UNIVERSITY OF CALGARY

Using 9.4T MRI To Investigate Parenchymal And Vascular Changes In An Animal Model Of Pediatric Mild Traumatic Brain Injury

by

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Abstract

Mild traumatic brain injury (mTBI) is the most common form of brain injury in Canada. No standard CT or MRI methods can identify or monitor mTBI. It is necessary to develop methods to identify patients at risk of long term problems and monitor treatment. Evidence exists for abnormal blood flow post-mTBI, therefore we hypothesized that 24 hr after injury there would be abnormal vascular regulation with no parenchymal changes. Juvenile rats bred in house and ordered from a supplier sustained a mTBI or a sham injury using a weight drop model. Animals were imaged 24hr later using a 9.4T MRI. No structural changes were evident using standard MRI sequences, supporting the model as being consistent with clinical case of mTBI. mTBI bred rats had a larger sagittal sinus size. The larger sinus size in mTBI compared to control rats potentially suggests cerebrovenous dysregulation which could relate to mTBI symptomology.
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<td>1H</td>
<td>Hydrogen 1</td>
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<tr>
<td>31P</td>
<td>Phosphorus 31</td>
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<tr>
<td>AD</td>
<td>Axial diffusivity</td>
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<tr>
<td>ADC</td>
<td>Apparent diffusion coefficient</td>
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<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ASL</td>
<td>Arterial spin labelling</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
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<tr>
<td>BG L</td>
<td>Basal ganglia left</td>
</tr>
<tr>
<td>BG R</td>
<td>Basal ganglia right</td>
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<tr>
<td>BOLD</td>
<td>Blood oxygen level dependent</td>
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<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>CC</td>
<td>Corpus callosum</td>
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<td>CCI</td>
<td>Controlled cortical impact</td>
</tr>
<tr>
<td>Cho</td>
<td>Choline</td>
</tr>
<tr>
<td>CPMG</td>
<td>Carr-Purcell-Meiboom-Gill sequence</td>
</tr>
<tr>
<td>CPP</td>
<td>Cerebral perfusion pressure</td>
</tr>
<tr>
<td>Cr</td>
<td>Creatine</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>Ctx L</td>
<td>Cortex left</td>
</tr>
<tr>
<td>Ctx R</td>
<td>Cortex right</td>
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CVR  Cerebrovascular reactivity
CVST Cerebral venous sinus thrombosis
DAI Diffuse axonal injury
DTI Diffusion tensor imaging
DWI Diffusion weighted imaging
FA Fractional anisotropy
FISP Fast imaging with steady state precession
fMRI Functional MRI
FPI Fluid percussion injury
GABA Gamma-aminobutyric acid
GCS Glasgow coma scale
Glu Glutamate
Glx Glucose-glutamine
GM Grey matter
Hc L Hippocampus left
Hc R Hippocampus right
ICP Intracranial pressure
IIH Idiopathic intracranial hypertension
IJV Internal jugular vein
K Potassium
Lac Lactate
MD Medial diffusivity
mI Myo-inositol
MIP Maximum intensity projection
MRI Magnetic resonance imaging
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>MRV</td>
<td>MR venography</td>
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<tr>
<td>mTBI</td>
<td>Mild traumatic brain injury</td>
</tr>
<tr>
<td>Na</td>
<td>Sodium</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetylaspartate</td>
</tr>
<tr>
<td>NNLS</td>
<td>Non-negative least-squares</td>
</tr>
<tr>
<td>NODDI</td>
<td>Neurite orientation dispersion and density imaging</td>
</tr>
<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
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<tr>
<td>PCS</td>
<td>Post-concussion syndrome</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>Pi</td>
<td>Inorganic phosphate</td>
</tr>
<tr>
<td>PWI</td>
<td>Perfusion weighted imaging</td>
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<tr>
<td>RARE</td>
<td>Rapid acquisition with relaxation enhancement</td>
</tr>
<tr>
<td>RD</td>
<td>Radial diffusivity</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SS</td>
<td>Sagittal sinus</td>
</tr>
<tr>
<td>SWI</td>
<td>Susceptibility weighted imaging</td>
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<tr>
<td>TBI</td>
<td>Traumatic brain injury</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>TS L</td>
<td>Transverse sinus left</td>
</tr>
<tr>
<td>TS R</td>
<td>Transverse sinus right</td>
</tr>
</tbody>
</table>
TTR  Time to right
WD  Weight drop
WM  White matter
Chapter 1: Background

1.1 Introduction to mTBI

Every year, there are approximately 1.4 - 3.8 million mild traumatic brain injuries (mTBIs) occurring in the United States (Faul et al. 2010). Epidemiological data regarding mTBI for Canada as a whole is lacking; however, Ryu et al. (2009) found the incidence of mTBI in Ontario to be between 493/100,000 and 653/100,000 people. Mild traumatic brain injury represents a significant and growing problem, with the symptoms and potential implications becoming more widely recognized. Most patients recover fully within approximately 2 weeks; however, 10-15% of patients, will go on to suffer from post-concussion syndrome (PCS) which is a collection of cognitive, somatic, and behavioural symptoms that persist beyond 1 month post injury (Barlow et al. 2010; Barlow et al. 2014; Ryan & Warden 2003). As mTBI often goes unreported, it is likely that the prevalence of mTBI is underestimated (Langlois et al. 2006). A CDC report estimated that in years 2009/2010, there were 5465/100,000 brain injuries in the USA. Approximately 70-90% these injuries considered to be mild (Faul et al. 2010; Cassidy et al. 2004; Kraus et al. 1990). McKinlay et al. (2008) suggested that by the time children turn 10, 1/10 of them will have sustained a mTBI.

Traumatic brain injury (TBI) exists on a spectrum, ranging from mild (which includes concussion) to severe. Mild traumatic brain injury (mTBI) is defined as: “a complex pathophysiological process affecting the brain, induced by biomechanical forces” (McCrory et al. 2013). A mTBI should be suspected if the patients meets one or more of the following
criteria: 1) Symptoms post-injury, such as cognitive (deficits in attention, concentration or executive function), somatic (nausea, dizziness, headache, fatigue, sleep disturbances), and/or emotional (irritability, disinhibitions, emotional lability) 2) Physical signs such as loss of conscious not exceeding 30 minutes, and/or memory loss for events immediately prior to or after the injury not exceeding 24 hours, 3) Behavioural changes, such as irritability, 4) Cognitive impairment, such as slower reaction times, 5) Sleep disturbances (McCrory et al. 2004; McCrory et al. 2013). Additionally, patients must have an initial score of 13-15 on the Glasgow Coma Scale (GCS), which is a measure of the level of consciousness after head injury (ACRM 1993; Teasdale & Jennett 1974). A score of 13-15 is the mildest score on the GCS, indicating either a mild impairment of consciousness (score of 13 or 14) or a normal level of consciousness (score of 15) (Teasdale & Jennett 1974).

Moderate to severe TBI is associated with visible damage on standard magnetic resonance imaging (MRI) and computed tomography (CT) scans, as well more serious pathophysiology such as hemorrhages, cerebral edema, cortical contusions, and hematoma (Edlow & Wu 2012). The outcome after TBI is typically worse than mTBI with many patients suffering from lifelong disability and/or permanent changes in behaviour, cognitive functioning, and/or emotional lability (Bodanapally et al. 2015). In comparison, mTBI shows no obvious pathology on standard clinical MRI and CT scans, making it a challenge to accurately determine if someone has sustained a mTBI. In addition, due to the lack of pathological markers after mTBI, it is difficult to identify and monitor injury progression and to determine when it is safe for the injured individual to resume normal activities

Post-concussion syndrome (PCS) occurs in a minority of patients; however, it can significantly affect the patients’ quality of life. A patient is considered to have PCS if their
symptoms persist past 3 months after injury (Reuben et al. 2014). These patients can go on to suffer from PCS months to years post-injury (Reuben et al. 2014). The children typically suffer from a range of emotional, behavioural, physical, and cognitive symptoms which include headache, irritability, difficulty concentrating, memory problems, fatigue, and anxiety (Ryan & Warden 2003; Broshek et al. 2015). Although only a minority of patients go on to develop PCS, it is important to develop a means to determine who is at risk of long term problems and to monitor treatment for these patients.

1.2 Biomechanics

Biomechanical forces play a critical role in mTBI. By definition, mTBI is a closed-head diffuse injury to the brain caused by acceleration-deceleration forces (Barth et al. 2001). Acceleration-deceleration forces can be caused either by direct contact forces, such as a hit to the head or body, or inertial forces (acceleration) (Meaney & Smith 2011). The acceleration-deceleration forces cause the brain to move within the skull, resulting in multi-directional injuries including in the direction of the force, as well as on the opposite side of the applied force. Due to the nature of the impact, both linear and rotational forces are simultaneously present to some extent (Ommaya et al. 2002). While both planes can cause damage, it has been shown that rotational force results in a more severe injury (Barth et al. 2001). Due to the composition of the brain, it is very vulnerable to shear strain caused by rotational motion, which results in deformation of tissue, stretching and shearing of axons, and stretching of vasculature (Post & Hoshizaki 2014; Gennarelli et al. 1972; Barth et al. 2001; Lowenhielm 1973). White matter (WM) in particular is highly susceptible to rotational motion (Barth et al. 2001). Animal
studies have found that rotational motion is associated with a higher degree of axonal pathology (Greer et al. 2013; Browne et al. 2011; Barth et al. 2001). It is important to note that the amount of acceleration as well as the direction of head rotation affects the outcome of the injury (Eucker et al. 2011). Axons that lie along the plane of the shear force are most susceptible to shear strain (Maxwell et al. 1993). Interestingly, in rats it has been shown that the direction of rotation after injury (either sagittal or axial) affects the outcome. Rats which experienced a lateral injury had greater deficits in the forced swim task (indicating increased behavioural despair) and elevated plus maze (higher anxiety), while those injured in the sagittal plane showed greater impairment in working memory (Mychasiuk et al. 2016). This could be a contributing factor to the heterogeneous outcomes after mTBI (Grant 2005).

It has been suggested that mTBI outcome may be affected by age-dependent physical differences between children and adults (Kirkwood et al. 2006). For example, the neck and shoulder muscles of children are not as well-developed as adults and are therefore not able to transfer the applied force throughout the entire body. As a result, there is greater movement of the head, which imparts more force on the brain (McCrory et al. 2012). In addition, since the immature brain is smaller, it has been suggested that more force is required to cause a mTBI in children, although once injury is sustained, the outcome is worse (Ommaya et al. 2002; Kirkwood et al. 2006).

1.3 Pediatric mTBI

Children have the highest incidence of TBI, with the age group of 0-4 being the most likely to sustain a TBI (Faul et al. 2010). Despite this, research on mTBI in both humans and
animal models is primarily conducted on adults, with less emphasis placed on the pediatric population. This results in a paucity of information on pediatric mTBI. The lack of information on pediatric mTBI leads to a lack of understanding of the pathogenic processes and makes it unclear if we should assess and treat children in the same way that we do adults with mTBI (McKenna et al. 2015).

The Kennard principle postulates that the earlier in life a brain injury is sustained, the better the outcome due to neuroplasticity (Kennard et al. 1936). However, contrary to this principle, it has been found that a developing brain is more vulnerable to injury than an adult brain (Kirkwood et al. 2006; Levin 2003; Giza & Prins 2006). The developing brain has high metabolic demands and disruption of these processes can alter normal development. These disruptions can take the form of energy failure, disrupted synaptogenesis and cell division, and impaired ionic gradients (Prins & Matsumoto 2014). The high metabolic demands of the developing brain require an increase in cerebral blood flow (CBF) and a higher level of glucose utilization (Takahashi et al. 1999; Settergren et al. 1976). The use of glucose and other substrates in the brain, such as ketones, fatty acids, and glycerol, are used not only for energy, as in adults, but also for biosynthesis (Chugani & Phelps 1986; Settergren et al. 1976). Children are more likely to experience a worse outcome post-mTBI than adults, which can be attributed in part to the increased metabolic demands on the developing brain (McCarthy & Kosofsky 2015).

Children are more susceptible to diffuse brain injury after TBI than adults (McCrorey et al. 2004; Kirkwood et al. 2006). During childhood, the brain is undergoing significant myelination with maturation occurring in spurts (Jernigan et al. 1992). It was found by Hudspeth and Pribram (1990) that the period of most rapid myelination is between birth and 5 years of age; however, myelination continues throughout childhood. Unmyelinated axons are particularly vulnerable to
shear stress (Reeves et al. 2005). The larger proportion of unmyelinated axons in an immature brain could make children more vulnerable to traumatic forces, and potentially cause greater diffuse axonal injury.

A brain injury sustained in critical periods of skill development may disrupt acquisition of these skills and have long term effects on how well the child recovers (Anderson et al. 2005; Ewing-Cobbs et al. 2006; Levin et al. 1989). It has been suggested that injury during periods of myelination can disrupt the development of network connectivity between regions of the brain, particularly regions associated with cognitive function (Levin 2003). Interestingly, infants in the acute phase post-TBI showed average IQs; however, performance decreased from the acute assessment to the assessment at 30 months. This was not seen in the older children (Anderson et al. 2005). Children who sustained a TBI earlier in life (<3 years of age) showed a poorer long-term outcome than children >3 years of age (Anderson et al. 2005). This could be attributed to injury-induced disruption of myelination in regions associated with cognitive function. This may cause slowing of cognitive processing and attentional impairment which, in turn, affects future acquisition of cognitive and social skills (Anderson & Moore 1995). It has been hypothesized that TBI can cause damage to the brain networks responsible for social cognition (Yeates et al. 2004). Toddlers and infants show deficits in social functioning post-mTBI. Nine months post-injury, toddlers and infants with a mTBI showed more social-emotional behavioural difficulties than the control group, with a trend towards increasing difficulty in behaviour as time passed (Kaldoja & Kolk 2012).

