cord that are responsible for the production of rhythmic muscle activation, which control the limbs and alternate movements of locomotion (Duysens & Van de Crommert 1998; Lockyer et al. 2022; MacKay-Lyons 2002; Zehr 2005). Figure 2 displays the complexity of CPGs. Even though humans share similarities with quadrupeds there are notable neural differences. In both humans and animals, the spinal cord can

produce complex alterations of muscle contractions necessary for walking (Pearcey & Zehr 2020). However, as humans are bipedal there is more descending control required for successful movement. Therefore, the brain is important for gait initiation as well as obstacles avoidance and acts as a supervisor of movement rather than the main producer (Pearcey & Zehr 2020). CPGs generate the rhythm of locomotion and create the pattern of the motoneuron bursts (Duysens & Van de Crommert 1998). Direct human spinal cord research has been difficult and unobtainable due to the invasiveness of the research and ethical responsibilities. In animal models, it is possible to record CPG activity directly within the spinal cord, which is not possible in humans. Therefore, with human research, indirect experimental techniques are required to assess how much CPGs contribute to rhythmic human movement (Klarner & Zehr 2018).

Electromyography (EMG) provides a general output from the central nervous system as EMG assesses the electrical activity of muscles and nerves (Pearcey & Zehr 2020). Using EMG and measuring muscle activity, it is possible to better understand how supraspinal, spinal (CPGs) and sensory influences affect motor unit output (Pearcey & Zehr 2020). However, human research is problematic because spinal CPGs cannot be isolated since it is impossible to separate the spinal neurons from the rest of the nervous system. CPG's using animal models is much easier to study as a direct approach is possible.

Sherrington (1910) used animals to research the nervous system and concluded that locomotion originated from the peripheral nervous system rather than supraspinal. He concluded locomotion was due to a series of reflexes, specifically the crossed extension reflex; ipsilateral flexion and contralateral extension (Klarner & Zehr 2018; Sherrington 1910). In 1911 Graham Brown developed the half-center model, composed of flexor and extensor half-centers, which together oscillate to create the rhythmic pattern of locomotion (Brown 1911). This model suggests

that the pattern of rhythmic locomotion is created by reciprocal inhibition between the extensor and flexor interneuron pools (MacKay-Lyons 2002). Each half centre is composed of spinal neurons; the extensor group of neurons excites the extensor motoneurones, thus activating extensor muscles while simultaneously inhibiting the flexor neurons (Brown 1911; Klarner & Zehr 2018; Pearcey & Zehr 2020). When the extensor half begins to fatigue, this reduces the inhibition on the flexor half, thus exciting the flexor half center to excite flexor motoneurones and inhibit the extensor half center (Brown 1911; Klarner & Zehr 2018; MacKay-Lyons 2002). The alternation between flexion and extension creates the locomotor pattern. Brown's research proved that locomotion patterns continued without the influence of cortical input or peripheral input, concluding that spinal CPGs are present (Brown 1911; Pearcey & Zehr 2020). Figure 3 demonstrates that although cortical input and sensory feedback are not required, they are important for smooth successful locomotion in humans (Zehr 2005; Pearcey & Zehr 2020).

Figure 2: Schematic representation of interlimb connections in the spinal cord (Pearcey & Zehr 2020).

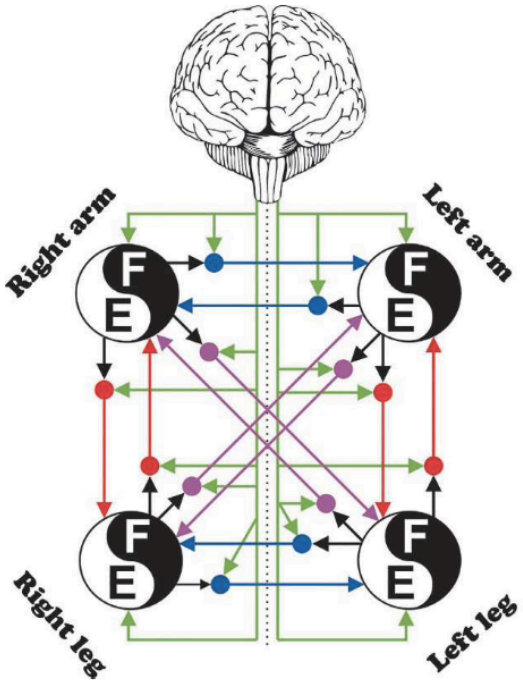
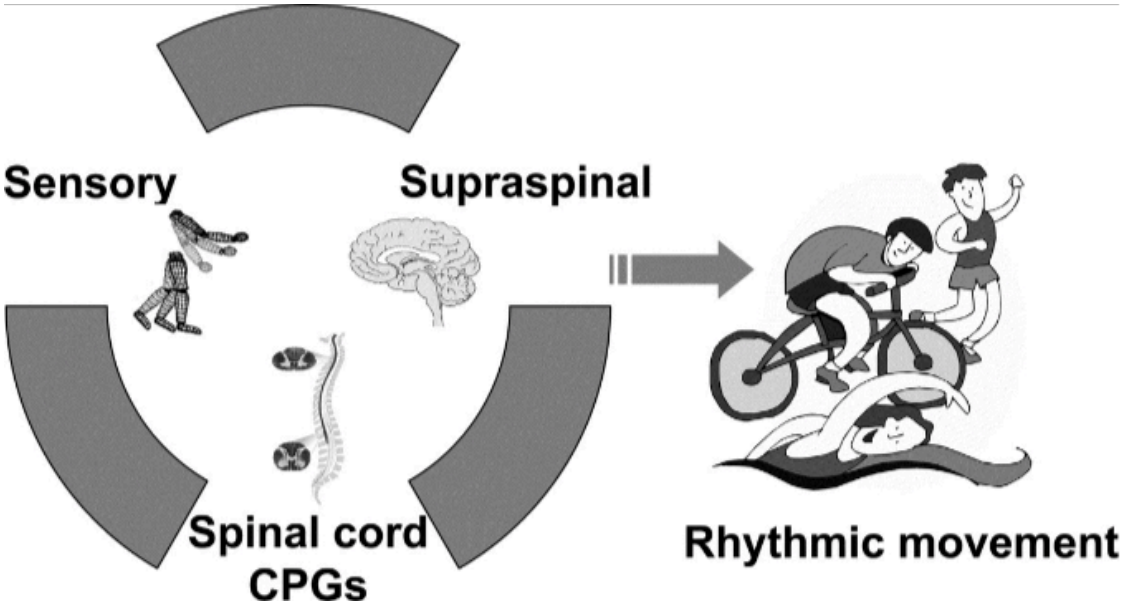


Figure 3: Hypothesised theory for rhythmic movement regulation in humans (Zehr 2005).



2.3.5 Spinal Reflexes

Within the body there are numerous spinal reflexes such as the cutaneous reflex, stretch reflex, recurrent inhibition, flexor reflex and Golgi tendon reflex and more (Hultborn 2006). Spinal reflexes can be monosynaptic, meaning there is only one synapse in the spinal cord. An example is 1a afferent directly synapsing onto the alpha motoneurone (Walkowski & Munakomi 2022). The stretch reflex, also known as the tendon reflex, is a monosynaptic reflex. This reflex arc has a direct synapse between the sensory neurons and alpha motoneurones. Briefly, this reflex is initiated by the stretch of the muscle, more specifically the intrafusal fibres where the muscle spindles are located (Walkowski & Munakomi 2022). The muscle spindles detect the muscle length and rate of stretch sending AP to the spinal cord via 1a afferents (Walkowski & Munakomi 2022). 1a afferents synapse onto an alpha motoneurone which sends AP to the muscle to cause a contraction. 1a afferent fibres enter the spinal cord on the dorsal side via the dorsal root of the spinal cord

(Walkowski & Munakomi 2022). The purpose of the stretch reflex is to contract the muscle in which the rapid stretch occurred, thus reducing the muscle length. The whole process happens within the spinal cord without cortical input.

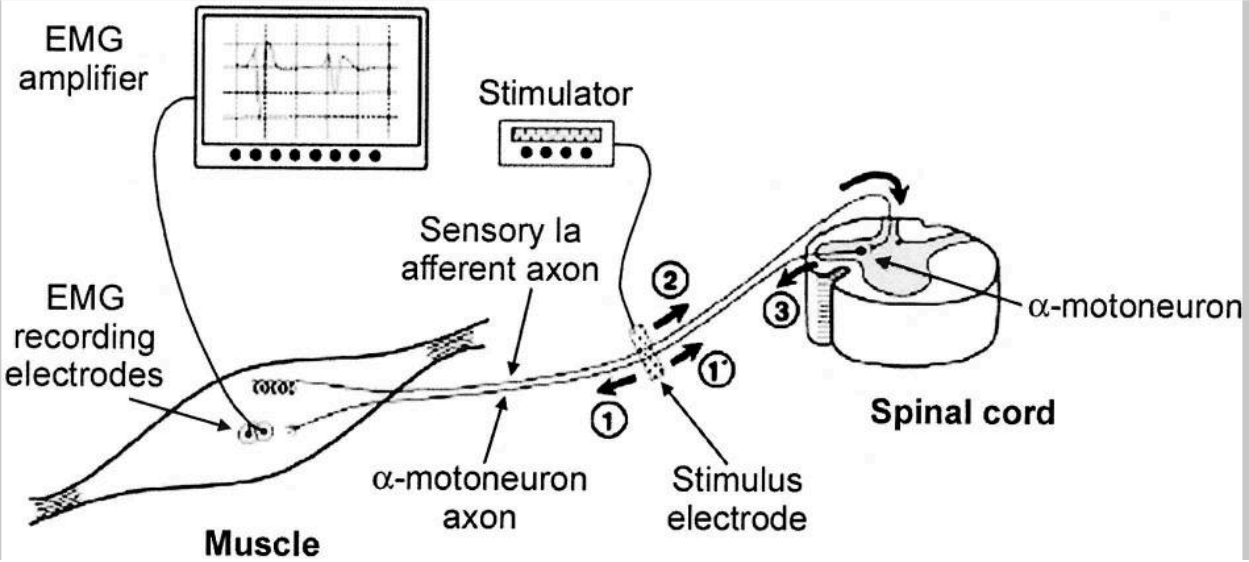
Although some spinal reflex arcs are simple monosynaptic reflexes, others can be quite complex and are polysynaptic. The crossed extension reflex or withdrawal reflex, occurs when a sensory stimulus is indicated on the foot when standing. This reflex utilizes, sensory neurons, motoneurones and interneurons (Herm et al. 2019). Briefly, when an individual senses a noxious stimulus, they react by removing their foot off the ground. This requires sensory neurones to communicate directly with motorneurones to cause flexion of the ipsilateral limb (Herm et al. 2019). Additionally, the sensory neurone activates interneurons which are required to send AP to the contralateral limb, activating extensor muscles (Herm et al. 2019). Extensor muscle activation is of the utmost importance to keep the individual upright while abruptly lifting the leg which received the noxious stimulus. Again, this entire reflex arc occurs within the spinal cord, proving that reflexes within the spinal cord can be complex and without cortical input. Various techniques have been developed in order to record and analyze spinal reflexes. When studying neural circuits, such as CPGs, spinal reflexes provide great insight as they allow for estimations about CPGs contribution on afferent feedback during rhythmic locomotion (Zehr 2005).

