CORTICAL EXCITABILITY AND INHIBITION IN POST-CONCUSSION SYNDROME

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Abstract

Post-concussion syndrome (PCS) is a poorly understood sequela of mild traumatic brain injury (mTBI), more commonly referred to as concussion. While PCS is known to affect a subset of individuals following injury, it remains unclear how and why specific individuals incur chronic symptoms. Concussions disrupt normal neurophysiologic function within the brain, however the neurophysiologic underpinnings of PCS are unclear. Using transcranial magnetic stimulation (TMS), it is possible to non-invasively investigate neurotransmission in clinical populations such as those with PCS by stimulating the primary motor cortex (M1) and recording motor outputs in a contralateral hand muscle. A study was conducted using TMS to measure corticospinal excitability, intracortical facilitation and inhibition, and transcallosal inhibition in M1 of a group with PCS and a non-injured, healthy control group. Greater corticospinal excitability, and specific reductions in intracortical and transcallosal inhibition were observed in the PCS group, providing evidence of impaired neurotransmitter receptor activity. Importantly, these findings differed from previous observations in recovered concussion groups using similar stimulation techniques. Furthermore, it was observed that these neurophysiological differences may relate specifically to the presence of depression symptoms rather than general concussion symptoms. The physiologic and clinical implications of the findings of this thesis are discussed, and novel research avenues warranting investigation are identified.

Acknowledgements

I would like to thank my supervisor, Dr. Aimee Nelson, for her guidance throughout my time at McMaster University. Her leadership and encouragement allowed the quality of my work to improve throughout my degree, and instilled in me to always remain accountable to myself in achieving my goals.

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Tables and Figures

List of Abbreviations

PCS – Post-concussion syndrome

TBI – Traumatic brain injury

TMS – Transcranial magnetic stimulation

M1 – Primary motor cortex

mTBI – Mild traumatic brain injury

NMDA – N-methyl-D-aspartate

AMPA - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

GABA – Gamma aminobutyric acid

TCA – Tricarboxylic acid

MRS – Magnetic resonance spectroscopy

TES – Transcranial electric stimulation

MEP – Motor evoked potential

RMT – Resting motor threshold

AMT – Active motor threshold

CSP – Cortical silent period

CS – Conditioning stimulus

TS – Test stimulus

SICI – Short-interval intracortical inhibition

ICF – Intracortical facilitation

iSP – Ipsilateral silent period

SIHI – Short-interval interhemispheric inhibition

LIHI – Long-interval interhemispheric inhibition

EMG – Electromyography

FDI – First dorsal interosseous muscle

PCSS – Post-concussion symptom scale

BDI-II – Beck's depression inventory

EEG - Electroencephalography

SSRI – Selective serotonin reuptake inhibitor

Declaration of Academic Achievement

The entirety of this thesis has been written by Mitchell Locke and all experiments and TMS analyses were conducted by Mitchell Locke. All silent period analyses were conducted by Mitchell Locke and Stephen Toepp. Study conceptualization was aided by supervisor Dr. Aimee Nelson and lab members Stephen Toepp, Claudia Turco, and Mitchell Savoie. Data collection was performed with the aid of Dr. Aimee Nelson and lab members Stephen Toepp, Claudia Turco, Diana Harasym, Patrick Dans, and Mitchell Savoie.

CHAPTER 1: THESIS INTRODUCTION

Post-concussion syndrome (PCS) describes the persistence of symptoms following a mild traumatic brain injury (mTBI), more commonly referred to as concussion. It remains unclear why specific individuals incur lasting symptoms. While novel blood-based markers have been identified to detect more severe TBI, there are currently no molecular biomarkers for concussion or PCS occurrence. Accordingly, clinicians must rely on symptom presentation and event history to inform diagnosis. Given the neurological basis of these diagnoses, exploration of neurophysiological function in PCS is warranted. The general aim of this thesis is to improve our understanding of PCS from a neurophysiological perspective. The primary investigation enclosed herein utilized transcranial magnetic stimulation (TMS) to non-invasively measure excitatory and inhibitory neurotransmission in the primary motor cortex (M1) of individuals with PCS, as well as transcallosal inhibition between motor cortices.

This research will impart a deeper scientific understanding of PCS and guide future research towards more targeted avenues in measuring and tracking neurophysiological function and recovery following mTBI. As will be discussed, there is a sparcity of literature pertaining to neurophysiological function in those who incur chronic symptoms following injury. Thus, this research will supplement existing evidence acquired acutely and in recovered cohorts of mTBI patients. Second, by characterizing inhibitory and excitatory neurophysiologic function in PCS, this research may provide insight into novel targets for clinicians to identify PCS, and possibly improve long-term outcome for future patients.

1.1 Significance of Thesis

This thesis will synthesize the existing literature surrounding the neurophysiologic implications of concussion injuries, with a focus on human evidence. Importantly, this thesis will explore cortical and corticospinal function in PCS as measured by TMS with two important scientific contributions. First, this study will add to the current understanding of the pathophysiology of PCS, which remains poorly understood. Second, this research may expose an objective, clinically useful measure of neural function with the potential to aid in the identification of this complex pathology. In these pursuits, this Master's thesis will illuminate clear gaps in our understanding of PCS, and reveal novel research avenues at both the basic and clinical levels.

CHAPTER 2: REVIEW OF THE LITERATURE

2.1 Epidemiology of Concussion

Mild traumatic brain injury (mTBI), more commonly referred to as a concussion, is becoming increasingly prevalent. This has been largely attributed to greater awareness of the seriousness of the injury, and thus athletes are more likely to report symptoms (Meehan et al., 2013). Previous estimates suggest an incidence of 250-600 injuries per 100 000 individuals, recognizing this is likely an underestimate due to presence of undiagnosed cases in the general public (Cassidy et al., 2004; Laker, 2011; Voss et al., 2015). Given this rate, one would expect an annual incidence up to 36 million TBIs worldwide based on the current population of 7.2 billion people. However, a recent study has put this estimate much higher, suggesting that as many as 42 million mTBIs alone occur each year after accounting for potential undiagnosed cases (Gardner & Yaffe, 2015). While there is significant variability in current estimates regarding the frequency of such injuries, it is clear that concussions are highly prevalent and pervasive on a global scale.

Understanding what a concussion is can be difficult due to ambiguity in the language used to define the injury. Originally, the term "concussion" was intended to refer to a mTBI acquired in a sport-specific context (McCrory et al., 2017). In the literature, however, "concussion" and "mTBI" are used interchangeably (McCrory et al., 2017). This lexical issue is problematic as TBI severity is not always clear (Voss et al., 2015). To add even greater confusion, "concussion" has been used as a broad term to describe any brain injury, abnormal brain function and various other phenomena (McCrory & Berkovic, 2001). For the purposes of this thesis, the term *concussion* will be

used to describe any closed-head injury resulting from a direct or indirect biomechanical force to the head or neck, resulting in neurological impairment. This injury must not display any macrostructural damage, such that standard structural neuroimaging does not show any damage. This definition adheres to that of the Berlin expert panel (McCrory et al., 2017).

2.2 Etiology and Pathophysiology: What is a concussion?

2.2.1 Mechanism of Injury

The hallmark of a concussion is abnormal brain function resulting in a conglomeration of symptoms, despite the absence of any observable structural damage by traditional imaging techniques (McCrory et al., 2017). However, this does not mean that neuronal damage is not occurring at a microstructural level. Using experimental animal models, mechanistic insights have been gathered to describe the neuronal, molecular, and synaptic disturbances underlying concussion which are described herein.

Trauma to the brain typically occurs due to direct or indirect biomechanical forces applied to the head or neck, leading to diffuse axonal injury caused by linear and rotational acceleration forces within brain structures (Meaney & Smith, 2012). Rotational acceleration is primarily responsible for shear-induced tissue damage, and is thus the predominant mechanism of neuronal injury in concussion (Adams et al., 1982). Since neuronal density is not constant across neural structures, rotational acceleration within the brain occurs at different rates, leading to axonal stretching and disruption of cell membranes (Barkhoudarian et al., 2016; Meaney & Smith, 2012).

An accumulation of evidence, both in animal models and humans, provides an overview of the neurometabolic events that follow a concussion. These events take place across a region of neurons resulting in diffuse axonal injury as previously mentioned. As will be discussed, a combination of ionic and metabolic disturbances contribute to altered synaptic activity at the level of the neuron. The neurometabolic cascade following impact applies at all levels of severity, though the degree of damage and subsequent neuronal changes are not necessarily equivalent across injury severity.

2.2.2 Hyper-acute Period (<6 hours post-injury)

When microstructural neuronal damage occurs, namely axonal stretching and deformation of cell membranes, previously regulated ion channels become dysfunctional (Farkas, 2006). Specifically, unregulated efflux of K^+ leads to depolarization of the damaged membrane causing increased pre-synaptic Ca^{2+} influx. Subsequently, this results in greater Ca^{2+}-mediated release of synaptic glutamate, the primary excitatory neurotransmitter in the central nervous system (Faden et al., 1989). Excess glutamate is then free to bind post-synaptic N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) channels leading to depolarization of the post-synaptic membrane. This leads to spreading depolarization resembling the cortical depression seen in the penumbra in stroke patients (Guerriero et al., 2015; Katayama et al., 2009; Barkhoudarian et al., 2016). Not only is there increased glutamate release, but there is also reduced removal of glutamate from the synaptic cleft due to reduced expression of astrocyte GLT-1 transporter (Goodrich et al., 2013). This enables greater post-synaptic excitation via prolonged exposure to glutamate. The glutamate that is able

to be taken up by neighbouring astrocytes is then recycled back to glutamine to be re-distributed to either the pre-synaptic neuron for conversion into glutamate, or to local inhibitory interneurons to be converted into gamma-aminobutyric acid (GABA), the primary inhibitory neurotransmitter of the nervous system. Inhibitory interneurons play an important role in modulating excitatory cortical activity by releasing GABA which binds to $GABA_A$ or $GABA_B$ receptors which hyperpolarize the membrane via Cl^- or K^+ influx, respectively (Guerriero et al., 2015). However, immediately following injury, reduced $GABA_A$ subunit expression impairs phasic inhibition, an important modulator of cortical neuron membrane excitability (Raible et al., 2012),

Neurometabollically, there is greater demand during this hyper-acute period as Na^+/K^+ ATPase pumps must operate maximally to restore resting membrane potential following unregulated K^+ efflux (Giza & Hovda, 2014). The majority of ATP necessary to support the demands of the Na^+/K^+ pump are produced via the tricarboxylic acid (TCA) cycle. This is important to note, because intermediaries of this process are critical for the chemical processes associated with glutamine-glutamate-GABA recycling (Walls et al., 2014). Thus, the ability of astrocytes to replenish pre-synaptic glutamate levels becomes impaired which contributes to subsequent problems following injury.

2.2.3 Acute Period (6 hours – 10 days)

As a result of continued excessive glutamate signalling, post-synaptic NMDA channels remain open allowing influx of Na^+ and Ca^{2+} into the post-synaptic neuron. Over this time, Ca^{2+} accumulation results in mitochondrial dysfunction resulting in reduced glycolysis (Lifshitz et al., 2004; Xiong et al., 1997). Consequently, a state of

hypoglycolysis limits the capability of the Na^+/K^+ pump to maintain ionic gradients due to reduced ATP availability (Barkhoudarian et al., 2016). Furthermore, there is reduced production of the intermediaries from the TCA cycle necessary for recycling of glutamate and GABA (Walls et al., 2014). Indeed, magnetic resonance spectroscopy (MRS) investigations of M1 in humans one to six days following a concussion revealed increased glutamine content and decreased glutamate (Henry, et al., 2010). Although the total amount of glutamate was shown to be lower, microdialysis studies which only indicate extracellular metabolite concentrations, showed increased extracellular glutamate up to four days following injury (Chamoun et al., 2010), supporting continued elevation of synaptic glutamate post-injury.

