Mild Traumatic Brain Injury and Advanced Magnetic Resonance Imaging Techniques

Dr Sharon Marie Jay

Thesis submitted for Master Medical Science Degree
University of Otago
Student ID 8354766

April 2017
Abstract

Introduction
Mild Traumatic Brain Injury (mTBI) has been described as a ‘silent epidemic’ and is a significant health burden to the global population. New Zealand specific data estimates mTBI to occur 749 in 100,000 person-years. While the majority of patients that suffer mTBI make a complete recovery between 3 and 12 months following the injury, approximately 15% of all mTBI will have ongoing symptoms one year after initial injury. This condition is known as Post Concussion Syndrome (PCS).

The sheer scale and cost of PCS, not just in terms of acute care and rehabilitation costs, but the emotional and financial costs to the patient and their family are significant; this makes injury prevention, detection and care a significant health priority. At present, no robust or reliable radiological or biochemical markers have been developed to diagnose mTBI. Moving forward, injury prevention, early diagnosis, treatment and identification of people at risk of developing PCS would mean better patient education and resource allocation for rehabilitation; this in turn may ease the burden of this condition.

The objective with this thesis is to identify potential brain differences associated with full or poor recovery post mTBI and to determine whether differences existed with a group of participants with chronic pain and a group of healthy controls.

Methods
This study used both structural (grey matter volume and cortical thickness) and DTI techniques to investigate the brains of participants who had experienced an mTBI and two control groups. Data previously collected from 138 participants were used for this study; forty-two subjects comprised the PCS group. Thirty subjects deemed fully recovered from mTBI comprised the non-PCS group. The two control groups were healthy controls (n=41) and those with a chronic pain condition (CP; n=25). The PCS, non-PCS and CP groups were all matched for age, gender, education, injury, severity and pre-injury
work status. The healthy control group was matched for mean age and sex distribution.

Results
There were no significant differences in brain volume, cortical thickness, or DTI metrics along the centres of principal white matter tracts between the PCS and non-PCS groups, nor when these two mTBI groups were compared to controls. However, the CP participants exhibited reduced cerebellar grey matter volume relative to all three groups (PCS, non-PCS, and healthy controls) and significant reduction in FA and increased MD in several white matter tracts; there were no cortical thickness differences between any of the groups.

Conclusion
Overall the heterogeneity of the literature help to frame my results within the larger field. Lack of significant differences is not an uncommon finding. In my hands, PCS and non-PCS were indistinguishable on T1-weighted images, as well as DTI. A prospective study following mTBI from acute to chronic state with serial imaging or other study techniques such as HARDI or diffusion kurtosis should be used in future research to evaluate mTBI.
Acknowledgements

I would firstly like to thank my supervisors; Dr Tracy Melzer, Dr Deborah Snell and Professor Tim Anderson. Their doors were always open for me and I can’t thank them enough for their guidance, encouragement and especially their patience. Thanks also for taking a chance on me.

I’d also like to thank my colleagues at the New Zealand Brain Research Institute for their help, advice and encouragement. I’d especially like to thank Maddie Pascoe, Mustafa Almuqbel and Mildred Tan for their patience and time in teaching me the computer and MRI software packages. Legends.

I would like to acknowledge the generous support I have received from the Brain Research New Zealand Centre of Research Excellence (CoRE) for assisting to make this research possible.

Finally, I cannot begin to thank my whanau and friends for their unfailing support of me and encouraging me in my journey. I am so grateful for all the kindness and love that you continue to show me. This accomplishment would not have been possible without all of you. Thank you all so very much.
Table of Contents

Abstract ........................................................................................................................................ i
Acknowledgements ................................................................................................................... iii
Table of Contents ........................................................................................................................ iv
List of Table and Figures ............................................................................................................ vi
List of Abbreviations .................................................................................................................... vii

1  Mild Traumatic Brain Injury ................................................................................................. 1
   1.1  Definition .......................................................................................................................... 1
   1.2  Epidemiology ................................................................................................................... 3
   1.3  Pathophysiology ............................................................................................................... 5
       1.3.1  Metabolic .................................................................................................................. 7
       1.3.2  Vascular changes ........................................................................................................ 9
       1.3.3  Blood brain barrier ................................................................................................... 9
       1.3.4  Inflammation ............................................................................................................ 10
       1.3.5  Neuronal damage ..................................................................................................... 11
   1.4  Diagnosis .......................................................................................................................... 12
   1.5  Traditional Neuroimaging ............................................................................................... 12
   1.6  Management .................................................................................................................... 15
   1.7  Prognosis ......................................................................................................................... 16
   1.8  Post Concussion Syndrome ............................................................................................ 18
   1.9  Diagnosis .......................................................................................................................... 21
   1.10 Management .................................................................................................................... 21
   1.11 Prognosis ........................................................................................................................ 22

2  Chronic Pain and its relation to mTBI ............................................................................... 23
   2.1  Introduction ..................................................................................................................... 23
   2.2  Definition of Chronic Pain ............................................................................................. 23
   2.3  Incidence .......................................................................................................................... 24
   2.4  Pathophysiology ............................................................................................................. 25
   2.5  Brain changes in Chronic Pain ....................................................................................... 26
   2.6  Chronic Pain and mTBI .................................................................................................... 28

3  MRI Basic Principles ............................................................................................................. 30
   3.1  Introduction ..................................................................................................................... 30
   3.2  Basic MR Physics ............................................................................................................ 30
   3.3  MRI in TBI ...................................................................................................................... 33
       3.3.1  Structural ................................................................................................................... 34
       3.3.2  Diffusion Tensor Imaging (DTI) ............................................................................... 34

4  Advanced Neuroimaging and mTBI Literature Review .................................................... 39
   4.1  Structural Findings in mTBI Literature ......................................................................... 39
   4.2  DTI in mTBI .................................................................................................................... 40
       4.2.1  Findings ..................................................................................................................... 40
       4.2.2  Variability in the literature ..................................................................................... 43

5  Methods .................................................................................................................................. 45
   5.1  Introduction ...................................................................................................................... 45
   5.2  Aims/hypotheses ............................................................................................................. 45
   5.3  Subjects ............................................................................................................................ 46
   5.4  MRI acquisition .............................................................................................................. 47
List of Table and Figures

Table 1-1 The definition of severity of TBI ................................................................. 1
Table 1-2 Graph showing the causes of mTBI in New Zealand................................. 5
Table 1-3 Symptoms of mTBI .................................................................................. 15
Table 1-4 Pathologic changes seen in Chronic Trauma Encephalopathy (CTE) .......... 17
Table 5-1 Study sample demographics ................................................................... 47
Table 5-2 MRI image acquisition parameters ....................................................... 48
Table 5-3 Pairwise comparisons tested ................................................................... 52
Table 6-1 Study sample demographics ................................................................... 54
Table 6-2 Injury severity and neuropsychological tests ......................................... 55
Table 6-3 Summary MRI measurements ................................................................... 56

