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Sex-based Differences in Corticospinal Excitability and Inhibition

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Kinesiology

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Abstract

The purpose of this study was to perform a novel exploration of sex-based differences in corticospinal excitability and inhibition, with consideration of hormone phases. Thirty participants (15 females) attended two visits during different phases (low vs high). Responses evoked by single- and paired-pulse transcranial magnetic stimulation were recorded using electromyography from a hand muscle. Excitability was assessed via the motor-evoked potential and intracortical facilitation. Inhibition was assessed via the cortical silent period (CSP), short-interval (SICI), and long-interval intracortical inhibition (LICI). Each measure was compared between phases and sexes. Neither sex differed significantly across phases for excitability, nor inhibition for males. Females displayed significantly greater inhibition in the low phase (CSP: $p=0.04$). Overall, males and females had similar excitability. Males displayed significantly greater inhibition vs females for SICI ($p=0.004$) and LICI ($p=0.008$) but not CSP ($p=0.28$). Findings suggest both sexes could be equally included in research, during different hormone phases.

Keywords

Sex-based differences; corticospinal excitability; corticospinal inhibition; transcranial magnetic stimulation; single-pulse TMS; paired-pulse TMS; hormone phases

Summary for Lay Audience

Males and females differ anatomically and physiologically throughout the body, including the brain. These differences suggest potential differences in the communication from the brain to the muscle that is needed to produce a movement. Transcranial magnetic stimulation is a non-invasive tool commonly used in neurophysiology research to investigate the responsiveness and resistance of a muscle to the communication from the brain. Males have shown similar responsiveness, but greater resistance compared to females. This greater resistance often results in slower movement or worsened reaction times. However, these measures of communication from the brain to the muscle have not been extensively studied between sexes. Therefore, the purpose of the present study is to explore the sex-based differences in the responsiveness and resistance of a muscle to the communication from the brain, while considering the male (24-hr) and female (28-day) hormone cycles. A group of 30 young healthy adults (15 females) visited the laboratory two times during different phases of their hormone cycle. Transcranial magnetic stimulation was applied over the part of the brain that controls the hand. This stimulation initiated the communication from the brain and was recorded at the muscle in the hand. These values were compared between low and high hormone phases for each sex and were compared between males and females. No differences in responsiveness were seen between phases for either males or females. Females displayed greater resistance in the low hormone phase compared to the high hormone phase, while there were no differences in resistance between phases for males. When comparing sexes, males and females displayed similar responsiveness. Males displayed greater resistance compared to females. These results suggest that males and females could be equally included in neurophysiology research, during low or high phases of their hormone cycle. This was one of the first studies to compare these sex-based differences in communication from the brain to the muscle using various measures of responsiveness and resistance. Further research should continue to investigate these sex-based differences.

Co-Authorship Statement

Anita D. Christie contributed to the study design, data analysis interpretation, and provided guidance and feedback on the construction of the manuscript. Alexandra N. Pauhl contributed to data collection. Alicia M. Kells contributed to the study design, data collection, data and statistical analysis, data interpretation, and wrote the manuscript.

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Chapter 1

1 General Introduction

To perform a voluntary movement, communication must travel from the brain to the muscle through the corticospinal pathway. The three major levels of this pathway are: the motor cortex of the brain, the spinal cord, and the muscle (Kalmar et al., 2019). Externally stimulating the cortical level allows for the evaluation of the function of the entire corticospinal tract in delivering information from presynaptic neurons at the cortical level to the muscle (Kobayashi & Pascual-Leone, 2003). Sex-related differences have been documented in neuroanatomy (Solomito et al., 2019) and neurochemistry (Hampson, 1990), which may result in differences in the communication along the corticospinal pathway. However, differences between males and females in measures of the corticospinal pathway have not been largely investigated. It is essential to strengthen the understanding of the possible sex-based differences in the measures of the corticospinal pathway, to allow for appropriate interpretation of results in neuromuscular research.

1.1 Corticospinal Pathway

The corticospinal tract is a major neural pathway within the central nervous system. During voluntary movements, this descending neural pathway is activated via a thought to perform a movement within the cerebral cortex, which then activates the motor cortex, through an electrical signal created by neurotransmitter and ion fluctuations (Kalmar et al., 2019). Essentially, neurotransmitter release by presynaptic neurons influences the influx and efflux of ions through voltage-gated ion channels on the post-synaptic neurons. These ion fluxes can result in the depolarization and resultant action potentials in the upper motor neurons (McCormick, 1992). The upper motor neurons transmit the action potentials from the cortex to the spinal cord, where they synapse with lower motor neurons on the contralateral side of the body (Kalmar et al., 2019). The action potentials then propagate down the lower motor neurons and cause release of acetylcholine, resulting in depolarization of the muscle fibers, and leading to the production of muscle

contraction (Kalmar et al., 2019). This pathway can also be activated involuntarily, through external stimulation applied to the motor cortex, using techniques such as transcranial magnetic stimulation (TMS).

1.2 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation is a common non-invasive tool in neurophysiology research, which is used to investigate the communication between the brain and muscles by evaluating signal transmission within the central motor pathway (Kobayashi & Pascual-Leone, 2003). The use of TMS is often chosen rather than electrical stimulation due to its painless characteristics (Barker et al., 1985). Essentially, a TMS machine transmits electrical energy through a coil, which will undergo electromagnetic induction, converting electrical energy into magnetic energy and creating a magnetic field (Barker et al., 1985; Kobayashi & Pascual-Leone, 2003). The rapid changes of the magnetic field will induce an electrical current in nearby excitable tissues, leading to the activation of neurons within the area of the brain that the TMS coil is placed over (Barker et al., 1985; Kobayashi & Pascual-Leone, 2003). This activation begins the propagation of the electrical signal down the corticospinal tract to elicit a response at the muscle, known as a motor evoked-potential (MEP), which can be recorded through electromyography (EMG).

There are various coils that could be utilized during the TMS process, which affect the size and depth of the magnetic field. For instance, there are circular coils, which produce a broad magnetic field that is relatively superficial. Figure-of-eight and butterfly style coils consist of two coils connected at the center. These coils generate a stronger magnetic field than circular coils and the strongest part of the field is at the point of contact of the two coils, producing a deeper and more focused stimulation area (Lefaucheur, 2019). Regardless of the coil type, to elicit a response in an extremity of the body, the placement of the coil should be on the contralateral side of the target muscle and over the motor cortex (Barker et al., 1985). A common muscle targeted during TMS is the first dorsal interosseus muscle in the hand, as it typically has a lower threshold for activation compared to other muscles, leading to the need of a lower stimulation intensity (Chen et al., 1998; Lefaucheur, 2019). However, it is important to consider that

placement of the coil may slightly differ between individuals as there are inter-individual differences in the precise anatomical location that represents the hand within the motor cortex.

Although TMS has been designed as a method for interrogating the excitability and inhibition of the corticospinal pathway, its applications have been expanded upon in recent years (Kobayashi & Pascual-Leone, 2003; Lefaucheur, 2019; Ziemann, 2017). The method of TMS is now being used for clinical-related situations such as cortical function mapping prior to surgery, diagnosis and investigation of neurological diseases and/or dysfunctions, and as a form of therapy (Kobayashi & Pascual-Leone, 2003; Lefaucheur, 2019; Ziemann, 2017). The focus of this thesis, however, is on methods of assessing corticospinal excitability and inhibition. Although other methods exist (Kobayashi & Pascual-Leone, 2003), the most commonly-employed techniques to assess corticospinal excitability and inhibition with TMS involve single-pulse and paired-pulse techniques. The single-pulse TMS technique involves single, supra-threshold stimulations that can elicit a response in the muscle (Kalmar et al., 2019). The single-pulse TMS technique will produce an excitatory response known as a motor-evoked potential (MEP). If the muscle is active during the stimulation, the single pulse will also produce a measure of inhibition known as a cortical silent period (CSP), reflected by the duration of the characteristic pause in muscle activity following the stimulation. The paired-pulse TMS technique involves two stimuli, a conditioning stimulus and a test-stimulus, which will trigger a faciliatory (intracortical facilitation [ICF]) or inhibitory (short-interval intracortical inhibition [SICI] or long-interval intracortical inhibition [LICI]) response at the intracortical level, depending on the inter-stimulus interval (Kujirai et al., 1993; Lefaucheur, 2019; Valls-Solé et al., 1992).

