

PHYSIOLOGICAL CONSEQUENCES OF MILD TRAUMATIC BRAIN INJURY IN  
INDIVIDUALS WITH ACUTE AND CHRONIC SYMPTOMS

by

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DISSERTATION APPROVAL PAGE

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## DISSERTATION ABSTRACT

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Doctor of Philosophy

Department of Human Physiology

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Title: Physiological Consequences of Mild Traumatic Brain Injury in Individuals with Acute and Chronic Symptoms

Mild traumatic brain injury (mTBI) is the subject of increasing public health concern. Symptoms following mTBI, as well as risk factors for prolonged recovery, have been well-described. The physiological mechanisms behind these physical symptoms, and time course of recovery after injury, however, remain largely unknown. The purpose of this dissertation, therefore, was to assess the acute and chronic impact of mTBI on motor cortex function and associated neurotransmitter concentrations. A secondary goal of this dissertation was to establish the relationship between motor cortex function and the risk factors of female sex and APOE genotype in acute and chronic mTBI patients.

Motor cortex function and neurotransmitter concentrations were assessed using transcranial magnetic stimulation and magnetic resonance spectroscopy, respectively. It was found that excitability, as assessed by the amplitude of the motor evoked potential (MEP), was lower in the Chronic group (participants with chronic symptoms from mTBI, lasting at least 3 months post-injury) compared to the control groups ( $p=0.02$ ), but no differences in glutamate, the primary excitatory neurotransmitter, were found in the motor cortex between any group ( $p=0.93$ ) or over time acutely following mTBI ( $p=0.70$ ). Intracortical inhibition, as assessed by the duration of the cortical silent period (CSP),

was higher in individuals acutely following mTBI (within 72 hours of mTBI diagnosis) and throughout two months of recovery ( $p=0.02$ ), but no differences in GABA, the primary inhibitory neurotransmitter, were found in the motor cortex between any group ( $p=0.06$ ) or over time following acute mTBI ( $p=0.57$ ). There were no differences in MEP amplitude, CSP duration, glutamate concentration, or GABA concentration between males and females, or carriers of the apoε4 allele at any time point following mTBI ( $p\geq 0.10$ ).

This dissertation represents a compilation of studies which are among the first to document motor cortex excitability, intracortical inhibition, glutamate, and GABA concentrations in individuals with acute and chronic symptoms from mTBI. The data suggest a possible functional change longitudinally following mTBI, despite an expected neurochemical profile. Results from these studies suggest that humans may not follow the same neurometabolic timeline as the rodent model following mTBI.

This dissertation contains previously published and unpublished co-authored material.

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# CHAPTER I

## INTRODUCTION

Traumatic brain injury (TBI) is classified as an injury to the brain caused by mechanical force acting on the body (McCrorry et al. 2013). This type of injury can lead to cognitive symptoms such as impaired memory, confusion, and difficulty concentrating (Cantu 2006) as well as deficits in physical function such as slow movement speed (Gray et al. 1998), impaired balance (Chou et al. 2004), and slowed reaction time (Howell et al. 2013a). An estimated 5.3 million Americans currently live with a TBI-related disability, and for some, the effects may be permanent (Center for Disease Control and Prevention 2010). Numerous factors have been suggested to contribute to recovery from mild traumatic brain injury (mTBI), and among the most extensively studied include age, sex, history of mTBI, and presence of the apolipoprotein E (*APOE*) genotype (Meehan et al. 2011; McClincy et al. 2006; Scopaz and Hatzenbuehler 2013; Eramudgolla et al. 2014; Broshek et al. 2005; Tierney et al. 2010). The physiology of TBI has been studied in animal models (Giza and Hovda 2001), but has not been extensively explored in humans. The overall goal of this dissertation is to assess the acute and chronic impact of mTBI on motor cortex function and associated neurotransmitter concentrations. A secondary goal of this dissertation is to establish the relationship between motor cortex function and *APOE* genotype in individuals who have suffered an mTBI.

Structural changes within the brain after an mTBI are not always evident with imaging techniques, making it difficult to identify and treat areas affected by the injury (van der Naalt et al. 1999; Voller et al. 1999). Recovery from mTBI, therefore, is commonly assessed by neuropsychological tests, the majority of which typically return to

baseline values within two weeks post-injury (Hinton-Bayre and Geffen 2002). It has been consistently demonstrated, however, that even in the absence of neuropsychological deficits, motor deficits such as gait instability (Slobounov et al. 2007), slow movement speed (Gray et al. 1998; De Beaumont et al. 2009) impaired balance (Chou et al. 2004), and slowed reaction time (Howell et al. 2013b; Halterman et al. 2006) can persist months to years after initial injury (Gray et al. 1998; Chou et al. 2004). Howell et al. (2013a) found that adolescents with mTBI demonstrated higher levels of medial/lateral displacement during an attention-divided gait task at 72-hours post-injury, a deficit that did not resolve to control levels throughout two months of testing. Similarly, adults tested within 48 hours of injury have also demonstrated altered gait, as evidenced by slower walking speed during dual-task walking (Catena et al. 2009). Although such motor impairments have been cited as early indicators of chronic difficulties associated with brain injury (Rabadi and Jordan 2001), the mechanisms leading to these persistent motor deficits remain largely unknown.

Widespread changes in metabolite concentration are a likely candidate for explaining physical symptoms, as well as cognitive and motor deficits recorded in humans post-mTBI. Changes in metabolite concentration begin immediately in the brain after a mechanically-induced injury to the head or neck. Researchers have used the term "Neurometabolic Cascade" to describe these changes in neurotransmitter concentration and the effects each subsequent phase has on the next (Giza and Hovda 2001). Acutely after injury, the brain experiences a state of massive excitation caused by an indiscriminate release of excitatory neurotransmitters such as glutamate. Glutamate transport decreases following mTBI, allowing excess glutamate to stay in the synapse and

prolong the excitotoxic environment in the brain. This acute, excitatory phase resolves fairly quickly in the animal model as the sodium/potassium pumps work to restore homeostasis (Giza and Hovda 2001). A compensatory "spreading depression" phase follows the acute excitatory phase, leading to a global decrease in glutamate and an energy crisis as ATP is used by the sodium/potassium pump. Studies show that neurons injured following mTBI demonstrate a reduction in dendrite length and inhibited neuronal signaling, suggesting that mTBI may lead to a change in the excitatory/inhibitory balance post-injury (Brizuela et al. 2017). Although glutamate levels resolve within 10 minutes post-injury in a rodent model (Giza and Hovda 2001), this timeline cannot be directly applied to metabolite recovery in a human following mTBI. Non-invasive techniques such as transcranial magnetic stimulation (TMS) and magnetic resonance spectroscopy (MRS) indirectly and directly quantify neurotransmitter activity in humans and provide mechanistic insight into motor and cognitive functional decline and recovery post-injury.

Transcranial magnetic stimulation (TMS) and magnetic resonance spectroscopy (MRS) studies provide non-invasive techniques that can be used to assess functional changes and neurotransmitter concentrations in the primary motor cortex following mTBI. Lower cortical excitability, as assessed by the TMS measure resting motor threshold (RMT), has been demonstrated in individuals acutely post-mTBI (Chistyakov et al. 2001; Henry et al. 2010). Using TMS, it has also been demonstrated that intracortical inhibition, as assessed by the duration of the cortical silent period (CSP), is augmented in individuals with mTBI (De Beaumont et al. 2009), and recent findings

suggest that this greater level of inhibition does not recover by two months post-injury (Miller et al. 2014).

In addition to TMS measures of cortical excitability and inhibition, quantities of the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter GABA, as obtained by proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS), provide further insight into motor, cognitive, and physiological consequences of mTBI. While TMS provides useful information regarding cortical excitability and inhibition, <sup>1</sup>H-MRS can directly and non-invasively evaluate relative concentrations of neurotransmitters thought to modulate TMS measures of cortical excitability and inhibition in specific brain areas of interest. Using this technique, it has been shown that, despite normal neuropsychological scores and anatomical MRI results, athletes with mTBI have altered neurometabolic profiles in comparison to controls 1-6 days post-injury (Henry et al. 2010). Specifically, these athletes were reported to have lower levels of glutamate, the primary excitatory neurotransmitter, in the primary motor cortex (M1). It has been suggested that physical symptoms of mTBI including headache, sensitivity to light, dizziness, and nausea may be explained by these alterations of metabolite concentration within the brain acutely post-injury (Henry et al. 2010).

An individual's physiological response to mTBI is often unpredictable, as there is a high degree of inter-individual variability in symptoms experienced (Guskiewicz 2001). While symptoms recover within approximately 2 weeks for most individuals (Hinton-Bayre and Geffen 2002), 10-15% of the population with mTBI will continue to experience symptoms chronically (Ruff et al. 1996). While acutely-injured individuals have consistently demonstrated motor deficits in the absence of neuropsychological



deficits, (Gray et al. 1998; Chou et al. 2004; Slobounov et al. 2007; Howell et al. 2013b) similar assessments in individuals with chronic symptoms have not been made.

Therefore, little is known about the physiological characteristics of chronic mTBI and how they compare with characteristics of acute injury. Further, it is not known how these prolonged deficits relate to individual risk factors, such as Apolipoprotein E (*APOE*) genotype.

Recent mTBI studies have focused on the predictive properties of specific genes, most notably, the *APOE* gene. The *APOE* gene is located on the long arm of chromosome 19 and provides the template for creating a protein (apoE) that plays a vital role in transporting cholesterol to neurons. This protein is mainly produced by astrocytes and microglia in the central nervous system (Lawrence et al. 2015). The apoE protein is also critically involved with repair of the neuronal cell membrane (Mahley 2016), dendrite and synapse formation (Nathan et al. 1994), and the maintenance of calcium homeostasis within the neuron (Muller et al. 1998), all processes essential to recovery after mTBI. There are three main isoforms, or, versions, of *APOE* inheritable by humans. The rarest form,  $\epsilon 2$ , occurs in roughly 7% of the population, while the  $\epsilon 3$  and  $\epsilon 4$  alleles occur at a frequency of 78% and 15% respectively (Roses 1996).

The  $\epsilon 4$  allele has received much attention from TBI researchers in recent years and is associated with poorer outcome after TBI, including greater severity of injury (Jiang et al. 2011), worse Glasgow Coma Scale scores (Teasdale et al. 1997), lower motor scores on the Functional Independence Measure (Lichtman et al. 2000), and cognitive impairment in elderly and adolescent populations (Eramudgolla et al. 2014; Lawrence et al. 2015). Possession of one or more  $\epsilon 4$  alleles is associated with a 10-fold

increased risk of mTBI history in athletes (Tierney et al. 2010) and, despite the relative rarity of the  $\epsilon 4$  allele in nature, this isoform is overrepresented in chronic traumatic encephalopathy and chronic TBI populations (McKee et al. 2009). The detrimental effects of the  $\epsilon 4$  allele tend to occur in a dose-dependent fashion, meaning that the highest risks and poorest outcomes occur in those  $\epsilon 4$  homozygotes who possess two  $\epsilon 4$  alleles ( $\epsilon 4\epsilon 4$  genotype) (Guo et al. 2000). It has been suggested that the  $\epsilon 4$  allele produces a less efficient form apoE protein in astrocytes and microglia, and its inadequacy is exaggerated in an environment like TBI where its role is more heavily required for recovery (Lawrence et al. 2015).

Animal studies provide insight into the role of *APOE* genotype and levels of excitatory and inhibitory neurotransmitter concentration in the cortex. Chen et al. (2010) observed that  $\epsilon 4$  carrier mice displayed reduced glutamate receptor function, while Dumanis et al., (2013) used magnetic resonance spectroscopy and found decreased global concentrations of the excitatory neurotransmitter glutamate and increased levels of glutamine in the brains of  $\epsilon 4$  carrier mice. In this study, concentrations of GABA, an inhibitory neurotransmitter, were not different among  $\epsilon 4$  carriers. However, to our knowledge, similar assessments of glutamate, GABA and  $\epsilon 4$  carrier status have not been made in humans. Such information would provide valuable insights into risk factors for chronic mTBI and the physiological consequences.

The overall goal of this dissertation is to assess the acute and chronic impact of mTBI on motor cortex function and associated neurotransmitter concentrations. A secondary goal of this dissertation is to establish the relationship between motor cortex function, sex, and *APOE* genotype in acute and chronic mTBI patients. Such information

will provide insight into potential precursors for brain injury and prolonged recovery from mTBI.

### **Specific aim 1**

The aim of the first study was to determine the level of excitability and inhibition in the motor cortex of individuals with acute and chronic symptoms from mTBI, compared with healthy controls.

#### *Hypothesis 1.1*

Based on evidence from the Neurometabolic Cascade, acutely-injured individuals will experience higher levels of motor cortex excitability and higher levels of intracortical inhibition than healthy control participants.

#### *Hypothesis 1.2*

Individuals with chronic symptoms from mTBI will experience similar levels of motor cortex excitability and higher levels of intracortical inhibition compared with healthy control participants.

### **Specific aim 2**

The aim of the second study was to determine the concentrations of excitatory and inhibitory neurotransmitter in individuals with acute and chronic symptoms from mTBI compared with healthy controls.

#### *Hypothesis 2.1*

Based on evidence from the Neurometabolic Cascade, acutely-injured individuals will have higher glutamate and GABA concentrations in the motor cortex than healthy control individuals.

#### *Hypothesis 2.2*

Individuals with chronic symptoms from mTBI will have similar levels of glutamate in the motor cortex, but elevated levels of GABA compared to healthy control participants.

### **Specific aim 3**

The aim of the final study was to determine if the risk factors of female sex and apoε4 genotype will contribute to motor cortex function following mTBI.

#### *Hypothesis 3.1*

Females with mTBI will report higher symptom scores than males with mTBI.

#### *Hypothesis 3.2*

Females with mTBI will have similar levels of motor cortex excitability and inhibition compared to males with mTBI.

