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Potential sex-related differences in neurophysiology post-concussion

Alexandra N. Pauhl, *The University of Western Ontario*

Supervisor: Christie, Anita D., *The University of Western Ontario*
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Abstract

The purpose of this study was to examine potential sex-related differences in neurophysiology in concussed and healthy individuals. There was a total of 21(9 F) participants in each group. The concussion (CONC) group reported to the lab within 72 hours, 1- and 2-weeks post-injury. The control (CONT) group followed a similar measurement schedule once recruited. Motor evoked potential (MEP) amplitude and cortical silent period (CSP) duration was measured in the first dorsal interosseous muscle using transcranial magnetic stimulation. There were no significant differences in MEP amplitude or CSP duration between the CONC and CONT group. As well, there was no significant effect of time on these cortical measures. However, males had significantly longer CSP durations compared to females, indicating greater cortical inhibition, regardless of group. An important and novel finding of this study was the lack of differences in these neurophysiological measures between males and females following concussion.

Keywords

Lay Summary

Brain excitability and inhibition can be affected by the chemical changes that occur post-concussion. Research currently demonstrates that brain excitability is generally the same between concussed and healthy individuals. However, it has been reported that concussed individuals have greater brain inhibition levels than healthy individuals, but these measures have not been examined between sexes. Therefore, the purpose of this study was to determine potential sex-related differences in brain activity post-concussion. Twenty-one (9 F) concussed and healthy control individuals were recruited to come into the lab 72 hours post-concussion, and again at 1- and 2-weeks post-injury. Communication between the brain and muscle was measured in a hand muscle using transcranial magnetic stimulation. I did not find any differences in brain excitability or inhibition between the concussion group and the healthy control group. As well, these measures did not change over the 2-week testing period. Overall, males had significantly greater brain inhibition levels compared to females, regardless of injury status. This was the first study to demonstrate no differences in brain excitability or inhibition between males and females due to concussion.

Co-Authorship Statement

Anita D. Christie provided feedback and guidance on the entire manuscript, along with overall study design and data analysis. Data were analyzed and interpreted by Alexandra N. Pauhl.

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Chapter 1

1 General Introduction

The most agreed upon definition of a concussion is the transient neurological dysfunction that occurs as a result of any biomechanical force, not necessarily to the head (Giza & Hovda, 2001; MacFarlane & Glenn, 2015; McCrory et al., 2017). Neurological signs and symptoms are often seen after the biomechanical force, which is typically the result of functional or microstructural injury to the neural tissue (Giza & Hovda, 2014). A concussion can also be classified as a mild traumatic brain injury (mTBI), which represents 70% to 90% of all treated TBI's (Gauvin-Lepage et al., 2019). The range of youth and adults who seek care after a TBI is approximately 100 to 300 per 100,000 people (Gauvin-Lepage et al., 2019). The annual incidence of mTBI in Canadians is approximately 600 per 100,000 people (Gauvin-Lepage et al., 2019; Imhoff et al., 2016), and is a major health problem in children and adolescents because they are highly represented in sport-related head injuries (Imhoff et al., 2016).

1.1 Pathophysiology of a concussion – a brief review

A biomechanical insult can cause stretching and shearing of the brain tissue, which can lead to axonal stretching, disruptions of cellular membranes, opening of voltage- and ligand-gated channels, and an efflux of potassium (K^+) and influx of calcium (Ca^{2+}). Increasing extracellular K^+ causes greater neuronal depolarization that results in the nonspecific release of the excitatory amino acid (EAA) glutamate from the pre-synaptic vesicles (Choe, 2016; Giza & Hovda, 2001), which leads to greater excitation by opening ligand-gated channels on the post-synaptic terminal (MacFarlane & Glenn, 2015). The binding of glutamate to the N-methyl-D-aspartate (NMDA) and D-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors on the post-synaptic cell causes a continuous feed-back loop of depolarization, which allows an unrestricted efflux of K^+ and influx of Ca^{2+} in the neurons (Choe, 2016; MacFarlane & Glenn, 2015).

In the acute period, membrane disruption and ionic flux leads to a metabolic mismatch (Choe, 2016). The mitochondria are working in overdrive to produce more adenosine triphosphate (ATP) to supply the ATP-dependent sodium (Na^+)/ K^+ membrane

pump (Giza & Hovda, 2014; MacFarlane & Glenn, 2015). This increase in ATP production leads to an increase in glucose use, leading to a state of hyperglycolysis and an increasing energy demand to supply the energy deficit (Giza & Hovda, 2001; MacFarlane & Glenn, 2015). Hyperglycolysis can cause increases in lactate production (Giza & Hovda, 2001). Lactic acid accumulation can lead to neuronal dysfunction through acidosis and further membrane damage (Choe, 2016; Giza & Hovda, 2001). Additionally, the unrestricted influx of Ca^{2+} leads to an accumulation of intracellular Ca^{2+}, which prompts the mitochondria to uptake as much Ca^{2+} as possible (Giza & Hovda, 2014; MacFarlane & Glenn, 2015). Increasing Ca^{2+} uptake into the mitochondria is an oxygen-dependent process and can cause oxidative stress, which leads to a decrease in ATP production (Tarasov et al., 2012). The impairment of the mitochondria through oxidative metabolism provides further stimulus for increased glycolysis, leading to greater lactate production and accumulation (Choe, 2016; Giza & Hovda, 2001).

High levels of intracellular Ca^{2+} may also trigger cell death due to a number of negative events, such as overactivation of phospholipases, calpain, and protein kinases (Giza & Hovda, 2001; MacFarlane & Glenn, 2015). As well, due to the mechanical stretching of axons upon injury, axolemmal permeability is increased and can lead to an influx of extracellular Ca^{2+} directly into the axon (Giza & Hovda, 2014; MacFarlane & Glenn, 2015). This increased intra-axonal Ca^{2+} flux can degrade axonal integrity and function through cytoskeletal damage. For example, neurofilament side-arms can be phosphorylated, which can lead to neurofilament instability, compaction, and collapse (Giza & Hovda, 2001, 2014; MacFarlane & Glenn, 2015). Microtubules can also break down and affect axonal transport, causing swelling and secondary axotomy (Choe, 2016; Giza & Hovda, 2001, 2014; MacFarlane & Glenn, 2015).