Animal models have shown that during this window there is a continued shift in the subunits of $GABA_A$ receptors. The timeline is less certain regarding these alterations but receptor subunits related to phasic inhibition have been shown to be down-regulated from 24 hours up to one week following TBI (Raible et al., 2012).

2.2.4 Subacute Period (< 1 month)

Following the first 10 days post-injury, metabolic processes are thought to resume normal function (Giza & Hovda, 2014). However, continued dysfunction of $GABA_A$ receptors has been observed in rodent TBI models, which further lends to the notion of greater excitability in comparison with reduced inhibition in the post-TBI brain (Kharlamov et al., 2011).

2.2.5 Chronic Period (> 1 month)

By this time, an accumulation of compensatory changes have occurred which may or may not completely account for the neuronal injuries. In humans, non-invasive brain stimulation studies have shown that months after recovery from a concussion, measures thought to reflect $GABA_B$ receptor activity are upregulated (De Beaumont et al., 2007; De Beaumont et al., 2011; De Beaumont et al., 2012; Tremblay et al., 2011). This has been theorized to be a compensatory mechanism to protect the brain from glutamate excitotoxicity (De Beaumont et al., 2012). If there is persistent dysfunction of $GABA_A$ receptors, this would further support the necessity for $GABA_B$ to act in a compensatory manner.

2.3 Symptoms

Concussions are heterogeneous in nature, thus symptoms can differ greatly between individuals (Lovell et al., 2010). Symptoms can be psychological, neurological, or physical in nature and not all individuals will experience all three. The most typically presented symptoms following injury include headaches, fatigue, difficulty focusing, mental fogginess, and dizziness (Lovell et al., 2006). Neuropsychological deficits typically resolve within the first week following injury (McCrea et al., 2004), which aligns with the expected cessation of neurometabolic dysfunction (Yoshino et al., 1991).

2.4 Post-Concussion Syndrome (PCS)

Although typical recovery following a concussion occurs within the first seven to 10 days following injury (McCrea et al., 2004), some individuals experience pervasive

symptoms regardless of injury severity. This is described as post-concussion syndrome (PCS) and develops in a subset of individuals following injury. Similar to concussion , estimates of PCS prevalence vary greatly ranging from 10-50% of injuries (Hou et al., 2012; Sigurdardottir et al., 2009; Sterr et al., 2006; Theadom et al., 2016). Those who experience PCS most frequently report symptoms such as headaches, fatigue, sleep disturbance, and poor memory (Hou et al., 2012).

2.5 PCS Identification

Clinicians are faced with a significant challenge when attempting to identify PCS. At this time, they must rely primarily on symptom presentation as there are no definitive markers for PCS, and the criteria used to define PCS varies greatly between clinicians (Rose, Fischer, & Heyer, 2015). Furthermore, due to poor understanding of the pathophysiological processes underlying PCS there are no specific therapeutic markers for the identification or treatment of this syndrome. Indeed, there are no approved treatments for the direct consequences of concussion injuries, and physicians are left with indirectly treating PCS at the symptom level (Hadanny & Efrati, 2016). Thus, it is clear that a need exists for clinically useful, measurable markers of this syndrome.

Many factors have been associated with increased risk of developing PCS as it is understood to occur due to an interaction of biological, psychological and social factors (Ryan & Warden, 2003; Wood, 2004). For instance, a recently proposed model highlights the importance of early psychological factors such as coping behaviour in predicting injury outcome (Hou et al., 2012). Another model has suggested neuroinflammation to be

at the core of PCS development (Rathbone et al., 2015). However, human evidence is still forthcoming (Rathbone et al., 2015). Thus far, efforts to elucidate a fluid-based biomarker have achieved minimal success in identifying a biological marker indicative of a vulnerable brain (Kulbe et al., 2016).

Further, injury-related factors have demonstrated little predictive value. Loss of consciousness at the time of injury does not relate to PCS occurrence (Sterr et al., 2006), nor does injury severity (Sigurdardottir et al., 2009). It remains unclear why some patients do not recover across a typical timeline and further exploration via other avenues is warranted. Given the neurophysiological nature of this injury, another option is to investigate neurophysiologic function in those with PCS.

2.6 Transcranial Magnetic Stimulation

TMS is a method of non-invasively stimulating the brain to indirectly measures cortical function and corticospinal excitability (Hallett, 2007). TMS creates a magnetic field by briefly passing an electric current through a magnetic coil held over an individual's head. The electromagnetic field which is produced creates a current within the brain according to Faraday's law of electromagnetic induction, and can excite cortical neurons directly below the coil (Wagner et al., 2009).

The effects of TMS can be better understood by comparing it to transcranial electric stimulation (TES). Stimulation via TES results in two types of descending volleys, referred to as direct waves (D-waves) and indirect waves (I-waves) which precede D-waves by intervals of 1.5 ms (Hallett, 2007). D-waves pertain to the direct

activation of descending corticospinal neurons, while I-waves reflect the synaptic activation of corticospinal neurons (Hallett, 2007). TMS preferentially induces a corticospinal volley via I-waves, suggesting that TMS activates superficial pyramidal neurons that have excitatory connections to the corticospinal neurons (Di Lazzaro et al., 2012). The coil orientation over the head can influence the effects of TMS. TMS results in the greatest activation when the current is in the posterior-anterior direction, created by a coil orientation approximately parallel to the central sulcus (Hallett, 2007).

Most typically, TMS is used over M1 to induce a descending corticospinal volley pertaining to a target muscle (Figure 1). The corresponding muscle response is referred to as the motor evoked potential (MEP) (Hallett, 2000). Single-pulse TMS can be used to measure the motor threshold for producing an MEP, which reflects the membrane excitability of the target neurons (Rossini et al., 2015). Motor threshold refers to the minimum stimulator output required to elicit a target muscle response 50% of the time

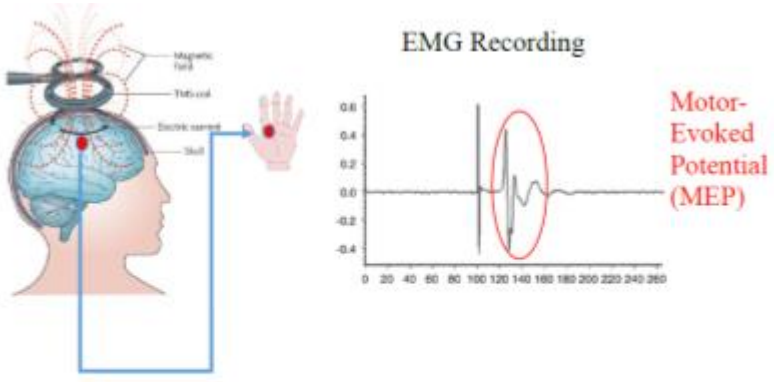

Figure 1 - TMS stimulation of M1 results in a descending corticospinal volley, leading to a muscle twitch in a target muscle called an MEP

(Rossini et al., 2015). Along with cortical excitability, various TMS techniques are available for assessing both glutamatergic (excitatory) and GABAergic (inhibitory)

function within M1, or from M1-M1 via transcallosal communication (see Table 1 for summary).

2.6.1 Cortical Silent Period

The cortical silent period (CSP) is an interruption of voluntary muscle activation caused by a single TMS pulse to the contralateral M1. The result is a silent period following the MEP, whereby voluntary muscle activation does not resume due to cortical inhibition (Figure 2). The first portion of the CSP is due to spinal cord refractoriness,

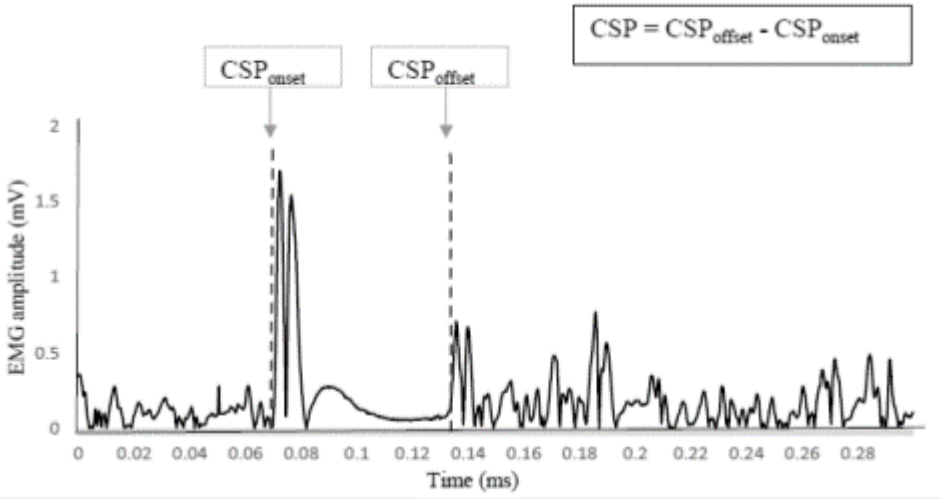

Figure 2 – Example silent period in voluntary muscle activation following a TMS-evoked MEP. A CSP is evoked by a TMS pulse to contra-M1, iSP is evoked by TMS to ipsi-M1 The blue bar represents the TMS pulse. (contra-M1 = contralateral M1, ipsi-M1 = ipsilateral M1)

while the later portion is due to cortical inhibition (Hallett, 2007). CSP is thought to be mediated by $GABA_B$ receptor activity (Stetkarova & Kofler, 2013; Werhahn et al., 1999), with a typical silent period lasting 100-300 ms, such that a longer silent period is indicative of greater $GABA_B$ mediated inhibition (Rossini et al., 2015; Ziemann et al., 2015).