The various TMS measures collected at the muscle are dependent on several characteristics including the number of stimulations (i.e. single-pulse or paired-pulse), the intensity of the stimulations, the inter-stimulus duration/interval, and if the measures are collected while the target muscle is at rest or in an active/contracted state (Kobayashi & Pascual-Leone, 2003; Lefaucheur, 2019). Each of these components can influence fluctuations of the fast-acting neurotransmitters glutamate and GABA (Lefaucheur, 2019;

McCormick, 1992), which bind to their corresponding receptors post-synaptically, influencing a conformational change that will elicit ionic fluctuations to produce the intended excitatory or inhibitory response in different neural circuits (McCormick, 1992). Due to the many variables contributing to the five measures of interest (i.e., MEP, CSP, ICF, SICI, LICI), the obtained values tend to be quite variable within and between individuals (Kobayashi & Pascual-Leone, 2003), leading to a minimum of 5 stimulations needed for reliable single-pulse measures (Christie et al., 2007) and a minimum of 8-10 stimulations needed for reliable paired-pulse measures (Lefaucheur, 2019).

1.3 Corticospinal Excitability and Transcranial Magnetic Stimulation Measures

Corticospinal excitability is associated with the responsiveness of the muscle to the communication from the brain, specifically through the descending activity of the corticospinal tract (Kobayashi & Pascual-Leone, 2003; Lefaucheur, 2019). These measures of corticospinal excitability have been associated with functional measures of cortical motor control and corticospinal conduction time (Lefaucheur, 2019). Corticospinal excitability can be calculated based on the amplitude of an MEP, which has similar characteristics of an action potential, and is triggered in response to ionic fluctuations in and out of neurons (Kobayashi & Pascual-Leone, 2003). One of the major excitatory neurotransmitters of the central nervous system, glutamate, is responsible for the ionic fluctuations that produce the corticospinal excitability measures of MEP and ICF (Liepert et al., 1997; Nakamura et al., 1997; Stagg et al., 2011). Glutamate can bind to an ionotropic receptor and allow for sodium to flow into the post-synaptic neuron (McCormick, 1992). This will lead to the neuron depolarizing, bringing about an excitatory response in the form of an action potential (McCormick, 1992). Prior to eliciting an excitatory measure, it is important to determine an individual's motor threshold, resting (RMT) or active, to ensure this measure is evoked with a standardized relative input to the motor cortex across individuals. An RMT is typically determined as the stimulation intensity that will result in 50% of trials responding with an MEP greater than 50 μV in amplitude, observed using EMG (Kobayashi & Pascual-Leone, 2003; Lefaucheur, 2019; Werhahn et al., 1999).

Motor-Evoked Potential (MEP)

An MEP is the response collected at the muscle following a stimulation to the motor cortex at an intensity that exceeds an individual's RMT (Kobayashi & Pascual-Leone, 2003). The stimulation must achieve or surpass the threshold for sufficient neurotransmitter fluctuations to depolarize the neurons of the motor cortex, resulting in an MEP and an associated movement at the muscle (Kalmar et al., 2019). The stronger the stimulation intensity, the larger the MEPs tend to be, as higher stimulus intensities will excite more neurons (Inghilleri et al., 1993; Lefaucheur, 2019). This emphasizes the crucial need to standardize stimulation intensities across individuals using an RMT. In addition, an MEP can be recorded at rest or in an active state. Indeed, it is important to consider that during an active state, MEP amplitudes will likely be larger due to the pre-activation of the spinal level motor neuron pools (Lefaucheur, 2019) suggesting contributions from both the spinal and cortical levels (Kobayashi & Pascual-Leone, 2003).

Intracortical Facilitation (ICF)

The ICF measure is collected from a paired-pulse TMS protocol. To elicit this response the conditioning stimulus should be sub-threshold (~80% of RMT), followed by a test-stimulus that is supra-threshold (~120% of RMT) and with an interstimulus interval (ISI) that can range from 7-20ms (Kujirai et al., 1993; Lefaucheur, 2019; Ziemann et al., 1996b). This measure, along with other paired-pulse measures, tend to be performed while the target muscle is at rest, as voluntary muscle activity could impact the observed effects of the intracortical circuits that are typically seen at rest (Lefaucheur, 2019; Ridding et al., 1995) An ICF is calculated based on the amplitude of the conditioned MEP resulting from the test-stimulus compared to a baseline (unconditioned) MEP, and usually results in a facilitation (i.e., the test-stimulus has increased relative to the baseline stimulus) (Kujirai et al., 1993; Lefaucheur, 2019). An ICF measure is the net effect of both excitatory and inhibitory neurotransmitters in the interneural circuit from preceding stimulation (conditioning stimulus), in addition to the neurotransmitters responsible for the conditioned MEP produced following the test-stimulus (Liepert et al., 1997; Reis et

al., 2008; Ziemann et al., 1996a). The ICF measure has been suggested to be one of the more variable paired-pulse TMS measures among a population, as the amplitude tends to vary within and between individuals (Lefaucheur, 2019).

1.4 Corticospinal Inhibition and Transcranial Magnetic Stimulation Measures

Corticospinal inhibition can be associated with the interruption of communication from the brain to the muscle through the corticospinal tract. Specifically, measures of inhibition have been linked to functional measures including movement speed (De Beaumont et al., 2012) and reaction time (Pearce et al., 2019). This inhibitory response occurs due to ionic fluctuations preventing the depolarization of neurons and therefore preventing the production of action potentials. The TMS-based measures of inhibition are attributed to the inhibitory neurotransmitter GABA, through action of either the $GABA_A$ (SICI) or $GABA_B$ (CSP and LICI) receptor subtypes (Nakamura et al., 1997; Werhahn et al., 1999). Corticospinal inhibition can be measured in two ways: the duration of a pause in electrical activity, or the amplitude of an MEP, depending on if the measure is attained by a single-pulse or paired-pulse TMS protocol (Lefaucheur, 2019). However, regardless of the measure, it is important to acknowledge the possible contributions from each level of the corticospinal tract that can influence the inhibitory responses collected at the muscle (Kalmar et al., 2019). Previously, it has been suggested that the first ~50ms of inhibition within the corticospinal pathway can be attributed to spinal mechanisms, while inhibition lasting longer than 50ms can be attributed to cortical mechanisms (Fuhr et al., 1999; Inghilleri et al., 1993). However, more recent work suggests that spinal inhibition may be involved in the CSP for durations as long as 150ms (Yacyshyn et al., 2016).

Cortical Silent Period (CSP)

A CSP is a pause in electrical activity at the muscle following a single-pulse supra-threshold stimulation that caused an MEP while the target muscle is contracted (Kobayashi & Pascual-Leone, 2003). The duration of the CSP can be measured by the distance from the end of an MEP and beginning of the pause in electrical activity, to the

resumption of electrical activity at the same level it was, prior to the stimulation (Kobayashi & Pascual-Leone, 2003). A typical CSP lasts a maximum of approximately 300ms, with longer durations indicating greater levels of inhibition (Inghilleri et al., 1993). The CSP duration can be influenced by the stimulation intensity, where CSP durations typically lengthen with increasing stimulus intensity (Inghilleri et al., 1993).

The CSP has been suggested to be mediated by $GABA_B$ receptors (Werhahn et al., 1999), which are metabotropic/G-protein coupled receptors (McCormick, 1992). Following the binding of GABA to these receptors, a protein is released which attaches to another receptor, resulting in the opening of potassium channels (McCormick, 1992). The subsequent release of potassium out of the neuron through these channels creates a hyperpolarized environment, preventing action potentials, and resulting in long-latency inhibition (McCormick, 1992; Nakamura et al., 1997).

Short-interval Intracortical Inhibition (SICI)

A SICI is a measure of inhibition attributed to a paired-pulse TMS protocol. To achieve this measure the conditioning stimulus must be sub-threshold (~80% of RMT), followed by a suprathreshold test-stimulus (~120% of RMT) (Kujirai et al., 1993; Lefaucheur, 2019). This conditioning stimulus results in a depression of the MEP amplitude during the test stimulus and the amount of depression provides an indication of inhibition. It has been previously observed that the intensity of the conditioning stimulus can alter the response of the test-stimulus (Kujirai et al., 1993). Therefore, a suggested range of intensity for the conditioning stimulus was determined to be 60%-80% of RMT, with 80% displaying the greatest inhibitory response (Kujirai et al., 1993). The duration between these stimuli can range between 1ms-6ms (Kujirai et al., 1993).