The initial insult can also cause disruptions in other metabolic pathways that can contribute to the damaging neurometabolic cascade of concussion. In a healthy brain, cerebral blood flow (CBF) is coupled with neuronal activity and cerebral glucose metabolism (Giza & Hovda, 2001). However, CBF can be reduced to 50% of typical capacity acutely post-injury (Yamakami & McIntosh, 1989). This reduction in autoregulation capacity in an environment of increased glucose use can contribute to the ongoing energy crisis (Choe, 2016; Giza & Hovda, 2001; MacFarlane & Glenn, 2015). Reductions in intracellular

magnesium (Mg^{2+}) have also been observed acutely post-injury (Vink et al., 1987). Magnesium is needed to facilitate glycolysis and oxidative metabolism, along with initiating protein synthesis and maintaining cellular membrane potential (Giza & Hovda, 2001; MacFarlane & Glenn, 2015). Decreased levels of Mg^{2+} may unblock NMDA receptors more easily and enhance the influx of Ca^{2+} and its adverse effects (Giza & Hovda, 2001). As well, neurotransmitter alterations can occur post-injury, such as dysfunctional excitatory glutamatergic systems, and a loss of inhibitory Gamma Aminobutyric Acid-producing (GABAergic) cells (Giza & Hovda, 2001, 2014). These alterations can cause an excitatory-inhibitory imbalance, which can produce atypical neurological functioning (Giza & Hovda, 2014; Guerriero et al., 2015).

1.2   Glutamate and Gamma Aminobutyric Acid (GABA) concentrations post-injury

The primary excitatory and inhibitory neurotransmitters in the brain are glutamate and GABA, respectively, and they are needed to create an excitatory-inhibitory balance within neurons (Giza & Hovda, 2014) which is required for maintaining typical neurologic function (Guerriero et al., 2015). Post-injury, there is an acute excitatory period followed by a subacute to possibly chronic depression period following a concussion (Giza & Hovda, 2001). The excitatory period is due to a continuous release of glutamate within the first few minutes post-injury (Giza & Hovda, 2001). Animal models have shown that glutamate transport is decreased post-injury (Cantu et al., 2015) and the excess extracellular glutamate creates an excitotoxic environment that causes greater neuronal injury and dysfunction (Giza & Hovda, 2014; Guerriero et al., 2015). One of the effects of neuronal dysfunction is GABA's inability to modulate excitatory pathways due to the lack of glutamate transport into astrocytes. In a healthy brain, glutamate is transported into astrocytes and converted into glutamine (Bartnik et al., 2005), glutamine is then transported to GABAergic neurons to convert glutamine back to glutamate, which is then converted to GABA through glutamate decarboxylase (Guerriero et al., 2015). Post-injury, GABA-producing cells are not as efficient as they are in a healthy brain, due to the reduced availability of the glutamate precursor, disrupting the balance between the excitatory and inhibitory neurotransmitters (Guerriero et al., 2015; Harris et al., 2012). This imbalance could have multiple consequences, such as greater susceptibility to a subsequent, more severe concussion,

accelerating the neurodegenerative process of aging/abnormal aging, memory and attention dysfunction, and prolonged symptoms (Henry et al., 2009; Kim et al., 2019; Tremblay et al., 2011).

Although glutamate signaling is increased and glutamate transport is decreased, research on rats using magnetic resonance spectroscopy (MRS) demonstrated a decrease in glutamate in the cortex and hippocampus as early as two to four hours and up to 14 days post-injury (Harris et al., 2012). This decrease in glutamate concentration as soon as two hours post-injury may be due to the short burst of glutamate release only seen within the first few minutes after insult, which seems to resolve after a few hours (Giza & Hovda, 2001). Along with decreased glutamate, an increase in glutamine in the cortex and hippocampus was observed throughout the 14 days (Harris et al., 2012), which can be explained by the uptake and conversion of glutamate to glutamine by astrocytes (Bartnik et al., 2005). Although there are considerable differences in the molecular and physiological make-up between animal models and humans, similar MRS findings still exist.

In humans, Henry et al. (2009) demonstrated decreased levels of glutamate at six days following a sports-related concussion (SRC) in the primary motor cortex (M1). In a later study, Henry and colleagues again demonstrated a depression of glutamate levels in the acute phase, but also saw a recovery of glutamate at six months post-injury (Henry et al., 2011). As well, a study investigating boxers greater than two years post-injury saw no statistical difference in glutamate levels compared to the control group in the prefrontal cortex (PFC) (Kim et al., 2019). These findings suggest that glutamate levels may be decreased in the acute phase post-injury and recover over time in the M1 and PFC. However, a recent pilot study did not demonstrate a statistical difference in glutamate levels in the M1 compared to the control group when examining mTBI individuals at 72 hours, two weeks, and two months post-injury (Yasen et al., 2018). Instead, Yasen et al. (2018) saw lower glutamate concentrations in the dorsolateral prefrontal cortex (DLPFC) in the acute phase, unlike previous studies that saw no significant difference in glutamate levels in the DLPFC (Henry et al., 2009, 2011). The explanation for these mixed findings between the M1 and DLPFC are still unknown, but biomechanical literature suggests that the M1 is consistently vulnerable to shear strain from the rotational forces coupled with concussion (Bayly et al., 2005; Henry et al., 2011; Sabet et al., 2008).

Additionally, Yasen et al. (2018) saw no significant difference between the mTBI and control group when comparing GABA levels and glutamate to GABA ratios in the M1. Although, a previous study examining M1 metabolism in athletes three years post-injury observed no correlation in glutamate to GABA ratios, when control athletes showed a significant positive correlation between the metabolites (Tremblay et al., 2011). Tremblay and colleagues concluded that even though absolute metabolite concentrations did not differ between the concussion and control group, the non-existent relationship between the metabolites in the concussion group may reflect an excitatory-inhibitory imbalance in the chronic post-injury stage (Tremblay et al., 2011). These findings support the idea that despite being asymptomatic, and having normal neurophysiological and anatomical MRI results, the brain may have altered neurometabolism that does not return to its pre-injury state (Henry et al., 2009).

In the DLPFC, GABA was found to be lower in people with mTBI compared with controls at 72 hours and two weeks post-injury (Yasen et al., 2018). As well, the ratio of glutamate to GABA was significantly higher in mTBI individuals at two weeks post-injury, representing an excitatory-inhibitory imbalance in this study (Yasen et al., 2018). To the authors' knowledge, this was noted as the first significant finding of lower GABA levels in the DLPFC. Lower GABA in the DLPFC was further confirmed by Kim et al. (2019) who found lower GABA in the PFC in boxers who experienced repetitive mTBI. Kim and colleagues also observed that lower levels of GABA was significantly correlated to memory dysfunction (Kim et al., 2019). Animal studies have correlated the chronic cognitive and behavioural changes seen in repetitive mTBI with the imbalance between glutamate and GABA concentrations (Guerriero et al., 2015). This may explain why Yasen et al. (2018) and Kim et al. (2019) had similar findings, as majority of Yasen's participants obtained their concussion playing high head impact sports (soccer, rugby, or hockey), which could contribute to repetitive blows to the head and result in an accidental examination of a repetitive mTBI population.