Sustaining an mTBI can be difficult for children to cope with as it can significantly affect their life due to missing school and/or behavioural and emotional changes, impacting both their intellectual and social development (Toledo et al. 2012). Children who have sustained moderate
and severe TBIs show significant deficits in social behaviour and problem solving (Janusz et al. 2002; Yeates et al. 2004; Muscara et al. 2009; Prigatano & Gupta 2006; Warschauisky et al. 1997; Dennis et al. 2001). Mild traumatic brain injury often has an impact on a child’s academic performance. It has been found that children in the subacute stage of mTBI (<4 weeks post-injury) have difficulties in math as a result of impaired visuospatial working memory (Van Beek et al. 2015).

Eleven percent of children have been reported to have PCS with cognitive, emotional, and somatic changes persisting to 3 months post-injury. 2.3% of children still show symptoms up to a year after injury (Barlow et al. 2010). Three months after injury, children scored significantly lower than controls on quality of life tests, with females reporting a lower quality of life when compared to males (Tham et al. 2015). Children suffering from PCS have been found to exhibit deficits in attentional-inhibitory control when studied >6 months post-injury (Dennis et al. 2001). Adolescents are also susceptible to persistent PCS. Adolescents have showed impairments in working memory post-mTBI which persisted for 6 months after injury (Baillargeon et al. 2012; Field et al. 2003). It has been suggested that some subclinical changes, such as impulsiveness, can persist for years post-mTBI in adolescents (Sroufe et al. 2010; Beers 1992). Adolescents who have sustained a mTBI can exhibit sleep disturbances up to 1 year post-injury. It has been suggested that this puts adolescents at a higher risk of developing sleep problems post-mTBI due to shortened sleep duration, delayed sleep timing, and reduced sleep quality (Tham et al. 2015).
1.4 Gender Differences

There is conflicting evidence on whether gender affects the outcome of mTBI in both children and adults. It has been suggested that females are at a higher risk of sustaining a mTBI (Dick 2009). It is hypothesized that women sustain more mTBIs because they have a smaller body size and reduced musculature in comparison to men (Webbe & Barth 2003). There is evidence that females suffer from worse outcomes post-mTBI than males, with a higher likelihood of persistently elevated symptoms such as headaches, irritability, and difficulty concentrating (Rabinowitz et al. 2015). Headaches are more commonly reported in females, with significantly longer recovery times (up to 3 months) in comparison to males (Bramley et al. 2015; Blume et al. 2012). It has been suggested that females also have a higher likelihood of developing post-concussion syndrome (PCS) (Vassilyadi et al. 2015; Dick 2009; Preiss-Farzanegan et al. 2009). However, as noted above there is conflicting evidence regarding sex differences as some studies suggest that there is no difference in likelihood of developing PCS between males and females or differences in working memory (Cancelliere et al. 2015; Ganti et al. 2014; Bazarian et al. 2010; Chen et al. 2012).

Contrary to the observation regarding the lack of gender differences in PCS, both structural and functional differences resulting from mTBI have been noted in males and females. An fMRI study examining functional working memory using tasks which tested working memory and attention/impulsivity, found that 1 month after mTBI males exhibited hyperactivation, while females showed hypoactivation in frontal and parietal regions relative to controls. Six weeks after the initial study the males showed a regression of the hyperactivation back to baseline; however, the hypoactivation seen in females continued to persist for 6 weeks after the initial study. This study suggests that females have a reduced working memory outcome.
after mTBI (Hsu et al. 2015). Evidence for sex differences in white matter (WM) after mTBI have also been shown using diffusion weighted imaging. Fakhran et al. (2014) demonstrated that male patients had significantly decreased fractional anisotropy (FA) values in the uncinate fasciculus when compared to female patients and controls, despite no differences in neuropsychological scores.

Interestingly, animal studies in the rat continue to provide evidence for differences in behaviour between the sexes. For example, control female rats are significantly less likely to engage in play behaviour with female mTBI rats. In males a similar trend was observed but it was not found to be significant, suggesting that female mTBI rats are more likely to be rejected from play behaviour than male rats (Mychasiuk et al. 2014b). Additionally, when examining attention deficit hyperactivity disorder (ADHD) symptomology post-mTBI, while all rats were initially hypoactive in the open field, at later time points males began to exhibit a trend towards hyperactivity while females continued to trend towards hypoactivity (Mychasiuk et al. 2015a). Thus far, gender differences post-mTBI have not been completely studied and results have been conflicting, particularly in humans. More studies are required before any firm conclusions can be drawn.

1.5 Imaging mTBI

1.5.1 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging is a very useful tool to non-invasively investigate the anatomy and functionality of the brain. It has several advantages over other nuclear imaging techniques, such as positron emission tomography (PET), as it does not expose the patient to
ionizing radiation and is generally non-invasive. As a result, it can be used repeatedly in patients and is safer, especially for children.

MRI relies on the use of a magnetic field to generate a signal which can then be converted into an image and analysed. All nuclei in biological tissue contain protons that have an inherent spin. When tissue is placed in a magnetic field, the dipoles in a small proportion of the protons orient themselves in the direction of the magnetic field causing a net magnetization (Hendee & Morgan 1984). Protons naturally orient themselves to exist in their lowest energy state, which in this case is in the direction of the magnetic field. The protons in the sample are precessing (a secondary spin) at the same frequency, however they are out of phase with each other. This precessional frequency is called the Larmour frequency and is dependent on the gyromagnetic ratio of the proton (the precessional frequency of an element when exposed to a magnetic field of 1T) and the strength of the magnetic field. It is then possible to create a low energy pulse (the radiofrequency pulse or RF pulse) which causes the protons to precess in phase and to absorb energy, tilting the protons out of their original orientation in the magnetic field. The protons begin to reorient themselves into alignment with the main magnetic field, a process called spin-lattice relaxation (T1 relaxation). Simultaneously, the protons lose energy to surrounding protons causing dephasing, which is termed spin-spin relaxation (T2 relaxation). As these relaxation processes occur, the signal decays, and this lack of signal causes an area of darkness on the image. Different tissues and fluids have different relaxation rates, providing a means to identify different tissues.

In order to obtain a signal from a tissue, a pulse sequence must be applied. Various different pulse sequences exist to obtain different measurements at the region of interest. Two important parameters in the pulse sequence are the echo time (TE), which is the time between the
RF pulse and the maximum signal produced in the echo, and the repetition time (TR), which is the amount of time between RF pulses. The signal intensity peaks when all spins come (briefly) into alignment (in phase) before beginning to decay. Another pulse is applied to cause the spins to align again to obtain another signal. MRI has a wide variety of uses when trying to non-invasively study tissue. Standard clinical MRI sequences are T1 or T2-weighted. This project uses a wider range of sequences to examine the brain for pathology not visible on standard scans.

1.5.2 Imaging Sequences

1.5.2.1 T1 & T2

T1 is a commonly used clinical scan. T1 scans have a short TE and TR. It provides valuable information on the surrounding tissue microstructures. T1 relies on longitudinal relaxation, also known as spin-lattice relaxation. The relaxation of the protons back to their equilibrium state is very influenced by tissue microenvironment. Fluids, such as cerebrospinal fluid (CSF), have a long longitudinal relaxation time and therefore appear hypointense. Conversely, tissues such as fat have a short longitudinal relaxation time and as a result appear hyperintense. T1-weighted images can provide information about the permeability of the blood brain barrier (BBB) when a contrast agent, such as gadolinium, is injected.

T2 scans are very commonly used in clinical imaging and are useful when looking for pathology. T2 scans usually rely on a long TE and long TR. T2 scans make use of transverse relaxation, which is caused by protons losing their energy to other spins which causes the spins to come out of alignment and the signal to decay. In T2 scans, highly mobile fluids have a slower decay, resulting in a hyperintense signal. Tissues which are more structured decay more quickly.
and therefore appear hypointense. When tissue is broken down, there is more fluid and therefore a longer T2. The same occurs with edema. As a result, lesions appear hyperintense on T2-weighted images. T2* is closely related to T2 imaging. It also measures transverse relaxation; however, it is also affected by local field inhomogeneities. T2* sequences are useful in the detection of hemorrhage, iron deposition, and calcification (Chavhan et al. 2009). In more severe injuries, T2* is able to detect lesions in TBI patients. It has been found to be more sensitive in detecting lesions than conventional T2 imaging (Gerber et al. 2004).

1.5.2.2 Susceptibility Weighted Imaging

Susceptibility weighted imaging (SWI) is a subset of T2* MRI and is based on the same principle of detecting changes in field inhomogeneity such as would be caused by microhemorrhages post-injury (Mechtler et al. 2014). The SWI MR technique is particularly responsive to the susceptibility difference between oxygenated and deoxygenated hemoglobin (Tong et al. 2008). This causes a phase difference between tissues and regions with deoxygenated blood which results in a cancellation of signal intensity (Tong et al. 2008). It is thought that these microhemorrhages represent hemorrhagic diffuse axonal injury (DAI) (Mechtler et al. 2014). Microhemorrhages appear as hypointensities on the image, and are not detectable using standard MRI and CT scans.

1.5.2.3 Perfusion Weighted Imaging

Perfusion weighted imaging (PWI) enables one to non-invasively measure cerebral blood flow (CBF) and cerebral autoregulation post-injury. PWI can be achieved by using magnetically labelled blood as an endogenous contrast agent, which is termed arterial spin labelling (ASL). When using ASL, the blood is magnetically labelled, causing contrast changes and allowing for
the measurement of cerebral perfusion (Mechtler et al. 2014). PWI is becoming increasingly popular in the study of mTBI and is very likely to become a mainstay of imaging in mTBI.

1.5.2.4 Diffusion Weighted Imaging & Diffusion Tensor Imaging

Diffusion weighted imaging (DWI) is based on the diffusion properties of water molecules as they move through the brain tissue. DWI allows for the calculation of the apparent diffusion coefficient (ADC) which is a measure of the magnitude of diffusion of water molecules through a tissue. Water can either be freely flowing (thus having a high degree of diffusion), or be forced to flow in one direction (low degree of diffusion). The image signal in DWI is mediated by membranes and other barriers (Alexander et al. 2011). The magnetic field gradients are applied sequentially, and any protons which have moved in this time interval will experience a different magnetic field. These spins will not get rephased and therefore will cause a loss in MRI signal (Erokwu et al. 2011). This loss is quantifiable and can be directly related to the ADC of the tissue. Areas where the water is free to move in all directions (such as CSF) appear hypointense with high ADC values, whereas regions where the water molecules are restricted in their diffusion appear hyperintense and have low ADC values (such as in grey and white matter). DWI is able to show DAI, as areas with acute cell death are restricted in their diffusion (Mechtler et al. 2014).

Diffusion tensor imaging (DTI) is similar to DWI as it measures the diffusion of water; however, instead of primarily measuring ADC it allows for the visualization, orientation, and calculation of anisotropy of white matter tracts (Mechtler et al. 2014). Anisotropy provides information about the direction of movement of water models. It is highly sensitive in studying axonal microstructure. There are different means of quantifying DTI data, including FA, medial...
diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). These values provide information on the direction of diffusion of water molecules, the average molecular displacement by diffusion, and information regarding white matter (WM), specifically in respect to axonal injury and demyelination (Mechtler et al. 2014; Alexander et al. 2011). Additionally, DTI allows for the generation of fibre tractography, which allows for the visualization of the WM tracts.

1.5.2.5 Functional Magnetic Resonance Imaging

It is possible to non-invasively measure brain function using functional MRI (fMRI). This is achieved by measuring the changes in oxygenation state of hemoglobin (Mechtler et al. 2014). This is inferred from the blood-oxygen-level-dependent (BOLD) signal. When regions of the brain are activated, they experience an increase in cerebral blood flow (CBF). This results in an increase in oxyhemoglobin in the capillaries, shifting the relationship between oxyhemoglobin and deoxyhemoglobin. The resulting decrease in deoxyhemoglobin results in an increase in the BOLD signal (Mechtler et al. 2014). fMRI can be done either at resting state, or during a task.

1.5.2.6 Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a non-invasive technique for investigating the biochemical characteristics of brain tissue (Gujar et al. 2005). MRS can provide information about neuronal integrity, cellular metabolism, membrane turnover, and anaerobic metabolism as well as provide information about neurotransmitters such as glucose-glutamine (glx), GABA, and glycine (Mechtler et al. 2014). MRS relies on using nuclei, such as carbon 13, nitrogen 15, fluorine 19, sodium 23, phosphorous 31, and hydrogen 1 to provide information on metabolites. Of these nuclei, hydrogen is the most commonly measured as it is the most abundant in the tissue (Bertholdo et al. 2013). The signals from the chosen nuclei present as peaks on a spectrum. The
height and width of the peak indicates the concentrations of that specific nuclei in a variety of metabolites, allowing for specific identification of a metabolite (Burtscher & Holtas 2001).

Phosphorus 31 (\(^{31}\)P) MRS provides valuable information on the state of metabolic energy in the brain, based on its ability to measure adenosine triphosphate (ATP) and phosphocreatine (PCr) (Burtscher & Holtas 2001). Phosphorus MRS has been used in the study of brain injury, primarily investigating TBI. This technique primarily measures intracellular free magnesium (Mg\(^{2+}\)) and the bioenergetic state of the brain. Magnesium is used in a number of metabolic reactions, including glycolysis, oxidative and substrate phosphorylation, protein synthesis, and phospholipid synthesis, making it a valuable measure of the metabolic state of the brain (Vink et al. 1988a).

