Cortical Excitability after Mild Traumatic Brain Injury in Children

by

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Abstract

Introduction: Mild traumatic brain injury is frequently complicated by post-concussive syndrome. It is unknown why these symptoms persist, but recent research suggests that cortical excitability may play a role.

Objectives: To determine if cortical excitability is different in pediatric mTBI, and if it correlates with symptom persistence.

Methods: This was a cross-sectional controlled cohort study. Cortical excitability was measured using a variety of TMS paradigms in children with (symptomatic) and without (asymptomatic) persistent symptoms at one month post injury. The primary outcome measure was the cortical silent period (cSP) (thought to represent GABAergic inhibition).

Results: 57 children with mTBI (44% male; age 14.23 (SD:2.49)) and 28 controls were compared. cSP was similar between groups (F(2, 70)=0.53, p=0.591). There were no other significant group differences in cortical excitability.

Conclusions: TMS was well tolerated in children with mTBI. Cortical excitability is similar to normal children at one-month following the injury.
Acknowledgements

Firstly, I would like to thank my supervisors Drs. Karen Barlow and Adam Kirton who have provided exemplary support for me as their graduate student. You have both guided me through a degree that has had some ups and downs, sticking by me and working for my best interests, even though I sometimes had a hard time seeing what my best interests were. You have both provided me with the tools and foundations to tackle whatever career obstacles I will inevitably face, and for that I am very grateful. I hope that one day I can use these tools to make real change in the way that the science community understands brain injury. I would also like to thank my supervisory committee: Dr. Jeff Dunn, Dr. Clare Gallagher, and Dr. Michael Esser. It is apparent to me that they have strove to challenge me with the sole purpose of complementing and expanding on the toolset planted by my supervisors. Together, these five individuals have helped guide me through some very important challenges, and helped me see when I have bitten off more than I can chew and where my weaknesses lie so that I can improve upon them, and for that I am very grateful.

Brenda Turley has also played a very important role for me, from putting up with my sarcasm and jokes, to always lending a hand no matter how busy she is, to always making sure that the lab felt welcoming no matter how many hours we were working. Without her, this project would have been infinitely more difficult, especially recruitment. As well, everyone who ran the sessions with me, and helped develop my protocol deserves a big thank you: Omar Damji, Ephrem Takele, Patrick Cienchanski, and Tina Samuel. And of course the rest of both my labs have been amazing in their support and always letting me bounce ideas off of them. Karolina, Andrea, Liz, Jacquie, Dr. Helen Carlson, Dr. Brian Brooks, Erica, thank you all for
listening to me ramble about whatever it was I was stuck on at the time, and all your help throughout.

Lastly, but definitely not least, I would like to thank my family and friends inside and outside of the lab. They have helped me keep my sometimes tenuous grip on the fact that there is a life outside of work and to keep me balanced, while still coaxing me to succeed.
Dedication

I would like to dedicate this thesis to my family, especially my parents, and all families that have had children go through a long recovery from concussions or mild traumatic brain injuries. When I suffered my worst mild brain injury at age thirteen, I missed school for a month, and was symptomatic for several months after that. I had been injured in September, and it took until January to feel like myself again. I don’t remember much about the time right after the injury, but further on, I remember is how helpless my parents felt, and how hard it was on them and our whole family. From then on I have worked towards understanding brain injury, in the hopes that, maybe someday, parents and children won’t have to feel quite so helpless in the face of an injury.
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<tr>
<td>TBI</td>
<td>Traumatic brain injury</td>
</tr>
<tr>
<td>mTBI</td>
<td>Mild traumatic brain injury</td>
</tr>
<tr>
<td>DAI</td>
<td>Diffuse axonal injury</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial magnetic stimulation</td>
</tr>
<tr>
<td>RMT</td>
<td>Rest motor threshold</td>
</tr>
<tr>
<td>AMT</td>
<td>Active motor threshold</td>
</tr>
<tr>
<td>SRC</td>
<td>Stimulus response curve</td>
</tr>
<tr>
<td>cSP</td>
<td>Cortical silent period</td>
</tr>
<tr>
<td>iSP</td>
<td>Ipsilateral silent period</td>
</tr>
<tr>
<td>SICF</td>
<td>Short interval intracortical facilitation</td>
</tr>
<tr>
<td>ICF</td>
<td>Intracortical facilitation</td>
</tr>
<tr>
<td>MEP</td>
<td>Motor evoked potential</td>
</tr>
<tr>
<td>NMDA</td>
<td>n-methyl-d-aspartate</td>
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<tr>
<td>GABA</td>
<td>$\gamma$-amino butyric acid</td>
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Epigraph

“Pediatric traumatic brain injury: Not just little adults”

Dr. Christopher C. Giza (2007)

The above quote is the title for a review by Giza, et al\textsuperscript{1}, which spoke to me. It very concisely summarizes why I feel that, though the body of literature in adults may be large and still growing, it is extremely important for children to be the focus of some research.
Chapter One: Introduction

1.1 Thesis overview

Traumatic brain injury (TBI) is one of the leading causes of disability and death in the first world\(^2\). Biomechanical forces applied to the head or body may be transmitted to the brain and cause injury which then initiates complex pathophysiological cascades leading to dysfunction and sometimes cell death. Mild TBI (mTBI) comprises 75% to 90% of TBIs\(^3\). Clinically, mTBI appears as a brief alteration in brain function, which may include loss of consciousness, amnesia, confusion, difficulty concentrating, and other signs. Recovery occurs quickly in most cases of mTBI, but many individuals can have symptoms (such as cognitive, somatic, behavioural, and sleep-related symptoms) that last several weeks or months\(^4\). The exact symptoms will vary between individuals. Half of children continue to have symptoms at one month post-injury\(^5\). The biological mechanisms underlying symptom persistence are unclear.

In cases where symptoms persist longer than 3 months, individuals are usually diagnosed as having post-concussion syndrome (PCS)\(^6\). Some theories suggest that prolonged symptom persistence is due, at least in part, to abnormal plasticity after the injury\(^7\). Neurons in the brain form small circuits, which are constantly undergoing plastic changes via the expression of neurotransmitter receptors and their components. These circuits are involved in a variety of processes, from interpretation of stimuli to voluntary motor movements. Damage to circuits could affect the natural hierarchical functioning of the brain and lead to the persistence of symptoms seen after mTBI. Changes in neuronal function may vary but are likely to affect action potential conduction and different stages of neurotransmission, such as vesicle release, receptor binding, and post-synaptic threshold.
Transcranial magnetic stimulation (TMS) provides a unique method for analysing the microcircuits and neurophysiology in the primary motor cortex, as it can safely and non-invasively measure the neurophysiology of small cerebral cortical regions and their response to stimulation. TMS relies on the Faraday’s principles of magnetic induction, which describes when a changing electric current passes through a coiled wire, inducing a magnetic field around that wire. The magnetic field passes through the skin and excites neurons in the cortical layers of the brain. Because no electrical stimulation reaches the participant’s scalp, it is uncommon for participants to find TMS painful. These attributes make TMS ideal for non-invasively testing neurophysiology in pediatric patients.

1.2 Rationale

It is still unknown why some people spontaneously recover quickly after mTBI, while others suffer persistent symptoms. Many studies have undertaken research to discover factors, such as metabolic, pre-injury, and post-injury factors, that may contribute to symptom persistence. However, there is only a small body of literature examining the possible electrophysiological mechanisms underpinning symptom persistence. Several modalities have been used to understand electrophysiological changes after mTBI, especially electroencephalography. TMS allows researchers to apply a stimulus and assess the response of local cortical circuits. To date, there has been no published research on the effects mTBI may have on TMS responses in children.

1.3 Thesis Objectives

The purpose of this study was to determine if there were recovery-related differences in cortical excitability between healthy children and children after mTBI and if these symptoms
were related to symptom persistence. This study had two specific aims: to explore if cortical excitability is different between healthy controls and children who have suffered an mTBI; and to investigate whether cortical excitability is related to symptom persistence.

1.4 Thesis outline

This thesis will begin by discussing TBI, including the clinical manifestations, biomechanics, and pathophysiology of the injury. Then it will address the common uses of TMS, including the cortical mechanisms underlying TMS, the typical pathway that the signals will travel, and the fundamental methods of TMS. The published literature using TMS in mild and moderate TBI populations will then be reviewed. Finally, the remaining chapters will be dedicated to the methodology, results, general discussion, limitations, and future directions of the current research.
Chapter Two: **Traumatic brain injury**

2.1 Chapter overview

Traumatic brain injury (TBI) is common, especially mild TBI (mTBI), and occurs more frequently in childhood than in any other time of life. This chapter describes the epidemiology of mTBI, its clinical manifestation, the pathophysiology of the acute injury, and the potential mechanisms of symptom persistence.

2.2 Mild traumatic brain injury

Traumatic brain injury is defined as an injury to the brain that occurs when biomechanical forces result in an alteration of brain function\(^\text{16}\). The incidence of TBI varies widely across studies, but it is commonly cited between 150 and 799 per 100,000 persons per year\(^\text{17-21}\). However, a prospective cohort found TBI incidences averaged over the first 20 years of life may be as high as 1750 per 100,000 persons per year\(^3\). Approximately 70-90\% of all TBIs are mild\(^\text{22}\). Recent estimates suggest that as many as 25\% of patients with mTBIs do not seek medical attention; therefore, the true proportion may be higher\(^\text{23}\).

Diagnosing mild injuries is often more difficult than more severe injuries. In mTBI, there may be no external signs of injury, and the person does not need to lose consciousness and the symptoms are often not specific to a brain injury. To be classified as mild they must not lose consciousness for longer than 30 minutes, the duration of memory loss of or around the time of the injury must not exceed 24 hours\(^\text{24-26}\), and their initial Glasgow coma scale must be 13-15 within the first 30 minutes after injury (Table 1). Additionally, neuroimaging is usually normal after mTBI\(^\text{24}\).
Table 1: American academy of rehabilitative medicine criteria for traumatic brain injury severity classification\textsuperscript{24–26}

<table>
<thead>
<tr>
<th>Severity</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
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<tbody>
<tr>
<td>Glasgow Coma Scale</td>
<td>13-15</td>
<td>9-12</td>
<td>3-8</td>
</tr>
<tr>
<td>Loss of consciousness</td>
<td>&lt; 30 minutes</td>
<td>30 minutes to 24 hours</td>
<td>&gt; 24 hours</td>
</tr>
<tr>
<td>Post traumatic amnesia</td>
<td>&lt; 24 hours</td>
<td>24 hours to 7 days</td>
<td>&gt; 7 days</td>
</tr>
</tbody>
</table>

Mild TBI partially overlaps with concussion, and though they are not identical, the terms are often used interchangeably. Concussion is a less severe brain injury, usually occurring in sports\textsuperscript{27,28}, for which any loss of consciousness does not exceed 5 minutes. Some definitions of concussion exclude patients with a Glasgow coma scale (GCS) of 13\textsuperscript{29}. In this thesis, “mTBI” will be used as a term that encompasses concussion.

Pre-injury risk factors for mTBI are widespread, but they are commonly linked to social environment, such as home or education, and participation in sports. Most studies of mTBI risk factors have been performed in adults. In young adults, the length of education and household income are inversely related to the number of mTBIs, and hospital admissions for acute intoxications are significantly more common in one or multiple mTBIs\textsuperscript{30}. In North America, ethnicity may also play a role in the risk of mTBI. A systematic review of incidence studies in adults found that African-American adult males are at up to a 2.8 times increased risk of mTBI than their Caucasian counterparts in North America. Ethnicity was not a major factor in non-North American studies, where the largest proportion of mTBIs are due to motor vehicle
collisions. Additionally, participation in certain sports is associated with mild injuries. National College Athletic Association (NCAA) women’s leagues with the highest risk for concussion are field and ice hockey, basketball, soccer, and lacrosse. NCAA men’s leagues at the greatest risk of concussion are American football, wrestling, and ice hockey. These leagues all show injury rates above 0.40 per 1000 athlete exposures at the college level. Athletes are also more likely to undergo repeated sub-concussive events i.e. hits to the head without overt clinical symptoms. This is exemplified by findings that one third of individuals who sustain a concussion will experience at least one additional TBI.

Children are at a greater risk of a TBI than adults, although the risk factors associated with injury are still related to social environment and participation in certain sports. Firstly, children and older adolescents are at approximately double the risk of sustaining a TBI compared to middle-aged adults. The social environments for children are less likely to include intoxicants like alcohol (common in adult TBI) but still revolve around their interactions with their peers and family. The most common mechanisms of injury are falls in young children, whereas motor vehicle accidents and assaults are more common in older adolescents. Family issues and past stress can increase a child’s risk of TBI. For example, more than four adverse life events, such as parental divorce, abuse, or deaths in the family, can increase the risk of TBI by as much as three times.

Sport-related injuries remain an important cause of mTBI and concussion in children. For example, at the high school level, American football, boys’ hockey, and boys’ lacrosse all have incidences above 0.40 injuries per 1000 athlete exposures. High school athletes are at a higher risk of concussion than university/college athletes, though this may not be true in
American football\textsuperscript{37}. Males are overrepresented in all age groups\textsuperscript{31}; an effect that is greatest during adolescence and young adulthood\textsuperscript{20}. This may be related to the increased sensation-seeking attitudes and lower impulse controls exhibited by males during adolescence compared to females or males at other stages of life\textsuperscript{38}.

### 2.3 Post-concussion syndrome

Outcome after TBI is predicted by pre-injury demographic factors, injury related factors, and post-injury factors, and therefore it shows a great deal of variation. Pre-injury demographic predictors include age, prior intellectual ability, behavioural or psychiatric conditions, socio-economic status, and family dynamics\textsuperscript{39}. Risk factors include: admission to hospital, presented with headaches in the emergency department, were female, required analgesics, or missed more than 2 days of school\textsuperscript{12}. Following an mTBI, there may be problems with fatigue, headache, drowsiness, difficulty sleeping, irritability, concentration difficulties, memory problems, and mood disturbance\textsuperscript{40}. Historically, these symptoms are thought to last between 7-10 days\textsuperscript{41}; however, the median persistence time of symptoms in children has recently been found at 29 days (95\% confidence intervals: 26.1, 31.9 days). Further, 11\% of children remain symptomatic at 3 months post injury\textsuperscript{5,42}.

Post-concussion syndrome (PCS) is a term used to describe the persistence of a cluster of symptoms after an mTBI. PCS definitions were described for adults first in the International Classification of Diseases (ICD-10) and the Diagnostics and Statistics Manual (DSM-IV) classification schemes\textsuperscript{6}. The ICD-10 criteria for PCS requires patients to experience head trauma, and symptoms from at least 3 of the 8 symptom categories (headache, dizziness, fatigue,
irritability, difficulty concentrating and performing tasks, memory impairment, insomnia, and reduced tolerance to stress, emotional excitement, or alcohol) must be present for 4 weeks or longer. The DSM-IV criteria uses the term post-concussional disorder (PCD), and requires a history of head trauma causing concussion, with difficulty in attention or memory. Here, at least 3 symptoms must last at least 3 months. Symptoms for the DSM-IV include: becoming fatigued easily; disordered sleep; headache; vertigo or dizziness; irritability or aggression; anxiety, depression, or affective lability; changes in personality; and apathy or lack of spontaneity. Further, the DSM-IV stipulates that symptoms must be either new or a worsening of pre-existing symptoms, that they significantly impair social or occupational function, which are suggested by objective neuropsychological evidence of dysfunction. There is a potential for over-diagnosing of PCS when using ICD-10 criteria and under-diagnosis with the DSM-IV criteria within adult populations.

Both the ICD-10 and DSM-IV definitions can lead to challenges in diagnosing PCS in children since they were not specifically designed for use in this population. Recently, clinical diagnostic criteria for PCS in children were developed as a compromise between the ICD-10 and DSM-IV, with more emphasis on the ICD-10. Special considerations for the age and activities of these children were included, e.g. children do not typically drink alcohol. The recently proposed criteria for PCS require that children experience an mTBI with symptom onset within 7 days of injury, and that symptoms last for 4 or more weeks after injury. Symptoms include at least three symptoms (headache, dizziness, fatigue, irritability, insomnia, difficulty concentrating, memory problems, emotional lability, or mood disturbance); and symptoms should persist for at least 4...
weeks after injury. These modified criteria have a positive predictive value of 86.7% and negative predictive value of 79.6% at 4 weeks after mTBI.

PCS in children has a significant impact on quality of life. The major issue with post-concussive symptoms though is that they are not unique to mTBI and there is no diagnostic test. Headaches are the most common symptom after mTBI, but are also common in depression, post-traumatic stress disorder, and even healthy people. Similarly, sleep disturbances and difficulties with memory and concentration are found in depression and other psychiatric disorders. Further complicating PCS is the potential prevalence of multiple comorbid conditions that are easily confused with symptoms after TBI. Examples of such comorbid conditions include depression and post-traumatic stress disorder, attentional problems, and others.

Assessments for PCS in children attempt to account for the commonness of post-concussive symptoms. There are several assessments for PCS, which usually take the form of questionnaires, such as the post-concussion symptom inventory (PCSI), Rivermead post-concussion symptom questionnaire (RPQ), health and behaviour inventory (HBI). The RPQ was psychometrically tested for use in adults and is less easily applied to children. The HBI is administered to the parents and children, with high agreement between parents and children for cognitive and somatic symptoms up to the first year after injury. The PCSI has been modified for specific age groups (5-7, 8-12, and 13-18). There is a similar parent report form. There is adequate correlation between parent and child report. The PCSI has 26 questions in 4 domains: physical/somatic, sleep/fatigue, emotional, and cognitive. Because pre-injury symptoms may have an effect on the number of post-injury symptoms, it is important that studies control for pre-injury symptoms. Therefore, studies retrospectively record the pre-injury
symptoms\textsuperscript{57}. However, caution must be used when interpreting pre-injury symptom ratings, as individual’s and parent’s may increasingly downplay pre-injury symptoms the further from the injury the ratings are taken\textsuperscript{58}.

The long-term complaints following paediatric TBI include behavioural problems, neuropsychological deficits (e.g. problems with attention, executive function, memory, and speed of processing), motor deficits, and decreased social skills\textsuperscript{59}. Pre-injury psychological factors play a role in the persistence of symptoms\textsuperscript{60,61}. Psychosocial factors become more important when symptoms persist for long periods of time. McNally et al\textsuperscript{56} followed 8 to 15 years old children with mTBI until 12 months after the injury. They used a series of questionnaires and interviews to determine pre-injury and post-injury child and family factors that may affect recovery from symptoms, including child intelligence and functioning, and family stressors. This research found that injury characteristics predicted parent and child ratings of PCS, but with decreasing accuracy through the follow up periods. By 3 months post-injury, demographic and pre-injury symptom ratings were more predictive of symptom persistence than injury factors\textsuperscript{56}.

In summary, mild TBI is common in adults and children, however the risk during childhood is some of the highest it will be during an individual’s life. Children are also at a high risk of persistent symptoms, known as PCS. There are multiple sets of criteria for PCS, including specific ones for children. Additionally, research assessments designed for evaluating symptoms have been developed specifically for use in children.
2.4 Biomechanical mechanisms of the insult

TBI begins with an insult to the brain and complex pathological processes and enzymatic cascades ensue. Consider the brain to be a suspended semi-solid with defined borders from the surrounding cerebrospinal fluid, all contained within the rigid skull. When a force is applied directly to the head or to the head via the neck, either linear or acceleration-deceleration forces are generated within the brain. The brain may collide with the inside of the skull and bony buttresses. Deformation may occur when it impacts the skull (even with “sub-concussive” forces). Injury occurs when these forces exceed the structural limitations of any elements undergoing strain (blood vessels, neurons, glia, etc.). The brain is not a homogeneous mass but has grey and white matter, blood vessels, and sinuses with cerebrospinal fluid. As grey and white matter have differing water content and densities (which change throughout brain development as myelination occurs), these structures move according to their inertia. This results in shear stresses on the axons, blood vessels, and oligodendrocytes as they cross the interfaces of these regions with different densities. During shear stress, these forces cause the cellular membranes to leak, known as mechanoporation, which results in a breakdown of the electrochemical gradient (needed for effective signal conduction) and disruption to cellular organelles. If the shear stress is great enough, it may traumatically detach axons from their somas, destroying that neuron’s ability to pass on signals. The process of mechanoporation occurs during the insult and is part of the primary injury. Additionally, the shear forces may cause gaps between cells in the blood brain barrier.
2.5 Pathophysiology of mild traumatic brain injury

2.5.1 Overview of brain pathology

When traumatic neuronal injury occurs there is an indiscriminate release of neurotransmitters, activating enzyme pathways and other cellular processes that subsequently cause secondary damage to the brain, known as “secondary injury”. This indiscriminate release of neurotransmitters begins a process called excitotoxicity. Excitotoxicity occurs when large amounts of glutamate from presynaptic neurons which binds to n-methyl-d-aspartate (NMDA) receptors on post-synaptic neurons and causes neuronal activation. With uncontrolled release of glutamate, the ensuing neuronal activation is excessive, and can damage the cell, see Figure 1.

Together, mechanoporation and excitotoxicity result in a shift in ionic gradients across membranes, an increase in cellular metabolism, and increases in multiple reactive oxygen species\textsuperscript{65–67}. More specifically, open NMDA receptors are permeable to calcium. Unregulated activation of NMDA receptors during excitotoxicity causes the intracellular calcium concentration to rise above normal levels. Because of the pre-existing ionic concentration gradients across the membrane, mechanoporation permits ion flux into or out of the cell down their electrochemical gradient. This is problematic because under normal circumstances, when a neuron is activated by glutamate binding to NMDA receptors, synaptic potentials travel through the soma and can activate voltage-gated sodium channels to begin an action potential if the threshold is surpassed. However, after mechanoporation, the sodium ion concentration gradient is dysregulated, substantially raising the threshold for an action potential and making it less likely that action potentials will be propagated. In response to increased intracellular sodium ion
concentration, sodium-potassium ion pumps will attempt to restore homeostasis. This process requires energy, and the mitochondria must create more adenosine triphosphate (ATP) via aerobic respiration. The ion pumps are quickly overwhelmed, leading to axonal and somal swelling resulting in cerebral oedema.

Dysregulation of intracellular calcium concentrations contributes to further damage. In a healthy cell, the intracellular calcium concentration is tightly controlled either by binding proteins or sequestration in specific organelles. When calcium ions enter the cell through the above dysregulated mechanisms, it plays a key role in the activation of enzymatic cascades and other signalling pathways associated with TBI. As the intracellular calcium concentration increases in the soma, the ion is sequestered in the mitochondria. The electrochemical gradient of protons in the mitochondria is overwhelmed by the influx of calcium ions, and the electron transport chain becomes less efficient, decreasing ATP production. This means that the mitochondria are unable to replenish the ATP required to maintain the ion pumps and re-establish homeostatic concentration gradients. Anaerobic respiration is less efficient at producing ATP, leading to an “energy crisis” in the cell. In the axon, calcium activates calpain enzymes, which initiates breakdown of the cytoskeleton and cellular transport mechanisms65,66. This cytoskeletal breakdown is further compounded by the neuronal swelling. Calcium dysregulation after TBI, therefore, plays an important role in secondary cell damage and cell death.

TBI results in different cellular injury phenotypes even in adjacent neurons65,66. Neurons and other cells may die through several different mechanisms: 1) as a direct result of the insult; 2) caspase-dependent apoptosis 3) progression of cytoskeletal breakdown. Calcium-activated calpain-mediated cytoskeletal breakdown can cause the neuron to sever its axon, causing the cell
to die or undergo Wallerian-like degeneration. When the neuron has no downstream connections, it will slowly decrease the size of its dendritic tree, and atrophy\textsuperscript{68}. Many injured neurons will repair any damage and resume functioning. Damaged or dead neurons are not all closely grouped but may be spread throughout the brain in what is called diffuse axonal injury (DAI). This distribution (focal or diffuse) depends on the primary insult, the biomechanical properties of the brain, the size and propagation of force waves through the tissues, and the resultant location of stress points.

Less is known about the long-term recovery processes following TBI especially in children where the normal developmental processes of receptor expression, synaptic pruning dendritic morphogenesis, and myelination are occurring. There is strong pathological evidence for a prolonged immune response in TBI, with identifiable microglial activation, astrocyte activation, and microvascular changes in the blood brain barrier years after injury. The release of ATP from damaged neurons initiates the activation of the innate immune system, resulting in the release of inflammation-promoting mediators such as cytokines, chemokines, and reactive oxygen and nitrogen species. Pro-inflammatory processes are intended to clear the central nervous system of potentially harmful substances and cellular debris. Anti-inflammatory processes follow this, performing reparative and regenerative functions. However, an unbalanced or prolonged inflammatory response in either the pro- or anti-inflammatory direction can be harmful, leading to excessive cell death and glial scar formation and may contribute to poorer long term outcomes\textsuperscript{69–74}. 
Figure 1: A summary of prevailing pathophysiological processes after traumatic brain injury in an injured brain cell.