#### *Hypothesis 3.3*

There will be a higher proportion of ε4 carriers in the group with chronic symptoms from mTBI compared to the allele frequency of ε4 in the general population.

#### *Hypothesis 3.4*

Apoε4 carriers will have lower levels of excitability and similar levels of intracortical inhibition than non-carriers in all groups.

#### *Hypothesis 3.5*

Apoε4 carriers will have lower levels of glutamate and similar levels of GABA than non-carriers in all groups.

### **Study participants**

Individuals with mTBI who participated in the studies outlined in this dissertation were diagnosed with concussion (a form of mTBI) by specialized health professionals

(certified athletic trainer or physician) at the time of injury. All injuries met the definition of concussion provided by the 4<sup>th</sup> International Consensus Statement on Concussion in Sport (McCrory et al. 2013). ImPACT post-injury tests were used as a diagnosis for individuals who had completed baseline ImPACT testing prior to mTBI.

Participants were excluded if they had (i) a history of two or more mTBIs or an mTBI within a year prior to testing, (ii) loss of consciousness for more than 1 minute (mTBI group), (iii) history of cognitive deficiencies independent of the injury, such as memory loss or difficulty concentrating, (iv) history of attention deficit hyperactivity disorder, neurological impairment, musculoskeletal impairments or seizures or (v) contraindications to the use of transcranial magnetic stimulation (TMS) or magnetic resonance imaging (MRI) including medications that could affect neurotransmitter function. All procedures used in this investigation were reviewed and approved by the Institutional Review Board of the University of Oregon, and participants provided written, informed consent prior to beginning study procedures.

Table 1.1 describes the type of mTBI (sports- or non-sports-related) sustained by individuals in the Acute and Chronic mTBI groups. Sports-induced mTBIs were sustained during play in the following sports: rugby (6), soccer (6), basketball (2), ultimate Frisbee (2), wrestling (1), hockey (1), and snowboarding (1).

**Table 1.1. MTBI Type**

	<b>Acute</b>	<b>Chronic</b>
<b>Sports</b>	16	3
<b>Non-sports</b>	13	10

Symptoms scores were obtained from participants at their first visit to the laboratory (within 72 hours for acutely-injured participants). Various symptom inventories were obtained from participants, including a symptom inventory adopted from McCrory et al (2013), a survey adapted from the Post-Concussive Symptom Score section of the ImPact test and from the SCAT3 (Broglio et al. 2007) and the Rivermead Post-Concussion Symptoms Questionnaire (King et al. 1995) For all inventories used, the Acute and Chronic groups reported more symptoms than their respective control groups ( $p \leq 0.01$ ).

A total of 35 individuals (5 Control, 7 Acute, 18 Chronic Control, and 5 Chronic) participated in all three studies included in this dissertation. Twenty-seven (11 Control, 13 Acute, 2 Chronic Control, and 1 Chronic) participated in study 1 only. Seven (1 Acute, 5 Control, and 1 Chronic Control) participated in studies 1 and 2 only. Fifteen (7 Acute, 6 Control, 2 Chronic Control) participated in studies 1 and 3 only. One (Chronic) participated in study 2 only, and one (Chronic) participated in studies 2 and 3 only.

### **Flow of dissertation**

Chapter II focuses on the post-mTBI recovery of motor cortex function as assessed by TMS. Chapter III discusses the presence of glutamate and GABA in the motor cortex following mTBI. Chapter IV evaluates the TMS and MRS data according to apoε4 carrier status.

Chapters II and III include previously published co-authored material. Portions of Chapters II and III were previously published in *Medicine and Science in Sports and Exercise* and *Journal of Neurophysiology*, respectively. Chapter IV includes unpublished co-authored material.

## CHAPTER II

### MOTOR CORTEX FUNCTION FOLLOWING MTBI

Some material from this chapter was previously published in volume 49, issue 6 of *Medicine and Science in Sports and Exercise* in June 2017. Alia Yasen contributed to the concept of the study, recruited participants, collected and analyzed data, and helped prepare the initial manuscript. Dr. Anita Christie contributed to the concept of the study, aided in the interpretation of the data, and critically reviewed and revised the manuscript.

#### **Introduction**

Mild traumatic brain injury (mTBI) has been described as a diffuse injury that can impact both cognitive and motor function (Howell et al. 2013a; Howell et al. 2013b; Miller et al. 2014; Parker et al. 2007). Common cognitive symptoms include, but are not limited to, confusion, difficulty focusing attention, impaired memory and reduced visual processing speed (Cantu 2006; Parker et al. 2007; Guskiewicz et al. 2003). Recovery from sport-related mTBI, in particular, is typically assessed using computerized neurocognitive testing techniques and is reported to occur within 10 days post-injury (Guskiewicz et al. 2003; Bleiberg et al. 2004). A growing body of literature, however, suggests that even in the absence of neurocognitive deficits, motor impairments such as reduced movement speed, and difficulties with gait and balance control, can persist for up to two months post-injury (Gray et al. 1998; De Beaumont et al. 2009; Miller et al. 2014; Howell et al. 2015). Although such motor impairments have been cited, in some cases, as early indicators of chronic difficulties associated with brain injury (Rabadi and Jordan 2001), the underlying mechanisms of these functional deficits are not yet fully understood.

Mild traumatic brain injury is accompanied acutely by alterations in neurotransmitter release as well as ionic and metabolic changes (Giza and Hovda 2001) that can impact cortical neuronal function. Using transcranial magnetic stimulation (TMS), a non-invasive method of probing cortical function, a growing body of literature (De Beaumont et al. 2009; Miller et al. 2014; De Beaumont et al. 2007; Tremblay et al. 2011) suggests that motor cortex function is affected by TBI. Specifically, inhibition, as measured by the cortical silent period, is increased (De Beaumont et al. 2009; Miller et al. 2014; De Beaumont et al. 2007; Tremblay et al. 2011) and is related to slowness of movement up to 30+ years post-injury (De Beaumont et al. 2009). These results suggest that high levels of cortical inhibition may be a prime mechanistic candidate for the motor deficits observed acutely and up to two months of recovery following mTBI (Catena et al. 2009; Parker et al. 2007; Parker et al. 2006)

The purpose of this study was to determine the level of excitability and inhibition in the motor cortex of individuals with acute and chronic symptoms from mTBI, compared with healthy controls. It was hypothesized that acutely-injured individuals would experience higher levels of motor cortex excitability and higher levels of intracortical inhibition than healthy control participants, and that individuals with chronic symptoms from mTBI would experience similar levels of motor cortex excitability and higher levels of intracortical inhibition compared with healthy control participants.

## **Materials and methods**

### *Participants*

Ninety-one individuals were assigned to one of four groups: (i) without history of mTBI (Control, n=27, 15 females), (ii) within 72-hours of diagnosis of mTBI (Acute,



n=29, 15 females), (iii) with history of mTBI and no remaining symptoms (Chronic Control, n=24, 11 females), and (iv) with chronic symptoms from mTBI, lasting at least 3 months post-injury (Chronic, n=13, 6 females). All participants provided written informed consent and were asked to complete a brief medical history and TMS safety screening questionnaire to determine eligibility to participate before enrollment.

Exclusion criteria for all participants included: 1) history of cognitive deficiencies, such as permanent memory loss or concentration abnormalities (unrelated to the mTBI injury), 2) loss of consciousness from the mTBI lasting more than one minute, 3) history of attention deficit hyperactivity disorder, or 4) contraindications to the use of TMS (Rossi et al. 2011). All procedures were reviewed and approved by the University of Oregon Institutional Review Board prior to any data collection. Three individuals (two from the Chronic group and one from the Chronic Control group) were excused from this portion of the study due to contraindications with TMS.

#### *Measurement schedule*

Each participant completed at least one testing session, during which measures of motor cortex excitability and inhibition were obtained using TMS. Participants in the Acute group were tested within 72 hours of sustaining their mTBI ( $\pm 2$  days). Participants in the Chronic group were tested an average of 4.0 years post-injury ( $\pm 2.0$  years), and those in the Chronic Control group were tested an average of years 3.9 years post-injury ( $\pm 2.8$  years).

A total of 9 Acute and 14 Control participants came in for additional testing. These acutely-injured individuals came in for testing within 72 hours of injury and again

at 1 week, 2 weeks, 1 month, and 2 months post-injury. The 14 Control participants followed a similar timeline, once recruited.

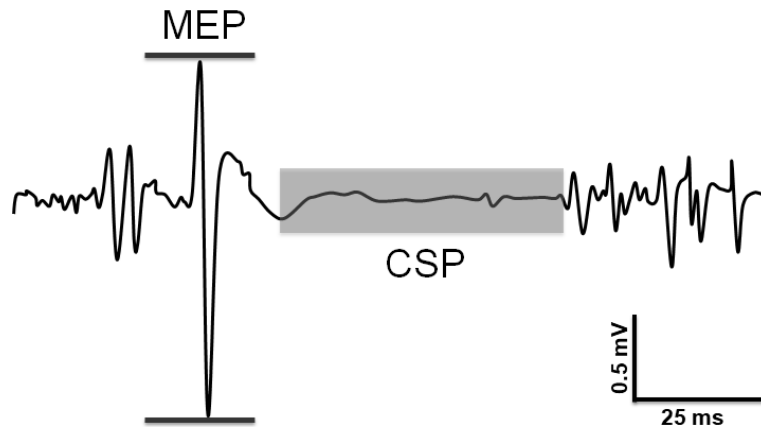
#### *Electromyography (EMG)*

A preamplified bipolar, Ag-AgCl EMG electrode (DE-2.1, Delsys Inc., Boston, MA), with an inter-electrode distance of 1 cm was placed over the first dorsal interosseous (FDI) of the dominant hand. This electrode was connected to a portable amplifier (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 sampled at 5 kHz with a 16-bit A/D converter (NI USB-6251, National Instruments, Austin, TX).

#### *Motor evoked potential (MEP) and Cortical Silent Period (CSP)*

TMS was performed using a flat, 70-mm figure-of-eight coil positioned over the optimal site of the contralateral motor cortex to elicit motor evoked potentials (MEP) in the dominant FDI. The optimal site was defined as the position that yielded the largest MEP consistently. Once the optimal site was located, the resting motor threshold (RMT) was determined as the lowest intensity required to evoke a response of at least 50 $\mu$ V in at least 5 out of 10 trials (Orth and Rothwell 2004; Werhahn et al. 1999). Eight cortical silent periods were then evoked by single-pulse stimulations delivered at 120% of the RMT while participants maintained an isometric of the dominant FDI at 50% of their maximal voluntary contraction (MVC). Visual feedback, including a target line at 50% MVC, was provided to the participants and the experimenter ensured that participants had reached the target level of EMG prior to providing the TMS pulse. A rest period of 30 seconds was provided between stimulations.

Motor cortex excitability was assessed through the peak-to-peak amplitude of the active MEP (Figure 2.1, horizontal lines). Inhibition was assessed through the CSP duration and was manually identified for each trial as the time between the end of the MEP and the resumption of voluntary EMG activity (Figure 2.1, shaded area). All trials were analyzed, using a custom-written MATLAB (Mathworks Inc., Natick, MA) program, by the same trained investigator, who was blinded to the participants' group at the time of data analysis. Such manual selection of EMG onset times has been shown to have similar reliability as automated procedures (Ives and Wigglesworth 2003).



**Figure 2.1. Sample recording of evoked responses.** Excitability was assessed through the peak-to-peak amplitude of the MEP (horizontal lines). Inhibition was assessed through the cortical silent period (CSP) duration (shaded area).

### *Statistical analysis*

Two-factor repeated measures ANOVAs (mTBI group, time) were calculated for the sub-set of Acute and Control participants who came to lab for multiple days of testing. One-factor (mTBI group) ANOVAs were performed to assess differences in MEP amplitude and CSP duration between all four groups. Where necessary, post-hoc pairwise comparisons with Bonferroni adjustments were made.

## Results

### *Participants*

Participant characteristics are presented in Table 2.1. There were no group differences in height ( $p=0.10$ ) or weight ( $p=0.2$ ), but the Chronic group was significantly older than the other three groups ( $p=0.003$ ).