Similar to an ICF response, when measuring a SICI the conditioned MEP resulting from the test-stimulus is compared to a baseline MEP value to evaluate the change (Kujirai et al., 1993). This inhibitory response tends display a reduction in MEP amplitude from baseline to test-stimulus due to the inhibitory effects of GABA through the $GABA_A$ receptor (Di Lazzaro et al., 2007; Nakamura et al., 1997). The $GABA_A$ receptor is an ionotropic receptor (McCormick, 1992; Nakamura et al., 1997). Once GABA binds to

this receptor, the receptor will allow for chloride, a negatively charged ion, to pass through ion channels into the neuron (McCormick, 1992). The flow of this ion will hyperpolarize the neuron and reduce the likelihood of producing action potentials, therefore resulting in short-latency inhibition (McCormick, 1992).

Long-interval Intracortical Inhibition (LICI)

A LICI is also a measure of inhibition collected through paired-pulse TMS however, it differs from the paired-pulse measures previously discussed. To produce a LICI, both the conditioning stimulus and the test-stimulus are at the same supra-threshold intensity (Valls-Solé et al., 1992; Wassermann et al., 1996). The ISI ranges between 50ms-200ms (Valls-Solé et al., 1992; Wassermann et al., 1996) allowing for the use of the $GABA_B$ receptors to produce the inhibitory response (Werhahn et al., 1999). Measurement of the inhibitory response for a LICI can be performed in two ways. As the conditioning stimulus is suprathreshold, the conditioned MEP resulting from the test-stimulus can be compared to either a baseline MEP value (Kujirai et al.,1993; Lefaucheur, 2019) or to the conditioning stimulus (Valls-Solé et al., 1992; Wassermann et al., 1996). Both techniques will typically produce a decrease in the MEP amplitude relative to the baseline MEP amplitude, with the degree of decline in the MEP indicating the level of inhibition (Kujirai et al.,1993; Lefaucheur, 2019).

1.5 Sex-based Differences in Corticospinal Measures

Sex-based Neuroanatomical Differences

Males and females differ vastly in neuroanatomy (Solomito et al., 2019), and in neurochemical and hormonal profiles (Hampson, 1990). Neuroanatomical differences between sexes include the volume, makeup, and connections of the brain. For instance, it has been previously observed that males tend to have a larger brain volume compared to females (Lüders et al., 2002) with greater relative volume of white matter and lower volume of gray matter (Cosgrove et al., 2007). In addition, it has been demonstrated that females may have better communication amongst various regions of the brain, including interhemispheric connections, compared to males, due to the differences in gray matter

(Solomito et al., 2019). These differences suggest that males and females may also differ in communication along the corticospinal pathway, however such differences have not been extensively studied.

Sex-based Hormonal Differences

Sex hormones such as estrogens, progestins, and androgens are hormones that influence sexual development and reproduction (Hall & Hall, 2020). However, these hormones can also greatly impact other physiological and anatomical components of the body. The hypothalamus-pituitary-gonadal (HPG) axis is the connection between neural and endocrine tissues used to regulate sex hormone production (Bliss et al., 2010), prior to the circulation of hormones throughout the body via the vascular system (Hall & Hall, 2020). The dominant sex hormones for males and females differ (Hall & Hall, 2020). Males tend to have higher levels of testosterone (Bhasin et al., 2011; Braunstein et al., 2011), while females tend to have higher levels of estrogen (Dighe et al., 2005).

A major difference between males and females is their hormonal cycles which can in turn influence their neurochemical levels. Males function with a 24-hour hormone cycle, with higher levels of testosterone in the morning and lower levels of testosterone in the late afternoon/ early evening (Bremner et al., 1983). Females have a monthly menstrual cycle (~28 days) consisting of a follicular phase (pre-ovulatory phase) and a luteal phase (post-ovulatory phase). The follicular phase is responsible for clearing and rebuilding the endometrium lining as well as preparing an oocyte for ovulation (Hall & Hall, 2020). The luteal phase is responsible for continuing to prepare the endometrium lining (Hall & Hall, 2020). Estrogen and progesterone concentrations are lower in the follicular phase and higher in the luteal phase (Elliott-Sale et al., 2021; Joshi & Kapur, 2019; Roeder & Leira, 2021). It is imperative to acknowledge the differences in hormone levels and the distinct length of each cycle, as hormones can influence various biological processes (Elliott-Sale et al., 2021) such as levels of neurotransmitters (Barth et al., 2015).

The flow and fluctuation of neurotransmitters, such as glutamate and GABA, can be influenced by hormone levels (Barth et al., 2015), among other variables. For instance, testosterone has shown to be associated with GABA receptor function in male mice

(Bitran et al., 1993), and therefore will impact inhibitory responses. Further, testosterone levels have displayed a positive correlation to GABA in the posterior cingulate cortex in females with depression (Flores-Ramos et al., 2019). In addition, it has been previously demonstrated that progesterone indirectly increases GABAergic activity, resulting in increased inhibition and decreased excitability (Joshi & Kapur, 2019; Roeder & Leira, 2021). Further, estrogen has been shown to activate glutamatergic receptors and reduce GABA release, enhancing excitability (Joshi & Kapur, 2019; Roeder & Leira, 2021; Smejkalova & Woolley, 2010). However, little is known about the effects of these hormones specifically on the motor regions of the brain and the excitability and inhibition of the corticospinal pathway.

Sex-based Differences in Corticospinal Measures

These recognized differences help to suggest the possibility for sex-related differences in corticospinal excitability and inhibition measures obtained from TMS. However, research in this area is severely lacking. It has been previously shown that while using single-pulse TMS there are relatively no differences in excitability between sexes (Pauhl et al., 2022; Pitcher et al., 2003). However, one study found that males had greater inhibition, based on longer duration CSPs, compared to females (Pauhl et al., 2022). Sex-based differences in corticospinal excitability and inhibition have yet to be extensively investigated using paired-pulse TMS paradigms. Moreover, there has been minimal exploration on the potential impact hormone phases may have on these measures, in both sexes. Despite the number of differences between males and females, there has been a lack of equal representation and consideration for both sexes within neurophysiology research, particularly with a focus on corticospinal excitability and inhibition. It has been previously noted, that the lack of equal representation and avoidance of including females in studies, in some facets of research, may be due to the difficulty and complexity of certain methodological considerations that need to be implemented for female participants (i.e., controlling for hormone cycles) (Elliott-Sale et al., 2021). However, these complexities and the differences previously observed between males and females, emphasize the need for investigation of potential differences between sexes to ensure ecological validity.

1.6 Purpose and Hypotheses

The purpose of this thesis is to examine sex-based differences in corticospinal excitability and inhibition in healthy young adults, using various TMS techniques. The hypotheses of the present thesis are 1) that there will be no differences in corticospinal excitability between males and females, 2) males will have greater corticospinal inhibition, 3) males and females will have no difference in excitability but greater inhibition during the high hormone phase of their hormonal cycle.

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Chapter 2

2 Sex-based Differences in Corticospinal Excitability and Inhibition

2.1 Introduction

In current neurophysiology research, and more specifically in studies with a focus on corticospinal excitability and inhibition, there is a lack of equal representation and consideration for both males and females. Some studies tend to refrain from investigating an equal sample of both males and females due to the suggested complex methodological considerations that need to be controlled for in females, such as their hormone cycle (Elliott-Sale et al., 2021). However, it is essential to acknowledge the need of both males and females in research, to ensure the results obtained are ecologically valid and applicable.

The corticospinal tract allows for communication, via electrical signals, between the brain and muscle, leading to the production of a voluntary movement (Kalmar et al., 2019). This tract is therefore an essential aspect of the central nervous system. Although differences in structure and function of components of this pathway exist between males and females (Hampson, 1990; Solomito et al., 2019), research documenting sex-based differences in the communication between the brain and muscle is severely limited.

Males and females differ in neuroanatomy (Solomito et al., 2019) and neurochemistry (Hampson, 1990), which may impact function of the motor cortex. For instance, males typically have a greater brain volume compared to females (Lüders et al., 2002). In addition, the composition of gray and white matter within the brain also differs between sexes (Cosgrove et al., 2007). Specifically, while males have more white matter (Cosgrove et al., 2007), females tend to have more gray matter (Cosgrove et al., 2007), which could contribute to better communication and connections among the different regions of the brain observed in females (Solomito et al., 2019).

Moreover, females and males differ in neurochemical and hormonal profiles (Hampson, 1990). Females depend on a ~28-day hormonal cycle, known as the menstrual cycle,

which has fluctuations of lower levels of estrogen and progesterone throughout the follicular phase (~day 1-13) and higher levels through the luteal phase (~day 15-28) (Elliott-Sale et al., 2021; Joshi & Kapur, 2019; Roeder & Leira, 2021). The hormonal cycle for males is a 24-hour cycle, with higher levels of testosterone in morning and lower levels in the afternoon (Bremner et al., 1983). These hormone cycles are known to have many impacts on different biological functions (Elliott-Sale et al., 2021). In the cortical region of the brain, estrogen, progesterone, and testosterone impact the level and function of excitatory and inhibitory neurotransmitters, such as glutamate and gamma aminobutyric acid (GABA) (Barth et al., 2015).