Significant steps in the pathophysiological cascades are numbered. (1) The indiscriminate release of glutamate at excitatory synapses leads to glutamate binding at (2) n-methyl-d-aspartate receptors on the post-synaptic neuron (shown in red), which opens the receptor pore, and increases ion flux: calcium and sodium influx, and potassium efflux. This is compounded by (3) any tears in the cell membrane, which similarly allow ions to travel down their concentration gradients. To compensate (4) sodium and potassium pumps (shown in purple) use adenosine triphosphate (ATP), converting it to adenosine diphosphate (ADP), to actively pump sodium out of the cell in exchange for potassium. (5) Excess influxed calcium is sequestered in the mitochondria. The change in relative charge concentration across the mitochondrial membranes reduces the effectiveness of oxidative phosphorylation (the red circle) which leads to (6) a decrease in ATP production. Ineffective oxidative phosphorylation leads to (7) increased lactate production through anaerobic respiration, which may lead to acidosis and edema. When calcium accumulation overwhelms the mitochondria, the increase in cytosolic calcium ions, especially in axons, (8) activates calpain and similar enzymes. (9) Activated calpains degrade cytoskeletal elements, which can lead to severing the axon.
2.6 Neurotransmission alterations

Beyond the secondary injury phase, changes in neurotransmitter receptor expression can be seen following TBI. In this way, TBI can be considered a dynamic process and not just a static insult to the brain. Cortical circuits that remain intact enough to propagate signals may show short-term and long-term changes in neurotransmission. The excitatory-inhibitory balance is altered by the number and type of neurons that survive, the absolute and relative number, and receptor subtype expression. The exact mechanisms and durations of neurotransmitter dysfunctions following TBI remain poorly elucidated, although these changes may be more prominent in children and have the potential to lead to longer lasting deficits due to concurrent developmental processes.

Changes in neuronal excitation can be immediate, but then tend to decrease over time. As glutamate and calcium levels normalize, longer-term changes in receptor expression occur. For example, in a juvenile mouse TBI model, the relative expression of the NMDA receptor NR1, and NR2 subunit subtypes A and B differ after TBI. Subtype NR1 and NR2B subunit expressions remain unaffected, while NR2A subunit expression in the synapse is reduced. Changes in receptor subunit or subtype expression can cause decreased ligand affinity, change protein transportation targets, and alter the functional mechanism (e.g. ionotopic instead of metabotropic) of the receptor.

GABAergic interneurons (most commonly inhibitory) may be particularly vulnerable to injury. GABA receptors show changes in their relative subtype expression following TBI. For example, some GABA A subtype (GABAa) receptor subunits are downregulated (e.g. ε and θ subunits in the thalamus and hypothalamus) whereas other are upregulated (α4 subunit increase
in the hippocampus\textsuperscript{79}. As implied above, the receptor subunit isoform (i.e. $\alpha_4$ versus $\alpha_3$) lead to differences in complex formation, ligand affinity, and protein transportation targets\textsuperscript{80}.

Although the GABA\textsubscript{A} receptor is more commonly studied, there are multiple subtypes of GABA receptors and the GABA B subtype (GABAb) receptors are also noteworthy. GABAb receptors are metabotropic, therefore these receptors have longer lasting effects than GABA\textsubscript{A} receptors and alterations in these receptors may have an important role in network oscillations. The resultant inhibitory alteration depends on the type of GABAergic interneurons affected, receptor subtype expression, their location in the brain, and the time after injury.

Neurotransmitter systems also do not work in isolation. GABA\textsubscript{A} and GABAb receptors cross talk, and the two proteins show a strong distribution overlap. For example, in the dentate gyrus, GABAb receptors enhance the tonic inhibition induced by extra-synaptic GABA\textsubscript{A} receptors\textsuperscript{81}. This crosstalk is not limited to inhibitory signalling, since GABAb receptors will crosstalk with metabotropic glutamate receptors, NMDA receptors, and tyrosine kinase receptors\textsuperscript{80}.

In the context of TBI, any of the above changes contribute to the evolving processes after injury, and it is unknown when changes in protein expression stop. However, protein expression changes on any of the levels (receptor, subunit, subtype, isoform) affect how the cell interacts with its neighbouring cells, which in turn change the excitability of those cells.

2.7 Cortical network changes after traumatic brain injury

Much like how neurotransmitter systems do not work in isolation, cells operate in microcircuits, connectomes and networks. As an organism is dynamic and must learn and adapt
to their environment, these circuits cannot be static. Neuroplasticity is the ability of the neurons to adapt to changes in the environment. It encompasses the formation of new synapses, activating latent synapses, and strengthening (or lessening) the modulatory influence of an existing synapse\textsuperscript{82}. Synapse formation and recruitment of latent synapses are costly in terms of cellular resources and take longer to achieve than strengthening an already active synapse. Strengthening already established synapses is more efficient, especially in a mild injury\textsuperscript{1}, since it can be achieved by adding more presynaptic neurotransmitter vesicles or increasing the probability of vesicle release in the pre-synaptic neuron. These processes will increase the post-synaptic potential strength, increasing the inhibitory or excitatory effect of synaptic transmission. Axonal growth and the formation of new synapses are related to the action of growth factors such as nerve growth factor induced gene A, homer, activity regulated cytoskeletal-associated protein, and brain-derived neurotrophic factor (BDNF)\textsuperscript{83}. BDNF, for example, increases the number of dendritic spines, and aids in the formation of new synapses.

Long-term potentiation (LTP) is a building block of plasticity specific to the NMDA receptor. Synapses that have undergone LTP tend to have stronger electrical responses to stimuli than other synapses and are associated with increases in NMDA receptor concentration at the post-synaptic membrane. This can be artificially induced with high frequency stimulation \textit{in vitro}\textsuperscript{84,85} and LTP-like effects can be induced in humans using high frequency stimulation in repetitive TMS (rTMS)\textsuperscript{86,87}. The opposing form of neuroplasticity to LTP is long term depression (LTD). Decreasing the NMDA receptor concentration at the post-synaptic neuron is the crux of LTD.
Plasticity is not solely a function of the relationship between two cells but involves groups of interconnected cells. These microcircuits include many cells with different cell types, protein expression patterns, and functions. Interneurons are short ranging neurons that modulate the activation of other cell types. They may receive inputs from different neurons or brain regions than that of the “target” neuron. If an inhibitory interneuron synapses just above the axon terminal on the pre-synaptic neuron, it decreases the likelihood of neurotransmitter release, as shown in Figure 2. If these neurons had undergone LTP, the pre-synaptic inhibition will largely counteract it. Thereby, GABA-mediated inhibition is thought to filter neuroplasticity at excitatory inputs. In addition, GABAb autoreceptors on the interneuron bind GABA released from the interneuron, (Figure 2C), causing it to become hyperpolarised and less likely to release additional inhibitory neurotransmitter. With rarer GABAergic inhibition from the interneuron, the pre-synaptic neuron returns to normal resting membrane potential and normal probability of vesicle release.
2.8 Special considerations of paediatric mild traumatic brain injury

The changes in receptor expression induced by the insult and ongoing adaptations afterwards may have more dramatic effects in children than in adults. Children have ongoing neurodevelopmental processes that may be disturbed by the injury. Modulating the cross-talk between neurotransmitter systems at different stages in development can alter the outcome of the patient, and restorative neuroplasticity may leave minor gaps in the microcircuits as they reform and solidify. These differences are dependent on the degree to which the systems are modulated i.e., changing receptor subtype expression may direct new receptors to other regions of the cell resulting in a different effect, or change ligand affinity so as to modulate the effect of a quanta of

Figure 2: GABAergic filtering of synaptic plasticity.

(A) shows a pre-synaptic/post-synaptic neuron pair (black), which will propagate signals from left to right, with a small blue arrow indicating long-term potentiation (LTP). (B) expands the same circuit to include an inhibitory interneuron (blue) synapsing onto the pre-synaptic terminal which negates the LTP effects. However, (C) shows auto inhibition of the same interneuron, which restores the effects of LTP.
neurotransmitter. The alterations invoked by a brain injury during development can have effective changes that potentially evolve and last much longer than if they were to occur in an adult.

These processes are not solely pertaining to signalling between neurons either: conduction within neurons is refined and developed in different brain regions at different rates. Myelin is vulnerable to damage during a biomechanical insult\textsuperscript{88,89}, and damage to myelin is associated with decreases in conduction speeds\textsuperscript{90}. This may disturb the synchrony of inputs to circuits. Asynchronous activation of inhibitory systems could lead to disinhibition, while asynchronous input to excitatory systems may resemble inhibition, when it is actually an inability to breach activation thresholds.

2.9 Summary

Changes occurring after TBI are more complex than the changes occurring in individual cells or small groups of synapsing cells. Collections of microcircuits form networks, sometimes ranging long distances across the brain. Information is conveyed in these networks using a careful balance between excitation and inhibition through successive levels of processing. A TBI can therefore affect the excitability of neuronal circuits and larger networks. Measuring these changes in cortical excitability has the potential to provide much needed information about plasticity and the reparative mechanisms taking place following an injury.
Chapter Three: **Interrogating the brain using transcranial magnetic stimulation**

3.1 **Chapter overview**

A modality that has recently gained popularity in neuroscience is transcranial magnetic stimulation (TMS) to investigate neurophysiology. TMS uses rapidly changing magnetic fields to non-invasively and safely investigate regional excitability of cortical neuronal populations. TMS includes three types of stimulation: 1) single pulse, which involves separating each pulse by several seconds, 2) paired pulse, which places two pulses in rapid succession to induce an interaction between circuits, and 3) repetitive TMS (rTMS), which involves multiple trains of stimuli. Single and paired pulse TMS are used to evaluate neurophysiology, while repetitive TMS may be used to modulate regional cortical function with resulting therapeutic potential. This chapter will review the mechanisms underlying TMS, and how evoked potentials are formed in the cortex. The review will be limited to single and paired pulse TMS, as they are more appropriate to investigate the existing changes in the cortex after TBI, where very little is known.

3.2 **Basic mechanisms**

3.2.1 **The motor pathway**

The motor system is most often chosen in single and paired pulse TMS studies because it provides one of the most directly measurable outputs from the cortex. The primary motor cortex, Brodmann’s area 4 in humans, receives inputs from many cortical (pre-motor areas, posterior parietal cortex, somatosensory cortex and others) and subcortical (thalamus, cerebellum, and others) regions. The motor cortex has a topographical organisation. The hand has a large representation because of its many small muscles that need individual and synergistic activation to generate movements with high dexterity. These muscles will have a higher motor neuron to
muscle fibre ratio, which increases the control and dexterity of each movement. To control more motor neurons, there must be more neurons from the cortex generating signals, which increases the motor representation area of that muscle. Additionally, motor representations that are required for more precise movements are more easily activated\textsuperscript{93,94}. Therefore, the motor representation of the hand is of special interest in TMS because of the low threshold for activation and the large representation for muscles in the hand. Individual muscles or small muscle groups in the hand can therefore be targeted with TMS.

The major output from the motor cortex is via the large pyramidal neurons in layer V. For example, when glutamate binds to post-synaptic NMDA excitatory receptors on these neurons; the ionotropic NMDA receptors will undergo a conformational change. In the stable open conformation, NMDA receptors are permeable to sodium, potassium, and calcium\textsuperscript{95}. As sodium enters the cell, it raises the potential of the neuron relative to the extracellular space, from the resting potential, which is approximately -70mV. When the membrane potential is sufficiently increased, it will begin an action potential in the layer V pyramidal neuron.

The action potential from the layer V large pyramidal cell will exit the grey matter of the motor cortex, travel down their axons through the white matter, and pass through the internal capsule. These axons travel through the midbrain region, pass through the cerebral peduncle, then the pontine nuclei in the pons. The axons pass through the medullary pyramids, where 90% of the tracts originating in the motor cortex cross to the contralateral side of the spinal cord at the pyramidal decussation, earning the name pyramidal tract neurons.
Once in the spinal cord, the descending tracts are now known as the lateral corticospinal tract. These axons form monosynaptic connections with the motor neurons in the spinal grey matter. The motor neurons transmit the action potential out the ventral horn, through the intervertebral foramen, where they form neuromuscular junctions. Here they release acetylcholine onto nicotinic receptors of the muscle cell membrane, or sarcolemma, generating a muscle potential. When the change in potential reaches the sarcoplasmic reticulum, calcium ions are released, which interact with muscle machinery, and cause muscle contraction.

3.2.2 Generating descending potentials with transcranial magnetic stimulation

TMS works based on Faraday’s law of magnetic induction to elicit responses in cortical neurons. This law states that when a change in the electromotive force (voltage) through a wire is made, the magnetic field surrounding the wire changes and vice versa. Therefore, in TMS, as the current passes through the coil of wire, it generates a magnetic field. The magnetic field can pass painlessly through the scalp and cerebrospinal fluid, to the superficial layers of the brain\(^96\). The range of the magnetic field is short and typically only reaches 1-2cm below the scalp before stimulatory capacity decreases\(^97\). This means that in the motor cortex the magnetic field will most often only be strong enough to evoke potentials in layer II/III of the motor cortex. At higher intensities it is possible that the magnetic field will be strong enough to evoke potentials in layer V pyramidal neurons. When the induced magnetic field reaches neurons, the change in potential induces a secondary current, or eddy current.

Evoking an action potential depends on three neuronal factors:

1) Location: The greatest probability of evoking a response will be if the magnetic field changes the potential at the axon hillock, due to its increased concentration of voltage-
gated sodium channels. However, the dendrites are not an ideal location to evoke a response using TMS because they rely on the passive diffusion of the potential to the axon hillock.

2) Orientation: Lenz’s law, which complements Faraday’s law, describes how electromagnetic conduction obeys the physical laws of energy conservation. It states that a change in one current (which induces a magnetic field) will be in the opposite direction of a secondary current that it induces. These two currents must oppose one another for energy to be conserved. Therefore, *the greatest change in potential will occur in axon segments that are perpendicular to the induced magnetic field*; if an axon is exactly parallel with the induced magnetic field, there will be no change in potential. Axons however are rarely straight, and their bends are advantageous when generating an evoked potential with TMS.

3) Excitatory state: Preparatory events in the motor cortex or other brain regions, prime the neuron or circuit increasing the likelihood of an action potential. Therefore, it is important that the participant not be able to guess when the next stimulation will be made.

For these reasons, it is important to position the coil correctly. When stimulating the hand, the optimum position for the coil is to be laying tangentially on the scalp, at 45 degrees from the midline. When the magnetic field reaches the correct area of the layer II/III neuron with a sufficiently low threshold, in the correct orientation, that neuron will likely generate an action
potential\textsuperscript{97}. However, these neurons do not project directly to the motor neurons in the spinal cord.

Layer II/III in the motor cortex consists primarily of interneurons that project between themselves or to layer V\textsuperscript{98} and so the pyramidal neurons are indirectly stimulated. Typical stimulation applied to the motor cortex must go through at least 3 synapses: between the layer II/III neurons and the pyramidal tract neurons, the pyramidal tract neurons and the motor neurons, and the motor neurons and muscle. If there are any more synapses, it is most likely that they will occur in layer II/III as interneurons modulate each other before the output reaches the pyramidal tract neurons\textsuperscript{98}. Strong stimuli may directly stimulate the pyramidal neurons.

3.2.3 Direct and indirect waves

Magnetic stimulation generates a unique series of descending potentials as the magnetic fields interact with cortical neurons. Using epidural recordings in human patients who had undergone neurosurgery for pain, Di Lazzaro et al\textsuperscript{99} discovered that TMS did not typically evoke only one wave of action potentials. The spinal epidural recordings showed that there are actually a series of waves occurring at \textasciitilde 1.5ms intervals – the approximate amount of time it requires for an action potential to travel across a synapse. The first wave is called the direct wave, or D-wave, and is thought to originate from axon hillock of the pyramidal tract neurons. The D-wave is difficult to elicit with TMS, and usually seen only with stronger stimuli. Subsequent waves are thought to originate in the layer II/III interneurons and these indirect waves are labelled according to their occurrence:

- the first wave is labelled I1, and follows the D-wave by \textasciitilde 1.5ms,
- the second wave is called I2, and follows the D-wave by \textasciitilde 3.0ms.
the third wave is called I3, and follows the D-wave by ~4.5ms

I-waves are thought to originate from a mixture of three mechanisms: the periodic activation of excitatory post-synaptic potentials through interneurons in parallel, multiple independent activations through interneurons in parallel, and powerful synchronous depolarisations of many pyramidal tract neurons leading to oscillatory activity. These D- and I-waves are all combined together in the EMG trace of the MEP. TMS is thought to manipulate the I-waves, and change the overall output of the MEP, however, this is difficult to prove definitively.

3.3 Transcranial magnetic stimulation fundamental methods

3.3.1 Phase of stimulation

To magnetically stimulate the brain, three pieces of equipment are crucial: a power capacitor, an inductor (coil), and a switch (Figure 3). At all times the power across the capacitor is of equal but opposite charge to that of the inductor. If all current flow is zero, all of the energy resides in the capacitor, and if the current is at maximum all of the energy is in the transducer, Figure 3. An effective switch will ensure that this change occurs very quickly. It is also through this transfer of energy that the phase of the stimulation is determined. The stimulation is also affected by the phase of the current through the coil, and the generation of the magnetic pulse through the coil.
The capacitor is used to store voltage potential between two plates, which generates potential energy that can be released by closing the circuit with the switch. Then, once the circuit has been opened (turned off), the capacitor is used to recapture most of the voltage potential at the end of the cycle period. In the magnetic coil, the current waveform and voltage waveform are both sinusoidal shaped, but phase shifted approximately one quarter cycle. In biphasic stimulation the cycles of these two waveforms interact such that before the switch is closed, the current is zero and the voltage is at its maximum. Therefore, the energy is in the magnetic field. When the switch is turned on, at one quarter of the way through the cycle, current will be at its maximum, and voltage will be zero. At half way through the cycle, current is zero, and the voltage has changed sign (direction), such that the energy is in the capacitor. At three quarters through the cycle, the energy is transferring back to the capacitor. Finally, the cycle completes when the voltage is at its maximum and the current is zero. This process is used in order to save energy and prevent damage to the circuit components. Because they recycle voltage, biphasic

**Figure 3: Setup and phase of TMS stimulation**

(A) shows the three basic element required to create a magnetic pulse used for stimulation. (B) shows the differences in voltage (V) and current (I) through two phases of stimulation.
stimulators require a lower stimulation intensity than monophasic stimulators, do not overheat as often, and create the telltale D- and I-waves only found in TMS.

In a monophasic stimulator, the cycle is only the same as a biphasic stimulator up to the first quarter of the cycle. After that, the current is allowed to slowly dissipate, rather than recharging the capacitor, giving monophasic stimulators a single polarity, unlike biphasic ones.

3.3.2 Types of coils and coil orientation

Often TMS researchers have manipulated the orientation and complexity of the coil used to induce the magnetic field. Choosing the type and size of coil to use as an inductor are important factors with respect to the focality and depth of stimulation. These choices are determined by the area chosen to stimulate and its location in the cortex. For example, to effectively stimulate the motor representation of the legs, greater depth and stronger stimuli are needed because the motor representation is further from the scalp. Conversely, the hand requires a more focused stimulation at less depth, because the representation is relatively superficial, and the goal is usually to stimulate single muscles.

There are two main types of TMS coils: a circular coil and a figure-8 coil. These two shapes show different patterns of magnetic field generation, causing them to stimulate the brain differently. In the circular coil, the single coil tends to be larger with more turns. The induced magnetic field is larger at the center of the coil, and the current is largest at the edge of the coil. If placed over the vertex to stimulate the motor representation of the leg, the coil will produce asymmetric magnetic fields. In this instance motor cortical stimulation will be greatest where the current flow through the wire is anterior to posterior at the vertex. However, if placed more
laterally, the magnetic fields will be symmetrical in each hemisphere. Because of their large size, these lack the focality of other coils types\textsuperscript{101}.

A figure 8 coil is two smaller circular coils joined in the middle so that the current flow is in the same direction at the junction. Therefore, as current passes this point, the induced magnetic fields are summed. In figure 8 coils, the current travelling through the outer loops is small enough that those induced magnetic fields can be ignored. This creates a very focal stimulation pattern with clearly defined borders in an oval shape under the junction. These traits make the figure 8 coil ideal for stimulating small areas such as the motor representation of the hand\textsuperscript{101}.

Within figure 8 coils, there are several types of coils with slightly different traits. The flat figure 8 coil has less penetration than the circular coil, as a compromise between focality and efficient use of power. A double cone coil has two small circular coils at an obtuse angle to each other, and increases the penetration of the magnetic field but has increased field strength at the coils, and therefore has decreased focality. There are more complicated coil configurations as well, but these are not commonly used. There is also an interplay between the type of coil and its orientation. Many TMS paradigms are affected by the direction of the induced magnetic field over the area being stimulated, and their direction will change based on the configuration of the coil.
3.3.3 Electromyography

Surface electromyography (EMG) is the study of potential shifts in the muscle fibers and requires at least two electrodes placed on the skin. Though it is possible to use more electrodes, TMS typically only requires two when stimulating small muscles. These electrodes are placed on the belly of the muscle and on a bony point adjacent the muscle, such as muscle insertions or origin if possible. Before placing the electrodes, the skin is often abraded or rubbed with alcohol to improve the adhesion of the electrodes and reduce the resistivity of the skin.

![Electromyography tracing](image)

**Figure 4: Electromyography tracing of a normal motor evoked potential induced by TMS, as recorded from the EMG with amplitude and latency.**

The asterisk indicates the stimulation artefact due to the magnetic field passing over the recording electrodes. Image courtesy of Dr. Kirton, and adapted.

*Abbreviations: FDI: first dorsal interosseous (muscle); TMS: transcranial magnetic stimulation; ms: milliseconds*

A single motor neuron divergently innervates multiple muscle fibres. When a motor neuron receives an action potential sufficiently strong to evoke another action potential, it will simultaneously activate multiple muscle fibres. In each muscle fibre, excitatory input causes an increase in intracellular calcium, and a shift in the muscle fibre potential. Similar to an action
potential, this process leads to muscle fibre depolarization. Superficially placed EMGs detect the summated change in potential in multiple motor units. The change in muscle membrane potential causes a shift in voltage between the pair of surface electrodes, which is translated to an electrical signal, and transmitted through wires to be translated into an electromyogram or EMG trace. TMS pulses that are above the participant’s threshold will show a positive deflection, immediately followed by a negative deflection, indicating a MEP, see Figure 4.

There are other EMG methods, which include intramuscular EMG and single fibre EMG. These methods are collectively called needle EMG, and involve insertion of different types of needles into the muscle. These methods give more precise information about the function of individual motor units, rather than combining all motor units involved in a movement, but because they are invasive, they are rarely used in TMS.

3.4 Neurophysiological outputs in transcranial magnetic stimulation

3.4.1 The motor evoked potential

All TMS paradigms rely on the MEP as the basic measurement of magnetic stimulation. The measurement of the MEP is an indirect measurement of cortical excitability and is minimally processed. MEPs are the compounded muscle potentials of many motor units. The goal with TMS is to excite neurons of the corticomotoneuron tracts, or their first order interneurons. Processing of the MEP will occur in three locations: at the stimulated interneurons in layers II/III, the pyramidal tract neurons, and at spinal cord interneurons (those excited by the primary motor cortex and other motor areas).
3.4.1.1 Amplitude

The MEP provides two types of information: the amplitude and the latency. The amplitude of the MEP is the ability of the pyramidal tract neurons in the primary motor cortex to effectively and synchronously summate in response to stimulation. The amplitude is a direct function of the stimulus intensity relative to the threshold. Most often the amplitude is measured as maximum difference in EMG voltage after stimulation.

Programming scripts can be written to automatically return the peak-to-peak amplitude of each stimulation by measuring the greatest positive and subsequent negative spike within an approximate range after the stimulus was given. However, it is also important to ensure that in resting paradigms, the participant is at rest, and that during active paradigms, they are contracting. Therefore, as an assurance, the background EMG activity may also be taken so that individual traces can be excluded if they violate the rules of the test.

When measuring MEPs, investigators must be aware that EMG electrodes will register muscle potentials from nearby muscles. Using intrinsic hand muscles, such as the first dorsal interosseous and abductor pollicis brevis, helps to minimize the effect of non-target muscles on the MEP amplitude because they are relatively isolated from other muscles.

3.4.1.2 Latency

While the amplitude shows the excitability of the neurons in the center of activation, the MEP latency is also important for understanding brain neurophysiology. The MEP latency describes the integrity of the pyramidal tracts. It is the time from the stimulation to the beginning of the upward deflection of the MEP. However, the MEP latency includes peripheral conduction times, which may increase the variability of measurements. Therefore, a mathematical equation
has been derived to measure the central motor conduction time (CMCT) and eliminate the peripheral latency. The peripheral latency is determined using either peripheral stimulation with additional calculations or using vertebral stimulation to excite the motor neurons at the ventral horn or intervertebral foramen. The peripheral latency is then subtracted from the MEP latency to give the CMCT. Both CMCT and MEP latency are fairly stable across healthy populations; therefore, if there is an increase in the amount of time for the MEP to reach the EMG electrodes, then the pyramidal tract axons are likely damaged or demyelinated.

3.4.2 Single pulse transcranial magnetic stimulation

Single TMS pulses are separated by 4-5 seconds to ensure that there is only minimal interaction between stimuli, letting researchers examine how the motor circuits summate or conduct single potentials. They are used to measure the motor thresholds, MEP responses to changing stimulation strength, and some inhibition processes.

3.4.3 Motor thresholds

The motor threshold is a measure of the integrated excitability of the motor tract, or the stimulation intensity required to generate motor responses of a given amplitude, and is described as a percentage of the maximal stimulator output. During a TMS session, the motor threshold is the first measurement. This is done to individualise the amount of stimulation that the participant receives to control for their motor cortex’s excitability, and so that all stimuli are given as a percentage of the motor threshold.