2.3.6 Hoffman-Reflex

The Hoffman-Reflex, also known as the H-reflex, is very similar to the stretch reflex. The major difference being that the H-reflex applies an electrical stimulus to the superficial peripheral nerves, thus bypassing any spindle activity making afferent input tightly controlled. Using this technique, assessments can be made of 1a afferent to motoneurone synaptic transmission which

can be influenced by presynaptic inhibition (Renshaw 1940; Capaday 1997; Zehr 2005). Specifically, as seen in Figure 4, nerves are stimulated via electrical stimulus. Starting at a low-intensity, AP are elicited first in sensory 1a afferents because of the large axon diameter in comparison to the smaller efferent neurons (Palmieri et al. 2004). These AP travel towards the spinal cord, entering through the dorsal root, giving rise to excitatory postsynaptic potentials in the alpha motoneurone. If depolarized enough, the alpha motoneurone generates an AP and transmit the signal toward the neuromuscular junction (Palmieri et al. 2004). The result will create a twitch response seen in EMG. However, the synapse of the afferent neuron onto the efferent neuron will cause a delay (Capaday 1997). The resulting H-reflex is a compound AP of several muscle fibres within the same area (Palmieri et al. 2004)**.** The muscle compound action potential (M-wave) is produced when the stimulation intensity is high enough to cause direct activation of the alpha motoneurones (Palmieri et al. 2004). AP will travel directly towards the muscle, known as orthodromic propagation, resulting in the M-wave (Palmieri et al. 2004). The M-wave will appear before the H-reflex as there are no nerve-to-nerve synapses (Palmieri et al. 2004). However, AP will also travel along the alpha motoneuron towards the spinal cord, known as antidromic propagation. Antidromic AP propagation is a non-biological behavior and only occurs with electrical stimulation. This is problematic as antidromic AP in the alpha motoneuron will eventually 'collide' with AP travel in the orthodromic direction in the 1a afferent neuron, thus causing a cancellation effect. Therefore, as the stimulation intensity increases the M-wave will reach its maximum amplitude and the H-reflex will decrease in size until it is fully cancelled out (Palmieri et al. 2004).

Figure 4: H-reflex pathway displaying antidromic and orthodromic AP as well as an EMG recording of both the M-wave and H-reflex. (Palmieri et al. 2004).



2.3.7 Spinal Control of Locomotion

Although a seemingly simple concept, the H-reflex is complex and has been used in countless studies to gain a better understanding of spinal pathways and excitation. The H-reflex is used extensively to examine spinal control of locomotor output. There is evidence that the H-reflex gives insights into more than just a simple monosynaptic reflex pathway (Misiaszek 2003). Some researchers have viewed oligosynaptic inputs contributing to the H-reflex through excitatory postsynaptic potentials and the H-reflex has been used to study inhibitory pathways (Misiaszek 2003). An individual's state: such as if they are laying down, standing, moving, doing tonic contractions, will determine the excitability of various reflex pathways. Reflex pathways are altered during locomotion compared to a tonic contraction or rest (Zehr 2005). For example, the stretch reflex in the lower limbs will be inhibited as a person walks in comparison to standing to allow for smooth locomotion (Misiaszek 2003). Utilizing the H-reflex allows researchers to gain

a better understanding of the degree of coupling between CPG components (Leppanen 2006). However, there are many external factors which influence reflex pathways. Maintaining similar levels of background EMG eliminates the factor of intensity on reflex pathways. If assessments of task-dependency and reflex pathways want to be made, in order to gain a better understanding of CPG activity, external factors must be kept consistent or be minimized (Zehr 2005). Studies have used the H-reflex to evoke a response during rhythmic motor outputs at specific phases of the locomotor cycle to better understand the influence of spinal CPGs (Pearcey & Zehr 2020). Examining reflex modulation during rhythmic movement is the main source of our understanding on CPGs in humans (Zehr 2005). The amplitude of the H-reflex is a way to quantify changes in reflex patterns with a change in locomotor output, allowing researchers to make indirect assumptions on the neural control mechanisms and how they are altered with varying locomotor outputs (Zehr 2005).

2.4 Introduction to the Corticospinal Tract

The nervous system is comprised of multiple complex and interconnected pathways. Within the nervous system, there are both ascending and descending tracts which facilitate communication between the central nervous system and periphery (Natali et al. 2022). The largest of the descending pathways is the corticospinal tract (Chen & Rothwell 2012). The information which descends from the cortical regions toward the periphery produces and refines the voluntary control of movement (Javed et al. 2022). As such, the corticospinal tract has been extensively studied to better understand the way in which humans move through the world. In addition, the connections between various regions of the brain, as well as the relationship between the central nervous system and the periphery, have been researched to a great extent.

2.4.1 Primary Motor Cortex

The primary motor cortex (M1) is a region of the brain located in the frontal lobe (Chen & Rothwell 2012). The primary motor cortex contains the cell bodies of the corticospinal neurons (Chen & Rothwell 2012). The cell bodies and axons make up the upper motoneurones of the corticospinal tract. These upper motoneurones synapse onto lower motoneurones in the spinal cord, also known as alpha motoneurones (Natali et al. 2022; Chen & Rothwell 2012). Upper motoneurones are involved in communication from the brain to the spinal cord, whereas lower motoneurones communicate between the spinal cord and muscle (Natali et al. 2022). The cerebral cortex is comprised of six layers, the neurons of the corticospinal tract are found within the fifth (V) layer (Welniarz et al. 2017). The large cell bodies of the corticospinal tract are pyramidal shaped and approximately 10-20% of the pyramidal neurons have monosynaptic connections with lower motoneurones (Chen & Rothwell 2012). In addition, the primary motor cortex also makes numerous synaptic connections with other brain regions, especially motor-related brain regions, to plan and execute movement both efficiently and effectively (Patton and Amassian 1954). The primary motor cortex has several intraneuronal connections that can be either facilitatory or inhibitory.

2.4.2 Anterior and Lateral Corticospinal Tracts

The corticospinal tract can be split into two components, known as the anterior and lateral corticospinal tracts. The main difference between the two components is that the anterior corticospinal tract does not cross over at the medulla and is thus known to have ipsilateral innervation (Natali et al. 2022). The anterior corticospinal tract is the smaller of the two, comprising approximately 5-15% of the total corticospinal tract (Jang 2014). In addition, the

anterior corticospinal tract is typically shorter than the lateral corticospinal tract, only descending to the upper thoracic spinal cord, rarely below (Jang 2014). Additionally, the anterior corticospinal tract indirectly innervates trunk and axial muscles (Natali et al. 2022).

The lateral corticospinal tract is the more abundant of the two as it makes up approximately 90% of the corticospinal tract (Javed et al. 2022). Within this tract, the upper motoneurones cross over at the medulla to synapse onto lower motoneurones which innervate muscles on the contralateral side of the body (Natali et al. 2022). Thus, the left motor cortex controls the right side of the body and vice versa. In order to assess and better understand the excitability of the corticospinal pathway, researchers use methodologies involving brain stimulation. One type of brain stimulation is transcranial magnetic stimulation (TMS).

2.5 Techniques Used to Examining the Neural Control of Movement

2.5.1 Transcranial Magnetic Stimulation (TMS)

TMS is a painless, non-invasive brain stimulation technique which can cause excitation or inhibition of neurons through a magnetic field (Chen & Rothwell 2012). TMS uses electromagnetic induction to produce a magnetic field that stimulates cells within the brain and can be used to measure corticospinal excitability (Di Lazzaro et al. 2001; Fatih et al. 2021). TMS applies a brief, yet powerful, magnetic field which is generated over the skull, typically exciting superficial neurons. Since the pyramidal cells of the corticospinal tract are located within layer V of the M1, TMS will activate superficial interneurons which synapse onto the pyramidal cells (Chen & Rothwell 2012). However, depending on the stimulation intensity and current direction, TMS can directly activate the corticospinal neurons found within layer V of M1 (Chen & Rothwell 2012). The pyramidal neurons found within M1 are more easily excitable when the electrical current flows parallel to the axons as opposed to perpendicular (Ueno and Sekino 2021). Therefore, the

way in which the TMS coil is placed on the skull can impact the ability of the coil to activate the neurons.

2.5.2 TMS Coils

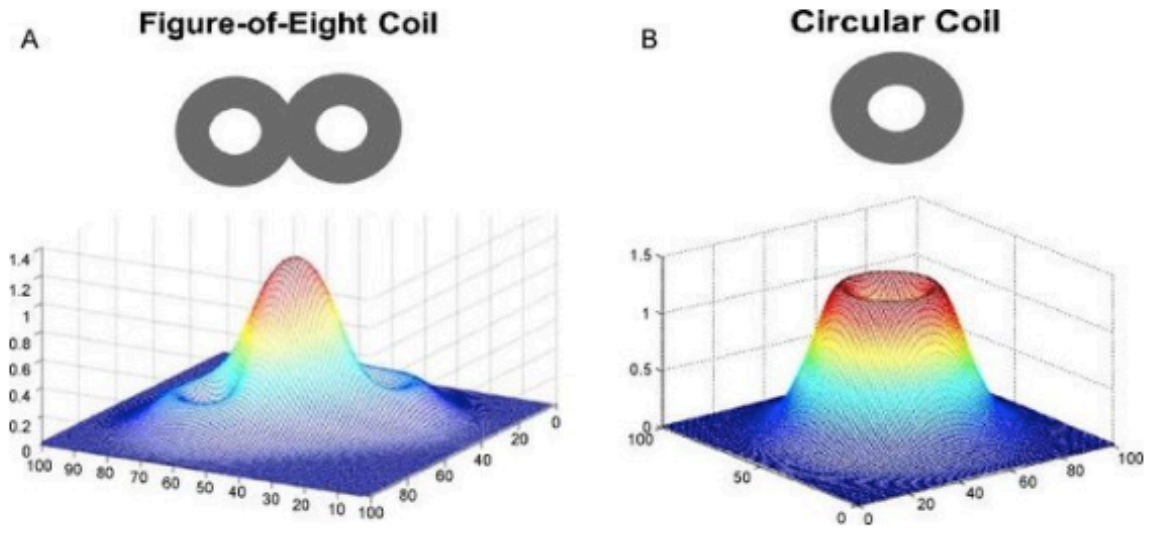
There are 3 main types of coils used in TMS: circular, figure 8 and double cone. Each coil type has a distinct set of advantages and disadvantages. TMS was developed by Anthony Barker in 1985 using a circular coil (Barker et al. 1985). The circular coil has a large magnetic field area in which the motor cortex is stimulated, thus, having very broad activation area. Although the circular coils have the largest activation area, there is less intensity directly in the centre. Therefore, to stimulate specific regions of the brain is difficult with a circular coil (Ueno & Sekino 2021). However, circular coils are advantageous, and typically used in research, as they allow for reproducible results as the large magnetic field, in which the circular coil produces, allows for small changes in coil location to be less problematic. A study by Badawy et al. (2011) found that when using paired pulse TMS no changes in results were noticed when using a circular coil compared to a figure of 8 coil.

In contrast, the figure of 8 coil is capable of producing localized stimulation. Due to the shape of the figure of 8 coil, 2 vortices are produced in the magnetic field, resulting in a large local activation region in the center of the figure of 8 (Ueno et al. 1988). Figure 5 below displays the magnetic field which is produced in the circular and figure of 8 coils. When the figure of 8 coil was developed, research concerned with the functional organization of the human brain increased (Ueno et al. 1990). In addition, research regarding neuronal plasticity has been enhanced with the use of the figure 8 coil because of the localized stimulation. A disadvantage is that due to the small

resolution of 5-mm in which the figure of 8 coil can stimulate, it is easy in repeated studies to stimulate different areas of the motor cortex (Ueno et al. 1990).

A final coil which is widely used in research is the butterfly or double cone coil. The coil, unlike the others stated above, is not flat but instead bent. An acute angle is formed between the two panels, allowing for the coil to surround the human head (Ueno & Sekino 2021). An advantage of the cone coil is that it enhances the depth in which the induced electric field can reach within the brain (Ueno & Sekino 2021). This can be very advantageous for studies regarding lower limbs as the motor processing for the lower limbs is found deep within the motor homunculus requiring deep stimulation in order to be activated. The placement of the coil as well as the type of coil used can have an impact on the motor evoked potential (MEP) observed.

Figure 5: Experimental set-up: a) figure-of-eight coil magnetic field that is generated and b) circular coil magnetic field (Giordano et al. 2012).