Figure 1-1 Mechanism for injury in mTBI .............................................................. 6
Figure 1-2 Showing the schematic metabolic changes within the neuron after mTBI... 8
Figure 1-3 Showing the cellular metabolic changes over time from injury ............ 8
Figure 1-4 The mechanical disruption of the blood vessel allowing systemic
inflammatory cells to leak into the brain parenchyma through gaps in the previously
impermeable endothelial cells ............................................................................. 10
Figure 1-5 Inflammatory changes involved in mTBI .......................................... 11
Figure 1-6 The Canadian CT Rule in decision making of neuroimaging after mTBI .. 13
Figure 1-7 Flow diagram in the decision making in mTBI in children ................... 14
Figure 3-1 Illustrating the precession of spin around Bo ...................................... 30
Figure 3-2 Diagram illustrating NMV and the resultant flip angle after resonant RF
pulse is applied .................................................................................................. 31
Figure 3-3 Schematic graph showing T1 recovery and T2 relaxation time .......... 32
Figure 3-4 Figure showing the MRI Neuroimaging techniques available and examples
of their functional use ..................................................................................... 33
Figure 3-5 Diffusion Direction .............................................................................. 36
Figure 3-6 Diagram of a diffusion-weighted spin echo sequence ................................ 36
Figure 3-7 Schematic illustration of tensor ellipsoid ........................................... 37
Figure 5-1 Example of design matrix .................................................................. 52
Figure 6-1 PCS>CP comparison showing reduced grey matter volume in CP subjects 56
Figure 6-2 Non-PCS>CP comparison showing reduced cerebellar volume in CP patients
......................................................................................................................... 57
Figure 6-3 Controls>CP comparison showing reduced cerebellar volume in CP patients
......................................................................................................................... 57
Figure 6-4 mTBI groups and RBANS score showing reduced grey matter volume in
those with lower RBANS score ....................................................................... 58
Figure 6-5 Study specific Mean FA as a base with overlay of white matter skeleton
mask in green .................................................................................................... 59
Figure 6-6 FA Comparison of positive association mTBI and CWIT IV ..................... 60
Figure 6-7 FA Comparison of CP> controls ....................................................... 61
Figure 6-8 FA comparison CP> controls ......................................................... 62
Figure 6-9 FA comparison CP> controls ........................................................... 62
Figure 6-10 MD Comparison CP< controls ....................................................... 63
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>Accident Compensation Corporation</td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent Diffusion Coefficient</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>CBT</td>
<td>Cognitive Behavioral Therapy</td>
</tr>
<tr>
<td>CP</td>
<td>Chronic Pain</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebral Spinal Fluid</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>CTE</td>
<td>Chronic Trauma Encephalopathy</td>
</tr>
<tr>
<td>CWIT</td>
<td>Colour Word Interference Test</td>
</tr>
<tr>
<td>D</td>
<td>Diffusion Coefficient</td>
</tr>
<tr>
<td>DAI</td>
<td>Diffuse Axonal Injury</td>
</tr>
<tr>
<td>DSM IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders 4th Edition</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>ED</td>
<td>Emergency Department</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
</tr>
<tr>
<td>FDR</td>
<td>False discovery rate</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional MRI</td>
</tr>
<tr>
<td>FSL</td>
<td>FMRIB Software</td>
</tr>
<tr>
<td>FWE</td>
<td>Family-wise error rate</td>
</tr>
<tr>
<td>GM</td>
<td>Grey matter</td>
</tr>
<tr>
<td>GP</td>
<td>General Practitioner</td>
</tr>
<tr>
<td>HADS</td>
<td>Hospital Anxiety and Depression scale (Anxiety or Depression)</td>
</tr>
<tr>
<td>IASP</td>
<td>International Association for the Study of Pain</td>
</tr>
<tr>
<td>ICD-10</td>
<td>International Classification of Disease, 10th Revision</td>
</tr>
<tr>
<td>ICV</td>
<td>Intracranial Volume</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin-1</td>
</tr>
<tr>
<td>LOC</td>
<td>Loss of consciousness</td>
</tr>
<tr>
<td>MD</td>
<td>Mean diffusivity</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MDT</td>
<td>Multidisciplinary team</td>
</tr>
<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic Resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>mTBI</td>
<td>Mild Traumatic Brain Injury</td>
</tr>
<tr>
<td>NGF</td>
<td>Nerve Growth Factor</td>
</tr>
<tr>
<td>NMV</td>
<td>Net Magnetisation Vector</td>
</tr>
<tr>
<td>Non-PCS</td>
<td>Non Post-Concussion Syndrome</td>
</tr>
<tr>
<td>NZBRI</td>
<td>New Zealand Brain Research Institute</td>
</tr>
<tr>
<td>PCS</td>
<td>Post-Concussion Syndrome</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PTA</td>
<td>Post Traumatic Amnesia</td>
</tr>
<tr>
<td>PTSD</td>
<td>Post-traumatic Stress Disorder</td>
</tr>
<tr>
<td>RBANS TS</td>
<td>Repeatable Battery Assessment of Neuropsychological Status Total Score</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>RPQ</td>
<td>Rivermead Post-Concussion Symptom Questionnaire</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Positron Emission Computed Tomography</td>
</tr>
<tr>
<td>SPM12</td>
<td>Statistical Parametric Mapping 12</td>
</tr>
<tr>
<td>SSH</td>
<td>Starship Hospital</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitors</td>
</tr>
<tr>
<td>T</td>
<td>Tesla</td>
</tr>
<tr>
<td>TBSS</td>
<td>Tract Based Spatial Statistics</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour Necrosis Factor α</td>
</tr>
<tr>
<td>VBM</td>
<td>Voxel Based Morphometry</td>
</tr>
<tr>
<td>WM</td>
<td>White matter</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
1 Mild Traumatic Brain Injury

1.1 Definition

Traumatic brain injury (TBI) is defined as an alteration in brain function caused by an external force (1)(2). Clinical criteria are used to characterize TBI patients by severity into mild, moderate or severe TBI subsets. There is much variation and heterogeneity in the published literature as to what defines each of these terms. Several definitions of mild traumatic brain injury (mTBI) have been suggested. For the purpose of this study, the definition of mTBI will be that used by the ACC Evidence Based best practice guideline on TBI (2). This is to ensure consistency because the mTBI participants in this study have been recruited from the ACC Concussion clinic, which also uses the ACC definition of mTBI.

The clinical criteria used to determine severity of TBI are: presenting level of consciousness measured on the Glasgow Coma Scale (GCS), duration of loss of consciousness (LOC) and post traumatic amnesia (PTA) (2)(3). The GCS was developed by Teasdale and Jennett and published in 1974 as a tool for the neurological assessment of coma and impaired consciousness (4). There are three aspects of behavior assessed: eyes, verbal and motor, and then a cumulative score given out of 15 (range 3-15). This scale is simple, reproducible and widely used in the routine medical observations performed by medical professionals. The PTA duration is used in conjunction with the GCS to define severity of TBI. PTA is any amnesia or memory loss (anterograde or retrograde) from the time of injury. The duration a patient experiences PTA is used in the injury classification (Table 1.1).

Table 1-1 The definition of severity of TBI

<table>
<thead>
<tr>
<th></th>
<th>LOC</th>
<th>GCS</th>
<th>PTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>&lt;30 minutes</td>
<td>13-15</td>
<td>0-1 day</td>
</tr>
<tr>
<td>Moderate</td>
<td>30minutes-24hours</td>
<td>8-12</td>
<td>1-7 days</td>
</tr>
<tr>
<td>Severe</td>
<td>&gt;24 hours</td>
<td>3-8</td>
<td>&gt;7 days</td>
</tr>
</tbody>
</table>

LOC=Loss of consciousness, GCS=Glasgow Coma Scale, PTA=Post traumatic amnesia. Modified from (2)(6)(7)
It is also important to acknowledge that other factors that may influence consciousness level are excluded, such as drug and alcohol use, medical drug sedation or other organ injury. These may artificially cause a reduced GCS unrelated to the TBI (2)(5). This is also why the GCS is not the sole measure of severity in TBI. Of note the other main definitions referenced are by the Mild Traumatic Brain Injury Committee of the Head Injury Interdisciplinary Special Interest Group of the American Congress of Rehabilitation Medicine 1993 (6), the WHO Collaborating Centre Task Force on mTBI 2004 (7), and the Position Statement by the Demographics and Clinical Assessment Working Group of the International and Interagency Initiative toward Common Data Elements for Research in TBI and Psychological Health 2010 (1).

The American Congress of Rehabilitation Medicine define a patient with mTBI as

“a person who has had a traumatically induced physiological disruption of brain function, as manifested by at least one of the following:

1. Any period of loss of consciousness;
2. Any loss of memory for events immediately before or after the accident;
3. Any alteration in mental state at the time of the accident (e.g feeling dazed, disoriented, or confused); and
4. Focal neurological deficit(s) that may or may not be transient, but where the severity of injury does not exceed the following:
   - LOC of approximately 30 minutes or less;
   - After 30 minutes, an initial GCS 13-15; and
   - PTA not greater than 24 hours.” (6)

The WHO task force describes mTBI as

“an acute brain injury resulting from mechanical energy to the head from external physical forces. Operational criteria for clinical identification include;

(i) 1 or more of the following: confusion or disorientation, loss of consciousness for 30 minutes or less, PTA for less than 24 hours, and/or other transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery;
(ii) GCS of 13-15 after 30 minutes post-injury or later upon presentation for healthcare.

These manifestations of mTBI must not be due to drugs, alcohol, medications, caused by other injuries or treatment for other injuries (e.g. systemic injuries, facial injuries or intubation), caused by other problems (e.g. psychological trauma, language barrier or coexisting medical conditions) or caused by penetrating craniocerebral injury” (7).

The Common Data Elements working group proposed the following definition: “TBI is defined as an alteration in brain function, or other evidence of brain pathology, caused by an external force.” In the explanatory notes it does describe the alteration in brain function and any period of LOC, PTA, neurological deficits and any alteration in mental state at the time of injury. However, this definition does lack precision in defining the terms of each component (1).

The terms mTBI, head injury and concussion are often used interchangeably. mTBI is the preferred term as it more accurately encompasses the brain dysfunction following trauma (8). At present mTBI is very much in the spotlight, both in mainstream media and published literature. This is no doubt due to the recent advances in the identification and diagnosis of Chronic Traumatic Encephalopathy (CTE) (9)(10)(11), with huge investment in research by professional sports leagues (12) and by the military, with specific interest in combat injuries (13)(14)(15). In New Zealand the new concussion guidelines in our national game, rugby (16), have helped raise awareness of concussion to an all-time high.

1.2 Epidemiology

TBI is a significant health burden to the global population and a significant cause of morbidity and mortality in all age groups. In fact it has been suggested that TBI is a ‘silent epidemic’ (17) and this can be illustrated by the WHO estimating that by 2020 TBI will become the leading cause of death and disability (18). Using internationally published data and extrapolating, the estimated incidence of mTBI is 100-300 per 100,000 population. However
taking into account not all mTBI is treated in the hospital setting, and hence not all cases are reported, the true rate is estimated to be closer to 600 per 100,000 (19). It is estimated that as many as 10 million people globally are affected annually by TBI (18).

A 2010-2011 New Zealand population-based incidence study using hospital, general practice and Accident Compensation Corporation (ACC) data in the Waikato region estimated mTBI to occur in 749 per 100,000 person-years (20). From this it is estimated there are 35,000 head injuries in New Zealand per year (21). In the year July 2015 to July 2016, ACC had 13,811 new claims for concussion/brain injury and had 17,913 active cases during this period. The total cost of all ACC payments was more than $85 million dollars over this period (22). This includes both acute treatment costs and rehabilitation.

ACC figures from 2003 similarly showed that 61.9% of people with mTBI were male (2). The young and the old are also over represented. ACC statistics show that the incidence of mTBI peaks in the 15-19 and over-60 years-old age groups, with the highest rates occurring in the 15-19 years age group (2). A Waikato population based study of 2010 again found similar results with those aged 15-34 years representing 40% of all mTBI cases. Males were again over represented with almost twice the rates of mTBI (RR 1.73) in females. This New Zealand data mirrors similar findings in overseas data; there is a clear male, young and old predominance (3)(5)(23).