By convention, the smallest rest motor threshold (RMT) requires MEPs with peak-to-peak amplitude of at least 50µV. Other tests for RMT often reach as high as 1000µV, which some investigators also call the “test stimulus” and use this stimulation intensity as the second
stimulus for paired pulse TMS. To control for the variability of responses, it is convention to use the minimum required stimulus to generate more than 5 of 10 consecutive MEPs, but 3 of 5 or 3 of 6 MEPs is also common in older research.103

In order to perform these analyses in real time, two horizontal lines are placed on the EMG trace frames at the desired peak-to-peak amplitude. The TMS operator can then visually inspect each frame as they pass and keep count. Instead of using EMG recordings, studies have also use observed movements103, however, due to the increased threshold in children, the isolated muscle contraction described by EMG is more useful.

The motor threshold can also be determined under the active condition (active motor threshold, AMT). The MEPs generated under the active condition are typically much larger than at rest, so the threshold is increased to compensate. Commonly, 200µV is used, and again, the convention is that the peak-to-peak amplitude is greater than the desired amplitude in 5 of 10 consecutive stimulations. In order to separate the presence of a MEP from background activity on the EMG trace during contraction, investigators will often look for a large spike in the EMG followed by a brief silent period. The silent period will be discussed in greater detail below, but during AMT, silent periods are a brief suppression of EMG activity immediately following the MEP. The silent periods in AMT are much shorter than real cortical silent periods (cSPs) because the stimulation is much weaker. In fact, the AMT, despite requiring a higher response amplitude, is typically only about ~80% of the RMT at 50µV, as the neurons are primed with activity.
The contraction during AMT measurement is an important source of variability between studies. Not only does the amount of contraction of the target muscle change the amplitude of the MEPs, but there are multiple methods for determining contraction strength. A common method is to determine the force of the contraction: maximal force is calculated against a mechanical resistance, and then to perform AMT the participant maintains a fraction of that maximal force. However, this method may be less precise than using an EMG oscilloscope to track contraction effort.

An EMG oscilloscope measures only the electrical activity that is between the two electrodes, therefore the contraction is isolated to the muscle targeted by the stimulation, as compared to the force which is the synergistic contraction of more than one muscle. To continuously track EMG activity for the contraction, EMG leads that are attached to the target muscle are routed through the EMG oscilloscope, and a real-time EMG can be shown to the participant. The participant then contracts at a fraction of their maximal effort for the duration of the testing. In younger children special attention must be paid to any paradigms requiring long contractions, as the children are likely to become tired and lose concentration. The TMS operators must give very clear instructions with frequent encouragements.

Measuring the motor thresholds in TMS is crucial in order to individualise the strength of all subsequent paradigms. There is a great deal of inter-individual variability in the intensity of stimulation required to evoke a response. Determining the motor thresholds allows the researcher to control for this phenomenon by expressing stimulation parameters for other paradigms relative to the motor thresholds.
3.4.4 Stimulus response curves

Expanding on the concept of recording MEP amplitudes, the stimulus response curve (SRC) was developed. SRCs require multiple stimuli at different levels of intensity to measure the motor cortex’s ability to effectively summate responses to increasing stimuli. To put this in terms of the motor thresholds, stimuli will often range from just below the threshold (e.g. 80-90% of the threshold) to well above the threshold (e.g. 150%). In analyses, the EMG traces can be sorted by their stimulus intensity, and the mean peak-to-peak amplitude for each stimulus intensity can be determined, see Figure 5. These mean amplitudes can then be plotted against the individualised stimulus intensity (percentage of the motor threshold) to form a recruitment curve for further analysis. From the plotted MEP amplitudes, the curve fit, the slope, the maximum, the area under the curve, and the V50 can all be determined:

- The curve fit of the SRC is a measure of excitability because, as the stimulus intensity increases, neurons with higher thresholds or that are more spatially distant from the center of activation are recruited to generate increasingly large MEPs. This effect will eventually saturate, and curve will level off, generating a sigmoidal shape that can be fit to a function.

- The slope is used to find the peak rate of recruitment, which only requires the maximum slope in the middle of the sigmoidal curve. It measures the rate of recruitment of higher threshold neurons.

- The maximum of the recruitment curve can only be calculated if the curve shows as a sigmoid, otherwise, it is impossible to know what the maximum would be because the top of the curve cannot be extrapolated. The maximum of the SRC describes the maximal
stimulatory capabilities of the region that is being activated, and the ability to effectively summate action potentials.

- The area under the curve can be calculated as the integral between the limits of the minimum and maximum of the stimulus intensities. It is a concise way to describe the curve. This is used as a general measure of excitability and recruitment.

- Finally, the V50 is the calculated stimulus intensity to generate a MEP halfway between the maximum and minimum of the curve.

![Stimulus Response Curve](image)

**Figure 5: Example of a normal Stimulus Response Curve from one patient expressed as the mean EMG response (mV) compared to stimulus intensity strength as a percentage of the rest motor threshold.**

Error bars show standard deviation. Ten stimuli were given at each intensity in a pseudorandom order. The green line describes the slope of the curve, and the orange line is the maximum. The greyed area is the area under the curve.

**Abbreviations:** RMT: rest motor threshold; EMG: Electromyography.

These paradigms can also be measured while the muscle is actively being contracted, which increases the amplitude of each MEP. During contraction, more of the higher threshold neurons that are not stimulated until late into the rest SRC paradigm are partially depolarized.
Therefore, the pyramidal neurons have a reduced threshold because they are primed for further action, and can be activated more easily. Additionally, first order excitatory interneurons that have higher thresholds or are not as optimally positioned/oriented can also be stimulated during active SRCs. In the active condition, the AMT is used as the basis of stimulus intensity.

Under the active condition, the tight regulation of the active neurons is essential to avoid over-excitation. Therefore, an additional measurement can also be calculated from the active condition of a stimulus response curve is the silent period. These silent periods are evoked closer to their threshold, so they are much shorter than standard cSPs. The silent periods can be determined for each EMG trace and, similar to the MEP amplitude, sorted according to states. These can then be used to generate silent period recruitment curves\(^{104,105}\).

### 3.4.5 Cortical silent period

Using single pulse TMS, inhibitory interneurons can be targeted during a muscle contraction. When a strong stimulus is given during contralateral contraction, the interneurons in that region of the stimulated motor cortex inhibit the pyramidal tract neurons to protect these neurons from fatigue or overexcitement and damage. On the EMG trace, this appears as flattened activity that can last for over 100ms in normal participants. This is called the cortical silent period (cSP). The first 50ms of the cSP may reflect the Hoffman reflex in the spinal cord, while the later period is due to cortical inhibition through GABA release from the inhibitory interneurons onto GABA\(_{\beta}\) receptors on the pyramidal tract neurons. Evidence for the GABAergic mechanism of cSP has been determined through pharmacological study of healthy participants. Tiagabine inhibits GABA reuptake into the neurons and astrocytes and baclofen is a GABA\(_{\beta}\) receptor agonist. Both tiagabine and baclofen increase the duration of the cSP\(^{106,107}\).
is due to the stimulation of high threshold interneurons, as indicated by the increase in cSP duration with increased stimulation intensity\textsuperscript{108}.

The cSP is defined as the disruption in an EMG waveform due to magnetic stimulation during active contraction (Figure 6). However, there is a good deal of variability in the methods for eliciting a cSP. A conventional cSP paradigm uses stimulation greater than the 50\textmu V RMT during a contraction to ensure that the duration is controlled by cortical mechanisms (>50ms). Many studies use the 1000\textmu V RMT or 120-130\% of the 50\textmu V RMT\textsuperscript{109–114}, which are usually similar in terms of the percentage of the maximal stimulator output. The duration of the cSP is sensitive to the stimulation intensity used\textsuperscript{104,105} but is far less sensitive to contraction strength\textsuperscript{108}. Much like AMT, the convention is around 20\% of the maximum voluntary contraction, measured by EMG oscilloscope.

The beginning of the cSP can be measured either from the beginning or the end of the MEP. When the MEP is included in the cSP, this method does not control for variances in the width of the MEP and may artificially increase the duration of the cSP by including time that is not actually operating under GABAergic mechanisms.
The end of the cSP is then typically defined as the return to normal EMG activity. However, the definition of normal EMG activity varies. Often, the pre-stimulation (background) EMG activity is used. When analysing the cSP, the EMG is rectified and the mean background EMG amplitude is calculated. Normal EMG activity is given a range, which is often 1 standard deviation from the mean in either direction. Therefore, a line is superimposed on the rectified EMG at these levels, and the return to the normal range of EMG activity is the first pixel to cross this line, which gives the end of the cSP\textsuperscript{94}.

Garvey et al have used an automated graphical method to automatically calculate the duration of the cSP\textsuperscript{115}. The definitions for onset and end are similar to those listed above, and this technique shows promise. However, the AMT (MEP > 100 µV in the average of 5 consecutive trials) used was not determined using typical methods, and stimuli were 150% AMT, which is weaker than most other publications recommend (120% of the 50µV RMT or 100% of...
the 1000µV RMT). Additionally, muscle activation was described as contraction to the participant’s full capacity, which is far greater than most studies use to achieve muscle activation. With prolonged maximal contraction, there is little doubt that cortical fatigue would play a role in the duration of the cSP calculated using the methods described in this publication. Further testing, using more common methods of eliciting cSP are needed to validate the use of the graphical method for automating cSP calculation.

3.4.6 Ipsilateral silent period

Silent periods can be used to measure a different form of inhibition. Stimulation to the cortex ipsilateral to the contraction evokes an ipsilateral silent period (iSP). With stimulation, neurons that send axons across the corpus callosum to the analogous muscle representation in the contralateral motor cortex are excited. In the contralateral motor cortex, these neurons synapse onto inhibitory interneurons, which inhibit pyramidal tract neurons, causing a silent period. It is believed that these inhibitory interneurons release GABA onto GABAb receptors on the pyramidal tract neurons, although this has yet to be confirmed. These projections to contralateral inhibitory interneurons are crucial for independent movement of analogous limbs and for bimanual coordination, and provide a measure of corpus callosal tract integrity. The onset and end of an iSP are similar to those of a cSP. The onset is marked as the beginning of the suppression in EMG activity, and the end is determined by a return to normal EMG activity.

3.5 Paired pulse transcranial magnetic stimulation

Paired pulse paradigms combine two stimuli in rapid succession such that the first stimulus (conditioning stimulus) has an effect on the output of the second stimulus (test stimulus). These stimuli do not need to be of the same intensity. Therefore, during paired pulse
paradigms with TMS, there are three facets that can be manipulated: the conditioning stimulus intensity, the inter-stimulus interval (ISI), and the test stimulus intensity. By changing the stimulus intensity and ISI, investigators can target specific populations of neurons by their refractory properties, neurotransmitter and receptor dynamics, and compounding D- and I-waves.

Optimally, paired pulse paradigms pseudo-randomise an unconditioned test stimulus with conditioning stimulus to prevent the participant from expecting a specific paradigm. Often in the laboratory, paired pulse paradigms that require similar parameters, such as intracortical facilitation (ICF) and short interval intracortical inhibition (SICI), are pseudo-randomized together with unconditioned test stimuli in order to prevent participants anticipating which stimulation they are receiving, which may change the state of the motor cortex. 93,94,100,117.

The amplitude of MEP responses to stimuli vary greatly between individuals in all TMS paradigms, therefore, paired pulse paradigms attempt to control for this variability. The ratio of the mean amplitude of the conditioned stimulus to the mean amplitude of the unconditioned test stimulus are calculated. This comparison determines whether the microcircuits performed facilitation or inhibition of the test stimulus. Therefore, paired pulse paradigms are typically shown as a ratio, where a value of 1 indicates that the conditioning stimulus has no effect on the test stimulus. Ratios below 1 indicate inhibition, and values above 1 indicate facilitation. The ratio can also relate the size of the modulation due to the conditioning stimulus by the magnitude of difference from 1.

3.5.1 Short interval intracortical facilitation

Short interval intracortical facilitation (SICF) summates the I-waves of the conditioning (first) stimuli and subsequent conditioned stimuli. The ISIs follow the ~1.5ms intervals of I-
waves such that the second stimulus is compounding on the I-waves from the first stimulus as they propagate through the $n^{th}$ order neurons, where $n$ is the I-wave number targeted by the second stimulus. Therefore, the discrete intervals to evoke SICF in adults are 1.1-1.5ms, 2.3-3.0ms, and 4.1-4.5ms$^{93,118,119}$ for first, second, and third order interneurons, respectively. These ISIs separate a conditioning stimulus that is slightly below the 50µV RMT, and a test stimulus intensity equal to the 1000µV RMT. The stimulus intensities remain constant between the different ISIs. These three ISIs, are then pseudo-randomised with the test stimulus alone. The subthreshold conditioning stimulus partially depolarises the nth order axon segments involved with I-waves and makes them hyper-excitible. When the test stimulus arrives at those neurons, more of them will generate action potentials together, which are seen in the EMG trace as a larger MEP.

### 3.5.2 Intracortical facilitation

Intracortical facilitation (ICF) is believed to act through NMDA receptors$^{120}$ but is not completely understood. Epidural recordings of ICF do not show an increase in descending potential amplitude, or an increase in the number of descending potentials$^{121}$.

ICF is elicited with a conditioning stimulus below the 50µV RMT and a test stimulus above the same threshold; usually 120% of the 50µV RMT or at the 1000µV RMT. These stimuli are separated by 8-25ms$^{93,94,100,117}$. Often this paradigm is combined with the short interval intracortical inhibition (SICI)$^{117}$ because they both use the same stimulation intensity parameters, and only vary the ISIs. Additionally, these ISIs are too close together to be noticed by the participant.
3.5.3 Short interval intracortical inhibition

Another example of compounding the I-waves is found in short interval intracortical inhibition (SICI). When performed at very short ISIs (1ms), SICI is largely due to the refractoriness of the pyramidal tract neurons, but when performed at slightly longer ISIs (2ms), SICI is thought to be due to the synaptic inhibition of the I3 wave by GABAa receptors. Without the I3 wave, the MEP amplitude is significantly reduced\textsuperscript{122}.

SICI can be elicited using a conditioning stimulus below the 50µV RMT and a test stimulus above the same threshold, usually 120\% of the 50µV RMT or at the 1000µV RMT. However, the ISI is 1-5ms long\textsuperscript{117}. Often, 2ms is chosen because it is between the SICF intervals, and has an established neurotransmitter-based mechanism\textsuperscript{93,123}. Similar to ICF, when SICI is performed in an active state, the effect is abolished.

3.5.4 Long interval intracortical inhibition

Long-interval intracortical inhibition (LICI) is another technique to target inhibitory interneurons long after D- and I-waves have subsided. It is thought that this paradigm functions through GABAb receptors on the pyramidal tract neurons, but through different mechanisms than cSP\textsuperscript{124}. Unlike cSP, LICI decreases with increasing second stimulus intensity, indicating that it is mediated by low threshold interneurons\textsuperscript{86}. LICI is elicited using two above threshold stimuli, separated by 50-200ms. At high intensities, these two pulses are more likely to overheat the machine, so measures must be taken to ensure that the magnet can be cooled, either as part of the coil design or with ice between paradigms.
3.6 Safety considerations in children

There are some general safety requirements for the safe use of TMS in humans, as well as some considerations specific to children. In order to use TMS safely, the coil must be properly insulated from the participant’s head and sufficiently grounded to avoid electrocution should a short be found (ground leakage < 300uA, enclosure leakage < 100uA with ground, resistance between ground pin and metal enclosure < 0.1 Ohms), as well as to not get overheated (contact surfaces <41C). To prevent overheating, some coils come with built-in air-cooling systems. However, these systems add weight to the coil, and make holding it manually very difficult. Most coils for single and paired pulse TMS only need cooling if the participant has an especially high threshold, therefore they do not require built-in systems.

In addition to system safety, it is important to ensure that the participant will be safe in proximity to a powerful magnetic pulse. The contraindications for TMS are similar to those for magnetic resonance imaging. These include ferromagnetic or implanted electronic medical devices (e.g. pacemakers). Any earrings or external metal should also be removed. With single and paired pulse paradigms, the risk of seizure is low. There was a single reported case of seizure after severe TBI in a repetitive TMS study but to date there have been no reports of seizure after mTBI.

Specific to children, their ability to sit still long enough to ensure that the same spot is stimulated and their ability to tolerate the TMS must be considered. Subjective sensations often include mild tingling or a tactile tapping sensation. These are both well tolerated and children find them fascinating. However, a systematic rating scale has been developed to subjectively rate
the tolerability of TMS sessions in children\textsuperscript{96}, which includes rating subjective sensations during and after the TMS sessions, such as nausea, headache, or unpleasant tingling sensations. A systematic review of safety literature found that TMS is safe and well tolerated in children and adolescents\textsuperscript{126}.

It may be difficult to perform TMS in children with brain injuries due to their inability to understand the situation and communicate any distress. In order to make the TMS session more enjoyable for the children, our lab has a selection of movies for the children to watch. While this may slightly change the excitability of the motor cortex due to visual stimulation and visuomotor interactions, it is valuable in keeping the children sitting still during the session. If they are less prone to move and shift during the stimulation paradigms, then the operator will be more accurate with the muscle representation less, and the quality of the data improves.

3.7 Summary

There are many different methods for eliciting similar TMS paradigms. TMS can target different pyramidal neurons and interneuronal populations using different stimulation intensities at the conditioning stimulus or the test stimulus, or by varying the ISI. The effect of these paradigms is strongly dependent on the equipment and experimental design.
Chapter Four: Transcranial magnetic stimulation after traumatic brain injury

4.1 Chapter overview

TMS has been used to examine various corticospinal and intracortical motor circuits after adult TBI. Currently, studies interrogating the electrophysiological effects of paediatric TBI on the cortex using TMS have not been published. The existing TMS studies have examined mild, moderate, and severe TBI, in both the athlete and general population at different time points post-injury. TMS findings in adults after TBI primarily fall into three categories: acute moderate and mild TBI, chronic moderate and mild TBI, and severe TBI. The populations and timing of the studies vary considerably, as do the TMS methodologies. Overall, TMS studies of TBI seem to suggest that the recovery process is associated with an increase in cortical inhibition and the available evidence for mTBI is summarized and in Table 2.

4.2 Investigating mild traumatic brain injury using transcranial magnetic stimulation

4.2.1 Motor thresholds

Changes in the excitation-inhibition balance can be measured using the rest motor threshold (RMT, measuring the most excitable neurons in the area) and the stimulus response curves (SRCs). Studies that report changes in RMT after mTBI, even in the acute stages after injury, are contradictory. In almost all of the studies, RMT is measured as the percentage of stimulator output where 5 out of 10 stimuli that elicit a MEP greater than 50µV, but in the early studies by Chistyakov et al, 3 out of 5 MEPS greater than 100µV was used for RMT. About half of the small number of studies describe no difference in thresholds, while the other half describe an elevation of the RMT after mTBI. For example, increased RMTs were found in mTBI patients with or without sleep disturbances and also in the chronic phase over 3 years.
after injury. Conversely, other studies have failed to show a difference in either mild or moderate TBI in both acute and chronic phases. This is highlighted in the study by Miller, et al, which examined patients repeatedly between 72 hours and 2 months after injury, and demonstrated no differences in RMT. Although some studies suggest a slight shift to increased inhibition, caution should be exercised as RMT is highly variable between individuals, and therefore it may not be sensitive enough to detect the often-subtle changes associated with a mild injury.

**4.2.2 Motor evoked potential amplitude and stimulus response curves**

Some small studies found MEP amplitude was increased within subject testing at 1, 3, 5, and 10 days following an mTBI in athletes. However, this was not substantiated in larger studies, or in a repeated measures study examining changes over the first two months post-injury. Chistyakov, et al report an interesting finding of MEP amplitude fatigability at two weeks post-injury. To do this, they used suprathreshold rTMS trains of 50 stimuli and found that MEP amplitude progressively decreases within trains of stimulation, and showed marked irregularity in the shape of the MEP waveform. This effect was resolved in a follow-up subsample at 3 months post-injury.

SRC investigations have been normal following mTBI. Together, MEP amplitude and SRC indicate that the neuron population that excites the target muscle pathways is still typically capable of eliciting a normal output following stimulation. It is possible that the variability of these measures is increased after mTBI, although this needs corroboration.
4.2.3 Motor evoked potential latency and corticomotor conduction time

The white matter tract injuries associated with TBI may be detected using TMS techniques exploring conduction times i.e. MEP latency and corticomotor conduction times (CMCT). In the acute stages after mTBI, subtle conduction abnormalities have not been identified using TMS in the setting of normal RMT and MEP amplitudes\textsuperscript{131,135}. However, Chistyakov et al\textsuperscript{136}, found increased latency in a larger adult study in association with increased RMT and MEP amplitude variability at 2 weeks after TBI. This conduction delay could be due to axonal dysfunction in the motor tracts, as longer axons are more vulnerable to stretch and torsion forces during the insult. In a younger group of patients with sport-related injuries Miller et al\textsuperscript{113} did not find any differences in MEP amplitudes or latencies between controls and mTBI participants at 72 hours, with no main effect of time at 1, 2, 4, and 8 weeks post-injury. This could be because Miller studied a group with milder injuries at a younger age than the Chistyakov study. The interpretation of such studies is difficult due to the heterogeneity of the populations studied.

The lack of conclusive findings is also found in CMCT studies. A study by Chistyakov et al\textsuperscript{137}, divided the participants into 4 groups: concussion, focal injury, diffuse injury, and combined focal and diffuse injury. The focal, diffuse, and combined injury groups consisted of both mild and moderate TBI participants. This study found that only the more severe injuries (diffuse and focal injuries) had increased CMCT compared to controls.

4.2.4 Cortical silent period

Miller et al. explored inhibition in the acute stages of mTBI and provided strong evidence for increased inhibition by demonstrating a prolonged cSP. cSP was prolonged at 72 hours post-
injury and remained increased at 8 weeks. This is in keeping with other adult TBI studies. It is reasonable to consider any changes in cortical excitability persisting for 3 months or longer as reflecting a chronic change.

Increases in cSP have been found between 3 months and 30 months post-injury. This implies that there is a change in cortical inhibition after mTBI, and further, that these changes may be persistent years after the injury and may be correlated with the number of concussions. In contrast, 22 years after a concussion, Pearce et al. found a shortened cSP (during an “active contraction”). These findings implicate a further change in cortical inhibition over time. As inhibitory interneurons are vulnerable in TBI, a prolonged cSP (i.e. increased inhibition) seems counterintuitive. These changes could represent alterations in GABAb receptor expression (similar to that found in murine models of TBI), but it is also possible that cortical inhibition was increased before the acute injury interrogated by Miller et al. These increases in inhibition could be due to previous concussive or sub-concussive events. Further validation and exploration of these findings together with ipsilateral silent period interrogation are needed.

4.2.5 Intracortical facilitation

The TMS data depicting motor cortex neurophysiology is still incomplete. SICF has not been studied after TBI, and ICF has only been investigated in 3 studies. Two studies found that there were no differences in ICF after mTBI, and the third study found that 22 years after the injury, ICF was increased compared to controls.
4.2.6 Short interval intracortical inhibition

Changes in SICI are variable after TBI, and methodologies vary significantly across studies. A wide range of populations as well as time points post-injury were studied making it difficult to draw conclusions about changes in intracortical inhibition over time\textsuperscript{104,111,112,129,132,138,139}. For example, Pearce et al found chronically decreased SICI following mTBI\textsuperscript{104}. Conversely, SICI was increased in individuals with excessive daytime sleepiness acutely after mild and moderate TBI\textsuperscript{138}. Further investigation is warranted.

4.2.7 Long interval intracortical inhibition

LICI is thought to be related to the GABAb receptor-mediated inhibition of the cortical interneurons and pyramidal tract neurons by the conditioning TMS stimulus. Tremblay, et al\textsuperscript{140} found that LICI was increased in the first 12 months following an mTBI and other studies indicate that this inhibition decreases in the long term\textsuperscript{104,110}. Although LICI and cSP share a common pathway (via GABAb receptors), they examine slightly different mechanisms of intracortical inhibition\textsuperscript{93,94,124}. Nevertheless, increases in LICI are consistent with increases in cSP following adult TBI supporting a role for GABAb receptor-mediated intracortical inhibition in the recovery from TBI\textsuperscript{113,135}.

4.2.8 Transcranial magnetic stimulation and functional testing

In order to use TMS neurophysiological measures to understand the clinical manifestations of TBI, it is used in conjunction with functional tests. For example, in mTBI cognitive deficits may only found in the most difficult tasks, and may persist after symptom resolution\textsuperscript{30,141,142}. The similar persistence of TMS abnormalities (specifically cSP) shows that TMS may provide a correlate to strengthen neuropsychological testing after TBI. One TMS
study did find differential recovery in symptoms, neuropsychological test scores, and TMS parameters after sports concussion within the first ten days after injury, but these results need corroboration\textsuperscript{143}. Also, this study does not factor in the possibility for prolonged symptoms in PCS as the study ended at ten days after injury. In the rTMS study by Chistyakov, normalization of the MEP waveform was associated with symptom improvement from two weeks to three months after injury\textsuperscript{136}. However, this study suffers from attrition (from n=39 at 2 weeks to n=15 at 3 months), and therefore require corroboration.

4.3 Summary

There is a paucity of literature examining cortical microcircuits and/or cortical excitability using TMS, especially in children. The types of single and paired pulse techniques used is diverse as are the populations studied. Some studies suggest that TBI may have a prolonged effect on cortical inhibition because GABAb receptor-related TMS paradigms are increased. As GABAb has a modulatory effect, this may indicate that the motor microcircuit is shifted towards inhibition, preventing further over-activation such as with indiscriminate glutamate release after TBI. To date there have been no studies of cortical microcircuit changes using TMS after TBI in children.
Table 2: Summary of the literature using single and paired pulse TMS paradigms after mild and moderate TBI.