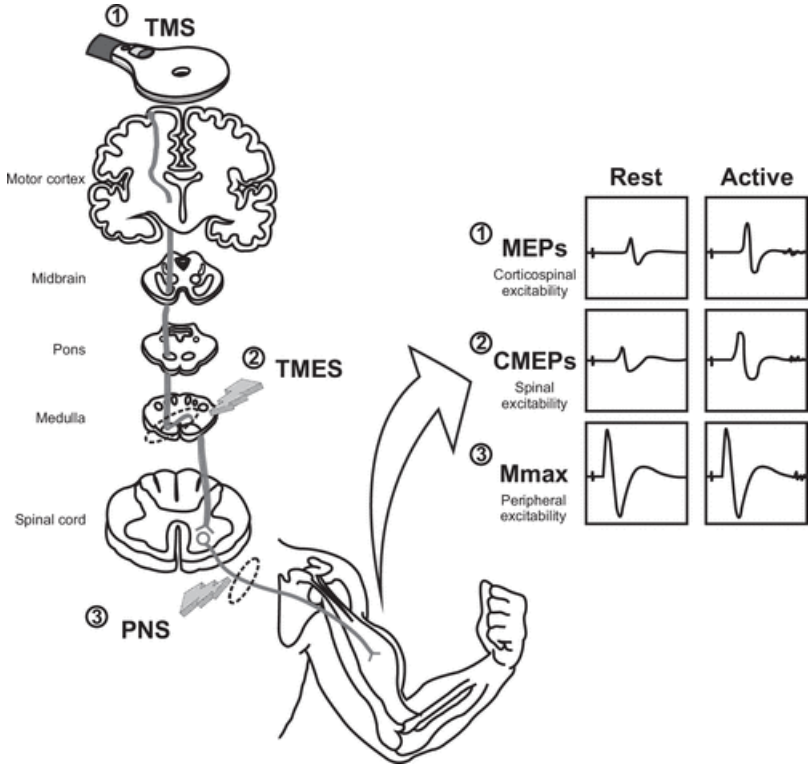
2.5.3 Transmastoid Electrical Stimulation (TMES)

Transmastoid electrical stimulation, TMES, is a non-invasive electrical stimulation technique that activates the upper motoneurne axons at the level of the cervicomedullary junction (Taylor 2006). TMES elicits a single descending volley which activates the corticospinal tract which synapses onto motoneurones. This elicits responses in muscles of the upper limb called cervicomedullary motor evoked potentials (CMEP) and gives an indication of spinal excitability (Taylor & Gandevia 2004; Taylor 2006). Unlike TMS, which gives an indication of corticospinal excitability, TMES is delivered below at the brainstem thus only providing insight into spinal and peripheral excitability (Taylor 2006).

2.5.4 Motor Evoked Potential (MEP) and Cervicomedullary Motor Evoked Potential (CMEP)

When the corticospinal neuron membrane potentials are depolarized enough to reach threshold, the neuron will become excited, AP will descend the upper motoneurone and synapse onto lower motoneurones. Using electromyography (EMG), the response from TMS can be observed as a motor evoked potential (MEP) and recorded from the muscle of interest (Nakamura et al. 1997). Similarly, TMES can be observed as a cervicomedullary motor evoked potentials (CMEP) (Taylor & Gandevia 2004). Figure 6 displays the techniques and pathways of the corticospinal tract in addition to the waves which are produced.

Figure 6: Stimulation techniques (left) 1) Transcranial Magnetic Stimulation (TMS), 2) Transmastoid Electrical Stimulation (TMES) and 3) Peripheral Nerve Stimulation (PNS) and their evoked responses 1) Motor Evoked Potential, 2) Cervicomedullary Motor Evoked Potential (CMEP) and Maximal Compound Action Potential (Mmax) (Lockyer et al. 2021).



The characteristics of the MEP and CMEPs can be analyzed in order to obtain information about corticospinal and spinal excitability respectively. TMS is able to elicit both an excitatory and inhibitory effect on the motor system (Nakamura et al. 1997). It is the interactions between the intra- and intercortical circuits as well as the balance between the excitation and inhibition neurons which result in the final MEP, which is recorded with EMG and subsequently analyzed (Hallett 2007). Maintaining the delicate balance between excitation and inhibition is critical for all humans and animals (Sukenik et al. 2021).

The amplitude of the MEP, as well as the silent period following the MEP, are commonly analyzed to assess corticospinal excitability. The MEP amplitude displays the excitatory effect while the silent period after the MEP is evidence of an inhibitory effect on the system (Rothwell et al. 1991). There are many factors which influence MEP amplitude, such as background activity of motoneurones, fatigue, the task an individual is completing and even what the individual is thinking about (Latash 2012).

When the motor cortex is stimulated via TMS, there is a noticeable pause in EMG activity following the MEP that is recorded from a muscle during voluntary contraction. This lack of EMG activity following stimulation is known as the silent period (Fuhr et al. 1991; Zeugin & Ionta 2021). The silent period is thought to be a combination of spinal and cortical inhibition (Zeugin & Ionta 2021). The duration of the silent period is variable and can be influenced by the amount of stimulation, level of fatigue of the participant and background EMG (Zeugin & Ionta 2021). The larger the silent period the greater the inhibition (Zeugin & Ionta 2021)

MEPs can be analyzed using peak-to-peak amplitude or area. A study by McDonnell et al. (2004) compared peak-to-peak amplitude verses area as a way to analyze MEPs. This study was done on the first dorsal interosseous muscle during rest (McDonnell et al. 2004). There was no significant difference when analyzing MEP area verses peak-to-peak amplitude (McDonnell et al. 2004). However, it is more common when analyzing MEPs to use peak-to-peak amplitude (Ammann et al. 2020; Bestmann & Krakauer 2015; Di Lazzaro & Rothwell 2014). Minimal locomotor studies been completed which analyze the area of the MEP in addition to peak-to-peak amplitude. Since a MEP amplitude gives indications of corticospinal excitability, the whole pathway must be considered including supraspinal, spinal, neuromuscular junction and muscle

fibre excitability. A change in the MEP amplitude can mean a change at any level of the corticospinal pathway (Rossini et al. 2015).

Due to CMEPs being largely monosynaptic, CMEPs can provide insight into motoneurone pool excitability in addition to changes in corticospinal excitability (Taylor 2006). CMEPs and MEPs can be assessed during a variety of types of motor outputs such as rest, tonic contractions and even dynamic motor outputs (Taylor & Gandevia 2004; Taylor 2006). It is important to note that CMEPs give an indication of corticospinal pathway excitability, however, CMEPs are not influenced by the cortex as the stimulation occurs at the brainstem (Figure 6). On the other hand, MEPs give an indication of the entire corticospinal pathway excitability. Utilizing both stimulation techniques allows for the cortex and spinal cord to be isolated from the rest of the corticospinal pathway allowing for individual analysis of cortical excitability. However, a disadvantage of TMES is the possibility of nerve root stimulation (Taylor & Gandevia 2004). Ideally when using TMES, EMG is only recording stimulation of the corticospinal tract and not any peripheral nerve excitability. Nerve root stimulation is possible because nerve roots are more easily activated compared to the spinal nerves (Taylor & Gandevia 2004). Nerve roots have a quicker onset latency time because of the direct connection with muscle fibres, unlike the spinal nerves which synapse onto the lower motoneurones. When utilizing TMES it is important to assess the CMEP onset latency time to determine if there is any nerve root stimulation or if the signal is purely spinal (Taylor & Gandevia 2004). Ideally, the signal should not have any peripheral nerve stimulation as researchers use TMES to get an indication of spinal excitability not peripheral nerve excitability. For the biceps brachii the onset latency should occur around 8.5ms after the stimulus artifact (Petersen et al. 2002; Taylor & Gandevia 2004).

2.5.5 Stimulation Intensity

The number of stimulations that TMS elicits can vary. Pulses can be delivered in a single stimulation, as paired-pulses or in trains of pulses. To assess cortical facilitation and inhibition, paired-pulse TMS is commonly used (Fatih et al. 2021; Nakamura et al. 1997). The use of paired-pulse stimulation has allowed researchers to identify various facilitatory and inhibitory intra- and intercortical circuits which depend on the interstimulus interval and the stimulation intensity of the TMS (Chen 2004; Hallett 2007). The first stimulation is called the conditioning stimulation (CS) and the second is the test stimulation (TS).

2.6 Cortical Control of Locomotion

There are several non-invasive techniques utilized to assess cortical structures and determine their involvement in the control of locomotor outputs (Barthélemy et al. 2011). Some of these non-invasive techniques are TMS, TMES, neuroimaging and electroencephalogram (EEG) which can be used independently or in combination (Barthélemy et al. 2011). Movement is rapid, so using techniques such as positron emission tomography (PET) and Magnetic resonance imaging (MRI) scans are good for spatial assessments of the cortex, however, the scans take several minutes to complete making real time assessments unobtainable. Coupling EEG with EMG gives researchers an indication of cortical activity as well as muscle activity (Barthélemy et al. 2011).

TMS has been widely used to assess cortical and corticospinal excitability during movement. Utilizing TMS, MEP amplitudes are phase-dependent during the gait cycling, where they are the largest during muscle activation and smallest during antagonist muscle activation (Barthélemy et al. 2011; Capaday et al. 1999). When a single-pulse TMS stimulation was delivered at suprathreshold intensity, there were notable muscle differences in MEP amplitudes (Capaday et al. 1999; Yang & Gorassini 2006). The ankle dorsiflexors tibialis anterior experienced an

excitatory effect during walking which was similar to a seated tonic contraction (Capaday et al. 1999). This same effect was not seen in the ankle plantar flexors, (ie. soleus). In fact, there was an inhibitory effect during walking of the soleus in comparison to a seated tonic contraction (Capaday et al. 1999). These results show task-dependent muscle differences in corticospinal excitability. Therefore, corticospinal excitability is altered during locomotion as there is greater excitability of the ankle dorsiflexors when walking compared to a stationary tonic contraction (Capaday et al. 1999; Yang & Gorassini 2006).

However, determining cortical excitability is difficult with the use of single pulse TMS. Petersen et al. (2001) used both TMS and electrical stimulation over the motor cortex to assess motoneuronal activity during walking. Excitability to both the ankle plantarflexors and dorsiflexors were reduced when using subthreshold TMS resulting in suppression of the muscle activity when walking (Petersen et al. 2001). However, when electrical stimulation over the motor cortex was used, there was not a suppression of muscle activity in the plantarflexors or dorsiflexors (Petersen et al. 2001). Therefore, the inhibition had to occur at the supraspinal level. Utilizing these two stimulation techniques, they had evidence that motor cortex activity is directly involved in the control of muscles during gait (Petersen et al. 2001; Yang and Gorassini 2006). Coupling TMS and TMES allows researchers to isolate supraspinal excitability by creating a MEP/CMEP ratio. This allows the location of corticospinal excitability changes to be narrowed down. However, it is important to note that the supraspinal component of the corticospinal tract is composed of the cortex as well as the brainstem. Paired-pulse TMS techniques allow various cortical circuits to be analyzed. A growing body of literature has examined cortical circuits during both arm and leg cycling (Alcock et al. 2019; Compton et al. 2022; Forman et al. 2014; Sidhu et al. 2013; Sidhu et al. 2018).

2.7 Cortical Circuits

Cortical circuits can be within the same hemisphere (intra-) or between two hemispheres (inter-) (Lee et al. 2007), faciliatory or inhibitory (Chen 2004; Lee et al. 2007). There are several inhibitory intra- and intercortical circuits respectively, such as short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI) and interhemispheric inhibition (IHI). To assess various cortical circuits, paired pulse TMS can used. By altering the intensity of the stimulation and the duration between the stimulations allows for assessments of various cortical circuits (Chen 2004).

2.7.1 Long-interval Intracortical Inhibition (LICI)

LICI is an inhibitory cortical circuit spanning one hemisphere (Fatih et al. 2021). To assess LICI a paired-pulse TMS stimulation paradigm is used. Suprathreshold stimulations for both the CS and TS must be used with an interstimulus interval of 60-150ms (Valls-Solé et al. 1992). Some research has even found that with an interstimulus interval of 50-200ms, inhibition of cortical activity can be observed (Sanger et al. 2001). When TMS is delivered at high stimulation intensities with an interstimulus interval of approximately 100ms this will result in the TS being delivered in the silent period following the CS, therefore causing a reduction of the TS MEP amplitude compared to that of the CS or when compared to a single TS (Valls-Solé et al. 1992).