Unfortunately, there are only a handful of studies that research metabolite concentrations following concussion in humans. Each study had different times of recruitment since injury and the number of times they took measures. Time of injury to recruitment varied from as early as 72 hours to three years post-injury and some studies had

multiple time points of measure (Henry et al., 2011; Yasen et al., 2018), while other studies only measured once (Henry et al., 2009; Kim et al., 2019; Tremblay et al., 2011). Generally, the brain region of interest remained similar throughout the studies and consist of the M1 (Henry et al., 2009, 2011; Tremblay et al., 2011; Yasen et al., 2018) and the PFC (Kim et al., 2019), which contains the DLPFC that some studies specifically measure (Henry et al., 2009, 2011; Yasen et al., 2018). Overall, the variability between studies assessing neurometabolic concentrations in humans post-concussion could add to the lack of consensus throughout the recent literature.

1.3   Corticospinal excitability and cortical inhibition post-injury

Transcranial magnetic stimulation (TMS) is a tool used to measure corticospinal excitability and cortical inhibition through motor evoked potential (MEP) amplitudes and cortical silent period (CSP) durations. Although there are other measures of corticospinal excitability and cortical inhibition (e.g. intracortical facilitation, long-interval intracortical inhibition, short-interval intracortical inhibition), these measures are reported to not be different between concussed and control individuals (De Beaumont et al., 2007, 2009; Tremblay et al., 2011, 2014). Also, it has been demonstrated that the MEP amplitude is primarily mediated by glutamate, while the CSP duration is primarily mediated by GABA (Tremblay et al., 2013; Werhahn et al., 1999). Therefore, these measures allow us to indirectly measure the neurotransmitters primarily involved in concussion.

Post-injury changes in corticospinal excitability are not consistent across studies. For example, studies that involved concussed individuals within 72 hours post-injury demonstrated no significant differences in MEP amplitude between the concussion and control group (Edwards & Christie, 2017; Miller et al., 2014). However, Edwards & Christie (2017) showed overall greater excitability in concussed individuals throughout two months of testing compared with controls. Conversely, smaller MEP amplitudes indicating lower excitability has been demonstrated at 72 hours post-injury, but not at 24 hours, five days, and 10 days post-injury (Livingston et al., 2010). Lower excitability was also demonstrated in a chronic symptomatic group when compared to healthy controls and chronic asymptomatic individuals (Yasen et al., 2020). These differences across studies may be due to different times of recruitment post-injury, as animal models have demonstrated that the hyper-

excitability period may only last a couple of hours after impact, and is then followed by a depression period (Giza & Hovda, 2001). However, this timeline is still unknown in humans and can vary between individuals.

CSP durations post-injury have consistently been longer compared to healthy controls, indicating greater inhibition. This may be due to the depression period following the initial impact, but how long this depression may affect the brain is unknown. Several studies have observed longer CSP durations at 72 hours post-injury, which typically do not change throughout two-months of testing (Edwards & Christie, 2017; Miller et al., 2014; Yasen et al., 2017). Although, conflicting evidence exists in the chronic post-injury phase (Tremblay et al., 2014; Yasen et al., 2020). These results suggest that cortical inhibition seems to be greater in concussed individuals in the acute and sub-acute post-injury stages.

Within human studies of corticospinal excitability and cortical inhibition post-concussion, there is a general lack of female representation. Some studies have only examined male varsity athletes (De Beaumont et al., 2007, 2009; Tremblay et al., 2011, 2014), while other studies examined a mixed population with approximately 50% being female (Edwards & Christie, 2017; Miller et al., 2014; Yasen et al., 2017, 2020). However, post-injury sex differences in neurophysiological measures of corticospinal excitability and cortical inhibition have not been examined. Therefore, it cannot be concluded that the absence or presence of females in these studies are the reason for the variance.

1.4   Sex differences in concussions

Over the decades males seem to consistently have a higher prevalence for concussion compared to their female counterparts (Mollayeva et al., 2018). However, this may not be an accurate reflection of concussion prevalence as it was determined through hospitalization rates and could therefore include moderate to severe traumatic brain injuries. In Canada, the greatest prevalence of concussion is seen in adolescents aged 10-19 years old and the incidence declines as age increases (Bang et al., 2020). Bang et al. (2020) speculated that the higher rates in this age group were due to adolescents being involved in more organized sports; specifically for males, playing more contact sports and having greater risk-taking behaviours. However, a study analyzing data from the National Collegiate Athletic Association from 2004-2005 to 2008-2009 found that in sex-comparable sports, females

reported a greater number of concussions than males (Covassin et al., 2016). Despite greater hospitalization rates in concussed males overall, when matched for sport, incidence appears to be higher in females.

Regardless of prevalence, it has been consistently demonstrated that females generally take longer to recover from concussion than males (Bazarian et al., 2010; Casey et al., 2016; Gallagher et al., 2018; King, 2014). This longer time to recover in females may be due to the different type and number of post-concussion symptoms (PCS) reported. Males tend to report more cognitive symptoms, while females report more neurobehavioural and somatic symptoms (Frommer et al., 2011). Similarly, the prevalence of depression and anxiety is much higher in females than males (Altemus, 2006) and studies have correlated increasing symptoms of depression with decreasing cortical glucose metabolism (Baxter et al., 1989). Compared to their male counterparts, researchers found that females generally report greater PCS scores and are more likely to miss more than seven days of normal activities due to concussive symptoms (Bazarian et al., 2010). Females have also been shown to have greater mean PCS scores at three-months post-injury compared to males and have a greater risk of prolonged or persistent post-concussion symptoms (PPCS) (Casey et al., 2016; King, 2014). Research has investigated that these differences in symptom reporting scores and overall recovery of concussion are often attributed to sex-based differences in honesty in reporting symptoms (Niemeier et al., 2014). However, these differences in symptoms may also be due to sex differences in hormonal systems, head and neck musculature, neural anatomy and physiology, and cellular responses (Broshek et al., 2005).

Animal studies have produced contrary findings regarding the potential role of female hormones, estrogen and progesterone, in concussion. Where the effects of estrogen on neural protection may be either protective or detrimental, progesterone seems to reduce TBI neural impairment (Roof & Hall, 2009). In a human study, it was found that females experienced a longer length of recovery and greater symptom severity than males (Gallagher et al., 2018). However, symptom severity was lower in females who used hormonal contraception compared to females who did not, but the length of recovery did not differ between the two female groups. In males however, there was a significant correlation between symptom severity and length of recovery in males (Gallagher et al., 2018). Gallagher et al. (2018)

concluded that self-reported symptom severity may have predictive validity regarding length of recovery in males, but not in females.

Symptom severity differences may also be due to sex differences in head and neck musculature. It has been reported that females generally have decreased neck strength compared to males, along with greater head-neck acceleration and displacement upon insult (Collins et al., 2014; Tierney et al., 2005). As well, greater head-neck acceleration and displacement has been associated with longer recovery time and cognitive deficits (Tierney et al., 2005). Females may also have greater self-awareness of their cognitive deficits compared to males, which may yield greater caution to return to activities and reports of greater symptoms (Niemeier et al., 2014).