In New Zealand the leading causes of TBI are: falls 38%, mechanical forces 21%, motor vehicle accident (MVA) 20% and assaults 17% (20) (Table 1.2). Mechanism of injury patterns are similar in reported literature from around the world (3)(23). One difference, however, is that in countries with a high socioeconomic status, motor vehicle accidents involve automobile passengers whereas in lower socioeconomic areas they are more likely to be bicycle or motorcycle passengers and drivers, as well as pedestrians hit by these vehicles (23).
1.3 Pathophysiology

By nature, it is difficult to study \textit{in vivo} human models of mTBI, and thus most information on the pathophysiology has traditionally come from animal models. With advances in neuroimaging and software design, this is an emerging area of brain research.

mTBI is not a single event or pathological process. It is roughly divided up into two phases; the primary insult, which occurs at the point of impact causing immediate damage or deformation to the brain tissue, and the secondary disease process which occurs after the point of impact and can last minutes to months following the primary insult. This secondary process involves metabolic, inflammatory and other changes at the cellular level. The only way to prevent primary impact damage is to either prevent the injury itself or to minimize the impact, for example by the use of safety helmets. The current focus in clinical treatment and research lies in trying to limit or prevent secondary damage once the primary insult has occurred.
It is also important to recognize the diversity of the mechanisms of injury within mTBI. Falls, MVA’s, and assaults may all have similar clinical outcomes, but the mechanical forces that cause the injuries can be very different. For example, the direct blunt force blow of a hammer or fist striking one part of the head is very different than the force of a global acceleration-deceleration injury sustained in an MVA. Yet patients with mTBI as a result of direct blunt force and global acceleration-deceleration will exhibit similar symptoms. This may indicate distinct cellular pathophysiology pathways that ultimately culminate in similar clinical outcomes of mTBI. With our current understanding, ultimately these culminate in similar clinical outcomes of mTBI and as such are treated similarly at present.

Biomechanical models of mTBI suggest symptoms result from rotational force on the brain tissue of the cerebral hemispheres in the anterior-posterior plane around the brainstem, which is a fixed point and acts as a fulcrum (Figure 1.1) (24). Focal brain injuries are more common after a linear acceleration/deceleration whereas diffuse injuries are more common after a rotational force injury. It is more likely though that all injuries are a combination, to varying degrees, of both mechanisms (25).
The mechanical event or injury leads to a secondary insult, which is a complex cascade of events leading to neuronal dysfunction. The true nature of the cascade is difficult to determine with certainty due to the difficulty of collecting real time, in vivo data and the differences between human and animal models. There are several theories about mTBI pathophysiology that will be outlined below.

One of the hallmarks of mTBI is ongoing neurological signs and symptoms after the initial biomechanical force, but an absence of macroscopic neural damage. It therefore follows that microstructural changes result in the damage to cell or physiological function. However, these microstructural changes-most likely multifactorial or from a series of interrelated events-have not been visible on traditional computed tomography (CT) or magnetic resonance imaging (MRI). From the literature, microstructural damage can be divided into the following mechanisms: metabolic, vascular/loss of autoregulation, damage to the blood brain barrier, inflammatory/immune mediated responses and neuronal damage.

1.3.1 Metabolic
Biochemical injury results in loss of the cell membrane integrity and disrupts cellular ion balance. Specifically, resting state ionic balance is disrupted by the efflux of potassium and influx of sodium and calcium ions (Figure 1.2). The elevated loss of intracellular potassium appears to be concomitant with glutamate release. Cell membrane pumps requiring ATP are activated to try and reverse the ionic disturbance. This process initiates cellular hyperglycolysis and a reduction in cell energy stores and subsequent accumulation of ADP. This state of impaired metabolism can last 7-10 days post injury (26)(27)(28).
Cellular calcium is transported into the mitochondria, however this can lead to reduced metabolism due to mitochondrial dysfunction and calcium overload. Mitochondrial calcium overloading is responsible for changes in inner membrane permeability with consequent malfunctioning, uncoupling of oxidative phosphorylation and organelle swelling. These dysfunctional mitochondria become the source of reactive oxygen species (ROS) inducing a phenomenon known as oxidative stress. Overproduction of ROS and an inability to clear them leads to irreversible cell change (26)(27). Figure 1.3 displays the timeline of cellular metabolism after mTBI.

**Figure 1-2** Showing the schematic metabolic changes within the neuron after mTBI


**Figure 1-3** Showing the cellular metabolic changes over time from injury

*Potassium (K+), glucose and glutamate peak and fall within minutes of the injury. Calcium (Ca2+) peaks within minutes and can sustain this peak for several days post injury. Note the reduction in Cerebral Blood Flow (CBF) within minutes and lasting up to 10 days post injury. Giza C, Hovda D. The New Neurometabolic Cascade of Concussion. Neurosurgery. 2014;75:S24–33, by permission of Oxford University Press (26)*
There is also research into the newly reported lymphatic pathway of the brain after mTBI, specifically with impairment of the ‘glymphatic’ pathway after TBI. This recently described pathway, which normally uses a brain wide paravascular pathway and cerebral spinal fluid (CSF) channels as a way for clearance of solutes becomes reduced by up to 60% in mice after TBI (29). This system may also be affected in humans post TBI.

1.3.2 Vascular changes
The importance of adequate cerebral perfusion and oxygenation in the mechanism and the management of TBI is well documented. The Brain Trauma Foundation Guidelines for the Management of Severe Traumatic Brain Injury evaluated the current evidence and made recommendations to prevent hypotension and hypoxia as a tool to limit secondary brain injury (30).

Microvascular changes in the function of brain capillaries have also been described in the pathophysiology of TBI. In rat models the consequences of these changes result in reduced cerebral blood flow and reduced oxygen delivery after the injury (31). The initial metabolic changes and depletion of cellular energy stores are coupled with the normal or reduced cerebral blood flow in the initial stages post injury, which then exacerbates the metabolic changes (26).

1.3.3 Blood brain barrier
The blood brain barrier (BBB) is a physical interface between the brain parenchyma and the vascular system. It is made up of near impermeable endothelial cell junctions that strictly regulate the flow of substrates into the brain. It serves as the highest level of defense to protect the brain from systemic insults. However, after brain injury this physical barrier can be mechanically disrupted and can become leaky (Figure 1.4). This allows the influx of systemic inflammatory cells into the brain and hence potentiating an inflammatory response post TBI and worsening the clinical outcome (27)(32).
1.3.4 Inflammation

Cell damage from the primary injury triggers cellular inflammatory responses from glial cells to repair damaged tissue. This process is called ‘reactive gliosis’ which aims to remove cellular debris and encourage tissue repair. Reactive gliosis involves the activation of glial cells to release inflammatory mediators such as prostaglandins, free radicals, cytokines and chemokines. These messengers in turn activate surrounding glia and neurons via autocrine and paracrine functions. As well as acting on nearby neural cells, they also act systemically to recruit neutrophils, macrophages and lymphocytes from the peripheral immune system into the damaged brain tissue. However excessive or sustained production of pro-inflammatory cytokines is deleterious in the recovery from TBI (32)(33).

Inflammatory cytokines are secreted by the glia and neurons and either work in cell to cell communication or, when derived from glial cells, are mediators for cell growth and repair (Figure 1.5). In both animal and human models examination has found high levels of Interleukin-1 (IL-1) in human CSF and brain parenchyma post TBI, and IL-1 has been shown to exacerbate neuronal injury in animal models. Interleukin-6, Interleukin-10 and Tumour necrosis factor-α (TNF α) are other important cytokines in TBI (32)(34), with similar deleterious effects.
1.3.5 Neuronal damage

Biomechanical forces directly act on neurons and damage their delicate microstructure resulting in loss of structural integrity. Microtubule disruption due to axonal stretch can interfere with axonal transport and can result in axonal disconnection leading to axonal atrophy and shrinkage of the neuron, not always cell death as once thought (26). This is termed Diffuse Axonal Injury (DAI) and is particularly evident after rapid acceleration/deceleration injury mechanisms (25). There are some that regard mTBI as a mild form of DAI (27).

There is still uncertainty across the literature as to the exact pathophysiology of mTBI and there are several potential pathophysiological mechanisms and theories as to the cause mTBI as detailed above. Perhaps there are several concurrent mechanisms or a combination of many that cause mTBI. Also worth considering is the possibility that different mechanisms of injury have different pathophysologies. The debate in this area continues and perhaps advanced neuroimaging techniques can provide clues to the pathophysiology of mTBI.
1.4 Diagnosis

Like most areas of clinical medicine, information is gained by taking an accurate history from the patient but also from eyewitnesses. Often the patients themselves have little or no recall of the events of the mechanism of trauma. Hence there is great value in an eyewitness account of events. In this way, the diagnosis of mTBI is based on the history of the event and clinical examination, which itself is normal in most cases as most mTBI patients have often returned to GCS 15/15 (a normal score) by the time they present for medical attention. More subtle symptoms and signs may include the pale, diaphoretic, nauseated or ataxic patient (35). Visual disturbance such as diplopia or photosensitivity are also common (36).

No robust or reliable radiological or biochemical markers have been developed to diagnose mTBI (3)(37). Candidate biomarkers including glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase L1 (UCH-L1) have shown some use in animal and early human studies, but are not yet reliable enough for clinical use (3)(38). Traditional neuroimaging techniques such as Computed Tomography (CT) and magnetic resonance imaging (MRI) are in most cases reported as normal. However, some new MRI techniques show promise and these will be discussed in the MRI section of this thesis (Section 3).