2.6.2 Short Interval Intracortical Inhibition and Intracortical Facilitation

Short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) are acquired using a paired-pulse paradigm whereby two pulses are delivered in sequence through the same TMS coil over the same cortical location (Ziemann et al., 1996). Paired-pulse studies are thought to reflect the influence of inhibitory interneurons, which as discussed earlier, modulate the excitatory signal of pyramidal neurons. The first pulse, known as the conditioning stimulus (CS), is delivered at a subthreshold level (i.e. a stimulus intensity that is not sufficient for eliciting an MEP). The test stimulus (TS) follows the CS, and is delivered at a suprathreshold intensity (i.e. sufficient for inducing an MEP in the target muscle). Using this technique, interneuron influences from the CS reduce the MEP amplitude from the TS when the interval between the two pulses is small (1-6 ms) (Kujirai et al., 1993). When the interval is slightly longer (8-30 ms), the evoked MEP becomes larger (Kujirai et al., 1993). These phenomena are SICI and ICF, respectively. SICI is thought to reflect $GABA_A$ receptor activity (Di Lazzaro et al., 2000,

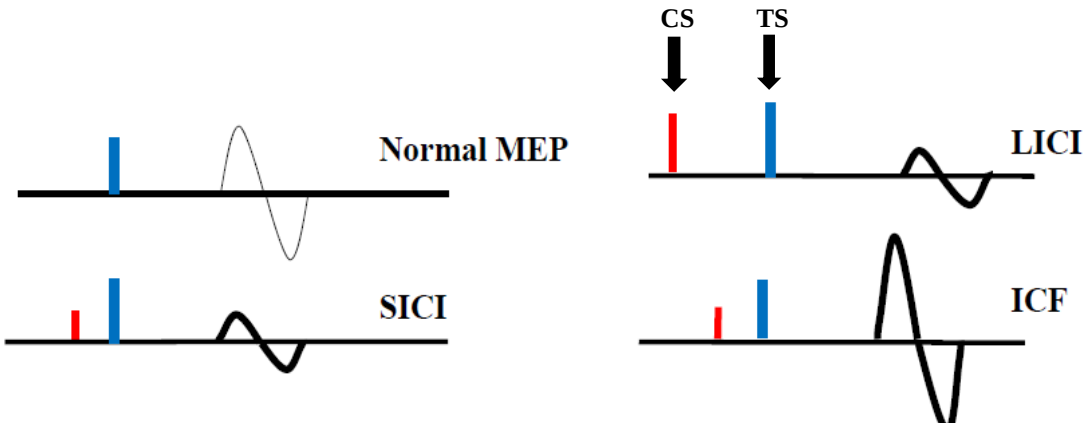

Figure 3 – Representation of how the MEP is changed by each intracortical paired pulse paradigm. LICI and SICI both demonstrate inhibition through reduction of MEP amplitude, while ICF demonstrates facilitation through a larger MEP. Red bars represent the conditioning stimulus, while blue bars represent the test stimulus

2007), while ICF has been suggested to result from recruitment of additional glutamatergic circuits, and is therefore NMDA mediated (Ziemann et al., 1997)

2.6.3 Interhemispheric Inhibition

Interhemispheric inhibition (IHI) is achieved using a paired-pulse paradigm, however this technique requires suprathreshold stimulation delivered to M1 within both hemispheres (Ferbert et al., 1992). To evoke IHI, a CS delivered to the ipsilateral M1 precedes a TS to the contralateral M1, thereby reducing the MEP amplitude elicited by the TS. IHI is observed when the interval between stimuli is 10 ms or 40-50 ms, referred to as short-latency (SIHI) and long-latency (LIHI), respectively (Ni et al., 2009). This pathway is most likely mediated by excitatory transcallosal pathways synapsing onto local inhibitory circuits within the contralateral M1. While the exact mechanism of SIHI is not currently known, the observed inhibition for LIHI is likely mediated by $GABA_B$ receptors based on pharmacological evidence (Irlbacher et al., 2007).

2.6.4 Ipsilateral Silent Period

Ipsilateral silent period (iSP), similar to CSP, is an interruption of volitional muscle activation by a single suprathreshold TMS pulse (Meyer et al., 1995). In this technique, however, the TMS pulse is delivered to ipsilateral M1 relative to the target muscle. Though the mechanism is not fully understood, it is thought to operate via excitatory transcallosal pathways interacting with local inhibitory neurons, similar to the mechanism of IHI (Chen et al., 2003). Importantly, the mechanism of iSP has not been pharmacologically tested, and although its inhibitory influence is similarly to IHI, it likely

operates through different neuronal populations (Chen et al., 2003; Perez & Cohen, 2009).

TABLE 1 – PARAMETERS FOR TMS MEASURES

Measure	*TMS paradigm*	*EMG*	*Receptor activity*
CSP	Single, suprathreshold pulse, contra-M1. Target muscle tonically active	Inhibition, interrupted voluntary EMG activity	$GABA_B$
SICI	Paired-pulse to contra-M1. Subthreshold CS precedes suprathreshold TS, ISI 1-6 ms. Target muscle at rest.	Inhibition, decreased MEP amplitude	$GABA_A$
ICF	Paired-pulse to contra-M1. Subthreshold CS precedes suprathreshold TS, ISI 8-30 ms. Target muscle at rest.	Facilitation, increased MEP amplitude	Glutamate (NMDA)
IHI	Paired-pulse. Suprathreshold CS to ipsi-M1 precedes suprathreshold TS to contra-M1 by 10 or 40-50 ms. Target muscle at rest.	Inhibition, decreased MEP amplitude	Excitatory transcallosal neurons, $GABA_B$ (LIHI only)
iSP	Single, suprathreshold pulse to ipsi-M1. Target muscle tonically active.	Inhibition, interrupted voluntary EMG activity	Excitatory transcallosal neurons, $GABA_B$*

Summary of TMS measures and their suspected biological mechanism of action (contra-M1 = contralateral motor cortex, ipsi-M1 = ipsilateral motor cortex).
*not pharmacologically tested

2.7 Effect of concussion on TMS measures of corticospinal function

The pathophysiology of PCS has primarily been observed in animal models. However, using non-invasive stimulation (i.e. TMS), it possible to test neurophysiological function following concussion in humans. Over the past decade, a substantial amount of

evidence has been compiled regarding acute changes in cortical activity following injury as well as some abnormal activity that may be persistent even after recovery. However, at this time there remains a paucity of evidence regarding how TMS evoked responses are changed in those with chronic symptoms.

2.7.1 Cortical Silent Period

Following concussion, one of the most consistently researched TMS measures is CSP duration. Specifically, it has been shown that CSP duration is increased acutely (Chistyakov et al., 2001; Edwards & Christie, 2017; Miller et al., 2014; Pearce et al., 2015), sub-acutely (Chistyakov et al., 2001; Edwards & Christie, 2017; Miller et al., 2014), and months to years following injury (De Beaumont et al., 2007; 2011; 2012; Edwards & Christie, 2017; Tremblay et al., 2011). Prolonged CSP has even been demonstrated in retired athletes 30 years after their last concussion who are completely asymptomatic (De Beaumont et al., 2009). Not all studies have been able to replicate these findings. Others found no difference in CSP length between controls and injured groups 4 weeks following injury (Powers, Cinelli, & Kalmar, 2014; Yasen et al., 2017), and approximately 1 year after injury (Davidson & Tremblay, 2016; Tremblay et al., 2014). Two studies observed reduced CSP (Pearce et al., 2014; Pearce et al., 2018). Importantly, all of the above findings in the chronic phase were conducted in asymptomatic (i.e. recovered) cohorts. One study investigated CSP in a chronically symptomatic group of adults (mean months post-injury = 15.4 months) and observed increased CSP length as well (Pearce et al., 2019). Although not unanimous, the evidence

suggests that CSP length is likely greater following a concussion, indicating greater $GABA_B$ receptor activity in M1 regardless of recovery status.

2.7.2 Short Interval Intracortical Inhibition

SICI, thought to reflect $GABA_A$ receptor activity, appears to be normal both acutely (Pearce et al., 2015; Powers et al., 2014) and in asymptomatic individuals long-term (De Beaumont et al., 2007; Tremblay et al., 2011). One study did report reduced SICI in retired athletes 21 years after their last concussive injury (Pearce et al., 2014). Another study found SICI to be increased in a PCS group compared to recovered concussion and control groups (Pearce, Tommerdahl, & King, 2019).

2.7.3 Intracortical Facilitation

In the sub-acute phase, ICF has been shown to be reduced (Powers et al., 2014) or increased (Bashir et al., 2012). In the following months, ICF is normal relative to non-injured controls (De Beaumont et al., 2007, 2009). These findings suggest ICF is a relatively unaffected TMS measure following injury, albeit there is less evidence surrounding this measures.

2.7.4 Interhemispheric Inhibition

IHI has not yet been investigated following concussion.

2.7.5 Ipsilateral Silent Period

ISP has only been investigated in recovered cohorts following injury. One study found reduced iSP months after injury in a young athletic population (Davidson & Tremblay, 2016), while another study observed normal iSP years after the injury event in elite and recreational athletes (Lewis et al., 2017). A reduction in iSP may suggest both

impaired excitatory glutamatergic drive and impaired local inhibitory function (Davidson & Tremblay, 2016), however more research is required to make any statements regarding the specific transcallosal impairments following concussion.

TABLE 2 – TMS FINDINGS IN THE CHRONIC PHASE FOLLOWING CONCUSSION/TBI

Measure	*Asymptomatic (> 3 months)*	*PCS (> 3 months)*
RMT	Ø: (Davidson & Tremblay, 2016; De Beaumont et al., 2007; Pearce et al., 2014, 2018) ↑: (J. Tallus, Lioumis, Hämäläinen, Kähkönen, & Tenovuo, 2012)	↑: (J. Tallus et al., 2012)
SICI	Ø: (De Beaumont et al., 2007, 2009; De Beaumont, Tremblay, et al., 2012; Pearce et al., 2018; Tremblay et al., 2014)	↑: (Pearce et al., 2019)
ICF	Ø:(De Beaumont et al., 2007, 2009)	~
CSP	↑: (De Beaumont, Henry, et al., 2012; De Beaumont et al., 2007, 2009; L. et al., 2011; Tremblay et al., 2011) Ø: (Davidson & Tremblay, 2016; Tremblay et al., 2014) ↓: (Pearce et al., 2014, 2018)	↑: (Pearce et al., 2019)
iSP	↑: (Davidson & Tremblay, 2016)	~
IHI	~	~

↑ = increase, ↓ = decrease, Ø = no change with history of injury. ~ indicates unknown.

2.7.6 Summary

TMS is becoming a common neurophysiological technique in research for measuring cortical function following concussive injury. This modality is effective for observing functional changes through measures with tested pharmacological underpinnings. The evidence using TMS to measure CSP generally agrees that inhibitory function in M1, specifically $GABA_B$-mediated inhibition, is enhanced and remains enhanced following injury despite recovery of symptoms. A current limitation is that

TMS-alone has rarely been used to investigate long-term changes in cortical and corticospinal function in a chronically symptomatic population.

CHAPTER 3: AN EXPLORATION OF BIOLOGICAL MARKERS OF POST-CONCUSSION SYNDROME USING TRANSCRANIAL MAGNETIC STIMULATION

3.1 Abstract

Concussions can result in chronic symptoms following injury, resulting in post-concussion syndrome (PCS), though it is unclear why this occurs for specific individuals. Identifying a biomarker for PCS may assist with this diagnosis and be used to assess changes over the course of recovery. Transcranial magnetic simulation (TMS) is often used to assess corticospinal excitability and inhibition in the primary motor cortex following concussion, however research has focused on the asymptomatic population. The present study tested 15 individuals with PCS following a medically diagnosed concussion and 13 healthy individuals. Symptoms were measured using the post-concussion symptom scale (PCSS) and Beck's Depression Inventory (BDI-II). TMS measures of corticospinal excitability were assessed, as well as intracortical facilitation, intracortical inhibition (short-interval intracortical inhibition, cortical silent period (CSP)), and transcallosal inhibition (interhemispheric inhibition, ipsilateral silent period). Results revealed reduced CSP in PCS compared to healthy controls ($p = 0.02$), a pattern that opposes previous observations in symptomatic and recovered concussion groups. This may indicate reduced GABA-mediated inhibition in PCS. Furthermore, multiple linear regression analyses showed that BDI-II ($\beta = -2.67$, $SE = 1.08$, $p = 0.02$) but not PCSS scores ($\beta = 0.32$, $SE = 0.25$, $p = 0.20$), predicted CSP. This data suggests that CSP length may be sensitive to the presence of depression symptoms in PCS, and may be useful in identifying sub-populations of PCS with different symptomatologies.

3.2 Introduction

Concussions are one of the most prevalent injuries globally, affecting more than 40 million individuals each year (Gardner & Yaffe, 2015). While most recover within the first 10 days following injury, symptoms persist in 10-50% of individuals for months or years following injury (Hou et al., 2012; Sigurdardottir et al., 2009; Sterr et al., 2006; Theadom et al., 2016). When symptoms persist beyond three months, the diagnosis is termed post-concussion syndrome (PCS).