Differences in the cerebral cortex between males and females may also contribute to differences in the experience of concussion between sexes. In males, the average neuronal density and nerve cell numbers are significantly higher than females (de Courten-Myers, 1999). These findings correspond with males generally having larger fibre cross-sectional areas, fibre diameter, and greater numbers of axons (de Courten-Myers, 1999; Solomito et al., 2019). However, females possess significantly more neuropil compared to males, indicating greater neuronal and glial processes (de Courten-Myers, 1999). Females make greater use of both hemispheres crossing larger areas of the brain to perform a variety of tasks, whereas males tend to use a single hemisphere to complete similar tasks (Solomito et al., 2019). If axonal dysfunction reduces or slows hemispheric communication, then females may be affected to a greater extent and perceive the injury differently as their ability to process information and neural networks become more disrupted than males (Solomito et al., 2019). Therefore, males may be able to withstand larger axonal stresses than females, and the dependency females have on greater interhemispheric connections may cause different symptoms between males and females (de Courten-Myers, 1999; Solomito et al., 2019).

1.5 Purpose and hypothesis

The overall purpose of this study was to determine whether there are sex-based differences in neurophysiology in the acute and sub-acute stages post-concussion compared to healthy controls. Specifically, I aimed to determine differences in corticospinal excitability and cortical inhibition in concussed males and females across two weeks post-injury. I

hypothesized that corticospinal excitability would not be different between concussed individuals and healthy controls, or between sexes and would not change across time. I also hypothesized that cortical inhibition would be greater in concussed individuals than controls across two weeks post-injury but would be similar between sexes.

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Chapter 2

2 Neurophysiological sex differences post-concussion

2.1 Introduction

Concussion is a major health problem in children and young adults, as they are highly represented in sport-related head injuries (Imhoff et al., 2016). A concussion is a sub-category of mild traumatic brain injury (mTBI), which represents 70% to 90% of all treated TBIs (Gauvin-Lepage et al., 2019). Upon a biomechanical insult, axonal stretching, disruptions of cellular membranes, and opening of voltage- and ligand-gated channels can occur, causing a harmful neurometabolic cascade. This cascade can result in a metabolic mismatch between the increasing energy demand and decreasing supply, decrease in cerebral blood flow (CBF), and an excitatory-inhibitory neurotransmitter imbalance (Choe, 2016; Giza & Hovda, 2001, 2014; Guerriero et al., 2015; MacFarlane & Glenn, 2015). In humans, post-injury recovery can be observed and measured through an acute stage (24-72 hours), sub-acute stage (four to ~14 days), and for some individuals, a chronic stage (> 10-14 days for adults, > four weeks for children) (McCrory et al., 2017).

Glutamate and gamma aminobutyric acid (GABA) are the primary excitatory and inhibitory neurotransmitters, respectively, that maintain the excitatory-inhibitory balance within neurons in the brain (Giza & Hovda, 2014). This neurotransmitter balance is required for maintaining typical neurological function. Therefore, an excitatory-inhibitory imbalance could have multiple consequences such as greater susceptibility to a subsequent and more severe concussion, memory and attention dysfunction, and prolonged symptoms (Henry et al., 2009; Kim et al., 2019; Tremblay et al., 2011). Although the effects of concussion on neurotransmitter concentrations have been established in rodents (Giza & Hovda, 2014), there is a lack of consensus in the literature of these effects in humans. For example, animal studies show an increase in glutamate concentrations in the acute post-injury stage due to the short burst of glutamate within the first few minutes after insult, which is resolved after a few hours (Giza & Hovda, 2001). In humans, however, the results are less consistent. Some studies have demonstrated that there is a decrease in glutamate levels in the primary motor cortex (M1) in the acute post-injury stage, but at six months post-injury glutamate levels had recovered (Henry et al., 2009, 2011). However, a more recent pilot study did not find

statistical differences in glutamate levels in the M1 in the acute through chronic post-injury stages (Yasen et al., 2018). In this study, lower glutamate concentrations were demonstrated in the dorsolateral prefrontal cortex (DLPFC) in the acute post-injury stage (Yasen et al., 2018), unlike previous studies that showed no significant glutamate differences in the DLPFC (Henry et al., 2009, 2011). Comparable findings have been demonstrated in the chronic post-injury period, where glutamate levels in the PFC were shown to be similar to healthy controls (Kim et al., 2019).

In the acute and sub-acute post-injury stages, GABA concentrations in the M1 have been found to be similar in individuals with mTBI compared with controls (Yasen et al., 2018). However, GABA was lower in the DLPFC in the mTBI group compared to the control group at 72 hours and two weeks post-injury (Yasen et al., 2018). This finding was further confirmed by Kim and colleagues who demonstrated lower GABA in the PFC in boxers who experienced repetitive mTBI (Kim et al., 2019). Lower GABA levels found in these mTBI groups may relate to the loss of GABA-producing interneurons, a decrease in GABA synthesis, or alterations in the cycling of glutamate to glutamine to GABA in astrocytes (Rae, 2014). Also, Yasen et al. (2018) did not observe significant differences in glutamate to GABA ratios in the M1 (Yasen et al., 2018), but in the DLPFC the glutamate to GABA ratio was significantly higher at two weeks post-injury, representing an excitatory-inhibitory imbalance (Yasen et al., 2018). Similarly, Tremblay and colleagues demonstrated no relationship between glutamate and GABA concentrations in concussed individuals, which was suggested to reflect an excitatory-inhibitory imbalance in the chronic post-injury stage (Tremblay et al., 2011). These findings may support the notion that even if post-concussion patients are asymptomatic, their brain can still have altered neurometabolism that does not return to pre-injury state (Henry et al., 2009).

Evidence from previous studies using transcranial magnetic stimulation has shown alterations in corticospinal excitability and cortical inhibition, possibly due to the excitatory-inhibitory imbalance (Yasen et al., 2020). Using motor evoked potential (MEP) amplitude to determine corticospinal excitability, studies have demonstrated mixed findings. Lower excitability has been reported in individuals with mTBI (Livingston et al., 2010) but other studies found no significant differences in MEP amplitude between a mTBI and control group (Miller et al., 2014; Yasen et al., 2020), or found that a concussion group generally had

greater excitability (Edwards & Christie, 2017). These differences across studies may reflect differences in post-injury testing time, as in animal models, excitability changes within minutes to hours post-injury (Giza & Hovda, 2001).