Glutamate is a major excitatory neurotransmitter of the central nervous system and is responsible, in part, for the excitatory response of the corticospinal pathway (Liepert et al., 1997; McCormick, 1992, Nakamura et al., 1997). Estrogen has been noted to promote and increase the function of glutamatergic receptors (Joshi & Kapur, 2019; Roeder & Leira, 2021; Smejkalova & Woolley, 2010), while progesterone results in decreases in excitability (Joshi & Kapur, 2019; Roeder & Leira, 2021). To our knowledge, there have not been any studies that have investigated the influence of testosterone on the excitatory response in males. However, when exploring the influence of time of day on excitability, in a predominately male sample, no differences were observed (Ter Braack et al., 2019), suggesting high or low levels of testosterone may not impact excitability.

The major inhibitory neurotransmitter of the central nervous system, GABA, is responsible, in part, for inhibition of the corticospinal pathway (Di Lazzaro et al., 2007; McCormick, 1992, Nakamura et al., 1997, Werhahn et al., 1999). It has been suggested that progesterone can indirectly increase the GABAergic activity, and therefore increase inhibitory effects in the cortex (Joshi & Kapur, 2019; Roeder & Leira, 2021). Further, testosterone has previously displayed a positive correlation with GABA; however, this was found in the cingulate cortex of females with depression (Flores-Ramos et al., 2019). However, this could potentially have the same effect in males, as a male mice study has shown that testosterone is associated to the GABA receptor function (Bitran et al., 1993).

It is essential to explore the corticospinal pathway, as corticospinal excitability has been associated with the functional measures of conduction time and cortical motor control (Lefaucheur, 2019), while measures of inhibition are associated with movement speed (De Beaumont et al., 2012) and reaction time (Pearce et al., 2019). Despite the many differences between males and females, sex-based comparisons of excitability and inhibition of the corticospinal pathway, have yet to be extensively studied.

Single-pulse and paired-pulse transcranial magnetic stimulation (TMS) paradigms are commonly used to assess excitability (i.e, motor-evoked potential [MEP], intracortical facilitation [ICF]), and inhibition (i.e., cortical silent period [CSP], short-interval intracortical inhibition [SICI], long-interval intracortical inhibition [LICI]) of the corticospinal pathway (Kobayashi & Pascual-Leone, 2003; Lefaucheur, 2019). Using TMS, the limited studies comparing males and females to date suggest there are no sex-related differences in excitability (Pauhl et al., 2022; Pitcher et al., 2003). However, one study has demonstrated greater inhibition, in males, compared to females (Pauhl et al., 2022).

However, each of these previous studies only used single-pulse TMS protocols, limiting information on sex-based differences in other measures (i.e., paired-pulse) of excitability and inhibition. Further, there is minimal research investigating the potential influence of low and high hormone phases on corticospinal excitability and inhibition within males and females. A study by Ansdell et al. (2019) observed no effects of the menstrual cycle on excitability within the MEP measure. The authors did, however, detect greater inhibition within the SICI measure in the mid-luteal phase compared to the early and late follicular phases (Ansdell et al., 2019). No other paired-pulse measures were explored, nor did the authors explore the male hormone cycle (Ansdell et al., 2019), highlighting the need for further investigation with more measures and different cycle time points for males and females.

Therefore, the purpose of this study is to investigate sex-based differences in corticospinal excitability and inhibition in healthy young adult males and females, using various measures of corticospinal excitability and inhibition, during low and high

hormone phases. The hypotheses are 1) there will be no differences in corticospinal excitability between males and females, 2) males will have greater corticospinal inhibition, 3) males and females will have no difference in excitability but greater inhibition during the high hormone phase of their hormonal cycle.

2.2 Methods

Participants

Thirty young, healthy adults (18-35 years), 15 males and 15 females participated in the present study. Female participants could be naturally cycling (n=11) or taking an oral contraceptive (n=4); however, it was necessary for them to have a regular menstrual cycle (i.e. consistent number of days per cycle). Individuals with a history of cognitive deficiencies, attention deficit hyperactivity disorder, neurological impairments, musculoskeletal impairments, or seizures, were ineligible to participate. Exclusion criteria also included contraindications to the use of TMS, based on the TMS screening questionnaire (Rossi et al., 2011), and any use of medications that may impact cognitive or neuromuscular function. Participants were asked to refrain from any exercise, consumption of caffeine and alcohol, and use of recreational drugs for at least 12 hours prior to their testing session. This study was approved by the Health Sciences Research Ethics Board (#122659) at the University of Western Ontario. Written informed consent was obtained from each participant upon arrival of their first testing session.

Experimental Protocol

The protocol consisted of two separate visits to the laboratory, each in a different phase of the participant's hormone cycle (i.e., time of day for males, phase of menstrual cycle for females). One session was performed during the low hormone phase (i.e., afternoon for males, mid-follicular phase for females) and the other session was performed during the high hormone phase (i.e., morning for males, mid-luteal phase for females). The phase of menstrual cycle was determined on a self-report basis. Females were asked to provide the length of their regular menstrual cycle and the day their last menstruation commenced. The day of the lab visit was determined by investigators, by counting the

days of the participant's cycle, specifically targeting the middle of each phase (i.e., mid-follicular: ~day 7, mid-luteal: ~day 21). Female participants were asked upon arrival of their visit, if there were any changes to their menstrual cycle, so investigators could ensure the testing session was occurring during the correct phase. The two visits for females were performed at the same time of day, to avoid any possible confounding influences of time of day between visits. During each visit, measures of corticospinal excitability (MEP and ICF) and corticospinal inhibition (CSP, SICI, and LICI) were obtained. Each participant was asked to self-report their age, height, and weight upon their first visit. The protocol consisted of the same techniques for each visit, however the order of delivery was randomized between participants and between sessions.

Force

Each visit commenced with the participant placing their dominant hand into a custom-made apparatus used to measure the force during index finger abduction, through a transducer (MBP-5; Interface, Scottsdale, AZ, USA). The components of the apparatus were adjusted to fit the individual's hand and the thumb and other fingers were restrained to isolate involvement of the first dorsal interosseous (FDI) muscle. The participant was then prompted to maximally abduct their index finger, to complete a maximum voluntary contraction (MVC) of their FDI. These MVCs were performed for a minimum of 3 trials, as long as their lowest and highest score were within 10% of one another. Each contraction lasted 4-5 seconds and was followed by 1-2 minutes of rest. Visual feedback of the contraction was provided on a computer screen in front of the participant, using DASYLab software (Data Acquisition System Laboratory, DasyTec, USA Inc., Amherst, NH). The highest value of the 3 trials was deemed the maximum and was used to set target force levels while evoking MEPs and CSPs.

Electromyography

With the hand positioned in the force-measuring apparatus, the skin above the area of the FDI and wrist were prepped using NuPrep® and alcohol wipes to lightly exfoliate and remove any dead skins cells on the surface of the skin, helping to produce a clear signal. Surface electromyography (EMG) electrodes (Bagnoli-4 EMG System; Delsys Inc.,

Natick, MA) were then placed on the cleaned skin over the FDI to measure the electrical responses of the muscle. These electrical signals were filtered by 20-450 Hz as well as amplified and collected at a sampling rate of 10,000 Hz using a 16-bit analog-to-digital converter (NI USB-6343; National Instruments, Austin, TX, USA) and stored on a computer for offline analysis (Data Acquisition System Laboratory, DasyTec, USA Inc., Amherst, NH). A ground electrode was placed on the wrist to minimize the amount of surrounding electrical noise that could impact the target electrical signal.

Neuronavigation

A neuronavigation device (ANT Neuro Visor2™, eemagine GmbH, Berlin, Germany) was utilized to ensure the same placement of the coil throughout the protocol, as well as between visits for the same individual (Lefaucheur et al., 2010). Participants were equipped with a headband with reference biomarkers, to track the participant's head in space. Using a 'pointer' with biomarkers, three landmarks were determined for the camera: the naison, left ear, and right ear. Next, the pointer was used to trace the circumference and top of the head, allowing the points to be captured by the camera. The neuronavigation system then used the points collected to declare the shape of the head and fit a standard magnetic resonance imaging image to the shape of the individual's head. The TMS coil had biomarkers on it allowing for it to be tracked throughout the protocol, once placed on the head. Every stimulation and its location were recorded throughout the protocol. Participant files were saved at the end of the testing session, and were retrieved for the next visit, helping to target the same stimulation spot from the previous visit.