Animal models have observed a neurometabolic cascade of events that transpire post-injury (Barkhoudarian, Hovda, & Giza, 2011; Guerriero et al., 2015), although the underlying cause of PCS remains unclear. Pre-injury factors may help predict PCS development in humans. For example, PCS is more common in females, in those whom have had multiple concussions, or those with psychological diagnoses (Tator et al., 2016). Factors directly related to the injury do not appear to relate to symptom persistence. Loss of consciousness at the time of injury does not correlate with PCS occurrence (Sterr et al., 2006), and a comparison of mild and severe injury groups revealed similar PCS prevalence one year post-injury (Sigurdardottir et al., 2009). This provides evidence that injuries of any severity can result in chronic effects, further necessitating an objective measure to identify individuals who remain chronically affected post-injury.

At this time, there is no objective biological marker to identify and potentially track recovery changes in an individual with PCS. Fluid-based markers such as tau protein have shown some potential (Zetterberg & Blennow, 2015), however peripheral

blood measures do not necessarily reflect central nervous system metabolite concentrations. Neuroinflammation has also been hypothesized to contribute to PCS development, although human evidence is still forthcoming (Rathbone et al., 2015). An alternative is to examine neurological function specifically in those who develop PCS. The aforementioned neurometabolic cascade that occurs post-injury is known to impact neurotransmission (Guerriero et al., 2015), and this has implications for neural function which can be assessed using transcranial magnetic stimulation (TMS). TMS is a non-invasive brain stimulation tool, commonly used to measure corticospinal excitability and cortical activity in the primary motor cortex (M1). Given that the motor system is commonly affected by concussion injury, M1 and descending corticospinal pathways are logical anatomical candidates for identifying a possible biomarker of PCS.

TMS has been used to investigate cortical changes both acutely and in the long-term following concussion, however these studies have tended to investigate asymptomatic individuals. Nevertheless, even in the recovered population, changes in cortical function within M1 are noted. Most typically, TMS has been used to induce a cortical silent period (CSP), whereby M1 stimulation briefly interrupts voluntary tonic contraction in the opposite limb (Wilson et al., 1993). Asymptomatic groups with a history of concussion have a longer CSP than their non-injured counterparts, and this pattern has been observed up to 30 years post-injury (De Beaumont et al., 2007; 2009; 2011; 2012; Tremblay et al., 2011) It is less clear how TMS measures such as CSP differ in those with PCS (Table 1).

The goal of the present study was to identify whether TMS biomarkers differentiate between a group of individuals living with PCS versus a control group without history of concussion. TMS was used to assess corticospinal excitability, intracortical facilitation and inhibition, and transcallosal inhibition between motor cortices. Given the theorized compensatory role of $GABA_B$ upregulation in recovered concussion cohorts (De Beaumont et al., 2012), it was predicted that CSP length would not differ between groups, reflecting the absence of such compensatory mechanism. It was also predicted that transcallosal measures (IHI and iSP) would also be reduced in the PCS group, reflecting functional disruption of the corpus callosum.

3.3 Methods

3.3.1 Participants

Fifteen individuals with PCS (mean age 28.8 ± 8.7 years) and 13 healthy controls without history of concussion (mean age 26.8 ± 7.7 years) participated (Table 2). Individuals with PCS were recruited from a Hamilton clinic database, McMaster University, and the Hamilton community. Control group participants were also recruited from McMaster University and the Hamilton community. To ensure that our sample was a chronically symptomatic group, concussion participants must have remained symptomatic for a minimum of 6 months following a medically diagnosed concussion without further head injury in the interim. Symptom persistence was confirmed in all concussion patients based on a post-concussion symptom scale (PCSS) score equal to or greater than 12 (mean score = 59.9 ± 27.4) (Lovell et al., 2010). All participants

participated in the Beck's Depression Inventory (BDI-II) and only individuals who scored below 29 were included (i.e. severe depression was excluded) (Beck et al, 1988). Participants were excluded if they were on any medications with known interactions with GABA or NMDA receptors including; psychiatric medications, any other depressants or stimulants, and medications that may reduce the threshold for seizure. All participants were confirmed right-hand dominant using a modified handedness questionnaire (Oldfield, 1971), were screened for contraindications to TMS, and provided informed written consent prior to participation. This study was approved by the Hamilton Integrated Research Ethics Board and conformed to the standards of the *Declaration of Helsinki*.

3.3.2 Electromyography

EMG recordings were acquired from the first dorsal interosseous muscle (FDI) of the right and and left hand using 9 mm Ag-AgCl surface electrodes. A wet ground was secured around the right forearm of the participant. All EMG recordings were amplified 1000x (Model 2024F; Intronix Technologies Corporation, Bolton, Ontario, Canada) and filtered using high and low band-pass filters of 20 Hz and 2.5 kHz, respectively. Data was digitized using an analog-to-digital interface at 5 kHz (Power 1401; Cambridge Electronics Design, Cambridge, UK) and subsequently analysed using Signal software (Signal, version 6.02; Cambridge Electronics Design). Each trial was analysed for excessive EMG using an 8 ms window immediately prior to the first TMS artefact. Any trial with peak-to-peak muscle activity greater than 50 μV within that window was excluded from analysis. Any data set in which > 25% of trials had contaminated EMG

activity was omitted from analysis. This occurred on three occasions, resulting in the removal of one participant from each of short-interval intracortical inhibition, cortical silent period, and ipsilateral silent period analysis. For two participants, multiple measures had systematic background noise present at a frequency of ~ 60 Hz. To account for this, an additional notch filter was applied with a center at 60 Hz and a width of 8 Hz.

3.3.3 Maximum Voluntary Contraction

MVC was acquired from the right FDI only. Participants were asked to contract right FDI maximally against an immovable, fixed beam. Three trials of 5 s were performed with ~ 1 minute of rest in between. Signals were rectified and displayed on an oscilloscope (Tektronix TDS2004c, USA) to provide the participants with visual feedback.

3.3.4 Transcranial Magnetic Stimulation

Two custom figure-of-eight coils (50 mm diameter) connected to two Magstim 200^2 stimulators (Magstim, Whitland, UK) were used for TMS delivery. A Bistim module was attached to one of the two stimulators to be connected for paired-pulse stimulation paradigms. One coil was always used to deliver TMS over left M1, and the second coil was used over right M1. Coils were positioned at a 45º angle from the sagittal plane to induce a posterior-to-anterior current within the cortex. To ensure consistent TMS delivery, coil location and orientation were digitally registered on a standard magnetic resonance imaging (MRI) image using Brainsight neuronavigation (Rogue Research, Montreal, Quebec City, Canada). TMS coils were held over right and left ‘motor hotspot’,

defined as the cortical locations that optimally elicit large and consistent motor-evoked potentials (MEP) in the contralateral FDI for each hemisphere. These locations were used for all TMS measures. Resting motor threshold (RMT) was obtained at the motor hotspot for each hemisphere as a metric for corticospinal excitability. RMT was measured using ML-PEST software (TMS Motor Threshold Assessment Tool; MTAT, version 2.0) which uses a predictive algorithm to accurately determine RMT after 20 stimuli (Ah Sen et al., 2017). The initial settings were set to *a priori* and the initial stimulus intensity was set to 37% of the maximum stimulator output. Active motor threshold (AMT) was acquired for the right FDI only while the muscle was contracted to 10% of the participant's MVC. AMT is defined as the minimum stimulus intensity required to induce a MEP greater than 200 μV in 50% of trials (Rossini et al., 2015), and was measured using the same methodology as RMT (Ah Sen et al., 2017). Additionally, the TMS intensity required to evoke an average MEP with a peak-to-peak amplitude of 1 mV was acquired for both FDI muscles and is referred to as '1 mV' herein. These set-up measures were all used to inform stimulation parameters for the remaining TMS procedures.

3.3.5 Paired-pulse TMS

Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were recorded from the right FDI with the conditioning stimulus (CS) intensity set to 90% AMT and the test stimulus (TS) set to 1 mV. The interstimulus interval (ISI) was set at 2 ms and 15 ms for SICI and ICF, respectively. Fifteen TS alone trials were randomized among 30 CS-TS trials (15 for SICI and 15 for ICF), with an inter-trial interval of 5 s.

3.3.6 Silent period

To measure cortical silent period (CSP) from the right FDI, participants maintained tonic muscle activation of the right FDI at 10% MVC for the duration of data acquisition. TMS was delivered to left M1 at the intensity of 1 mV in right FDI. Fifteen trials were delivered with an inter-trial interval of 5 s.

To measure ipsilateral silent period (iSP), TMS was delivered to the right M1 at 1 mV for the left FDI muscle and participants maintained a tonic muscle contraction of 50% MVC in the right FDI. Fifteen trials were acquired, separated into three collections of 5 trials, each separated by ~ 1 minute to reduce the influence of muscle fatigue.

3.3.7 Interhemispheric inhibition

TMS delivered to the right hemisphere (CS) preceded TMS delivered to the left hemisphere (TS) by 10 ms or 40 ms to acquire short-interval interhemispheric inhibition (SIHI) and long-interval interhemispheric (LIHI), respectively in the right FDI. The intensity of the CS and TS were set to evoke a MEP of ~1 mV peak-to-peak amplitude in the left and right FDI muscle, respectively. Fifteen TS alone trials were randomized among 30 CS-TS trials (15 for SIHI, 15 for LIHI), with an inter-trial interval of 5 s.

3.4 Data Reduction and Analysis

Figure 4 displays an example analysis for CSP (A) and iSP (B). CSP duration was measured in each of 15 trials and subsequently averaged for each participant. CSP was

independently analysed by two blinded raters and the two scores were averaged. Analysis was performed using a semi-automated approach adapted from previous work (Kimberley et al., 2009; Murase et al., 2005). EMG recordings were first rectified, and the mean EMG amplitude was determined from a 25 ms window immediately preceding the TMS pulse. CSP onset was determined as the beginning of the TMS-evoked response. CSP offset was determined as the beginning of the first consecutive 2 ms period of EMG activity exceeding the mean following the silent period. Although previous research using this method determined CSP offset based on 50% of the mean EMG activity, this was altered to accommodate the lower tonic muscle activation in this study (Kimberley et al., 2009; Murase et al., 2005). The difference between CSP offset and CSP onset determined CSP length. Of the 28 participants, 26 datasets were analysed. One dataset was removed due to excessive muscle activity in the "resting" left FDI, and the other was removed because excessive electrostatic noise interfered with accurate CSP determination. For data included in CSP analysis, 24 of 390 (6.2%) trials were removed due to excessive muscle activation of the left FDI or the absence of an observable CSP. Fifteen trials were gathered for each participant to determine iSP. Trials were rectified, then averaged and the mean EMG amplitude was measured during a 90 ms pre-stimulus window immediately preceding the TMS pulse. A horizontal cursor was placed at the pre-stimulus mean amplitude and iSP onset was determined as the first consecutive 5 ms period following MEP onset in the left FDI (Davidson & Tremblay, 2016). ISP offset was determined as the initiation of the first consecutive 2 ms period of EMG activity above the mean following iSP onset. If EMG activity did not fall below the mean for a

consecutive 5 ms period following MEP onset, then it was determined that iSP could not be adequately identified in that individual's data set. This was the case for three of the 28 data sets. Another data set was omitted due to electrostatic noise interference, and a fifth data set was removed due to EMG contamination in left hand recording.