Findings for changes in cortical inhibition, using the cortical silent period (CSP) duration, are more consistent across studies. Although there are some studies with participants in the chronic post-injury phase reporting no differences in CSP duration compared with controls (Tremblay et al., 2014, Yasen et al., 2020), the CSP duration is typically reported to be longer, indicating greater inhibition in concussed individuals in the acute and sub-acute post-injury phases. Numerous studies have observed that CSP duration is longer at 72 hours post-injury and remains longer up to two months post-injury (Edwards & Christie, 2017; Miller et al., 2014; Yasen et al., 2017), as well as nine months to 30 years post-injury (De Beaumont et al., 2007, 2009; Tremblay et al., 2011). Although the majority of these studies included both male and female participants, potential sex-based differences in the neurophysiology were not examined.

It has been consistently demonstrated that females generally take longer to recover from concussion than males (Bazarian et al., 2010; Casey et al., 2016; Gallagher et al., 2018; King, 2014) and report a greater number of concussions than males in sex-comparable sports (Covassin et al., 2016). Longer recovery in females has been linked to the different type and number of post-concussion symptoms (PCS) reported. Researchers found that females generally report greater PCS scores and are more likely to miss more than seven days of normal activities due to concussive symptoms (Bazarian et al., 2010). It has also been observed that females are at a greater risk of experiencing persistent post-concussion symptoms, as they have demonstrated greater mean PCS scores at three-months post-injury compared to males (Casey et al., 2016; King, 2014). Although the reasons for these sex-based differences are not yet fully understood, it has been suggested they may be due to honesty in reporting symptoms, different hormonal systems, head and neck musculature, neural anatomy and physiology, and cellular responses (Broshek et al., 2005). It is unknown if the neurophysiological consequences of concussion described above may contribute to these sex differences in concussion symptoms and recovery.

Therefore, the purpose of this study was to determine sex-based differences in neurophysiology in the acute and sub-acute post-injury stages. Corticospinal excitability and cortical inhibition were assessed in concussed (CONC) and healthy control (CONT) males and females. It was hypothesized that: i) corticospinal excitability would be similar between groups and sexes, and ii) cortical inhibition would be greater in the CONC group compared to the CONT group, but similar between sexes.

2.2 Methods

Participants

Participant data collection was conducted as part of a study in the Neurophysiology Laboratory at the University of Oregon. A total of 48 participants were included in this study with 24 participants (12 males and 12 females) in each of the CONC and CONT groups. Individuals in the CONC group were diagnosed by a specialized health professional (certified athletic therapist or physician). Individuals in the CONT group were sex-, age-, height-, weight-, and activity-matched to each participant in the CONC group.

All participants provided written informed consent and were asked to complete a brief medical history and transcranial magnetic stimulation (TMS) safety screening questionnaire (Rossi et al., 2011). Exclusion criteria for all participants included: i) a history of two or more concussions or a concussion (in addition to the current one for the CONC group) within a year prior to testing, ii) history of cognitive deficiencies such as memory loss or difficulty concentrating (unrelated to concussion), iii) history of attention deficit hyperactivity disorder, neurological impairments, musculoskeletal impairments, or seizures, or iv) contraindications to the use of TMS. Additional exclusion criteria specifically for the CONC group included loss of consciousness for more than one-minute. All procedures were reviewed and approved by the Institutional Review Board of the University of Oregon.

Measurement schedule

Each participant completed three testing sessions, during which measures of corticospinal excitability and cortical inhibition were obtained using TMS-evoked measures from the first dorsal interosseous (FDI) muscle of the dominant hand. Individuals in the CONC group reported to the laboratory within 72 hours of sustaining their injury and again at 1 week and 2

weeks post-injury. Control participants followed a similar timeline. Measures of height and weight were obtained during the first session.

Electromyography (EMG)

Surface EMG electrodes were placed over the FDI and recorded all evoked potentials. Prior to electrode placement, the skin was lightly abraded with NuPrep® and cleaned with alcohol to reduce signal impedance. A pre-amplified bipolar, Ag-AgCl electrode (DE-2.1, Delsys Inc., Boston, MA), with an inter-electrode distance of 1 cm was placed over the FDI of the dominant hand. This electrode was connected to a portable amplifier (Delsys Bagnoli, Delsys Inc., Boston, MA), which further amplified and band-pass (20-450 Hz) filtered the EMG signal. A ground electrode was secured to the posterior aspect of the distal ulna. The EMG signal was observed on an oscilloscope (TDS 2014C, Techtronix, Beaverton, OR), sampled at 5 kHz with a 16-bit A/D converter (NI USB-6251, National instruments, Austin, TX) and stored on a personal computer for offline analysis using Dasylab software (Data Acquisition System Laboratory, DasyTec, USA Inc., Amherst, NH).

Motor evoked potential (MEP) and cortical silent period (CSP)

MEPs were elicited from the FDI muscle using TMS (MagStim 200^2, MagStim Company, Ltd, Whitland, UK) with a flat 70-mm figure-of-eight coil positioned over the optimal site of the contralateral motor cortex. With the participant at rest, the optimal site was determined by moving the coil around the head and stimulating at 60% of the stimulator output (SO). The optimal site was defined as the position that consistently yielded the largest response in the FDI, as indicated by the peak-to-peak amplitude of the MEP. Once the optimal site was located, the resting motor threshold (RMT) was determined by reducing the SO in a step-wise fashion to find the lowest stimulus intensity required to evoke a response of at least 50 µV in at least five out of 10 trials (Orth & Rothwell, 2004; Werhahn et al., 1999). The SO was set to 120% of the RMT for the remainder of the study protocol stimulations.

Individuals maximal voluntary contraction (MVC) was calculated by isometrically contracting the FDI as hard as possible while the EMG amplitude produced was evaluated on an oscilloscope. Participants were asked to contract the FDI to 50% of their MVC, determined through visual feedback, while a single-pulse TMS stimulation was delivered to