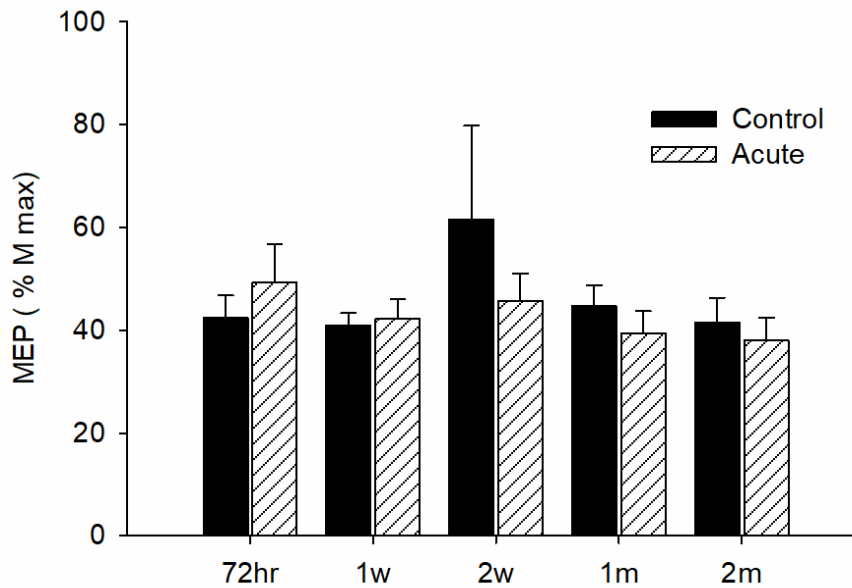
**Table 2.1. Participant Characteristics**

	<b>Control</b>	<b>Acute</b>	<b>Chron. Control</b>	<b>Chronic</b>	<b><i>p</i></b>
<b><i>n</i></b>	27 (15 f)	29 (15 f)	20 (9 f)	11 (6 f)	
<b>Age (years)</b>	21.6 ± 3.9	21.0 ± 3.8	20.2 ± 2.1	26.7 ± 9.5	0.003
<b>Height (cm)</b>	169.5 ± 10.2	174.4 ± 11.1	173.1 ± 8.3	164.6 ± 7.3	0.10
<b>Mass (kg)</b>	65.0 ± 10.0	73.2 ± 14.2	71.7 ± 14.3	62.0 ± 12.4	0.20

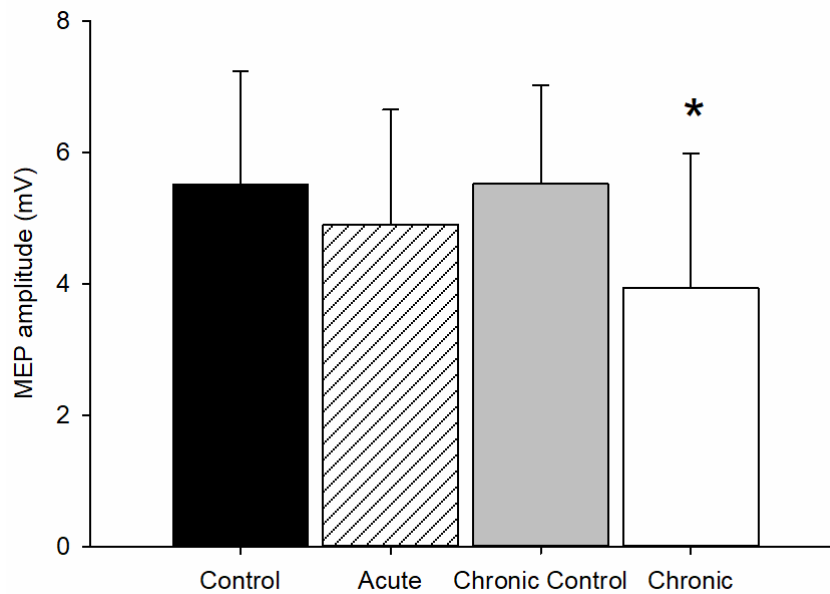
### *Motor evoked potential (MEP)*

There was no group difference in MEP amplitude between the subset of Acute and Control participants who completed 5 sessions of testing over a 2-month period ( $p=0.20$ , Figure 2.2). There was no significant effect of time on MEP amplitude ( $p=0.10$ ) and there was no group by time interaction ( $p=0.24$ ).

For the comparison between all four groups, the MEP amplitude was significantly different between groups ( $p=0.05$ , Figure 2.3). Post-hoc comparisons revealed that the Chronic group had a significantly smaller MEP amplitude compared to the Control ( $p=0.02$ ) and Chronic Control ( $p=0.02$ ) groups, indicating lower cortical excitability in the Chronic group. Data from three participants in the Chronic Control group were found to be outliers and removed from the analysis (data from 20 total Chronic Control participants were used in this analysis).



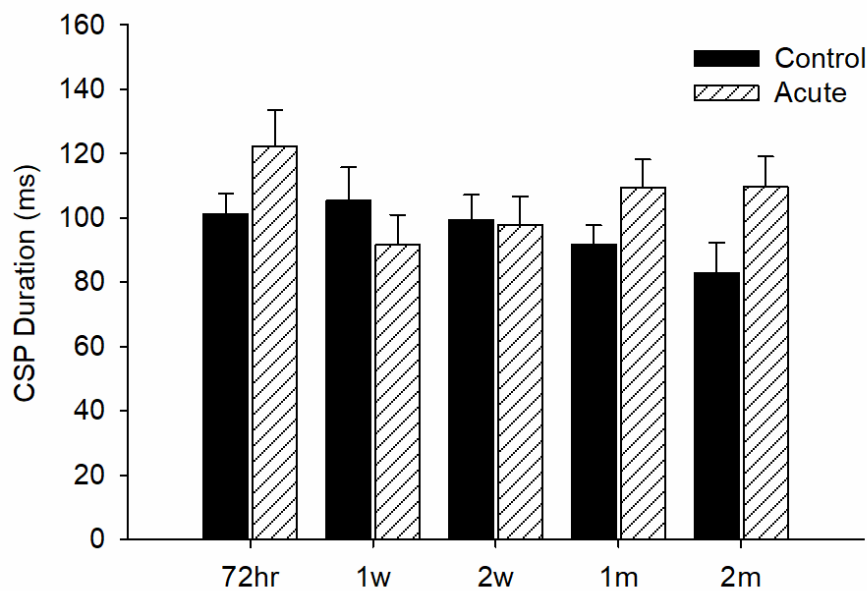
**Figure 2.2. MEP amplitude across time.** There was no effect of group ( $p=0.20$ ) or time ( $p=0.10$ ) on MEP amplitude over the course of recovery. There was no interaction between group and time ( $p=0.24$ ).



**Figure 2.3. MEP amplitude group comparison.** The MEP amplitude was significantly different between groups ( $p=0.05$ ). Post-hoc comparisons revealed that the Chronic group had a significantly smaller MEP amplitude compared to the Control ( $p=0.02$ ) and Chronic Control ( $p=0.02$ ) groups.

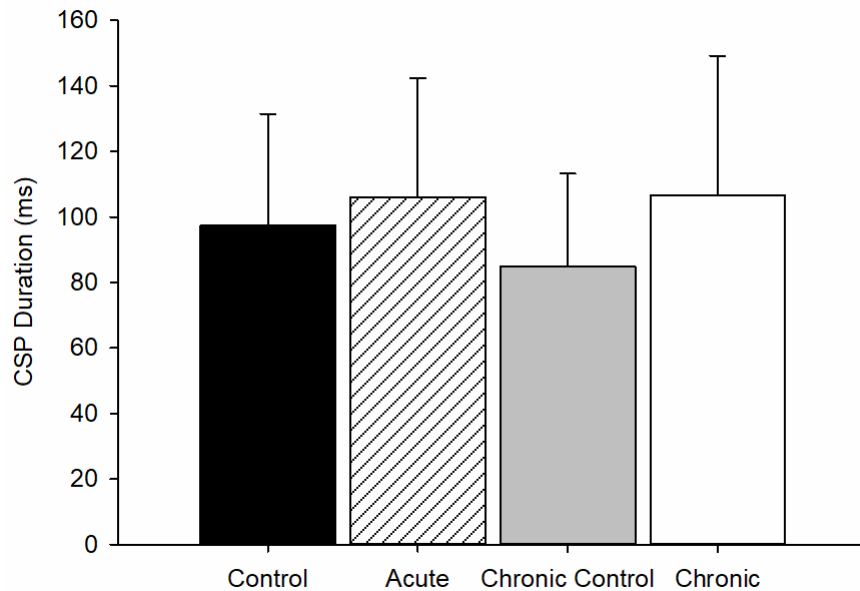
### Cortical silent period (CSP)

For the subset of participants who were tested across two months, the CSP duration was significantly longer in the Acute group compared to controls ( $p=0.02$ , Figure 2.4). Post-hoc analyses revealed the CSP duration was significantly longer in the Acute group at 72-hours, 1 month, and 2 months post-injury. There was no significant effect of time on CSP duration ( $p=0.20$ ). There was a significant group-by-time interaction ( $p\leq 0.001$ ) with longer CSP durations in the Acute group compared to controls at 72 hours ( $p=0.03$ ), 1 month ( $p=0.003$ ), and 2 months post-injury ( $p=0.005$ ).



**Figure 2.4. CSP duration across time.** The CSP duration was significantly longer in the Acute group compared to controls ( $p=0.02$ ), specifically, at 72-hours, 1 month, and 2 months post-injury. There was no significant main effect of time on CSP duration ( $p=0.20$ ) There was a significant group-by-time interaction ( $p\leq 0.001$ ) with longer CSP durations in the Acute group compared to controls at 72 hours, 1 month, and 2 months post-injury.

The comparison between all four groups indicated that the CSP duration was not significantly different between groups ( $p=0.14$ , Figure 2.5) indicating no effect of mTBI on intracortical inhibition.



**Figure 2.5. CSP duration group comparison.** The CSP duration was not different between groups ( $p=0.14$ ).

## Discussion

The purpose of this investigation was to determine the level of excitability and inhibition in the motor cortex of individuals with acute and chronic symptoms from mTBI, compared with healthy controls. The results demonstrated that individuals with chronic symptoms from mTBI had lower MEP amplitudes than individuals in the two control groups, indicating lower cortical excitability in the Chronic group. No differences in intracortical inhibition as assessed by the cortical silent period were observed between groups, suggesting that mTBI did not affect intracortical inhibition.

### *Excitability*

It was originally hypothesized that the Acute group would display higher levels of cortical excitability compared with the control group at 72 hours post-injury. We found that excitability, as assessed by the MEP amplitude, was not different between the subset of Acute and Control participants at any point throughout the 2-month recovery period. While we did not report any differences in MEP amplitude in our Acute group, this finding is consistent with other studies that have also reported no differences in cortical excitability 72-hours to 2-weeks post-mTBI (Miller et al. 2014; De Beaumont et al. 2007). In the rodent model, the acute, excitatory phase of the Neurometabolic Cascade following mTBI tends to resolve fairly quickly (Giza and Hovda 2001). While the time course of this cascade is unknown in humans, it is possible that the initial release of excitatory neurotransmitters in the brain following mTBI may resolve in the human brain by 72-hours post-injury. It is possible that differences in excitability in our Acute group were missed because too much time had elapsed between the initial injury and the data collection.

To our knowledge, this is the first study to report differences in cortical excitability in individuals with chronic symptoms from mTBI compared to healthy individuals. Results from this study indicated that chronically-symptomatic individuals showed smaller MEP amplitudes, and therefore, lower cortical excitability, than those in the two control groups, but similar levels of cortical excitability to those with acute mTBI. Based on animal literature regarding the relative timeline of metabolic changes in the brain post-mTBI, it was originally hypothesized that individuals with chronic symptoms from mTBI would be outside the window of time for excitotoxic changes to



occur in the brain, and therefore, show no differences in cortical excitability compared to control groups. Following the initial stage of the Neurometabolic Cascade, however, the brain enters a “spreading depression” phase that may have longer-lasting effects than the acute excitotoxic phase (Giza and Hovda 2001). It is possible that individuals whose symptoms do not recover from mTBI after months or years may still be in this altered “depressed” stage of metabolic and ionic imbalance.

It should be addressed that the average participant in the Chronic group was significantly older (26.7 years) than participants in the other three groups (< 22 years). Eisen et al., (1991) previously reported smaller MEP amplitudes in older individuals, but the average age of participants in this study was 63 years. Although participants in the Chronic group for this study were, on average, older, the average age was still well below what is considered to be an “elderly” or “older adult” population. Therefore, while the difference in age may have impacted the MEP results in this study, it is unlikely.

### *Inhibition*

Based on previous studies documenting higher levels of intracortical inhibition both acutely and longer-term following mTBI (Miller et al. 2014; De Beaumont et al. 2007), it was hypothesized that both the Acute and Chronic groups would have higher levels of intracortical inhibition. Similar to this previous research, we found that the CSP duration was longer in the subset of Acute and Control participants during 2 months of recovery. When using the acute, 72-hour time point as a comparison, however, we did not report any difference in CSP duration between all four participant groups. Although this comparison was not statistically different, the CSP duration was slightly higher in the Acute and Chronic groups compared to the two control groups, following a similar trend

as the longitudinal data from the subset of participants who came for multiple days of testing. The addition of more Acute and Control participants as well as the Chronic Control and Chronic groups introduced slightly more variability into the CSP data, which may explain why the difference in CSP duration did not reach statistical significance. It is also possible that additional factors may be responsible for TMS measures of inhibition, such as concentrations or availability of inhibitory neurotransmitter. To our knowledge, no studies have reported levels of intracortical inhibition in individuals with chronic symptoms from mTBI. Although our Chronic group did not demonstrate longer CSP durations than their control group, data from the longitudinal data from the Acute group suggests that there is increased inhibition during the subacute phase, which may help to explain some of the motor symptoms observed during this period of time following mTBI (Howell et al. 2013b; Howell et al. 2015). However, these data do not suggest a long-term difference in inhibition, as there were no differences in the Chronic group compared to controls. Therefore, cortical inhibition may not explain the persistent symptoms of chronic mTBI. Future studies should address why symptoms persist in some individuals, but not others, as well as the time course for changes in neurochemical activity following mTBI in humans

## **Conclusion**

We have shown that while cortical excitability does not seem to be altered acutely following mTBI, intracortical inhibition remains elevated into the subacute phase following mTBI. In individuals with chronic symptoms from mTBI, excitability was significantly different from healthy controls. While the Chronic group displayed lower cortical excitability compared to controls, no differences in intracortical inhibition in this

group were reported. Further research is needed to investigate the neurotransmitters responsible for TMS measures of cortical excitability and inhibition.

### **Bridge to Chapter III**

In Chapter II, alterations in motor cortex excitability were identified in individuals with chronic symptoms from mTBI, but there were no differences in cortical inhibition following mTBI. In Chapter III, potential differences in the neurotransmitters responsible for the TMS measures of excitability and inhibition, glutamate and GABA, are examined in individuals following mTBI.

## CHAPTER III

### GLUTAMATE AND GABA CONCENTRATIONS FOLLOWING MTBI

Some material from this chapter was accepted for publication in June 2018 in the *Journal of Neurophysiology*. Alia Yassen contributed to the concept of the study, recruited participants, collected and analyzed data, and helped prepare the initial manuscript. Dr. Jolinda Smith programmed the MRI functional localizer scan and provided support for the magnetic resonance spectroscopy sequences. Dr. Anita Christie contributed to the concept of the study, aided in the interpretation of the data, and critically reviewed and revised the manuscript.