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<td>Mild</td>
<td>x x</td>
<td>x</td>
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<tr>
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<td>Mild</td>
<td>Retrospective</td>
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<tr>
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<td>Mild</td>
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<td>x</td>
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</tr>
<tr>
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<td>Mild</td>
<td>Asymptomatic</td>
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<td>Mild</td>
<td>Concussion</td>
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<td>↑</td>
<td>x</td>
</tr>
<tr>
<td>Tremblay133</td>
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<td>Mild</td>
<td>x</td>
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<td>Tallus130,144</td>
<td>2012, 2013</td>
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<td>Mild</td>
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<tr>
<td>De Beaumont133</td>
<td>2007</td>
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<td>Mild</td>
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<td>↑</td>
<td>x</td>
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<tr>
<td>Chistyakov145</td>
<td>2001</td>
<td>Acute</td>
<td>Mild - Mod</td>
<td>Minor</td>
<td>x</td>
<td>x</td>
<td>x x</td>
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<td></td>
<td></td>
<td>Diffuse Injury</td>
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<td>↓</td>
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</tr>
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<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td>↑</td>
<td>↓</td>
<td>↑ x</td>
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<tr>
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<td>Mild - Mod</td>
<td>Fatigue</td>
<td>x</td>
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<td>x x</td>
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<td></td>
<td>Objective EDS</td>
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<tr>
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<td>1999</td>
<td>Acute</td>
<td>Mild</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mod</td>
<td>↑</td>
<td>↑</td>
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<td></td>
<td></td>
<td></td>
<td>Diffuse injury</td>
<td>↑</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Focal injury</td>
<td>↑</td>
<td></td>
<td>x</td>
</tr>
</tbody>
</table>

↑ indicates a significant increase in the paradigm after TBI, while ↓ indicates a decrease. “x” indicates that no significant differences were found. 0 indicates that the paradigm was performed, but results were not reported.
Abbreviations: TMS: transcranial magnetic stimulation; MEP: Motor evoked potential; SRC: stimulus response curve; CMCT: corticomotor conduction time; CSP: cortical silent period; SPT: silent period threshold; iSP: ipsilateral silent period; TCI: transcallosal inhibition; TCT: transcallosal conduction time; SICI: short interval intracortical inhibition; ICF: intracortical facilitation; LIC1: long interval intracortical inhibition; EDS: excessive daytime sleepiness; Vari: MEP Amplitude Variability; BG: Between groups comparisons
5.1 Research questions and rationale

Mild TBI and post-concussion syndrome are a significant cause of morbidity in children and adolescents. We know very little about the reason for persistence of symptoms in some individuals, or rapid recovery in others. Some insights about the changes that occur in the brain’s small circuitry after an mTBI may come from TMS. The TMS literature in adults indicates that cortical excitability shifts towards inhibition after a mTBI\textsuperscript{114,137}, especially with repeated concussions\textsuperscript{133}. In keeping with this, although the methodologies vary widely and most studies are small, there is evidence that symptom improvement may be related to the return of normal electrophysiology\textsuperscript{135,136}. Inhibition has been studied mainly by using the cSP until recently\textsuperscript{114,137}, however advances in TMS methodologies now allow us to examine excitation and inhibition in more detail\textsuperscript{109,110,132,147}. To date, TMS studies after mTBI have only been conducted in adults. Children require independent research in this field because not only do they behave differently clinically compared to adults, their cortical responses to magnetic stimuli also differ after a mTBI\textsuperscript{1}. The purpose of this study, therefore, is to explore whether cortical excitability plays a role in recovery from an mTBI in children.

5.1.1 Primary aims:

- To explore cortical excitability in children following mTBI. Hypothesis: Cortical excitability (measured using cSP) will be inhibited following mTBI when compared to healthy controls.
- Research question: Does the cSP in children with an mTBI differ when compared to healthy controls?
• To investigate whether cortical excitability is related to symptom persistence. Hypothesis: Cortical inhibition will be greater in the symptomatic group when compared to children who are asymptomatic and normal controls.

• Research questions: Does cSP in children who remain symptomatic at one month post-injury differ from children who have recovered and from normal controls? Does cSP correlate with the degree of symptoms (total score on the PCSI) in children with mTBI at one month post-injury?

5.1.2 Secondary aims

To explore other parameters of cortical excitability and inhibition in mTBI. Exploratory analyses are required to develop a more complete picture of the neurophysiology of the motor cortex after injury. There is evidence that other paradigms in TMS (SICI, LICI, ICF) may be different between children who have suffered an mTBI and healthy controls, based on the adult literature\textsuperscript{109,110,132,147}. These other neurophysiological measures will provide a more complete picture of the state of the motor cortex, and also need to be studied in children after an mTBI.

• Research questions: does cortical excitability in children with an mTBI differ when compared to healthy controls? Does cortical excitability in children who remain symptomatic at one month post-injury differ from children who have recovered and from normal controls?

5.2 Study design

This was a cross-sectional controlled cohort observational study of mTBI in children at one-month post-injury. This study was performed as part of the Play Game Trial
and was granted ethical clearance by the University of Calgary Conjoint Health Research Ethics Board (REB13-0372). Parental consent and participant assent/consent was obtained before participation in the study.

5.3 Recruitment

5.3.1 Inclusion criteria

Inclusion criteria were 8–18 years of age and were symptomatic following mTBI at one month post-injury. Participants were eligible if they were diagnosed with an mTBI or concussion, as defined by the American Academy of Neurology. Mild TBI was operationally defined as an impact to the head or body resulting in at least one of the following: (1) an observed loss of consciousness (less than 30 minutes), (2) a Glasgow Coma Score of 13-15, or (3) at least one acute symptom suggesting neurological dysfunction attributable to the injury (e.g., headache, confusion, vomiting, amnesia, balance problems). Concussion is considered to be part of the spectrum of mTBI. Symptomatic status was determined by an increase in PCSI score at one month post-injury compared to pre-injury ratings.

5.3.2 Exclusion criteria

Exclusion criteria included loss of consciousness exceeding 30 minutes, Glasgow Coma Scale (GCS) score <13/15, suspected child abuse, alcohol or drug use at the time of injury, a previous mTBI within the last 3 months, failure to recover from a mTBI (greater than 3 months before), inability to complete questionnaires/evaluation (e.g. non-English language), significant past medical history, contraindications to TMS (metal in the head or electronic medical implants, such as pacemakers), and pregnancy. Significant medical history included, seizures, neurological...
abnormalities, and/or psychiatric disorder resulting in admission to hospital. Neuroactive drugs in this case are drugs that may have significant effects on cortical excitability, such as anti-seizure medication, anti-depressants, or anti-psychotics.

5.3.3 Ascertainment

Children and adolescents presenting to the Alberta Children’s Hospital in Calgary, Alberta, Canada with an mTBI were eligible for study. Children were contacted by telephone at approximately ~28 days from the date of injury, to determine recovery status.

Controls

There were two control groups recruited concurrently with the symptomatic participants: 1) An asymptomatic mTBI group: children with a mTBI satisfying the above criteria who were similar in age and sex but who had recovered at one month post-injury; and 2) healthy controls satisfying exclusion criteria and without a history of TBI. Healthy children were recruited as a sample of convenience, using siblings and friends of the injured children who were similar in age. Both control groups (healthy and asymptomatic) were recruited so that age and gender would be similar across groups.

5.4 Procedure

Participants were contacted at 28 days post-injury. During this phone call, they and their parents were asked to assent and consent, respectively, to the study. When assent and consent were obtained, a TMS session was scheduled. Demographic, acute injury details, past medical and family history were only obtained after consent was given. Additionally, on the day of the TMS session, participants had a magnetic resonance imaging (MRI) scan performed, and a
health assessment was performed by qualified personnel as separate portions of the Play Game study.

5.4.1 Post-concussion symptom inventory

The PCSI is a standardized questionnaire of the symptoms commonly experienced following a concussion. It has 26 questions in 4 domains: somatic, cognitive, affective, and sleep, where symptoms are rated for 0 to 6. Scores of 0 indicate that the symptom is not a problem for the child, while scores of 3 are classified as moderate, and scores of 6 are a severe problem. Participants retrospectively reported pre-injury symptoms. Then post-injury PCSI was collected. The post-injury PCSI is the same except an extra question “In general, to what degree do you feel ‘different’ than before the injury (not acting like yourself)?” rated 0-4. Children were considered symptomatic if they rated two or more symptoms increased by two point and the question of whether they felt different was 1 or more. The asymptomatic group had an mTBI one month previously but were no longer symptomatic.

5.5 Transcranial magnetic stimulation protocol

5.5.1 Setup

The participants were instructed to select a movie to watch during the TMS session to help ensure that they were comfortable and able to sit still during the session. Basic TMS principles and common experiences during the session were also explained to the children, beforehand, to put them at ease.
We attached Ag/AgCl EMG electrodes (Amplified 1000 times, band pass filtered 20-2000Hz, 5000Hz sampling rate) bilaterally to the first dorsal interosseus (FDI) muscle, which have been well studied in TBI\textsuperscript{133,149,150}. With the head in a neutral position and in a comfortable seating, the coil was manually held and the participant was encourage to relax. Using a Magstim BiStim 200 Transcranial Magnetic Stimulator (The Magstim Company Limited, Carmarthenshire), biphasic stimuli were applied to the motor cortex contralateral to the participant’s dominant hand. To find the “hotspot” for the FDI of the dominant hand, stimuli were applied in a small grid with a figure-8 coil (70mm inner diameter, each circle) resting tangential to the skull, at 45° from the midline. The hotspot is the area where a stimulus evokes the strongest and most consistent responses in the FDI EMG. The grid to determine the hotspot was approximately halfway between the nasion and occipital protuberance, ~3-4cm lateral to the vertex. Hotspot determination was aided by Brainsight neuronavigation software (Rogue Research Inc., Montreal, Canada) combined with a structural MRI. Structural MRIs were obtained earlier that day for another portion of the Play Game study, when available. If unavailable, the Montreal Neurological Institute MRI brain atlas for 13 to 18 year old children was used to position the TMS coil...

Maximal voluntary contraction, or effort, was then determined using an EMG oscilloscope (GwINSTEK GDS-1022, 25 MHz, 250M Sa/s, Good Will Instrument Co, New Taipei City, Taiwan). Participants were given compressible stress toys and instructed to squeeze the toy between their index finger and thumb of the dominant hand in a way that they could maintain for a long duration.
5.5.2 Stimulation protocol

5.5.2.1 Motor threshold

All stimuli were delivered over the hemisphere contralateral to the dominant hand. Two levels of RMT were used for this study: the lowest stimulus intensity that could elicit an EMG response of either 50µV (the 50µV RMT) and 1000µV (the 1000µV RMT) peak-to-peak amplitude in 5 out of 10 consecutive trials. The 1000µV RMT stimulus intensity was used as a standardized suprathreshold stimulation intensity, which ensures that a strong MEP can be compared to conditioned stimuli in the offline analyses. Stimulus intensity was represented as a percentage of the maximal stimulatory output (MSO). To determine each motor threshold, stimulus intensity began at the same amount needed to find the hotspot. Then the stimulus intensity was adjusted by 2% of the MSO until the stimulus failed to reach the minimum MEP amplitude for that RMT in 5 of 10 consecutive trials. The stimulator strength was subsequently increased by 1% of the maximum stimulator output to determine the final RMT. AMT was the lowest stimulus intensity that could elicit an EMG response of 200µV in 5 of 10 consecutive trials while performing an isometric contraction at 20% of the maximum voluntary effort. AMT was determined similarly to the RMTs.

5.5.2.2 Stimulus response curves

Once these were determined, a rest SRC of six levels was performed, with stimulus intensities pseudorandomized in 10% intervals between 100% and 150% of the 50µV RMT. Then the same script was used to test active SRC during an isometric contraction of 20% of the participants maximal effort, and stimuli were pseudo-randomized from 100% - 150% of the
AMT. From the active SRC, silent periods were also calculated, using cSP definitions for onset and end of the silent period.

5.5.2.3 Cortical silent period: primary outcome measure

cSP was the primary outcome measure for this study. cSP was chosen because it is the most consistently altered TMS paradigm found in adult TBI studies (see Chapter 4). To determine cSP, the participant isometrically contracted their hand at 20% of their maximal effort. They received 15 stimuli equal to their 1000µV RMT, while being encouraged to maintain their contraction throughout the trial. Stimuli were separated by 3s.

5.5.2.4 Ipsilateral silent period

iSP was performed as the participant isometrically contracted their FDI at 50% of their maximal strength in the hand ipsilateral to the coil (non-dominant), and a pulse is applied at their 1000µV RMT at the same hotspot as the other tests.

5.5.2.5 Paired pulse transcranial magnetic stimulation

Paired pulse TMS was used to evaluate cortical excitability and inhibition control mechanisms with 4 different tests, see Table 3.

1. SICF was performed using a conditioning stimulus at 90% of 50µV RMT followed by a test stimulus at the 1000µV RMT. The ISIs for SICF were 1.5ms, 2.6ms, and 4.3ms.
2. SICI and ICF were randomized together with the test stimulus. The pairs of stimuli were separated by ISIs of 2ms for SICI and 10ms for ICF. These combined paradigms were performed with both hands relaxed, with a conditioning stimulus of 90% RMT, and a test stimulus equal to the 1000µV RMT.
3. SICI and ICF were pseudo-randomised together again, using the same ISIs, but during an isometric contraction of 20% of maximum strength. In these paradigms, the conditioning stimulus was 70% AMT, followed by a test stimulus at 1000µV RMT.

4. LICI was performed using two stimuli (condition and test) at the 1000µV RMT separated by 100ms.
Table 3: Depiction of paired pulse paradigm methods used in the current study

<table>
<thead>
<tr>
<th>Paradigm</th>
<th>Conditioning Stimulus</th>
<th>Interstimulus interval</th>
<th>Test Stimulus</th>
<th>Waveform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Stimulus</td>
<td>-</td>
<td>-</td>
<td>1000μV RMT</td>
<td><img src="image1" alt="Waveform" /></td>
</tr>
<tr>
<td>SICF</td>
<td>90% RMT (70% AMT active)</td>
<td>1.5, 2.6, or 4.3ms</td>
<td>1000μV RMT</td>
<td><img src="image2" alt="Waveform" /></td>
</tr>
<tr>
<td>ICF</td>
<td>90% RMT (70% AMT active)</td>
<td>10ms</td>
<td>1000μV RMT</td>
<td><img src="image3" alt="Waveform" /></td>
</tr>
<tr>
<td>SICI</td>
<td>90% RMT (70% AMT active)</td>
<td>2ms</td>
<td>1000μV RMT</td>
<td><img src="image4" alt="Waveform" /></td>
</tr>
<tr>
<td>LICI</td>
<td>1000μV RMT</td>
<td>100ms</td>
<td>1000μV RMT</td>
<td><img src="image5" alt="Waveform" /></td>
</tr>
</tbody>
</table>

The grey lines in all cases are the same test stimulus superimposed for reference. Short vertical lines are the stimulus artefact from the magnetic field registering in the electrodes. The black lines show the effect of the conditioning stimulus.

Abbreviations: RMT: Rest motor threshold; AMT: active motor threshold; SICF: short interval intracortical facilitation; ICF: intracortical facilitation; SICI: short interval intracortical inhibition; LICI: long interval intracortical inhibition.
5.5.3 Safety and tolerability

At the end of each TMS session, children were asked a series of questions from the paediatric TMS tolerability questionnaire\(^{96}\). Children ranked 8 common activities, including their TMS session, in order of most enjoyable (1) to least enjoyable (8). The activities included, but were not limited to: a long car ride, a shot at the doctor’s office, watching TV, and going to a birthday party. Children also noted the presence and severity of any unpleasant tingling, headaches, neck pain, nausea or vomiting, and lightheaded or faint feelings they experienced during the session on the same questionnaire.

5.6 Data Processing

Data were processed while blinded to participant information, such as group, name, gender, and study identification number. Blinding was performed by a lab member who removed study identification numbers, and replaced them with a second, independent set of identification numbers.

Offline, every blinded EMG trace was checked against paradigm specifications in Signal. Any resting paradigms that showed background EMG above 100\(\mu\)V were removed on a frame-by-frame basis. Similarly, any EMG traces during active paradigms that showed consistent activity less than 100\(\mu\)V or a MEP was not visible were excluded on a frame-by-frame basis.

Data were processed using Matlab (MATLAB and Statistics Toolbox Release 2014b, The MathWorks, Inc., Natick, Massachusetts, United States.). In both SRCs and all paired pulse paradigms, peak-to-peak amplitudes were determined using automated scripts. For SRCs, the amplitude of each MEP was sorted into the stimulation intensities, and the mean amplitude at each stimulation intensity was measured, giving six mean amplitudes per person. The participant
means were plotted against stimulus intensity relative to the motor threshold used to stimulate. From the plotted means, the slope, maximum, and area under the curve were calculated for each individual. In group analysis, the mean amplitudes at each stimulus intensity were averaged, and a graph was plotted.

For paired pulse paradigms, the peak-to-peak amplitudes were calculated under each ISI. To do this, the mean peak-to-peak amplitude was calculated under each condition, and the conditions were sorted then averaged. Within each paired pulse test, conditioned stimuli were normalised to test stimuli amplitudes by taking the ratio of the conditioned MEP to the unconditioned (test stimulus alone, stimulated at 1000µV RMT) MEP amplitude. If the mean of the test stimuli alone for any paired pulse paradigm were not greater than 100µV, it was ruled that the cortex did not receive sufficient magnetic power to evoke responses and the entire paradigm was discarded for that participant. In paired pulse configurations, some magnetic coils suffer a decrease in power, and these were discarded to avoid biasing the data towards a facilitation effect.

In silent period paradigms the duration of the disrupted waveform was analysed. The duration of the cSP and iSP was considered the onset of the disrupted waveform after the MEP to when EMG activity returned to 25% of the mean rectified background EMG activity before the stimulus artefact in offline analysis.

A spreadsheet connecting the two identification numbers was kept separate from any other data to aide in unblinding. Demographic data was kept in a third database, and PCSI data was kept in a fourth, online database. The multiple sets of data were all combined into one spreadsheet at the end of the study, and analysed.
5.7 Sample size calculation

Sample sizes were calculated using data from the study from Miller et al\textsuperscript{153} because they recruited mTBIs of various aetiologies and investigated cSP at 1 month post-injury. The means (standard deviations) for the healthy controls were 85.7 (23.2) and for the mTBI group was 111.5 (36.4). These values were entered into G*Power\textsuperscript{154} to calculated required sample size. The calculated sample size was 24 for a critical $F(2, 69) = 3.13$, when alpha was set to 5% and power was set to 80%.

5.8 Statistical analyses

Groups were described using tables and graphs. Normality was tested using Shapiro-Wilks. If the data in the groups were not normally distributed, they were transformed according to the skew of the data to a normal distribution. All non-normal data were right skewed, and so were log\textsubscript{10} transformed.

Graphs were created in Sigmaplot 13.0 (Systat Software, Inc., San Jose California USA, \url{www.sigmaplot.com}). Line graphs show the means with standard deviations. Boxplots show the group median as a black horizontal line inside the box. The top edge of the box is the 75\textsuperscript{th} percentile, and the bottom of the box is the 25\textsuperscript{th} percentile, with the group mean in the middle of the box. Outside the box, the whiskers denote the ends of the inner fence, or normal range of data. To calculate the inner fence, 1.5 times the interquartile range is subtracted or added to the first or third quartile, respectively. Outliers are shown as points.
Pre-injury variables were compared between healthy controls and mTBI participants, and healthy controls, asymptomatic mTBI, and symptomatic mTBI using chi squared tests for the frequency of learning and attention difficulties, and number of previous injuries. Similar comparisons were made for injury characteristics such as loss of consciousness, amnesia, and cause of injury, and for TMS side effects, such as headaches, neck pain, tingling, light headedness, and nausea.

A series of student t-tests were performed to compare the cSP of healthy controls to all children who incurred a paediatric mTBI one month previously. Significance was set to 0.05. Similarly, secondary analyses were carried out using student t-tests on iSP, SICI, ICF, LICI, and the slope, maximum, and area under the curve of the SRC of healthy controls to all children who incurred a paediatric mTBI. One sample t-tests were performed in all paired pulse TMS paradigms independent of group to determine if there was an effect of inhibition or facilitation. Mixed models ANOVAs were used to analyse SICF and SRC. In SICF, ISI was treated as a within subject variable, and in SRC, stimulus intensity was treated as a within subject variable. The two groups were used as between subjects’ variables in these analyses. Experiment-wise error rates were not corrected for because these were exploratory analyses.

In the mTBI group, relationships between age, handedness, gender, and presence of learning difficulties or attention deficit hyperactivity disorder (ADHD) were related to cSP individually using univariate analyses. The relationship between cSP and post-injury PCSI was explored using regression. Measures that were significantly related to cSP were treated as covariates.
The symptomatic and asymptomatic groups were compared to healthy controls using one-way analyses of variance (ANOVAs) for cSP. ANOVAs were used in secondary analyses to compare the iSP, SICI, ICF, LICI, and the slope, maximum, and area under the curve of the SRC between healthy, asymptomatic, and symptomatic groups. Mixed models ANOVAs were used to analyse SICF and SRC. In SICF, ISI was treated as a within subject variable, and in SRC, stimulus intensity was treated as a within subject variable, and the healthy, asymptomatic, and symptomatic groups were used as a between subjects variable. In all comparisons, Tukey’s post-hoc tests were used to correct for multiple comparisons between groups.
Chapter Six: **Results**

### 6.1 Demographic information

A total of 28 healthy controls were recruited to the study, and 57 mTBI participants (25 asymptomatic, symptomatic; See Table 4). The groups were similar in age (mean (standard deviation): healthy controls: 14.20 (3.01), asymptomatic: 14.16 (2.46), and the symptomatic group was 14.29 (2.56)) and sex (control group was 53% female, the asymptomatic group was 52% female, and the symptomatic group was 59% female. The number of previous concussions did not differ between the asymptomatic and symptomatic groups ($\chi^2 (4) = 8.72, p = 0.069$). 50% of the symptomatic mTBI group had had at least one previous concussion. The median [range] PCSI scores before the injury were similar between groups (healthy controls: 0 [0-29], asymptomatic: 0 [0-9], symptomatic: 4 [0-46]). School and learning difficulties were more common in the symptomatic group, 31%, and 16% were received requiring learning support. 9% were diagnosed with ADHD and a further 4% had attention problems pre-injury. None of the children were taking medication around the time of injury or at the time of assessment.