elicit a MEP and CSP. Participants were instructed to maintain the contraction through the stimulation and briefly afterwards. Five trials were performed with ~15-30 seconds of rest between trials. Corticospinal excitability was assessed through the peak-to-peak amplitude of the MEPs produced. Cortical inhibition was assessed through the duration of the CSPs produced. All trials were recorded for offline analysis.
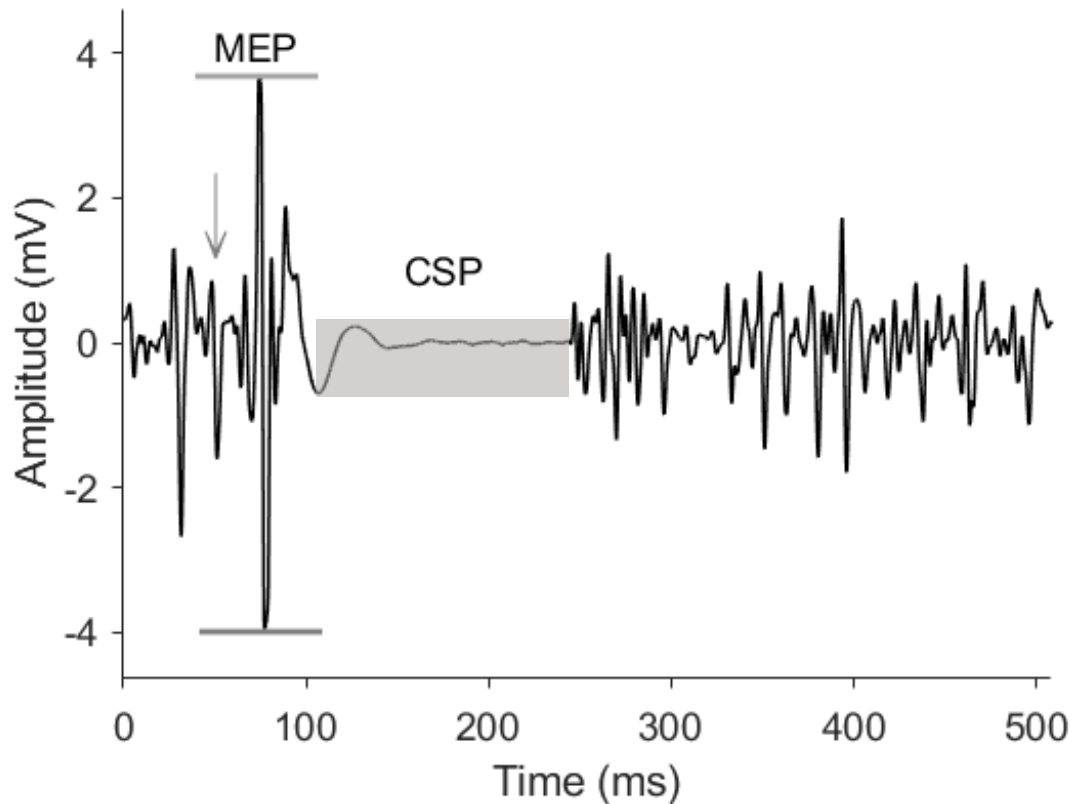

Figure 1. Sample EMG recording. Corticospinal excitability was assessed through peak-to-peak amplitude of the MEP (horizontal lines). Cortical inhibition was assessed through the CSP duration (shaded area). The arrow indicates stimulation from TMS.

Data analysis

Sample recordings of a MEP and CSP are shown in Figure 1. All trials were analyzed with a custom-written program using MATLAB software (Mathworks Inc, Natick, MA). The peak-to-peak MEP amplitude was determined by marking the onset and offset of muscle response and calculating the magnitude of the range between the highest and lowest EMG value in the selected period. The silent period was determined as the time between the end of the MEP, evoked during a voluntary contraction at 50% MVC, and the resumption of EMG activity.

These points were manually selected by a trained operator. The five trials were then averaged to obtain the MEP amplitude and CSP duration at each time point.

Statistical analysis

Two-factor (group, sex) analysis of variance (ANOVAs) were used for group characteristic comparisons of age, height, and weight. Two-way repeated measures ANOVAs were used to determine the effect of sex (male vs. female), group (CONT vs. CONC), time (72 hours, 1 week, and 2 weeks), and interaction effects for MEP amplitude and CSP duration. Data are presented as mean ± standard deviation (SD) and statistical significance was set at $p \leq 0.05$. Incomplete data sets due to failure to attend all three lab visits for the CONT (*n*=1 F) and CONC (*n*=2 F) group were therefore excluded from analysis, along with their matched counterpart. This resulted in a total of 42 participants in the CONT (9 F) and CONC (9 F) groups, respectively.

2.3 Results

Participants

Participant characteristics are presented in Table 1. There were no group differences in age, height, or weight ($p \geq 0.49$), indicating that the groups were well matched. There were significant sex differences in height and weight ($p < 0.001$), as males were generally taller and heavier than females.

Table 1. Group Characteristics

	Control (n=21; 9 F)		Concussion (n=21; 9 F)	
	Males	*Females*	*Males*	*Females*
Age (years)	21.75 ± 5.6	20.56 ± 1.1	21.58 ± 5.0	20.11 ± 2.4
Height (cm)*	179.98 ± 5.7	163.20 ± 5.1	179.45 ± 7.7	166.68 ± 8.4
Weight (kg)*	77.98 ± 6.4	64.00 ± 8.2	76.85 ± 13.4	62.57 ± 6.8

Values are presented as mean ± SD. *Males significantly greater than females ($p < 0.001$).

Motor evoked potential (MEP) amplitude

The MEP amplitudes at each time point are shown in Figure 2. There was no difference in MEP amplitude between the CONT and CONC groups ($p = 0.55$). There was also no difference between males and females ($p = 0.77$) in MEP amplitude. Over the 2-week testing period, there was no significant effect of time on MEP amplitude ($p = 0.21$) and there were no significant interactions ($p \geq 0.24$).
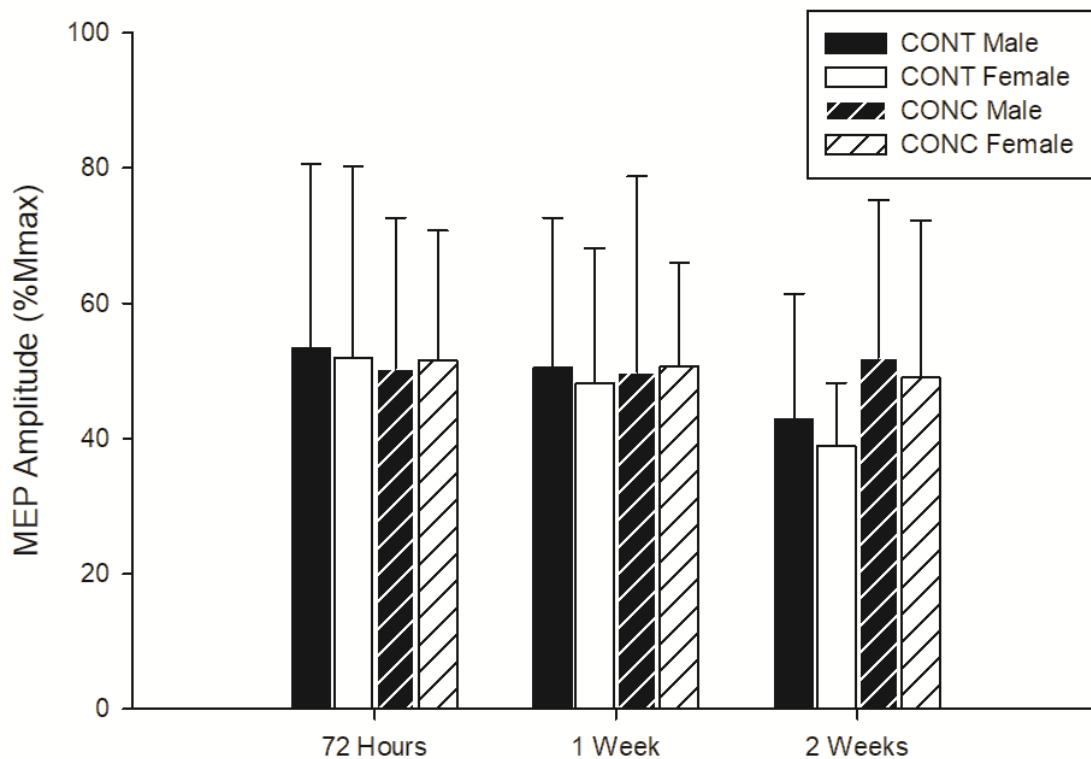

Figure 2. MEP amplitude across the 2-week testing period. The MEP amplitude was similar between groups ($p = 0.55$) and sexes ($p = 0.77$) across all time points.