Hydrogen 1 (\(^{1}\)H) is far more abundant in the brain than any other nuclei, including \(^{31}\)P. This high natural abundance results in a stronger signal, and better spatial resolution than any other nuclei (Sappey-Marinier et al. 1992). Proton MRS is able to look at markers of neuronal function, myelin, cell membranes, and metabolic active compounds, making it a valuable tool in studying cerebral pathophysiology (Burtscher & Holtas 2001). Typical metabolites measured by proton MRS include: N-acetylaspartate (NAA), creatine (Cr), myoinosital (Ins), choline (Cho), lactate (Lac), and some neurotransmitters such as glutamate (Glu) (Mechtler et al. 2014). Each metabolite is a useful indicator of cellular integrity and function. Measurements are taken either examining a single metabolite, or measuring a ratio of metabolites. Cr is often used as a denominator in MRS measurements, as it is assumed that it remains constant in healthy and disease states; however, recent studies have started to question the validity of this assumption (Li et al. 2003).
1.6 Pathophysiology with an Emphasis on MR Related Data

mTBI is a heterogeneous injury, with factors such as age, premorbid conditions, and sex affecting the outcome. For many years mTBI was considered a harmless injury, however, recent research has found strong evidence for dysfunction in cellular metabolism, axonal damage and dysfunction, and alterations in the cerebral vascular system.

1.6.1 Parenchymal Changes

Typically, given the subtle nature of the injury, patients with mTBI do not present with parenchymal damage on standard clinical MRI sequences such as T1 and T2. Patients with mTBI appear normal on T2-weighted images and do not exhibit any structural abnormalities or blood brain barrier disruptions on T1-weighted images (Zhou et al. 2013). Patients with moderate-to-severe TBI can show visible lesions on T2-weighted images, which tend to correspond with poorer outcomes (Galloway et al. 2008). While useful in the study of moderate and severe TBI, pathology related to mTBI is not visible on T1 and T2-weighted images, and thus the usefulness these scans is limited when studying mTBI. Animals models that investigate mTBI using controlled cortical impact (CCI) and fluid percussion injury (FPI) techniques, which involve direct impact to the brain resulting in focal damage, show obvious pathology on T1 and T2-weighted sequences, as well as altered T1 and T2 values at the site of injury and in the peri-lesional area (Huang et al. 2013; Henninger et al. 2005, 2007; Higashi et al. 2014). Focal injuries result in disruptions in blood brain barrier permeability as detected by T1-weighted imaging at the site of the contusion and in the peri-lesional area (Schneider et al. 2002). In closed-head models of mTBI, where the impact is applied to the animals head, rather than the brain, no changes are demonstrated in measurements of T2 or T1, acutely or chronically, post-injury
which supports the validation of these models as better approximations of human mTBI (Henninger et al. 2005, 2007; Higashi et al. 2014).

Susceptibility weighted imaging enables the investigation of microhemorrhages in the brain. Although microhemorrhages are more commonly seen in TBI patients than in mTBI patients, Beauchamp et al. 2013 found that 19% of pediatric mTBI patients, who had normal clinical MRI and CT scans, exhibited microhemorrhages which were detectable by SWI. The lesions were mostly seen in the frontal lobes, however, lesions were also seen in the temporal, parietal and occipital lobes. Interestingly, the number of lesions corresponded with poorer outcomes in these patients (Beauchamp et al. 2013).

A study investigating repetitive mTBI on a CCI rat model found that, after a single hit, there were no SWI lesions; however, rats which received a second hit did show visible hemorrhages around the injury site 24 hours post-hit which persisted for 14 days after the second injury (Huang et al. 2013). SWI is a useful sequence which is capable of providing evidence for hemorrhagic DAI, and can possibly help aid in determining injury severity.

1.6.2 Cellular metabolism

Immediately post-injury, a cascade of cellular events occur. Initially, due to shear and strain stresses on the brain, there is an unregulated flux of ions through the ion channels resulting in transient membrane dysfunction (Farkas et al. 2006). This uncontrolled flux of ions can result in an indiscriminate release of excitatory neurotransmitters. In order to restore homeostasis, the brain must increase the activity of Na+/K+ pumps, depleting energy stores which likely results in cerebral hypofunction (Kawamata et al. 1992; Yoshino et al. 1991). Reductions in ATP and
adenosine diphosphate (ADP) have been observed 24h post-injury in humans (Gujar et al. 2005; Burtscher & Holtas 2001). Despite this imbalance, after a single mTBI these results are transient and will recover to normal function.

1.6.2.1 Magnesium and pH

The metabolic state of the brain can be measured using $^{31}$P MRS through the measurement of magnesium (Mg2+) levels. A pig model of diffuse brain injury found a significant acute loss of intracellular Mg2+, which took 7 days to resolve. However, the study found no changes in pH, phosphocreatine, inorganic phosphate, ATP, and lactate, as well as no abnormalities on standard MRI sequences (Smith et al. 1998). Other models of TBI have also shown decreases in free Mg2+, cytosolic phosphorylation ratio and an increased rate of mitochondrial oxidative phosphorylation, although there was no change in pH (Heath & Vink 1995; Vink et al. 1988a). Examination of rats subjected to mild, moderate, and severe fluid percussion injury found that all levels of injury had decreases in intracellular Mg2+ (Vink et al. 1988b). McIntosh et al. (1987) found that in animals subjected to mild, moderate, and severe injury, animals in the moderate and severe groups exhibited a decrease in intracellular pH which returned to baseline after 90 mins. Transient increases in lactate were significantly correlated with the decrease in pH. Study of lateralized TBI in rats found that in the acute stage there was a decrease in the ratio of phosphocreatine to inorganic phosphate (PCr/Pi), which recovered after 40 minutes, then declined for a second time at 2 hours. The second decline was associated with a drop in tissue pH, although there was no change in ATP (Vink et al. 1987). Despite the wealth of information provided by $^{31}$P MRS, it has yet to be widely applied to the study of mTBI; however, it shows promise in assessing the bioenergetic state of the brain post-mTBI.
1.6.2.2 *N*-acetylaspartate

*N*-acetylaspartate (NAA) is an important marker which provides information on myelin repair, neuronal loss, and metabolic depression. NAA is fairly homogenously distributed throughout the brain and is primarily found in mature neuronal cell bodies and axons (Pouwels and Frahm 1998; Rudkin and Arnold 1999). In addition to its predominance in neurons and their processes, NAA is also synthesized in mitochondria, which allows for the assessment of neural metabolism (Moffett et al. 2007). For these reasons, it is a valuable marker of brain pathology, providing information on neuronal loss or damage and neural metabolism. Numerous studies examining cerebral NAA after pathology have contributed to the determination of the role that NAA plays in the brain. While decreases in NAA are seen in many diseases that affect the brain, such as multiple sclerosis (MS), stroke, hypoxia, brain tumours, dementia, and TBI (Arnold 1999; Igarashi et al. 2015; Brandao & Caires 2013; Shiroishi et al. 2015; Rose et al. 1999; Wang et al. 2009; Shannon et al. 2015), increases in NAA have also been observed, in both developing infants and after axonal recovery (Kreis et al. 1993; Jackson & Connelly 1999).

Animal models have found fairly consistent decreases in NAA post-TBI and mTBI. In both rat and pig mTBI and TBI studies, using a model that induces a focal injury, it has been found that NAA decreases ipsilateral to the injury at 24h and can persist for a week (Signoretti et al. 2010; Smith et al. 1998). Often decreases in NAA/Cr are observed after injury in both TBI and mTBI studies in rat and pig models. This drop occurs as early as 1h post-injury, and can persist for a week (Xu et al. 2011; Cecil et al. 1998). Not only can these changes be seen in the acute and subacute phase of injury, but there is evidence that they can last into the chronic stages. Five months post-TBI, it was found that levels of the neurotransmitter *N*-acetylaspartylglutamate (NAAG), which has been shown to suppress excitotoxicity, regulate
long-term potentiation and depression, and inhibit synaptic release of GABA, were decreased. In addition, levels of NAAG+NAA continued to be decreased over this time period as well (Neale et al. 2007; Immonen et al. 2009). Other animal studies have consistently found decreases in NAA post-mTBI and TBI (McAllister et al. 2001). Longitudinal studies have found prolonged decreases in NAA which is suggestive of cellular damage in the brain (Immonen et al. 2009).

Human studies have also found NAA to be reduced post-mTBI in both children and adults, both in acute and chronic stages (Cohen et al. 2007; Johnson et al. 2012a,b; Henry et al. 2010; Babikian et al. 2006; McAllister et al. 2001). Ratios of NAA/Cr have also been found to be altered acutely and subacutely post-mTBI (Henry et al. 2011; Vagnozzi et al. 2010; McAllister et al. 2001). Decreases in NAA and NAA/Cr have shown to be significantly correlated with decreases in cognitive performance post-TBI in children shortly after injury (Babikian et al. 2006). Conversely, another study examining NAA concentrations in humans within 2 weeks of their mTBI found no significant difference of NAA concentration in WM or grey matter (GM) between mTBI patients and age and gender matched healthy controls (Gasparovic et al. 2009).

1.6.2.3 Creatine

Creatine (Cr) is a marker of brain energy metabolism. Cr concentration is homogenous throughout the brain and is relatively resistant to change (Burtscher & Holtas 2001). However, there can be alterations in Cr, along with other metabolites, during disease. Alterations in Cr have been seen in TBI, hypoxia, MS, and tumours, challenging the notion that Cr levels are resistant to change (Garnett et al. 2000; Inglese et al. 2003; Hattingen et al. 2008; Guzman-De-Villoria et al. 2014). Cr is increased in hypometabolic states and decreased in hypermetabolic
states (Castillo et al. 1996). Cr is rarely measured alone and is typically measured as a ratio with NAA.

Despite the claim that Cr levels are relatively resistant to change, there is evidence for altered Cr levels post-mTBI. A study by Signoretti et al. (2010) found that after a weight-drop injury, rats exhibited a decline in Cr, which reached minimum values at 24h; however, Cr levels recovered to baseline after 5 days. The same study found similar results when measuring creatine phosphate (CrP) and NAA, with minimum levels seen at 24h and a full recovery by 5 days.

Reductions in Cr have been observed in the dorsolateral prefrontal cortex of adults with post-concussion syndrome 1+ years after mTBI (Dean et al. 2015). In contrast, mTBI patients imaged within 2 weeks after the injury were shown to have increases in Cr in the white matter and splenium, which was surprising as it was hypothesized that Cr would be reduced in the brain after injury (Gasparovic et al. 2009).

1.6.2.4 Choline

Choline (Cho) is a precursor to phosphatidylcholine which is a constituent of the cell membrane, and is therefore considered to be a marker of cell turnover (Miller 1991). It is also a precursor for acetylcholine which is a neurotransmitter involved in memory, cognition, and mood (Castillo et al. 1996). Choline levels are altered in various disease states. In pathologies that involve demyelination, choline levels increase due to the liberation of membrane phospholipids (Matthews et al. 1991). In healthy individuals, choline is generally not visible on MR due to its storage in cell membranes and myelin; however, it becomes visible in pathological situations due to breakdown of these structures (Burtscher & Holtas 2001).
Abnormalities in Cho levels post-mTBI have been observed in humans. In the thalamus, it was found that there were decreases in Cho/Cr in the subacute stages of injury, approximately 1 month post-mTBI (George et al. 2014).

1.6.2.5 Lactate

Lactate (Lac) is the end product of anaerobic metabolism. It accumulates when the energy needs are too high for oxidative metabolism to fulfil. Elevations in lactate may also occur as a result of inflammation due to cellular infiltrates, such as infiltrating macrophages (Rudkin & Arnold 1999). Increased neuronal activity causes an increase in glycolysis, producing pyruvate, which is rapidly converted to lactate (Magistretti et al. 1999). Under normal conditions, lactate does not accumulate in significant concentrations and is therefore not detected using MRS (Soares & Law 2009). Lactate is seen when oxidative metabolism is unable to meet energy demands (Burtscher and Holtas 2001). Increases in lactate are seen in conditions such as status epilepticus, TBI, tumours, mitochondrial disease, hypoxia, and ischemia (Canas et al. 2010; Lama et al. 2014; Garber 2004; Sijens et al. 1996; De Stephano et al. 1995; McKenna et al. 2015).

Lactate levels are not often studied in mTBI; however, 2 animal studies have shown that mTBI rats exhibit an increase in lactate concentration immediately post-injury, with Lac levels returning to baseline 20-45 minutes after the injury (Kawamata et al. 1992; Cohen et al. 1991). This is likely a result of the hyperactivity in the brain seen immediately post-mTBI due to mitochondrial dysfunction (Barkhoudarian et al. 2011).
1.6.2.6 *Glutamate and Glutamine*

Deformation of the membrane causes an indiscriminate release of glutamate post-mTBI (Barkhoudarian et al. 2011). Glutamate, the primary excitatory neurotransmitter in the brain, binds to N-methyl-D-aspartate (NMDA) and D-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Kierans et al. 2014; Barkhoudarian et al. 2011). Typically, in spectroscopy studies glutamate is measured in conjunction with glutamine due to spectral overlap, represented as Glx (Burtscher and Holtas 2001; Gardner et al. 2014). Glutamine is a precursor and storage form of glutamate and is located inside astrocytes (Govindaraju et al. 2000).

Glutamate has been associated with post-traumatic excitotoxicity in both mTBI and TBI. Glutamate (Glu) is reduced in mTBI patients, with decreases in the Glu/Cr ratio being observed within 6 days post-injury (Henry et al. 2010, 2011). Kierans et al. 2014 found adult patients had an increase in Glx/Cr in the putamen 3-55 days post-mTBI, providing support for the concept that there is an excitatory response post-mTBI. However, since Glx is a combination of glutamate and glutamine it is difficult to interpret the Glx/Cr results in relation to neuronal excitotoxicity (Kierans et al. 2014).