3.5. Statistics

All measures were assessed for normality using Shapiro-Wilk tests. Outliers were assessed using SPSS software (IBM) and extreme outliers were removed, defined as greater than 1.5x the interquartile range. Conover's ANOVA was performed on all paired-pulse TMS measures to determine whether CS pulses significantly influenced the TS-evoked MEPs (Conover & Iman, 1982). For SICI and ICF, a two-way Conover's ANOVA with between-subject factor GROUP (two levels: PCS, CONTROL) and within-subject factor PATTERN (two levels: TS, CS-TS) was performed. For SIHI and LIHI, a two-way Conover's ANOVA with between-subject factor GROUP (two levels: PCS, CONTROL) and within-subject factor PATTERN (three levels: TS, CS, CS-TS) was performed. Between-group differences of normally distributed data were analysed using Welch's t-test, and non-normal data was analysed using a Mann-Whitney U test. A two-way mixed model intraclass correlation was performed to assess inter-rater reliability for CSP analysis. To further explore the impact of PCS on TMS measures that were different between groups, multiple linear regression analysis was performed that included data from both groups (PCS and CONTROL) using a model including PCSS and BDI-II

scores. All data is presented as means ± standard deviation, and statistical significance was considered as $p < 0.05$.

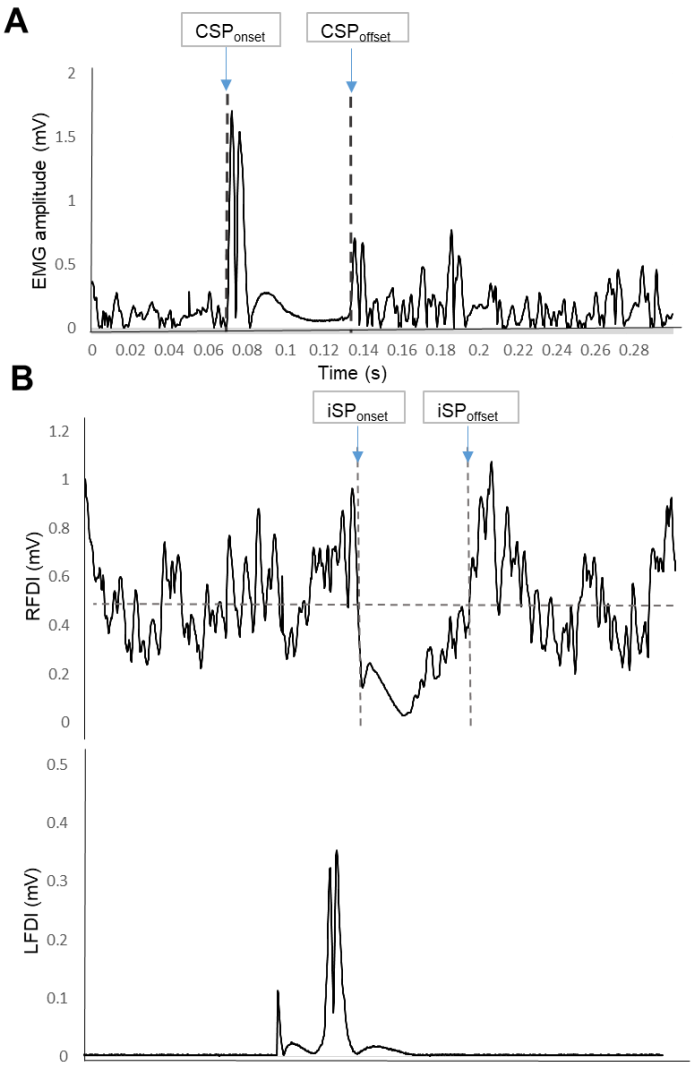

Figure 4. (A) exemplar CSP analysis. CSP length was quantified using the described technique (see methods); CSP length = CSP offset – CSP onset. (B) exemplar iSP analysis. ISP was quantified using the described technique (see methods), where interrupted voluntary EMG activity in the right FDI can be seen in the top row and the MEP evoked in the left FDI can be seen on the bottom. iSP length = iSP offset – iSP onset.

3.6 Results

All participants successfully completed the experiment with no adverse events. Participant demographics are reported in Table 3. Table 4 indicates the statistics from Conover's ANOVA's used to assess the presence of paired-pulse measures, and Table 5 provides the between-group statistical analyses for all TMS measures.

Figure 5 plots the individual PCSS and BDI scores within each group. Separate Mann-Whitney U t-tests revealed that the PCS group scored significantly higher on the PCSS ($p < 0.01$) and the BDI-II questionnaires ($p < 0.01$). Furthermore, correlational analysis revealed a significant positive correlation between PCSS and BDI-II scores ($\rho = 0.74$, $p < 0.01$).

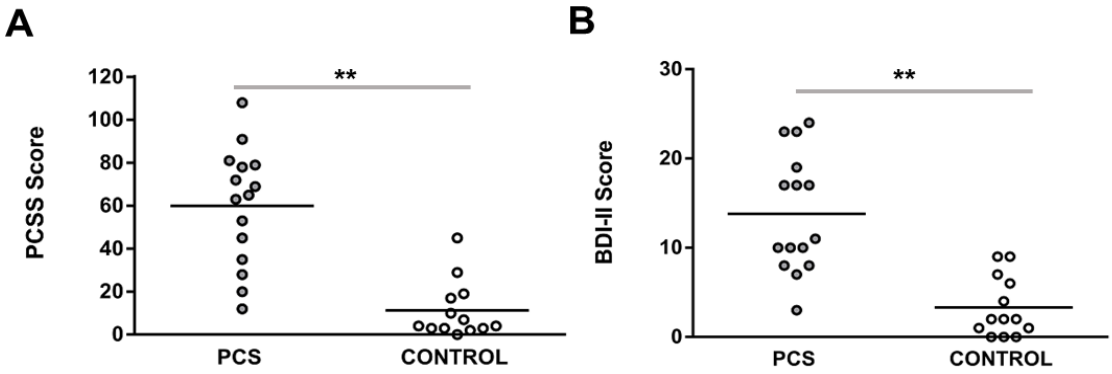

Figure 5. (A) Post-concussion symptom scale scores by group. (B) Beck Depression Inventory scores by group. For both measures, mean bars are displayed. ** indicates $p < 0.01$

3.6.1 TMS measures of intracortical and transcallosal activity

No significant between-group differences were observed for any measure of corticospinal excitability, with the exception of RMT in right FDI whereby thresholds were lower in the PCS group ($p = 0.03$).

TABLE 3. PARTICIPANT DEMOGRAPHICS FOR I) PCS GROUP, II) CONTROL GROUP, AND III) GROUP-AVERAGED DEMOGRAPHICS

i) INDIVIDUAL PCS PARTICIPANTS

Participant	*Age*	*Sex*	*Months Since Last*	*PCSS*	*BDI-II*	*# of concussions*
1	37	M	9	12	3	5
2	24	M	23	65	23	4
3	24	F	8	20	10	1
4	21	M	20	69	23	6
5	19	F	18	108	8	4
6	43	F	8	81	11	1
7	19	F	59	72	17	1
8	32	F	26	63	17	1
9	41	F	12	28	10	3
10	21	M	30	35	10	1
11	44	F	20	78	17	1
12	24	F	15	91	19	3
13	25	F	30	45	7	3
14	29	F	16	53	24	1
15	29	F	22	79	8	1

ii) INDIVIDUAL CONTROL PARTICIPANTS

Participant	*Age*	*Sex*	*PCSS*	*BDI-II*
1	23	F	4	9
2	22	M	17	9
3	23	M	3	0
4	20	F	4	2
5	21	F	10	1
6	32	F	3	0
7	21	M	3	6
8	22	F	2	2
9	23	F	19	7
10	30	F	45	2
11	39	F	7	1
12	45	F	29	4
13	27	F	0	0

iii) GROUP-AVERAGED DEMOGRAPHICS

	PCS	*Control*	*p (Hedge's g)*
n	15 (11 female)	13 (10 female)	
Age$	28.80 ± 8.69	26.77 ± 7.75	0.52 (0.25)
PCSS**$	59.9 ± 27.4	11.2 ± 13.2	**< 0.01 (2.22)**
BDI-II**$	13.8 ± 6.6	3.3 ± 3.4	**< 0.01 (1.96)**
Time since last	21.1 ± 12.7	N/A	
# of concussions (self-reported)	1: n = 8 2+: n = 7	N/A	

**p < 0.01. $ indicates a Mann-Whitney U t-test was performed, otherwise a Welch's t-test was run.

For SICI, a two-way Conover's ANOVA was performed to determine whether significant inhibition was observed for each group. Only a main effect of PATTERN ($F_{(1,25)} = 61.44$; $p < 0.01$) was observed, indicating that inhibition was seen in both groups. The magnitude of SICI was not different between groups ($p = 0.59$). For ICF, a two-way Conover's ANOVA revealed a main effect of PATTERN (19.43; $p < 0.01$), without differences between groups ($p = 0.73$).

Figure 6 displays the group-averaged and individual CSP data. CSP was significantly reduced in the PCS group ($p = 0.02$) based on Welch's t-test. Importantly, MEPs evoked during CSP acquisition did not differ between groups ($p = 0.204$).For CSP assessments, inter-rater reliability was excellent ($r = 0.986$) between the two blinded raters. It has been shown that at increased stimulation intensity, MEP amplitude and CSP increase (Wilson et al., 1993).

Separate two-way Conover's ANOVAs of SIHI and LIHI demonstrated that SIHI and LIHI were observed in both groups (Table 4), and did not significantly differ between groups (Table 5). A trend towards reduced iSP length was observed in the PCS group ($p = 0.08$). Interestingly, 3 PCS individuals did not evoke observable silent periods, and these data were not included in the analyses, in further support that iSP may be reduced in PCS. MEP amplitudes were not significantly different ($p = 0.14$).

TABLE 4. PAIRED-PULSE MEASURES

Measure (group = n)	*TS Mean ± SD*	*CS Mean ± SD*	*CS-TS Mean ± SD*	*Conover's ANOVA*
SICI (PCS = 14, CON = 13)[#]	PCS = 1.07 ± 0.23 CON = 1.07 ± 0.23	~	PCS = 0.50 ± 0.26 CON = 0.55 ± 0.27	**PATTERN: $F_{(1,25)} = 61.44$; $p < 0.01$**, d = 2.15 PATTERN*GROUP: $F_{(1,25)} = 0.06$; p = 0.81 GROUP: $F_{(1,25)} = 0.20$; p = 0.66
ICF (PCS = 15, CON = 12)[#]	PCS = 1.06 ± 0.22 CON = 1.07 ± 0.22	~	PCS = 1.38 ± 0.51 CON = 1.28 ± 0.38	**PATTERN: $F_{(1,25)} = 19.43$; $p < 0.01$**, d = 0.76 PATTERN*GROUP: $F_{(1,25)} = 0.77$; p = 0.39 GROUP: $F_{(1,25)} = 0.02$; p = 0.90
SIHI (PCS = 15, CON = 13)	PCS = 1.01 ± 0.26 CON = 1.03 ± 0.27	PCS = 0.96 ± 0.67 CON = 1.02 ± 0.58	PCS = 0.57 ± 0.22 CON = 0.65 ± 0.43	**PATTERN: $F_{(1,26)} = 15.53$; $p < 0.01$** CS v TS: p = 0.27, d = 0.06 **TS v CS-TS: p < 0.01, d = 1.39** **CS v CS-TS: p < 0.01, d = 0.77** PATTERN*GROUP: $F_{(1,26)} = 0.20$; p = 0.82 GROUP: $F_{(1,26)} = 1.14$; p = 0.30
LIHI (PCS = 15, CON = 13)	PCS = 1.01 ± 0.26 CON = 1.03 ± 0.27	PCS = 0.89 ± 0.56 CON = 1.03 ± 0.43	PCS = 0.70 ± 0.31 CON = 0.70 ± 0.33	**PATTERN: $F_{(1,26)} = 7.68$; $p < 0.01$** CS v TS: p = 0.39, d = 0.16 **TS v CS-TS: p < 0.01, d = 1.12** CS v CS-TS: p = 0.07 d = 0.61 PATTERN*GROUP: $F_{(1,26)} = 0.31$; p = 0.65 GROUP: $F_{(1,26)} = 0.94$; p = 0.34

The following data was excluded from analysis: SICI (PCS=1), EMG contamination; ICF (CON=1), extreme outlier; CSP (PCS = 2), no observable silent period (1), EMG contamination (1); iSP (PCS=5), no observable silent period (3), EMG contamination (2). [#]indicates data was omitted from analysis. Bolded text indicates $p < 0.05$.