#### **Introduction**

Mild traumatic brain injury (mTBI) is classified as an injury to the brain caused by mechanical force acting on the body (McCrory et al. 2013). It can lead to cognitive symptoms such as impaired memory, confusion, difficulty concentrating (Cantu 2006) and deficits in physical function such as slow movement speed (Gray et al. 1998), impaired balance (Chou et al. 2004), and slowed reaction time (Howell et al. 2013a). Widespread changes in metabolite concentration are a likely candidate to explain physical symptoms post-mTBI (Henry et al. 2010).

Work in rodent models of mTBI suggests that immediately after a mechanical injury to the head, the brain enters a state of excitation as excitatory neurotransmitters, such as glutamate, are released (Giza and Hovda 2001). Glutamate transport decreases following mTBI, allowing excess glutamate to stay in the synapse and prolong the excitotoxic environment in the brain. In the rodent TBI model, the glutamate transporter GLUT-1 remains down-regulated up to 7 days post-injury (Cantu et al. 2015). This event,

and the resulting changes in metabolic environment in the brain that follow, is known as the Neurometabolic Cascade.

Following the initial excitatory phase, an inhibitory, “spreading depression” phase occurs as the brain attempts to maintain homeostasis (Giza and Hovda 2001). Studies show that neurons injured following mTBI demonstrate a reduction in dendrite length and inhibited neuronal signaling, suggesting that mTBI may lead to a change in the excitatory/inhibitory balance post-injury (Brizuela et al. 2017). Indirect evidence from transcranial magnetic stimulation studies suggests the potential for similar alterations in neurotransmitters in humans, as cortical excitability and inhibition have been shown to be altered following mTBI (Livingston et al. 2010; Miller et al. 2014). However, studies involving direct measurements of neurotransmitters in humans are limited.

Proton magnetic resonance spectroscopy ( $^1\text{H-MRS}$ ) is a non-invasive, reliable (Yasen et al. 2017) technique that can be used to determine relative concentrations of target metabolites in vivo in the human brain. Using this technique, it has been shown that, despite normal neuropsychological scores and anatomical MRI results, athletes with mTBI have altered neurometabolic profiles in comparison to controls (Henry et al. 2010). Specifically, these athletes were reported to have lower levels of glutamate, the primary excitatory neurotransmitter, in the primary motor cortex (M1). Measures of GABA, the primary inhibitory neurotransmitter, have not shown to be different in M1 post-mTBI (Tremblay et al. 2014). These previous studies, however, involved measurements taken from those with acute injury (Henry et al. 2010) and asymptomatic controls (Tremblay et al. 2014). Assessments from individuals with chronic symptoms from mTBI will substantially enhance our understanding of the neurophysiological recovery from mTBI.

The purpose of this study, therefore, was to determine concentrations of glutamate and GABA in the brain acutely after mTBI and in those who experience chronic symptoms ( $\geq 3$  months) following mTBI. It was hypothesized that these concentrations would follow a pattern similar to that of rodent models, with higher concentrations of excitatory neurotransmitters acutely post-injury and higher concentrations of inhibitory neurotransmitters in those with chronic symptoms from mTBI.

## **Materials and methods**

### *Participants*

Fifty-three individuals were assigned to one of four groups: (i) without history of mTBI (Control, n=11, 6 females), (ii) within 72-hours of diagnosis of mTBI (Acute, n=9, 5 females), (iii) with history of mTBI and no remaining symptoms (Chronic Control, n=20, 10 females), and (iv) with chronic symptoms from mTBI, lasting at least 3 months post-injury (Chronic, n=13, 6 females). All injuries met the definition of concussion provided by the 4<sup>th</sup> International Consensus Statement on Concussion in Sport (McCrory et al. 2013). All participants provided written informed consent and were asked to complete a brief medical history and TMS safety screening questionnaire to determine eligibility to participate before enrollment.

Exclusion criteria for all participants included: 1) history of cognitive deficiencies, such as permanent memory loss or concentration abnormalities (unrelated to the mTBI injury), 2) loss of consciousness from the mTBI lasting more than one minute, 3) history of attention deficit hyperactivity disorder, or 4) contraindications to the use of MRS. All procedures were reviewed and approved by the University of Oregon Institutional Review Board prior to any data collection. Three individuals (one each from

the Control, Chronic Control, and Chronic groups) were excused from this portion of the study due to contraindications with MRS, leaving a total of 50 participants.

#### *Measurement schedule*

Each participant completed at least one testing session, during which measures of glutamate and GABA concentrations were obtained using proton magnetic resonance spectroscopy ( $^1\text{H-MRS}$ ) from the primary motor cortex (M1).  $^1\text{H-MRS}$  was performed in a 3T whole-body MR scanner (Skyra: Siemens, Erlangen, Germany) using a 32-channel receive-only phased-array head coil. Individuals in the acute mTBI group were tested within 72 hours of sustaining their injury ( $\pm 2$  days). Participants in the Chronic group were tested an average of 3.4 years post-injury ( $\pm 2.1$  years), and those in the Chronic Control group were tested an average of 4.3 years post-injury ( $\pm 2.9$  years).

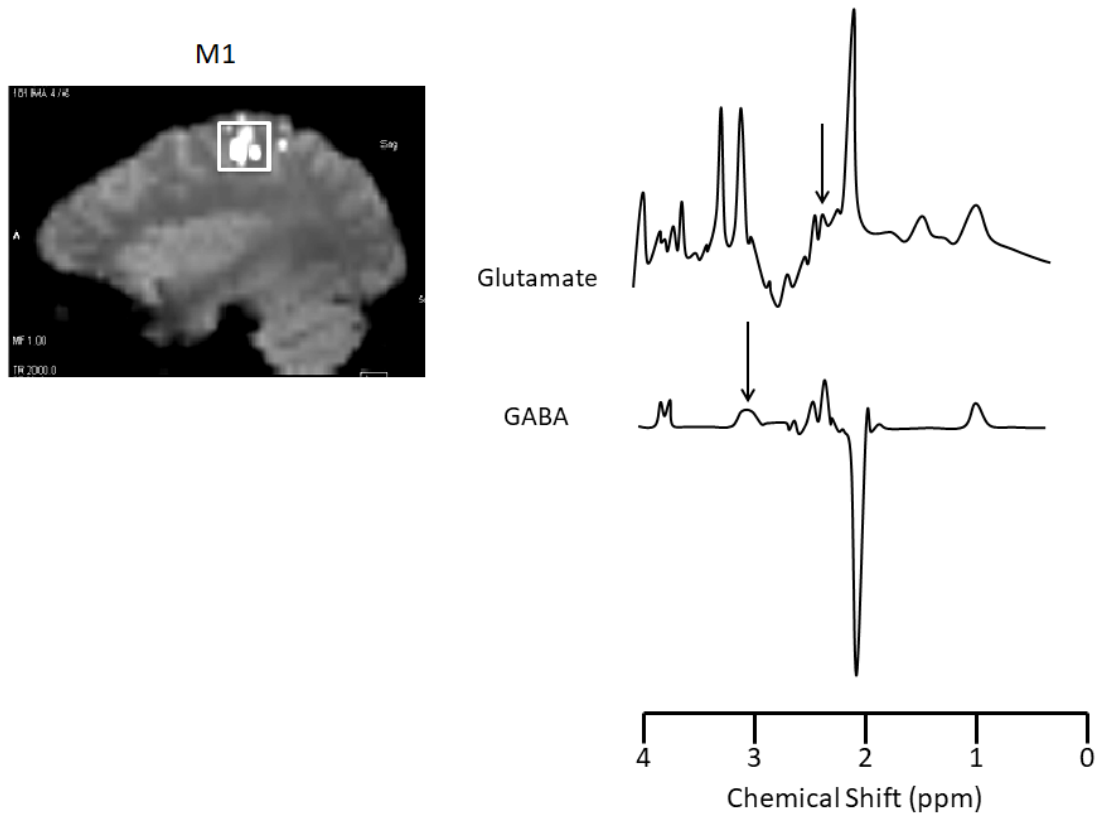
A total of 9 Acute and 9 Control participants came in for additional testing. These acutely-injured individuals came in for three testing sessions, first, within 72 hours of injury, then again at 1 month and 2 months post-injury. The 9 Control participants followed a similar timeline, once recruited.

Anatomical MRI images of the brain were acquired and reviewed by the MRI technologist, and no incidental findings or brain abnormalities were reported for any of the participating subjects.

#### *Functional localizer task*

A functional localizer task was administered in order to place a voxel at M1. During the M1 localizer task, participants were asked to tap the index finger of their dominant hand on the MRI bed upon visual presentation of a target word. Participants alternated between tapping for 24 seconds and resting for 24 seconds for approximately 3

minutes. Once the localizer task was complete, a voxel measuring  $20 \times 20 \times 20$  mm was placed in the region activated by the localizer contrast representing M1 (Figure 3.1, left).



**Figure 3.1. Functional localizer scans for voxel placement and Glutamate and GABA sample spectra from M1.** Left: M1 localizer scan (voxel size:  $20 \times 20 \times 20$  mm). Right: The area under glutamate (2.4 ppm; TR/TE=1500/30ms, 256 acquisitions) and GABA (3.0 ppm; TR/TE=2000/68ms, 128 acquisitions) peaks were calculated and expressed relative to total creatine. Target peaks for glutamate and GABA are indicated by an arrow.

#### *Glutamate and GABA estimation*

A 6-minute single-voxel PRESS sequence (TR/TE=1500/30ms, 256 acquisitions) (Henry et al. 2010) was used to assess glutamate and a 9-minute adapted MEGA-PRESS sequence for GABA (TR/TE=2000/68ms, 128 acquisitions) (Mullins et al. 2013). Sample spectra are shown in Figure 3.1., right. The area under glutamate (2.4 ppm) and GABA (3.0 ppm) peaks were calculated and expressed relative to total creatine using LCMoDel



(Provencher 1993). Creatine values did not change over time in M1 ( $p=0.25$ ). Therefore, we are confident that any differences in metabolic ratios expressed over creatine between groups or over time are a result of changes in glutamate or GABA rather than creatine.

### *Statistical analysis*

Two-factor repeated measures ANOVAs (mTBI group, time) were calculated for the sub-set of Acute and Control participants who came to lab for multiple days of testing. Unpaired t-tests were used to compare age, height, and weight between all four groups. One-factor (mTBI group) ANOVAs were used to assess differences in glutamate and GABA concentration between mTBI groups. Where necessary, post-hoc pairwise comparisons with Bonferroni adjustments were made.

## **Results**

### *Participants*

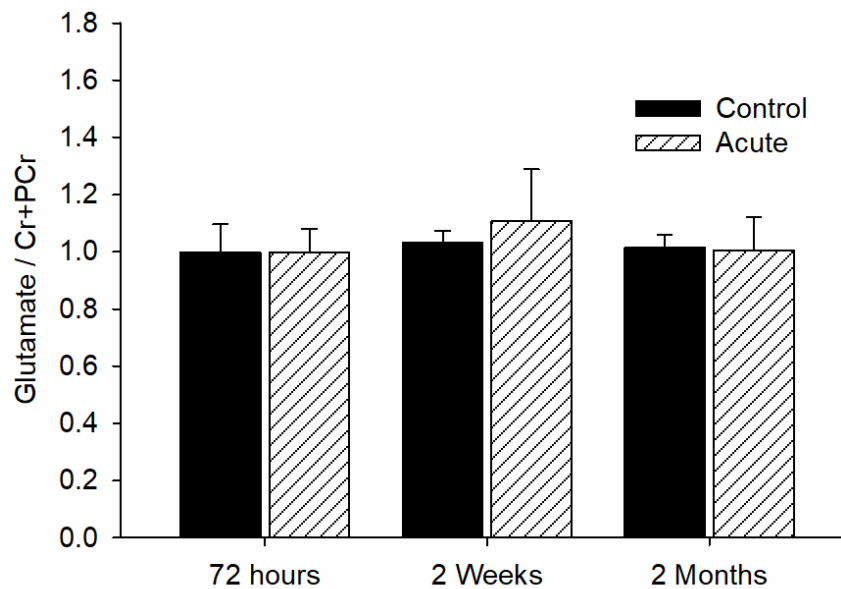
Participant characteristics are presented in Table 3.1. There were no group differences in height ( $p=0.21$ ) or weight ( $p=0.28$ ), but the Chronic group was significantly older than the other three groups ( $p=0.009$ ).

**Table 3.1. Group Characteristics**

	<b>Control</b>	<b>Acute</b>	<b>Ch. Cont.</b>	<b>Chronic</b>	<b><i>p</i></b>
<b><i>n</i></b>	10 (6 f)	9 (5 f)	19 (10 f)	12 (6 f)	
<b>Age (years)</b>	21.1 ± 0.9	20.8 ± 2.3	20.6 ± 2.0	26.7 ± 9.5	0.009
<b>Height (cm)</b>	169.9 ± 10.7	174.4 ± 11.1	173.7 ± 8.3	164.6 ± 7.3	0.21
<b>Mass (kg)</b>	66.1 ± 9.8	73.2 ± 14.2	72.5 ± 14.3	62.0 ± 12.4	0.28

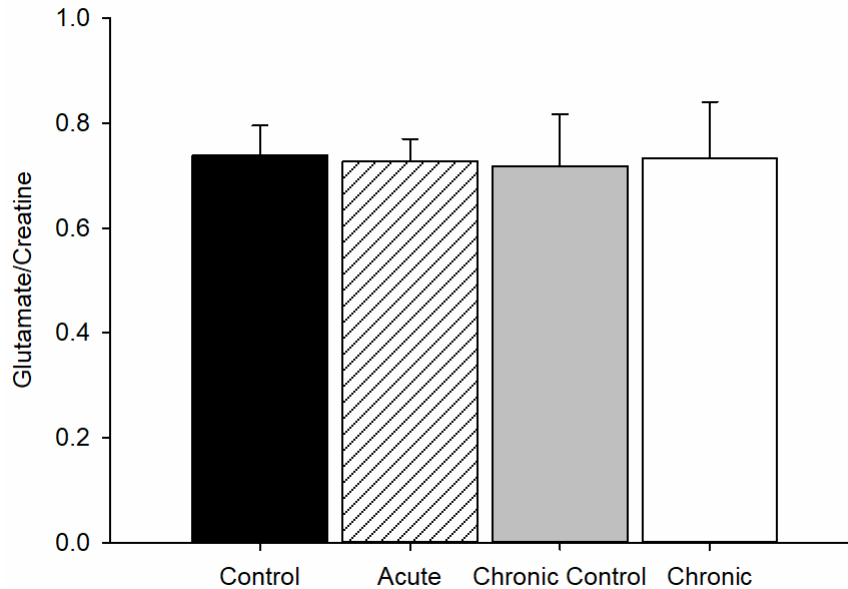
## Glutamate

For the subset of Acute and Control participants who participated in three sessions of testing over a 2-month recovery period, glutamate concentrations were similar between groups ( $p=0.70$ ; Figure 3.2) but significantly different across time ( $p=0.03$ ). While the 2-week time point was slightly higher than the 72-hour time point, this difference did not reach statistical significance ( $p\geq 0.06$ ). Post-hoc analysis did not reveal a significant difference between any other time points ( $p\geq 0.10$ ). There was no significant interaction between group and time ( $p=0.13$ ).