1.5 Traditional Neuroimaging

Computed Tomography of the head is the front-line investigation tool in most Emergency Departments (ED). With the large number of people presenting to hospital ED following mTBI, there remains a lot of uncertainty as to who to image. The decision to image must balance the financial cost and potential harm from radiation exposure, especially with children, against the risk of not missing a surgically significant pathology such as an extradural haematoma. Many guidelines have been proposed for patient selection to produce the highest yield of significant pathology. The most commonplace method used in hospital ED is the Canadian CT Rule (39). This is based on evidence gained by a prospective cohort study of ten large ED in Canada, totaling 3121 patients with GCS 13-15 after mTBI. The main outcome measures were the need for
neurosurgical intervention and clinically significant brain injury on CT. It identified high and medium risk factors as predictors for clinically significant brain injury. Predictors are summarized in Figure 1.6.

**CT Head Rule is only required for patients with minor head injuries with any one of the following:**

**High risk (for neurological intervention)**
- GCS score <15 at 2 h after injury
- Suspected open or depressed skull fracture
- Any sign of basal skull fracture (haemotympanum, ‘raccoon’ eyes, cerebrospinal fluid otorrhoea/rhinorrhoea, Battle’s sign)
- Vomiting >two episodes
- Age >65 years

**Medium risk (for brain injury on CT)**
- Amnesia before impact >30 min
- Dangerous mechanism (pedestrian struck by motor vehicle, occupant ejected from motor vehicle, fall from height >3 feet or five stairs)

Minor head injury is defined as witnessed loss of consciousness, definite amnesia, or witnessed disorientation in patients with a GCS score of 13–15.

*Figure 1.6: The Canadian CT Rule in decision making of neuroimaging after mTBI*

*Made with data from (39)*

The high-risk factors were 100% sensitive (95% CI 92-100%) in identifying the patients requiring neurosurgical intervention. These criteria would mean 32% of the mTBI population would undergo CT scanning. Further analysis with all seven factors (both high and medium risk) showed 98.4% sensitivity (95% CI 96-99%) and 49.6% specificity (95% CI 48-51%).

It is important to note that alcohol was not an identified factor in this tool and patients on anticoagulation were also excluded from the study. There must be a lower threshold of clinical concern for these two factors as alcohol can mask neurological signs and anticoagulation may potentiate any intracerebral bleeding events. These should be judged on a case by case basis with clinical correlation required. The Canadian CT rule is also not for use in children under the age of 16 years; there are separate clinical guidelines for children. In New Zealand we favour the use of the Starship Children’s Health (SSH) Clinical Guidelines (40) or the ACC Diagnostic Management and Selection for
Imaging of Children and Young People aged <17 years (2). This is outlined in Figure 1.7.

MRI is another noninvasive tool for imaging the brain after mTBI. The use of MRI is not routine in day to day practice, but is useful in cases where the CT scan is normal but the patient has significant or unexplained symptoms. New MRI techniques such as diffusion-weighted, susceptibility-weighted and functional imaging, as well as MR spectroscopy, are starting to be reported in the mTBI literature. Another imaging technique, positron emission tomography, is also an emerging area of research (41)(42).

Regardless of neuroimaging tool used, the most common mTBI outcome is an absence of positive clinical findings and for scans to be reported as normal. Abnormal imaging results such as traumatic subarachnoid blood, small intraparenchymal contusion or even skull fractures do not preclude a diagnosis of mTBI, as the diagnosis is based on clinical history and physical examination.
rather than imaging results. A negative scan does not give any prognostic benefit; patients with normal scans can still have significant mTBI symptoms and develop chronic sequelae.

1.6 Management

The majority of mTBI cases do not require admission to hospital. They can be managed in a patient’s home environment with supportive and expectant management. Symptoms can be characterized as somatic, emotional or neurocognitive; common symptoms of mTBI are tabulated in Table 1.3. Many of these symptoms are extremely common. For example, it is estimated that the incidence of headache alone is as high as 90% at one month and 25% at 1 year post-injury (24).

Table 1-3 Symptoms of mTBI

<table>
<thead>
<tr>
<th>Somatic</th>
<th>Emotional</th>
<th>Neurocognitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>Feeling frustrated</td>
<td>Poor attention/concentration</td>
</tr>
<tr>
<td>Balance disturbance</td>
<td>Anxiety</td>
<td>Slowed response</td>
</tr>
<tr>
<td>Dizziness</td>
<td>Depression</td>
<td>Poor memory</td>
</tr>
<tr>
<td>Restlessness</td>
<td>Irritability</td>
<td>Slowed thinking</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>Emotionally labile</td>
<td></td>
</tr>
<tr>
<td>Visual change e.g. diplopia</td>
<td>Fatigue/low energy</td>
<td></td>
</tr>
<tr>
<td>Insomnia/Sleeping more</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noise sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photophobia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tinnitus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The initial recovery phase is that of complete rest in a low stimulus environment. This phase should last until all the symptoms have resolved and this duration varies person to person. Once symptoms have completely resolved, gradual return to work, school or sport is recommended. Exposure to normal stimuli is introduced in a stepwise manner (21)(43).
Pharmacotherapy is also frequently administered, in the form of simple analgesia and antiemetics with care to avoid opiates, if possible. For those with dizziness, the use of promethazine and vestibular therapy have been shown to be effective treatments (24).

Upon diagnosis, information about what to expect and how to manage symptoms is given to the patient and their family (44)(45)(46)(47). The recovery period for mTBI is followed by the rehabilitation phase. There is no single plan or course of rehabilitation for everyone, rather rehabilitation is individualized to the patient (5). Follow up and further management is with the patient’s General Practitioner (GP) and the ACC Concussion Clinic. The Concussion Clinic provides early intervention rehabilitation and is made up of interdisciplinary services providing assessments and therapies tailored to the individual’s recovery needs (48).

1.7 Prognosis

Most patients that suffer mTBI make a complete recovery by 3 to 12 months following the injury (49). However a proportion of patients will have ongoing symptoms; this is known as Post-Concussion Syndrome (PCS)(36). It is estimated that patients with PCS make up approximately 15% of all mTBI patients at 1 year after their initial injury (35). PCS will be discussed further in section 1.8.

The young and old suffer disproportionately from mTBI. Those less than 20 years-of-age have been found to have a longer recovery and are more likely to experience persistent symptoms than older adults (2)(36). This could be for several reasons, including less skull thickness, incomplete myelination and greater head to body ratio in younger individuals. Within the brain parenchyma, perhaps the immature brain is more vulnerable to biochemical damage (36). The very old are also at higher risk of developing PCS after TBI, with a tenfold increase in risk of adverse outcome compared to the age group 15-25 years (2). Those experiencing a mTBI over the age of 65 are more likely to develop chronic sequelae and have lower functional independence (36)(49). It has been suggested that this could be due to the age related changes in the brain such as reduced brain volume (50).
Repetitive head trauma can result in progressive tauopathy known as Chronic Trauma Encephalopathy (CTE). CTE results in symptoms of memory loss, difficulties with higher executive function, mood and behavior changes, as well as movement and coordination disorders (10)(51). Recent public interest in CTE may give the appearance of this being a relatively new disease entity, but it was described in boxers in the early to mid-1900s (52)(53). Whereas the symptoms of mTBI, and even PCS, are temporary and directly attributable to the injury, CTE is a chronic neurodegenerative condition that manifests often decades after the initial insult. At this stage, it is thought to be related to multiple events of mTBI, and even sub-concussive events, not just one episode (9). As with mTBI the pathophysiology of the condition is also not very well understood. It has been suggested that it is a combination of axonal stretching and subsequent neurodegenerative changes (9). There are many varied post mortem neuropathological changes in CTE, these are illustrated in Table 1.4 below.

Table 1.4 Pathologic changes seen in Chronic Trauma Encephalopathy (CTE)

<table>
<thead>
<tr>
<th>Overall changes</th>
<th>Ventricular changes</th>
<th>Atrophy</th>
<th>Pallor</th>
<th>Microscopic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in brain mass</td>
<td>Enlarged lateral ventricles</td>
<td>Generalised atrophy and reduced brain weight</td>
<td>Locus coeruleus</td>
<td>Tau-immunoreactive neurofibrillary tangles</td>
</tr>
<tr>
<td>Cavum septum pellucidum</td>
<td>Enlarged third ventricles</td>
<td>Frontal and temporal lobes</td>
<td>Substantia nigra</td>
<td>β-Amyloid deposits</td>
</tr>
<tr>
<td>Septal fenestrations</td>
<td></td>
<td></td>
<td></td>
<td>Loss of myelinated fibres</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TDP-43 proteinopathy</td>
</tr>
</tbody>
</table>

While the incidence is thought to be rare, the only real indication is from a post mortem study of retired professional American Football players. Based on
player deaths and those who had a post mortem performed, prevalence is estimated at 3.7% (10). However, it is difficult to generalize these findings as only athletes with signs of the disease had post mortems performed and this is also not a representative sample of the mTBI population.

1.8 Post Concussion Syndrome

PCS frequency and natural history are not certain, but it is estimated that PCS affects approximately 15% of all mTBIs one year after their initial injury (35)(54).