1.6.2.7 *Myoinositol*

Myoinositol (mI) is an osmolyte and an astrocyte marker and increases in mI are attributed to astrocytosis. In humans, mI levels have been shown to be increased 3-55 days post-mTBI in the grey matter, as well as mI/Cr levels (Kierans et al. 2014).
1.6.3 White Matter Abnormalities

It has been shown that mTBI causes significant axonal stretching and shearing, resulting in diffuse axonal injury (DAI) in the brain. DAI induces altered axonal transport, disruptions in axonal cytoskeleton, and axonal swelling (Toledo et al. 2012).

The use of unconventional MRI sequences has begun to reveal white matter abnormalities after mTBI in both humans and animals. Diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI) have proven to be particularly useful in the study of white matter injury. Studies employing DTI have consistently found reductions in structural integrity in a variety of areas including the genu, body and splenium of the corpus callosum, the anterior and posterior capsule, superior longitudinal fasciculus, superior fronto-occipital fasciculus, and centrum semiovale in both adults and children post-mTBI (Veeramuthu et al. 2015; Dean et al. 2015; Keightley et al. 2014a). The majority of studies find a decrease in fractional anisotropy (FA) and an increase in mean diffusivity (MD) and radial diffusivity (RD) (Xiong et al. 2014; Fakhran et al. 2014). The largest decrease in FA in humans is seen in the subacute phase of mTBI in the superior longitudinal and fronto-occipital fasciculus, and the splenium, with slow recovery in the chronic phase (Messe et al. 2012).

Studies in adults have found that MD, which is an indicator of edema and necrosis, is reduced after mTBI in several locations including, the internal and external capsule, the corona radiata, the fronto-occipital and inferior fasciculus, and the corpus callosum (CC) (Veeramuthu et al. 2015; Narayana et al. 2015). Similarly, children with TBI have shown decreases in membrane density in the corpus callosum (Keightley et al. 2014a). A study by Van Beek et al. (2015) found that pediatric mTBI patients showed decreased MD values and lower RD values in
the CC genu, and increased FA values in the CC splenium compared to control children 30 days or less post-mTBI. Voxelwise analysis of DTI has provided evidence of a widespread increase in FA through brain white matter tracts in children even up to 5 months post-mTBI (Mayer et al. 2012). Despite these findings, there is evidence that directly contradicts these studies. Grossman et al. (2013) found that adults, 1 month post-injury, had regionally decreased FA and mean kurtosis and increased MD in the thalamus, the internal and external capsule, the corpus callosum, cingulum, optic radiations, centrum semiovale, total deep GM, and total WM. These studies in both children and adults highlight the vulnerability of WM to stretching and shearing forces. Some studies suggest that the reduced structural integrity correlates with poorer functional performance in attention and working memory areas (Dean et al. 2015; Veeramuthu et al. 2015).

ADC values show a decrease in diffusivity in human mTBI patients in the cingulum bundles and both the left and right dorsolateral prefrontal cortex (Wu et al. 2010; Zhang et al. 2010). However, some studies report no significant changes in ADC post-mTBI in adolescents and adults (Chu et al. 2010; Chen et al. 2012).

Animal models of mTBI have also revealed disruptions in tissue microstructure post-injury. These disruptions are seen acutely and can persist 30 days post-injury or longer (Henninger et al. 2007; Bennett et al. 2012; Rubovitch et al. 2011). In mouse mTBI models, it has been found that AD, which is associated with axonal degradation, is lowered post-injury and persists for 7 or more days in the corpus callosum and external capsule (Bennett et al. 2012). Additionally, the same study found that MD was normal at 24 hours post-mTBI, but decreased by day 7 in the corpus callosum and external capsule (EC). In the ipsilateral cortex, MD was decreased 24 hours post-injury but normalized by day 7 (Bennett et al. 2012). Rats typically
show a decrease in FA acutely post-injury (Hylin et al. 2013). In a study of closed-head mTBI in a rat model, Henninger et al. (2007) found a significant increase in ADC both at 1 and 7 days post-mTBI, despite normal appearing DWI images. Blast-induced mTBI has been studied in a mouse model. It was found that there was a decrease in RD, which persisted for 1 month post-injury (Rubovitch et al. 2011). Evidence for progressive axonal injury has also been found. A mouse model of mTBI, which used a fluid percussion injury (FPI) to generate a focal insult, found axonal swelling and axotomy in the neocortex 15 minutes post-FPI. At 12 hours, there was an increase in the number of swollen axons and an increase in the number of axotomized neurons, suggesting progressive axonal injury after mTBI (Greer et al. 2013).

Measurements attained using DTI vary greatly amongst studies, likely due to the mechanism of injury. Despite these seemingly contradictory findings, there is strong evidence that WM is disrupted post-mTBI both acutely and chronically. Most studies using DWI and DTI have found alterations in WM integrity. This indicates that there is microstructural damage post-mTBI and suggests that DWI and DTI are useful MR sequences for examination of mTBI.

1.6.4 Functional Changes

Functional MRI provides a means of looking for functional changes following mTBI. Patients who have sustained a mTBI show both regions of increased activity, and regions of reduced activity. Regions of increased activity include the cerebellum, insula, and the parietal lobe. Regions with reduced activity included the frontal lobe, anterior cingulate, and the temporal lobe (Eierud et al. 2014). Despite these abnormalities in functional activity, adult patients with mTBI do not perform differently than controls while doing cognitive tasks as early as 72 hours
post-injury to 2 months after injury (Chen et al. 2004; Dettwiler et al. 2014; Jantzen et al. 2004). This is likely because the brain is engaging compensatory mechanisms to maintain the same level of performance (Chen et al. 2004). Interestingly, a similar study performed in children found that children with mTBI had reduced brain activation and reduced working memory accuracy, suggesting that they are unable to recruit compensatory mechanisms post-mTBI, or at least not as successful at compensation as adults (Keightley et al. 2014b). However, contrary to this finding, studies have shown alterations in activation in the absence of neuropsychological abnormalities in children and adolescents within 3 months of injury (Saluja et al. 2015; Krivitzky et al. 2011). Although it is not clear if children are unable to activate compensatory mechanisms, there is strong evidence for alterations in neural activation in both adults and children.

Similar to humans, animal models have shown alterations in functional activity after mTBI. Henninger et al. (2007) found that rats exhibited impairments in functional activation post closed-head mTBI. These rats were subjected to tasks, such as hypercapnia and forepaw stimulation, which cause changes in functional activation in normal animals. A normal response would result in an increase in BOLD; however, the mTBI rats had significant decreases in BOLD signal in various regions of interest (ROI), including the hippocampus and regions of the sensorimotor network. A reduced BOLD response was also seen after forepaw stimulation; however, this response normalized within 7 days of the injury (Henninger et al. 2007). Despite the lack of literature on animal mTBI fMRI, it appears that there are changes in brain function post-mTBI.
1.6.5 Cerebrovascular dysregulation

Cerebrovascular dysregulation has been observed after mTBI in both animal models and human patients. There is considerable evidence suggesting that cerebrovascular reactivity and cerebrovascular autoregulation are impaired after mTBI (Len & Neary 2011).

1.6.5.1 Cerebral Blood Flow

Maintaining a consistent cerebral blood flow (CBF) is critical. A lack of blood flow in the brain can cause irreversible brain damage in only 4-5 minutes (Golding et al. 1999a). Neurons rely almost solely on aerobic metabolism and thus require a constant supply of blood. Interruption of blood flow can cause areas of the brain to have energy needs that exceed the available energy supply. Alterations in CBF could also indicate altered metabolism in the brain due to changes in function (Doshi et al. 2015). CBF regulation is very complex. Two contributors to CBF which are implicated in mTBI are cerebral perfusion pressure (CPP) and cerebrovascular reactivity. These mechanisms work together to maintain a consistent CBF. Cerebral perfusion pressure is a net pressure gradient which causes cerebral flood flow to the brain and can be defined as the difference between arterial and venous pressure (Len & Neary 2011). Using transcranial Doppler techniques, it has been shown that healthy children have a higher basal CBF than adults, peaking at 5-6 years (Bode & Wais 1988).

There have been a number of studies published which have found impairments in CBF post-mTBI. Impairments in CBF post-mTBI affects the supply of energy to neurons, which can result in brain energy demand exceeding supply. It is possible that this could contribute to some of the symptomology post-mTBI (Giza & Hovda 2001; Leddy et al. 2007). Adult athletes have been found to have reduced CBF 3-6 days post-mTBI (Militana et al. 2015). A separate study
found that college football players had decreased CBF in the acute phase post-injury (1 day); however, CBF recovered by 1 month (Meier et al. 2015). Becelewski & Pierzchala (2003) measured CBF in the middle cerebral artery using transcranial Doppler sonography and found children to have an acute increase in CBF immediately following mTBI, with a subsequent decrease to below baseline levels. In contrast, the same study found that adults suffered an immediate decrease in CBF after injury (Becelewski & Pierzchala 2003). Other studies have reported a decrease in CBF in the thalamus immediately post-mTBI in children, which can persist even after symptom resolution (Maugans et al. 2012). Children have also been found to have regional decreases in CBF in the bilateral frontotemporal regions after injury (Wang et al. 2015). Examining adults with mTBI, Grossman et al. (2013) found decreases in the thalamus post-mTBI both acutely and after follow-up visits. In contrast, Doshi et al. (2015) found that patients imaged <48 hours post-injury had increases in regional CBF in the caudate, putamen, pallidum, and the frontal and occipital lobes, which contradicts most other studies. Animal models have provided evidence for decreased CBF post-mTBI. CBF has been found to be decreased immediately post-mTBI and persist for more than 20 days after injury in both a focal mTBI and closed-head mTBI (Golding et al. 1999b; Henninger et al. 2007).

Most studies using PWI have found regional decreases in CBF (Wang et al. 2015; Militana et al. 2015; Becelewski & Pierzchala 2003; Maugans et al. 2012). However, a study by Doshi et al. (2015) has provided evidence to the contrary, finding that mTBI patients had regional increases in CBF post-mTBI. Animal studies using PWI have found cerebrovascular dysregulation both acutely and chronically post-mTBI. PWI detected hypoperfusion in rats after a mild closed-head injury, despite normal appearing T2 and DWI scans (Henninger et al. 2007).
Due to conflicting evidence, it is still controversial whether or not alterations to CBF occur post-mTBI.

1.6.5.2 Cerebral autoregulation

Cerebral autoregulation is the ability of the brain to maintain a relatively constant cerebral blood flow (CBF) despite changes in blood pressure (Paulson et al. 1990). It is very important for the body to maintain constant CBF across various physiological conditions. Typically, cerebral autoregulation is most active when there is a change in arterial blood pressure or changes in cerebral perfusion pressure. Cerebral autoregulation is influenced by arterial blood pressure. When there is a drop in the arterial blood pressure, the blood vessels in the brain will react by dilating the cerebral arteriolar and capillary beds. Conversely, the vasculature in the brain reacts to an increase in arterial blood pressure by constricting the cerebral arteriolar and capillary beds. This allows the brain to maintain a constant CBF. Cerebral autoregulation can reach a limit where it can no longer act. In this case, there is no regulation of CBF and any increases in CPP will likely result in an increase in CBF, and visa versa. It is possible that post-TBI, patients are less able to respond to changes in CPP, which could then lead to ischemic injury (Lewelt et al. 1980). Interestingly, it has been suggested that cardiac output can influence CBF and thus cerebral autoregulation (Van Lieshout & Secher 1985). There is preliminary evidence which suggests the cardiovascular system is also affected by mTBI. Recently concussed athletes have been shown to have an increased heart rate after exercise, which can affect cardiac output, compared to control subjects, when tested <2 days after injury. This could suggest the presence of dysfunction between the nervous system and cardiovascular system post-mTBI (Gall et al. 2004). This has also been seen in patients post-TBI (King et al. 1997).
Cerebral autoregulation can be vulnerable to traumatic injury as it has been found that traumatic injuries to the head can cause significant changes in intracranial blood pressure (Len & Neary 2011). It is established that after TBI patients experience impaired, or a complete loss of, cerebral autoregulation (Len & Neary 2011). Recent studies of mTBI have started to build evidence for impairments in cerebral autoregulation. A case study of one patient with mTBI found a complete lack of cerebral autoregulation. When challenged by an increase in arterial blood pressure, the patient’s CBF increased in a pressure-passive manner (Strebel et al. 1997). Another study found that 49 hours after injury, mTBI patients who underwent an experimental transient decrease in arterial blood pressure caused by the inflation and subsequent deflation of blood pressure cuffs around the thighs, were unable to maintain a constant level of cerebral blood flow during this challenge, unlike control subjects, suggesting impairments in the cerebral autoregulation (Junger et al. 1997). Examination of cerebral autoregulation post diffuse mTBI in rats found that the rats had impaired cerebral autoregulation 4 hours after the injury (Prat et al. 1998)

1.6.5.3 Cerebrovascular Reactivity

Cerebrovascular reactivity (CVR) is a compensatory mechanism which dilates cerebral blood vessels in response to hypercapnia in order to maintain homeostasis (Svaldi 2015). The body is highly sensitive to changes in CO$_2$, which is a potent vasodilator with even a 1 mmHg increase in the partial pressure of CO$_2$ resulting in a 5% increase in CBF. The body reacts quickly to changes in the partial pressure of CO$_2$ (Len & Neary 2011). Given the sensitivity of the cerebrovascular system to CO$_2$, it has been suggested that cerebrovascular responses to CO$_2$ are greater than cerebral autoregulatory responses to CPP (Len & Neary 2011). Cerebrovascular reactivity and cerebral autoregulation are independent processes and do not correlate with each
other (Len & Neary 2011). Decreases in CVR have been seen in younger people (<30 years of age) post-mTBI; however, interestingly in an older population (>30 years) there was no change post-mTBI (Becelewski & Pierzchala 2003). In asymptomatic adolescent football players, it was found that CVR decreased in comparison to baseline within the first 6 weeks of football season (Svaldi et al. 2015). Often impairments in CVR are not seen unless the patient is subjected to a hyper- or hypocapnic challenge. Adult athletes were found to have significant impairment in CVR after hyper- and hypocapnic challenges one week or less following mTBI (Len and Neary 2011; Gardner et al. 2014).