In the central nervous system Gamma-aminobutyric acid beta ($GABA_B$) acts as a primary inhibitory neurotransmitter (Krnjević 1997). Within the cortex there are many inhibitory circuits which are analyzed to give insights into inhibitory neurotransmitters. Various studies have determined that LICI is mediated by $GABA_B$ receptors (Florian et al. 2008). The interstimulus interval used to produce LICI corresponds to the timing of $GABA_B$'s inhibitory post-synaptic

potentials (Fatih et al. 2021). Thus, $GABA_B$ neurotransmission causes the suppression of cortical excitability (Fatih et al. 2021). Therefore, by using paired-pulse TMS with an interstimulus interval of ~100-200ms, researchers can assess LICI and, in turn, analyze the activity of cortical circuit GABAergic interneurons (Chen & Rothwell 2012). LICI is only one of the many paired-pulse paradigms that is studied and although more research is being done, LICI is still not well understood.

2.8 Tonic Motor Output

The reliability of intracortical inhibition was tested by Presland et al. (2022) for the biceps femoris with both concentric and eccentric contractions. TMS, both paired and single-pulse, was delivered at low contraction force produced excellent reliability for all contraction types of the biceps femoris muscle (Presland et al. 2022). The authors concluded that across all contraction types there was excellent reliability of TMS outcomes, however the reliability was not tested during a locomotor output (Presland et al. 2022). The majority of studies which have investigated LICI use methodologies in which the participant is at rest or performing a tonic contraction. A study by Clark et al. (2010) investigated the effects of limb immobilization, using a cast, on LICI. The authors used paired-pulse TMS, with an interstimulus interval of 100ms, during a resting state as well as during a 15% maximum voluntary isometric contraction before and after limb immobilization (Clark et al. 2010). The results of the study found that LICI did not have any notable changes at rest. However, during a 15% MVC there was a significant loss of strength and LICI was increased (Clark et al. 2010). A study by Latella et al. (2019) compared the effects of concentric vs eccentric contractions of the biceps brachii and LICI. Using paired-pulse TMS with an interstimulus interval of 100ms, the authors compared innervations between the two contraction types at pre-exercise, immediately post-exercise, and 1 hour post exercise (Latella et al. 2019). It

was determined that LICI only increased after eccentric contractions and that the effects lasted for an hour (Latella et al. 2019). LICI has been studied in several other reports involving the elderly during fatigue (Otieno et al. 2021) and the effects of exercise on LICI (O'Leary et al. 2018). However, all these studies analyzed LICI during rest and/or during a low intensity tonic contraction. Even if the main focus of the research was to determine how exercise or contraction types influenced cortical inhibition, during the TMS testing session the participant was in a resting state or completing low intensity tonic contraction. If researchers wish to investigate how exercise or various locomotor outputs influence LICI, then the experimental conditions in which LICI is tested should be during locomotor output. However, research analyzing LICI during movement is very limited. It is important to assess LICI during dynamic motor output because very rarely in life are human performing tonic contractions. As humans are dynamic beings it is vital that research protocols which mimic activities of daily life.

2.9 Assessing Corticospinal Excitability during Locomotion

As mentioned earlier, corticospinal excitability is conventionally assessed during tonic contractions or when the participant is in a resting state. However, as humans are dynamic beings, it is of the upmost importance to understand how the nervous system is altered during movement. There is a growing body of literature which has assessed corticospinal excitability during various locomotor outputs (Alcock et al. 2019; Compton et al. 2022; Forman et al. 2014; Lockyer et al. 2021; Power et al. 2018; Sidhu et al. 2013; Sidhu et al. 2018). Initial research investigated how altering various factors of arm cycling would alter corticospinal excitability. It is now understood that corticospinal excitability during arm cycling is phase-, cadence-, task- and muscle-dependent (Alcock et al. 2019; Compton et al. 2022; Forman et al. 2014; Forman et al. 2015; Lockyer et al. 2021; Power et al. 2018).

Forman et al. (2014) set out to determine if corticospinal excitability was task-dependent. In this study, they looked at arm cycling as a model of locomotion and focused on the biceps brachii during arm cycling as well as a tonic contraction. The tonic contraction was position and intensity matched to arm cycling. One result of the study was that supraspinal excitability increased during the flexion phase of arm cycling (6 o'clock) with no changes in spinal excitability (Forman et al. 2014). This was an unpredicted result as it was hypothesised that supraspinal excitability would be less during arm cycling compared to a contraction and spinal excitability would be greater due to the presence of CPGs found within the spinal cord. As these results were not predicted, studies investigating various intra- and intercortical circuits have been done to investigate the mechanisms leading to higher supraspinal excitability during arm cycling compared to a position and intensity matched tonic contraction.

2.9.1 Cortical Circuit Excitability Alteration during Arm and Leg Cycling

Sidhu et al. (2013) was the first to assess cortical circuit excitability during a locomotor output. In this study the researchers investigated short-interval intracortical inhibition (SICI) during leg cycling. It was determined that for the knee extensor muscles SICI was present during leg cycling. Additionally, SICI was reduced during muscle activation whereas during deactivation, SICI was enhanced. These results in indicate that cortical circuits are in fact present during leg cycling but are variable within the movement.

A study by Alcock et al. (2019) compared SICI during arm cycling to a tonic contraction. This was the first study to assess SICI during arm cycling. Sidhu et al. (2013) assessed SICI during leg cycling focusing on the knee extensors, whereas Alcock et al. (2019) investigated SICI during arm cycling assessing the elbow flexors. As there are muscle dependent differences in corticospinal

excitability during locomotor output, results determined for knee extensors cannot be assumed for elbow flexors. Contrary to Alcock et al. (2019) hypothesis it was determined that there was no difference in the amount of SICI to the biceps brachii during arm cycling compared to a tonic contraction. Therefore, these results do not help describe why supraspinal excitability was greater during arm cycling (Forman et al. 2014).

To further investigate supraspinal excitability Compton et al. (2022) investigated interhemispheric inhibition (IHI) during arm cycling and a tonic contraction. Again, contrary to the hypothesis and not supporting Forman et al. (2014) findings, it was determined that IHI was greater during arm cycling (Compton et al. 2022). The greater the inhibition, the less supraspinal excitation is present. Meaning that this circuit provides more inhibition during arm cycling in comparison to a tonic contraction. Similarly to Alcock et al. (2019) these results do not help describe why supraspinal excitability was greater during arm cycling (Forman et al. 2014).Would LICI be the circuit that explains the findings of Forman et al. (2014)?

Sidhu et al. (2018) is the only one who has performed a study which focused on LICI during cycling. The authors wanted to determine how fatigue alters cortical excitability during a dynamic movement (Sidhu et al. 2018). The authors used an interstimulus interval of 100ms and used the area of the MEP to analyze the data (Sidhu et al. 2018). It was determined that during a fatiguing leg cycling protocol there was a noticeable decrease in cortical excitability due in part to an increase in LICI (Sidhu et al. 2018). This is one of the first studies to analyze LICI during a dynamic movement to determine if similar trends are observed compared to tonic contractions or resting state. However, this study was analyzing the lower limbs, specifically the vastus lateralis. No studies to date has analyzed LICI with regard to the upper limb muscles.

2.10 Conclusion

Using TMS it is possible to view inter- and intracortical pathways, such as LICI, and determine the neuron-mediated inhibition present through $GABA_B$. Most research to date has observed LICI during tonic contractions, or during a resting state. While this gives great insight into the various cortical pathways, more research needs to be done during a dynamic movement to determine if similar inhibition is noticed.

The following project will explore a cortical circuit, LICI, during a locomotor output, arm cycling. Additionally, the following project will investigate the task-dependency of LICI during arm cycling in comparison to a tonic contraction. This research will contribute to the limited existing literature regarding cortical circuits during locomotor outputs with the potential for application in neurorehabilitation.

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Chapter 3 Manuscript

Long-interval intracortical inhibition to the biceps brachii is present during arm cycling but is not different than a position-matched tonic contraction

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3.0 Abstract

Previous work has shown that supraspinal excitability is higher during arm cycling than a position- and intensity-matched tonic contraction, yet the mechanism(s) are unclear. The purpose of the present study was to investigate if long-interval intracortical inhibition (LICI) is present during arm cycling and if the amount of LICI is different between arm cycling and a position-matched tonic contraction. A paired-pulse transcranial magnetic stimulation (TMS) paradigm was used to assess LICI. A supramaximal stimulation intensity, which elicits a silent period of approximately 150ms (conditioning pulse), was delivered, followed by a second TMS pulse (test pulse) of the same intensity with an interstimulus interval of 100ms. The position in which the first stimulation occurred was 4 o'clock, relative to a clock face, to ensure the test pulse occurred on the ascending limb of the biceps brachii EMG profile during the elbow flexion phase of arm cycling. During single-pulse trials the TMS stimulation was delivered with a time delay of 100ms after the 4 o'clock position. Motor-evoked potentials (MEP) are expressed as a ratio of the conventional test pulse-evoked MEP over the conditioned pulse-evoked MEP and a new position-matched ratio of test pulse-evoked MEP over the single pulse-evoked MEP during arm cycling and a position-matched tonic contraction. MEPs were analyzed using peak-to-peak amplitude. Using the new position-matched ratio, LICI was present during arm cycling ($p<0.001$) but there was no statistically significant difference between LICI during arm cycling and tonic contraction when utilizing either ratio (conventional $p=0.23$ and new $p=0.12$).

Keywords Paired-pulse TMS · Cortical · Task-dependent · Arm cranking · Locomotion

3.1 Introduction

Locomotor outputs are controlled by a combination of descending input, sensory feedback and networks of cells in the spinal cord called central pattern generators (CPGs) (MacKay-Lyons, 2002; Natali et al. 2022). In the absence of descending or sensory input, CPGs are able to produce rhythmic muscle activation, which create complex patterns of locomotor outputs such as crawling, swimming and walking (MacKay-Lyons 2002; Power et al. 2018). Arm cycling is also partially produced by spinal CPGs and as such is used as a model of locomotion to examine various changes in neural excitability (Zehr et al. 2016). Unlike quadrupeds, however, humans require descending input in order to preform successful locomotion (Christensen et al. 2001; Petersen et al. 2001; Power et al. 2018; Zehr et al. 2016). Given the corticospinal tract is involved in the voluntary control of human motor output, corticospinal excitability is frequently assessed to further understand the neural control of human movement (Brouwer & Ashby 1990; Natali et al. 2022; Power et al. 2018).

The excitability of the corticospinal pathway and cortical circuits have mainly been assessed during rest or tonic contractions (Rothwell 1997; Rothwell et al. 1991). However, over the last two decades more research has been done to assess the excitability of the corticospinal pathway during various locomotor outputs (Carroll et al. 2006; Forman et al. 2014; Lockyer et al. 2021; Sidhu et al. 2014). Forman et al. (2014) demonstrated that corticospinal excitability was *greater* during arm cycling compared to an intensity-matched tonic contraction as shown by a larger motor evoked potential (MEP) amplitude. They also showed there was no difference in cervicomedullary evoked potential (CMEP) amplitude, indicating no task-dependent difference in spinal excitability (Forman et al. 2014). A larger MEP amplitude but no difference in CMEP amplitude indicates that supraspinal excitability was increased during arm cycling compared to an

intensity-matched tonic contraction (Forman et al. 2014). The authors did not assess cortical excitability and the mechanisms responsible for the higher supraspinal excitability during arm cycling remain unclear, though several cortical circuits have since been examined (Alcock et al. 2019; Compton et al. 2022).

There are numerous faciliatory and inhibitory intra- and intercortical circuits, which can be assessed using transcranial magnetic stimulation (TMS) (Chen and Rothwell 2012; Nakamura et al. 1997). Cortical circuits have been studied during locomotor output, including short-interval intracortical inhibition (SICI) of the upper (Alcock et al. 2019) and lower limbs (Sidhu et al. 2013) and interhemispheric inhibition (IHI) of the upper limbs (Compton et al. 2022). Alcock et al. (2019) determined that SICI was not significantly different during arm cycling compared to a position- and intensity-matched tonic contraction while Compton et al. (2022) determined that IHI was *greater* during arm cycling compared to tonic contractions. While contributing to our understanding of the cortical control of locomotor output, neither study aided in the explanation of the Forman et al. (2014) results, namely an increase in supraspinal excitability during arm cycling.