Cortical silent period (CSP) duration

The CSP durations at each time point are shown in Figure 3. There was no difference in CSP duration between CONT and CONC groups ($p = 0.54$). There was a significant sex difference ($p = 0.04$), as males had longer CSP durations than females. Across time, changes in the CSP duration approached, but did not reach, statistical significance ($p = 0.06$). There were no significant interactions ($p \geq 0.36$).
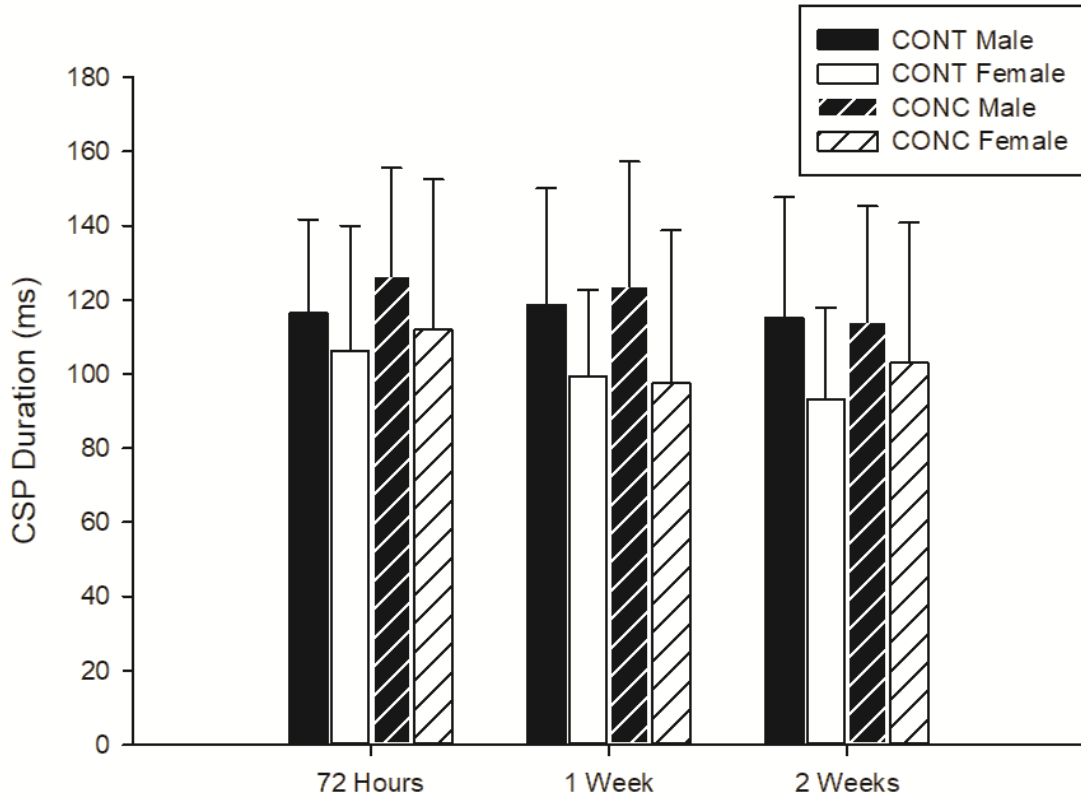

Figure 3. CSP duration across the 2-week testing period. The CSP duration was similar between groups ($p = 0.54$) across all time points. Overall, males had significantly longer CSP durations than females ($p = 0.04$).

Chapter 3

3 Discussion and Summary

3.1 Discussion

The purpose of this study was to determine sex-related differences in neurophysiology in the acute and sub-acute post-concussion stages. As hypothesized, there were no differences in MEP amplitude between groups or sexes, and there was no effect of time over the 2-week testing period. Therefore, there were no group or sex differences in corticospinal excitability. Although CSP duration was not different between groups, it was significantly longer in males compared to females regardless of group, indicating greater cortical inhibition in males. As well, the effect of time on CSP duration approached statistical significance ($p = 0.06$), as there was a general decrease in inhibition over time. To the author's knowledge, this is the first study to examine potential sex-related differences in neurophysiology post-concussion.

This study's findings of no group differences in corticospinal excitability are consistent with previous studies of concussed individuals within 72 hours to two weeks post-injury (Edwards & Christie, 2017; Miller et al., 2014; Yasen et al., 2020). A novel contribution of the current study was the demonstration that the lack of difference in corticospinal excitability following concussion was observed in both males and females. Similar MEP amplitudes in males and females has been demonstrated previously in healthy individuals (Cantone et al., 2019; Pitcher et al., 2003). However, to the author's knowledge, this is the first study to extend these findings to concussed individuals. Although the timeline of the neurometabolic cascade in humans is unknown, reports of similar corticospinal excitability levels between the concussion and control group at 72 hours post-injury may be due to the recovery of the excitatory neurotransmitter burst seen only within the first few minutes post-injury in animal models (Giza & Hovda, 2001). Therefore, it is possible that 72 hours is too long of a post-injury period to capture differences in corticospinal excitability in humans.

There is a general consensus across the literature pertaining to cortical inhibition, demonstrating that concussed individuals have longer CSP durations, which remain longer up to two months post-injury (Edwards & Christie, 2017; Miller et al., 2014; Yasen et al., 2017),

as well as at nine months and 30 years post-injury (De Beaumont et al., 2007, 2009; Tremblay et al., 2011). Therefore, the current study hypothesized that the CONC group would have longer CSP durations than the CONT group, and this longer duration would not change over time. However, this study's findings contradict previous studies and my hypothesis, demonstrating that CSP durations were similar between the CONC and CONT group, and CSP durations generally decreased over the 2-week testing period. However, the change over time was not specific to the CONC group and did not reach statistical significance. Similar to the current findings, a more recent study demonstrated no significant differences in CSP durations between acutely-injured individuals and uninjured controls (Yasen et al., 2020). In the study conducted by Yasen et al. (2020) and the current study, the mean CSP duration was typically longer in the CONC group than the CONT group but did not reach statistical significance. Together these studies suggest that while higher levels of inhibition in concussed individuals is a common finding, it is not an inevitable consequence of concussion and should therefore be studied further.