Investigation of cerebrovascular reactivity using fMRI in a rat model of mTBI found that there was a blunted response to both hyper- and hypocapnia post-mTBI (Henninger et al. 2007). This decrease in CVR was transient and did recover over time. Response recovery time was region specific, with the primary somatosensory cortex recovering on day 1, and the thalamus recovering by day 21 (Henninger et al. 2007). CVR is often impaired in mTBI patients and it has been suggested that it could be a valuable biomarker of mTBI in the acute phase of the injury (Chan et al. 2015).

1.6.5.4 Venous System

The study of venous involvement post-mTBI has largely been neglected. To our knowledge, there are only two studies examining venous involvement post-mTBI. The internal jugular veins (IJVs) are the main cerebrovenous drainage system in the supine position, whereas
in the upright position, the secondary draining veins (vertebral venous system) are predominant (Valdueza 2000). Pomschar et al. (2013) used MR venography (MRV) to examine the effects of mTBI on the cerebral venous system. They found decreased cerebrovenous flow through the IJVs post-mTBI in the supine position in conjunction with an increase in venous drainage through the secondary draining veins (Fig. 1.6-1). This was seen in asymptomatic patients who sustained a mTBI months to years prior to this study. One other study has examined the venous system using susceptibility-weighted imaging. This study demonstrated decreased susceptibility values in mTBI patients in the venous system, which involved the left thalamostriate vein and right basal vein of Rosenthal, within 48 hours of the injury suggesting increased venous oxygenation (Doshi et al. 2015). The authors suggest that this could be due to either a reduction in oxygen absorption into the tissue, or a reduction in oxygen demand. Despite a paucity of information regarding cerebrovenous involvement post-mTBI, these preliminary studies suggest that there could be cerebrovenous dysregulation after injury, emphasizing the need for further studies in this area.

Figure 1.6-1. MRV images of control patient and mTBI patient showing a significant increase in venous outflow through secondary vessels (Pomschar et al. 2013).
1.7 Animal Models

There are several animal models of mTBI. Although each model has individual strengths and weaknesses, some animal models are better able to represent human mTBI. Below are the three most common animals models used in the study of mTBI.

1.7.1 Fluid Percussion Injury

Fluid percussion injury (FPI) requires surgical intervention. The injury is achieved by the injection of fluid directly into the epidural space (Bolouri et al. 2008). It produces a brain injury through direct insult to the brain and as a result this method causes significant focal damage and is often accompanied by subdural-, subarachnoid-, and/or intracerebral hemorrhages (Cernak et al. 2004). The insult can be applied in either be dorsal or lateral ventral planes.

1.7.2 Controlled Cortical Impact

The controlled cortical impact (CCI) model is similar to the FPI in that it typically involves a craniotomy and direct injury to the brain. The skull is removed and a metal piston strikes the dura of the brain. This causes a significant focal contusion, which is also visible on imaging and histology, which is not typical of what is seen in human mTBI (Dewitt et al. 2013).

Despite being straightforward, the FPI and CCI models are not ideal for modelling mTBI for several reasons. First, the injury results in a focal insult which is visible on both imaging and histology. In human mTBI, the injury is closed-head, resulting in diffuse injury with no focal damage. Second, these models typically involve the animal fixed in place in a stereotaxic frame which does not allow the animal’s body to move after impact. During human mTBI the entire body is affected by the impact, generating considerable biomechanical forces which are critical
in the development of mTBI. When seeking a good, translational animal model, it is important that the animals experience similar biomechanical forces to those occurring in human mTBI. In this case, since the FPI and CCI models are not closed-head injuries, nor do they incorporate biomechanical forces during the hit, they are not ideal models for studying mTBI.

1.7.3 Weight Drop Model

The weight-drop (WD) model could be considered the gold standard for studying closed-head mTBI. In this model a free falling weight is dropped through a column directly onto the animal’s head. Typically brass weights are used and the height of the drop and the weight are determined by species, age, and the desired severity of the injury. A couple of variations on this model exist, with the initial WD model developed by Marmarou et al. (1994). Metal caps were cemented directly onto the skull vault of the rat. The rats were placed on a foam bed with a known spring constant, and the weight was dropped through a column and would strike the metal cap. The cemented metal caps were used primarily for 2 reasons: to protect from lacerations and fractures, and to help to evenly disperse the impact energy over the surface of the brain (Marmarou et al. 1994; Cernak et al. 2004). The WD model generates graded DAI without significant focal lesions that more closely models the human injury (Cernak et al. 2004).

Fig. 1.7-1 Modified weight-drop model developed by Mychasiuk et al. 2014a.
Since its development, variations on the Marmarou weight-drop model have been created. Many studies forgo the skull cap and drop the weight directly onto the animal’s head. Using the skull cap requires additional intervention (surgery) which adds confounding factors. Another model has been developed which does not use the stereotaxic frame to hold the rat in place. Instead, the animal rests unrestricted on sheet of aluminium foil. Upon receiving the hit, the animal breaks through the aluminium foil, experiencing a 180° sagittal rotation, and lands on a foam pad (Fig. 1.7-1) (Mychasiuk et al. 2014a).

The WD model has significant advantages over both FPI and CCI models. Using this model, a closed-head injury is inflicted generating diffuse injury in the absence of focal damage. Additionally, further refinement of this model has resulted in the ability to recreate the rotational biomechanical forces which are experienced in human mTBI. For these reasons, when attempting to model a closed-head mTBI, the WD model is superior to both CCI and FPI and is more representative of the injury.

**Summary**

Mild traumatic brain injury is a subtle injury and, as a result, it can be difficult to identify and manage. This presents a challenge to healthcare providers as they must rely on subjective measures to identify the injury. The development of a biomarker would allow clinicians to objectively identify mTBI and determine which individuals might be at risk of developing long-term problems. This would also allow for the development of optimal management strategies, such as to determine when a child is safe to resume playing a sport. Ideally, a biomarker would be non-invasive and easy to identify. Seeking an imaging biomarker using MRI could be an effective and non-invasive way to measure mTBI objectively.
Chapter 2: Objectives

2.1 **Aim 1:** To determine if we can detect parenchymal changes in the brain using a 9.4T MRI after a modified closed-head impact in a juvenile rat model.

   2.1.1 **Hypothesis:** Standard imaging sequences will not reveal observable pathophysiological changes in this model in the acute stage (24 hours) post-mTBI.

2.2 **Aim 2:** To investigate pathophysiological vascular changes in the brain post-mTBI using a 9.4T MRI.

   2.2.1 **Hypothesis:** There will be a cerebrovascular pathphysiology detectable by MRI in the acute stage (24 hours) post-mTBI.
Chapter 3: Methodology

3.1 Study Design

Aim 1: We used male and female juvenile Sprague-Dawley rats which were either bred in house or ordered at P21 from Charles Rivers. The rats received a mTBI at P30. Immediately post-mTBI time to right was recorded. Twenty-four hours after the mTBI, the rats underwent a beam walking task and the number of hind foot slips were counted. Immediately after the behavioural testing was concluded, the animals were imaged using a 9.4T MRI in the Experimental Imaging Centre. To look for parenchymal changes, the following sequences were run: rapid acquisition with relaxation enhancement (RARE), spin echo, multi-gradient echo, and diffusion weighted imaging (DWI).

Aim 2: To investigate vascular changes post-mTBI, specific MRI sequences were run to measure CBF and to provide data on venous anatomy. The sequences used included: continuous arterial spin labeling (cASL), and fast imaging with steady state precession (FISP).

Immediately post-imaging, the rats were sacrificed and perfused. The brains were then removed and preserved in 10% formalin. All data were analyzed using ImageJ, SPIN, and the Bruker ParaVision software Version 4.0.
3.2 Animals

In house bred rats - The breeding protocol followed was similar to that of Mychasiuk et al. 2015b. Sprague-Dawley breeding pairs were ordered from Charles-Rivers. All animals were given ad-libitum access to food and water. Females were mated, pair-housed, and kept in a temperature controlled (21°C) animal facility. Animals were maintained on a 12:12 light:dark cycle. Each female was mated to a different male. The day prior to delivery, dams were removed to individual cages. The pups were weaned at P21. Pups used for this study were taken from different litters, with a maximum of 1 male and 1 female from each litter. All animals were kept in the animal care facility until P30 when the experiment took place.

Ordered rats - A subset of animals were ordered at P21 from Charles-Rivers. These animals were ordered as there was not enough time to follow the breeding and raising protocol implemented for the bred in house group. The animals were housed in pairs and kept in a temperature controlled (21°C) animal facility and were given ad-libitum access to food and water. Animals were maintained on a 12:12 light:dark cycle. The rats were kept in the animal house until the 9<sup>th</sup> day (P30) when they received the mTBI. The procedure was identical to the in house bred rats.

3.3 mTBI Procedure

Male and female juvenile rats (P30) underwent either a mild traumatic brain injury (mTBI) (n=16) using a modified weight-drop model or a sham injury (n=9), as described by Mychasiuk et al. (2014a). Rats were anesthetized using isoflurane (3-4% in 100% oxygen) until they were no longer responsive to a toe pinch. They were then quickly placed on a sheet of
aluminium foil, which was suspended 10 cm above a sponge cushion. To induce mTBI, a brass weight of 150g was dropped through a plexiglass tube from a height of 0.5m. The weight was tethered on knotless Nylon 3.6 angler fishing line to prevent re-hits. Upon impact, the aluminum foil broke causing the rat to undergo a 180° rotation in the sagittal plane, landing on the sponge cushion. Mild traumatic brain injury rats received topical application of lidocaine to the site of injury immediately post-hit. The rats were then immediately placed in a clean cage on a warming pad to recover. Sham rats received the same treatment; however, they did not receive the impact. In order to verify that the rats underwent a successful mTBI, time to right was recorded immediately post-hit or post-sham treatment and was measured until the animal moved from the supine to prone position.

3.4 Behavioural Testing

Beam walking

Twenty-four hours post-injury (P=31), a beam-walking task was performed using a tapered beam (165cm) that narrowed as the animal moved forward. The animal’s home cage was placed at the far end of the suspended beam as incentive. Animals were given one trial to learn the task, which was not recorded, then 4 subsequent trials which were carried out and video recorded. The video recordings were reviewed and number of hind foot slips counted. This task was used to assess motor impairment in the rats post-mTBI to verify injury.
3.5 MRI Acquisition

MR data were acquired using a 9.4T MRI Bruker Avance-console and a 35mm quadrature volume coil. Animals were imaged 24 hours to 30 hours post-mTBI. Rats were anesthetized using isoflurane (1.8-2.5% in 100% oxygen). Sequences used were: rapid acquisition with relaxation enhancement (RARE)(TE/TR: 16/4000 ms, RARE factor: 8, averages: 5, FOV: 25.6x25.6 mm, matrix: 256x256, voxel size: 0.1x0.1x1mm), spin echo (TE/TR: 6/3000 ms, averages: 4, FOV: 25.6x25.6 mm, matrix: 128x128, voxel size: 0.2x0.2x1mm), multi-gradient echo (TE/TR: 3/300 ms, echo spacing: 4, averages: 10, pulse angle: 30 degrees, FOV: 25.6x25.6, matrix: 128x128, voxel size: 0.2x0.2x1mm), continuous arterial spin labeling (cASL) (TE/TR: 2.66/3000 ms, averages: 16, RARE factor: 36, FOV: 30x30 mm, matrix: 128x128, voxel size: 0.23x0.23x1mm), fast imaging with steady precession (FISP) (TE/TR: 1.5/3 ms, averages: 8, pulse angle: 15 degrees, FOV: 30x30, matrix: 128x128, voxel size: 0.23x0.23x1 mm), DWI using the DtiEpi sequence (TR/TE: 5000/40 ms, averages: 4, directions: 1, b values: 82, 306, 675, 914, 1189, echo delay: 7.08 ms, FOV: 25.6x25.6, matrix: 128x128, voxel size: 0.2x0.2x1mm), and FLOWMAP (TR/TE: 20/6, averages: 16, FOV: 25.6x25.6, matrix: 512x512, zero fill factor: 1, voxel size: 0.05x0.05x1mm).

3.6 Data Analysis

MR data were analyzed using ImageJ and Bruker ParaVision software 4.0. Fig. 3.6-1. T2-weighted RARE anatomical image with ROIs labelled.
Regions of interest (ROIs) included: cortex, dorsal hippocampus, basal ganglia, and corpus callosum (Fig. 3.6-1).

3.6.1 Sinus Area

T2-weighted images acquired from the RARE sequences were used to calculate sinus area in left and right transverse sinuses as well as in the superior sagittal sinus. Images were thresholded using the automatic threshold function on ImageJ, generating a black and white image. Prior to the area calculation of the sinuses, it was necessary to set the scale of the image. Once the scale was set, the area was calculated using the Analyze Particles function. ROIs were drawn by hand on each sinus, encompassing the entire area of the sinus. The area measurement was generated in mm². Areas from 3 consecutive slices at approximately bregma 3.8, -4.8 and -5.8 were calculated and averaged.