Gamma-aminobutyric acid beta ($GABA_B$) acts as a primary inhibitory neurotransmitter (Krnjević 1997) in the central nervous system. Long-interval intracortical inhibition (LICI) is an inhibitory cortical circuit (Fatih et al. 2021) mediated by $GABA_B$ receptors (Florian et al. 2008). In order to assess LICI, a paired-pulse TMS paradigm must be used whereby TMS is delivered at high stimulation intensities (both supramaximal), with an interstimulus interval of 50-200ms (Fatih et al. 2021; Sanger et al. 2001; Valls-Solé et al. 1992). Provided LICI is present, these stimulation parameters cause a reduction of the second MEP (test) amplitude compared to that of the first MEP (conditioning) or when compared to a single pulse. The interstimulus interval used to produce LICI

corresponds to the timing of $GABA_B$ inhibitory post-synaptic potentials (Fatih et al. 2021). Thus, $GABA_B$ neurotransmission causes the suppression of cortical excitability (Fatih et al. 2021). Therefore, by using paired-pulse TMS with an interstimulus interval of ~100-200ms, researchers can assess LICI, and in turn, analyze the activity of GABAergic interneurons in the cortex (Chen & Rothwell 2012).

A review by Lockyer et al. (2021), describes challenges with assessing LICI during locomotor output given the long interstimulus interval required. During locomotor output the position in which the first stimulation is delivered, which has specific joint-angle afferent feedback and EMG activation may be different than the second stimulus because of the long interstimulus interval (Lockyer et al. 2021). However, the importance of ensuring that the stimulations are position-matched has yet to be studied mainly because when assessed at rest or during a tonic contraction the joint angle remains the same. LICI has only been assessed once during a locomotor output (i.e., leg cycling; Sidhu et al. 2018). Sidhu et al. (2018) determined that LICI was present during leg cycling with respect to the knee extensors. However, corticospinal excitability is phase-, task-, intensity-, direction- and muscle-dependent (Lockyer et al. 2021; Power et al. 2018) indicating that results determined for knee extensors cannot be assumed for elbow flexors. In addition, no comparisons between cycling and tonic contractions were made in the study by Sidhu et al. (2018). Determining an effective way to assess LICI during movement and determining the task-dependency of LICI during arm cycling could shed light on pervious findings of greater supraspinal excitability during arm cycling (Forman et al. 2014).

The current study assessed LICI using two different ratios based on arm position during arm cycling. The first ratio was that conventionally used during tonic contractions and the second ratio was a new position-matched method to determine the most effective way to analyze LICI, an

important distinction given that the conventional ratio does not involve a change in joint angle and/or EMG profile of the muscle being examined. In contrast, the second ratio involves assessing LICI with TMS evoked-MEPs occurring at the same joint angle and EMG profile during arm cycling (see METHODS for details). The purpose of this study was to determine if LICI is 1) present during arm cycling and 2) task-dependent (i.e. arm cycling vs tonic contraction). It was hypothesized that 1) LICI would be observed during arm cycling and 2) the amount of LICI would be less during arm cycling compared to a tonic contraction.

3.2 Methods

3.2.0 Participants

Fourteen young, healthy adults (5 females and 9 males) participated in the study (height 170.78 ± 6.07 cm; weight 78.66 ± 8.06 kg; age 26.2 ± 4.30 years). All participants had no history of neurological disease or upper musculoskeletal injury. The experiment was verbally explained, and written consent was obtained for each participant. In addition, each participant completed the magnetic stimulation safety checklist (Rossini et al. 2015), a Canadian Society for Exercise Physiology (CSEP) Get Active Questionnaire, and to identify the dominant limb the Edinburgh handedness questionnaire was used (Veale 2014). The study's experimental procedure was 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. 20231547-HK). All of the risks were outlined and explained to participants, and the protocol used is in accordance with the Tri-Council Guidelines in Canada.

3.2.1 Experimental Setup

All trials were completed with the use of an arm cycle ergometer (SCIFIT ergometer, model PRO2 Total Body, Tulsa, OK, USA). Participants sat in an upright, comfortable position at an appropriate distance from the hand cranks to ensure no reaching or trunk rotation occurred during arm cycling (Figure 1). The seat height was adjusted to ensure that the shoulders of the participant were level with the arm crank shaft. Wrist braces were worn by all participants to minimize wrist flexion and extension in an attempt to limit the movement of the wrist, as there is a heteronymous reflex connections that are present between the wrist flexors and biceps brachii muscles (Manning and Bawa 2011). Participants maintained a neutral forearm position while arm cycling. Crank

positions are made relative to a clock face with 12 o'clock being dead centre at the top and 6 o'clock being at the bottom dead centre. The biceps brachii is most active during elbow flexion. During arm cycling, elbow flexion occurs between 3 o'clock to 9 o'clock, with peak activation occurring at approximately 6 o'clock. TMS was delivered to the dominant motor cortex when the dominant arm passed the 4 o'clock position during arm cycling. 4 o'clock was chosen as the stimulation site because when the stimulation was delivered, with an interstimulus interval of 100ms, the participants still had high biceps brachii activation, ensuring the biceps was near peak activation. A study by Chaytor et al. (2020) determined the EMG activity of various elbow flexors and extensors, such as the biceps brachii, during cycling position changes. The biceps brachii has the highest EMG activity during the 3 o'clock to 9 o'clock position (Chaytor et al. 2020). However, after 6 o'clock the EMG activity of the biceps brachii begins to decrease (Chaytor et al. 2020). Therefore, in the present study by triggering TMS at 4 o'clock after 100ms the participant will still be in the ascending range of the EMG activity of the biceps brachii. Participants were instructed to cycle at 60 revolutions/minute (RPM) at a constant workload of 25 watts.

To compare cycling and tonic contraction trials, cycling background EMG (bEMG) was assessed from the dominant biceps brachii. To determine bEMG the blank trials were averaged and a 50ms average was taken, beginning at 4 o'clock and ending 50ms after the 4 o'clock position. The bEMG was used to attempt to make the tasks of arm cycling and tonic contraction intensity-matched, which we have previously done (Alcock et al. 2019; Compton et al. 2022; Forman et al. 2014, 2016). The average smoothed bEMG value is an indication of contraction intensity which was displayed on a computer screen, visible to the participant, as a horizontal line with a +/- 5% (Figure 1). All trials of the tonic contractions used locked arm cranks of the arm cycle ergometer

previously used for the cycling trials. The arm cranks were locked in the 5 o'clock position. Participants were instructed to produce a tonic contraction where the dominant biceps brachii smoothed EMG activity matches the displayed horizontal target EMG, which is a representation of the cycling bEMG, staying within the range displayed.

3.2.2 Electromyography

Electromyography (EMG) recordings used Ag-AgCl surface electrodes from the biceps and triceps brachii muscles of the dominant arm (KendallTM 130 Foam Electrodes with conductive adhesive hydrogel, Covidien IIC, Massachusetts, USA). Electrodes were placed over the muscle belly of the dominant biceps brachii and triceps brachii, parallel to the muscle fibres placed at a distance of 2cm apart. The ground electrode was placed on the lateral epicondyle of the dominant humorous. Before the placement of electrodes, the skin of the dominant arm was prepped by shaving to remove any dead skin cells as well as any hair. The skin was abraded with Nuprep and cleaned with a 70% isopropyl alcohol swab. Prior to the placement of electrodes, the skin on the arm was dried. The EMG data was collected online and analog-to-digitally converted with the use of CED 1401 interface and the associated Signal 5.12 (Cambridge Electronic Design Ltd., Cambridge, UK) software. A sampling frequency of 5000 Hz was used and amplified (gain of 300). A 3-Pole Butterworth filter was used with cut-off frequencies of 10-1000 Hz.

3.2.3 Transcranial Magnetic Stimulation

TMS was delivered to the motor cortex using a BiStim module connected to two Magstim 200 stimulators (Magstim, Whitland, Dyfed, UK) and a circular coil (13.5-cm outside diameter). Vertex was determined by measuring the intersection points between the midpoint of the tragus to

tragus and the midpoint of the nasion to inion. The intersection point was marked, vertex, with the current flow preferentially activating the dominant motor cortex. The coil was placed firmly on the participants head, parallel to the ground. During arm cycling, stimulation intensity began at 50% of magnetic stimulator output (MSO). The stimulation intensity increased until a 150ms silent period was noticed after the MEP because after 100ms the silent period is mediated by $GABA_B$ receptor-activated intracortical inhibition (Inghilleri et al. 1993, Siebner et al. 1998). The stimulation intensity was determined once 6 consecutive stimulations were completed that produced a MEP with a silent period of at least 150ms.

3.2.4 Brachial Plexus Stimulation (Erb's Point)

Brachial plexus stimulation (also known as Erb's point stimulation) was used to measure maximal compound motor unit action potential (Mmax). To stimulate Erb's point a cathode and anode (Meditrace Ag–AgCl pellet electrode, disc-shaped 10 mm diameter, Graphic Controls Ltd., Buffalo, NY, USA) were used where the cathode was placed on the skin over the supraclavicular fossa and the anode was placed over the acromion process. Stimulations were delivered as a singlet using the constant-current electrical stimulator (square wave pulse, 200μs duration at 100–300 mA; model DS7AH, Digitimer Ltd, Welwyn Garden City, UK). Participants cycled at 60 RPM at a constant workload of 25 watts. The stimulation intensity of Erb's point incrementally increased until there was a plateau in M-wave, thus reaching Mmax of the biceps brachii. To ensure a supramaximal stimulation, the intensity was 120% of Mmax.

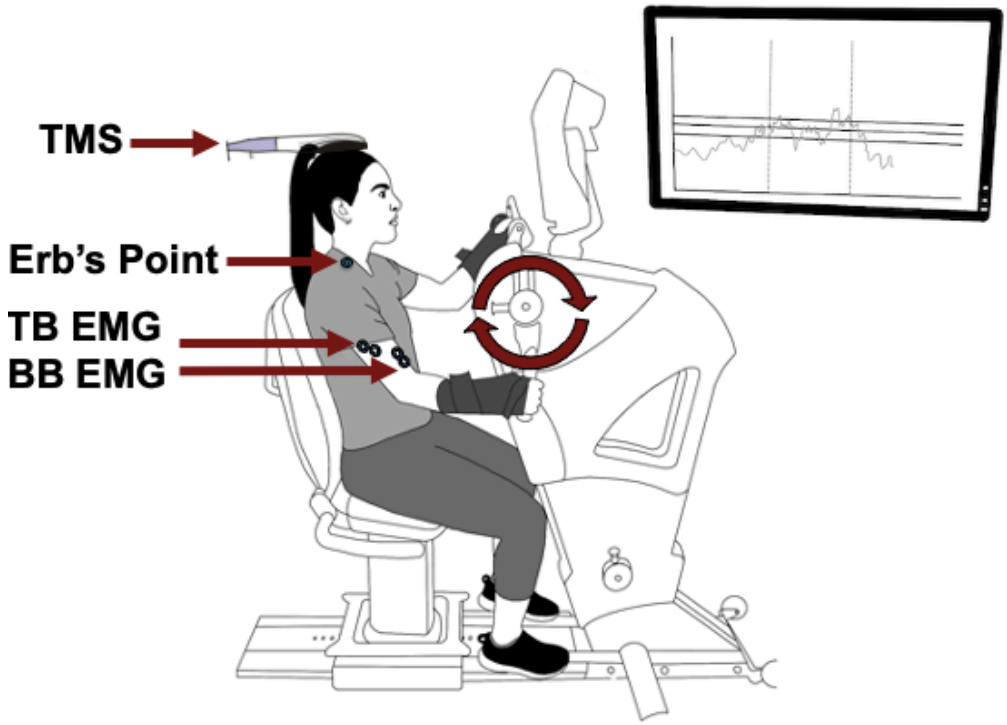

Figure 1: Participant sitting at arm cycle ergometer. Labels indicate TMS paddle, Erb's point stimulating electrodes, biceps and triceps brachii EMG recording electrodes. Arrows indicating the direction of rotation. Screen used for matching biceps EMG during tonic contractions with horizontal lines representing target ± 5%.