The International Classification of Disease(ICD)-10+ revision (1992) defines criteria for diagnosis of PCS as (8)(24):

A. History of head trauma with loss of consciousness preceding symptoms onset by a maximum of 4 weeks.
B. Symptoms in 3 or more of the following:
   a. Headache, dizziness, malaise fatigue, noise tolerance
   b. Irritability, depression, anxiety, emotional lability
   c. Subjective concentration, memory, or intellectual difficulties without neuropsychological evidence of marked impairment.
   d. Insomnia
   e. Reduced alcohol tolerance
   f. Preoccupation with above symptoms and fear of brain damage with hypochondriacal concern and adoption of sick role

Another definition was presented in the Diagnostic and Statistic Manual for Mental Disorders- IV edition in 2000 (DSM-IV). The DSM-IV criteria for PCS are;

A. “A history of head trauma that has caused significant cerebral concussion. Note: the manifestations of concussion include the loss of consciousness, PTA, and less commonly posttraumatic onset of seizures. The specific method of defining this criterion needs to be established by further research.
B. Evidence from neuropsychological testing of quantified cognitive assessment of difficulty in attention (concentrating, sifting focus of attention, performing simultaneous cognitive tasks) or memory (learning or recall information).

C. Three (or more) of the following occur shortly after the trauma and last at least three months:
   i. Becoming fatigued easily
   ii. Disordered sleep
   iii. Headache
   iv. Vertigo or dizziness
   v. Irritability or aggression on little or no provocation
   vi. Anxiety, depression, or affective instability
   vii. Changes in personality (e.g. social or sexual inappropriateness)
   viii. Apathy or lack of spontaneity

D. The symptoms in criteria B and C have their onset following head trauma or else represent a substantial worsening of preexisting symptoms.

E. The disturbance causes significant impairment in social or occupational functioning and represents a significant decline from a previous level of functioning. In school age children, the impairment may be manifested by a significant worsening in school or academic performance dating from the trauma.

F. The symptoms do not meet criteria for Dementia Due to Head Trauma and are not better accounted for by another mental disorder (e.g. Amnesic Disorder Due to Head Trauma, Personality Change due to Head Trauma).”

While similar these two definitions have some striking differences. The most obvious is that the DSM IV requires symptoms to persist for at least 3 months, as well as having more criteria needed for diagnosis. There have also been studies comparing the difference between the diagnostic accuracy of both definitions. Boake et al. (55) performed the first prospective study comparing
the prevalence and specificity of diagnosing PCS by comparing 178 TBI patients with 104 patients with only extracranial injuries. They found prevalence of PCS was higher using the ICD-10 definitions - 64% ICD-10 vs 11% DSM IV. However, neither definition was specific to TBI as over one third of subjects in the extracranial group also met criteria for PCS diagnosis.

The DSM IV has now been replaced by the DSM V (56) and PCS has been removed and replaced within a category of Mild or Major Neurocognitive Disorders due to Traumatic Brain Injury. It is more focused on cognitive and neuropsychiatric consequences of TBI and the effect on everyday life (57).

There appears to be a variety of interacting factors, both biological and psychological, underlying PCS. There has been considerable debate in the literature as to the origin of PCS symptoms, with many suspecting a psychological rather than biological origin (47)(54)(58)(59)(60). One of the arguments for the psychological basis is that the symptoms of PCS are not specific to this disorder. Rather they can be seen in many other conditions such as chronic pain (CP), baseline psychological conditions such as depression or post-traumatic stress disorder (PTSD) and even to varying degrees amongst the healthy population (8)(43)(61)(62)(63). CP will be discussed in the following chapter (Chapter 2).

mTBI represents a collection of symptoms that are both wide and variable in frequency and severity. Baseline inter-individual differences or pre injury factors may also influence outcomes and development of PCS (36). Pre injury baseline characteristics including young or older age, female gender, lower educational background, poor socioeconomic status, positive psychiatric history or emotional personality traits and alcohol and drug use have been identified as factors that can increase the severity of or yield poorer recovery from mTBI (36)(54)(62)(58). For example, those who used oral analgesics pre injury were more likely to develop PCS; this has been suggested to be evidence for poor coping style (64).

While mTBI itself is more common in males, the development of post mTBI symptoms are said to be higher in females (2)(36)(65). Bazarian et al. (64)
investigated the independent association of sex with outcome after mTBI. Using a study population of 1126 mTBI patients, males were found to have lower odds of having symptoms post mTBI at 3 months than females (OR 0.62, 95% CI: 0.50, 0.78; p<0.0001). However, it is uncertain whether this is a true finding or if it merely represents reporting bias in that females are more likely to report symptoms than males. The human results contrast with experimental animal evidence, with female rats having been shown to have a better cognitive performance compared to male rats following mTBI (66). It was postulated that progesterone and its metabolites are neuroprotective. However, in humans, even when accounting for confounding factors, females have higher rates of post mTBI symptoms (58).

1.9 Diagnosis
There are currently no reliable biochemical or radiological markers for the diagnosis of PCS. As such it is purely a clinical diagnosis based on the clinical criteria outlined above.

1.10 Management
There are several studies which suggest education and the delivery of knowledge and information to a patient about mTBI and PCS helps improve recovery time (3)(43)(54). For example, a face to face consultation and an information sheet with an explanation of mTBI symptoms, including what to expect, how to manage these symptoms, and reassurance that the natural history of mTBI is that symptoms will improve over time, has been shown to improve outcomes (58)(67). This knowledge gives patients reassurance and power to monitor their condition.

Patients with long term PCS ideally require management by an interdisciplinary approach, such as that provided through ACC Concussion Clinics. In these settings, neurocognitive testing and cognitive rehabilitation may be employed. Cognitive Behavioral Therapy (CBT) has been useful in the treatment of mood and anxiety symptoms (43). Pharmacological treatments include a trial of Selective Serotonin Reuptake Inhibitors (SSRI) for depressive symptoms, though duration of use is unclear (43)(67).
1.11 Prognosis

PCS natural history is not well understood. This is possibly due to symptom overlap with numerous mental health conditions and the lack of long term follow up data. However, a prospective long-term study by Zumstein et al. (68) followed 86 mTBI patients, 46 % of the initial study group of 176 patients, 10 years after the initial mTBI. Thirty-seven percent of those patients followed showed a decline in general health and quality of life.

PCS is a significant and debilitating condition for those affected. While some strategies and treatments are available there remains much to learn of the pathophysiology and management of this condition.
2 Chronic Pain and its relation to mTBI

2.1 Introduction

Pain is a normal response to acute injury, but in a small group of patients, pain persists beyond the usual period of healing, and is termed “chronic pain” (CP). Chronic pain has many similarities to mTBI and especially PCS. For example, as with mTBI there are many definitions of CP and these variations make comparisons across studies difficult. The pathophysiology for the development of CP is also not well understood; just like PCS. Questions abound as to why some mTBI patients go onto develop PCS, and why only some acute pain sufferers go onto develop CP? Chronic pain and PCS share clinical features, especially with respect to the time course and psychological factors involved in the development of each condition. Given these similarities, I have used a group with chronic pain to compare with mTBI patients, in addition to a healthy control group. The rationale for this comparison was to evaluate the PCS and CP groups to look for MRI similarities and this will be further discussed in section 2.6. This chapter will discuss in more detail chronic pain and its relationship to mTBI.

2.2 Definition of Chronic Pain

Definitions of CP vary. One of the most comprehensive comes from the International Association for the Study of Pain (IASP) which defines CP as: “pain without biological value that has persisted beyond the normal tissue healing time (usually taken to be three months)” (69). This definition goes further to define the origin of different types of chronic pain including (70):

- Chronic Primary pain: a pain in one or more anatomical regions, which is multifactorial in contribution and causes significant emotional distress.
- Chronic Cancer pain: pain caused by cancer itself or its treatment.
- Chronic Post-Surgical and Post-Traumatic pain: pain developing after a surgical procedure or tissue injury from any trauma and persisting beyond the healing process of 3 months.
- Chronic Neuropathic pain: pain caused by a lesion or disease of the somatosensory nervous system including skin, musculoskeletal and
visceral organs and may involve the peripheral or central nervous system.

- Chronic Headache and Chronic Orofacial pain: headache or orofacial pain on at least 50% of days during 3 months.
- Chronic Visceral pain: pain that is persistent or recurring from internal organs.
- Chronic Musculoskeletal pain: pain arising from bones, joints, muscles, the spine or other related soft tissue.

The current International Classification of Disease (ICD-10) includes chronic pain but doesn’t reflect the epidemiology. The IASP is working in collaboration with the WHO on the upcoming ICD-11 definitions to extend the pain categories similar to those listed above. The ICD-11 is expected to be released in 2018 (71).

The New Zealand Pain Society supports the IASP definition (72). This definition is used at the Pain Management Service, from where the participants of this study have been recruited from.

2.3 Incidence

As with mTBI, Definitions of CP vary. One of the most comprehensive comes from the International Association for the Study of Pain (IASP) which defines CP as: “pain without biological value that has persisted beyond the normal tissue healing time (usually taken to be three months)” (69). Definitions of CP vary. One of the most comprehensive comes from the International Association for the Study of Pain (IASP) which defines CP as: “pain without biological value that has persisted beyond the normal tissue healing time (usually taken to be three months)” (69). In 1999, a UK population-based study analysed a random postal questionnaire of 3605 people (73). It found 46.5% equivalent of the population self-reported chronic pain symptoms. This figure is significantly higher than that quoted in the literature and illustrates the variation in incidence. A similar screening population-based screening study in Europe (2006) surveyed 46,394 participants in different countries and reported a mean prevalence rate of chronic pain of 19%, with a range of 12-30%
between countries (74). These studies illustrate the wide variability in reported prevalence. This variation may well reflect the different definitions of chronic pain as well as the body of patients of patients who ‘suffer in silence’ and may not seek medical attention. New Zealand specific data from the New Zealand Pain Society estimates one in six New Zealanders suffer with chronic pain (72).