Injury characteristics are shown in Table 5. The majority of mTBIs occurred during sports activities (74%) and were similar between asymptomatic and symptomatic groups ($\chi^2 (6) = 4.69, p = 0.585$). Fifteen percent of the mTBI group lost consciousness. The median post-injury PCSI score in the symptomatic group was 34 [range: 0-122].
### Table 4: Pre-injury characteristics of study sample

<table>
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<tr>
<th></th>
<th>Healthy</th>
<th>mTBI</th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
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</thead>
<tbody>
<tr>
<td><strong>Total n</strong></td>
<td>28</td>
<td>57</td>
<td>25</td>
<td>32</td>
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<tr>
<td><strong>Gender, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>15 (53.6)</td>
<td>32 (56.1)</td>
<td>13 (52.0)</td>
<td>19 (59.4)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>13 (52.0)</td>
<td>25 (44.6)</td>
<td>14 (56.0)</td>
<td>13 (40.6)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>19 (59.4)</td>
<td>32 (56.1)</td>
<td>25 (100)</td>
<td>32 (100)</td>
</tr>
<tr>
<td><strong>Mean Age at assessment (years) (SD)</strong></td>
<td></td>
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<tr>
<td>Healthy</td>
<td>14.20 (3.01)</td>
<td>14.23 (2.49)</td>
<td>14.16 (2.46)</td>
<td>14.29 (2.56)</td>
</tr>
<tr>
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<td>13 (2.8)</td>
<td>15 (3.0)</td>
<td>12 (4.0)</td>
<td>14 (4.0)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>10 (3.1)</td>
<td>14 (2.8)</td>
<td>14 (4.0)</td>
<td>16 (4.0)</td>
</tr>
<tr>
<td><strong>Any School/Learning Difficulties, n (%)</strong></td>
<td></td>
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<tr>
<td>Healthy</td>
<td>6 (21.4)</td>
<td>13 (22.8)</td>
<td>3 (12.0)</td>
<td>10 (31.3)</td>
</tr>
<tr>
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<td>13 (22.8)</td>
<td>25 (44.6)</td>
<td>14 (56.0)</td>
<td>13 (40.6)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>10 (31.3)</td>
<td>32 (56.1)</td>
<td>25 (100)</td>
<td>32 (100)</td>
</tr>
<tr>
<td><strong>ADHD, n (%)</strong></td>
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<td></td>
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<tr>
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<td>4 (7.0)</td>
<td>1 (4.0)</td>
<td>3 (9.4)</td>
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<tr>
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<td>4 (7.0)</td>
<td>12 (21.1)</td>
<td>2 (8.0)</td>
<td>10 (31.3)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>10 (31.3)</td>
<td>25 (44.6)</td>
<td>14 (56.0)</td>
<td>13 (40.6)</td>
</tr>
<tr>
<td><strong>Learning Difficulties, n (%)</strong></td>
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<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>1 (3.6)</td>
<td>6 (10.5)</td>
<td>1 (4.0)</td>
<td>5 (15.6)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>6 (10.5)</td>
<td>25 (44.6)</td>
<td>14 (56.0)</td>
<td>13 (40.6)</td>
</tr>
<tr>
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<td>14 (41.2)</td>
<td>32 (56.1)</td>
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<td>32 (100)</td>
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<td><strong>Learning Support, n (%)</strong></td>
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<td>1 (3.6)</td>
<td>5 (8.8)</td>
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<td>16 (50.0)</td>
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<tr>
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<td>16 (50.0)</td>
<td>32 (56.1)</td>
<td>25 (100)</td>
<td>32 (100)</td>
</tr>
<tr>
<td><strong>Number of Previous Brain Injuries, n (%)</strong></td>
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<td></td>
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<tr>
<td>0</td>
<td>28 (100)</td>
<td>36 (63.2)</td>
<td>20 (80.0)</td>
<td>16 (50.0)</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>12 (21.1)</td>
<td>2 (8.0)</td>
<td>10 (31.3)</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>6 (10.5)</td>
<td>2 (8.0)</td>
<td>4 (12.5)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>-</td>
<td>3 (5.3)</td>
<td>1 (4.0)</td>
<td>2 (6.3)</td>
</tr>
<tr>
<td><strong>Pre-Injury Child PCSI, [range]</strong></td>
<td>0 [0-29]</td>
<td>2 [0-46]</td>
<td>0 [0-9]</td>
<td>4 [0-46]</td>
</tr>
</tbody>
</table>

*Abbreviations: mTBI: mild traumatic brain injury*
### Table 5: Injury and post-injury characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total n</th>
<th>mTBI</th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
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<tr>
<td><strong>Cause of Injury, n (%)</strong></td>
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<td></td>
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<tr>
<td>Sports</td>
<td>57</td>
<td>42 (73.7)</td>
<td>21 (84.0)</td>
<td>21 (65.6)</td>
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<tr>
<td>Fall</td>
<td>25</td>
<td>7 (12.3)</td>
<td>3 (12.0)</td>
<td>4 (12.5)</td>
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<tr>
<td>MVC</td>
<td>32</td>
<td>1 (1.8)</td>
<td>0 (0)</td>
<td>1 (3.1)</td>
</tr>
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<td>Pedestrian-MVC</td>
<td>21</td>
<td>1 (1.8)</td>
<td>0 (0)</td>
<td>1 (3.1)</td>
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<td>Assault</td>
<td>65.6</td>
<td>1 (1.8)</td>
<td>0 (0)</td>
<td>1 (3.1)</td>
</tr>
<tr>
<td>Other</td>
<td>12.5</td>
<td>2 (3.5)</td>
<td>0 (0)</td>
<td>2 (6.3)</td>
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<td>Unknown</td>
<td>6.3</td>
<td>3 (5.3)</td>
<td>1 (4.0)</td>
<td>2 (6.3)</td>
</tr>
<tr>
<td><strong>Loss of Consciousness, n (%)</strong></td>
<td>n = 52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 (15.8)</td>
<td>3 (12)</td>
<td>6 (18.8)</td>
<td></td>
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<tr>
<td><strong>Anterograde Amnesia, n (%)</strong></td>
<td>n = 55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 (24.6)</td>
<td>8 (32.0)</td>
<td>6 (18.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Retrograde amnesia, n (%)</strong></td>
<td>n = 55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 (19.3)</td>
<td>7 (28.0)</td>
<td>4 (12.5)</td>
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<tr>
<td><strong>Median Time Since Injury (days), [range]</strong></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>40 [28-57]</td>
<td>40.5 [28-57]</td>
<td>40 [32-54]</td>
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</tr>
<tr>
<td><strong>Post- Injury Child PCSI, [range]</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12 [0-122]</td>
<td>2 [0-26]</td>
<td>34 [0-122]</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: PCSI: Post-concussion symptom inventory; MVC: Motor vehicle crash*
6.2 Transcranial magnetic stimulation

6.2.1 Motor thresholds

Patients whose thresholds were too high to perform certain paradigms are shown in Table 6. If a participant’s RMT at 50µV or AMT were above 66% of the maximal stimulator output, they could not complete the stimulus response curve paradigms as the stimulator could not provide a magnetic pulse at 150% of the threshold. If a participant had an RMT at 50µV above 88% of the maximal stimulator output, then they could not complete short interval intracortical inhibition (SICI) or intracortical facilitation (ICF). The numbers of individuals whose thresholds were too high for certain paradigms are listed in Table 6.

Motor thresholds were similar between healthy controls (n = 27) and mTBI participants (n = 54), see Table 7 and Figure 7, and likewise similar between controls (n = 27), asymptomatic (n = 22), or symptomatic (n = 32) groups for any motor threshold (p = 0.20-0.69). One asymptomatic participant’s motor thresholds were performed but not documented.
Table 6: Numbers of participants completing paradigms

<table>
<thead>
<tr>
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<th>Groups</th>
<th>χ²</th>
<th>p</th>
</tr>
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<tbody>
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<td></td>
<td>Healthy</td>
<td>mTBI</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Total n</td>
<td>28</td>
<td>57</td>
<td>25</td>
</tr>
<tr>
<td>All Paradigms Performed</td>
<td>24</td>
<td>48</td>
<td>23</td>
</tr>
<tr>
<td>Unable to Stimulate at 120% 50µV RMT</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>RSRC Not Performed</td>
<td>3</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Unable to Stimulate at RMT 1000µV</td>
<td>3</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>ASRC Not Performed</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hemisphere Stimulated</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>25</td>
<td>47</td>
<td>22</td>
</tr>
<tr>
<td>Right</td>
<td>3</td>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>

Chi-squared tests performed between controls, asymptomatic, and symptomatic mTBI.

Abbreviations: mTBI: mild traumatic brain injury; RMT: rest motor threshold; RSRC: rest stimulus response curve; ASRC: active stimulus response curve
Figure 7: Logs of motor thresholds under rest and active conditions for all mild traumatic brain injury (mTBI) participants and sorted based on compared to controls.

There are no differences between groups. (A, C, and E) are the logs of the rest motor threshold (RMT) at 50µV (control n = 27; mTBI n = 54; t (52.02) = 0.55, p = 0.583), RMT at 1000µV (control n = 25; mTBI n = 47; t (40.35) = 1.15, p = 0.257), and the active motor threshold at 50µV (control n = 27; asymptomatic n = 23; symptomatic n = 31; F (2, 78) = 0.38, p = 0.687), RMT at 1000µV (control n = 25; asymptomatic n = 22; symptomatic n = 25; F (2, 69) = 1.63, p = 0.204), and the active motor threshold (control n = 27; asymptomatic n = 24; symptomatic n = 32; F (2, 80) = 0.65, p = 0.522), respectively, when all mTBI participants are grouped together compared to controls. (B, D, and F) are the logs of the RMT at 50µV (control n = 27; asymptomatic n = 23; symptomatic n = 31; F (2, 78) = 0.38, p = 0.687), RMT at 1000µV (control n = 25; asymptomatic n = 22; symptomatic n = 25; F (2, 69) = 1.63, p = 0.204), and the active motor threshold (control n = 27; asymptomatic n = 24; symptomatic n = 32; F (2, 80) = 0.65, p = 0.522), respectively, when mTBI participants are sorted into asymptomatic or symptomatic status compared to controls.
6.2.2 Stimulus response curves

The resting SRCs for both the two group analysis and the three group analysis were $\log_{10}$ transformed. The resting SRC amplitude showed a main effect of stimulus intensity ($F(2.2, 158.2) = 281.36, p < 0.01$). However, there was no significant difference between the healthy controls ($n = 24$) and all mTBI participants ($n = 50$) ($F(1, 72) = 2 \times 10^{-3}, p = 0.96$) and no significant group X stimulus intensity interaction ($F(5, 68) = 1.28, p = 0.28$). The areas under the SRCs ($t(59.28) = -0.21, p = 0.838$), slope ($t(44.23) = -0.78, p = 0.440$), and maximum ($t(40.08) = -0.44, p = 0.661$) for the healthy controls and combined mTBI group are shown in Table 7 and Figure 8, and they were not different between groups.

The resting SRCs for the healthy controls ($n = 24$), asymptomatic ($n = 24$), and symptomatic groups ($n = 26$) are shown in Figure 9, and showed a significant increase in MEP amplitude with increasing stimulation intensity ($F(2.19, 155.26) = 320.34, p < 0.01$). There was no effect of group ($F(2, 71) = 1.34, p = 0.267$), nor a group X stimulation intensity interaction ($F(10, 136) = 1.32, p = 0.227$). There were no significant between groups differences for the areas under the SRCs ($F(2, 71) = 0.42, p = 0.656$), slopes ($F(2, 71) = 0.53, p = 0.593$), or maximums ($F(2, 71) = 1.52, p = 0.225$).

The active SRCs for the healthy controls ($n = 27$) and the combined mTBI groups ($n = 55$) are shown in Figure 10. Again, there was an expected significant increase in MEP amplitude with increasing stimulation intensity ($F(2.19, 80) = 314.76, p < 0.01$), but no effect of group ($F(1, 80) = 0.09, p = 0.766$), nor a group X stimulation intensity interaction ($F(5, 76) = 1.73, p = 0.138$). The areas under the curves ($t(59.28) = -0.21, p = 0.838$), slopes ($t(59.81) = 0.09, p =
0.925), and maximums (t (55.86) = -0.34, p = 0.734) are not significantly different between healthy controls and the mTBI group.

The active SRCs for the healthy controls (n = 27), asymptomatic (n= 24), and symptomatic mTBI (n = 31) groups are shown in Figure 11, and show a similar pattern as above. There was a significant increase in MEP amplitude with stimulation intensity (F (2.21, 174.89) = 345.04, p < 0.01), but the curves were similar between groups (F (2, 79) = 0.09, p = 0.913), and did not show a group X stimulus intensity interaction (F (10, 152) = 1.25, p = 0.263). The calculated values from this SRC were normally distributed, and therefore were not transformed. There were no significant group differences for area under the curve (F (2, 79) = 0.19, p = 0.824), slope (F (2, 79) = 0.25, p = 0.776), and slope (F (2, 79) = 0.21, p = 0.814).
Figure 8: Rest stimulus response curve measures and supplemental analyses for controls compared to combined mTBI at one month after injury.

(A) Line graph shows the log$_{10}$ of the stimulus response curve with the mean (±standard deviation) of the amplitude of motor evoked potentials at all stimulus intensities, with no differences between groups (controls n = 24; mTBI n = 50; F (1, 72) = 2E$^{-1}$, p = 0.96).

(B) Boxplots show the distribution of the log$_{10}$ of the rest area under the curve (t (59.28) = -0.21, p = 0.838).

(C) Boxplots show the distribution of the log$_{10}$ of the peak slope of the curve (t (44.23) = -0.78, p = 0.440).

(D) Boxplots show distribution of the log$_{10}$ of the maximum (t (40.08) = -0.44, p = 0.661).
Figure 9: Rest stimulus response curve measures and supplemental analyses for controls compared asymptomatic and symptomatic mTBI participants one month after injury.

(A) Line graph shows the log$_{10}$ of the stimulus response curve with the mean (±standard deviation) of the amplitude of motor evoked potentials at all stimulus intensities, with no between group differences (controls n = 24; asymptomatic n = 24; symptomatic n = 26; $F(2, 71) = 1.34, p = 0.267$). (B) Boxplots show the distribution of log$_{10}$ of the rest area under the curve ($F(2, 71) = 0.42, p = 0.656$). (C) Boxplots show distribution of the log$_{10}$ of the peak slope of the curve ($F(2, 71) = 0.53, p = 0.593$). (D) Boxplots show distribution of the log$_{10}$ of the maximum ($F(2, 71) = 1.52, p = 0.225$).
Figure 10: Active stimulus response curve measures and supplemental analyses for controls compared to combined mTBI at one month after injury.

(A) Line graph shows the stimulus response curve with the mean (±standard deviation) of the amplitude of motor evoked potentials at all stimulus intensities (control n = 27; mTBI n = 55; F (1, 80) = 0.09, p = 0.766). (B) Boxplots show the distribution of rest area under the curve (t (59.28) = -0.21, p = 0.838). (C) Boxplots show the distribution of the peak slope of the curve (t (59.81) = 0.09, p = 0.925). (D) Boxplots show the distribution of the maximum (t (55.86) = -0.34, p = 0.734).
Figure 11: Active stimulus response curve measures and supplemental analyses for controls compared asymptomatic and symptomatic mTBI participants one month after injury.

(A) Line graph shows the stimulus response curve with the mean (±standard deviation) of the amplitude of motor evoked potentials at all stimulus intensities (control n = 27; asymptomatic n = 24; symptomatic n = 31; F (2, 79) = 0.09, p = 0.913). (B) Boxplots show the distribution of rest area under the curve (F (2, 79) = 0.19, p = 0.824). (C) Boxplots show distribution of peak slope of the curve (F (2, 79) = 0.25, p = 0.776). (D) Boxplots show distribution of the maximum (F (2, 79) = 0.21, p = 0.814).
6.2.3 Cortical silent period

The cSPs in the healthy and combined mTBI groups are in Figure 12 and Table 7. These data were normally distributed in all groups. There were no differences between healthy (n = 25) and mTBI groups (n = 48) (t (45.37) = 0.43, p = 0.667), or between healthy (n = 25) group, asymptomatic (n = 23), and symptomatic groups (n = 25) (F (2, 70) = 0.53, p = 0.591).

CSP correlated with age (R² = 0.142, p = 0.001). A bivariate correlation of age and cSP duration was significantly negatively correlated (r = -027, p = 0.072). The number of previous injuries (R² = 0.009, F (1, 71) = 1.64, p = 0.204), presence of learning difficulties (R² = 0.003, p = 0.270), and ADHD (R² = 0.002, p = 0.354) did not correlate with cSP. A linear regression was used to compare cSP (dependent variable) and PCSI (independent variable) was ran (R² = 0.270, p = 0.045) with age at assessment as a covariate. Age explained a significant amount of the variance (F (1, 37) = 6.26, p = 0.017), while PCSI score did not (F (33, 37) = 1.37, p = 0.175).

The cSP was dependent on the strength of the stimulation. In the active SRC, the silent period durations were log-transformed (Figure 12). In healthy and mTBI group, there was an effect of stimulation intensity (F (2.25, 77) = 348.53, p < 0.001), but no effect of group (F (1, 77) = 0.17, p = 0.682), or a group X stimulus intensity interaction (F (5, 73) = 0.47, p = 0.798). The same pattern was found in analyses of healthy controls, asymptomatic participants, and symptomatic participants with increasing stimulation intensity (F (2.25, 170.75) = 395.32, p < 0.01), but no effect of group (F (2, 76) = 0.19, p = 0.830), or a group X stimulus intensity interaction (F (10, 146) = 0.91, p = 0.526).
6.2.4 Ipsilateral silent period

The iSP (Figure 13) was normally distributed in all groups. Between group comparisons were not significant for the healthy controls (n = 24) compared to the combined mTBI group (n = 46) (t (40.29) = 0.53, p = 0.601) or for the healthy controls (n = 24), asymptomatic (n = 23), and symptomatic group (n = 23) comparisons (F (2, 67) = 0.55, p = 0.582).
<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>mTBI</th>
<th>t-test</th>
<th>P</th>
<th>Healthy</th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
<th>F-test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log(_{10}) RMT</td>
<td>1.67 [1.62 - 1.71]</td>
<td>1.65 [1.62 - 1.68]</td>
<td>t (52.02) = 0.55</td>
<td>0.583</td>
<td>1.67 [1.62 - 1.71]</td>
<td>1.64 [1.6 - 1.68]</td>
<td>1.66 [1.61 - 1.71]</td>
<td>F (2, 78) = 0.38</td>
<td>0.687</td>
</tr>
<tr>
<td>50(\mu)V</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Log(_{10}) RMT</td>
<td>1.76 [1.7 - 1.81]</td>
<td>1.72 [1.69 - 1.75]</td>
<td>t (40.35) = 1.15</td>
<td>0.257</td>
<td>1.76 [1.7 - 1.81]</td>
<td>1.75 [1.7 - 1.8]</td>
<td>1.7 [1.67 - 1.74]</td>
<td>F (2, 69) = 1.63</td>
<td>0.204</td>
</tr>
<tr>
<td>1000(\mu)V</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log(_{10}) AMT</td>
<td>1.53 [1.49 - 1.58]</td>
<td>1.54 [1.5 - 1.57]</td>
<td>t (58) = -0.16</td>
<td>0.876</td>
<td>1.53 [1.49 - 1.58]</td>
<td>1.52 [1.47 - 1.56]</td>
<td>1.55 [1.50 - 1.60]</td>
<td>F (2, 80) = 0.65</td>
<td>0.522</td>
</tr>
<tr>
<td>200(\mu)V</td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RSRC Area</td>
<td>1866.49 [1057.82 - 2675.17]</td>
<td>1746.94 [1443.17 - 2050.71]</td>
<td>t (30.08) = 0.29</td>
<td>0.777</td>
<td>1866.49 [1057.82 - 2675.17]</td>
<td>1571.14 [1096.73 - 2045.56]</td>
<td>1909.21 [1501.10 - 2317.32]</td>
<td>F (2, 71) = 0.42</td>
<td>0.656</td>
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<td>Under Curve</td>
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<td></td>
</tr>
<tr>
<td>Log(_{10}) RSRC</td>
<td>-1.43 [-1.6 - -1.26]</td>
<td>-1.35 [-1.46 - -1.24]</td>
<td>t (44.23) = -0.78</td>
<td>0.44</td>
<td>-1.43 [-1.6 - -1.26]</td>
<td>-1.39 [-1.59 - -1.19]</td>
<td>-1.32 [-1.44 - -1.20]</td>
<td>F (2, 71) = 0.53</td>
<td>0.593</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSRC Max</td>
<td>0.29 [0.14 - 0.45]</td>
<td>0.33 [0.24 - 0.42]</td>
<td>t (40.08) = -0.44</td>
<td>0.661</td>
<td>0.29 [0.14 - 0.45]</td>
<td>0.25 [0.1 - 0.4]</td>
<td>0.41 [0.31 - 0.50]</td>
<td>F (2, 71) = 1.52</td>
<td>0.225</td>
</tr>
<tr>
<td>ASRC Area</td>
<td>3.6 [3.52 - 3.68]</td>
<td>3.61 [3.54 - 3.68]</td>
<td>t (59.28) = 0.21</td>
<td>0.838</td>
<td>4146.49 [3660.92 - 5172.05]</td>
<td>4710.75 [3556.76 - 5864.75]</td>
<td>4660.78 [3977.68 - 5343.89]</td>
<td>F (2, 79) = 0.19</td>
<td>0.824</td>
</tr>
<tr>
<td>Under Curve</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASRC Slope</td>
<td>-1.1 [-1.21 - -0.99]</td>
<td>-1.11 [-1.2 - -1.02]</td>
<td>t (59.81) = 0.09</td>
<td>0.925</td>
<td>0.09 [0.07 - 0.11]</td>
<td>0.09 [0.07 - 0.11]</td>
<td>0.10 [0.08 - 0.12]</td>
<td>F (2, 79) = 0.25</td>
<td>0.776</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASRC Max</td>
<td>0.72 [0.63 - 0.80]</td>
<td>0.73 [0.67 - 0.8]</td>
<td>t (55.86) = -0.34</td>
<td>0.734</td>
<td>5.81 [4.79 - 6.83]</td>
<td>6.03 [4.82 - 7.24]</td>
<td>6.26 [5.30 - 7.22]</td>
<td>F (2, 79) = 0.21</td>
<td>0.814</td>
</tr>
<tr>
<td>CSP</td>
<td>114.58 [95.53 - 133.63]</td>
<td>109.77 [97.43 - 122.12]</td>
<td>t (45.37) = 0.43</td>
<td>0.667</td>
<td>114.58 [95.53 - 133.63]</td>
<td>115.89 [97.78 - 134.00]</td>
<td>104.15 [86.34 - 121.96]</td>
<td>F (2, 70) = 0.53</td>
<td>0.591</td>
</tr>
</tbody>
</table>

Paradigms include: Motor thresholds, shown as the log of the percentage of the maximal stimulator output (MSO); silent periods, with values in milliseconds; and logs of the stimulus response curve values area under the curve, slope, and log of the maximum, which are all functions of the motor evoked potential amplitude. The Slope of the ASRC is transformed by Log\(_{10}\) in the Healthy controls and mTBI combined group only; the healthy controls, asymptomatic, and symptomatic grouping is not Log\(_{10}\)transformed.

Abbreviations: RMT: rest motor threshold; AMT: active motor threshold; CSP: cortical silent period; ISP: ipsilateral silent period; MSO: maximal stimulator output; RSRC: rest stimulus response curve; AUC: area under the curve; ASRC: active stimulus response curve
Figure 12: Cortical silent period paradigms.

(A) Boxplot of the cortical silent period duration for the healthy controls (n = 25) against combined mTBI (n = 48) in milliseconds (t (45.37) = 0.43, p = 0.667). (B) Line graph shows the log of the duration of the cortical silent period with increased stimulation intensity for healthy controls (n = 27) and the combined mTBI group (n = 55), taken during active stimulus response curve trials, at a contraction of 20% of the participant’s maximum voluntary effort (F (1, 77) = 0.17, p = 0.682). (C) Boxplot of the cortical silent period duration for the healthy controls (n = 25) against asymptomatic (n = 23) and symptomatic (n = 25) mTBI in milliseconds (F (2, 70) = 0.53, p = 0.591). (D) Line graph shows the log of the duration of the cortical silent period with increased stimulation intensity for healthy controls (n = 27), and asymptomatic (n = 24) and symptomatic mTBI group (n = 31), taken during active stimulus response curve trials, at a contraction of 20% of the participant’s maximum voluntary effort (F (2, 76) = 0.19, p = 0.830).
Figure 13: Ipsilateral silent period paradigms.
(A) Boxplot of the ipsilateral silent period duration for the healthy controls (n = 24) against combined mTBI (n = 46) in milliseconds (t (40.29) = 0.53, p = 0.601). (B) Boxplot of the ipsilateral silent period duration for the healthy controls (n = 24) against asymptomatic (n = 23) and symptomatic mTBI (n = 23) in milliseconds (F (2, 67) = 0.55, p = 0.582).
6.2.5 *Short interval intracortical facilitation*

There was no significant facilitation in healthy controls at an ISI of 1.5ms (t (11) = -1.7, p = 0.118), and healthy controls were significantly inhibited at 2.6ms (t (11) = -3.87, p = 0.003) and at 4.3ms (t (11) = -2.56, p = 0.026). The mTBI group was significantly inhibited at 1.5ms (t (22) = -4.91, p < 0.001), 2.6ms (t (22) = -6.65, p < 0.001), and 4.3ms (t (22) = -3.37, p = 0.003).

Based on mixed models ANOVAs of healthy controls (n = 12) to combined mTBI (n = 23), there is a significant effect of ISI on the log of the ratio of MEP amplitudes (F (2, 66) = 15.76, p < 0.01). In post-hoc analyses, 1.5ms ISI was different from 2.6ms ISI (p < 0.001), and 2.6ms ISI was different from the 4.3ms ISI (p < 0.001). There are no group differences (F (1, 33) = 0.39, p = 0.535) or any group X ISI interaction (F (2, 66) = 0.24, p = 0.787).

The asymptomatic group (1.5ms: t (11) = -4.71, p = 0.001, 2.6ms: t (11) = -5.85, p < 0.001, 4.3ms: t (11) = -2.44, p = 0.033) and symptomatic group (1.5ms: t (10) = -2.47, p = 0.03, 2.6ms: t (10) = -3.81, p = 0.003, 4.3ms: t (10) = -2.7, p = 0.022) showed inhibition at all three ISIs. Comparisons between the healthy controls (n = 12), asymptomatic (n = 12) and symptomatic (n = 10) mTBI showed a similar pattern: a significant effect of ISI (F (2, 64) = 16.9, p < 0.001), but no significant effect of group (F (2, 32) = 0.38, p = 0.690) nor a significant group X ISI interaction (F (4, 64) = 0.37, p = 0.826). SICF is shown in Figure 14.

6.2.6 *Intracortical facilitation*

ICF (Figure 15 and Table 8) was not seen in the healthy controls (t (14) = -0.45, p = 0.656) or in mTBI participants (t (40) = 1.65, p = 0.106) under the resting condition. Log10 of healthy controls (n = 15) and combined mTBI groups (n = 41) data showed no significant (t (29.35) = -1.33, p = 0.193). The asymptomatic group showed facilitation (t (18) = 2.4, p =
0.028), while the symptomatic group did not (t (21) = 0.26, p = 0.797). ICF did not show differences between the healthy controls (n = 15), asymptomatic mTBI (n = 19), and symptomatic mTBI groups (n = 22) (F (2, 53) = 1.81, p = 0.173).

Under the active condition, facilitation was not evident in the healthy controls (t (23) = -0.67, p = 0.507) or combined mTBI group (t (44) = 0.04, p = 0.97). There were no differences in ICF MEP amplitude ratios between controls (n = 24) and combined mTBI (n = 45) (t (26.87) = -0.66, p = 0.516). Facilitation was not shown in either the asymptomatic group (t (21) = -0.98, p = 0.337) or symptomatic group (t (22) = 0.54, p = 0.597). There were no differences between controls (n = 24), asymptomatic (n = 22), and symptomatic groups (n = 23) in active ICF (F (2, 66) = 0.49, p = 0.617), as shown in Figure 16 and Table 8.