To my knowledge, the result of significantly longer CSP durations in males compared to females is a novel finding. However, as there was no group-by-sex interaction, these results suggest that sex-based differences in cortical inhibition do not explain previous reports of greater PCS scores and longer recovery times in concussed females than males (Bazarian et al., 2010). The sex-related differences in recovery time may therefore be related more to other physiological and psychosocial factors. For example, it has been shown that females have greater interhemispheric communication (Solomito et al., 2019) and greater self-awareness of cognitive deficits, yielding overall reports of greater symptoms and taking longer to recover by creating greater caution to return to regular activities (Niemeier et al., 2014). Further, pre-menstrual symptoms tend to overlap with concussion symptoms (e.g. feeling upset, anxious, or irritable, headaches, tiredness or trouble sleeping) and studies have demonstrated negative effects on emotional processing in the early follicular (~ days 24-28) and late luteal (~ days 1-8) stage in the menstrual cycle, when hormone levels are declining or low (Amin et al., 2006; Protopopescu et al., 2005). Progesterone levels did not have a significant correlation with brain activation, suggesting that estrogen may play a larger role in cognitive processing than progesterone (Amin et al., 2006; Hampson, 1990). Therefore, varying hormone levels across the menstrual cycle may affect cognitive and motor

performance in healthy females (Hampson, 1990) and may therefore play a role in the severity and timeline of recovery of post-concussive symptoms.

3.2   Conclusion

In this study, it was demonstrated that there were no significant differences in corticospinal excitability or cortical inhibition in concussed individuals compared with controls. An important and novel finding was the lack of differences in these neurophysiological outcomes in males and females following concussion. Although CSP duration was longer in males than females, this was not specific to the CONC group. Further research is required to better understand sex-related differences in the consequences of and recovery from concussion.

3.3   Limitations

Due to COVID-19, I was unable to perform my own data collection. Therefore, the data presented are from two studies that were conducted at an earlier date in a different facility. Although I performed all data and statistical analysis presented, the studies were not designed specifically to examine sex-based differences and I did not collect the data. Human EMG and TMS limitations across multiple testing sessions are inherent in that electrode and/or stimulator placement may not be exactly the same across testing sessions. As well, cortical measures vary within individuals from day to day (Malcolm et al., 2006). Therefore, the natural physiology of the human body may contribute to variability across groups and sexes.

Post-concussion symptom severity scores were obtained from some participants in the dataset, but not all. Therefore, symptom severity scores were not analyzed for the current study. As the number of symptoms reported and recovery of symptoms has been reported to differ between sexes (Bazarian et al., 2010; Casey et al., 2016; Frommer et al., 2011; King, 2014), obtaining symptom scores across the testing time would allow a better understanding of the relationship between changes in neurophysiology and changes in symptoms during concussion recovery in males and females. As well, for the female participants, I did not track which menstrual cycle stage they were in when they received their concussion and throughout their recovery time. Female hormone levels fluctuate throughout stages, which may affect cognitive and motor processes (Amin et al., 2006; Hampson, 1990; Protopopescu

et al., 2005). Therefore, it may be beneficial to collect these measures to gain more insight into potential sex-related differences post-concussion.

Failure to attend every testing session removed three participants and their control counterparts from statistical analysis. This resulted in an unequal number of males and females in each group (M = 12; F = 9). In human studies on corticospinal excitability and cortical inhibition post-concussion, there has been a lack of female representation. Similar to this study, the majority of studies have less than 50% female participants (Edwards & Christie, 2017; Miller et al., 2014; Yasen et al., 2017, 2020). However, these studies did not analyze sex differences. Therefore, more research is required to fully understand the effects of sex on neurophysiology in concussed individuals compared to healthy controls.

3.4 Future Directions

As stated above, this is the first study to examine sex differences in neurophysiology in concussed individuals. The lack of significant group-by-sex interactions suggests that differences in corticospinal excitability and cortical inhibition likely do not contribute to the previously documented sex-related differences in symptoms and recovery (Bazarian et al., 2010; Casey et al., 2016; King, 2014). Therefore, further work is necessary to understand the physiology underlying greater symptom scores and longer recovery times in females. An unexpected result in this study was the finding of males having overall greater cortical inhibition compared to females, regardless of injury status. This finding highlights the importance of including analyses of sex differences in neurophysiology research. As well, if recruiting females, it may be beneficial to collect data on menstrual cycle stages upon injury and throughout testing periods. It has been documented that different stages in the menstrual cycle effects brain areas typically involved in cognitive and motor processes (Amin et al., 2006; Hampson, 1990; Protopopescu et al., 2005).

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Curriculum Vitae

Name:	Alexandra N. Pauhl

Post-secondary Education and Degrees:

Brock University
St. Catharines, Ontario, Canada
Bachelor of Kinesiology – Honours (2015-2019)
Supervisor: Dr. Craig Tokuno

University of Western Ontario
London, Ontario, Canada
Master of Integrative Biosciences in Kinesiology (2019-present)
Supervisor: Dr. Anita D. Christie

Related Work Experience:

Graduate Teaching Assistant (2019-2021)
University of Western Ontario
- Practical Aspects of Athletic Injuries 3336A/B

- Cognitive Ergonomics 3457A

Summer Graduate Research Assistant (2020/2021)
University of Western Ontario
Neurophysiology Laboratory

Event Coordinator and Presenter (2019-2020)
Western University
Concussion Legacy Foundation

Undergraduate Research Assistant (2018-2019)
Brock University
Balance and Motor Control Laboratory

Student Athletic Therapist (2017-2019)
Ridley College
Women's U19 Prep Hockey

Awards and Honours:

Graduated with First-Class Standing
Brock University (2019)

Dean's Honour List
Brock University (2016)

Top 15% in the program (Kinesiology)
Brock University (2016)

In-course scholarship

Brock University (2016)

Publications:
Carter, K.M.*, Pauhl, A.N.*, Christie, A.D. (2021). The Role of Active Rehabilitation in Concussion Management: A Systematic Review and Meta-Analysis. *Medicine and science in sport and exercise*. Advance online publication. https://doi.org/10.1249/MSS.0000000000002663

*First co-authors.

Presentations:
Pauhl A.N., Carter K.M., Christie A.D. "The Role of Active Rehabilitation in Concussion Management: A Systematic Review and Meta-Analysis". 3MT Poster Presentation at the Western Kinesiology Graduate Student Annual Research Conference. Virtual, 2021.

Pauhl A.N. "The effects of habitual physical activity on concussion recovery". Oral Presentation at the Fowler-Kennedy Sport Medicine Clinic Research Rounds. Virtual, 2021.