2.4 Pathophysiology

The majority of patients with acute pain will fully recover with less than 5% transitioning into chronic pain (75). The mechanisms that drive the progression to chronic pain are not well understood. Several hypotheses have been put forward, including social, psychological, neural or genetic factors, but no firm causation has been established.

Once peripheral pain has been elicited, it is mediated and modulated by spinal and supraspinal mechanisms. Some suggest that neuroplasticity of the central nervous system facilitates transition from acute to chronic pain (76)(77). One potential pathway implicates astrocytes. Astrocytes within the central nervous system play an important role in releasing neurotransmitters and other cellular signaling mechanisms involved at the cellular level in generating chronic pain (78). Upregulation or over expression of neurotransmitters can cause exaggerated pain states. Astrocytes release neurotransmitters, such as the excitatory amino acid glutamate, which in excess increases neuronal excitability in chronic neuropathic pain (79). Astrocytes have also been shown to secrete 1β, a pro-inflammatory cytokine, and nerve growth factor (NGF) which are both involved in the cellular level in the generation of chronic pain (78). These detailed cellular mechanisms involved in the generation of chronic pain are not discussed further as they are beyond the scope of this thesis.

Baseline psychosocial characteristics of patients are also predictive of transition to chronic pain. Linton performed a systematic review of thirty-seven studies to summarize the psychological variables in the development of neck and back pain and concluded: “Psychosocial variables are clearly linked to the transition from acute to chronic pain disability.” (Level A evidence) (80). The strongest psychosocial predictor is a history of depression or low mood. Meyer et al.
examined chronic pain and depression using a Health Outcome Survey of 55,690 subjects 2 years after initial survey and identified a “dose-response” relationship between CP and depression (81). That is, low levels of depression were associated with low levels of chronic pain and high levels of depression were associated with high chronic pain. Other predictive psychosocial factors include patient attitude to pain, somatization, health seeking behavior as well as the strength and duration of the acute pain episode (80)(82)(83)(84)(85).

2.5 Brain changes in Chronic Pain

In recent times, there has been much research with MRI into the brain structure and function of patients with chronic pain. Little is known about the pathophysiology of chronic pain and neuroimaging is being used to explore the possible underlying changes or mechanisms. There is debate as to whether the structural brain changes found on MRI are the cause or the result of the chronic pain. The current evidence suggests these morphological brain changes are the latter, due to neuroplasticity resulting from a noxious stimuli such as chronic pain. Interestingly there have been studies to show that after removal of the chronic stimulus, for example an osteoarthritic hip joint replaced with a total hip joint replacement, as symptoms improve the morphological brain changes also improve and almost return to pre morbid normal limits (76)(86)(87).

Several studies which provide evidence to show structural brain changes in patients with CP, in particular a reduction of grey matter volume. One examined the brain morphology of 10 patients with fibromyalgia compared to 10 healthy subjects. The authors showed significantly less total brain and grey matter volume and a 3.3 times greater age-associated decrease in grey matter compared to controls (88). However, these grey matter changes are not necessarily permanent. For example in a pilot study published in 2009 by Rodriguez-Raecke et al. (86), 10 patients with osteoarthritis of the hip were followed pre and post hip replacement with structural MRI brain scans. At baseline pre-operatively when compared to normal controls, the patients with chronic hip pain had reduced grey matter in the anterior cingulate cortex, right insula cortex, and operculum, dorsolateral prefrontal cortex, amygdala, brainstem and cerebellum. Interestingly they also found that when the same
participants were scanned a second time when pain free post operatively, the resolved pain patients showed increased grey matter volume, in particular in the dorsolateral prefrontal cortex, anterior cingulate cortex, amygdala and brainstem. They suggest that, rather than be caused by brain damage, the morphological changes as a result of the noxious stimuli are reversible so that when the causative stimulus is removed the morphological changes improve (87). Rodriguez-Raecke et al. repeated the study in 2013, this time with 20 patients and monitored structural changes up to a year post hip replacement. They found the same changes, with increased grey matter in the previously listed areas but also in the premotor cortex and the supplementary motor area (87).

All eight studies using volumetric analysis to investigate the structure of the brain in chronic pain showed a reduction in grey matter volume in chronic pain compared to age/sex matched normal controls. Regardless of the location of the pain or pain syndrome mechanism, the most common findings were of reduced grey matter volume in cingulate gyrus, orbitofrontal cortex, the insula and the dorsal pons. The reasons for this loss of grey matter volume are unknown but have been thought to be due to cell atrophy, reduced cell size, or a loss of the synaptic architecture (76).

Functional MRI (fMRI), Positron Emission Tomography (PET), Electroencephalogram (EEG) and Magnetoencephalography (MEG) studies have also been used to further illustrate that the areas of the brain involved with acute pain to various stimuli are the primary and secondary somatosensory, insula, anterior cingulate, prefrontal cortices and the thalamus. These supratentorial anatomical areas are involved in the processing and modulation of pain. The brainstem, particularly the periaqueductal grey matter and the brainstem reticular formation play a role in the descending regulation of pain (89)(90)(91). In normal subjects the acute pain perception pathways were ‘partially’ different from those of participants with chronic pain (92). The review identified that the regions of the brain engaged with chronic pain were the cognitive/emotional regions namely the prefrontal cortex via the spinobrachial, spinothalamic and spinoreticular pathways. It is suggested that chronic pain is less about sensory processing and more likely about increased
emotional and cognitive processing (92). This has also been shown in chronic Complex Regional Pain Syndrome (CRYPS) (93). Early work such as the Gate Control Theory of pain, proposed by Melzack and Wall in 1965, theorised that the interrelated parts of the brain were responsible for the perceptions of pain-thalamus, limbic system, hypothalamus, reticular formation, parietal and the frontal cortex-but also surmised that the emotive responses played a role in the pain response (94).

A longitudinal observational study by Vachon-Presseau et al., used anatomical and diffusion tensor imaging (DTI) MRI to follow acute back pain over 3 years to either recovery or chronic pain, also with a normal control group for comparison. The authors found that a higher incidence of connections within the dorsal medial prefrontal cortex-amygdala-nucleus accumbens was predictive of chronic pain at 1 year post onset of pain (95). This finding suggests that CP is a maladaptive response with addictive corticolimbic properties responsible for its development. They also found smaller morphometric measures of the amygdala and hippocampal in patients with chronic pain (95).

2.6 Chronic Pain and mTBI

As detailed in chapter 1 the symptoms of Post-Concussion Syndrome (PCS) are not particular to mTBI and have cross over into a variety of conditions including chronic pain.

The underlying time course of each condition is similar; both mTBI and the majority of CP patients start with an initialising event-an injury or stimulus. Only a small percentage in each of the mTBI or acute pain groups go onto develop chronic symptoms. Significantly, there are psychosocial factors associated with this progression and baseline pre-morbid psychological conditions and personality traits that will impact the development of both conditions. Symptoms of PCS are similar to those with CP. Studies with direct comparison between PCS and CP show similar impairment across physical, cognitive and somatic domains (61)(63)(96).
Not surprisingly, given the current theory of similar underlying psychological factors in the development of both CP and PCS, there have also been similar psychological and cognitive therapies used for the treatment of both conditions. For example, CBT is used for the treatment of cognitive symptoms in both conditions (43)(97).

Can one take the comparison between PCS and CP a step further and ask if there are common mechanisms contributing to the chronicity of each condition? Furthermore, MRI has already been used to investigate the brain changes associated with chronic pain. Perhaps comparison with MRI changes in PCS might aid understanding of the pathophysiology of both conditions. Thus, I postulate that CP patients comprise a suitable active control group in the investigation of MRI brain changes in mTBI patients.
3 MRI Basic Principles

3.1 Introduction

Magnetic resonance imaging (MRI) is a non-invasive imaging technique that uses magnetic fields and radiofrequency waves to create detailed images of the body. Specifically, MRI utilizes the electromagnetic properties of hydrogen protons, found in the form of water making up 60% of body weight, to form an image. Unlike x-ray or CT, MRI uses no ionizing radiation. The higher magnetic field results in stronger Magnetic Resonance (MR) signals. In clinical practice 1.5T magnets are common in hospitals, but now 3T scanners are becoming more widely used. Today, an MRI scanner is found in almost every major hospital in the western world and is a vital diagnostic tool to physicians. This chapter will outline basic MR Physics and the role of MRI in TBI with further discussion of the physics in the MR techniques used in this study.

3.2 Basic MR Physics

Each hydrogen nucleus contains a positively charged proton, which possesses a constant intrinsic nuclear spin around its own axis. This produces a small magnetic moment, which means that in the presence of an external magnetic field, protons will tend to align with the magnetic moment and begin to precess, which is a circular path around the axis of the external magnetic field (Figure 3.1) [98][99][100].