6.2.7 Short interval intracortical inhibition

SICI appeared in the healthy controls (t (14) = -3.54, p = 0.003) and mTBI group (t (40) = -3.11, p = 0.003). \( \log_{10} \) transformed SICI (Figure 15 and Table 8) showed no differences between healthy controls (n = 15) and combined mTBI groups (n = 41) (t (22.74) = -1.63, p = 0.117). Resting SICI paradigms also showed significant inhibition in asymptomatic (t (18) = -1.67, p = 0.112) and symptomatic (t (21) = -2.63, p = 0.016) groups. There were no differences between healthy controls, (n = 15), asymptomatic mTBI (n = 19), and symptomatic mTBI groups (n = 22) (F (2, 53) = 1.74, p = 0.186).

Under the active condition inhibition was still present in the healthy controls (t (23) = -2.4, p = 0.025) and mTBI group (t (44) = -2.45, p = 0.018), and there were no differences between healthy controls (n = 24) and combined mTBI (n = 45) (t (28) = -1.52, p = 0.14). The
asymptomatic group (t (21) = -2.22, p = 0.038) showed inhibition, but the symptomatic group did not (t (22) = -1.36, p = 0.188). Comparisons showed the healthy controls (n = 24), asymptomatic (n = 22), and symptomatic groups (n = 23) were similar (F (2, 66) = 1.75, p = 0.181, Figure 16 and Table 8).

6.2.8 Long interval intracortical inhibition

All groups showed inhibition under LICI (healthy controls: t (20) = -6.6, p < 0.001, mTBI group: t (38) = -6.74, p < 0.001, asymptomatic group: t (18) = -5.56, p < 0.001, and symptomatic group: t (19) = -4.1, p = 0.001). LICI trended toward significance between the healthy controls (n = 21) and the combined mTBI groups (n = 39) (t (37.75) = -1.76, p = 0.086, Figure 17). There was also a trend towards significance between healthy controls (n = 21), asymptomatic mTBI (n = 19), and symptomatic mTBI (n = 20) (F (2, 57) = 2.84, p = 0.067).
Figure 14: Short interval intracortical facilitation.

(A) Line graph shows means with standard deviations of the log$_{10}$ of the resting short interval intracortical facilitation for the healthy controls ($n = 12$) against combined mTBI ($n = 23$) ($F_{(1, 33)} = 0.39, p = 0.535$). (B) Line graph shows means with standard deviations of the log$_{10}$ of the resting short interval intracortical facilitation for the healthy controls ($n = 12$) against asymptomatic ($n = 12$) and symptomatic ($n = 10$) mTBI ($F_{(2, 32)} = 0.38, p = 0.690$). All graphs are plotted as the log$_{10}$ of the ratio of the conditioned stimulus to the unconditioned stimulus (the test stimulus alone), which is indicated by the black line. Values above the black line indicate facilitation, values below the black line indicate inhibition. Means are plotted as a function of the inter-stimulus interval used to elicit each response.
Table 8: Paired pulse transcranial magnetic stimulation results one month after paediatric mild traumatic brain injury.

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>mTBI</th>
<th>t-test</th>
<th>p</th>
<th>Healthy</th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
<th>F-test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
<td>Mean 95% CI</td>
<td>F-test</td>
<td>p</td>
</tr>
<tr>
<td>Log₁₀ SICI</td>
<td>-0.31</td>
<td>[-0.5  -0.12]</td>
<td>-0.15</td>
<td>[-0.24 -0.05]</td>
<td>t (22.74) = -1.63</td>
<td>0.117</td>
<td>-0.31</td>
<td>[-0.5  -0.12]</td>
<td>-0.11</td>
</tr>
<tr>
<td>Log₁₀ Active SICI</td>
<td>-0.09</td>
<td>[-0.17 -0.01]</td>
<td>-0.03</td>
<td>[-0.06 -0.01]</td>
<td>t (28) = -1.52</td>
<td>0.14</td>
<td>-0.09</td>
<td>[-0.17 -0.01]</td>
<td>-0.04</td>
</tr>
<tr>
<td>Log₁₀ ICF</td>
<td>-0.03</td>
<td>[-0.15 -0.1]</td>
<td>0.07</td>
<td>[-0.02 -0.15]</td>
<td>t (29.35) = -1.33</td>
<td>0.193</td>
<td>-0.03</td>
<td>[-0.15 -0.1]</td>
<td>0.13</td>
</tr>
<tr>
<td>Log₁₀ Active ICF</td>
<td>-0.02</td>
<td>[-0.09 -0.05]</td>
<td>0</td>
<td>[-0.02 -0.02]</td>
<td>t (26.87) = -0.66</td>
<td>0.516</td>
<td>-0.02</td>
<td>[-0.09 -0.05]</td>
<td>-0.01</td>
</tr>
<tr>
<td>Log₁₀ LICI</td>
<td>-0.83</td>
<td>[-1.09 -0.57]</td>
<td>-0.56</td>
<td>[-0.73 -0.39]</td>
<td>t (37.75) = -1.76</td>
<td>0.086</td>
<td>-0.82</td>
<td>[-1.07 -0.56]</td>
<td>-0.7</td>
</tr>
</tbody>
</table>

Paired pulse paradigms include intracortical facilitation (ICF) and long and short intracortical inhibition (LICI and SICI, respectively). Paired pulse paradigms are expressed as the log to the base 10 of the ratio of the conditioned stimulus to the test stimulus alone (unconditioned), where values less than zero indicate inhibition, and values greater than one indicate facilitation.

Abbreviations: ICF: intracortical facilitation; SICI: short interval intracortical inhibition; LICI: long interval intracortical inhibition.
Figure 15: Resting intracortical facilitation and short interval intracortical inhibition

(A) Boxplots show distributions of the log₁₀ of the resting short interval intracortical inhibition for the healthy controls (n = 15) against combined mTBI (n = 41) (t (22.74) = -1.63, p = 0.117). (B) Boxplots show distributions of the log₁₀ of the resting intracortical facilitation for the healthy controls (n = 15) against combined mTBI (n = 41) (t (29.35) = -1.33, p = 0.193). (C) Boxplots show distributions of the log₁₀ of the resting short interval intracortical inhibition for the healthy controls (n = 15) against asymptomatic (n = 19) and symptomatic (n = 22) mTBI (F (2, 53) = 1.74, p = 0.186). (D) Boxplots show distributions of the log₁₀ of the resting intracortical facilitation for the healthy controls (n = 15) against asymptomatic (n = 19) and symptomatic (n = 22) mTBI (F (2, 53) = 1.81, p = 0.173). All graphs are plotted as the log₁₀ of the ratio of the conditioned stimulus to the unconditioned stimulus (the test stimulus alone), which is indicated by the black line. Values above the black line indicate facilitation, values below the black line indicate inhibition.
Both paradigms were performed during a contraction at 20% of the maximal voluntary effort. (A) Boxplots show distributions of the log_{10} log of the resting short interval intracortical inhibition for the healthy controls (n = 24) against combined mTBI (n = 45) (t (28) = -1.52, p = 0.14). (B) Boxplots show distributions of the log_{10} of the resting intracortical facilitation for the healthy controls (n = 24) against combined mTBI (n = 45) (t (26.87) = -0.66, p = 0.516). (C) Boxplots show distributions of the log_{10} of the resting short interval intracortical inhibition for the healthy controls (n = 24) against asymptomatic (n = 22) and symptomatic (n = 23) mTBI (F (2, 66) = 1.75, p = 0.181). (D) Boxplots show distributions of the log_{10} of the resting intracortical facilitation for the healthy controls (n = 24) against asymptomatic (n = 22) and symptomatic (n = 23) mTBI (F (2, 66) = 0.49, p = 0.617). All graphs are plotted as the log_{10} of the ratio of the conditioned stimulus to the unconditioned stimulus (the test stimulus alone), which is indicated by the black line. Values above the black line indicate facilitation, values below the red line indicate inhibition.

Figure 16: Active intracortical facilitation and short interval intracortical inhibition
Boxplots show distributions of the log\(_{10}\) of the resting long interval intracortical inhibition for the healthy controls \((n = 21)\) against combined mTBI \((n = 39)\) \((t (37.75) = -1.76, p = 0.086)\). (B) Boxplots show distributions of the log\(_{10}\) of the resting long interval intracortical inhibition for the healthy controls \((n = 21)\) against asymptomatic \((n = 19)\) and symptomatic \((n = 20)\) mTBI \((F (2, 57) = 2.84, p = 0.067)\). All graphs are plotted as the log\(_{10}\) of the ratio of the conditioned stimulus to the unconditioned stimulus (the test stimulus alone), which is indicated by the red line. Values above the red line indicate facilitation.
Boxplots are shown describing the distribution of the subjective ratings of the TMS session compared to other activities a child is likely to know (play a game, get a shot at the doctors, etc.). Ratings are out of 8 points, with ratings closer to one indicating that the participant ranked their session more favourably. Rating frequencies were not different between groups ($\chi^2 (16) = 18.85, p = 0.277$)

Figure 18: Subjective comparative rating of TMS sorted by symptom resolution
Table 9: Occurrence and subjective ratings of minor adverse events during the transcranial magnetic stimulation session.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Healthy</th>
<th>mTBI</th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
<th>$\chi^2$</th>
<th>p (2)</th>
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<tbody>
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<td></td>
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<td></td>
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</tr>
<tr>
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<td>27</td>
<td>53</td>
<td>24</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
<td>0</td>
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<td>0.181</td>
</tr>
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<td>0</td>
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</tr>
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<tr>
<td><strong>Neck Pain</strong></td>
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<tr>
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<td>48</td>
<td>23</td>
<td>25</td>
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<td>4</td>
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<tr>
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<td>49</td>
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<td>8</td>
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<td>$\chi^2$ (1) = 3.89</td>
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<tr>
<td><strong>Lightheadness/Faint feeling</strong></td>
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<td>56</td>
<td>25</td>
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<tr>
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<td>3</td>
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<td>0</td>
<td>1</td>
<td></td>
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</tr>
<tr>
<td>$\chi^2$ (2) = 4.10</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Subjects rated the presence of any symptoms that appeared during or immediately following the session as none, mild, moderate, or severe. Between groups comparisons for frequency (Pearson’s chi squared test) are shown. The left p value, p (2 groups) represents the significance value for the Pearson’s chi squared test between the healthy controls and the mTBI combined group. The right p value, p (3 groups) is the analogous comparison between healthy controls, asymptomatic mTBI and symptomatic mTBI.
6.3 Safety and Tolerability

A box plot of the subjective ratings of the TMS session compared to other activities children normally encounter can be found in Figure 18. There were no differences in the frequencies of subjective TMS ratings between healthy controls and the combined mTBI group ($\chi^2 (8) = 11.83$, $p = 0.159$), or for comparisons between the healthy controls, asymptomatic mTBI, and symptomatic mTBI ($\chi^2 (16) = 18.85$, $p = 0.277$). The frequencies of mild adverse events experienced by participants, sorted by group, are shown in Table 9. There were no differences in event occurrences between healthy, asymptomatic, and symptomatic groups.
Chapter Seven: Discussion

7.1 Overview

This is the first study to investigate cortical excitability using TMS in children after they have experienced an mTBI. We examined children at one month post-injury to determine if cortical excitability can provide insights into why some children continue to have symptoms following an injury and why others recover quickly.

7.2 Tolerability of transcranial magnetic stimulation in paediatric traumatic brain injury

This research shows that at one month post-injury children with mTBI were able to tolerate TMS, even if they were symptomatic. However, this finding is limited in its generalizability. While it may be safe to assume that children that have had more time post-injury will be able to undergo TMS with minimal complaint, studying more acute injuries must still be approached cautiously. Acutely, children are likely to suffer from headaches before the session begins, which may be exacerbated by TMS.

7.3 Cortical excitability after mild traumatic brain injury

The results from this study present several expected and unexpected results. Routine neuroimaging is usually normal after mTBI in children\textsuperscript{155} and it is likely that, similar to adult studies, any neurophysiological deficits are likely to be subtle. As was expected from previous literature\textsuperscript{110,132,143}, motor thresholds are not different between groups since motor thresholds are relatively coarse methods for measuring cortical excitability. The stimulator is limited at approximately 2T for safety, because this avoids overheating or potentially painful stimuli. High thresholds in children are to be expected. Consequently, some participants were excluded due to thresholds beyond the capacity of the stimulator\textsuperscript{156}. The similar thresholds in each group indicate
that they received similar levels of stimulation during the various paradigms. Therefore, each group is receiving similar magnetic power. This increases the internal validity of our study.

The SRCs measure the recruitment of neurons to efficiently summate action potentials. This was similar between groups. Therefore, in paediatric mTBI the motor cortex continues to show normal recruitment patterns at one month post-injury.

Before discussing the cSP results it is important to consider whether our methodology is valid. The methods used to evoke cSPs in our study are similar to previously published literature\textsuperscript{94}. A cSP above 50ms is of cortical origin\textsuperscript{157}. All groups in our study show values that are normal in the literature for children\textsuperscript{115}. Therefore, the cSP data in our study are valid for investigating cortical inhibition.

The cSP in healthy controls does not differ from mTBI participants, and is similar regardless of recovery status. The same result is found in silent period recruitment curves obtained during active SRC. These results indicate that both the peak and graded recruitment of cSP in the motor cortex are also normal after injury, regardless of symptom persistence.

Some adult literature suggests that there is a shift in cortical excitability towards inhibition after injury\textsuperscript{110,113,140}. For example, Miller et al found that a prolonged cSP, which indicates stronger inhibition, was present within the first week of an mTBI in adults and remained prolonged when tracked longitudinally until 2 months after injury\textsuperscript{113}. This controlled study had 15 participants who, although older (average age was 20.8 years), were similar in that 70% had a sport-related concussion, and all had less than two previous concussions. By one month post-injury, most of the participants had recovered (as indicated by head injury recovery
scores). This research is supported by a few other small studies which found increases in cSP in the chronic phase from an mTBI\textsuperscript{110,111,133,137}.

However, increased cSP was not found across all studies. In a study by Chistyakov et al\textsuperscript{137}, increased cSP was not found 2 weeks after injury in the minor TBI, or concussion, group. Interestingly in this same study, more severe injury (mild or moderate TBI) did show significantly prolonged cSP durations. As the injury in sports-related TBI is often less severe than other mechanisms of injury\textsuperscript{29}, these differences may be due to the differences in injury severity. Overall, given the similarities between our study and Miller’s work, it is surprising that our study finds conflicting results, especially in our symptomatic group. This suggests that children do indeed differ in their response and recovery from mTBI.

Of note, age is also significantly correlated to cSP duration, regardless of group. Children are reported to have shorter cSP values than adults although the numbers studied are small\textsuperscript{115,156}. Within the pediatric samples, Garvey et al did not find age-related cSP differences in 34 healthy children\textsuperscript{156}. However, cSP is affected by stimulation strength (Figure 12)\textsuperscript{108,158}, and Garvey et al\textsuperscript{156} did not use strong enough stimulations to examine the same population of neurons that are activated by stimulation at the 1000µV RMT as in our study. As age was controlled for in the methods, it does not explain why we failed to find any group differences.

Thirty-seven percent of the mTBI group had sustained at least one previous concussion and 15% had two or more concussions. De Beaumont et al found that cortical inhibition was increased in active collegiate football players with repeated mTBI\textsuperscript{133}. This difference between our studies maybe due to age, population, or a lack of power as only nine children in our study
had 2 or more concussions. Nevertheless, this finding is interesting. Although the populations are very different, and football players are likely to have many sub-concussive events, the difference may also be due to differing adaptations to injury. The adult brain, for example, may attempt to compensate for repeated episodes of mTBI (and thereby increased episodes of increased glutamate release) by increasing cortical inhibition. Whereas, the developing brain may not compensate in the same way as it requires a careful balance of inhibition to excitation in order to facilitate new learning\textsuperscript{159,160}. This difference is worthy of further study.

The SICF paradigm showed an inhibitory response in the current study, which is contrary to the proposed mechanisms\textsuperscript{93}. This was the first study to examine SICF in children. The single motor unit and epidural recording data that determined I-wave intervals\textsuperscript{99,161} have only been performed in adults, and the ISIs derived from them may not be suitable for children. Our SICF results in 12 normal children suggest that further research is necessary to optimize the ISIs necessary to elicit SICF (also known as I-wave facilitation) in children. It is possible that the mechanisms that control SICI are overwhelming the I-wave facilitation necessary for SICF as the stimulation parameters and ISIs are similar i.e. the ISI for SICI is 2ms and for SICF are 1.5ms, 2.6ms, and 4.3ms. The mechanisms underlying SICI are thought to rely on the inhibition of I\textsubscript{2} and I\textsubscript{3} waves\textsuperscript{94}.

Although non-significant, the findings in other TMS paradigms (iSP, ICF, SICI, LICI) still provide useful information and allow us to pose interesting questions. Firstly, the mostly non-normal distributions of the current (pre-transformed) findings leads us to believe that the reduced sample size in these paradigms was not sufficient for the amount of between subject variability. Therefore, we transformed the data to a normal distribution to allow the groups to be compared
using the more powerful parametric statistical analyses, but did not find statistically significant differences. This raises the question of whether these non-significant findings are a result of reduced sample size or some physiological mechanism. Further research with larger sample sizes are required to confirm this. Given this limitation, there are also several possible physiological interpretations for non-significant TMS results such as: there is not a large, circuit-wide change in the motor cortex cortical excitability; cortical excitability has changed since the injury, but recovery has occurred; there may be differences in the neurophysiology, but the TMS paradigms lack sensitivity; and finally there may be differences in cortical areas outside of the motor cortex, such as supplementary motor areas\textsuperscript{162,163}.

In summary, TMS is well-tolerated by children recovering from a mild traumatic brain injury. Age was found to correlate with the main outcome measure, the cortical silent period, regardless of group. Cortical inhibition is not different in children with mTBI compared to normal children and does not explain the persistent symptoms of post-concussion syndrome. Further research is needed to examine whether these findings are upheld when using the potentially more sensitive paired pulse TMS paradigms in larger cohorts of children.
Chapter Eight: Limitations

Limitations to the current research include the small sample size, design, and technical considerations of TMS.

The mTBI group in this study is not representative of a normal mTBI population. At one month after the injury, the proportion of symptomatic and asymptomatic children should be approximately even or skewed towards recovery\textsuperscript{5}. However, the groups in this study were selected to be skewed towards the symptomatic group. Additionally, the asymptomatic participants were selected to be similar to the symptomatic group for age and sex. In reality, older adolescents, are more likely to experience an mTBI\textsuperscript{20,33}, and are more likely to remain symptomatic. Further, although males are also more likely to incur an mTBI\textsuperscript{3,11,20,33}, while females are more likely to have persistent symptoms\textsuperscript{164,165}.

The desired sample size was obtained (except for one asymptomatic participant). However, our study is likely to be underpowered. The sample size calculations performed for this study were based on mean cSP differences between groups in an adult population at one month after injury\textsuperscript{113}. The variability of cSP duration in the current study, and other paediatric studies, is much larger than in adult populations\textsuperscript{156}. Further, we unexpectedly found that cSP was inversely correlated with age within 8 and 18 year olds. Both of these factors are likely to significantly affect the power of our study, even though the groups were matched for age. A larger sample size would also help to control for the clinical (often unmeasured) heterogeneity of mTBI\textsuperscript{68} and would also allow us to further investigate the influence of previous concussions on cortical inhibition in children\textsuperscript{133}.
It was disappointing that the sample size in the paired pulse paradigms was decreased due to small test MEP amplitudes. Paradigms were excluded (unconditioned stimulus MEPs and corresponding conditioned stimulus MEPs) because the mean test stimuli were not above 10% of the desired 1000µV value. Therefore, they were excluded because these test MEPs were unreliable. As the neurons receive reduced magnetic field strength during conditioning, these findings may not have been measuring the same cortical mechanisms as those producing normal sized test MEPs.

With a heterogeneous injury, using each individual to control for their own variance increases the external validity of any conclusions because the statistical model focuses on the differences caused by the injury and less on the natural inter-individual variability. This will be even more powerful if the measurements are taken before and after the injury. The current research lacks pre-injury TMS data, which could be used to largely eliminate any pre-existing differences between individuals. This is very difficult to perform in TBI, as it is impossible to know when humans will incur an injury.

The technical limitations to studying TBI with TMS is that only two neurotransmitters (GABA and glutamate) have been strongly linked to the TMS paradigms used in the motor cortex. Although specialized neuroimaging such as conventional magnetic resonance imaging\(^{166}\), magnetic resonance spectroscopy\(^{167,168}\), arterial spin labelling\(^{169}\), and functional magnetic resonance imaging\(^{170,171}\) can detect differences following mTBI, these findings don’t involve the motor cortex. It is possible that the motor cortex is less adversely affected following mTBI and that TMS paradigms outside of the motor cortex may be better suited for exploring mTBI.
Although the exact mechanisms are not known, TMS paradigms such as cSP, SICI, and LICI target GABAergic inhibition mechanisms, and ICF is thought to target NMDA receptor-mediated actions. However, investigating only these two systems is an oversimplification of the processes occurring in the brain, and cannot be taken as fully explaining the state of even the motor cortex. There are many other receptor types in the brain that mediate communication between neurons especially as afferent communication from the brain stem: serotonin, noradrenaline, dopamine, and acetylcholine.

Additionally, despite stimulating in an output centre of the cerebral cortex, the stimuli are still modulated in the spinal cord. When the pyramidal tract neurons synapse onto motor neurons, they can be modulated by interneurons in the spinal cord that receive action potentials from the reticulospinal and vestibulospinal tracts, and the surrounding motor cortex. It is important, therefore, that the participants are in a comfortable and consistent body position to avoid activation of these modulating spinal cord neurons. However, stimulating in the motor representation of the hand, especially intrinsic hand muscles, provides the greatest likelihood for stimulating neurons that do form direct connections to their respective motor neurons with minimal modulation.
Chapter Nine: **Future directions**

Larger paediatric TBI studies are required to further study the consequences of an injury especially in the setting of the developing brain.\(^{143}\) The study of athletic populations may allow us to control for changes over time and perform pre-injury assessments. Factors such as aging, pubertal development, exercise training and the use of prescribed medications (i.e. for ADHD)\(^ {172}\) could then be explored. High school or extracurricular sports teams that are at high risk of mTBI, could have a TMS session performed prior to the start of the season, then again at a certain points after a concussion or mTBI. Comparisons could be made with their pre-injury data but also with age-matched teammates and would allow us to control for any changes in cSP over time.

Additionally, studies should investigate more severe forms of paediatric TBI, such as moderate or severe TBI. The recovery from more severe forms of TBI is longer, and is more likely to involve gross motor deficits. Therefore, changes that are too subtle to be found in paediatric mTBI are likely to be exaggerated in moderate or severe TBI\(^ {158}\) (Table 2). This research could be useful in determining which TMS paradigms are going to be the most useful in understanding mTBI.

Finally, the TMS paradigms used in the current research are only a small selection of the possible paradigms currently being used to study other neuropathologies. 1) In paired pulse paradigms with one coil, further research could investigate the interactions between single and paired pulse paradigms\(^ {173,174}\). 2) Two coils can be used to stimulate different areas of the cortex to test long range communication. Interhemispheric interactions (inhibition) can be tested when the second coil is placed on the contralateral primary motor cortex prior to a test stimulus in the
motor cortex of interest. Other regions of cortex that share significant connections with the primary motor cortex can also be stimulated: the contralateral or ipsilateral posterior parietal cortex, the contralateral sensory cortex, or the cerebellum. 3) The triple stimulation technique is a collision technique that prevents motor neurons from discharging more than once and synchronizes the response for each magnetic stimulus. This complex technique involves a single magnetic stimulus to the cortex and two electrical stimuli to the peripheral nerve supplying the target muscle, which, when they collide isolate single activations of the motor neurons. However, its use in children has not been investigated.

In summary, cortical excitability does not differ after pediatric mTBI. Our findings suggest that the electrophysiological response of the brain to mTBI is indeed different in children when compared to adults. Further research is necessary with larger samples to confirm these findings especially using more sensitive paired pulse techniques.
APPENDIX A: TRANSCRANIAL MAGNETIC STIMULATION NEUROPHYSIOLOGY OF

PAEDIATRIC TRAUMATIC BRAIN INJURY

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9.1 Abstract

Traumatic brain injury is common yet the clinician has difficulty predicting its outcome whether mild or severe, and there are relatively few treatments to offer. Transcranial magnetic stimulation (TMS) has the potential to offer unique insights into the reparative processes that occur following traumatic brain injury (TBI), and repetitive TMS may offer a new treatment modality. This chapter reviews the pathophysiology of paediatric TBI emphasizing the role of neurodevelopment and neuroplasticity in recovery. It summarizes the TMS literature to date, providing insights into the network adaptations and neurophysiological changes that occur, focusing on mild TBI and post-concussion syndrome. Few of the studies performed previously have included children. Our pilot controlled cohort study examining cortical excitability in thirty children with mild TBI is reviewed and suggests that children do respond differently and that changes in cortical excitability may explain symptom persistence in this challenging problem.
Traumatic brain injury has been studied since antiquity. The Edwin Smith Papyrus outlines how academics in Ancient Egypt and Mesopotamia studied how traumatic brain injury affected the brain. With the evolution of the scientific method, and the advent of non-invasive stimulation techniques, we are uniquely positioned to investigate the electrophysiological and neuronal network changes that occur following traumatic brain injury (TBI) in ways that have previously been unattainable. This chapter will first introduce the reader to the clinical, and physiological aspects of TBI and the unique challenges that are associated with pediatric TBI. It will review neuroplasticity and how the cortical networks change after TBI. The literature exploring the insights that TMS can provide in TBI is summarized, and finally some early results from the first study of cortical excitability in TBI in children.