3.6.2 T2/T2* & R2/R2*

T2 was calculated using a Carr-Purcell-Meiboom-Gill (CPMG) spin echo sequence. Images were opened using ImageJ and a T2 map was generated using the MRI Analysis Calculator which was a downloaded plugin for the program. In order to generate the T2 map, the program required all TE values (in seconds) from the sequence to be entered. Once the T2 map was generated, ROIs were drawn on a single slice and T2 values were obtained.

Fig. 3.6-2. Exponential T2 decay curve for the left basal ganglia.
Multicomponent T2 measurements were carried out using MatLab. Images were opened on MatLab and ROIs drawn. The T2 decay curve (example: Fig. 3.6-2) was acquired for each ROI and was fit using a non-negative least-squares (NNLS) algorithm to calculate the T2. T2 values were separated into 3 bins which are as follows: 6-20 ms, 20-200ms, and 200-2000 ms. Separating the T2 values into 3 separate bins provides information on multiple T2 components, short, medium, and long. This allows for the differentiation of different tissues within the ROI, as different tissues have different relaxation times. This method is superior to generating a T2 map as it breaks the ROI into specific components, rather than generating an average T2 for the ROI.

T2* measurements were obtained using a multi-gradient echo sequence. ROIs were drawn and signal intensities were acquired for each echo time using ImageJ. These values were then entered into Sigma Plot and plotted against the echo time. A single exponential non-linear regression was carried out and T2* values obtained. The equation was used to calculate R2*.

\[ \frac{1}{T2^*} = \frac{1}{R2^*} \]

Where SI(t) is the signal intensity of the multiple TE data and t is the echo time.

3.6.3 T1

T1 values were obtained from the FISP sequence. These files were opened on the Bruker ParaVision software. ROIs were drawn. An exponential growth curve was generated and then, using the Image Sequence Analysis function on the ParaVision software, T1 inversion recovery values were calculated in milliseconds. These values were then incorporated into the CBF calculation.
3.6.4 CBF

Continuous arterial spin labeling (cASL) was used as a measure of CBF using the following perfusion equation [where \( f \) = tissue perfusion rate in mL/100g/min, \( \lambda \) = the blood-brain partition coefficient, assumed to be 0.9, \( T1_o \) = the measured T1 in the presence of flow (s), \( \delta \) = a constant value of 0.039s\(^{-1}\), \( Mbcon \) = intensity of control images (++)+(--). \( Mbinv \) = intensity of inverted images (+-)+(-+), \( \alpha \) = the spin-tagging efficiency (0.75)].

\[
f = \lambda \left( \frac{1}{T1_o} + \delta \right) \frac{Mbcon - Mbinv}{2 \alpha Mbcon} \times 6000
\]

Mbcon and Mbinv signal intensity values were obtained using the ImageJ plugin, Calculator Plus. Mb is the longitudinal magnetization of the water protons in the brain slice (Meng & Lei 2009). Using this plugin, the 2 sets of control images (+gradient +offset and –gradient –offset) were added together. The same was done with the inverted images. The images generated by the additions were then subtracted from each other, and that resulting image was divided by the added control image. Once this final image was generated, which was representative of the Mb fraction, ROIs were drawn and signal intensity obtained. Using excel, the values obtained were inserted into the equation along with the T1 values calculated from the FISP sequence and CBF was calculated. As all measurements were in milliseconds, it was necessary to multiply the resulting value by 6000 to get the value in mL/100g/min. The CBF was calculated to be mL/100g/min as that is currently the standard for reporting CBF (Sours et al. 2015).
3.6.5 DWI

A subset of animals underwent diffusion weighted imaging (DWI). Diffusion weighted images were opened in Bruker ParaVision software and ROIs drawn. Using the Image Sequence Analysis function, ADC was automatically calculated for each ROI.

3.6.6 SWI

Multi-gradient echo images were converted into DICOM files using SPIN. After the conversion, MIP/SWI processing was carried out. Maximum intensity projection (MIP) with phase multiplications was chosen (SWI processing). The magnitude and phase images were selected from the 3rd echo and processed to create an SWI image for visual analysis.

3.7 Statistics

A between group comparison was conducted between the control and mTBI groups for all measurements. This was achieved through the use of a t-test. Mean and standard deviation (SD) was calculated for each group. The bred in house rats and ordered rats were analysed separately. The rats were analysed separately as the groups were raised in different conditions and therefore could not be grouped together.

In this study groups were divided into 4 as animals were separated by sex as well as experimental condition.

A power analysis was conducted to determine the number (n) required for each group in order to achieve a power of 0.8, which represents the probability of detecting the true effect specified, in this case differences in sagittal sinus size. G*Power v. 3.9.1.2. was used to carry out
the power calculation. Effect size was determined between control and mTBI groups for the mean sagittal sinus size (+/- SD) and entered into the input parameters with alpha error probability set at 0.05, power set to 0.80, and an effect size of -1.23. Output parameters suggested sample sizes of 7 for each group, which would generate a power of 0.82.

Correlational analysis was conducted amongst the various parameters using SigmaPlot. Both the Pearson Product Moment and Spearman Rank Order correlational analyses were conducted, based on the distribution of the data. The Pearson Product Moment was used when the data were normally distributed, as in the case of the correlating the superior sagittal sinus size and time to right. In cases where the data were not normally distributed, the Spearman Rank Order analysis was used, as in the case of ADC vs. CBF, ADV vs. time to right, and CBF vs. time to right.
Chapter 4: Results

4.1 Individual rat information

Individual data for each rat used in the study is presented below. Animals 1 to 24 were bred in house while animals 25 to 32 were ordered from Charles Rivers. As there were 5 control rats and 12 mTBI rats in the bred in house group, the control group does not meet the desired n of 7. Similarly, both Charles Rivers rat groups were too small to meet the desired power (obtained 4 mTBI and 4 controls but aimed for 7 each). Table 4.1-1 provides information on the rats which were used in the study and Table 4.1-2 provides information on the rats which were excluded from the study, including reasons for exclusion.

Table 4.1-1. Details on each rat included in the analysis. Weight data for the first 10 rats was not available and is therefore marked N/A on the chart.

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<th>Injury Date</th>
<th>Imaging Date</th>
<th>Animal Number</th>
<th>Sex</th>
<th>Treatment</th>
<th>Weight</th>
<th>Time to right post-injury</th>
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<td>June 13/2013</td>
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<td>July 30/2013</td>
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<td>Time</td>
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<td>1m 14s</td>
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<td></td>
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<td>1m 3s</td>
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<td>0m 49s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Female</td>
<td>mTBI</td>
<td>126g</td>
<td>1m 1s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Female</td>
<td>Sham</td>
<td>125g</td>
<td>1m 9s</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.1- 2. Table listing the rats excluded from analysis.

<table>
<thead>
<tr>
<th>Injury Date</th>
<th>Animal Number</th>
<th>Sex</th>
<th>Treatment</th>
<th>Weight</th>
<th>Time to right post-injury</th>
<th>Reason for exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 12/2013</td>
<td>4</td>
<td>Female</td>
<td>Sham</td>
<td>N/A</td>
<td>1min 37sec</td>
<td>Highly abnormal scan (Fig. 4.1-1)</td>
</tr>
<tr>
<td>Feb 10/2014</td>
<td>F1</td>
<td>Female</td>
<td>Sham</td>
<td>78g</td>
<td>0m 55s</td>
<td>Not imaged: Ran out of time to image in the day.</td>
</tr>
<tr>
<td>Feb 11/2014</td>
<td>M1</td>
<td>Male</td>
<td>mTBI</td>
<td>96g</td>
<td>0m 32s</td>
<td>Not imaged: 9.4T MRI was broken.</td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>Male</td>
<td>Sham</td>
<td>97g</td>
<td>0m 43s</td>
<td>Not imaged: 9.4T MRI was broken.</td>
</tr>
<tr>
<td></td>
<td>M3</td>
<td>Male</td>
<td>Sham</td>
<td>91g</td>
<td>0m 42s</td>
<td>Not imaged: 9.4T MRI was broken.</td>
</tr>
<tr>
<td>Sept 8/2014</td>
<td>20</td>
<td>Male</td>
<td>Sham</td>
<td>94g</td>
<td>0m 34s</td>
<td>After perfusion, upon removal of the brain a large clot was found anterior to the cerebellum on the dorsal surface of the brain.</td>
</tr>
</tbody>
</table>
4.1.1 Bred in house rats

There was a significant difference in time to right between control and mTBI rats (p<0.05), with mTBI rats exhibiting a greater latency (mTBI: 69s±39 (mean±S.D.) vs. sham: 27s±12). Number of footslips for this group could not be assessed as there was a record management accident in the lab that led to missing data from the first 12 rats.

4.1.2 Rats ordered Charles-Rivers

Data for the ordered rats were analysed in the same manner as the in house bred rats. This study was conducted in 2 separate sessions (2 different cohorts), with 2 control and 2 mTBI rats per session (Total: 4 mTBI rats, 4 control rats). There was no significant difference in time to right between control and mTBI rats. Both cohorts of rats did not exhibit significant differences.
in number of footslips on the beam-walking task. However, the second cohort of rats did show a non-significant increase in the number of foot slips compared to controls. Despite this, the results were not significant as the sample size for this cohort was small (mTBI: 2, control: 2). When both cohorts of ordered rats were combined, there was no significant difference in the number of footslips. The results for the first cohort of Charles Rivers rats were surprising as there were no complications with the weight drop, such as a double hit due to tangling in the fishing line or failure of the aluminium foil to properly break, and all mTBI rats experienced a $180^\circ$ rotation after they broke through the aluminium foil.
4.2 Aim 1 Results

4.2.1 T2/T2*

Analysis of each ROI for the in house bred rats did not reveal significant differences between mTBI and control rats in T2 and T2* measurements (Fig. 4.2-1,2; Table 4.2-1).

Similar to the bred in house rats, the ordered rats showed no significant differences for all ROIs in T2 and T2* measurements (Fig. 4.2-1,2; Table 4.2-1).

Table 4.2-1. Average T2 values and standard deviations for control and mTBI rats in all ROIs in both bred in house rats (left) and ordered rats (right). Ctx L: cortex left; Ctx R: cortex R; Hc L: hippocampus left; Hc R: hippocampus right; BG L: basal ganglia left; BG R: basal ganglia right; CC: corpus callosum.

<table>
<thead>
<tr>
<th>Bred in house</th>
<th>Control</th>
<th>mTBI</th>
<th>Ordered rats</th>
<th>Control</th>
<th>mTBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROI</td>
<td>T2 Average (ms) ± Std. Dev.</td>
<td>T2 Average (ms) ± Std. Dev.</td>
<td>ROI</td>
<td>T2 Average (ms) ± Std. Dev.</td>
<td>T2 Average (ms) ± Std. Dev.</td>
</tr>
<tr>
<td>Ctx L</td>
<td>46.4±0.9</td>
<td>45.8±1.6</td>
<td>Ctx L</td>
<td>45.9±1.0</td>
<td>46.4±0.3</td>
</tr>
<tr>
<td>Ctx R</td>
<td>46.8±1.3</td>
<td>46.0±0.6</td>
<td>Ctx R</td>
<td>46.2±0.9</td>
<td>45.6±0.4</td>
</tr>
<tr>
<td>Hc L</td>
<td>50.2±1.5</td>
<td>50.0±1.0</td>
<td>Hc L</td>
<td>49.2±1.1</td>
<td>50.1±0.5</td>
</tr>
<tr>
<td>Hc R</td>
<td>48.8±2.2</td>
<td>48.7±1.2</td>
<td>Hc R</td>
<td>48.7±1.4</td>
<td>49.4±0.4</td>
</tr>
<tr>
<td>BG L</td>
<td>42.2±1.1</td>
<td>41.8±0.8</td>
<td>BG L</td>
<td>42.3±0.3</td>
<td>42.6±0.4</td>
</tr>
<tr>
<td>BG R</td>
<td>41.6±0.9</td>
<td>41.3±0.6</td>
<td>BG R</td>
<td>41.9±0.1</td>
<td>42.1±0.7</td>
</tr>
<tr>
<td>CC</td>
<td>44.8±1.3</td>
<td>45.0±2.0</td>
<td>CC</td>
<td>46.2±4.0</td>
<td>45.2±1.4</td>
</tr>
</tbody>
</table>
Fig. 4.2-1. Graphs showing T2 values in bred in house (top) (mTBI=12; control=5) and ordered rats (bottom) (mTBI=4; control=4). There is no significant difference between control and mTBI rats for both populations.
Fig. 4.2-2. T2* values for control and mTBI rats. The graph on the left shows T2* values for the bred in house rats (mTBI=12; control=5), while the graph on the right shows T2* values for ordered rats (mTBI=4; control=4).
4.2.2 $R2/R2^*$

Similarly, measurements of $R2$ and $R2^*$ in bred in house mTBI and control rats were not significantly different. $R2$ and $R2^*$ measurements were also not significant in any ROIs in the ordered rats (Fig. 4.2-3,4).

Fig. 4.2-3. $R2$ values for control and mTBI rats for bred in house (top) (mTBI=12; control=5) and ordered (bottom) rats (mTBI=4; control=4).
Fig. 4.2-4. R2* values for control and mTBI rats for bred in house (top) (mTBI=12; control=5) and ordered (bottom) rats (mTBI=4; control=4).
4.2.3 T1

Both visual inspection and quantitative measurements of T1 values did not reveal any differences between mTBI and control rats for the bred in house cohort (Table 4.2-2).