**Figure 3.2. Glutamate concentrations/creatinine in M1 over time.** Glutamate concentrations were similar between groups ( $p=0.70$ ) and across time ( $p\geq 0.10$ ). There was no significant interaction between group and time ( $p=0.13$ ).

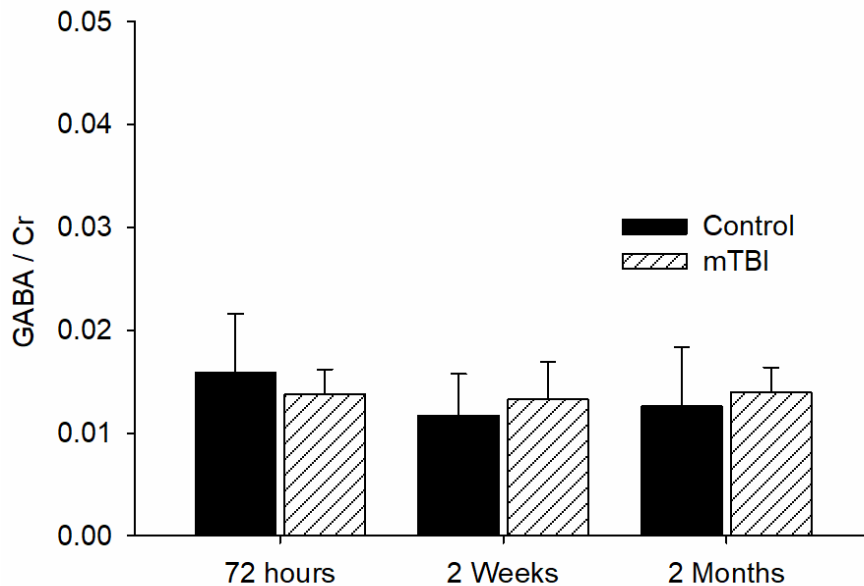
Glutamate/creatinine values in M1 for all four groups are shown in Figure 3.3. Results from the ANOVA test indicated that glutamate/creatinine values were similar between groups ( $p=0.93$ ).



**Figure 3.3. Glutamate concentrations/creatinine in M1.** Glutamate/creatinine concentrations were similar between groups ( $p=0.93$ ).

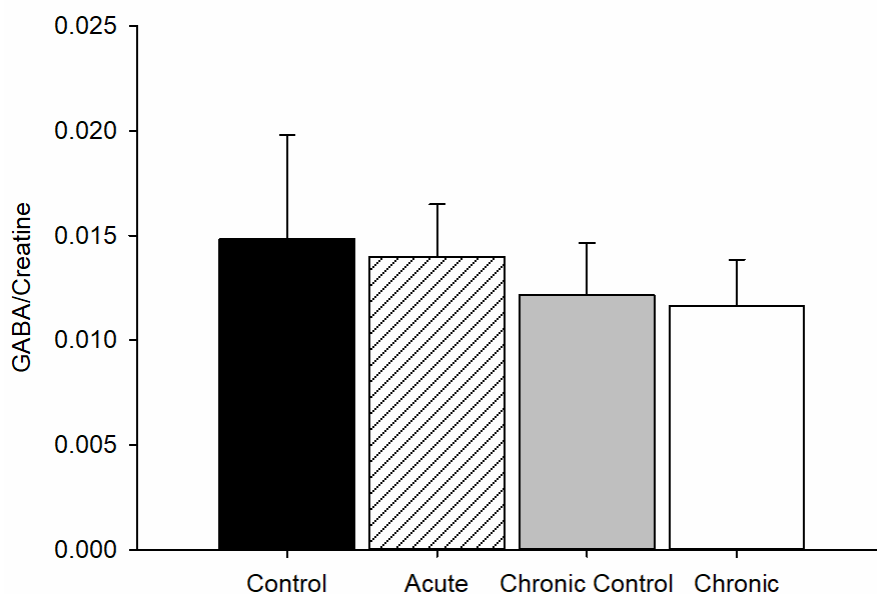
### GABA

GABA concentrations for the subset of Acute and Control participants were similar between groups ( $p=0.57$ ; Figure 3.4) and across time ( $p=0.13$ ). There was a significant interaction between group and time ( $p=0.05$ ), however, post-hoc comparisons did not reveal any significant difference in GABA between groups at any of the three time points ( $p\geq 0.40$ ).



**Figure 3.4. GABA concentrations/creatinine in M1 over time.** GABA concentrations were similar between groups ( $p=0.57$ ) and across time ( $p=0.13$ ). There was a significant interaction between group and time ( $p=0.05$ ), however, post-hoc comparisons did not reveal any significant difference in GABA between groups at any of the three time points ( $p \geq 0.40$ ).

GABA/creatinine values in M1 for all four groups are shown in Figure 3.5. Results from the ANOVA test indicated that GABA/creatinine values were not quite statistically different between groups ( $p=0.06$ ). Post-hoc analysis indicated that the trend for significance was between the Acute and Chronic groups, where the Chronic group had lower levels of GABA in M1 compared to Acute participants ( $p=0.04$ ).



**Figure 3.5. GABA concentrations/creatinine in M1.** Concentrations of GABA/creatinine were not quite statistically different between groups ( $p=0.06$ ).

## Discussion

The purpose of this study was to determine concentrations of glutamate and GABA in the brain acutely after mTBI and in those with chronic symptoms ( $\geq 3$  months) post-mTBI. These results suggest that individuals with mTBI have similar levels of glutamate and GABA in M1 compared with healthy controls, and these concentrations do not seem to change over time during recovery.

Evidence from animal studies suggests that the concentration of glutamate is higher in the brain immediately post-mTBI, but typically resolves within hours (Giza and Hovda 2001). In the current study we found no difference in concentrations of glutamate in M1 in those with and without mTBI at any time point over a period of recovery. These results are similar to those of Tremblay et al., (2014), who found no difference in M1 concentrations of glutamate in athletes 3 years after an mTBI. However, further

conflicting evidence suggests lower levels of glutamate in M1, in athletes with mTBI, one week post-injury (Henry et al. 2010). Taken together, the data from these studies suggest that potential changes in glutamate concentrations are highly dependent on the time post-injury and the time course of changes may be different for different regions of the brain. Further work is necessary to establish such a time course in humans.

Results from the present study suggest no differences in GABA concentration in M1 at any time point during a period of recovery from mTBI. Few  $^1\text{H}$ -MRS studies have recorded GABA concentrations post-mTBI, but Tremblay et al., (2014) found no differences in M1 GABA concentrations in their mTBI group 3 years post-injury. To our knowledge, no studies have reported GABA concentrations in individuals with chronic symptoms post-mTBI. Like glutamate, it is possible that changes in GABA concentrations in individuals post-mTBI may be region-specific.

One limitation with  $^1\text{H}$ -MRS is that this technique provides an assessment of the total concentrations of metabolites in the brain and is not capable of measuring intra- and extra-cellular volumes separately. Therefore, it is possible that differences in the release or action of neurotransmitters like glutamate and GABA may exist in individuals following mTBI, but these alterations are not able to be detected using the  $^1\text{H}$ -MRS technique.

## **Conclusion**

Differences in glutamate and GABA concentration in the brain post-mTBI did not follow the pattern typically seen in the animal literature. In humans, changes in glutamate and GABA concentrations in the brain may depend on the amount of time that has elapsed post-injury, and may be region-specific.

## **Bridge to Chapter IV**

In Chapters II and III, potential differences in TMS and MRS measures of cortical excitability and inhibition were investigated in individuals with mTBI. In Chapter IV potential differences in these same measures of excitability and inhibition in M1 in individuals with risk factors for prolonged recovery and poorer outcomes following mTBI will be examined.

## CHAPTER IV

### RISK MODIFIERS FOR PROLONGED RECOVERY FOLLOWING MTBI

This chapter contains unpublished co-authored material. Alia Yasen contributed to the concept of the study, recruited participants, collected and analyzed data, and helped prepare the initial manuscript. Dr. Geeta Eick designed the genotyping and PCR reaction protocols, and aided in the interpretation of the data. Dr. Kirstin Sterner contributed to the concept and design of the study. Dr. Anita Christie contributed to the concept of the study, interpretation of the data, and critically reviewed and revised the manuscript.

#### **Introduction**

Numerous factors have been found to contribute to recovery from mTBI, and among the most extensively studied are the influence of sex and genetic factors that may predispose individuals to negative outcome following mTBI.

#### *Influence of sex on mTBI recovery*

Females now comprise 43% of the collegiate athlete population, an eightfold increase since Title IX went into effect in 1972 (Irick 2014). As the rate of female student-athlete participation in sports increases, a greater understanding of the role of sex in recovery from mTBI is needed.

Mortality rates in individuals after mTBI have been shown to be significantly higher in females compared with males (Klauber et al. 1981; Farace and Alves 2000) despite the fact that males are more at-risk for sustaining mTBI (Broshek et al. 2005). While many studies report significantly poorer post-injury outcomes in females following mTBI, the overall findings are inconsistent. Some research suggests that females suffer from more severe outcomes after mTBI, including more post-injury symptoms (Covassin



et al. 2013), longer reaction times (Colvin et al. 2009), and more significant declines in cognitive function from baseline pre-injury levels (Broshek et al. 2005; Covassin et al. 2013). However, other human and animals studies have reported no sex differences in recovery outcomes after mTBI (Zuckerman et al. 2012), and some have concluded that males have more long-lasting and severe cognitive outcomes than females (Groswasser et al. 1998).

While changes in motor cortex function (addressed in Chapter II) have been reported in individuals with mTBI, very little is known about how mTBI may differentially affect motor cortex function in males and females. Assessing neurophysiological differences post-injury can provide valuable insights into potential sex-specific approaches to treatment.

#### *APOE and mTBI*

The *APOE* gene has received much attention from mTBI researchers in recent years, specifically in regards to the association between the  $\epsilon 4$  allele with poorer outcome after TBI. Researchers have reported greater severity of injury (Jiang et al. 2011), worse Glasgow Coma Scale scores (Teasdale et al. 1997), lower motor scores on the Functional Independence Measure (Lichtman et al. 2000), and cognitive impairment in elderly and adolescent populations (Eramudgolla et al. 2014; Lawrence et al. 2015) in those individuals who possessed at least one  $\epsilon 4$  allele in their genotype. Possession of one or more  $\epsilon 4$  alleles is associated with a 10-fold increased risk of mTBI history in athletes (Tierney et al. 2010). Despite the relative rarity of the  $\epsilon 4$  allele in nature, this isoform is overrepresented in chronic traumatic encephalopathy and chronic TBI populations (McKee et al. 2009).

The detrimental effects of the  $\epsilon 4$  allele tend to occur in a dose-dependent fashion, meaning that the highest risks and poorest outcomes occur in those  $\epsilon 4$  homozygotes who possess two  $\epsilon 4$  alleles ( $\epsilon 4\epsilon 4$  genotype) (Guo et al. 2000). It has been suggested that the  $\epsilon 4$  allele produces a less efficient apoE protein in astrocytes and microglia, and its inadequacy is exaggerated in an environment like TBI where its role is more heavily required for recovery (Lawrence et al. 2015).

Animal studies provide insight into the role of *APOE* genotype and levels of excitatory and inhibitory neurotransmitter concentration in the cortex. Chen et al. (2010) observed that  $\epsilon 4$  carrier mice displayed reduced glutamate receptor function in the brain. Further, Dumanis et al., (2013) used magnetic resonance spectroscopy (MRS) and found decreased global concentrations of the excitatory neurotransmitter glutamate and increased levels of glutamine in the brains of  $\epsilon 4$  carrier mice. In this study, concentrations of GABA, an inhibitory neurotransmitter, were not different among  $\epsilon 4$  carriers. While the  $\epsilon 4$  allele has been associated with alterations of glutamate and GABA in the rodent brain, the relationship between this risk factor and neurochemical changes following mTBI in humans remains unknown.

### **Purpose**

The purpose of this study was to examine the potential influence of sex and genetic factors on neurophysiological recovery from mTBI. To accomplish this, two sub-studies are included, one addressing each risk factor. The aim of the first sub-study was to compare excitability and inhibition of the motor cortex in males and females with mTBI acutely following injury and throughout two months of recovery.

The aim of the second sub-study was to establish the difference in motor cortex function between apø4 carriers and non-carriers. It was hypothesized that carriers would have lower levels of excitability and excitatory transmitter (glutamate) and similar levels of intracortical inhibition and inhibitory neurotransmitter (GABA) than non-carriers.