9.2 Epidemiology and clinical spectrum of traumatic brain injury

Traumatic brain injury (TBI) is common, especially mild TBI (mTBI), and occurs more frequently in childhood than at any other time of life. Traumatic brain injury is defined as an injury to the brain that occurs when biomechanical forces result in an alteration of brain function\(^{16}\). The incidence of TBI varies widely across studies, occurring between 341 and 1750 per 100,000 persons per year\(^{3,17–19}\) and depends on the methodologies and populations investigated. Approximately 70-90% of all TBIs are considered mild\(^{22}\). As many as 25% of mTBIs do not receive medical attention, the true incidence is likely to be much higher\(^{23}\). Young children and older teens are at greater than double the risk of sustaining a TBI when compared to adults\(^{33}\). Additionally, more than four adverse life events, such as parental divorce, abuse, or deaths in the family, can increase the risk of TBI by as much as three times\(^{34}\).
The causes of TBI are varied. Falls are the most common cause in young children, whereas motor vehicle accidents and assaults are more common in older teens and adults\textsuperscript{20}. Sport-related injuries are an important cause of mild TBI and concussion\textsuperscript{3}. One third of individuals who sustain a concussion will incur at least one additional TBI\textsuperscript{12}. Concussion is more likely to occur in certain sports i.e., ice hockey, women’s soccer, and men’s football, which all have injury rates above 0.40 per 1000 athlete exposures\textsuperscript{1}. In high school, football has the highest injury rate at 0.47 injuries per 1000 athlete exposures. As athletes are more likely to undergo repeated sub-concussive events i.e. hits to the head without overt clinical symptoms, these figures are concerning\textsuperscript{12}. With such high injury rates in these contact sports, the potential long-term consequences of repetitive injury such as possible psychiatric illness and chronic traumatic encephalopathy\textsuperscript{176,177} become very important. Other sports such as mountain biking and sports involving motorized vehicles are associated with more severe forms of TBI.

The severity of a TBI ranges from mild to severe and is assessed using clinical measures such as the presence or absence of a loss of consciousness, focal neurological signs, behavioural disturbances and the length of amnesia following the injury (post traumatic amnesia), see Table S10. Whereas a severe TBI will be associated with a prolonged period of coma (lasting 7 days or more), an mTBI may or may not be associated with a loss of consciousness. Somewhat confusingly, these criteria may not be used in sports medicine\textsuperscript{3}. Here, the term “concussion” is usually used to imply milder form of mTBI\textsuperscript{27,28} and may exclude patients with a Glasgow coma scale of 13 and/or a loss of consciousness for longer than 5 minutes. For these reasons, the inclusion criteria in studies examining concussion and/or mTBI should be explicit, and the reader
should be aware of the patient population and their potential differences when drawing any conclusions.

**Table S10: American academy of rehabilitative medicine criteria for traumatic brain injury severity classification**

<table>
<thead>
<tr>
<th>Severity</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glasgow Coma Scale</td>
<td>13-15</td>
<td>9-12</td>
<td>3-8</td>
</tr>
<tr>
<td>Loss of consciousness</td>
<td>&lt; 30 minutes</td>
<td>30 minutes to 24 hours</td>
<td>&gt; 24 hours</td>
</tr>
<tr>
<td>Post traumatic amnesia</td>
<td>&lt; 24 hours</td>
<td>24 hours to 7 days</td>
<td>&gt; 7 days</td>
</tr>
</tbody>
</table>

**9.2.1 Outcome**

Outcome after TBI is predicted by pre-injury demographic factors, injury related factors, and post-injury factors. Pre- injury demographic predictors include age, prior intellectual ability, and the presence of behavioural or psychiatric conditions, socio-economic status and family dynamics. Outcomes strongly correlate with injury severity (e.g. duration of unconsciousness). Post-traumatic injury predictors include success with rehabilitation programmes and interventions. In children, TBI patients showed persistently lower quality of life than extracranial injury controls of a similar age. The long-term impairments following paediatric TBI include behavioural problems, neuropsychological deficits (e.g. problems with attention, executive function, memory, and speed of processing), motor deficits, and decreased social skills. The pattern of interaction between child behaviour problems and family functioning
months after injury is predictive of the burden on the family, parental stress and child behaviour problems at 1 year\textsuperscript{178}. Recovery takes longer as the injury severity increases\textsuperscript{179}.

Following an mTBI, there may be problems with fatigue, headache, drowsiness, difficulty sleeping, irritability, concentration difficulties, memory problems, and mood disturbance\textsuperscript{40}. These symptoms have typically been considered to last between 7-10 days\textsuperscript{41}, however, the median persistence time of symptoms in children has recently been shown to be 29 days (95% confidence intervals: 26.1, 31.9 days), and 11% of children remain symptomatic at 3 months post injury\textsuperscript{5,42}. Post-concussion syndrome (PCS) is diagnosed when there is a constellation of three or more post-concussive symptoms lasting for over three months\textsuperscript{42}.

9.3 Pathophysiology of traumatic brain injury

TBI begins with an insult to the brain, and resultant complex pathological processes and cascades ensue. Consider the brain to be a high density semi-solid with defined borders suspending in a liquid, the cerebrospinal fluid (CSF), all of which are contained within a rigid skull. When a force is applied, it causes the whole structure to accelerate or decelerate. When the skull stops or reverses direction (e.g. in a whiplash movement), the brain continues to move and may collide with the inside skull and its bony buttresses. The brain will deform when it impacts the skull (even with “sub-concussive” forces). Injury occurs when such deformations exceed the biomechanical structural limits of any tissues undergoing strain (blood vessels, neurons, pial tissues, etc.). The brain is not a homogeneous mass but has grey and white matter, blood vessels and CSF spaces. As grey and white matter have differing water content and densities (which change throughout brain development as myelination occurs), these structures move according to their inertia. This results in shear stresses on the axons, blood vessels and oligodendrocytes as
they cross the different densities. These forces cause the membranes to leak, known as mechanoporation, and results in a breakdown of the electrochemical gradient needed for effective signal transmission and disruption to cellular organelles.

An indiscriminate release of neurotransmitters occurs following TBI, activating enzyme pathways and other cellular processes that subsequently cause secondary damage to the brain. The pathophysiology of TBI has been reviewed by Giza et al.\textsuperscript{65,66}. This indiscriminate release of neurotransmitters begins a process called excitotoxicity. Excitotoxicity occurs when presynaptic neurons release large amounts of glutamate that binds to n-methyl-d-aspartate (NMDA) receptors on post-synaptic neurons and causes uncontrolled neuronal activation. These pathways combined with mechanoporation result in a shift in ionic gradients across membranes, an increase in cellular metabolism, and increases in reactive oxygen species. The net effect may lead to cytoskeletal and intracellular transport system degradation.

Calcium plays a key role in the activation of the many enzymatic cascades and intracellular disruptions associated with TBI. Calcium ion accumulation stems from excitotoxicity as NMDA receptors are permeable to calcium ions. In cells that have undergone mechanoporation, calcium will flow down its large concentration gradient into the cell. Increased intracellular calcium at the synaptic terminals can also lead to uncontrolled neurotransmitter release and further excitotoxicity. Calpain enzymes become activated, and may lead to breakdown of the cytoskeleton. These processes are activated to varying degrees and so can result in different cellular injury phenotypes even in adjacent neurons.

Indiscriminate activation of intracellular processes leads to the consumption of adenosine triphosphate (ATP), the primary unit of energy in the cell. Axons and cell bodies of injured neurons may swell due to ionic influx resulting in cerebral oedema. Multiple secondary injury
cascades and reduced blood flow to the brain exacerbate the energy crisis. Although many neurons will recover, others may be too severely damaged to recuperate, and will undergo apoptosis or necrosis. Damaged or dead neurons may not all be closely grouped, but may be spread throughout the brain in what is called diffuse axonal injury (DAI). This distribution (focal or diffuse) depends on the primary insult, the biomechanical properties of the brain, the size and propagation of force waves through the tissues, and the resultant location of stress points.

Recovery from TBI is less well understood than the acute response to injury. There is strong pathological evidence for a prolonged immune response in TBI, with microglial activation, astrocyte activation, and microvascular changes in the blood brain barrier identifiable months and years after injury. This may contribute to poorer long term outcomes.\textsuperscript{69–73} The release of ATP from damaged neurons initiates the activation of the innate immune system, resulting in the release of inflammation-promoting mediators such as cytokines, chemokines, and reactive oxygen and nitrogen species. Pro-inflammatory processes are intended to clear the central nervous system of potentially harmful substances. Anti-inflammatory processes follow this, performing reparative and regenerative functions considered to be beneficial to neuronal survival. An unbalanced or prolonged inflammatory response can be harmful.\textsuperscript{74}

\section*{9.3.1 Changes in cortical excitability following TBI}

It is advisable to consider TBI as a dynamic process, and not just a static insult to the brain.\textsuperscript{75} Cortical networks that remain intact enough to propagate signals may show short-term and long-term changes in excitability. The excitatory-inhibitory balance may be altered due to both the number and type of neurons that survive as well as changes in the number and type of receptor expression. The exact mechanisms and durations of neurotransmitter dysfunctions...
following TBI remain poorly elucidated, although these changes are more prominent in children, and have the potential to lead to longer lasting deficits due to the concurrent developmental processes.

Changes in neuronal excitation occur and can be immediate. This is reflected by the increased incidence of seizures in the acute post-injury period. Excitation tends to decrease over time. As glutamate and calcium levels normalize, longer-term changes in receptor expression occur. For example, in a juvenile mouse TBI model the relative expression of the NMDA receptor NR1, and NR2 subunit subtypes A and B differ after TBI. Subtype NR2B and NR1 remain unaffected, while NR2A subunit expression in the synapse is reduced. Changes in receptor subunit or subtype expression can cause decreased ligand affinity, change protein transportation targets, and alter the functional mechanism (e.g. ionotropic instead of metabotropic) of the receptor.

GABAergic interneurons (most commonly inhibitory) may be particularly vulnerable to injury. GABA receptors may also show changes in their relative subtype expression following TBI. For example, some GABA A subtype receptor subunits are downregulated (e.g. ε and θ subtypes in the thalamus and hypothalamus) whereas other are upregulated (α4 subunit increase in the hippocampus). However, there are multiple subtypes of GABA receptors, and though the GABAa receptor is more commonly studied, GABA B subtype receptors are also noteworthy. Although less studied, GABAb receptor alterations may have an important role in network changes as they are metabotropic and so have longer lasting effects. The resultant inhibitory alteration depends on the type of GABAergic interneurons affected, receptor subtype expression, their location in the brain, and the time after injury.
9.3.2 Neuroplasticity following TBI

One of the most interesting aspects of recovery following a TBI that can be investigated using TMS is neuroplasticity (see also Chapter 3). Neuroplasticity is the ability of the neurons to adapt to changes in their environment. It encompasses the formation of new neuronal connections, uncovering latent connections, and strengthening (or lessening) the modulatory influence of an existing connection. Connection formation and recruitment of latent connections are costly in terms of cellular resources and also takes longer to achieve than strengthening an already active connection. Therefore, strengthening an established connection is more efficient, especially in a mild injury. This can be achieved by adding more presynaptic neurotransmitter vesicles or post-synaptic receptors. In contrast, as severe injuries have a greater number of cell deaths, new connections will be required. Axonal growth and the formation of new synapses are related to the action of growth factors such as nerve growth factor induced gene A, homer, activity regulated cytoskeletal-associated protein, and brain-derived neurotrophic factor (BDNF). BDNF, for example, increases the number of dendritic spines, and aids in the formation of new synapses.

Long-term potentiation (LTP) is a building block of plasticity. Synapses that have undergone LTP tend to have stronger electrical responses to stimuli than other synapses and is associated with increases in NMDA receptor concentration at the post-synaptic membrane. This can be artificially induced with high frequency stimulation in vitro. The opposing form of neuroplasticity to LTP is long-term depression (LTD). Decreasing the NMDA receptor concentration at the post-synaptic neuron is the crux of LTD. LTP-like and LTD-like effects
have been found non-invasively in humans using high and low frequency stimulation in repetitive TMS (rTMS) respectively\textsuperscript{86,87}.

Plasticity is not solely a function of the relationship between two cells but involves groups of cells. These microcircuits include many different cell types, protein expression patterns, and functions. Interneurons are thought to play a key role in network adaptation. Interneurons are short-ranging neurons that modulate the activation of other cell types. They may receive inputs from different neurons or brain regions than that of the “target” neuron. If an inhibitory interneuron synapses just above the axon terminal on the pre-synaptic neuron, this will

![Diagram of synaptic plasticity](image)

**Figure S19: GABAergic filtering of synaptic plasticity.**

(A) shows a pre-synaptic/post-synaptic neuron pair (black), which will propagate signals from left to right, with a small blue arrow indicating long-term potentiation (LTP). (B) expands the same circuit to include an inhibitory interneuron (blue) synapsing onto the pre-synaptic terminal which negates the LTP effects. However, (C) shows auto inhibition of the same interneuron, which restores the effects of LTP.
decrease the likelihood of neurotransmitter release, see Figure S19. By this mechanism and similar mechanisms, a pre-synaptic/post-synaptic neuron synapse will be inhibited, and may produce weaker than normal signals. If these neurons had undergone LTP, this inhibition will counteract the LTP. This GABA-mediated inhibition, therefore, filters neuroplasticity at excitatory inputs\(^8\). Autoreceptors on the interneuron moderate this activity. At the interneuron-presynaptic neuron synaptic cleft, GABAb autoreceptors on the interneuron bind GABA released from the interneuron. When this happens, the interneuron becomes hyperpolarised and therefore less likely to release neurotransmitter. As a result, the presynaptic neuron does not become hyperpolarized, and is now more likely to generate an action potential.

Changes occurring after TBI are of course more complex than the changes occurring in individual cells or small groups of synapsing cells. Collections of microcircuits form networks, sometimes ranging long distances across the brain. Information is conveyed in these networks using a careful balance between excitation and inhibition through successive levels of processing. Dysfunction in single or groups of cells after TBI may affect the excitability of neuronal circuits, and such disruption will affect the larger network. Therefore, when measuring cortical network activity after TBI, the TMS investigator must be cognizant that the reparative mechanisms of the network may alter the network’s response to stimulation. This is true in single and paired pulse TMS, where the investigator is attempting to evaluate the current state of the network, and modulate its plasticity, such as in rTMS. rTMS will be attempting to modulate plasticity in a system that is already undergoing an increased amount of neuroplasticity compared to the normal brain, and outputs will reflect the interaction between the ongoing neuroplasticity and that evoked by rTMS.
9.4 Insights about TBI using TMS: a literature review

TMS has been used to examine various networks after TBI including changes in the excitability of corticospinal, intracortical, and interhemispheric motor networks. The existing TMS studies have examined mild, moderate and severe TBI, in both the athlete and general population at different time points post injury. As most studies do not include children, it is not known whether their findings can be generalized to the paediatric age range. TMS findings in adults after TBI primarily fall into three categories: acute mTBI, chronic mTBI, and severe TBI. As the populations vary considerably, as does the time of study after the injury, it is hard to draw definite conclusions about exactly how cortical excitability and cortical inhibition change during recovery. Overall, the literature suggests that recovery is associated with an increase in cortical inhibition. The evidence is summarized in the following sections and in tables 2 and 3.

9.4.1 Mild traumatic brain injury

Changes in the excitation-inhibition balance can be measured using the rest motor threshold (measuring the most excitable neurons in the area) and the stimulus response curves (SRCs)\(^9\). Studies that report changes in RMT after mTBI, even in the acute stages after injury, are contradictory. About half of the small number of studies describe no difference in thresholds, while the other half describe an elevation of the RMT. For example, increased RMTs were found in mTBI patients with or without sleep disturbances\(^{12,129}\) and those whose injury was over 3 years prior\(^{130}\). Conversely, other studies have failed to show RMT differences in either mild or moderate TBI in both acute and chronic phases\(^{113,131–133}\). This is highlighted in the study by Miller, et al\(^{113}\), which examined patients repeatedly between 72 hours and 2 months after injury.
Although some studies suggest a slight shift to increased inhibition, RMT is highly variable between individuals and also may not be sensitive enough to detect the often-subtle changes associated with a mild injury.

Excitation can be measured using MEP amplitude, which is the ability of corticospinal tract neurons to effectively summate, and create a unified descending volley of action potentials. This measure is a function of the RMT and is affected by the skull thickness. Some small studies found MEP amplitude was increased within the same subject over the first ten days following an mTBI in athletes\textsuperscript{131,134,135}. However, this was not substantiated in larger studies\textsuperscript{104,112}, or in a repeated measures study examining changes over the first two months post-injury\textsuperscript{113}. Stimulus response curves (SRC) or input/output (IO) curves expand these investigations to measure recruitment of higher threshold corticospinal tract neurons and the synchrony of descending action potential volleys\textsuperscript{180}. SRCs are normal following mTBI\textsuperscript{133,135}, although they show increased variability\textsuperscript{137}. Together, these two measures, MEP amplitude and SRC, indicate that the neuron population that excites the target muscle pathways is still fully capable of eliciting a normal output following stimulation.

The white matter tract injuries often associated with TBI may be detected using techniques exploring conduction times e.g. MEP latency and corticomotor conduction times (CMCT). In the acute stages after mTBI, subtle conduction abnormalities have not been identified using TMS in the setting of normal RMT and MEP amplitudes\textsuperscript{131,135}. In a larger study of individuals two weeks after injury, Chistyakov et al\textsuperscript{136} found an increase in MEP latency associated with increased RMT and MEP amplitude variability. This conduction delay could be due to axonal dysfunction in the motor tracts, as longer axons are more vulnerable to stretch and
torsion forces during the insult. However, this was not substantiated by Miller et al.\textsuperscript{113} who did not find any differences in MEP amplitudes or latencies between controls and mTBI participants at 72 hours, and 1, 2, 4, and 8 weeks post-injury. Ultimately, the interpretation of such studies is difficult due to the heterogeneity of the populations studied (Miller et al. studied younger people with sport-related concussion) and the severities of injury even in mild TBI. This is corroborated by CMCT studies. CMCT mathematically eliminates the peripheral motor conduction times from the MEP latency. A study by Chistyakov et al.\textsuperscript{137}, divided the participants into 4 groups: concussion, focal injury, diffuse injury and combined focal and diffuse injury. The focal, diffuse, and combined injury groups consisted of both mild and moderate TBI participants. This study found that only the more severe injuries (diffuse injuries) had increased CMCT compared to controls.

The role of inhibition following TBI has also been explored. Inhibition can be measured using single or paired pulse paradigms, such as cortical and ipsilateral silent period, and short and long interval intracortical inhibition respectively. GABAergic inhibitory mechanisms can be confirmed by increasing GABA in the synapse e.g. Tiagabine inhibits GABA reuptake into the neurons and astrocytes and by using GABAb receptor agonists such as baclofen. Both tiagabine and baclofen increase the duration of the cortical silent period\textsuperscript{106,107}. Miller et al. explored inhibition in the acute stages of TBI and provided strong evidence for increased inhibition by demonstrating a prolonged CSP in mTBI. CSP was prolonged at 72 hours post-injury and remained increased until at least 8 weeks\textsuperscript{113}. This is in keeping with other adult TBI studies\textsuperscript{110–112,137}.\textsuperscript{137}
Table S11: Summary of the literature using single and paired pulse transcranial magnetic stimulation paradigms after mild and moderate traumatic brain injury (TBI).

↑ indicates a significant increase in the paradigm after TBI, while ↓ indicates a decrease. “x” indicates that no significant differences were found.

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year(s)</th>
<th>Time after injury</th>
<th>TBI Severity</th>
<th>Notes</th>
<th>TMS paradigms</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Single Pulse TMS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>RMT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Peak to Peak</td>
</tr>
<tr>
<td>Powers</td>
<td>2014</td>
<td>Acute</td>
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<td>Asymptomatic</td>
<td>x</td>
</tr>
<tr>
<td>Livingston</td>
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<td>Mild</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day 3</td>
</tr>
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<td></td>
<td></td>
<td>Day 5</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Day 10</td>
</tr>
<tr>
<td>Chistyakov</td>
<td>1998</td>
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<td>Mild</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Miller</td>
<td>2014</td>
<td>Acute</td>
<td>Mild</td>
<td>x x</td>
<td>x</td>
</tr>
<tr>
<td>Pearce</td>
<td>2014</td>
<td>Chronic</td>
<td>Mild</td>
<td>Retrospective</td>
<td>x</td>
</tr>
<tr>
<td>De Beaumont</td>
<td>2012</td>
<td>Chronic</td>
<td>Mild</td>
<td>Asymptomatic</td>
<td>x x</td>
</tr>
<tr>
<td>De Beaumont</td>
<td>2011</td>
<td>Chronic</td>
<td>Mild</td>
<td>Asymptomatic</td>
<td>↑</td>
</tr>
<tr>
<td>Tremblay</td>
<td>2011</td>
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<td>Mild</td>
<td>Concussion</td>
<td>0</td>
</tr>
<tr>
<td>Tremblay</td>
<td>2014</td>
<td>Chronic</td>
<td>Mild</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Tallus</td>
<td>2012, 2013</td>
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<td>Mild</td>
<td>Symptomatic</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Asymptomatic</td>
<td>↑</td>
</tr>
<tr>
<td>De Beaumont</td>
<td>2007</td>
<td>Chronic</td>
<td>Mild</td>
<td>Single</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Multiple</td>
<td>↑</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Acute</td>
<td>Lesion</td>
<td>Minor</td>
<td>x</td>
</tr>
<tr>
<td>-----------</td>
<td>-------</td>
<td>---------</td>
<td>-------------------</td>
<td>-------</td>
<td>---</td>
</tr>
<tr>
<td>Chistyakov</td>
<td>2001</td>
<td>Acute</td>
<td>Mild - Mod</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mild</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Moderate</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Concussion</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Focal Lesion</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diffuse Injury</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Combined</td>
<td>↑</td>
</tr>
<tr>
<td>Nardone</td>
<td>2011</td>
<td>Acute</td>
<td>Mild - Mod</td>
<td>Fatigue</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Subjective EDS</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Objective EDS</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hypersomnia</td>
<td>x</td>
</tr>
<tr>
<td>Chistyakov</td>
<td>1999</td>
<td>Acute</td>
<td>Mild - Mod</td>
<td>Diffuse injury</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Focal injury</td>
<td>↑</td>
</tr>
</tbody>
</table>

Post-concussive symptoms have usually resolved by three months in 80-85% of children with mTBI. It is therefore reasonable to consider any changes in cortical excitability persisting for three months or longer as reflecting a chronic change. Increases in CSP have been found between 3 months and 30 months post injury. This implies a change in cortical inhibition, and further, that these changes may be persistent years after the injury. They may also correlate with the number of previous concussions. In contrast, 22-years after a concussion, Pearce et al found a shortened CSP (during an “active contraction”). These findings implicate a further change in cortical inhibition over time. The biological advantage and neurophysiological substrates underpinning this however are unknown. There may be a slow evolution in the regulation of the GABAb receptor protein expression over time, and/or a decrease in GABA release, which may eventually “overcompensate” for the injury.

As inhibitory interneurons are vulnerable in TBI however, a prolonged CSP seems counterintuitive. These changes could represent alterations in GABAb receptor expression (similar to that found in murine models of TBI), although it is also possible that the individuals with concussion studied by Miller et al were different even before the injury i.e., cortical inhibition was increased before the acute injury. These increases in inhibition could be due to previous concussive or sub-concussive events. Further validation and exploration of these findings together with ipsilateral silent period interrogation are needed.

Intracortical inhibition has been studied using the paired pulse paradigms short interval and long interval intracortical inhibition (SICI and LICI, respectively). SICI is thought to be the effect of interneuronal GABAa receptor-mediated inhibition of the pyramidal tract neurons. Changes in SICI are again variable after TBI, and methodologies vary significantly across
studies. A wide range of populations as well as time points post-injury were studied making it
difficult to draw conclusions about changes in intracortical inhibition over
time\textsuperscript{104,111,112,129,132,138,139}. For example, Pearce et al found chronically decreased SICI following
\textit{mTBI}\textsuperscript{104}. Conversely, SICI was increased in individuals with excessive daytime sleepiness early
after mild and moderate TBI\textsuperscript{138}. The mechanisms underlying the modulation of SICI after TBI is
poorly understood and warrants further investigation.

LICI is thought to be due to the GABAb receptor-mediated inhibition of the pyramidal
tract neurons by the conditioning TMS stimulus. Tremblay et al found that LICI was increased in
the first 12 months post \textit{mTBI} and that this inhibition may decrease in the long term\textsuperscript{140}. Although
LICI and CSP share a common pathway (via GABAb receptors), they do examine slightly
different mechanisms of intracortical inhibition\textsuperscript{93,94,124}. Never-the-less, increases in LICI are
consistent with increases in CSP following adult TBI supporting a significant role for GABAb
receptor-mediated intracortical inhibition in the recovery from TBI\textsuperscript{113,135}. Further studies
exploring the role of intracortical inhibition in the recovery from a TBI over time may well
provide useful insights into potential therapeutic interventions in TBI in the future.

Chistyakov et al\textsuperscript{136} report an interesting finding of fatigability which was present at two
weeks post injury, and resolved by three months. To do this, they used suprathreshold \textit{rTMS}
trains of 50 stimuli and found that MEP amplitude progressively decreases within trains of
stimulation, with a marked irregularity in the shape of the MEP waveform. Although this study
does suffer from attrition, these abnormalities improved at three months and may correlate with
symptom improvement\textsuperscript{136}.
Combining TMS with functional tests can be more informative in mTBI, as cognitive deficits may only found in the most difficult tasks, and may persist after symptom resolution\textsuperscript{30,141,142}. The similar persistence of TMS abnormalities (specifically cSP) may provide a correlate to these findings after mTBI. One study did find a differential recovery in symptoms, neuropsychological test scores and TMS parameters after sport-related concussion within the first ten days after injury, but these results need corroboration\textsuperscript{143}.