Upon examination of T1 values in the ordered rats, there were no significant differences between mTBI and control rats in all ROIs (Table 4.2-2). There were no significant differences between control rats in the in house bred group and the control rats in the ordered group in all ROIs (p>0.05).

Fig. 4.2-5. Example of a T1 weighted-image of a control rat from the bred in house rats.
Table. 4.2-2. Average T1 values and standard deviations for control and mTBI rats in all ROIs for both bred in house (left) and ordered (right) rats.

<table>
<thead>
<tr>
<th>Bred in house</th>
<th>Control</th>
<th>mTBI</th>
<th>Ordered rats</th>
<th>Control</th>
<th>mTBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROI</td>
<td>T1 Average (ms) ± Std. Dev.</td>
<td>T1 Average (ms) ± Std. Dev.</td>
<td>ROI</td>
<td>T1 Average (ms) ± Std. Dev.</td>
<td>T1 Average (ms) ± Std. Dev.</td>
</tr>
<tr>
<td>Ctx L</td>
<td>1757.8±49</td>
<td>1750.0±106</td>
<td>Ctx L</td>
<td>1791.2±30.8</td>
<td>1762.8±37.8</td>
</tr>
<tr>
<td>Ctx R</td>
<td>1743.1±46</td>
<td>1748.3±118</td>
<td>Ctx R</td>
<td>1768.0±23.5</td>
<td>1722.0±53.1</td>
</tr>
<tr>
<td>Hc L</td>
<td>1767.4±23</td>
<td>1777.4±41</td>
<td>Hc L</td>
<td>1754.9±49.1</td>
<td>1786.0±47.2</td>
</tr>
<tr>
<td>Hc R</td>
<td>1799.8±14</td>
<td>1797±38</td>
<td>Hc R</td>
<td>1774.1±45.9</td>
<td>1774±57.1</td>
</tr>
<tr>
<td>BG L</td>
<td>1575.3±33</td>
<td>1569.6±89</td>
<td>BG L</td>
<td>1535.3±21.1</td>
<td>1526.1±21.2</td>
</tr>
<tr>
<td>BG R</td>
<td>1595.1±36</td>
<td>1591.4±72</td>
<td>BG R</td>
<td>1543.3±7.3</td>
<td>1527.3±42.5</td>
</tr>
<tr>
<td>CC</td>
<td>1693.6±53</td>
<td>1699±50</td>
<td>CC</td>
<td>1636.5±48.4</td>
<td>1644.8±35.8</td>
</tr>
</tbody>
</table>

4.2.4 DWI

In the in house bred cohort, mTBI and control rats that underwent DWI showed no significant differences in ADC in all ROIs (Fig. 4.2-6). Although it appears that there may be a significant difference in the corpus callosum, the variability was so large that it did not reach significance. Despite the lack of significance, there was considerable variability within the ADC values. ADC values were plotted in a scatterplot as individual data points and separated by gender (4.2-7). No gender differences were seen in ADC for all ROIs. The distribution between control and mTBI rats was not significantly different. All the outlying data points belonged to one female rat. No diffusion data were collected for the ordered rats.
Cortex and basal ganglia ADC data from male and female rats from the bred in house rats was grouped together and plotted against CBF to look for correlations, however no correlation was found between ADC and CBF (Fig. 4.2-8).

Fig. 4.2-6. ADC values for all ROIs in bred in house rats (mTBI=12; control=5).

Fig. 4.2-7. ADC values plotted as individual data points for each animal for all ROIs. Control females are coloured in red and mTBI females coloured in pink. The outliers in the mTBI group all belong to the same female.
A correlational analysis of ADC vs. CBF in the bred in house rats was conducted using the Spearman Rank Order test as the data were not normally distributed. The correlational analysis was conducted for all ROIs in both control and mTBI groups (p>0.05). There was no significant correlation between any of the variables.

ADC was correlated with the time to right measurement using the Spearman Rank Order test. ADC from each ROI was compared to the time to right and no significant correlations were found for any measurement.

![ADC vs. CBF in the bred in house rat cortex](image)

Fig. 4.2-8. ADC values plotted against CBF in the bred in house rat cortex. Plotting ADC vs. CBF did not reveal any correlations between the 2 measurements. Black dots represent control animals while open dots represent mTBI animals. There are only 3 control animals as DWI data was only collected for 3 of the 5 controls.
4.2.5 SWI

SWI images were severely affected by motion artefact (Fig. 4.2-9). As a result, it was not possible to accurately determine the presence of microbleeds from motion artefact.

Fig. 4.2-9. Example of a SWI from a control rat. The image is significantly affected by motion artefact and therefore we were unable to differentiate hypointensities, which could represent microhemorrhages, from motion artefact.
4.3 Aim 2 Results

4.3.1 Sinus Area

There was a significant increase in cross sectional area of the sagittal (mTBI: 0.60±0.2 mm$^2$ (mean±S.D.) vs. sham 0.38±0.07 mm$^2$) and left transverse sinus (mTBI: 0.12± 0.04 mm$^2$ vs. sham: 0.07±0.02 mm$^2$) relative to controls (p<0.05) with a trend towards enlargement in the right transverse sinus, although it did not reach significance in bred in house rats (Fig. 4.3-2A).

There was no gender difference observed in sinus size in all 3 sinuses among the bred in house rats (Fig. 4.3-2A).

Analysis of sinus size in ordered rats revealed no significant difference in sinus size between the control and mTBI groups, both when compared only within the ordered rats, and when compared to the group of in house bred rats. Sinus sizes for all mTBI rats in the ordered group were found to be within the values for the control rats in the in house bred group (Fig. 4.3-2B).
Fig. 4.3-2. Sinus size for the sagittal and left and right transverse sinuses, for the bred in house (top) (mTBI=12; control=5) and ordered rats (bottom) (mTBI=4; control=4). Pink dots represent females and black dots represent male rats. SS= sagittal sinus, TS L= transverse sinus left, TS R= transverse sinus right.
Correlational analysis was carried out to investigate the possible relationships between superior sagittal sinus area and time to right in the bred in house rats (Fig. 4.3-3) and ordered rats. The Pearson Product Moment analysis was conducted as the data followed normal variance. There was no correlation between sinus area and time to right for either the in house bred or ordered rats (p>0.05).
4.3.2 CBF

CBF was calculated using subtracted images from the cASL sequence. A perfusion map was generated for visualization (4.3-4). There was large variability in CBF in both control and mTBI rats in the bred in house rats. Analysis revealed no significant difference in CBF for all ROIs (Fig. 4.3-5A).

The ordered rats showed large individual variability, similar to what was observed in the in house bred animals and there was no significance between mTBI and control groups (Fig 4.3-5B).

Fig 4.3-4. Perfusion map generated of a bred in house control rat brain.
Fig. 4.3-5. CBF in both bred in house rats (top) (mTBI=12; control=5) and ordered rats (bottom) (mTBI=4; control=4). The ordered rats showed considerably higher variability among individuals than the bred in house rats.
Correlational analysis was conducted for CBF and time to right in the bred in house rats and ordered rats. A Spearman Rank Order test was carried out as the data did not follow a normal distribution. There was no significant correlation between the CBF and time to right in both groups (p>0.05).
Chapter 5: Discussion

Mild traumatic brain injury is a major and growing health concern. The increased awareness of mild traumatic brain injuries highlights the need to find a reliable means of objectively identifying mTBI, which individuals may go on to develop PCS, and to develop a method to monitor injury progression. Special focus must be placed on the pediatric population as they respond differently to injury in comparison with adults, which are more commonly studied. Children have been shown to be more susceptible to diffuse injury than adults (Kirkwood et al. 2006). As there is a paucity of information in the pediatric population and preliminary research has indicated that they are more likely to suffer a poorer outcome than adults, there is an obvious need for more studies examining mTBI in children. In order to thoroughly study mTBI, one needs a reliable animal model. This study paired a reliable animal model of juvenile mTBI with novel MRI sequences to further examine mTBI in a juvenile population.

Time to right (TTR) and beam walking were chosen to ensure that the rats had sustained an mTBI. When this model was established, it was determined that impairments in TTR and beam walking indicated that the animal had experienced a mTBI. Since mTBI is not visible on standard MRI, it is important to include these measurements as a way to ensure the animal did sustain an injury. These methods of validation were chosen based on after effects of mTBI in humans. Typically, post-mTBI humans have balance and motor disturbances, thus the beam walking task was chosen to be a measure of this (Ryan & Warden 2003). A significant increase
in foot slips in mTBI rats compared to controls indicates motor impairment in rats (Mychasiuk et al. 2014a).

Some humans experience a loss of consciousness post-mTBI. This is difficult to measure in rats, however it is accepted that TTR is a measure consistent with a loss of environmental awareness and responsivity, albeit an imperfect one (Mychasiuk et al. 2014a). The original study validating the model showed that mTBI animals had a significantly increased latency in TTR, as well as a significantly increased number of hind foot slips on the beam walking test (Mychasiuk et al. 2014a). The data collected in the current study indicated a significant increase in TTR in the mTBI animals in the in house bred animal group. Interestingly, in both cohorts of ordered rats, there was no difference in TTR between control and mTBI rats, potentially suggesting that none of these animals experienced a loss of awareness of their environment. Assessment of beam walking data in the ordered rats did not show significance differences between control and mTBI rats when taken in context with the lack of difference in TTR suggests that the mTBI animals in the ordered group did not actually sustain an mTBI. Unfortunately, the data set for beam walking was incomplete for the in house bred rats and as a result it could not be included in the analysis. Due to the significant increased latency in TTR, it was determined that the bred in house rats did experience a successful mTBI.

As there was no difference in TTR or beam walking in the first cohort of ordered rats, the injury could not be validated, and thus we cannot verify that the rats had a successful mTBI, despite an uncomplicated hit resulting in a 180° rotation. As a loss of consciousness in humans does not always occur, lack of differences in TTR amongst groups does not uncategorically indicate a lack of injury, but rather may suggest a milder injury (Mychasiuk et al. 2014a). For this reason, beam walking is used in conjunction with time to right measurements to validate
injury. In all the experimental sets, except for the first cohort of ordered rats, there were
differences in one of these parameters, thus suggesting that the animals in this study experienced
successful mTBIs. The bred in house rats showed a statistically significant increase in time to
right when compared to the control rats. The second cohort of ordered rats did not statistically
show a significant increase in the number of foot slips; however, this is likely due to the low
number (only 2 rats per group). Examination of the number of foot slips reveals a visibly
elevated number of foot slips when the mTBI rats are compared to the controls. Therefore,
although this finding could not be reported as significant, there was a difference between the
mTBI rats and the control rats in the second ordered cohort. Since there was no significant
difference found in time to right or number of foot slips when the ordered rats were grouped
together, it was decided to not do any further studies using ordered rats.

Consistent with human studies (Mechtler et al. 2014), there were no visual or quantitative
differences in the parenchyma between the control and mTBI groups on T1 and T2-weighted
images. This shows that this model is a good animal model of mTBI as it is consistent with what
is seen in imaging of human mTBI. In other animal models of mTBI (CCI, FPI), there are visible
abnormalities seen on MR images. These include skull fracture, focal injury, edema, and
hemorrhage. Given that human mTBI is undetectable on standard imaging protocols, this
indicates that these are not ideal models of mTBI when trying to simulate a human mTBI. The
lack of abnormalities on standard imaging sequences for this modified weight-drop model along
with the behavioural results, supports the conclusion that this is a good model of mTBI.

Despite previous studies having shown that this is a good model of mTBI, the lack of
behavioural abnormalities in the rats that were not in house bred calls in to question the
reproducibility of the model. In this case, shipping stress may be a variable. Stress can cause
significant psychological and physiological effects in animals. These effects are caused by factors such as strange noises, temperature variations, and rough handling. Adult mice have been shown to have a significant increase in corticosterone levels post-shipping which persisted up to 48hr (Landi et al. 1982; Tuli et al. 1995). Increased corticosterone secretion has been shown to alter dendritic branching and spine density as well as affect neurogenesis and synaptic plasticity in animals. These effects can be seen even after a single exposure (Romeo et al. 2005). Acute stress can cause a stress response that persists past the application of the stressor, and the length of this response has been shown to be age dependent. Acute stress has been found to cause a more prolonged stress response, including corticosterone secretion, in prepubescent (P28) male rats compared to adult rats (Romeo et al. 2005a). It has been suggested that prepubescent animals are more susceptible to stress effects due to the ongoing development of certain brain regions, including the hypothalamus, hippocampus, amygdala, and prefrontal cortex, which are also the regions most affected by corticosterone (Romeo et al. 2004; Romeo et al. 2005b). Since stress has been shown to cause physiological and psychological alterations, it adds in an additional variable which could affect the results of the study. However, despite this, it is possible to argue that children are not raised in an environment completely free of stress and therefore shipping stress may make the model more translatable. Ideally, ordered rats could be used as it would decrease the amount of time it takes to raise the rats, make it easier to acquire more rats for the study, and it would save costs.

It was surprising that the ordered animals did not consistently exhibit behavioural abnormalities as the injury was uncomplicated and the animals underwent a visible 180° rotation. In addition, preliminary studies from the Esser lab used animals that were transported from Charles Rivers after weaning rather than being bred in house (Mychasiuk et al. 2014a). The
protocol followed in this study for the ordered rats was identical to the protocol Mychasiuk et al. (2014a) used in their validation study. In both studies, animals were ordered in at P21 and injured at P30. The difference between the 2 studies was that in the current study the animals were not handled prior to the injury. Comparing the results to the in house bred rats, the ordered rats did differ in upbringing. The in house bred rats were handled every day since birth and were therefore well habituated to humans and raised with minimal stress. The ordered rats experienced more stress as they were shipped from Charles-Rivers to the University of Calgary and were not handled until the day of the injury. The increased corticosterone secretion due to stress could possibly have an effect on neurogenesis and dendritic branching/spine density in the ordered rats, therefore altering how the animals respond to the injury (Romeo et al. 2005).