Transcranial Magnetic Stimulation

A figure-of-eight TMS coil, connected to a TMS stimulator (D-B80; MagPro X100; MagVenture, Inc; Alpharetta, GA, USA), was positioned over the region of the motor cortex on the head, specific to the hand representation on the contralateral side of the participant's dominant hand. Stimulations were applied with slight adjustments to the position of the coil until the largest MEP response was elicited. Once the optimal spot was achieved, the resting motor threshold (RMT) was determined as the lowest intensity

stimulation required to achieve an MEP of at least 50 μV in 5 out 10 trials (Werhahn et al., 1999). After determining the RMT, the testing intensity of the stimulation was set to 120% of the RMT (i.e., 20% above the RMT), to ensure all trials would be suprathreshold.

Each testing session consisted of single-pulse and paired-pulse stimulations. The order of these protocols was randomized between participants and between sessions. The single-pulse protocol consisted of asking the participant to contract their FDI to 50% of their MVC, which was marked on a graph on a computer screen in front of them. Once contracted, the participant was asked to maintain the 50% contraction for 5 seconds as a stimulation was applied and to continue contracting until they were told to relax. This was completed 10 times (Christie et al., 2007), with 10 seconds of rest between each trial. From these trials, the amplitude of the MEP was determined as an indication of corticospinal excitability, and the duration of the CSP provided an indication of inhibition.

The paired-pulse portion of the experiment was used to assess ICF, SICI and LICI. This portion consisted of 30 paired-pulse stimulations (10 trials for each measure (Lefaucheur, 2019)) randomized and divided into 3 blocks of 10, with 3 single-pulse baseline stimulations applied between each block. Following each paired-pulse stimulation and baseline stimulation, 10 seconds of rest was provided. To assess SICI a 2 ms interstimulus interval (ISI) was used, where the first stimulation was subthreshold at 80% of RMT and the second stimulation was suprathreshold at 120% of RMT (Kujirai et al., 1993). To assess ICF a 10 ms ISI was used, where the first stimulation was at 80% of RMT and the second stimulation was at 120% of RMT (Kujirai et al., 1993). To assess LICI a 100 ms ISI was used, where the first stimulation was suprathreshold at 120% of RMT and the second stimulation was also at 120% of RMT (Nakamura et al., 1997; Valls-Solé et al., 1992). The stimulation order was randomized between participants and between sessions. The second stimulation in each of the paired-pulse stimulations will be addressed as the conditioned MEP. The proportion of the conditioned MEP amplitude relative to baseline MEP amplitudes indicated excitability or inhibition.

Data Analysis

Data analysis was conducted using two custom written MATLAB (Mathworks Inc, Natick, MA, USA) programs. To obtain the MEP amplitudes, time points before and after the MEP were manually selected and the peak-to-peak amplitude was calculated from the minimum and maximum values within the selected window. The duration of the CSPs were manually selected by clicking on a time point at the end of the MEP and at the resumption of voluntary EMG activity. Such manual selection of EMG onset and offset times has been shown to be reliable (Ives & Wigglesworth, 2003). From each single-pulse stimulation trial, the peak-to-peak amplitudes (mV) of the MEP was determined and averaged across the 10 trials for each participant. The CSP values were determined from the same trials as the MEP, by measuring the duration (ms) between the end of the MEP and resumption of EMG activity following the characteristic pause. The duration of the CSP was averaged across the 10 trials for each participant. The SICI, ICF and LICI measures were determined by measuring the peak-to-peak amplitude of each of the 10 conditioned MEPs for each measure, relative to the average of the 3 baseline MEP amplitude values, obtained at the beginning of the block. The values obtained from the paired-pulse measures were represented as a percent of baseline MEP amplitudes (*Equation 1*) (Kujirai et al., 1993; McNeil et al., 2011).

Equation 1: $\text{Percent of Baseline} = \left(\frac{\text{Conditioned MEPs}}{\text{Baseline MEP}}\right) \text{x}100$

Percentages greater than 100% of baseline display facilitation, while percentages less than 100% of baseline display inhibition. The smaller the percentage value, the more inhibition there is.

Statistical Analysis

Independent samples t-tests were used to compare participant characteristics (i.e., age, height, and weight) between males and females. To assess differences within sexes across hormone phases, dependent samples t-tests were used. The values of each measure, within each sex, were then averaged between the low and high hormone phases. Independent samples t-tests were used to compare these values between males and

females. Averages were used rather than pooling the data together to prevent the risk of increased homogeneity. Further, averages were used to compare between males and females rather than just one time point (high or low hormone phase) due to the different main hormones that fluctuate throughout the differing hormone cycles between groups. Extreme outliers were determined as values 3 standard deviations away from the mean and were removed from statistical analysis. All values are presented in mean ± standard deviation (SD). Statistical significance was determined by $p \leq 0.05$. Cohen's d effect sizes were also calculated and classified as: $d > 0.8$ = large effect, $d > 0.5$ = medium effect, and $d > 0.2$ = small effect. Statistical analyses were complete using SPSS (IBM Corp. Released 2023. IBM SPSS Statistics for Macintosh. Version 29.0.2 Armonk, NY: IBM Corp) and figures were created using SigmaPlot (SigmaPlot® 12, Systat Software, Inc., Chicago, IL).

2.3 Results

Participants

Participant characteristics are presented in Table 1. Males were significantly taller and heavier than females ($p \leq 0.001$). The average time of day for males during the low hormone phase (afternoon) was ~3:00pm., and during the high hormone phase (morning) was ~9:30am. The average day within the menstrual cycle for the low hormone phase (mid-follicular) was ~day 7, and for the high hormone phase (mid-luteal) was ~day 21, or the equivalent days relative to their regular cycle length (i.e., middle of both phases). Ten of the fifteen female participants completed the study in the morning. Removal of female participants using oral contraceptives did not alter the results and they were therefore included in the following results.

Table 1. Participant Characteristics

	Males (n=15)	**Females** (n=15)
Age (years)	23.33±1.3	22.87±2.8
Height (cm)*	180.74±8.5	164.23±6.1
Weight (kg)*	77.61±9.0	63.91±11.3

Values are reported as mean ± SD. *Denotes a significant difference (p≤0.001) between sexes.

Hormone Phases

Excitability measures for both hormone phases for each sex are displayed in Figure 1. and inhibition measures are displayed in Figure 2. One MEP value for a male participant could not be calculated in the high hormone phase (MEP for males: n=14). One extreme outlier was identified and removed from analysis for each of: male LICI, female ICF, and female LICI. No significant differences and only negligible to small effect sizes, except for the medium effect size for ICF, were found between the low and high hormone phases for males, for each measure of excitability (MEP: p=0.67, d=0.12; ICF: p=0.24, d=0.32; Figure 1) and inhibition (CSP: p=0.13, d=0.41; SICI: p=0.33, d=0.26; LICI: p=0.70, d=0.11; Figure 2). Similarly, in females there were no significant differences between hormone phases and only negligible to small effect sizes for each measure of excitability (MEP: p=0.25, d=0.31; ICF: p=0.95, d=0.02; Figure 1) and inhibition (SICI: p=0.80, d=0.07; LICI: p=0.63, d=0.13; Figure 2), except the CSP measure. The CSP measure was significantly different between phases in females, with greater inhibition in the low hormone phase compared to the high hormone phase (p=0.04, d=0.60; Figure 2).