9.4.2 Severe traumatic brain injury

Studies using TMS to investigate severe TBI in both the acute and chronic states are rare. Most studies report RMT values in severe TBI (including minimally conscious states following TBI) to be similar to control populations\textsuperscript{149,181–183} except in one study. This study by Bernabeu et al\textsuperscript{158} classified the participants based on i) motor function (paretic vs. non-paretic), and ii) radiological findings (mild and moderate DAI, severe DAI, and combined DAI and focal lesions). Increased RMT and decreased area under the MEP curve was present in severe TBI, especially in the presence of paresis, and/or severe diffuse axonal injury. This is consistent with the clinical picture and the neuronal loss following severe diffuse axonal injury. The concurrent dysregulation of the SRCs demonstrates that neuronal recruitment was lower in those recovering from TBI who have the greatest functional motor impairment.

Conduction times may be more sensitive to the consequences of severe brain injury than RMT and MEP amplitude. MEP latency was increased following severe TBI\textsuperscript{182,184}. It is not clear whether CMCT or MEP latencies are better to document these abnormalities as the current studies are small and conflicting\textsuperscript{183,184}.
Bernabeu et al\textsuperscript{158} also investigated the chronic effects of diffuse axonal injury on intracortical excitability and inhibition. CSP prolongation, indicating an increase in GABA\textsubscript{b} receptor-mediated inhibition, also occurs in severe TBI. Such increased inhibition may be a neuroprotective mechanism by blunting the effects of indiscriminate excitatory glutamate release\textsuperscript{185}.

Corpus callosal injury is often found in severe TBI\textsuperscript{186–188}. In a normal motor cortex, the transcallosal fibers originating from one motor cortex will synapse onto inhibitory interneurons in the contralateral cortex thereby inhibiting contralateral motor output. Evidence of decreased transcallosal cortical inhibition has been found 2 years following adult severe TBI and correlated with injury severity. Takeuchi et al. found that there is a decreased magnitude of inhibition in the contracting hand (ipsilateral to stimulation) after severe TBI, but that the duration of the inhibition elicited via these transcallosal tracts (ipsilateral silent period, iSP) was not different\textsuperscript{78}. This suggests a decrease in the number of inhibitory interneurons stimulated in the contralateral hemisphere, which is in keeping with transcallosal tracts damage and might also explain the presence of the mirror movements that can be seen after severe TBI.
### Table S12: Summary of the literature using single and paired pulse transcranial magnetic stimulation paradigms after severe traumatic brain injury (TBI).

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year(s)</th>
<th>Acutenes s</th>
<th>TBI Severit y</th>
<th>Notes</th>
<th>TMS paradigms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mazzini</td>
<td>1999</td>
<td>Acute</td>
<td>Severe</td>
<td>Upper</td>
<td>RMT Peak to Peak MEP Amplitude</td>
</tr>
<tr>
<td>Jang</td>
<td>2005</td>
<td>Chronic</td>
<td>Severe</td>
<td></td>
<td>CSP Duration</td>
</tr>
<tr>
<td>Bagnato</td>
<td>2012</td>
<td>Chronic</td>
<td>Severe</td>
<td>VS</td>
<td>TCI onset</td>
</tr>
<tr>
<td>Fujiki</td>
<td>2006</td>
<td>Chronic</td>
<td>Severe</td>
<td>DAI</td>
<td>Paretic ↓  x  ↓  ↓</td>
</tr>
<tr>
<td>Takeuchi</td>
<td>2006</td>
<td>Chronic</td>
<td>Severe</td>
<td></td>
<td>Overall ↑  x  ↓</td>
</tr>
</tbody>
</table>

| Bernabéu     | 2009    | Chronic     | Severe        | Non-Paretic ↑  x  ↓  ↓ |
|              |         |             |               | Paretic ↑  ↑  ↑  x |
|              |         |             |               | Mild DAI ↑  x  ↓  x |
|              |         |             |               | Severe DAI ↑  x  ↓  x |
|              |         |             |               | Overall ↑  ↑  x  ↓ |

### Abbreviations: TMS: transcranial magnetic stimulation; RMT: Rest motor threshold; MEP: Motor evoked potential; SRC: stimulus response curve; CMCT: corticomotor conduction time; CSP: cortical silent period; SPT: silent period threshold; ISP: ipsilateral silent period; TCI: transcallosal inhibition; TCT: transcallosal conduction time; SICI: short interval intracortical inhibition; ICF:
intracortical facilitation; LICI: long interval intracortical inhibition; VS: vegetative state; DAI: diffuse axonal injury. Upper and lower (Mazzini 1999) refer to the upper or lower limbs, respectively.
9.5 Treatment studies using rTMS

The safety of performing rTMS in patients with TBI is still being investigated, but early reports suggest that it is probably safe. The literature contains one report of a seizure following TMS as well as an increase in post-concussion symptoms. These seem to be uncommon occurrences but further study is needed. The studies using rTMS as a treatment modality in TBI are few and have explored its use in post-concussion syndrome following mTBI, and in minimally conscious states and dysphagia following severe TBI.

rTMS over the dorsolateral prefrontal cortex (DLPFC) has been used to successfully treat patients with depression (see Chapters 15, 16). As many patients with persistent post-concussion symptoms have quite marked mood disturbance, it is logical to explore its similar use in PCS. In a pilot study, Koski et al examined the safety and use of rTMS stimulating the DLPFC to treat persistent PCS which had been present for 6 months or longer. At the end of 20 sessions over a four-week period, although one patient reported an increase in symptoms, participants reported a significant decrease in symptoms overall. Given these favourable results and good safety profile, larger studies exploring rTMS in mTBI studies are ongoing.

Minimally conscious states (including vegetative state) have an extremely poor prognosis following TBI. There are case series of rTMS being used to try and improve the conscious level in these patients following severe TBI but the results are disappointing. One patient was reported to show minimal improvement (changing from a vegetative to minimally conscious state), but the other 5 patients showed no response. Another study investigated whether rTMS to the mylohyoid motor cortex improves swallowing post-injury in severe TBI. The protocol was similar to those used in the treatment of stroke except that the target differed (i.e., contralateral...
low frequency and ipsilesional high frequency stimulations for 10s, repeated every minute for 20 minutes for ten days). Gains were made in two of the 3 dysphagia measures when compared to sham rTMS treatment\textsuperscript{195}. More research is needed but rTMS may be a useful adjunct therapy in the rehabilitation of people with TBI in the future especially for specific deficits.

9.6 TMS in pediatric mild traumatic brain injury

There is no literature exploring cortical excitability using TMS in children. We report our pilot study investigating cortical excitability in children during their recovery from a mild TBI. This is a controlled longitudinal cohort study. Children with mTBI are eligible for study if they are between 8 and 18 years of age, and are “symptomatic” at one month post-injury. They are compared to two control groups: i) children who have recovered by one month post-injury and so are “asymptomatic”, and ii) normal children without a history of mTBI. Exclusion criteria include an injury of greater severity, the use of any medications that could affect TMS, and/or a history of serious medical or psychiatric illness.

\textit{TMS methodology:} Biphasic stimuli were given using a Magstim BiStim 200 Transcranial Magnetic Stimulator (The Magstim Company Limited, Carmarthenshire) with a figure-8 coil (70mm inner diameter, each circle) to the hotspot for the first dorsal interosseous muscle. Electromyography (EMG) was recorded bilaterally from the first dorsal interosseous muscle using Ag/AgCl EMG electrodes (Amplified 1000 times, band pass filtered 20-2000Hz, 5000Hz sampling rate). Motor thresholds were adjusted to the minimum stimulation intensity required to elicit 5/10 consecutive MEPs of the required amplitude. Active contractions were measured using an EMG oscilloscope (GwINSTEK GDS-1022, 25 MHz, 250M Sa/s, Good Will Instrument Co, New Taipei City, Taiwan), and all contractions were 20\% of the maximal
voluntary contraction, unless stated. If a participant’s 50µV RMT was above 66% of the maximal stimulator output, they could not participate in the rest SRC trial. If their 1000µV RMT was above the maximal stimulator output, they could only perform the active SRC. Data were processed using Matlab (MATLAB and Statistics Toolbox Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States.). Graphs were created in Sigmaplot 13.0 (Systat Software, Inc., San Jose California USA, www.sigmaplot.com). Line graphs show the means with standard deviations. Boxplots show the group median as a black horizontal line inside the box. The top edge of the box is the 75th percentile, and the bottom of the box is the 25th percentile, with the group mean in the middle of the box. Outside the box are the “whisker” which denote the 95% confidence intervals. Outliers are shown as points.

*TMS outcome measures* included single TMS and paired pulse TMS paradigms. The primary outcome measure was CSP. Other measures included RMT (50µV and 1mV), AMT, active and resting SRC, short interval intracortical facilitation (SICF), active and resting SICI, active and resting ICF, LICI, and iSP. Secondary outcome measures included the tolerability of the TMS session using a questionnaire designed for TMS in children\textsuperscript{103}. Active and resting SRC were ten percent intervals from 100% to 150% of the resting and active motor threshold. For active SRC, we also measured the silent period after each stimulus to create a silent period recruitment curve. CSP was evoked in the active condition using a stimulus at the 1000µV RMT\textsuperscript{109,110}. CSP duration was from the onset of disrupted waveform after the MEP to the resumption of normal EMG. iSP duration was found similarly, with contraction at 50% of the maximal voluntary effort in the hand ipsilateral to the stimulation\textsuperscript{183}. SICI and ICF stimuli were separated by ISIs of 2ms for SICI and 10ms for ICF\textsuperscript{117}. The active state SICI/ICF used a
conditioning stimulus at 70% AMT, then a test stimulus at 1000µV RMT. Resting state SICI/ICF used a conditioning stimulus applied is 90% RMT, and a test stimulus at 1000µV RMT. LICI used conditioning and test stimuli at 1000µV RMT, separated by 100ms. SICF used a conditioning stimulus at the 1000µV RMT and a test stimulus at 90% of 50µV RMT, with ISIs at 1.5ms, 2.6ms, and 4.3ms\textsuperscript{152}. For paired pulse techniques, all ISIs were pseudorandomised with a test stimulus alone, which was compared offline.

Table S13: Demographic information of paediatric mild traumatic brain injury pilot study.

<table>
<thead>
<tr>
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<th>Healthy</th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
</tr>
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<tbody>
<tr>
<td>Sample Size</td>
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<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Age at assessment</td>
<td>Mean (SD)</td>
<td>14.1 (3.47)</td>
<td>14.04 (2.27)</td>
</tr>
<tr>
<td></td>
<td>Mean Rank</td>
<td>22.93</td>
<td>20.13</td>
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<tr>
<td>Hemisphere stimulated</td>
<td>Left:Right</td>
<td>14:1</td>
<td>15:0</td>
</tr>
<tr>
<td>Gender</td>
<td>Male: Female</td>
<td>6:9</td>
<td>6:9</td>
</tr>
<tr>
<td>Active SRC only (1000µV &gt; 100% MSO)</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>No Rest SRC</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Full TMS</td>
<td>14</td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

Abbreviations: SRC: stimulus response curves; TMS: Transcranial magnetic stimulation; MSO: Maximal stimulator output.

Preliminary analysis has been completed on 15 symptomatic and 15 asymptomatic mTBI participants, and 15 healthy controls. Groups are comparable in age and gender; see table 4.

Two asymptomatic controls had 50µV RMTs that exceeded the maximal stimulator output (2
Tesla), preventing further study. RMT thresholds exceeded 66% of maximal stimulator output in 1 healthy and 2 symptomatic participants, who therefore could not participate in resting SRC. Additionally, 2 participants from each group had 1000μV RMTs greater than the maximal stimulator output, and so could only perform active SRC.

9.6.1 Safety and Tolerability

No serious adverse events were reported. Tolerability scores for ranking TMS out of 8 activities were similar between groups (p = 0.623): mean symptomatic scores were 3.75 (SD±1.49); asymptomatic scores were 3.86 (SD±1.88); and normal controls 4.29 (SD±1.94). Healthy controls reported mild headache (1 participant), tingling sensations (1 participant), and nausea (1 participant). Asymptomatic participants reported 1 mild instance of neck pain, and 1 instance of mild tingling. Symptomatic participants reported one instance of each of mild neck pain, light-headedness and nausea, and 4 instances of mild tingling in the fingers or at the stimulation site. The frequency of reported minor adverse side effects were not different between participants.

9.6.2 Results

All three motor thresholds showed no differences between groups. CSP times did not differ between groups: 86.87 ± 37.85 ms in the symptomatic group, 115.82 ± 33.74 ms in the asymptomatic group, and 110.37 ± 51.59 ms in healthy control, (F (2, 37) = 1.82, p = 0.177), see Figure 2. These values are similar to healthy children in other studies115,116 and our lab (Kirton, personal communication, June 2, 2015). iSP at 1000μV RMT stimulation was similar between groups, F (2, 35) = 0.55, p = 0.582. The details of each of the TMS outcome measures are given in Table 5.
The SRC at rest showed a significant effect of stimulation intensity \( (F(1.53, 58.10) = 69.09, p < 0.01) \), but was not different between groups \( (F(5, 34) = 0.46, p = 0.90) \). Similarly, active stimulus response curves, showed an effect for stimulus intensity \( (F(1.63, 61.85) = 122.37, p < 0.01) \) but no effect of group, or interaction; shown in Table 6. Silent period recruitment curves during active SRC are shown in Figure 2. The duration of the silent period response curve during active stimulus response curve increased with increasing stimulation \( (F(1.42, 59.77) = 86.54, p < 0.01) \). There was not main effect of group \( (F(2, 42) = 0.247, p = 0.782) \).

SICF has not previously been explored in TBI. Groups were similar \( (F(2, 36) = 0.53, p = 0.600) \), and there was no effect of ISI \( (F(2, 72) = 1.38, p = 0.260) \), see Figure 3. ICF and SICI for the active or resting conditions was also similar between groups. However, the variability in resting SICI was higher than expected. As expected, during the active SICI/ICF paradigm, inhibition and facilitatory effects of these paired pulse paradigms is negated\(^93\), see table 6. All SICI and ICF paradigms are shown in Figure 4. LICI differed significantly in that symptomatic participants had a decreased inhibitory response \( (\chi^2 (2) = 7.75, p = 0.021) \).
Figure S20: Stimulus response curve measures and cortical silent periods.

(A) shows the rest stimulus response curve mean (±standard deviation) of the amplitude of motor evoked potentials expressed as percentages of the rest motor threshold. (B) shows the active stimulus response curve mean (±standard deviation) of the amplitude of motor evoked potentials expressed as percentages of the active motor threshold at a contraction of 20% of the participant’s maximum voluntary effort. (C) shows a boxplot for the distribution of the cortical silent period for the controls, asymptomatic and symptomatic mTBI groups. (D) shows the mean (±standard deviation) silent period curve calculated from the active stimulus response curve.
Table S14: Transcranial magnetic stimulation results one month after paediatric mild traumatic brain injury.

Single pulse TMS paradigms include: Motor thresholds, shown as a percentage of the maximal stimulator output (MSO); silent periods, with values in milliseconds; and stimulus response curve values area under the curve, slope, and max, which are all functions of the motor evoked potential amplitude. Paired pulse paradigms include intracortical facilitation (ICF) and long and short intracortical inhibition (LICI and SICI, respectively). Paired pulse paradigms are expressed as a ratio of the conditioned stimulus to the test stimulus alone (unconditioned), where values less than one indicate inhibition, and values greater than one indicate facilitation.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Healthy</th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
<th>Stat</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSP</td>
<td>110.37 (51.59)</td>
<td>115.82 (33.74)</td>
<td>86.87 (37.85)</td>
<td>F (2, 37) = 1.82</td>
<td>0.177</td>
</tr>
<tr>
<td>ISP 1mV</td>
<td>12.66 (8.83)</td>
<td>11.97 (5.69)</td>
<td>9.83 (6.00)</td>
<td>F (2, 35) = 0.55</td>
<td>0.582</td>
</tr>
<tr>
<td>ISP 75%MSO</td>
<td>17.04 (13.45)</td>
<td>14.76 (10.33)</td>
<td>10.28 (6.16)</td>
<td>χ (2) = 1.70</td>
<td>0.427</td>
</tr>
<tr>
<td>Rest ICF</td>
<td>1.53 (1.27)</td>
<td>1.68 (0.99)</td>
<td>1.34 (1.02)</td>
<td>χ (2) = 2.41</td>
<td>0.300</td>
</tr>
<tr>
<td>Active ICF</td>
<td>0.97 (0.16)</td>
<td>0.96 (0.09)</td>
<td>1.04 (0.25)</td>
<td>χ (2) = 0.596</td>
<td>0.742</td>
</tr>
<tr>
<td>Rest SICI</td>
<td>1.44 (1.20)</td>
<td>0.78 (0.36)</td>
<td>1.17 (0.79)</td>
<td>χ (2) = 1.80</td>
<td>0.406</td>
</tr>
<tr>
<td>Active SICI</td>
<td>0.87 (0.19)</td>
<td>0.88 (0.13)</td>
<td>0.99 (0.24)</td>
<td>F (2, 37) = 1.44</td>
<td>0.251</td>
</tr>
<tr>
<td>LICI</td>
<td>0.44 (0.45)</td>
<td>0.41 (0.74)</td>
<td>0.99 (0.80)</td>
<td>χ (2) = 7.75</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Abbreviations: RMT: rest motor threshold; AMT: active motor threshold; CSP: cortical silent period; ISP: ipsilateral silent period; MSO: maximal stimulator output; RSRC: rest stimulus response curve; AUC: area under the curve; ASRC: active stimulus response curve; ICF: intracortical facilitation; SICI: short interval intracortical inhibition; LICI: long interval intracortical inhibition.
Table S15: Mean (standard deviations) amplitudes in millivolts of the motor evoked potential (MEP) by the stimulation used for stimulus response curves.

Statistical comparisons from mixed models analysis of variance are shown by interaction, between subjects and within subjects.

<table>
<thead>
<tr>
<th>TMS Paradigm</th>
<th>Amplitude (mV)</th>
<th>Interaction</th>
<th>Between Subjects</th>
<th>Within Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stat</td>
<td>p</td>
<td>Stat</td>
</tr>
<tr>
<td>TMS</td>
<td>Stimulation</td>
<td>Healthy</td>
<td>Asymptomatic</td>
<td>Symptomatic</td>
</tr>
<tr>
<td>Rest stimulus response curve</td>
<td>100% RMT</td>
<td>0.13</td>
<td>0.15 (0.15)</td>
<td>0.15 (0.14)</td>
</tr>
<tr>
<td></td>
<td>110% RMT</td>
<td>0.30</td>
<td>0.47 (0.68)</td>
<td>0.40 (0.31)</td>
</tr>
<tr>
<td></td>
<td>120% RMT</td>
<td>0.87</td>
<td>1.06 (1.00)</td>
<td>0.98 (0.67)</td>
</tr>
<tr>
<td></td>
<td>130% RMT</td>
<td>1.52</td>
<td>1.57 (1.22)</td>
<td>1.77 (1.24)</td>
</tr>
<tr>
<td></td>
<td>140% RMT</td>
<td>2.05</td>
<td>2.12 (1.50)</td>
<td>2.36 (1.44)</td>
</tr>
<tr>
<td></td>
<td>150% RMT</td>
<td>2.57</td>
<td>2.40 (1.27)</td>
<td>2.67 (1.43)</td>
</tr>
<tr>
<td>Active stimulus response curve</td>
<td>100% AMT</td>
<td>1.22</td>
<td>2.03 (1.23)</td>
<td>1.72 (0.51)</td>
</tr>
<tr>
<td></td>
<td>110% AMT</td>
<td>1.62</td>
<td>2.58 (1.91)</td>
<td>2.12 (0.87)</td>
</tr>
<tr>
<td></td>
<td>120% AMT</td>
<td>2.41</td>
<td>3.77 (1.98)</td>
<td>3.43 (1.13)</td>
</tr>
<tr>
<td></td>
<td>130% AMT</td>
<td>3.71</td>
<td>5.05 (2.27)</td>
<td>4.63 (1.62)</td>
</tr>
<tr>
<td></td>
<td>140% AMT</td>
<td>4.87</td>
<td>5.99 (2.25)</td>
<td>5.79 (1.95)</td>
</tr>
<tr>
<td></td>
<td>150% AMT</td>
<td>6.13</td>
<td>7.05 (2.19)</td>
<td>6.75 (2.01)</td>
</tr>
</tbody>
</table>

Abbreviations: RMT: rest motor threshold; AMT: active motor threshold, performed with 20% maximal voluntary contraction.
Table S16: Mean (standard deviations) silent periods in milliseconds during 20% maximal voluntary contraction by the stimulation used.

Statistical comparisons from mixed models analysis of variance are shown by interaction, between subjects and within subjects.

<table>
<thead>
<tr>
<th>TMS Paradigm</th>
<th>Silent Period (ms)</th>
<th>Interaction</th>
<th>Between Subjects</th>
<th>Within Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMS Stimulation</td>
<td>Healthy</td>
<td>Asymptomatic</td>
<td>Symptomatic</td>
</tr>
<tr>
<td>Active stimulus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>response curve silent</td>
<td>100% AMT</td>
<td>15.26 (5.09)</td>
<td>16.36 (43.41)</td>
<td>8.84 (30.68)</td>
</tr>
<tr>
<td>silent period</td>
<td>110% AMT</td>
<td>18.58 (6.39)</td>
<td>25.41 (46.88)</td>
<td>16.41 (34.68)</td>
</tr>
<tr>
<td></td>
<td>120% AMT</td>
<td>31.77 (15.6)</td>
<td>42.21 (60.15)</td>
<td>31.09 (47.28)</td>
</tr>
<tr>
<td></td>
<td>130% AMT</td>
<td>52.82 (33.27)</td>
<td>55.75 (60.84)</td>
<td>50.95 (60.51)</td>
</tr>
<tr>
<td></td>
<td>140% AMT</td>
<td>72.47 (43.47)</td>
<td>72.74 (62.95)</td>
<td>60.42 (64.62)</td>
</tr>
<tr>
<td></td>
<td>150% AMT</td>
<td>95.76 (47.89)</td>
<td>94.07 (68.45)</td>
<td>71.81 (67.32)</td>
</tr>
</tbody>
</table>

Abbreviations: AMT: active motor threshold
Figure S21: Short interval intracortical facilitation conditioned stimulus to test stimulus ratios for each group.

The lines represent different groups, while each point on the x-axis represents the interstimulus interval at which the stimuli were given. All data are shown as the ratio of the amplitudes of conditioned stimuli to the test stimulus alone (unconditioned). The red line indicates 1, or the dividing point between inhibition (<1) and facilitation (>1).
Figure S22: Intracortical facilitation and short interval intracortical inhibition during rest and active states.

The resting short interval intracortical inhibition (SICI; A) and resting intracortical facilitation (ICF; B) are the top row, while the same measures in the active state are the bottom row (C&D). The red line on each graph indicates 1, or the dividing point between inhibition (<1) and facilitation (>1).
Figure S23: Long interval intracortical inhibition ratios for each group.

All data are shown as the ratio of the amplitudes of conditioned stimuli to the test stimulus alone (unconditioned). The red line on each graph indicates 1, or the dividing point between inhibition (<1) and facilitation (>1).
These preliminary data show that it is feasible and safe to examine cortical excitability in children following mild TBI. Although most measures did not differ in mTBI at one-month post injury, symptomatic children were found to have a decreased long interval intracortical inhibitory response (i.e., decreased LICI). This finding is consistent with chronic findings in the literature\textsuperscript{110,147}. These findings may suggest that GABAb receptor-mediated inhibition is decreased in symptomatic participants and that this returns to normal as symptoms decrease. Larger sample sizes are required to confirm this finding given the amount of between subject variability of TMS measures.

9.7 Summary

The study of cortical excitability in TBI is uniquely challenging in that it is a highly heterogeneous condition that is lacking in accurate quantification of its severity. TMS is also highly variable between individuals in both adults and children. The current literature is insufficient to draw conclusions about what changes in cortical excitability occur after TBI and their neurophysiological drivers. Although most studies suggest an increase in cortical inhibition, the studies are small, populations are heterogeneous, and TMS methodologies used vary considerably. Larger studies are required especially in the setting of the developing brain\textsuperscript{143}.

We report our findings in a well-controlled group of children with and without persistent symptoms one month following a mTBI. Our provisional results suggest that PCS symptoms are associated with a lack of cortical inhibition, although the sample is small and pre-injury values are unknown. The study of young athletic populations may offer a way to study changes in cortical excitability over time due to the concussion. However, these studies should control factors that change over time including age, pubertal development, exercise training and the use
of prescribed and non-prescribed medications. Future studies should be carefully controlled for severity of the injury (using sensitive measures), multiple injuries, functional outcome, cognition, and the presence or absence of medical conditions that may alter cortical excitability (e.g. attention deficits\textsuperscript{197}; migraine\textsuperscript{198,199}).

In summary, TBI may exhibit various changes in cortical excitability, even years after the injury, in sports or the general population, potentially showing a shift towards inhibition. Changes in cortical excitability may help to better explain the persistence of symptoms after TBI and poorer outcomes associated with paediatric TBI\textsuperscript{44,200}. 
References


129. Golaszewski, S. M. *et al.* Modulation of motor cortex excitability by different levels of


192. Theresa Pape. rTMS: A Treatment to Restore Function After Severe TBI.