3.2.5 Experimental Protocol

After the %MSO for the TMS paradigms and Erb's point stimulation intensities were determined, participants completed 2 cycling trials and 2 tonic contractions. First, the 2 cycling trials were completed where TMS was either delivered in single-pulse or paired-pulse (Lockyer et al. 2021). The order in which the single- or paired-pulse paradigms were completed was randomized. Since each participant cycled at a cadence of 60 RPM, there were 83.33ms between each cycle position. TMS was automatically triggered once the participant passed 4 o'clock on the cycle ergometer. Each arm cycling trial lasted 2 minutes, with stimulations approximately every 7 seconds. During each cycling trial 20 TMS stimulations were delivered, 20 single-pulse

stimulations during the single-pulse trial and 20 sets of paired-pulse stimulations (20 control stimulations and 20 test stimulations) during the paired-pulse trial, 2 Mmax stimulations were also delivered as well as 5 blanks which is where no stimulation were delivered. In the single-pulse trial, TMS was delivered to the dominant motor cortex, with a time delay of 100ms, and the paired-pulse cycling trial had an interstimulus interval of 100ms. Therefore, applying the first stimulation at 4 o'clock, with an interstimulus interval of 100ms, resulted in the second stimulation occurring between the 5 and 6 o'clock position. At this position the biceps brachii was still activated during the second stimulation, thus ensuring proper phase-dependent comparisons to our prior work (Forman et al. 2014).

The same stimulation intensity was used for cycling and tonic trials. During the tonic trials, participants attempted to match the intensity of the smoothed bEMG activity in the cycling conditions for 3 seconds, staying within the 5% range indicated on the screen. There were two tonic trials, single-pulse and paired-pulse TMS, which were randomized. For each of the two tonic trials, participants received 20 TMS (either single-pulse or paired-pulse, depending on the trial), 2 Mmax stimulations and 5 blanks. Each tonic contraction trial lasted approximately 2 minutes, a 3-second contraction followed by a 4-second rest, which repeated until all the stimulations were completed for that given trial.

3.2.6 Data Analysis

MEPs were analyzed using the peak-to-peak amplitude of the average MEP from the dominant biceps brachii for each trial. The peak-to-peak amplitude of the MEP was measured using cursers on the Signal 5.12 software (CED) placed after the stimulus artifact and near the

return of the voltage trace to baseline levels. The peak-to-peak amplitude of Mmax was assessed to give indications of muscle fatigue and peripheral nerve excitability. MEPs were made into ratios in order to assess if inhibition was in fact present. Ratios were the conventional ratio of paired-pulse test MEP/ paired-pulse conditioning MEP and the position-matched ratio of paired-pulse test MEP/ single-pulse test MEP. To determine if cycling and tonic trials had similar background EMG (bEMG) for both the biceps and triceps brachii, the EMG signal was rectified and smoothed using a 30ms window. The single-pulse trials were used to assess muscle activity where the EMG data was averaged over 50ms immediately prior to the stimulation artifact.

3.2.7 Statistical Analysis

All statistical analyses were performed in IBM's SPSS Statistics (SPSS 20 for Macintosh, IBM Corporation, Armonk, New York, USA). The normality of the data was tested using Shapiro-Wilk tests ($p > 0.05$). The data that was normally distributed was analyzed using the paired T-test. The data which was not normally distributed was bEMG of the biceps and triceps brachii as well as was the conditioning paired-pulse MEP produced in the arm cycling condition. Therefore, Wilcoxon signed rank test was used to assess any differences in bEMG between the two tasks (arm cycling and tonic contraction) for biceps and triceps brachii and to compare arm cycling vs tonic contraction using the conventional ratio. All data are presented as mean ± standard deviation, and the alpha level was set at $p < 0.05$.

3.3 Results

3.3.0 Ratios for Assessing LICI

To assess the amount of LICI present the conventional ratio used is paired-pulse test MEP/ paired-pulse conditioning MEP (PP Test/PP Conditioning). Therefore, the conventional LICI ratio shows the size of the test MEP as a percentage of the conditioning MEP, where a value below 100% displays a reduction in MEP amplitude, indicative of inhibition. Using the conventional method, LICI is not significantly different from 100% ($p= 0.463$), meaning the paired-pulse conditioning MEP is not significantly different from the paired-pulse test MEP. This is due to the fact that 42.9% (6/14) participants had a greater paired-pulse test MEP in comparison to the paired-pulse conditioning MEP, resulting in a non-significant finding of inhibition (Figure 2).

However, we propose a position-matched method of assessing LICI during dynamic motor output, paired-pulse test MEP/single-pulse test MEP (PP Test/ SP Test), enabling the comparison to be position-matched. The new position-matched ratio maintains the numerator of paired-pulse test MEP but has a new denominator of single-pulse test MEP. Utilizing this new position-matched ratio (test MEP a percentage of the single pulse test MEP), LICI is present during arm cycling ($p<0.001$). As seen in Figure 2, all participants had a value less than 100% indicating inhibition.

The traces shown in Figure 3 display the average of 20 MEPs for two participants. Figure 3a displays the average MEPs, of an individual showing facilitation during arm cycling as seen by the increase in amplitude from the first paired-pulse MEP (conditioning MEP) to the second MEP (test MEP). However, Figure 3b displays the average MEPs from an individual displaying inhibition and the presence of LICI. This is noted by the reduced MEP amplitude of the second, paired-pulse MEP (test MEP) compared to the conditioning MEP. In addition, Figure 3b displays the presence of LICI during tonic contraction, again noted by the reduction of the test MEP.

3.3.1 LICI is Not Task-Dependent

Regardless of the ratio used to assess LICI, either conventional or the position-matched, LICI is not task-dependent. There is no statistically significant difference between the conventional and new position-matched ratios between arm cycling and tonic contraction conditions ($p=0.778$ and $p=0.242$) (Figures 4 and 5).

3.3.2 Intensity-Match/Background EMG

Figures 6 and 7 display the group data of smoothed bEMG for the biceps and triceps brachii. BEMG data is only present for single-pulse trials due to the presence of the silent period following the conditioning MEP during paired-pulse trials, leading to no EMG prior to the secondary MEP (test MEP). The bEMG of the biceps brachii was a significantly higher during arm cycling compared to tonic contraction ($p= 0.005$). However, there was no significant difference ($p= 0.109$) between arm cycling and tonic contraction conditions for the triceps brachii.

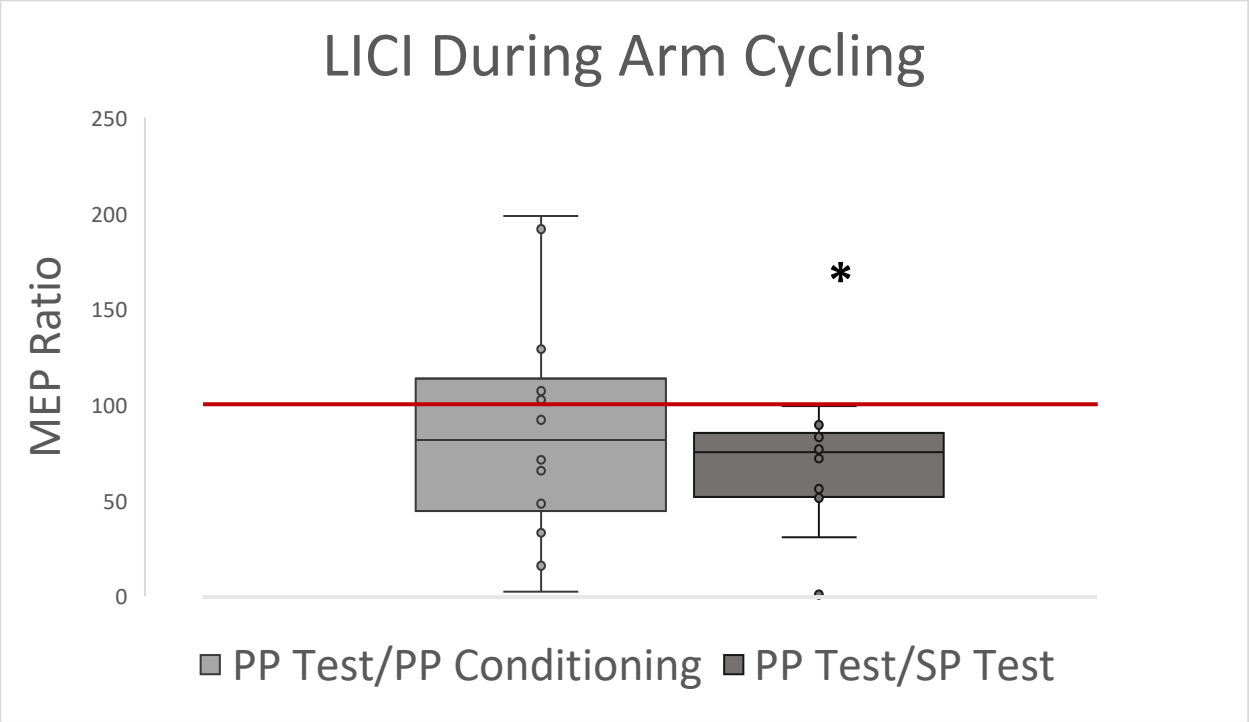

Figure 2: Comparison of both ways to assess LICI during arm cycling. Conventional ratio to assess LICI- paired-pulse test MEP/ paired-pulse conditioning MEP which is not significantly different from 100% (p= 0.463), indicating no inhibition present. New position-matched ratio of assessing LICI- paired-pulse test MEP/ single-pulse test MEP which is significantly different from 100% (p<0.001), indicating inhibition.

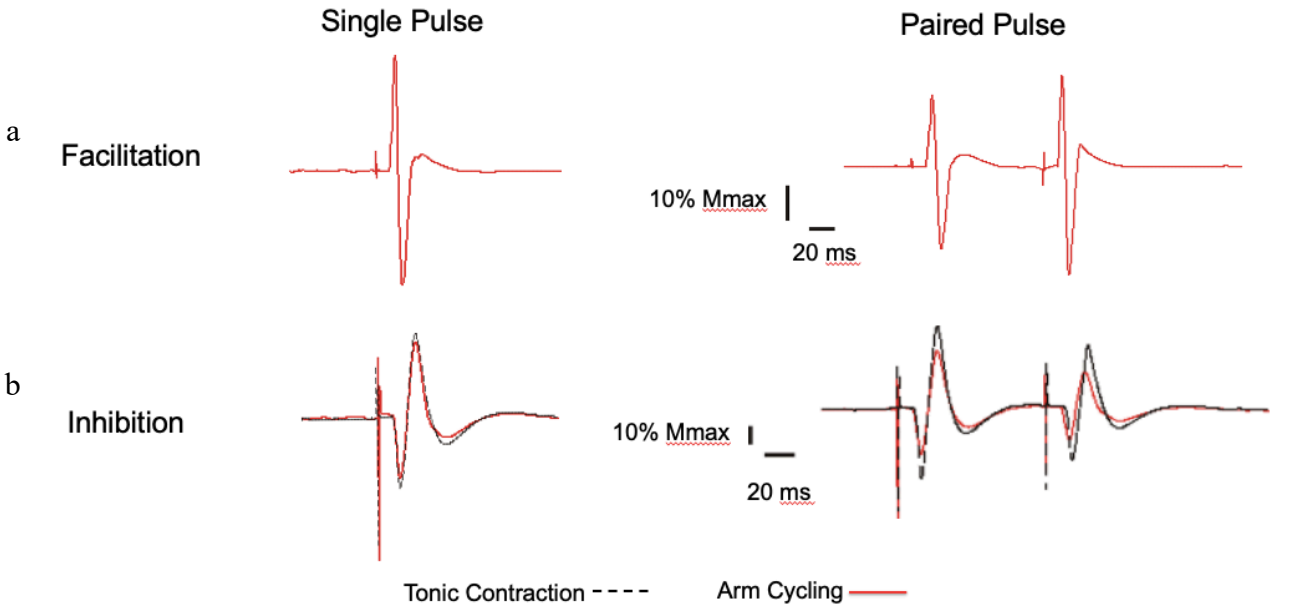

Figure 3: Raw MEP trace for two individuals, 3a showing the presence of facilitation when arm cycling as seen by the increase in MEP amplitude in the paired-pulse condition. 3b displays the presence of LICI when arm cycling as seen by the reduction in the second paired-pulse MEP. 3b also displays LICI present during tonic contractions again with a reduction in the second paired-pulse MEP.