## **Materials and methods**

### *Sub-study 1: The influence of sex on motor cortex function following mTBI*

#### Participants

Twenty individuals (ages  $21.2 \pm 4.4$  years; 10 female) who had suffered mTBI and 20 control individuals participated in this study. Individuals in the mTBI group were diagnosed by health professionals (physician or athletic trainer) as having a mild traumatic brain injury and reported to the laboratory within 72 hours post-injury. Of the 20 mTBIs sustained, 8 were sports-related and included injuries sustained from playing rugby (4), soccer (2), wrestling (1), and basketball (1). The 12 non-sports-related mTBIs were sustained as a result of bicycle accidents (4), household accidents (4), falls (3), and motor vehicle accidents (1). Eight individuals in the mTBI group had history of at least one previous mTBI. No other injuries requiring medical attention were reported. The control participants were age-, height-, weight-, sex-, and activity-matched to each concussed participant. Exclusion criteria included (1) a previous mTBI within a year prior to testing or two or more mTBIs prior to one year before testing, (2) loss of consciousness (LOC) for more than one minute (mTBI group), (3) history of cognitive deficiencies including memory loss or difficulty concentrating, (4) history of attention-deficit hyperactivity disorder, seizures, musculoskeletal impairments, neurological impairments, or (5) contraindications to the use of TMS. All participants completed a

brief medical history and TMS safety screening questionnaire (Wasserman 1998) to determine their eligibility to participate. Participants in the mTBI group who experienced a loss of consciousness for greater than 1 minute were excluded because of the role that prolonged LOC plays in mTBI management modification (McCrorry et al. 2013). All procedures were reviewed and approved by the Institutional Review Board at the University of Oregon and written informed consent was obtained prior to testing.

#### Testing Protocol

All participants completed 5 testing sessions, during which measures of cortical excitability and inhibition were obtained from the first dorsal interosseous (FDI) muscle of the dominant hand. All participants with mTBI were tested within 72 hours of sustaining their injury, and on four subsequent visits at: one week, two weeks, one month, and two months post-injury. Control participants followed a similar timeline.

#### Symptom inventory

Prior to each testing session, all participants completed a 22-item symptom inventory adopted from the IMPACT grading scale (McCrorry et al. 2009) and the recommendations outlined in a 2005 consensus statement (McCrorry et al. 2005). Severity of symptoms was rated on a 6-point Likert scale and summed across items to yield an overall score out of 132, with higher scores indicating worse symptoms.

#### Electromyography (EMG)

EMG activity was recorded with surface electrodes placed over the FDI muscle of the dominant hand. Prior to electrode placement, the skin over the FDI was cleaned to reduce signal impedance. A preamplified bipolar Ag-AgCl electrode (DE-2.1, Delsys Inc., Boston, MA), with an inter-electrode distance of 1 cm was connected to a portable

amplifier (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 sampled at 5kHz with a 16-bit A/D converter (NI USB-6251, National Instruments, Austin, TX) and stored for off-line analysis (Data Acquisition System Laboratory, DasyTec, USA Inc., Amherst, NH). At the beginning of each testing session, subjects performed two maximum voluntary contractions (MVCs), and the highest EMG amplitude of the two was recorded as their maximum.

#### Resting Motor Threshold (RMT) and Motor evoked potential (MEP)

Transcranial magnetic stimulation (TMS) was performed using a flat 70-mm figure-of-eight coil (Magstim 200, Magstim Ltd., Carmarthenshire, UK) positioned over the optimal site of the contralateral motor cortex to elicit MEPs in the dominant FDI. Once the optimal site was located, the RMT was determined as the lowest intensity required to evoke a response of at least 50 $\mu$ V in at least 5 out of 10 trials (Orth and Rothwell 2004; Werhahn et al. 1999). The stimulus intensity was decreased or increased in increments of 1 or 2% of stimulator output until an intensity that met the threshold criteria was found, while stimulation at 1% of stimulator output below that intensity did not meet the threshold criteria. Five stimuli were then delivered at an intensity of 120% of RMT to evoke MEPs with at least a 10 second inter-stimulus interval. Five stimulations have been shown to be sufficient to obtain reliable MEP amplitudes (Christie et al. 2007). These trials were recorded for offline analysis.

#### Cortical silent period (CSP)

Cortical silent periods were evoked by five single-pulse stimulations delivered at 120% of the RMT while participants maintained a voluntary isometric muscle contraction

of the dominant FDI at 50% of their maximum. Visual feedback of EMG activity was provided on an oscilloscope with target lines positioned at 50% of MVC. Five trials were completed with a rest period of 10-15 seconds between stimulations.

#### Data analysis

Sample recordings of an MEP and CSP are shown in Figure 2.1. All processing was completed by the same trained investigator, using custom programs written in Matlab software (Mathworks Inc., Natick, MA). The peak-to-peak amplitude of the MEP was determined by marking the onset and offset of the muscle response and calculating the magnitude of the range between the highest and lowest EMG value in the selected period. The CSP duration was manually determined as the time between the conclusion of the MEP and the resumption of voluntary EMG activity. Manual selection of EMG onset and offset times has been shown to be highly reliable when performed by the same experimenter (Ives and Wigglesworth 2003), and is a standard, reliable method for determining CSP duration (Kimberley et al. 2009). MEP amplitude and CSP duration were then averaged across the five trials of each measure for each participant. Experimenters were blinded to participant status during data analysis.

#### Statistical analysis

Two factor (group, sex) ANOVAs were performed to examine differences across groups in the descriptive characteristics of age, height and weight. Three-way repeated measures ANOVAs were performed to determine the effect of sex (female vs. male), group (mTBI vs. controls), time (72 hours, one week, two weeks, one month, and two months), and interaction effects for Symptom Score, RMT, MEP amplitude, and CSP duration. Post-hoc pairwise comparisons with Bonferroni correction were performed for

significant interactions. Data are presented as mean $\pm$ SEM. Effect sizes (Cohen's d) were calculated for the main effect of group and sex for RMT, MEP amplitude, and CSP duration. For all TMS measures, coefficients of variation were calculated to determine inter- and intra-subject variability.

*Sub-study 2: The influence of APOE genotype on motor cortex function following mTBI*

### Participants

Fifty-two participants between the ages of 18 and 60 participated in this study. Exclusion criteria for all participants included: 1) history of cognitive deficiencies, such as permanent memory loss or concentration abnormalities 2) history of attention deficit hyperactivity disorder, or 3) contraindications to the use of TMS or MRS. All procedures were reviewed and approved by the University of Oregon Institutional Review Board prior to any data collection. A total of 35 individuals consented to participate in both the TMS and MRS portions of the study, 16 participated in TMS only, and one individual participated in the MRS protocol only.

Of the fifty-two participants who provided saliva samples for genotyping, samples for twenty-three participants were collected remotely and mailed to the laboratory by the participant. These participants had previously completed the TMS and /or MRS protocol 1-5 years prior and agreed to provide a sample upon being contacted. These individuals used the same DNA self-collection kits as the participants who provided a sample on the day of testing.

### APOE genotype

All participants provided a saliva sample for genotyping using the Oragene OG-500 Self-Collection kits (DNA Genotek, Ontario, Canada). The saliva was stored at room

temperature and the DNA extracted according to the manufacturer guidelines and standard methodology. Genomic DNA was quantified using a Qubit 2.0 Fluorometer. Genotyping was performed on an Applied Biosystems StepOne Plus Real-time PCR machine using two predesigned Applied Biosystems TaqMan SNP Genotyping Assays (C-3084793-20 and C-904973-10) that targeted the *APOE* SNPs of interest (rs429358 and rs7412, respectively) (Applied Biosystems, Foster City, California). Briefly, 12.5 ng of DNA was added to an 8-strip tube containing 13.75  $\mu$ l of master reaction mix (12.5  $\mu$ l 2 $\times$  TaqMan Universal PCR Master Mix, and 1.25  $\mu$ l 20 $\times$  assay mix) along with negative (no template) controls in a final volume of 25  $\mu$ l.

#### Measurement schedule

Participants completed one session of TMS and/ or one session of MRS, during which measures of motor cortex excitability and inhibition were obtained using TMS, and measures of glutamate and GABA concentrations were obtained using proton magnetic resonance spectroscopy ( $^1\text{H}$ -MRS) from the primary motor cortex (M1).

#### Electromyography (EMG)

A preamplified bipolar, Ag-AgCl EMG electrode (DE-2.1, Delsys Inc., Boston, MA), with an inter-electrode distance of 1 cm was placed over the first dorsal interosseous (FDI) of the dominant hand. This electrode was connected to a portable amplifier (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 sampled at 5 kHz with a 16-bit A/D converter (NI USB-6251, National Instruments, Austin, TX).



### Motor evoked potential (MEP) and Cortical Silent Period (CSP)

TMS was performed using a flat, 70-mm figure-of-eight coil positioned over the optimal site of the contralateral motor cortex to elicit motor evoked potentials (MEP) in the dominant FDI. The optimal site was defined as the position that yielded the largest MEP consistently. Once the optimal site was located, the resting motor threshold (RMT) was determined as the lowest intensity required to evoke a response of at least 50 $\mu$ V in at least 5 out of 10 trials (Orth and Rothwell 2004; Werhahn et al. 1999). Eight cortical silent periods were then evoked by single-pulse stimulations delivered at 120% of the RMT while participants maintained an isometric of the dominant FDI at 50% of their maximal voluntary contraction (MVC). Visual feedback, including a target line at 50% MVC, was provided to the participants and the experimenter ensured that participants had reached the target level of EMG prior to providing the TMS pulse. A rest period of 30 seconds was provided between stimulations.

Motor cortex excitability was assessed through the peak-to-peak amplitude of the active MEP (Figure 2.1, horizontal lines). Inhibition was assessed through the CSP duration and was manually identified for each trial as the time between the end of the MEP and the resumption of voluntary EMG activity (Figure 2.1, shaded area). All trials were analyzed, using a custom-written MATLAB (Mathworks Inc., Natick, MA) program, by the same trained investigator, who was blinded to the participants' group at the time of data analysis. Such manual selection of EMG onset times has been shown to have similar reliability as automated procedures (Ives and Wigglesworth 2003).

### Functional localizer task

A functional localizer task was administered in order to place a voxel at M1. During the M1 localizer task, participants were asked to tap the index finger of their dominant hand on the MRI bed upon visual presentation of a target word. Participants alternated between tapping for 24 seconds and resting for 24 seconds for approximately 3 minutes. Once the localizer task was complete, a voxel measuring  $20 \times 20 \times 20$  mm was placed in the region activated by the localizer contrast representing M1 (Figure 3.1, left).

### Glutamate and GABA estimation

A 6-minute single-voxel PRESS sequence (TR/TE=1500/30ms, 256 acquisitions) (Henry et al. 2010) was used to assess glutamate and a 9-minute adapted MEGA-PRESS sequence for GABA (TR/TE=2000/68ms, 128 acquisitions) (Mullins et al. 2013). Sample spectra are shown in Figure 3.1., right. The area under glutamate (2.4 ppm) and GABA (3.0 ppm) peaks were calculated and expressed relative to total creatine using LCModel (Provencher, 1993). Creatine values did not change over time in M1 ( $p=0.25$ ). Therefore, we are confident that any differences in metabolic ratios expressed over creatine between groups or over time are a result of changes in glutamate or GABA rather than creatine.

### Statistical analysis

Unpaired t-tests were used to compare age, height, weight, motor evoked potential (MEP) amplitude, cortical silent period (CSP) duration, glutamate concentration, and GABA concentration in M1 between carriers and non-carriers of the apoε4 allele.

## Results

### *Sub-study 1*

Participant characteristics are presented in Table 4.1. There were no significant differences in age ( $F_{1,38}=1.48$ ;  $p>0.24$ ) or weight ( $F_{1,38}=0.002$ ;  $p>0.10$ ) across groups. Males were significantly taller than females ( $F_{1,38}=48.44$ ;  $p=0.0001$ ).

**Table 4.1. Group Characteristics (Sex)**

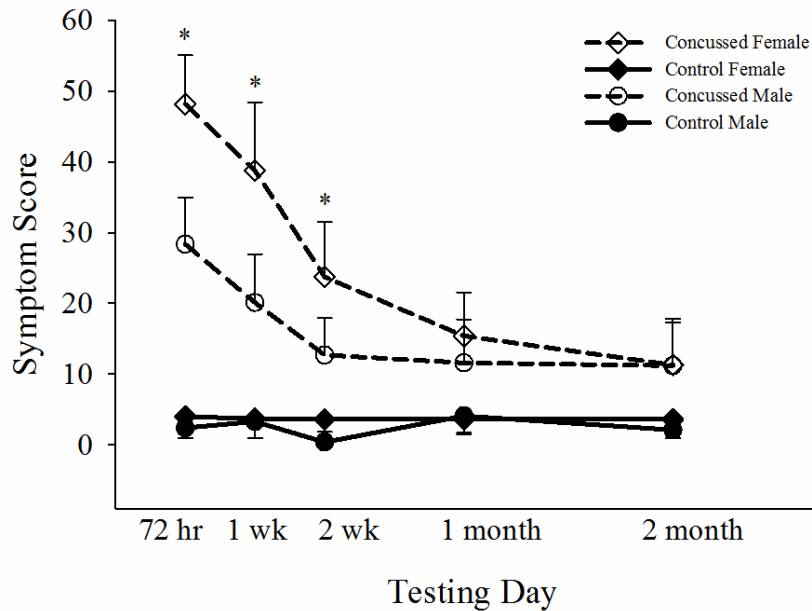
	<b>mTBI Female</b>	<b>mTBI Male</b>	<b>Control Female</b>	<b>Control Male</b>
<i>n</i>	10	10	10	10
<b>Age (years)</b>	20.2 ± 0.63	22.1 ± 1.86	20.6 ± 0.56	22.1 ± 2.02
<b>Height (cm)†</b>	165.1 ± 1.97	178.2 ± 2.13	164.6 ± 2.06	179.2 ± 1.99
<b>Mass (kg)</b>	69.0 ± 5.91	73.3 ± 3.63	66.7 ± 3.07	76.0 ± 3.28

†Significant difference between males and females ( $p < 0.0001$ )

### Symptom inventory

Figure 4.1 displays the results for symptom score. Symptom scores were higher overall in the mTBI group compared with controls ( $F_{1,35}=16.82$ ;  $p<0.001$ ;  $d=1.09$ ) and decreased over time ( $F_{4,140}=19.74$ ;  $p<0.001$ ). A significant group-by-time interaction was observed ( $F_{4,140}=18.96$ ;  $p<0.001$ ), suggesting that the groups changed differently over time. Specifically, symptom scores were higher in the mTBI group than the controls at the 72 hour, one week, and two week time points ( $p\leq 0.04$ ) with no differences between groups at the 1 month or 2 month time point ( $p\geq 0.20$ ). There was no significant main effect of sex ( $F_{1,35}=1.53$ ;  $p=0.22$ ;  $d=0.30$ ) and no significant group by sex interaction ( $F_{1,35}=0.94$ ;  $p=0.34$ ). However, the time-by-sex interaction was significant ( $F_{4,140}=2.61$ ;

$p=0.04$ ), as females had higher symptom scores than males at the 72-hour time point ( $p=0.05$ ). There were no differences between sexes at any other time point ( $p\geq 0.13$ ).