TABLE 5. TMS BETWEEN-GROUP DATA

Measure (group = n)	*PCS Mean ± SD*	*CON Mean ± SD*	*p-value (Hedge's g)*
RMT-RFDI* (PCS = 15, CON = 13)	38.47 ± 5.25	44.46 ± 8.72	**0.03 (0.85)**
RMT-LFDI (PCS = 15, CON = 13)	40.93 ± 8.28	42.77 ± 7.97	0.56 (0.23)
AMT-RFDI (PCS = 15, CON = 13)	29.47 ± 3.93	31.46 ± 3.78	0.18 (0.52)
1mV-RFDI (PCS = 15, CON = 13)	49.27 ± 11.19	55.54 ± 12.04	0.18 (0.54)
1mV-LFDI (PCS = 15, CON = 13)	50.27 ± 12.50	54.92 ± 15.31	0.61 (0.34)[$]
SICI (PCS = 14, CON = 13)[#]	0.49 ± 0.27	0.55 ± 0.32	0.60 (0.21)
ICF (PCS = 15, CON = 12)[#]	1.30 ± 0.32	1.21 ± 0.31	0.73 (0.30)[$]
CSP* (PCS = 13, CON = 13)[#]	112 ± 31 ms	141 ± 29 ms	**0.02 (0.96)**
SIHI (PCS = 15, CON = 13)	0.58 ± 0.24	0.64 ± 0.41	0.64 (0.18)
LIHI (PCS = 15, CON = 13)	0.69 ± 0.22	0.70 ± 0.32	0.92 (0.04)
iSP (PCS = 10, CON 13)[#]	20.2 ± 13.3	30.6 ± 13.2 ms	0.08 (0.78)

Paired-pulse measures were calculated as the ratio of the mean conditioned stimulus divided by the mean unconditioned stimulus (CS-TS/TS) for each participant. The following data was excluded from analysis: SICI (PCS=1), EMG contamination; ICF (CON=1), extreme outlier; CSP (PCS = 2), no observable silent period (1), EMG contamination (1); iSP (PCS=5), no observable silent period (3), EMG contamination (2). * indicates $p<0.05$. [#] indicates that some data was omitted from the analysis. [$] indicates a Mann-Whitney U t-test was used for statistical analysis, otherwise a Welch's t-test was performed.

Secondary analysis was performed using multiple linear regression to determine whether any significant findings may be associated with PCSS and BDI-II scores. Results indicated that greater BDI-II (β = -0.50, SE = 0.26, p = 0.06) but not PCSS scores (β = 0.07, SE = 0.06, p = 0.26) may be associated with greater cortical excitability (i.e. reduced thresholds) in left M1. Furthermore shorter CSP length was associated with BDI-II (β = -2.67, SE = 1.08, p = 0.02) but not PCSS scores (β = 0.32, SE = 0.25, p = 0.20).

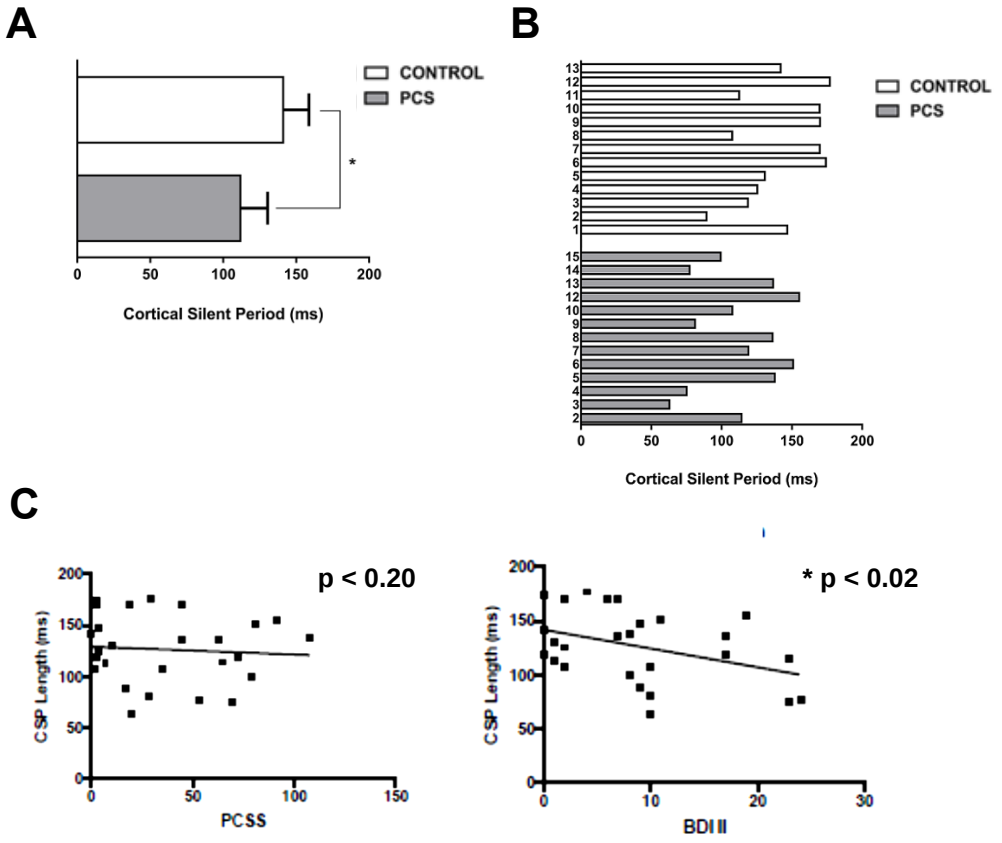

Figure 6. Cortical Silent Period data. (A) Group-averaged CSP length. 95% confidence interval bars displayed. (B) CSP individual data. For PCS participant 1, CSP could not be accurately quantified using the described criteria (see methods). For PCS participant 11, CSP could not be accurately quantified due to EMG contamination. These 2 individuals were omitted from any CSP analysis. (C) Results of the multivariate regression performed on CSP length. No relationship was observed between PCSS score and CSP length. Greater BDI-II scores predicted shorter CSP length. * indicates $p < 0.05$

For iSP length, neither PCSS (β = -0.08, SE = 0.13, p = 0.55) nor BDI-II scores (β = 0.05, SE = 0.55, p = 0.92) related to iSP length.

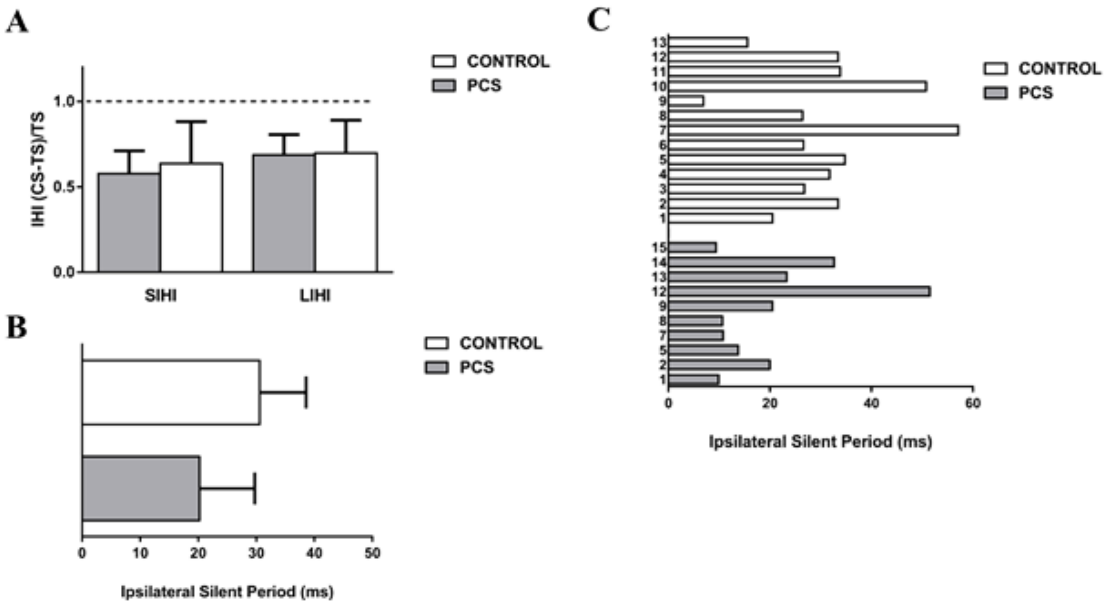

Figure 7. Transcallosal TMS measures. (A) Interhemispheric Inhibition (IHI). (B) Group-averaged iSP analysis. (C) individual iSP data. PCS participants 3, 6, and 10 did not evoke observable silent periods. PCS participants 4 and 11 could not be accurately quantified due to EMG contamination. These 5 individuals were omitted from all analyses. 95% confidence interval bars are displayed.

3.7 Discussion

This study provides evidence of neurological differences present in PCS compared to controls. There are three notable findings. First, we observed evidence that suggests iSP is reduced in PCS. Second, CSP was reduced in PCS which may be related to BDI-II scores but not PCSS scores. Third, reduced RMT was measured in the left hemisphere suggesting greater corticospinal excitability in PCS compared to controls.

This study is the first to measure iSP and IHI in a chronically symptomatic concussion population. Although the pharmacology underlying iSP and IHI are not entirely clear, they are thought to operate via excitatory transcallosal projections interacting with local inhibitory GABAergic interneurons (Daskalakis et al., 2002; Ferbert et al., 1992; Perez & Cohen, 2009), though it remains unclear whether they represent similar or distinct processes (Perez & Cohen, 2009). Although IHI was not different between the PCS and control groups, iSP tended to be reduced in the PCS group compared to controls, a trend with a large effect size ($p = 0.08$, $g = 0.78$). Notably, three individuals with PCS did not evoke a quantifiable silent period and these individuals were omitted from analysis rather than included as a 0 value to reduce any bias of the results. It is plausible that very brief (i.e. less than 5 ms) silent periods were present in these three individuals which could not be quantified by the methods described and thus prescribing 0 values would have skewed our results. However, we conclude that iSP was likely reduced in PCS despite the non-statistically significant result based on this point and the moderate-large effect size observed ($g = 0.78$). Based on the suggested neurophysiology underpinning iSP, differences in this metric may indicate either disrupted excitatory transcallosal neurotransmission or reduced activity of local GABAergic interneurons. Diffusion tensor imaging and diffusion-weighted imaging has shown microstructural damage to the corpus callosum acutely following injury (D'Souza et al., 2015; Messé et al., 2011; Smits et al., 2011), which is present to a greater degree in those who incur PCS (Messé et al., 2011). Therefore, one possible explanation for reduced iSP in PCS could be that reduced excitatory transcallosal signal resulted in less activation of local GABAergic

interneurons. The alternative explanation would be that reduced local inhibitory control of corticospinal projections was responsible for reduced iSP. The latter explanation could align with the present finding of reduced CSP in the PCS group, a measure also thought to reflect GABAergic activity (Ziemann et al., 2015), however it is unknown whether these measures reflect similar neuronal populations. Future studies should continue to assess transcallosal function in PCS using TMS in conjunction with advanced neuroimaging techniques to build upon the present findings.