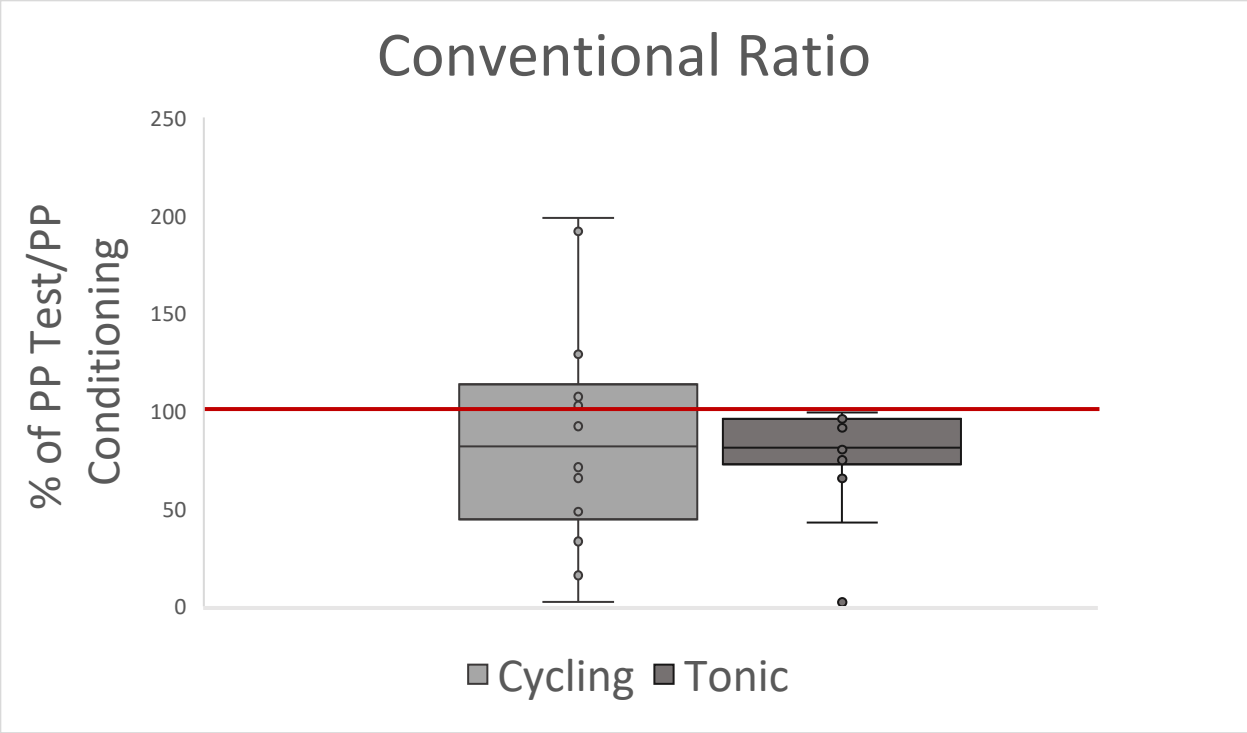

Figure 4: Paired-pulse test MEP amplitudes as a percentage of the conditioning MEP response in the biceps brachii for both cycling and tonic tasks (n = 14). No significant differences between conditions (p=0.778).

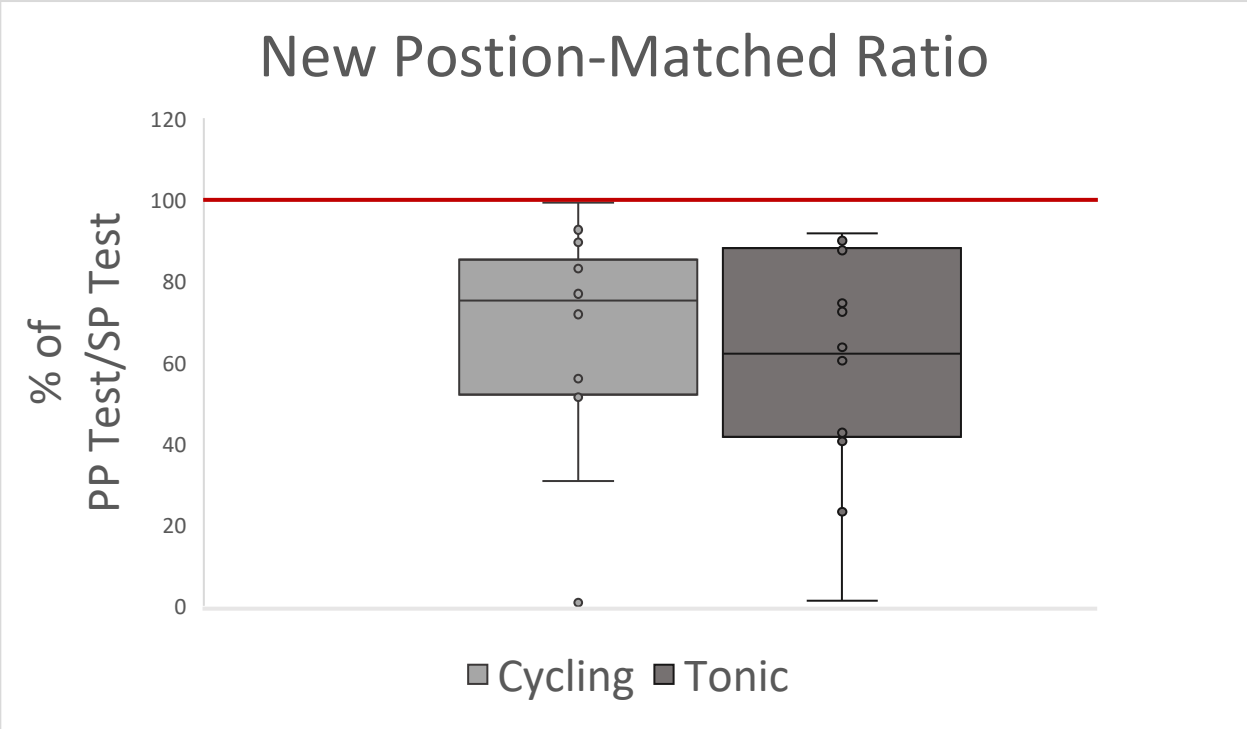

Figure 5: Paired-pulse test MEP amplitudes as a percentage of the single-pulse test MEP response from the biceps brachii for both cycling and tonic tasks (n = 14). No significant differences between conditions (p=0.242).

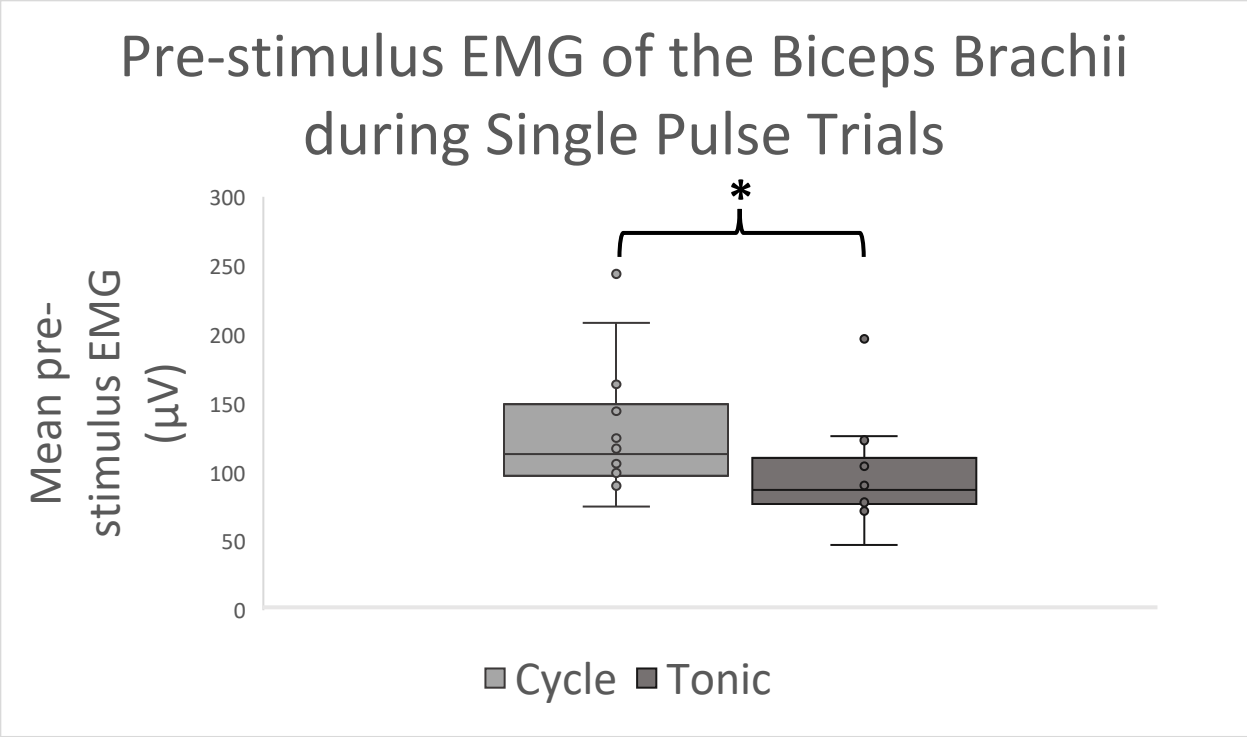

Figure 6: Background EMG (bEMG) assessment 50ms prior to the 4 o'clock position during single-pulse trials for the biceps brachii of the dominant arm.

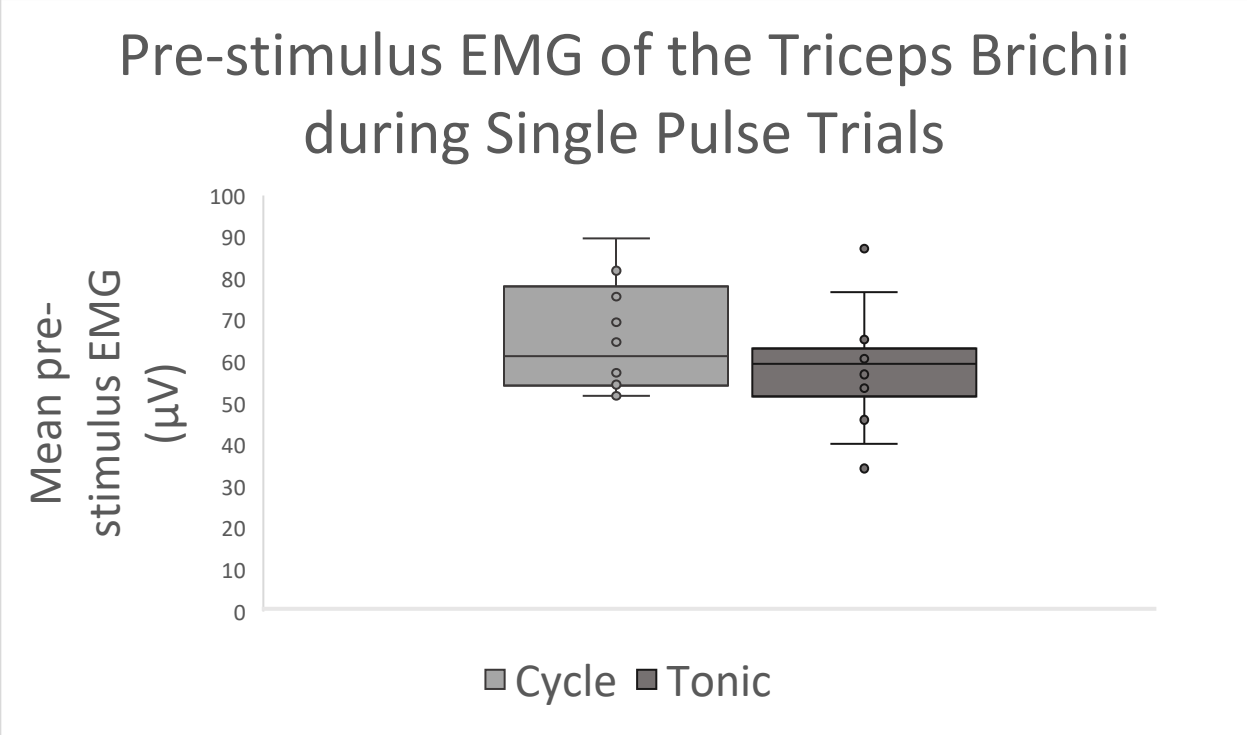

Figure 7: Background EMG (bEMG) assessment 50ms prior t the 4 o'clock position during single-pulse trials for the triceps brachii of the dominant arm.