![Figure 3-1 Illustrating the precession of spin around Bo](Adapted from [101][102])
There is a weak tendency for protons to align with the external magnetic field (longitudinal, or B0 direction) forming the net magnetization vector (NMV). When a radiofrequency plus (RF) is applied at the resonant frequency (the frequency of the precessing protons, also called the Lamor frequency), the NMV is moved out of the B0 plane (Figure 3.2). The NMV is rotated away from the direction of the large external magnetic field (B0); the resulting change in direction is called the flip angle. The flip angle itself depends on both the duration and amplitude of the RF pulse. The frequency required to cause this rotation away from the B0 direction is called the Lamor frequency. The Lamor frequency is given by the Lamor equation (98)(101)(99):

$$\omega = \gamma B$$

\(\omega\)= Lamor frequency in megahertz  
B= the strength of the magnetic field in T  
\(\gamma\)= the gyromagnetic ratio (a constant value specific to each type of nucleus, for hydrogen 42.5 MHz/T)

The Lamor equation is the cornerstone of MRI physics as it describes the relationship between the magnetic field and the spin (proton) precession.

Once the NMV is rotated away from the longitudinal plane into the transverse plane, the signal fades back to its equilibrium state via two processes. The first is called T1 relaxation or ‘spin-lattice interaction’. This is where excited protons lose excess energy to the surrounding environment (or lattice) and return to their original equilibrium orientation. The T1 time of a tissue is defined as the time it takes for 63% of the equilibrium longitudinal magnetization (NMV) to recover in a tissue. The second is called T2 relaxation,
or spin-spin relaxation. This occurs as energy is transferred between adjacent spins (protons). The T2 time is defined as the time it takes for the transverse magnetization to drop to 37% of its original size (Figure 3.3). Of note, for any tissue T2 is always shorter than T1. Different tissues take different amounts of time to return to equilibrium, hence they have different T1 and T2 times. For example, fluids have longer T1 and T2 times compared to more solid tissues that contain higher amounts of lipids or proteins, which have shorter T1 and T2. These differences give rise to a cornerstone of MR imaging and by tuning various imaging parameters one can exploit differences in T1 and T2 values to create images with different contrast (98)(99)(102)(103).

![Figure 3-3 Schematic graph showing T1 recovery and T2 relaxation time](modified from 100 (104)

After excitation, the precessing NMV is tipped into the transverse plane. This rotating magnetization, in accordance with Faraday’s law of induction, induces a current within the receiver coil and provides the raw signal for the MR signal. This detected signal is then reconstructed to form the image (104)(98)(101)(6).

The digital image is three dimensional and made up of voxels (a 3D pixel). Each voxel is assigned a value that corresponds to a signal intensity. A voxel is a cube usually measuring 1-2mm on a side and the higher the resolution of the MRI image, the smaller the size of the voxels (104)(101).
3.3 MRI in TBI

With the lack of traditional radiological evidence of brain injury combined with the advent of advanced MRI technologies, there has been increasing MR evidence of the structural, functional and metabolic imaging changes in patients with mTBI. The most robust findings have come from Diffusion Tensor Imaging (DTI), with findings in regional and whole brain degradation both in acute stages of injury with the detection of haemosiderin staining and microhaemorrhage and chronic changes detailed in Chapter 4 (Figure 3.4) (105).

With reference to advanced MRI techniques and their use in TBI the White Paper from the Journal of the American College of Radiology 2015 states “there is insufficient evidence supporting the routine clinical use of advanced neuroimaging for diagnosis and/or prognostication at the individual patient level (class IIb recommendation)” (42). At present these advanced MRI techniques are still being assessed for validity and effectiveness before use in routine clinical practice.
3.3.1 Structural
Structural MRI is a static image of the brain, providing high resolution, anatomical information. A number of MRI sequences can be used, however, in this thesis, I used T1-weighted images to investigate atrophy (106). Extensive preprocessing of the structural data facilitates segmentation into grey, white and CSF maps, allowing investigation of volumes (106). Thickness of the cortex can also be calculated. Common brain segmentation packages include SPM, FSL, (107)(108)(109) and FreeSurfer (110); all three have been used in this study.

3.3.1.1 Cortical Thickness
The cerebral cortex is comprised of layers of folded neurons. The thickness of the cortex has been used in the study of neurodegenerative conditions and emerging research in mTBI. However, the thickness of the brain cortex is not uniform due to different arrangement of cell architecture. For example, Brodmann’s area 3 (primary sensory area) on the posterior aspect of the central sulcus is only 2mm in average thickness, as it contains tightly packed granule cells, whereas Brodmann’s area 4 (primary motor area) on the anterior aspect of the central sulcus, is often more than 4mm in thickness as it contains giant pyramidal cells of Betz. Regional variations and the highly sensitive nature of brain anatomy make it a difficult task to manually trace. It takes several days to manually trace the entire brain and even then, this may measure volume rather than thickness. This is because to measure thickness the location and orientation of the grey/white matter and the pial surfaces must be precisely known (111)(112). New computer modelling software has become available to calculate cortical thickness. Over the years this software has been refined as initial programs struggled with accuracy due to the close proximity of the deep sulci not being penetrated by the models causing partial volume effects (112). Freesurfer is a software developed out of Harvard (111), specifically designed to calculate cortical thickness across the entire brain. This is one approach I will use in this thesis.

3.3.2 Diffusion Tensor Imaging (DTI)
DTI has been used to investigate microstructural white matter change after mTBI (105)(42)(113). Diffusion MR techniques were first described by Stejskal and Tanner in 1965 when they outlined a diffusion pulse sequence, which still
forms the basis of modern DTI (114). However, it wasn’t until the 1980s that the technology was routinely available on clinical MRI machines (115). The first published literature of the use of DTI and mTBI was in 2002 by Arfanakis et al. (116) and since then there has been a large amount of research performed. An advanced PubMed search with the terms ‘mild traumatic brain injury’ AND ‘diffusion tensor imaging’ yielded 247 results in the last five years alone.

Diffusion is a primary biological process that describes the microscopic movement of molecules due to thermal motion. This process is also referred to as Brownian motion, and forms the basis of DTI. Diffusion follows a Gaussian process, where molecules move randomly over a distance and can be described by the diffusion coefficient ($D$). $D$ is dependent on size of the molecules, temperature and the viscosity of the medium (117)(118)(119)(120).

Diffusion is affected by the inherent properties of the surroundings in which this process occurs. Isotropic diffusion is the process by where molecules travel equally in all directions. In the brain, isotropic diffusion occurs in the CSF of the ventricular system as there is little restriction of movement (Figure 3.5, left panel). In other tissues, such as highly ordered and organized white matter, the diffusion of water molecules is direction dependent, known as anisotropy diffusion (Figure 3.5, right panel). In the white matter of the brain, water molecules diffuse more easily parallel to the long axis of the axons rather than perpendicular to the tract, so in white matter the direction of water movement is the direction of the fiber bundle. Also in different body tissues water molecules interact with cell membranes which impede its movement. The movement of water between the intra and extracellular spaces, within the extracellular space itself, and tissue cellularity can also affect diffusion. The net movement of molecules across an area of tissue per second is called the Apparent Diffusion Coefficient (ADC), and is reported as cm$^2$/s or mm$^2$/s. Where there is free (unrestricted) diffusion, the ADC value is high, whereas with restricted diffusion the ADC is low (99)(101)(104)(121)(117)(119)(122).
3.3.2.1 Generation of Diffusion Data

In diffusion imaging an external parameter called the $b$ value reflects the strength, duration and spacing of the pulsed gradients used to generate the diffusion weighted images. The larger the $b$ value, the more diffusion weighting in the final image, however this comes with increased signal noise and possibly more mechanical vibration artefacts. The $b$ value range can vary substantially depending on acquisition, but most published literature uses a standard $b$ value of $1000\text{s/mm}^2$. The $b$ value is defined by the Stejskal and Tanner equation which uses the excitation pulse given between a pair of gradient pulses (Figure 3.6) (101)(113)(114)(121)(122).

Stejskal and Tanner equation;

$$b = \gamma^2 G^2 \delta^2 (\Delta - \delta/3)$$

$\gamma$= gyromagnetic ratio  
$G$= amplitude  
$\delta$= duration  
$\Delta$= time interval of the diffusion gradient

The equation can be graphically represented as follows:

![Diagram of a diffusion-weighted spin echo sequence](image)

*Figure 3-6 Diagram of a diffusion-weighted spin echo sequence*

The two trapezoidal waveforms on the $x$ gradient ($Gx$) either side of the $180^\circ$ RF pulse sensitize the MR signal to the diffusion in the $x$ direction. The degree of diffusion weighting ($b$-value) depends on the timing and
The tensor is the most common way to describe the multi-directional properties of diffusion. The diffusion tensor is represented as a 3x3 matrix which represents the movement of water in 3-D (Dxx, Dxy, Dyz, Dyz, Dyy, Dzx, Dzy, Dzz), with x, y and z being x-axis, y-axis and z-axis. To determine the tensor, diffusion must be measured in at least six different directions. An additional non-diffusion weighted image is also needed as a reference.

The diffusion tensor can be represented by an ellipsoid (Figure 3.7). The long axis represents the direction of greatest diffusion, called axial diffusivity ($\lambda_1$, principle eigenvalue and its direction is the corresponding eigenvector). The minor axes are used to describe radial diffusivity ($\left(\lambda_2 + \lambda_3\right)/2$). Using the axes, it is possible to calculate other values that can provide information about axonal structure and integrity.

Fractional anisotropy (FA) is a scalar measure that quantifies degree of angular variation of the diffusion of water. Values vary between 0 and 1; where 0 represents isotropic diffusion, i.e. a perfect sphere, and 1 represents diffusion in a single direction only, where the diffusion ellipsoid is a thin cigar-like shape. Therefore, areas of highly organized white matter have a high FA value, as seen in the corpus callosum, and areas without a preferred direction or structure, as seen in CSF, have a low FA value. The direction of the white matter tracts can be displayed using the directional information and the anisotropy measures of the individual voxels. This is known as tractography and allows the in vivo study of white matter tract anatomy.
The other most common measure that can be calculated from the diffusion tensor is the mean diffusivity (MD). MD gives average diffusion over all directions and is the same as the ADC. For the most part, FA and MD are inversely related. FA decrease and MD increase have been associated with loss of tissue integrity (117)(118).