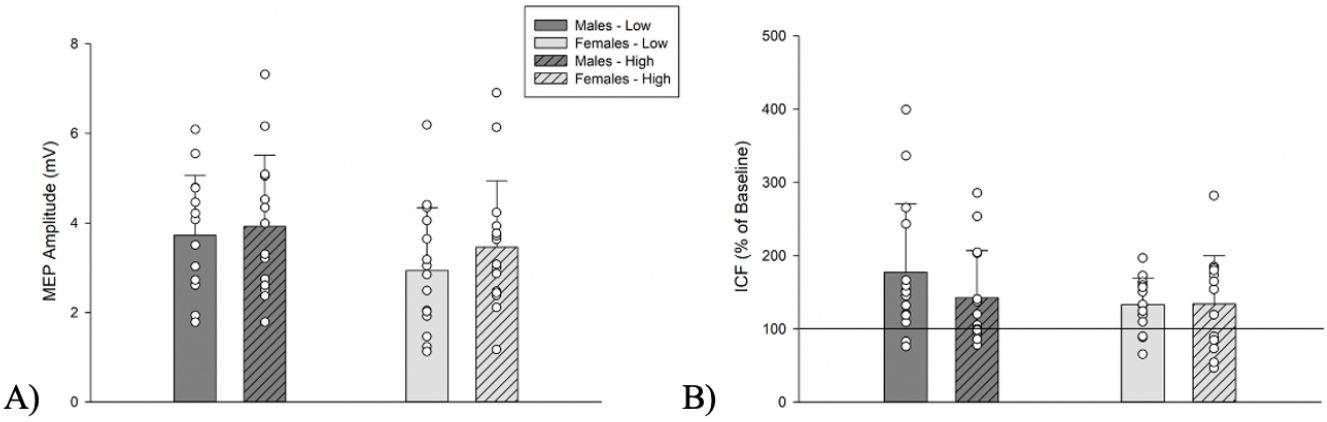

Figure 1. Low vs high hormone phases for males and females in excitability measures. A) MEP amplitudes were similar between hormone phases within males (n=14; p=0.67) and within females (p=0.25). B) ICF % of baselines were similar between

hormone phases within males (p=0.24) and within females (n=14; p=0.95). The black line at 100% denotes baseline, with values above the line representing facilitation.

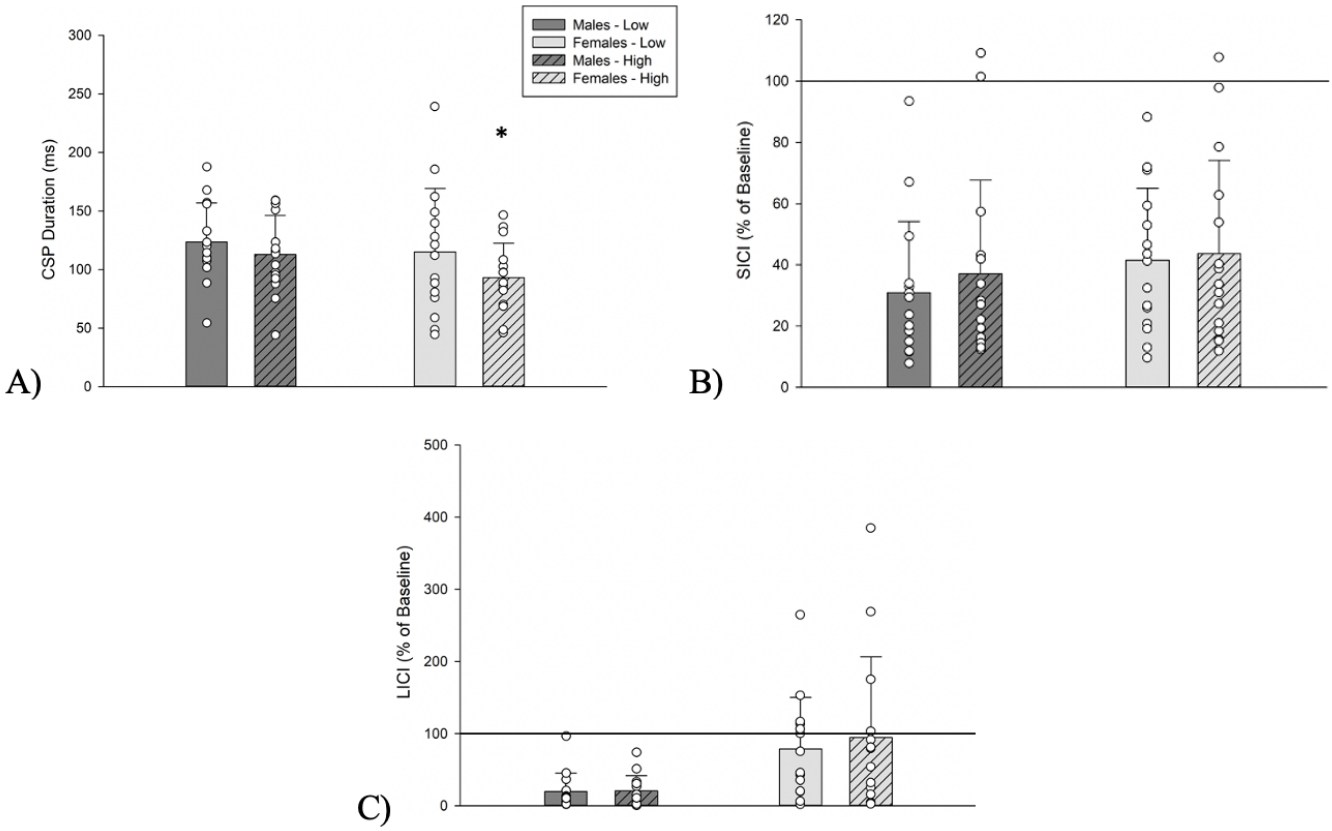

Figure 2. Low vs high hormone phases for males and females in inhibition measures. A) CSP durations were similar between hormone phases within males (p=0.13) and displayed greater inhibition in the low hormone phase compared to the high hormone phase within females (p=0.04); * denotes a significant difference (p≤0.05) between phases. B) SICI % of baselines were similar between hormone phases within males (p=0.33) and within females (p=0.80). The black line at 100% denotes baseline. C) LICI % of baselines were similar between hormone phases within males (n=14; p=0.70) and within females (n=14; p=0.63). The black line at 100% denotes baseline, with values below the line indicating inhibition.

Sex-based Differences

Excitability measures for both sexes are displayed in Figure 3., while inhibition measures are displayed in Figure 4. Comparisons between sexes were conducted after averaging

the values for each measure across the phases, within each sex. There was one missing MEP value, meaning one male participant's average was calculated from one timepoint. Three values for males in the SICI measure were identified as extreme outliers and were removed from analysis. Corticospinal excitability, as assessed with the MEP amplitude was not significantly different between sexes but had a medium effect size (p=0.09, d=0.65; Figure 3). No significant difference and a negligible effect size was found between sexes for the ICF measure (p=0.65, d=0.17; Figure 3). Corticospinal inhibition, as assessed with the CSP duration was not significantly different between sexes and had a small effect size (p=0.28, d=0.40; Figure 4).There was a significant difference between sexes, with a large effect size, for the SICI measure (p=0.004, d=-1.15) and the LICI measure (p=0.008, d=-1.12), with significantly greater inhibition in males compared to females (Figure 4).

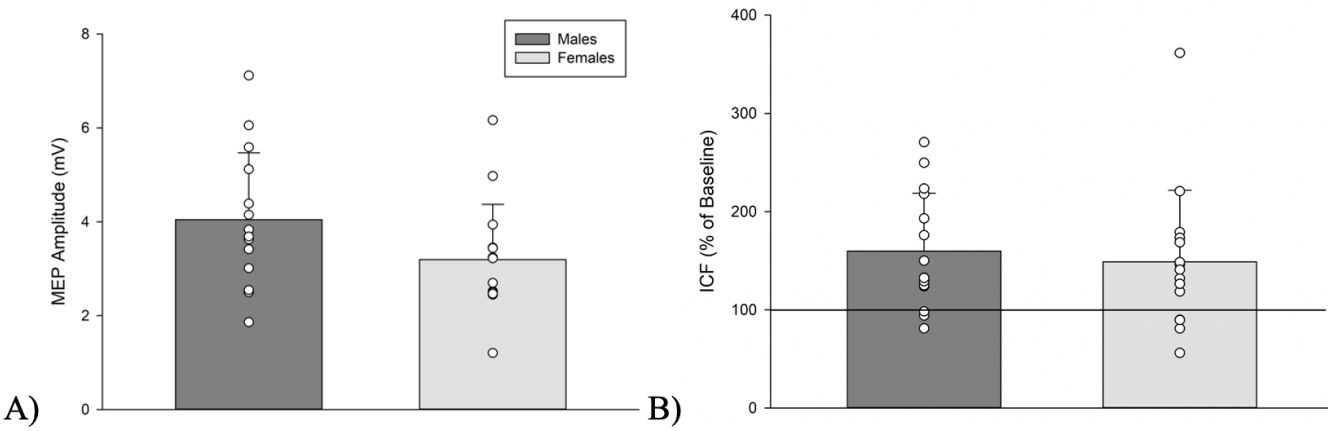

Figure 3. Differences between males and females in excitability measures. A) MEP amplitudes were similar between sexes (p=0.09). B) ICF % of baselines were similar between sexes (p=0.65). The black line at 100% denotes baseline.