3.4 Discussion

The objectives of the study were to determine if LICI was present during arm cycling and if LICI was task-dependent. Since LICI has been challenging to assess during movement due to the long interstimulus interval, a secondary objective was to assess potential difference in position-matching LICI analysis. This is the first study to show that LICI is present during arm cycling when the newly proposed position-match ratio was used. However, contrary to our hypothesis, the amount of LICI is not statistically different from a position-matched tonic contraction.

Research assessing the differences in corticospinal excitability between locomotor output and tonic contractions is expanding (Alcock et al. 2019; Compton et al. 2022; Forman et al. 2014; Lockyer et al. 2021). Work from our lab showed that corticospinal excitability to the biceps brachii was greater at the 6 o'clock position during arm cycling when compared to a position- and intensity-matched tonic contraction (Forman et al. 2014). However, Forman et al. (2014) found that there was no difference in CMEP amplitude at the 6 o'clock position, indicating that spinal excitability was not task-dependent. The authors concluded that the difference in corticospinal excitability, therefore, must be supraspinal (Forman et al. 2014). In order to further understand why supraspinal excitability is greater during arm cycling, various cortical circuits need to be analyzed. The first cortical circuit assessed during arm cycling was short-interval intracortical inhibition (SICI) (Alcock et al. 2019). It was determined that although SICI was present during arm cycling it was not task-dependent, thus failing to explain the original findings of Forman et al. (2014). Another cortical circuit that was analyzed was interhemispheric inhibition (IHI), which is an inhibitory circuit between the two cortices (Compton et al. 2022). It was determined that IHI was *greater* during arm cycling compared to a position- and intensity-matched tonic contraction.

Greater inhibition indicates less supraspinal excitation, again not aiding in the explanation of the Forman et al. (2014) study. To further assess cortical excitability during arm cycling and potentially explain the higher supraspinal excitability shown by Forman and colleagues (2014), we sought to examine another cortical circuit, long-interval intracortical inhibition.

Long-interval intracortical inhibition has only been assessed once during locomotor output (Sidhu et al. 2018). Sidhu et al. (2018) focused on fatigue impacts on cortical excitability during leg cycling. It was determined that during a fatiguing leg cycling protocol, there was a noticeable decrease in cortical excitability, thus displaying an increase in LICI (Sidhu et al. 2018). The study by Sidhu et al. (2018) was the first study to analyze LICI during a dynamic movement. To analyze LICI the authors utilized the position-matched ratio of paired-pulse test MEP/single-pulse test MEP (Sidhu et al. 2018). However, no discussion was made about the theory behind using the position-matched ratio nor was any comparison made between the conventional way to assess LICI compared to position-matching. Additionally, Sidhu et al. (2018) assessed the vastus lateralis during leg cycling, whereas the present study analyzed corticospinal excitability to the biceps brachii during arm cycling. Given there are muscle-dependent differences in corticospinal excitability the findings from Sidhu and colleagues are not easily extrapolated to work in the present study using the biceps brachii muscle during arm cycling (Lockyer et al. 2021; Power et al. 2018). In addition, the present study is the first to assess the task-dependency of LICI.

LICI has been very problematic to research during dynamic motor output due to the long interstimulus interval. During a tonic contraction, where position does not change, varying interstimulus intervals is not of concern. However, during a dynamic movement, such as arm

cycling, the longer the interstimulus interval, the greater the variance in cycle position. When a participant cycles at 60 RMP, a change in cycle position occurs every 83.33ms. Therefore, having longer interstimulus intervals will result in different cycle positions being analyzed. Thus, if a change in MEP amplitude is noticed, it is impossible to identify if the change was due to a position change rather than inhibition of the corticospinal tract alone due to the paired-pulse paradigm. Therefore, the position-matched analysis method to assess LICI allows for the ratio to be position-matched, reducing any outside factors which may influence the variability of the MEP amplitude. There is a growing body of literature to support the position changes in corticospinal excitability of the upper (Lockyer et al. 2021; Power et al. 2018) and lower limbs (Sidhu et al. 2012). Therefore, creating a 'new' ratio to assess LICI will create a ratio that is position-matched, thus minimizing external factors that influence corticospinal excitability and, therefore, MEP amplitude.

Figures 6 and 7 display the pre-stimulus EMG of the biceps and triceps brachii 50ms prior to the stimulus artifact of the single-pulse. Pre-stimulus EMG 50ms prior to the stimulus artifact has been used as a methodology to determine if the two tasks, arm cycling and a tonic contraction, are, in fact, intensity matched (Alcock et al. 2019; Compton et al. 2022; Forman et al. 2014; Forman et al. 2015). There was a significant difference in the biceps brachii pre-stimulus EMG ($p=0.005$), indicating the tasks were not intensity matched. This is in part due to the silent period following the conditioning stimulus, which made it difficult to determine background EMG to match from cycling to the tonic contraction. However, the bEMG for the biceps brachii was *greater* during arm cycling compared to a tonic contraction but there was no significant difference in MEP amplitude of the biceps brachii when using the conventional and new position-matched ratios between arm cycling and tonic contraction conditions ($p=0.778$ and $p=0.242$). During tonic

contractions there is a linear relationship between EMG activity and MEP amplitude (Darling et al. 2006; Yahagi et al. 2003). Hence, the greater the EMG the greater the MEP. However, a greater bEMG activity did not result in a greater MEP amplitude during arm cycling, which could indicate that LICI was actually *greater* during arm cycling. This is contrary to our original hypothesis that LICI would be greater during a tonic contraction meaning corticospinal excitability would be greater during arm cycling. Future studies could implement the ramp contractions to get a better estimation of intensity to compare the two tasks. As LICI is only one of numerous cortical circuits, these results mean LICI is not solely responsible for task-dependent difference between arm cycling and a tonic contraction. Future studies should also investigate cortical circuit interactions and their task-dependency.

3.4.0 Methodological Considerations

As mentioned by Lockyer et al. (2021), one method to assess task dependency of LICI would be to perform a tonic ramp contraction. This would resemble arm cycling more closely, allowing for closer comparisons rather than a simple plateau tonic contraction. A ramp tonic contraction that mirrors arm cycling has an increase in EMG followed by a decrease in EMG signal in a similar timeframe to arm cycling, which would allow for LICI comparisons between cycling and tonic contractions (Lockyer et al. 2021). Although the present study used plateau tonic contractions, this methodology has been used previously to assess task dependence (Alcock et al. 2019; Compton et al. 2022; Forman et al. 2014).

3.5 Conclusion

In the current study, we showed that LICI was present during arm cycling when using the new position-matched ratio, but was not different than a position-matched tonic contraction. However, there is the potential that LICI was *greater* during arm cycling due to an increase in bEMG but no change in MEP amplitude. Neither finding aids in explaining past results of supraspinal excitability being greater during arm cycling. It is important to remember the abundance of cortical circuits, in addition to the way in which they all interact. Therefore, future work should analyze other cortical circuits, such as short-interval facilitation (SICF) and intracortical facilitation (ICF), and various cortical circuit interactions during locomotor activity.

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Chapter 4 Summary and Future Directions

Summary and Future Directions

Gaining a better understanding of cortical circuit activity and excitability in young healthy individuals during locomotor output is of the utmost importance to advance our basic understanding of the neural control of locomotor outputs. The present study investigated how one cortical circuit, long-interval intracortical inhibition (LICI), to the biceps bacchii is altered during arm cycling in comparison to a tonic contraction. The findings aid in the literature of cortical circuits during locomotor outputs, which is currently very minimal. Additionally, increasing our knowledge of the task-dependency of cortical circuits allows for a better understanding and comparison between the abundance of research done during rest or tonic contractions and how that is altered during locomotor output. The results of the current study found that with regards to LICI there was no task-dependency between a tonic contraction and arm cycling. However, the way in which LICI was assessed was also investigated discovering that the conventional method used to assess LICI during tonic contractions may not be the most effective way to analyze LICI during movement due to the long interstimulus interval. Instead, creating a ratio which the two pulses are position-matched aids in minimizing external factors which influence motor evoked potential (MEP) amplitude, such as position, therefore allowing any change in MEP amplitude be solely due to a change in LICI.

Other factors also influence MEP amplitude such as intensity of the contraction. Although efforts were made to attempt to make arm cycling and a tonic contraction intensity matched, there was a significant difference in the biceps brachii background electromyography (EMG). In the current study a plateau tonic contraction was used. Future studies could implement a ramp contraction to get a better estimation of intensity to compare the two tasks. A ramp contraction would resemble arm cycling more closely, allowing for closer comparisons rather than a simple

plateau tonic contraction. A ramp tonic contraction that mirrors arm cycling has an increase in EMG followed by a decrease in EMG signal in a similar timeframe to arm cycling, which would allow for improved LICI comparisons between cycling and tonic contractions.

Additionally, no evaluation was done to assess overall effort of the two tasks. However, based on verbal feedback during the experiment, several individuals found the tonic contraction to be extremely difficult to match the background EMG produced in arm cycling trials. Participants arm cycled at 60 revolutions per minute at 25 watts, which is relatively easy, and the protocol was non-fatiguing. But when participants were attempting to match the background EMG activity produced during arm cycling, many struggled to produced similarly high levels of biceps EMG activity. Therefore, future work should assess perceived effort levels during locomotor outputs and tonic contractions as there is the potential for differences in relative effort even though there is no difference in EMG activity which would greatly impact MEP responses.

There is limited knowledge of corticospinal excitability and cortical circuits during locomotor outputs. The present study assessed LICI, adding to the literature surrounding arm cycling, corticospinal excitability and cortical circuits. There are now three cortical circuits which have been assessed during arm cycling: LICI, short-interval intracortical inhibition (SICI), and Interhemispheric Inhibition (IHI). All of the aforementioned circuits are inhibitory and have been assessed independently. Future work should analyze other cortical circuits, such as short-interval facilitation (SICF) and intracortical facilitation (ICF), and various cortical circuit interactions during locomotor activity. It is unlikely that one cortical circuit is solely responsible for changes in overall corticospinal excitability during cycling, but rather a combination of multiple circuits. Finally, although this research was conducted in young healthy adults, by improving our fundamental knowledge of the nervous system could potentially be translated to clinical

populations in the future. Arm cycling is a common tool used in neurorehabilitation for patients who have suffered a stroke or patients with various neurological disorders/ diseases. Therefore, by improving our basic understanding of the nervous system could help guide neurorehabilitation techniques.

Appendix

Appendix A

MEMORIAL UNIVERSITY

Interdisciplinary Committee on Ethics in Human Research (ICEHR)

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Tel: 709 864-2561 icehr@mun.ca
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ICEHR Number:	**20231547-HK**
Approval Period:	May 16, 2023 – May 31, 2024
Funding Source:	NSERC [RIS# 20161819]
Responsible Faculty:	Dr. Kevin Power School of Human Kinetics and Recreation
Title of Project:	*Is Long-Interval Intracortical Inhibition of the Biceps Brachii Task-Dependent*

May 16, 2023

Ms. Alysha Wira
School of Human Kinetics and Recreation
Memorial University

Dear Ms. Wira:

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 **May 31, 2024**. 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 R Drover

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 Initiatives and Services