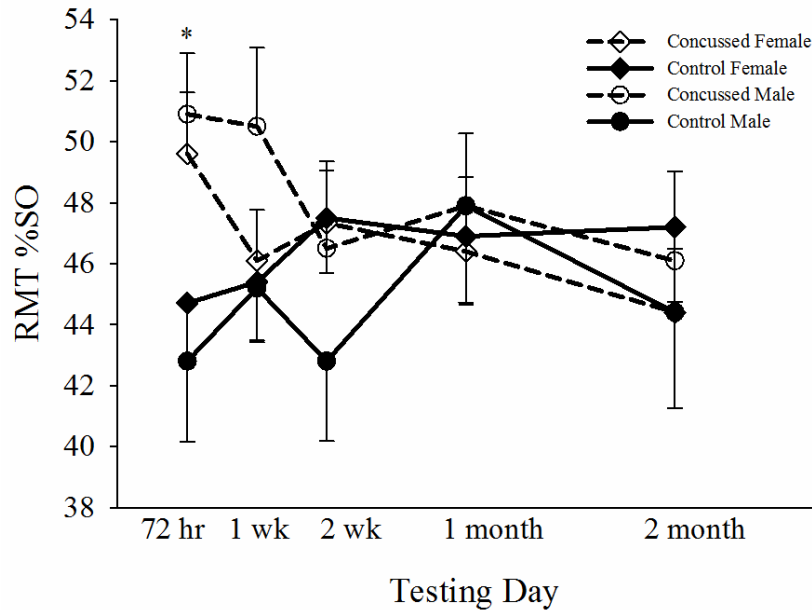


**Figure 4.1. Symptom score.** Symptom scores were higher overall in the concussed group compared with controls ( $p<0.001$ ) and decreased over time ( $p<0.001$ ). There was a significant group-by-time ( $p<0.001$ ) and sex-by-time interaction ( $p=0.04$ ). \*Significant difference between concussed and control participants ( $p\leq 0.01$ ). †Significant difference between males and females ( $p=0.05$ ).

#### Resting motor threshold (RMT)

Figure 4.2 shows the data for RMT. There were no significant effects of group ( $F_{1,35}=1.31$ ;  $p=0.26$ ;  $d=0.28$ ), sex ( $F_{1,35}=0.01$ ;  $p=0.91$ ;  $d=0.01$ ), or time ( $F_{4,140}=1.22$ ;  $p=0.31$ ) on RMT. There was a significant group-by-time interaction ( $F_{4,140}=3.94$ ;  $p=0.005$ ), as RMT values were higher in the mTBI group at 72 hours post-injury ( $p=0.02$ ), but were similar to controls at all other time points ( $p\geq 0.74$ ). The group-by-sex ( $F_{1,35}=0.59$ ;  $p=0.45$ ) and time-by-sex ( $F_{4,140}=1.41$ ;  $p=0.24$ ) interactions were not significant, indicating similar changes in RMT in males and females over time. Inter-

subject variability for RMT was low (CV=0.16), as was intra-subject variability across the 5 testing times for the mTBI group (CV=0.12; 0.10) and control (CV=0.08; 0.09) females and males, respectively.

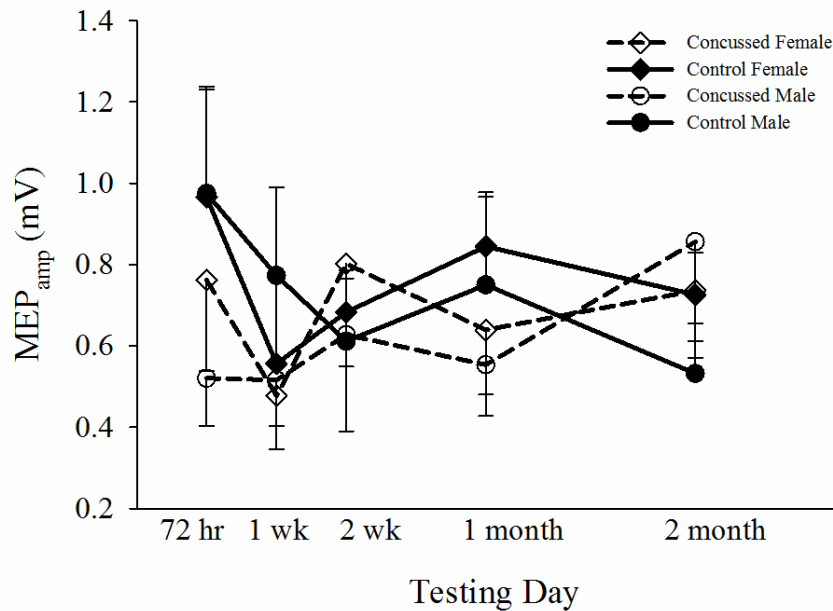


**Figure 4.2. Resting motor threshold.** There were no significant effects of group ( $p=0.26$ ), sex ( $p=0.91$ ), or time ( $p=0.31$ ) on RMT. There was a significant group by time interaction ( $p=0.005$ ). The group-by-sex ( $p=0.45$ ) and time-by-sex ( $p=0.24$ ) interactions were not significant. \*Significant difference between concussed and control participants ( $p=0.02$ ).

#### Motor evoked potential (MEP)

The results for MEP amplitude are shown in Figure 4.3. There were no significant effects of group ( $F_{1,34}=1.04$ ;  $p=0.31$ ;  $d=0.17$ ), sex ( $F_{1,34}=0.05$ ;  $p=0.82$ ,  $d=0.08$ ), or time ( $F_{4,136}=1.56$ ;  $p=0.33$ ) on MEP amplitude. The group-by-sex ( $F_{1,34}=0.13$ ;  $p=0.72$ ) and time-by-sex ( $F_{4,136}=0.51$ ;  $p=0.73$ ) interactions were also not significant. Inter-subject variability for MEP amplitude was high (CV=0.82), as was intra-subject variability for

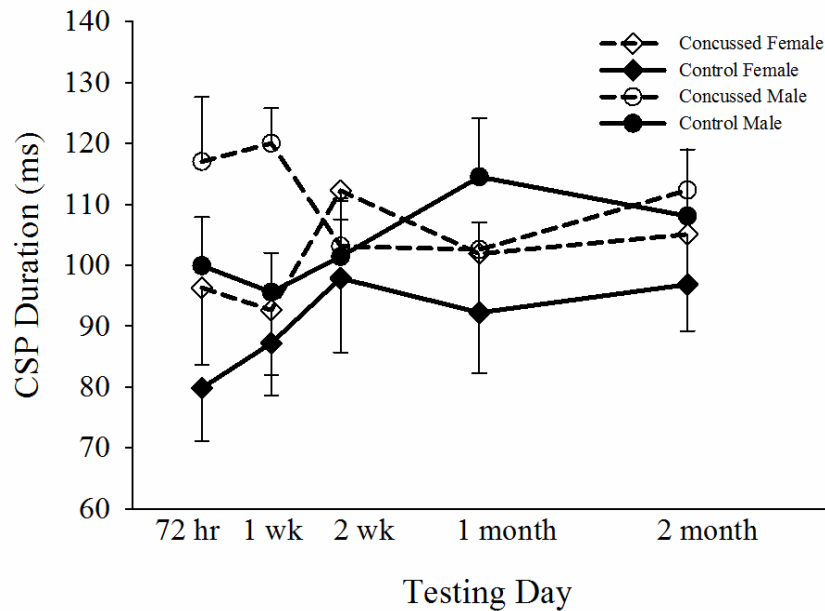
the mTBI group (CV=0.62; 0.63) and control (CV=0.61; 0.58) females and males, respectively.



**Figure 4.3. MEP amplitude throughout recovery.** There were no significant effects of group ( $p=0.31$ ), sex ( $p=0.82$ ), or time ( $p=0.33$ ) on MEP amplitude. The group-by-sex ( $p=0.72$ ) and time-by-sex ( $p=0.73$ ) interactions were not significant.

#### CSP Duration

Results for CSP duration are displayed in Figure 4.4. There were no significant effects of group ( $F_{1,35}=1.50$ ;  $p=0.23$ ;  $d=0.31$ ), sex ( $F_{1,35}=2.84$ ;  $p=0.10$ ;  $d=0.41$ ), or time ( $F_{4,140}=1.03$ ;  $p=0.39$ ) on CSP duration. The interactions between group and sex ( $F_{1,35}=0.04$ ;  $p=0.84$ ) and time and sex ( $F_{4,140}=2.03$ ;  $p=0.09$ ) were also not significant. Inter-subject variability for CSP duration was low (CV=0.28), as was intra-subject variability for the mTBI group (CV=0.22; 0.17) and control (CV=0.23; CV=0.18) females and males, respectively.



**Figure 4.4. CSP duration throughout recovery.** There were no significant effects of group ( $p=0.23$ ), sex ( $p=0.10$ ), or time ( $p=0.39$ ) on CSP duration. The group-by-sex ( $p=0.84$ ) and time-by-sex ( $p=0.09$ ) interactions were not significant.

*Sub-study 2*

Participants

Participant characteristics are presented in Table 4.2. There were no group differences in height ( $p=0.87$ ), weight ( $p=0.93$ ), or age ( $p=0.45$ ).

**Table 4.2. Group Characteristics (APOE Genotype)**

	Non-carrier	$\epsilon 4$ Carrier	<i>p</i>
<i>n</i>	40 (25f)	12 (5f)	
Age (years)	21.0 ± 2.3	21.8 ± 5.5	0.45
Height (cm)	173.5 ± 9.9	174.2 ± 8.9	0.87
Mass (kg)	71.9 ± 16.5	72.4 ± 12.7	0.93

Genotype frequencies are presented in Table 4.3. There were a total of 40 non- $\epsilon 4$  carriers and 12 carriers of the  $\epsilon 4$  allele. One carrier had the genotype  $\epsilon 2/\epsilon 4$  and was excluded from the TMS and MRS analyses. This is standard procedure, as the  $\epsilon 2$  allele exhibits protective effects that are thought to oppose the effects of the  $\epsilon 4$  allele (Hostage et al. 2013).

**Table 4.3. Genotype Frequencies**

<b>Genotype</b>	<b><i>n</i></b>
$\epsilon 2/\epsilon 2$	1 (0f)
$\epsilon 2/\epsilon 3$	4 (4f)
$\epsilon 2/\epsilon 4$	1 (1f)
$\epsilon 3/\epsilon 3$	35 (21f)
$\epsilon 3/\epsilon 4$	10 (4f)
$\epsilon 4/\epsilon 4$	1 (0f)

Table 4.4 shows the distribution of *APOE* genotype across mTBI group. Participants were categorized into one of four groups: (i) without history of mTBI (Control), (ii) within 72-hours of diagnosis of mTBI (Acute), (iii) with history of mTBI and no remaining symptoms (Chronic Control), and (iv) with chronic symptoms from mTBI, lasting at least 3 months post-injury (Chronic).