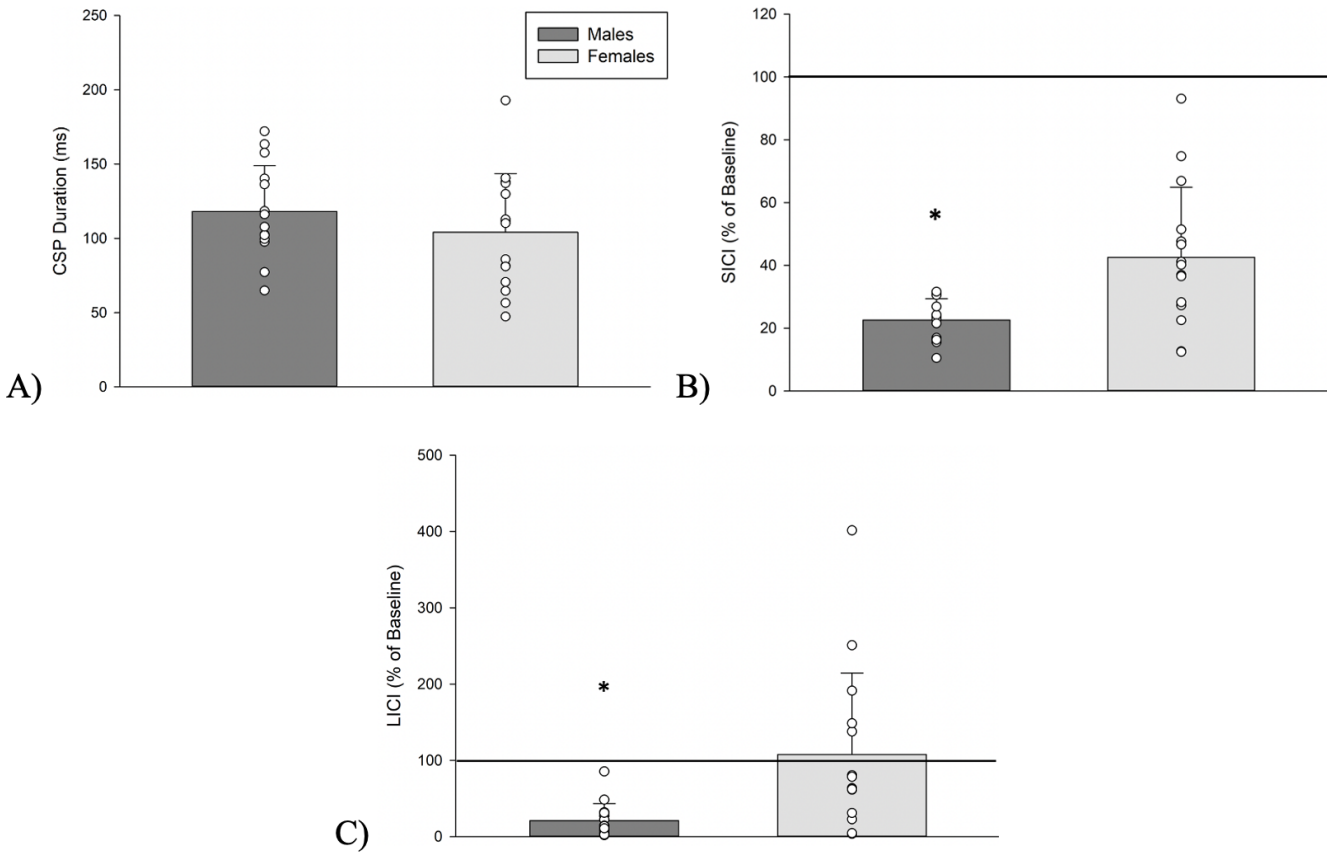

Figure 4. Differences between males and females in inhibition measures. A) CSP durations were similar between sexes (p=0.28). B) Males (n=12) displayed significantly greater inhibition compared to females (n=15) for the SICI measure (p=0.004). The black line at 100% denotes baseline; * denotes a significant difference (p≤0.05) between sexes. C) Males displayed greater inhibition compared to females for the LICI measure (p=0.008). The black line at 100% denotes baseline; * denotes a significant difference (p≤0.05) between sexes.

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Chapter 3

3 Discussion and Summary

3.1 Discussion

The present study explored sex-based differences in various corticospinal excitability and inhibition measures, with consideration of low and high hormone phases. As hypothesized, there were no sex-based differences in either of the excitability measures and no significant differences were observed between the low and high hormone phases, for either males or females. Further, as hypothesized, males had greater inhibition compared to females, but specifically only in the SICI and LICI measures. However, in contrast to the hypothesis, there were no significant differences in inhibition between the low and high hormone phases for either sex, except for the CSP measure in females, displaying greater inhibition in the low hormone phase.

Excitability

Similar to previous research, no cycle phase differences in excitability measures (MEP and ICF) were present for males and females in the present study. To our knowledge there has yet to be other studies specifically designed to evaluate the male hormonal cycle and its influences on the MEP and ICF measures. However, time of day influences on the MEP (Strutton et al., 2003; Ter Braack et al., 2019) and ICF (Doeltgen & Ridding, 2010) measures have been previously investigated in samples of majority males, displaying no differences between morning and afternoon values, supporting the present study's findings.

In addition, previous research supports the lack of cycle phase differences in excitability measures for females. For instance, no significant differences were observed in MEP measures between the early-follicular, late-follicular, and mid-luteal hormone phases for females in a previous study (Ansdell et al. 2019). Moreover, several studies found that the ICF measure was similar across phases of the menstrual cycle (Smith et al., 2002; Zoghi et al., 2015). However, Smith et al. (2002) saw that out of the three phases explored, the late-follicular phase (high estrogen) had a significantly greater influence on

both the excitability (ICF) and inhibition (SICI) measures collected, compared to the early-follicular phase (low estrogen). As suggested, this may be due to the higher levels of estrogen found in the late-follicular phase relative to the latter.

Estrogen has been associated with promoting glutamate and can demote GABA (Joshi & Kapur, 2019; Roeder & Leira, 2021; Smejkalova & Woolley, 2010) which could influence a more faciliatory effect across all measures, as observed by Smith et al. (2002). However, the late-follicular phase was not explored in the present study. The present study explored low (mid-follicular) and high (mid-luteal) hormone phases, specifically the middle of each phase where estrogen and progesterone are either both low or both raised (Elliott-Sale et al., 2021; Joshi & Kapur, 2019; Roeder & Leira, 2021; Smith et al., 2002; Stricker et al., 2006). As progesterone acts in opposition to estrogen by promoting GABA and suppressing glutamate (Joshi & Kapur, 2019; Roeder & Leira, 2021), it is possible there is a cancelling-out effect of hormonal impacts on overall excitability in the phases currently explored. For instance, it has been suggested that the ICF measure is influenced by both glutamate and GABA, as it is the net-effect of the neurotransmitters in the intraneuronal circuit (Reis et al., 2008; Ziemann et al., 1996). Therefore, the competing effects of high levels of both progesterone and estrogen on glutamate and GABA may have led to an attenuation of excitability responses, resulting in no significant differences between mid-phases, as observed in the current results.

Males and females did not differ in measures of excitability in the present study. This has been previously observed in literature when assessing the MEP measure (Pauhl et al., 2022; Pitcher et al., 2003) and the ICF measure (Zoghi et al. 2015). Previous research has suggested that there may be a spinal contribution to the ICF measure (Kujirai et al., 1993). When the excitability at the spinal level was explored using the Hoffmann reflex, there were also no differences observed between males and females (Hoffman et al., 2018). Collectively these studies support a lack of differences between males and females for excitability measures, regardless of the cortical or spinal contribution.

Inhibition

To our knowledge, no previous studies have explored the impact of the male hormone cycle on measures of inhibition (CSP, SICI, and LICI). However, studies investigating time of day influences on majority male samples for the CSP measure (5 males, 1 female) (Strutton et al., 2003) and the SICI measure (6 males, 4 females) (Doeltgen & Ridding, 2010) specifically in hand muscles, found no differences throughout the day, similar to our findings but in contrast to our hypothesis. However, other reports have shown a reduction in CSP duration and LICI in the afternoon compared with the morning, in a group of mostly males (10 males, 5 females) (Lang et al., 2011). It is possible there was a mis-capture of the exact lowest and highest peak time points of each individual male participant's cycle in the present study, as we did not account for circadian rhythms, which influence testosterone levels (Bremner et al., 1983). However, it is likely that controlling for time of day alone was sufficient to show different phases of the male hormone cycle, as testosterone progressively decreases throughout the day (Bremner et al., 1983).