This study found a significant increase in cerebral sinus size amongst control and mTBI rats in the rats who were bred in house. The cerebral sinuses are generally considered to be largely passively regulated draining vessels. They collect venous blood from venules, as well as CSF. The venous blood and CSF from all sinuses meet at the venous confluens at the back of the skull and drain out of the skull through a number of pathways. The main pathways are the internal jugular veins (IJVs) and the vertebral venous system (also called secondary veins). Outflow through these veins is posture dependent. In the supine position, outflow is primarily through the IJVs, whereas in the upright position, the outflow is mostly through the vertebral venous system (Valdueza et al. 2000).

It is widely believed that venous drainage system in the brain is passively regulated, with very little to no smooth muscle in the veins. The study of cerebral veins has largely been neglected; however, there is evidence for the presence of smooth muscle in cerebral veins. A study examining dogs found that there were rings of smooth muscle in the retroglenoid veins,
which are part of the vertebral venous system, as they exit the skull. It was shown that these smooth muscle rings would constrict in response to both exogenous norepinephrine as well as stimulation of adrenergic nerve terminals (Pearce & Bevan 1984). Constriction of rings of venous smooth muscle results in the diversion of blood flow to alternative pathways (Pearce & Bevan 1984). Other studies have revealed evidence suggesting autonomic and sympathetic regulation of cerebral veins. Stimulation of the cervical sympathetic system in cats resulted in a significant constriction of pial veins, which was more pronounced than the constriction seen in the pial arteries at the same time point (Auer et al. 1981). Edvinsson et al. (1983) found that pial veins constricted in response to noradrenaline. All cerebral veins were in proximity to adrenergic nerve fibres, suggesting that cerebral veins are also under adrenergic control, as well as sympathetic control (Edvinsson et al. 1983). Additionally, cerebral veins will constrict in response to topical administration of an adrenergic agonist (Edvinsson et al. 1982). This suggests that veins are under sympathetic control and could play a role in cerebrovascular regulation.

Human studies have also found evidence for smooth muscle in veins. Vignes et al. (2007) looked for the presence of smooth muscle in ex vivo human brains. They found abundant and thick smooth muscle fibres, forming what they called a “smooth sphincter” at the junction between the cerebral bridging veins and the superior sagittal sinus (SSS). Although the role of the smooth muscle sphincters is unclear, it has been suggested that they play a role in IJV and secondary venous drainage pathways in physiological conditions and that it is perhaps involved in autoregulatory processes (Vignes et al. 2007). This is not the only study to provide evidence for smooth muscle in human cerebral veins. The presence of smooth muscle was found in the great cerebral vein where it joins with the straight sinus; however, the role of this smooth muscle sphincter remains unknown (Dagain et al. 2008). The cerebrovenous system is often naturally
asymmetric and is considered to be more variable than the arterial system (Schaller 2004). Evidence has been found for active regulation of the cerebral venous system; however, the exact function of this has yet to be elucidated.

Primarily, in the study of TBI, it is the arterial system which is investigated, with the venous system being largely overlooked. A few studies have been conducted which have investigated the impact of TBI on the venous system. The venous system is lacking ubiquitous smooth muscle, as is found in the arterial system, and is thus more susceptible to edema and elevation of intracranial pressure (ICP) (Chen et al. 2015). Post-TBI, patients can suffer from severe edema. This edema can cause increases in ICP, leading to compression of the collapse of venules and capillaries (Chen et al. 2015). This puts the patient at an increased risk of thrombosis, as well as obstruction of venous flow. Obstruction in venous outflow can cause edema and possibly the enlargement that was seen in the present study. In addition, venous obstruction can affect the CSF system and may cause a decrease in CSF absorption if the pressure gradient is too large between the CSF system and the venous system (Owler et al. 2005). Cerebral veins have also been shown to be surrounded by pericytes, which are capable of causing venous constriction. Pericyte contraction can lead to constriction of the veins and thus abnormal venous drainage (Chen et al. 2015). Chen et al. (2015) also suggest that when there is venous outflow obstruction, it leads to intracranial narrowing and proximal vascular dilation, such as enlargement of the draining sinuses.

The observed enlargement of the sinuses seen in this model is not novel in the context of cerebral pathology, although it is the first time it has been seen in mTBI. Enlargement of the sinuses can be associated with various pathologies. Cerebral venous sinus thrombosis (CVST) is perhaps the most common of these pathologies. CVST is an uncommon form of stroke, where
there is thrombosis in either the cerebral veins or sinuses (Bousser & Ferro 2007). Headaches are the most commonly reported symptom, reflecting an increase in intracranial pressure (Canhao et al. 2005). CVST is commonly diagnosed using imaging, including MRI and CT. Standard MRI sequences, including T1, T2 weighted sequences are able to identify the thrombi (Bousser & Ferro 2007). Downstream occlusion of sinuses or veins by thrombi could lead to observable enlargement of the sinuses. Some cases of TBI have been shown to produce thrombi (Salunke et al. 2013; Bakar & Tekkok 2010; Matsushige et al. 2009; Ferrera et al. 1998). It is typically associated with more severe injury and has yet to be reported in mTBI. The observed enlargement of the sinuses in these experiments is unlikely to be caused by thrombi as they would be visible on T1 and T2-weighted sequences.

Alterations in the cerebrovenous system as well as sinus enlargement has also been observed in patients who suffer from migraines and headaches. A study examining migraine patients found very similar pathophysiology in migraine sufferers as in mTBI patients when using MRV. The migraineurs exhibited altered venous drainage, showing a significantly larger venous outflow in the secondary venous channels, compared to controls who had more flow through the IJVs. The authors suggested that the reason for this could be release of vasoactive substances or growth factors (Koerte et al. 2011). Wilson et al. (2013) found that patients who suffered from high altitude headache showed evidence of restricted venous drainage, resulting in venous engorgement and a subsequent increase in ICP. This venous distension in these patients was induced by hypoxia.

In has been found in patients with idiopathic intracranial hypertension (IIH), which is characterized by increased ICP without evidence of a mass or ventricular obstruction, that occluded or narrowed IJVs resulted in significantly increased flow through the secondary venous
channels (Alperin et al. 2005). The MRV images for the IIH showed a very similar pathology to what was seen in the venous draining system in mTBI patients (Pomschar et al. 2013). However, these patients do not always exhibit venous obstruction. Upon examination of a female population with IIH, it was found that the majority of the patients do not exhibit any signs of venous sinus thrombosis on MRV scans, with the cause of the increased ICP remaining a mystery (Lee & Brazis 2000). Alperin et al. 2005 suggested that the increase in ICP is due to increased extracranial resistance to venous outflow; however, it was not suggested what the cause of this would be. This could be a potential explanation for what is causing the enlargement of the sinuses in the present study.

Studies have shown that after TBI, microthrombi occluded up to 70% of the venules in a mouse model of TBI. Over time, the microthrombi grew to completely occlude the venules (Schwarzmaier et al. 2010). Similarly, microthrombi have been observed in humans with severe TBI (Stein et al. 2004). Numerous studies in humans using SWI have shown that some patients with mTBI have microbleeds bleeds post-injury (Liu et al. 2014; Wang et al. 2014; Kou et al. 2013). In the present study, there were no microthrombi observed on the susceptibility weighted images; however, these images were severely affected by motion artefact and, despite attempts to seek hypointensities which would potentially represent microthrombi, we were unable to make any conclusions from this analysis. It is therefore possible that the animals used in the present study could have microthrombi which could contribute to the observed enlargement of the sinuses; however, we were unable to confirm this.

Currently, it is unclear why there is enlargement of the sinuses in this model. ICP was not measured in this study, so it is difficult to determine if the enlargement is due to increased ICP, venous obstruction, or an increase in flow through the sinuses. A FLOWMAP sequence was used
to attempt to determine the velocity of venous blood and CSF flow through the sinuses; however, it was unsuccessful in elucidating this. Unfortunately, as the SWI images were severely affected by motion artefact, we were not able to draw any conclusions regarding microthrombi.

The observed pathophysiology is not unique to this model. There is evidence for altered venous drainage and cerebrovenous abnormalities in other pathologies and disease conditions such as idiopathic intracranial hypertension and migraines. Currently it is not obvious why this observed pathology is occurring. Looking at other disease states could provide clues to the cause(s) of this phenomenon. This finding is an unexpected observation with no clear cause. It requires further investigation to determine what is causing this pathophysiology.
Chapter 6: Limitations

This study had a number of limitations, mainly due to the lack of behavioural data to fully confirm the injury. Behavioural impairment could not be completely confirmed in the preliminary results as foot slip data were incomplete. The beam walking task has been established as an accurate measure of impairment following neurological insult (Schallert et al. 2002; Dixon et al. 1987). Initially used to assess impairment post-ischemic injury, the beam walking task has also now been validated for use in post-traumatic brain injury (Dixon et al. 1987). Animals with traumatic brain injury (mild to severe) exhibit increased latency to cross the beam, as well as an increase in the number of foot slips (Dixon et al. 1987, Mychasiuk et al. 2014a). Given the consistency in the results, the beam walking task has become an accepted method of assessing impairment post-mTBI as animals with a mTBI typically do not show any overt signs of pathophysiology. Since the foot slip data is incomplete, it could not be used in the present study to assess impairment in the rats. As a result, only time to right could be used to suggest impairment. Subsequent experiments will include the beam walking test to be used as a measure of impairment post-mTBI.

The animals in this study were struck on the dorsal plane of the head; therefore, they did not receive a lateral injury. Without conducting experiments using a lateral injury, all conclusions in this study must be considered model specific.
This study was largely an investigational study testing both a modified model of mTBI and the use of various imaging sequences to seek pathophysiology. The finding of an increase in sinus size needs to be investigated further. It would be worthwhile to investigate sinus enlargement in models that administer a lateral injury, rather than the dorsal injury used in this model. By investigating lateral injury, it will be possible to determine if this pathophysiology is seen solely due to the direction of injury (a dorsal strike) or if this enlargement of the sinus is found universally in all models of mTBI. Secondly, it is important to determine what is causing the observed sinus enlargement. There are various reasons that could be attributed to this pathophysiology and it is important to separate them out and determine why this is occurring. In the future, it would be important to measure intracranial pressure in these animals. Additionally, it would be valuable to work out the FLOWMAP sequence in order to measure the flow through the sinuses. Since altered venous drainage is observed in humans post-mTBI, in the future the upper neck of the rats should be imaged using magnetic resonance venography (MRV) to see if this pathophysiology (reduced flow through IJVs) is also seen in the animal model. Newly developed MR sequences would also be valuable to apply to the study of this model. A recently developed sequence, neurite orientation dispersion and density imaging (NODDI), could be useful when looking at white matter microstructure post-mTBI. This technique allows for the examination of dendrites and axons. The advantage to using NODDI over DTI is that it allows for the separation of the components of fractional anisotropy into neurite orientation dispersion
and the volume fraction of intracellular compartments, thus providing a more specific measure of white matter pathology (Zhang et al. 2012).

Conducting histology on the perfused brains would be very valuable. Immunohistochemistry would be particularly interesting. Recent animal studies of mTBI have found evidence of an immune response in the brain post single and repetitive mTBI (Hylin et al. 2013; Huang et al. 2013; Fidan et al. 2015; Aungst et al. 2014).

Additionally, it would be worthwhile to attempt to elucidate the cause of restricted venous drainage. Given that Pearce & Bevan (1984) found evidence of smooth muscle in the draining veins in the dog, it would be valuable to conduct a dissection on a rat in order to isolate the draining vessels of the brain and stain them for smooth muscle to see if this could also be found in rats. If there is smooth muscle there, it is possible that constriction of this muscle could cause a reduction of venous flow, resulting in the sinus enlargement observed in this study.
Chapter 8: Conclusions

Mild traumatic brain injury is a growing problem. The increased recognition has developed over the years has spurred interest in the study of mTBI. It is becoming increasingly clear that children do not react the same way to mTBI as adults. Despite these preliminary findings, the pathophysiology of mTBI is still not fully understood, especially in children. Animal models are critical to the study of brain injury, and as a result it is important to design a model that is representative of human injury. This study validated a new model of pediatric mTBI using imaging, showing that it modelled what is seen in clinical mTBI. This model can provide a reliable means of studying mTBI in children.

It is difficult to monitor and identify mTBI. In most cases this does not pose a significant problem; however, a small proportion of children go on to suffer from symptoms for months to years post-injury. It would be helpful to have a clinical marker which could identify which children are at risk of long-term problems, as well as a biomarker which could monitor treatment and aid in deciding when it is safe for the child to resume their normal activities. MRI provides a promising means of developing a clinical, non-invasive method of identifying and monitoring injury. Interestingly, using MRI, this study found abnormalities post-mTBI in the injured rats which could be indicative of pathophysiology. The significant enlargement of the superior sagittal and transverse sinus is a surprising finding, which is suggestive of cerebrovenous abnormalities post-mTBI. While the venous system is largely overlooked in the study of mTBI, Pomschar et al. (2013) also found evidence for venous involvement after injury. As the cerebrovenous system has been implicated in other disorders, such as migraines, the observed
enlargement of the draining sinuses may be linked to mTBI symptomology, particularly to headache. This is an interesting finding and it deserves to be investigated further.

Overall, this is a promising model of pediatric mTBI. The finding of the enlarged sinuses suggests that MRI could potentially be used effectively to search for biomarkers of mTBI, as well as, reveals an interesting and unexpected pathophysiology post-injury that has not been reported in the literature to date.
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