Reduced CSP length was seen in the PCS group compared to controls. This contrasts the majority of findings in recovered concussion groups demonstrating increased CSP (De Beaumont et al., 2007; 2009; 2011; 2012; Tremblay et al., 2011), though others observed no change in CSP (Davidson & Tremblay, 2016; Tremblay et al., 2014), or reduced CSP (Pearce et al., 2014, 2018). Of note, the two studies that found reduced CSP in asymptomatic concussion groups also reported slower reaction time and poorer dexterity in the concussion groups which correlated with reduced CSP (Pearce et al., 2014, 2018). Based on these functional deficits, it is possible that the concussion groups in the two aforementioned studies remained symptomatic in some aspect of motor control although no measure was acquired regarding the subjective symptom experience of these groups. To date, only two studies have measured CSP in a chronically symptomatic population. A recent study of 8-18 year olds found CSP to be similar in symptomatic, asymptomatic and non-injured youth 4-6 weeks following injury (Seeger et al., 2017). A lack of difference among groups may relate to the immature nervous system influencing corticospinal excitability or relate to a unique criteria for defining PCS as one

month post-injury in that study (Nezu et al., 1997). Most recently in adults, CSP was increased in PCS compared with a recovered group and a healthy control group (Pearce et al., 2019). Differences between Pearce et al. (2019), and our study may relate to the use of anti-depressants (five out of 20 participants in Pearce et al. (2019)), as chronic use of anti-depressants increase $GABA_B$ receptor activity which may increase CSP length (Ghose et al., 2011; Ziemann et al., 2015). In our study, those medically treated for depression in the past or at present were excluded. A further consideration is that Pearce et al. (2019) studied exclusively mild injuries whereas our investigation did not exclude participants based on injury severity. Clarifying the relationship between persistent symptoms and CSP length could provide valuable information about the pathophysiology of PCS and thus should be tested further.

Multiple linear regression was used to assess associations between TMS measures and our two symptom scales. While PCSS scores were not associated with any TMS measure, higher BDI-II scores predicted shorter CSP and trended to predict lower RMT. Importantly, although our PCS group was not being treated for depression, the mean BDI-II values were higher compared to controls and ranged from minimal to moderate depression (Figure 5). Reduced CSP has been found in major depressive disorder (MDD) patients compared to controls (Bajbouj et al., 2006; Levinson et al., 2010). Furthermore, as mentioned, classic anti-depressants such as selective serotonin reuptake inhibitors (SSRI's) are shown to increase CSP length (Robol, Fiaschi, & Manganotti, 2004). Collectively, these data suggest that CSP is sensitive to the depression symptoms in PCS. Importantly, there exists a well-documented issue surrounding the difficulty in

determining a differential diagnosis of PCS and MDD, as they often present similarly (Iverson, 2006). Therefore, future study of PCS should monitor but not exclude co-morbid depression, and include this as a co-variate to expand on our findings to better assess the clinical utility of CSP in PCS patients.

The modest evidence of greater corticospinal excitability as measured by RMT is in-line with the notion that concussion results in greater excitatory input due to greater glutamate release and binding with post-synaptic NMDA receptors (Guerriero et al., 2015). Indeed, RMT is reflective of synaptic glutamatergic synaptic activity of corticospinal neurons (Paulus et al., 2008). Magnetic resonance spectroscopy data has shown elevated glutamate-glutamine/creatine in M1 6 months following concussion and this could lend to greater glutamatergic neurotransmission (Chamard et al., 2015), however MRS is only indicative of total concentrations of a given metabolite and thus does not indicate synaptic glutamate levels. In contrast to our findings, one study reports increased RMT in adults with PCS (Tallus et al., 2012). Although reduced GABAergic inhibition in our PCS cohort may seem to offer an explanation for this discrepancy, this logic is weakened by evidence indicating that pharmacological manipulation of GABAergic neurotransmission did not affect motor thresholds (Kähkönen & Ilmoniemi, 2004). While the present discrepancy is unclear, corticospinal excitability seems to be affected in PCS and warrants further investigation.

3.8 Conclusions

This study is the first to use TMS to assess both intracortical and transcallosal neurophysiological function in a chronically symptomatic concussion group. Reductions in silent periods, both transcallosal and intracortical, suggest reduced GABAergic control in PCS patients. Further, when considering the predictive value of the BDI-II in conjunction with the existing literature in MDD, our findings support similarities in the pathophysiology of these two conditions. We propose that future investigations of PCS should monitor the presence of depression and include this as a co-variate to better elucidate the value of corticospinal and inhibitory cortical measures, in particular CSP, as specific markers of PCS.

CHAPTER 4: GENERAL DISCUSSION AND CONCLUSIONS

The purpose of this Master's thesis was to investigate the neurophysiology of PCS, a poorly understood sequela of concussion injury. This was tested in a single study using TMS to measure corticospinal excitability and inhibition in a group of individuals with PCS and in non-injured controls. Compared to controls, PCS demonstrated increased excitability in three main findings. First, reduced motor threshold in the left (dominant) hemisphere was observed. Second, reduced intracortical inhibition was found, as measured by the cortical silent period. Third, reduced transcallosal inhibition, as measured by the ipsilateral silent period. Secondary analysis revealed that BDI-II scores, but not PCSS scores, were related to CSP length. These findings indicate that TMS measures, in particular CSP, may offer clinical utility in the identification of PCS. The physiological and clinical relevance of these findings will be discussed herein.

4.1 Physiological Perspective

Our main findings of reduced CSP and iSP in PCS suggest reduced inhibition in the motor cortex. Specifically, it is thought that CSP involves local $GABA_B$ receptor activity (Stetkarova & Kofler, 2013; Werhahn et al., 1999), while the mechanism of iSP is less clear. It has been theorized that upregulated $GABA_B$ activity in recovered concussion groups is indicative of a compensatory mechanism against increased cortical excitability (De Beaumont et al., 2012). Thus, it is plausible that our findings indicate an alternate pattern of inhibitory dysregulation that is specific to the chronically symptomatic population. Impaired neurotransmission post-injury is thought to be related to impaired

cognition, slowed processing, and slowed reaction time (Giza & Hovda, 2014). Our study also observed SICI was not different between groups, suggesting that $GABA_A$ receptor function is different in PCS. Several previous studies have also observed no difference in SICI null findings in TMS work in both the acute and long-term phases following concussion (Table 1) there appears to be very specific inhibitory dysfunction associated with $GABA_B$ receptors in PCS.

TMS is limited to inferring M1 function, however neurophysiological function outside of M1 can be investigated by pairing electroencephalography (EEG) and TMS methodologies. By providing sufficient stimulation using TMS over the dorsolateral prefrontal cortex, a TMS-evoked potential can be detected up to 300 ms following stimulation using EEG. When performed in individuals with PCS and recovered individuals, differences have been observed in the acquired signals (Tallus et al., 2013). Notably, a larger N100 amplitude was found in symptomatic group, thought to indicate greater $GABA_B$ activity. While this appears to present a contradiction to conventional TMS findings, this difference at N100 was observed following stimulation of dorsolateral prefrontal cortex but not following M1 stimulation, highlighting brain region-specific effects of concussion (Tallus et al., 2013).

It must be discussed, however, that there appears to be a disconnect between animal and human literature regarding $GABA_A$ dysfunction following injury. As mentioned in Chapter 2, rodent models have shown reduced expression of $GABA_A$ subunits related to phasic inhibition in the first weeks following injury (Raible et al., 2012). In contrast, the TMS literature in acute and recovered concussion groups have

consistently shown no change in SICI, thought to reflect $GABA_A$ activity (Table 1). Notably, one study did observe greater SICI in a chronically symptomatic concussion group (Pearce 2019). While this may appear to contradict the animal literature, SICI likely relies on different sub-units from those involved in phasic inhibition. Phasic inhibition typically involves the α1 and ɣ2 subunits (Kharlamov et al., 2011), whereas SICI is most likely to involve α2 or α3 $GABA_A$ subunits (Di Lazzaro et al., 2007). This may explain why human studies using SICI do not reflect $GABA_A$ changes observed in animal models. Therefore, it cannot be said conclusively that $GABA_A$ receptors are not affected following concussion in humans, however it does appear that specific subunit types and thus certain receptor configurations may be spared.

4.2 PCS and Depression

Concussion and depression have long been intertwined. It has been estimated that up to 44% of concussions feature some degree of depression during the first 3 months post-injury (Iverson & Lange, 2011). Conversely, half of major depression patients fit the criteria for PCS without any head injury having occurred (Iverson, 2006). A careful review of the literature has concluded that psychological disturbances contribute to symptoms during the acute and chronic phase and are predictive of PCS outcomes (Silverberg & Iverson, 2011). This study's findings corroborate the influence of psychological factors in PCS. First, significantly greater BDI-II scores were observed in the PCS group, such that the group average score would be described as mild-moderate

depression (Beck et al, 1988). Furthermore, our findings of reduced CSP length in a PCS group may reveal a novel link between PCS and depression. Indeed, this aligns with the reduction in CSP observed in groups with depression (Bajbouj et al., 2006; Levinson et al., 2010). This commonality may highlight a physiological similarity between PCS and depression implicating GABAergic, specifically $GABA_B$, receptor dysfunction. Therefore, I would theorize that GABAergic dysfunction may partially explain why anti-depressant medications are effective treatments in PCS, though more research is clearly needed.

4.3 Clinical Relevance

The findings of this research have important clinical implications. At this time, clinicians are limited to providing pharmacological treatment for specific symptoms presenting post-injury as there are no approved treatments for the specific consequences of concussion (Hadanny & Efrati, 2016). The most commonly prescribed medications for PCS are anti-depressants, which include SSRI's (Mittenberg et al., 2003). Chronic administration of SSRI's are shown to increase CSP length (Robol et al., 2004). Given our finding of reduced CSP length in an unmedicated cohort of individuals with PCS, it is plausible that SSRI's may exert their benefits in part through the normalization of inhibitory cortical activity. Although speculative, this may offer a mechanistic explanation for some of the benefits SSRI's provide for individuals with PCS. This speculation is in-line with the idea that asymptomatic individuals have a compensatory

elevation of GABAergic activity. Such speculation leaves two unanswered questions. First, if there is a compensatory response of increased inhibitory activity post-concussion, why is it absent for specific individuals? Second, could those with PCS benefit from non-anti-depressive $GABA_B$ modulators? At present, there is no clear answer for the first question. As for the second question, there is some evidence of baclofen, a $GABA_B$ agonist, providing benefits in more severe TBI cases (Pérez-Arredondo et al., 2016) . However, the side effects of baclofen are a deterrent (Perez-Arredondo et al., 2016). Both questions require further inquiry using pre-clinical models, and addressing these gaps will contribute to the current understanding of the pathophysiology underlying PCS.