In contrast to our hypothesis, the present findings demonstrated a lack of differences between high and low hormone phases for the SICI and LICI measures, but greater inhibition in the low hormone phase compared to the high hormone phase for the CSP measure, in females. Smith et al. (2002) reported similar results, as they observed no significant differences between the early-follicular and mid-luteal phases, which are most similar to the time points used in the current study. A study conducted by Ansdell et al. (2019) explored the CSP and SICI measures over the early-follicular phase (low estrogen and progesterone), late-follicular phase (high estrogen, low progesterone), and mid-luteal phase (high estrogen and progesterone). In contrast to our findings, Ansdell et al. (2019) observed no differences in the CSP measure over the phases, but greater inhibition for the SICI measure in the mid-luteal phase (high hormone) compared to the other phases. Moreover, Smith et al. (1999) also found that there was greater inhibition for the SICI measure in the mid-late luteal phase compared to the mid-late follicular phase (i.e., any day within the second half of the follicular and luteal phases). In both of these studies, different phases were explored relative to the present study, which could lead to the differing results.

The measures of inhibition in the current study are mediated primarily by the neurotransmitter GABA (Di Lazzaro et al., 2007; Nakamura et al., 1997, Werhahn et al., 1999), which is promoted by progesterone and suppressed by estrogen (Joshi & Kapur, 2019; Roeder & Leira, 2021; Smejkalova & Woolley, 2010). As there is a greater increase in progesterone than estrogen during the mid-luteal phase (Ansdell et al., 2019; Smith et al., 2002; Stricker et al., 2006), it is possible that the increase in both hormones during the mid-luteal phase led to a cancellation of the effect of each hormone on GABA, and therefore inhibition. Therefore, these influential background neurotransmitters systems (Barth et al., 2015), and hormones (Bitran et al., 1993; Joshi & Kapur, 2019; Roeder & Leira, 2021; Smejkalova & Woolley, 2010) do not function alone, and may be neutralizing the expected effect. However, this concept is speculative and should be further investigated.

It is also possible that the hormones impact different GABA receptor pathways differently. For instance, Ansdell et al. (2019) found phase differences in SICI, which involves $GABA_A$ receptors, but not in the CSP, which involves $GABA_B$ receptors. (Nakamura et al., 1997; Werhahn et al., 1999). We are unaware of any other studies that have explored the LICI measure between hormone phases. Further exploration into this may help to explain if this concept is due to the neurotransmitter receptor used, as the LICI measure uses the $GABA_B$ receptor (Nakamura et al., 1997; Werhahn et al., 1999).

To our knowledge this is among the first studies to explore the direct comparison of paired-pulse inhibition measures, SICI and LICI, between males and females. In the present study, greater inhibition was observed in males compared to females, as supported by previous research (Pauhl et al., 2022). However, unlike the previous research supporting this concept specifically through the CSP measure (Pauhl et al., 2022), in the present study, we observed these significant differences only in the SICI and LICI measures, not the CSP measure. All measures of inhibition in the current study are thought to be mediated by GABA and testosterone has been shown to be a GABA receptor agonist (Bitran et al., 1993). It is therefore possible that the differences in testosterone between males and females (Bhasin et al., 2011; Braunstein et al., 2011) contribute to greater inhibition in males. Although the measures of CSP and LICI are

thought to be mediated by the same neurotransmitter and receptor ($GABA_B$) (Nakamura et al., 1997; Werhahn et al., 1999), and therefore should be similar measures, the present study did not observe the same significant difference between sexes for both measures.

Previous research suggests that the spinal level contributes to the first 50ms of cortically-evoked inhibition, with any inhibition exceeding this time attributed to the cortical level (Fuhr et al., 1991; Inghilleri et al., 1993). However, more recent findings have shown that there may be a spinal contribution for up to 150ms of inhibition (Yacyshyn et al., 2016). In the current study, the SICI and LICI were evoked using an inter-stimulus interval of 2ms and 100ms, respectively, while the CSP duration exceeded 150ms for several participants. It is therefore possible that the greater inhibition observed in males compared with females in the SICI and LICI measures were more spinally-mediated differences, rather than cortical. This finding aligns with studies demonstrating greater spinal inhibition, through Hoffmann reflex testing, in males compared to females (Johnson et al., 2012).

The LICI measure in particular produced a facilitatory effect in some participants, particularly in females (Figure 4). This may be related to the overall facilitatory effects of estrogen (Smith et al., 2002). Specifically, females typically have greater levels of estrogen than males (Dighe et al., 2005). Estrogen's actions of promoting glutamate and suppressing GABA (Joshi & Kapur, 2019; Roeder & Leira, 2021; Smejkalova & Woolley, 2010), may result in greater facilitation in females than males. Further, the potentially inhibitory effects of testosterone (Bitran et al., 1993) may make males more susceptible to inhibition than females. Further, of the paired-pulse measures employed in this study, LICI has the largest range of ISIs used across different studies, ranging from 50-200ms (Lefaucheur, 2019; Valls-Solé et al., 1992). It is therefore possible that there is a different optimal ISI to produce LICI in males and females.

3.2 Conclusion

Overall, males tended to have greater corticospinal inhibition than females, with no sex-related differences in excitability. The lack of differences in excitability and inhibition between hormone phases, other than one inhibition measure for females, suggest that

males and females can be equally included in neurophysiology research during the phases of their respective hormone cycles included in this study. Indeed, it is necessary to continue to consider the inter-individual and inter-measure variability when exploring corticospinal measures. Moreover, it is necessary to further investigate the differences in inhibition between sexes, to better-understand the underlying mechanisms.

3.3 Limitations

There were several limitations to the present study. This was one of the first studies to control for hormone phases for both males and females while collecting corticospinal measures, however no direct measures of hormones were obtained. There are several techniques that could be implemented to collect direct hormone levels such as collecting blood or saliva samples or using a urine test. Nevertheless, participants self-reported the regular length of their cycle and the day their last menstruation commenced, which allowed investigators to calculate the appropriate testing days. Additionally, participants confirmed the day of their cycle at the time of the visit, to ensure self-reported days were correct and no irregularities developed over the cycle. These strategies were suitable for the present study, as findings were similar to studies that collected direct measures of hormones (Ansdell et al. 2019; Bremner et al., 1983; Hoffman et al., 2018; Smith et al., 2002).

No direct observations were made for the spinal contributions to the target measures in the present study. Implementing a direct measure of the spinal level, such as using the Hoffmann reflex, would allow for comparisons and quantification of the contribution from the spinal cord to be obtained. However, the possibility of contributions from other areas of the corticospinal pathway were considered when interpreting the results of the present study.

Lastly, strategies to elicit the measures of corticospinal excitability and inhibition, can vary greatly between studies. For instance, the measures employed in the current study depend on stimulation thresholds (i.e., between the conditioning and conditioned stimulus), the ISI range used for each of the measures, and the state of the muscle (i.e., resting or active) (Kobayashi & Pascual-Leone, 2003; Lefaucheur, 2019). Precautions

were taken to attempt to minimize the variability within measures. For instance, the conditioning stimulus intensity was determined by previous studies investigating the intensities that could produce the best responses and could be applied for more than one measure (Kujirai et al., 1993). Further, the ISI values used for each measure were relatively close to the lower end of their respective range. The paired-pulse measures were completed at rest, while the single-pulse measures were completed at a 50% of MVC contraction, to help standardize the voluntary output across participants. Each of these variables remained the same across participants and visits, however it would be beneficial to determine if different combinations of these parameters could be sex-specific, to help minimize the variability within results. Nevertheless, the techniques used to evaluate the outcome measures may also differ amongst studies. In the current study, measures were manually selected for evaluation. While there are other objective techniques that could be implemented, manually choosing the measures allowed for careful consideration of the correct peaks for MEPs and durations of CSPs and has been suggested as a reliable technique for evaluating electromyography activity (Ives & Wigglesworth, 2003).

3.4 Future Directions

To our knowledge, this is the first study to investigate sex-based differences in various measures of corticospinal excitability and inhibition, while addressing hormone phases. Researchers should continue to investigate these measures to strengthen the findings. Incorporating multiple visits, such as two visits in each phase, may help increase the reliability of these measures in each phase. In addition, implementing direct measures of hormone levels may help in determining the exact levels of each hormone, in each phase and the effects on excitability and inhibition. Although self-reporting can be used, this direct measure of hormone levels may assist in the acknowledgement of individual hormone variability within each phase, in addition to between phases. Further, investigating potential sex-based differences in these measures for other specific groups, such as within the aging population, is a direction that may help to develop a greater understanding of the aging corticospinal pathway and the potential influence hormones may have on measures of corticospinal excitability and inhibition.

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