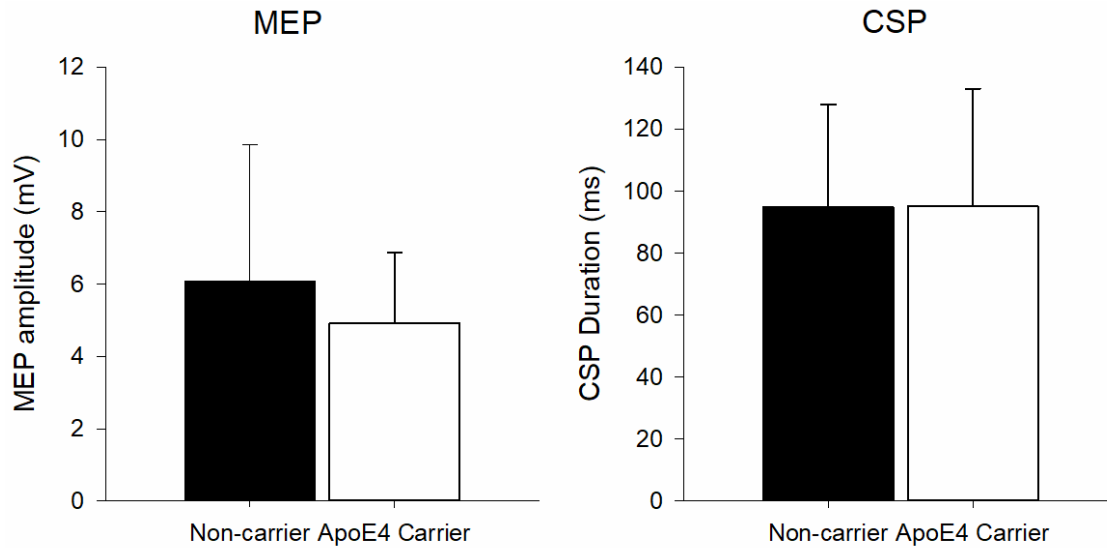
**Table 4.4. APOE Genotype Across mTBI Group**

	<b>Total samples</b>	<b>Total <math>\epsilon 4</math> carriers</b>	<b>Percent carrier</b>
<b>Control</b>	11 (5f)	2 (1f)	18%
<b>Acute</b>	14 (9f)	3 (0f)	21%
<b>Chronic Control</b>	21 (10f)	4 (2f)	19%
<b>Chronic</b>	6 (5f)	3 (2f)	50%



### Motor evoked potential and cortical silent period

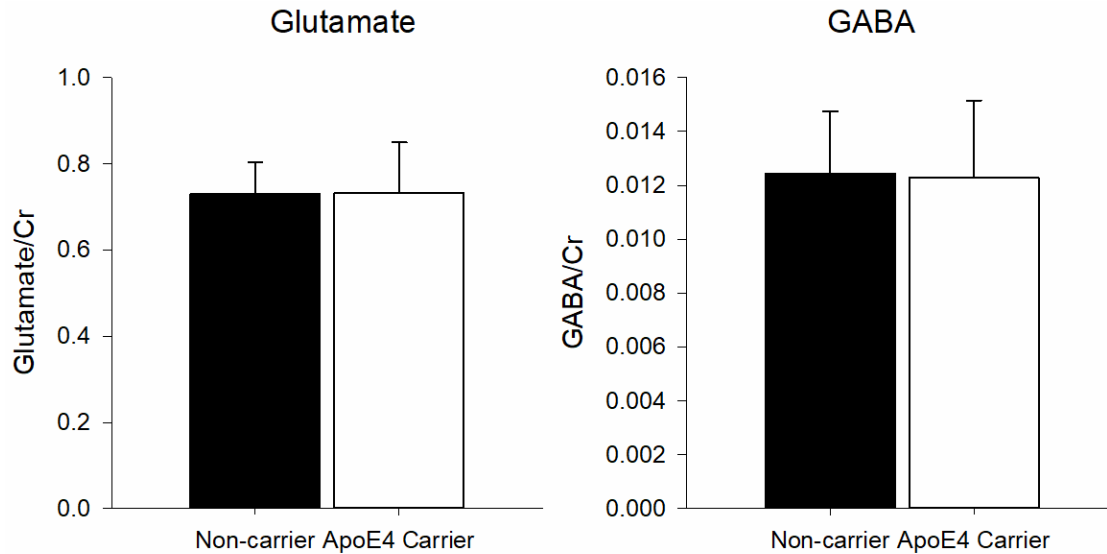
There was no significant difference in MEP amplitude ( $p=0.19$ ; Figure 4.5, left) or CSP duration ( $p=0.96$ ; Figure 4.5, right) between groups.



**Figure 4.5. MEP amplitude and CSP duration.** MEP amplitude was not significantly different between groups ( $p=0.19$ ). CSP duration was not significantly different between groups ( $p=0.96$ ).

### Glutamate and GABA concentrations

There were no differences in glutamate/creatinine concentrations ( $p=0.95$ ; Figure 4.6, left) or GABA/creatinine concentrations ( $p=0.90$ ; Figure 4.6, right) in M1 between groups.



**Figure 4.6. Glutamate and GABA concentrations in M1.** Glutamate concentration was not significantly different between groups ( $p=0.95$ ). GABA concentration was not significantly different between groups ( $p=0.90$ ).

## Discussion

The purpose of this study was to examine the potential influence of sex and genetic factors on neurophysiological recovery from mTBI. Despite differences in recovery of symptoms between males and females, we did not observe any sex-related differences in excitability or inhibition of the motor cortex. Results from this study also suggest that motor cortex function, as assessed by TMS measures of excitability and inhibition, and MRS measures of excitatory and inhibitory neurotransmitter is similar in those who possess an apoε4 allele and those who do not.

### *Influence of sex on motor cortex function*

We found that RMT values were significantly higher in the mTBI group at 72 hours post-injury, indicating lower levels of motor cortex excitability relative to the control group in both males and females. To our knowledge, this is the first study to provide a sex-based comparison of motor cortex excitability following mTBI. At all time

points beyond 72 hours, RMT decreased to control levels in both sexes, suggesting similar recovery in males and females. Although some reports suggest no difference in RMT acutely following mTBI (De Beaumont et al. 2007), our results are in agreement with reports demonstrating an acute reduction in excitability (Miller et al. 2014; Chistyakov et al. 1999). Such an acute alteration of excitability is also in agreement with data from rodent models, suggesting ionic and metabolic changes resulting in hyperexcitability, which is thought to resolve within days post-injury (Giza and Hovda 2001). We did not document any significant differences in RMT beyond the 72-hour testing point. Consistent with these results, additional studies involving sports related mTBI have indicated no differences in RMT weeks to months after injury (De Beaumont et al. 2007).

MEP amplitude may also be affected by metabolic changes resulting in hyperexcitability (Di Lazzaro et al. 2003). We did not see a significant effect of sex on MEP amplitude, a finding consistent with previous literature reporting no difference in MEP amplitude between healthy males and females (Pitcher et al. 2003). We also did not find a difference in MEP amplitude between control and mTBI individuals, which is consistent with previous literature reporting no difference in MEP amplitude at 2 weeks to 30 years post-injury (De Beaumont et al. 2009; Miller et al. 2014). Together, these results suggest that any changes in cortical excitability are resolved similarly in males and females within approximately one-week, consistent with the neurometabolic changes documented in rodent models (Clarkson et al. 2010).

Previous studies have shown that CSP duration is longer in individuals with mTBI compared with controls (De Beaumont et al. 2009; Miller et al. 2014), indicating

greater inhibition. Contrary to these previous reports (De Beaumont et al. 2009; Miller et al. 2014), we did not find any significant differences in inhibition between individuals with mTBI and control participants. Previous work has cited injury severity and number of previous mTBIs as factors explaining motor cortex dysfunction (De Beaumont et al. 2007). It is possible that the injuries sustained in our mTBI group, which excluded moderate and severe injuries, as well as a history of two or more mTBIs, were not severe enough to detect a difference in motor cortex inhibition.

Although there were no apparent sex differences in motor cortex excitability or inhibition, we did demonstrate a difference in recovery of symptoms. Participants with mTBI initially reported significantly more symptoms than the control group, but their symptoms resolved by two weeks post-injury. While the main effect of sex on symptom score was not significant, the significant time by sex interaction indicated that males and females recovered from symptoms differently. Females had significantly greater symptom scores than males at the 72-hour time point, with no differences by one week post-injury. Our observation of a sex-based difference in recovery of self-reported symptoms, but not measures of cortical excitability and inhibition, suggests that recovery of symptoms and cortical function may involve different physiological processes. Our data suggest that the metabolic changes (Giza and Hovda 2001) that would alter cortical excitability and inhibition may be similar between men and women, while additional factors contributing to symptoms may be different between sexes. Although there is no clear consensus on the reason for poorer post-injury outcomes in females, factors such as hormone levels, neck musculature, and overall cerebral organization have been suggested as contributors (Roof and Hall 2000; Andreason et al. 1994; Eckner et al. 2014). These

results provide indirect evidence for a similar neurometabolic response in males and females following mTBI and suggest that neurophysiological function does not likely contribute to the sex-based difference in symptom recovery.

#### *Influence of APOE on motor cortex function*

To our knowledge, this is the first study to compare motor cortex function between human carriers and non-carriers of the apoε4 allele. While previous studies have reported lower concentrations of glutamate in the brains of rodent apoε4 carriers (Dumanis et al. 2013), we did not report any differences in glutamate or motor cortex excitability between groups. It is possible that humans do not exhibit the decreased glutamate receptor function seen in animal carriers. It is also possible that differences in glutamate concentration may not be region-specific, as Dumanis et. al (2013) reported these differences in glutamate concentration globally in rodents, rather than in M1 specifically. Similar to animal literature, we did not report any differences in GABA concentration or intracortical inhibition between carriers and non-carriers.

Similar to previous studies, the apoε4 allele was overrepresented in our participants with chronic symptoms from mTBI (McKee et al. 2009). While half of our participants in the Chronic group were found to be carriers of the apoε4 allele, the sample sizes of each group were too small to determine if this difference was significant between groups. More participants are needed in each group to determine if allele frequencies significantly differ from Hardy-Weinberg proportions.

#### **Conclusion**

In these studies, we have demonstrated sex-based differences in symptoms scores, with no differences in cortical excitability or inhibition. Additionally, human carriers of

the apoε4 allele appear to have similar motor cortex function and concentrations of excitatory and inhibitory neurotransmitter than non-carriers.

While sex and the apoε4 allele have been associated with differences in outcome following mTBI, these risk factors did not seem to affect the function of the human motor cortex in this group of participants. It is possible that additional risk factors not addressed in this study, such as age at injury or mTBI history, may influence motor cortex recovery following mTBI. Further studies and more participants are needed to systematically determine the physiological basis for sex-related and gene-related differences in recovery of symptoms following mTBI.

## CHAPTER V

### CONCLUSION

#### Summary of findings

This dissertation represents a compilation of studies which are among the first to document motor cortex excitability, inhibition, glutamate, and GABA concentrations in individuals with acute and chronic symptoms from mTBI. The data suggest that motor cortex excitability may be lower in individuals with chronic symptoms from mTBI, but glutamate, the excitatory neurotransmitter responsible for measures of cortical excitability, was not significantly different from control levels following injury. Intracortical inhibition was higher in acutely-injured participants within 72 hours of injury and at specific time points throughout a 2-month recovery, despite GABA concentrations in the motor cortex being similar to control levels. The risk factors female sex and apoε4 genotype did not have an effect on motor cortex function in individuals with mTBI. Taken together, these TMS and MRS data suggest a possible functional change longitudinally following mTBI, despite an expected neurochemical profile.

Research on the apoε4 allele in animal models has suggested that carriers of this allele may have lower levels of glutamate in the brain compared with non-carriers (Dumanis et al. 2013). In this investigation, however, neurotransmitter concentrations were not affected by *APOE* genotype. It is therefore possible that differences in the action of glutamate, rather than simply the concentration of glutamate as detected by <sup>1</sup>H-MRS, exist in those ε4 carriers affected by mTBI. Indeed, it has been shown that glutamate receptors in ε4 rodent carriers are less effective than in non-carrier rodents (Chen et al. 2010). Such a difference in receptor function, rather than total concentration of glutamate

may explain why individuals in our Chronic group (with a high percentage of  $\epsilon 4$  carriers compared to other groups) demonstrated lower cortical excitability as assessed by the MEP amplitude, but similar concentrations of glutamate compared to healthy controls. Further research and more participants are necessary to determine if the lower levels of cortical excitability reported in the Chronic group were due to *APOE* genotype or some other factor related to their chronic mTBI.

Very little is known about the association between GABA and the apo $\epsilon 4$  allele. We found no relationship between intracortical inhibition, levels of GABA in M1, and the apo $\epsilon 4$  allele. Our findings are similar to Dumanis et al. (2013), who found differences in glutamate function in rodent  $\epsilon 4$  carriers, despite no differences in global GABA levels. It would be beneficial for future MRS studies to determine if the glutamate-to-GABA conversion is affected by the presence of the  $\epsilon 4$  allele.

Overall, results from these studies suggest that humans may not follow the same neurometabolic timeline as the rodent model following mTBI. It is likely that the TMS and MRS measures used to evaluate these changes in brain function following mTBI may not be ideal methods of determining the time course of a human Neurometabolic Cascade. Instead, measures sensitive to both the presence and action of neurometabolites in the brain should be taken from immediately following mTBI and throughout recovery. Caution should be taken when interpreting data from animal studies and applying to humans who have suffered mTBI.

### **Limitations**

Some factors may have limited the interpretation of the data in these studies. The majority of studies investigating neurochemical changes in the brain following TBI have



been conducted on animal models, often using post-mortem tissue samples. Human mTBI research presents a unique challenge, as it is impossible to control the impact and location of injury, and very difficult to monitor the time course of injury and recovery using TMS and MRS techniques. Although we excluded individuals from this study who were diagnosed with moderate or severe TBI, the exact location and mechanical impact of injury is difficult to monitor in human participants. Further, much of the research aiming to understand the recovery timeline of mTBI in humans relies on the brain-injured individual to accurately describe the method of injury and their symptoms following the event. Although participants were excluded from the study if they had experienced more than two diagnosed mTBIs in their lifetime, research suggests that repeated sub-concussive blows may have a physiological influence as great as the impact of a diagnosed mTBI (Johnson et al. 2014). It is possible that control participants involved in a sport such as soccer may have had neurophysiological profiles more similar to those of the mTBI groups involved in this study.

In the current investigation, the TMS measures of MEP amplitude and CSP durations were within normal ranges outlined in the literature (Oliviero et al. 2006; Sale and Semmler 2005). Although these have been documented to be reliable measures within participants across days (Edwards and Christie 2017), there was a high level of inter-individual variability with these measures, particularly the MEP. It is possible that this high level of variability may have reduced our ability to detect a statistically significant difference between groups when data from all four participant groups were compared.

Human  $^1\text{H}$ -MRS studies are limited in that quantities of neurotransmitter are sampled from small regions of the brain. It is possible that, although rodent studies show global changes in the brain following mTBI, human may experience more region-specific changes. Further, in animal models, quantities of neurotransmitter are typically calculated from whole-brain histological samples post-mortem.

### **Future research**

Further study is required in order to understand the symptoms underlying acute and chronic mTBI, and why symptoms recover for some individuals following mTBI but not others. It would be ideal to establish baseline pre-injury measures from participants, and then follow up with those individuals who experience mTBI later in life. Such longitudinal studies, however, are costly and may take years to complete. Additionally, as the apo $\epsilon$ 4 allele is relatively rare among the population, more participants with this risk factor need to be recruited in order to compare the interaction between apo $\epsilon$ 4 carrier status and measures of motor cortex excitability, inhibition, and glutamate and GABA levels in those with acute and chronic symptoms from mTBI.

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