4.4 Limitations and Challenges

No functional assessments of motor function were performed. While subjective symptom scores are likely sufficient for the confirmation of PCS, it may not have been sufficient for confirming specific motor system abnormalities. Indeed, only one item on the PCSS ("balance problem") directly pertains to sensorimotor function. The addition of a motor task such as the O'Connor Finger Dexterity test, which has been used to detect motor deficits post-concussion (Pearce et al., 2014; 2015), would have provided greater functional context to the TMS findings herein. Another limitation of this research is that findings are all within M1, therefore it is does not explain neurophysiological changes in other cortical areas. Further studies including both TMS and TMS-EEG can ameliorate this concern by providing a broader view of how cortical function is affected in PCS.

A general limitation of TMS research is the lack of clarity regarding the neuronal pathways that are accessed with different measures. While single pulse TMS is thought to act trans-synaptically to excite descending cortical pyramidal neurons, it is less understood how paired-pulse TMS exerts its influence. Specifically, it is not known whether paired-pulse inhibitory measures reflect inhibition occurring pre- or post-synaptically. This may explain why animal models of concussion have identified alterations of $GABA_A$ receptor function that have not been reflected in SICI, the TMS proxy for $GABA_A$ function, acquired in humans. This lack of specificity restricts TMS findings from describing pathophysiological processes at the cellular level for PCS, which should be further investigated in animal literature.

Several pragmatic challenges were overcome to successfully complete this thesis work. In order to have physician supervision, TMS equipment had to be transported off-site to an operational clinic for individual sessions. This required careful coordination of researchers and resources in order to accommodate the clinic's patient schedule and other ongoing studies utilizing the same TMS equipment. Another challenge was ensuring clear communication in the recruitment and screening process for participants with PCS. Due to memory issues and light sensitivity being experienced by many individuals, multiple avenues of communication were required to ensure each participant received the study information in a manner that met their particular needs. This meant careful organization and planning to ensure that all information was delivered similarly regardless of whether it be communicated over the phone, via e-mail, or through physical documentation.

4.5 Conclusions of Masters Thesis

The original aim of this thesis was to improve our understanding of PCS from a neurophysiological perspective. I demonstrated that there are unique changes in neurophysiological function in PCS that are different from previous findings in recovered cohorts. Specifically, I observed reduced intracortical inhibition that is likely related to $GABA_B$ receptor activity in M1. These changes also related specifically to the depression symptoms experienced by the individual. Indeed, the findings herein are consistent with previously observed reductions in CSP length in groups with depression. Thus, the presented findings highlight a potentially clinically useful marker that is sensitive to the presence of depression in chronically symptomatic concussion patients. In the future, I recommend a prospective study be conducted with a larger cohort to observe whether differences in cortical and corticospinal function emerge across varying degrees of recovery over time. Importantly, this thesis has contributed to our understanding of the neurophysiological consequences of concussion injury, and add to the existing literature regarding symptom persistence. Future research should continue to investigate neurological function in humans following concussion to continue to unravel the many questions surrounding this heterogeneous injury.

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Appendix 1. TMS SCREENING QUESTIONNAIRE

Date:_____ Participant ID:_____ Researcher: _____

Your responses to the following questions will be used solely for assessing your eligibility for participation in a TMS study. Additional details about the study are described in the informed consent form. To maximize safety, please answer the questions below. Please do not hesitate to ask any questions you may have regarding the items below. If you are not comfortable disclosing any of the required information, please inform the researcher that you do not wish to take part in the study. Your privacy will be respected, and after this documentation is analyzed, it will be destroyed.

1. YES / NO – Are you younger than eighteen years old?
2. YES / NO – Do you have a pacemaker?
3. YES / NO – Do you have any metal/electrical/magnetic implanted in your body except dental fillings?
4. YES / NO – Are you, or could you possibly be, pregnant?
5. YES / NO – Do you have any major medical problems or unstable medical problems?
6. YES / NO – Do you have any history of neurological or psychiatric illness?
7. YES / NO – Have you had any head injury or head surgery (**excluding concussion**)?
8. YES / NO –Experience frequent headaches or migraine?
9. YES / NO – Do you or any blood relatives (grandparent, parent, aunt/uncle, sibling, self) have a history of a seizure? If so, please circle all that apply.
10. YES / NO – Have you had unusual responses, e.g. faint when you go to the hospital or get blood drawn?
11. YES / NO – Are you currently taking any prescription or non-prescription medications (e.g. antihistamines, antibiotics) or use street drugs? If so, please discuss the exact medications you are taking with the researcher.
12. YES / NO – Have you taken any antibiotics in the past week?
13. YES / NO – Have you taken any medication other than contraceptives in the past 12 hours?
14. YES / NO – Have you used alcohol, nicotine, or drugs in the past 12 hours?
15. YES / NO – Have you experienced sleep deprivation within the past 48 hours?
16. YES / NO –Do you have any metal on your body (e.g. watch or jewelry, hair holders or pins, eye glasses, body piercings, wallet, keys?) If so, please remove.

17. YES / NO – Did you ever undergo TMS in the past?
18. YES / NO – Did you ever undergo MRI in the past?

Participant Signature: _____ Date:_____

Researcher Signature: _____ Date:_____

Appendix 2. BECK'S DEPRESSION INVENTORY (BDI-II)

Participant Name: Date:

Participant ID: Age:

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully and then pick out the **one statement** in each group that best describes how you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (changes in sleeping pattern) or Item 18 (changes in appetite).

1. Sadness
   - 0 I do not feel sad
   - 1 I feel sad much of the time
   - 2 I am sad all the time
   - 3 I am so sad or unhappy that I can't stand it.

2. Pessimism
   - 0 I am not discouraged about my future
   - 1 I feel more discouraged about my future than I used to be.
   - 2 I do not expect things
   - 3 I feel my future is hopeless and will only get worse

3. Past Failure
   - 0 I do not feel like a failure
   - 1 I have failed more than I should have
   - 2 As I look back, I see a lot of failures
   - 3 I feel I am a total failure as a person

4. Loss of Pleasure
   - 0 I get as much pleasure as I ever did from the things I enjoy
   - 1 I don't enjoy things as much as I used to
   - 2 I get very little pleasure from the things I used to enjoy
   - 3 I can't get any pleasure from the things I used to enjoy

5. Guilty feelings
   - 0 I don't feel particularly guilty
   - 1 I feel guilty over many things I have done or should have done
   - 2 I feel quite guilty most of the time
   - 3 I feel guilty all of the time

6. Punishment Feelings
   - 0 I don't feel I am being punished
   - 1 I feel I may be punished
   - 2 I expect to be punished
   - 3 I feel I am being punished

7. Self-dislike
   - 0 I feel the same about myself as ever
   - 1 I have lost confidence in myself
   - 2 I am disappointed in myself
   - 3 I dislike myself

8. Self-criticalness
   - 0 I don't criticize or blame myself more than usual
   - 1 I am more critical of myself than I used to be
   - 2 I criticize myself for all of my faults
   - 3 I blame myself for everything bad that happens

9. Suicidal thoughts or wishes
   - 0 I don't have any thoughts of killing myself
   - 1 I have thoughts of killing myself, but I would not carry them out
   - 2 I would like to kill myself
   - 3 I would kill myself if I had the chance

10. Crying
    - 0 I don't cry any more than I used to
    - 1 I cry more than I used to
    - 2 I cry over every little thing
    - 3 I feel like crying, but I can't

11. Agitation
    - 0 I am no more restless or wound up than usual
    - 1 I feel more restless or wound up than usual
    - 2 I am so restless or agitated that it's hard to stay still
    - 3 I am so restless or agitated that I have to keep moving or doing something

12. Loss of interest
    - 0 I have not lost interest in other people or activities
    - 1 I am less interested in other people or things than before
    - 2 I have lost most of my interest in other people or things
    - 3 It's hard to get interested in anything

13. Indecisiveness
    - 0 I make decisions about as well as ever
    - 1 I find it more difficult to make decisions than usual
    - 2 I have much greater difficulty in making decisions than I used to
    - 3 I have trouble making any decisions

14. Worthlessness
    - 0 I do not feel I am worthless
    - 1 I don't consider myself as worthwhile and useful as I used to
    - 2 I feel more worthless as compared to other people
    - 3 I feel utterly worthless

15. Loss of energy
    - 0 I have as much energy as ever
    - 1 I have less energy than I used to have
    - 2 I don't have enough energy to do very much
    - 3 I don't have enough energy to do anything

16. Changes in sleeping pattern
    - 0 I have not experienced any change in my sleeping pattern
    - 1a I sleep somewhat more than usual
    - 1b I sleep somewhat less than usual
    - 2a I sleep a lot more than usual
    - 2b I sleep a lot less than usual
    - 3a I sleep most of the day
    - 3b I wake up 1-2 hours early and can't get back to sleep

17. Irritability
    - 0 I am no more irritable than usual
    - 1 I am more irritable than usual
    - 2 I am much more irritable than usual
    - 3 I am irritable all the time

18. Changes in appetite
    - 0 I have not experienced any change in my appetite
    - 1a My appetite is somewhat less than usual
    - 1b My appetite is somewhat greater than usual
    - 2a My appetite is much less than before
    - 2b My appetite is much greater than usual
    - 3a I have no appetite at all
    - 3b I crave food all the time

19. Concentration Difficulty
    - 0 I can concentrate as well as ever
    - 1 I can't concentrate as well as usual
    - 2 It's hard to keep my mind on anything for very long
    - 3 I find I can't concentrate on anything

20. Tiredness or fatigue
    - 0 I am not more tired or fatigued than usual
    - 1 I get more tired or fatigued than usual
    - 2 I am too tired or fatigued to do a lot of the things I used to do
    - 3 I am too tired or fatigued to do most of the things I used to do

21. Loss of interest in sex
    - 0 I have not noticed any recent change in my interest in sex
    - 1 I am less interested in sex than I used to be
    - 2 I am much less interested in sex now
    - 3 I have lost interest in sex completely

Participant Signature:

Date:

Researcher Signature:

Date:

Appendix 3. POST-CONCUSSION SYMPTOM SCALE

Name: _____ Age/DOB: _____ Date of Last Injury: _____

# of prior concussions (diagnosed/self-diagnosed): _____

To what degree do you experience the following symptoms on more days than not?

No symptoms "0" Moderate "3" Severe"6"

Number of Months Since injury: _____

1.	Headache	0	1	2	3	4	5	6
2.	Nausea	0	1	2	3	4	5	6
3.	Vomiting	0	1	2	3	4	5	6
4.	Balance problems	0	1	2	3	4	5	6
5.	Dizziness	0	1	2	3	4	5	6
6.	Fatigue	0	1	2	3	4	5	6
7.	Trouble falling to sleep	0	1	2	3	4	5	6
8.	Excessive sleep	0	1	2	3	4	5	6
9.	Loss of sleep	0	1	2	3	4	5	6
10.	Drowsiness	0	1	2	3	4	5	6
11.	Light sensitivity	0	1	2	3	4	5	6
12.	Noise sensitivity	0	1	2	3	4	5	6
13.	Irritability	0	1	2	3	4	5	6
14.	Sadness	0	1	2	3	4	5	6
15.	Nervousness	0	1	2	3	4	5	6
16.	More emotional	0	1	2	3	4	5	6
17.	Numbness	0	1	2	3	4	5	6
18.	Feeling "slow"	0	1	2	3	4	5	6
19.	Feeling "foggy"	0	1	2	3	4	5	6
20.	Difficulty concentrating	0	1	2	3	4	5	6
21.	Difficulty remembering	0	1	2	3	4	5	6
22.	Visual problems	0	1	2	3	4	5	6

TOTAL SCORE _____