### 3.3.2.2 The Assessment of White Matter Tracts

There are a number of approaches to analyzing brain anatomy using DTI (122)(123). In the Region of Interest (ROI) approach, specific ROIs are identified, either manually or via automated methods. DTI metrics, usually FA and MD, are then extracted from these ROIs and entered into statistical models (124)(125)(126). The ROI method has the benefit of expert knowledge leading the identification of specific regions of interest in the brain, but there are a few limitations. Manual tracing is very time consuming with the potential for variation of inter- and intra-user differences in the processing of images and identification of regions (122)(124)(125)(127). Whole brain voxel based analysis (VBA) facilitates making statistical comparisons across the whole brain, without determining a priori ROIs. In VBA, spatial normalization is performed by warping the individual image into a standard space and then performing voxel wise analysis. The standard space template can be an average image of the whole group analysis, but is typically defined by the Montreal Neurological Institute (MNI) (128) atlas. An example of DTI VBA is Tract Based Spatial Statistics (TBSS), which is implemented as part of the FMRIB Software Library (FSL) (129). TBSS has been used in this thesis research (122)(130). A further method of analyzing diffusion MRI is fiber tractography, which specifically identifies individual white matter pathways. Tractography uses diffusion data to reconstruct white matter tracts in two ways; deterministic or probabilistic. Deterministic uses the principle direction of diffusion in each voxel to reconstruct the fiber pathways. Probabilistic tractography uses probability distributions to reconstruct tracts (117)(131).

Advanced MRI techniques are becoming popular in mTBI literature and the next chapter will review the body of literature and the current knowledge and common findings in this area.
4 Advanced Neuroimaging and mTBI Literature Review

4.1 Structural Findings in mTBI Literature

There is considerable literature evaluating the white matter changes that occur after mTBI using diffusion MRI, but there is far less published data studying grey matter volume and cortical thickness post mTBI. Overall there are only a few small studies looking specifically at structural changes post mTBI and the heterogeneity of these, both in TBI type and sample demographics, makes any consistent conclusions difficult.

Tate et al. (132) studied twelve military personnel that had suffered blast injuries and sustained mTBI on average three months post injury. Compared to a control group of their colleagues that did not have mTBI, there were statistically significant differences between the two groups with reduced cortical thickness in the superior frontal gyrus and the superior temporal gyrus in those that sustained blast mTBI. However, this group also suffered audiology complaints such as hearing loss or tinnitus. As the areas affected are involved in speech reception, the reduction in thickness could be due to the audiological damage rather than the mTBI injury, per se (132).

Ling et al. (133) performed a prospective study with 51 mTBI patients matched with healthy controls and acquired structural imaging twenty-two days post injury, and did not show any significant difference in cortical thickness or whole brain volume between the mTBI and the control groups. A smaller group of twenty-seven mTBI and matched controls returned at approximately four months post injury for repeat imaging and again there were no differences between the two groups (133). One factor to take into account is the short time course from injury to scan time. Perhaps four months is not long enough for cortical thickness or atrophy changes to develop. Another study by Wang et al. (134) also used similar methods and looked specifically at cortical thickness. They found reduced cortical thickness in the mTBI group three months post injury in the left rostral middle frontal gyrus, but this group was small (n=11), and all the mTBI patients had been in an MVA as their mechanism for injury. A ‘normal’ healthy control group would have been an advantage in such a study (134).
Zhou et al. (135) followed 28 mTBI patients and 22 controls one year post injury and found significant structural changes in the mTBI group. They found atrophy in the right precuneus. The right inferior and medial orbital olfactory frontal regions also showed atrophy but this did not reach statistical significance (135).

4.2 DTI in mTBI

4.2.1 Findings
Fractional anisotropy (FA) is the most common DTI metric in the mTBI literature (42)(113). There is a mixture of findings, but the majority suggests a decrease in FA in white matter tracts after mTBI. The decrease in FA suggests a loss of normal neuronal architecture, indicative of axonal disruption or increased oedema within the white matter tracts or the perivascular spaces (123). However many studies do not find any statistically significant differences post mTBI (42)(113)(123)(136)(137)(138). Overall the heterogeneity of the literature in terms of groups definitions, scan protocols, time intervals and small sample sizes combined with a likely positive reporting bias makes me question the strength of the mTBI literature in this area.

A quantitative literature review by Hulkower et al. (113) in 2013 looked at 100 studies examining DTI in TBI of all severities with a combined patient number of 2337 subjects (113). Abnormal FA in the corpus callosum was the most common abnormality in all methods of white matter tract analysis. A subgroup analysis to separate findings by TBI severity was not performed and all severity mild-severe were analyzed together, which is a weakness of the review. In 25 studies that used whole brain analysis, 17 showed abnormal FA in either the anterior/genu body or corpus callosum overall. Abnormal FA was also reported in papers that used Region of Interest (ROI) and tractography. Other areas of significant FA abnormality include the internal capsule, the fronto-orbital fasciculus, fornix, cingulum bundle and superior and inferior longitudinal fasciculus. In 96 out of 100 studies, FA values were reduced in patients with TBI, with four of the studies finding elevated FA values (113). While these four studies reporting increased FA may appear to be false positives, increased FA may be indicative of damage, specifically in areas of crossing fibres where a single fibre population is affected (139).
Hulkower et al. also looked at abnormal mean diffusivity (MD) and found similar results in the same anatomical white matter tracts observed for FA. An interesting finding was in almost all ROI and tractography studies, MD values were abnormally low in mTBI relative to controls. This seems counterintuitive given the findings of low FA. On reviewing the supplementary table and some study references themselves which report high MD, I suggest this is most likely error and the findings mislabeled in the Hulkower paper. Looking at several of the referenced studies, the reported change in MD should read as increased (140)(141)(142)(143). Increased MD values months after mTBI could represent neuronal cell loss (144). Thirteen studies that used whole brain analysis reported varying results but with a maximum of only six studies finding increased MD in analyses (113). This further adds to the heterogeneity of the literature findings.

Within the Hulkower et al. quantitative analysis 72 of the studies looked at functional outcome of subjects and the relation to DTI findings. Measures and methods of neuropsychological testing varied between studies, but overall, there was a positive correlation between cognitive measures and FA, and a negative correlation between cognition and MD. With regard to general clinical outcomes, such as Glasgow Coma Scale (GCS), global outcome measures and Post-Concussion syndrome (PCS) symptoms, the majority again found a positive correlation with FA and negative correlation with MD (113), suggesting an underlying relationship between white matter health, cognitive measures, and clinical outcome.

A meta-analysis by Aoki et al. (123) in 2012 included 13 studies with a total of 280 mTBI patients and 244 controls; 15 individual comparisons were investigated (123). All studies used FA, but only seven reported on MD. The corpus callosum showed a decrease in FA ($p=0.023$) and increased MD ($p=0.015$) compared to controls. Specifically, when analyzing sub regions of the corpus callosum, only the splenium showed a significant decrease in FA ($p=0.025$) and increase in MD ($p=0.0013$) in the mTBI group relative to controls. The corpus callosum may be at increased risk of damage during mTBI in that it is the largest volume white matter tract in the brain, and the splenium may be
particularly vulnerable; the splenium’s posterior position adjacent to the immobile falx cerebri may make it more susceptible to shear/strain injury (105)(123).

Aoki and Inokuchi (145) undertook a second meta-analysis published in 2016. The difference compared to their previous meta-analysis is that they performed a primary meta-analysis to analyze FA regional differences between control and mTBI in seventeen TBSS studies with 529 mTBI patients and 373 control patients (145). The secondary analysis used whole brain voxel based and TBSS studies (583 mTBI patients and 414 controls patients). Both the primary and secondary analysis showed reduced FA in three regions- the left thalamus extending into the splenium of the corpus callosum, the forceps minor and the superior longitudinal fasciculus (145).

Khong et al. performed a systematic review of DTI and PCS in 2016 (138). A literature search yielded 205 studies, of which 10 were included in the systematic review. The study used PCS defined as “patients who experience persistent symptoms for 3 months or longer post-injury” (page 2) (138) and excluded studies that didn’t define PCS or mTBI or had fewer than six participants. These 10 studies included 235 patients with PCS (141 male and 44 female) and control groups were: orthopaedic injuries in one study, uninjured military controls in two studies, and the other seven studies used healthy controls. DTI analysis methods included whole brain analysis in four, ROI analysis in four, and a voxel-wise approach to identify ROIs for ROI analysis in two studies. The median time from injury to MRI scan was 20.5 months (range 7 days to 259 months). Seven out of the 10 studies used MRI data collected after the diagnosis of PCS, whereas three collected images in the acute stage before the development of PCS. Thus, this review may be able to comment on the use of DTI as a diagnostic tool as well as a predictor of the development of PCS in the acute stage after the head injury. Seven out of 10 studies found reduced FA in patients with PCS post mTBI compared to controls. The most consistent finding was abnormal DTI metrics in the corpus callosum. However, three out of 10 studies did not find any changes in FA. Seven studies reported increased MD and three did not. Interestingly only one study found no change in either FA or MD. The conclusion of this systematic review was
that “no DTI biomarker for PCS is identified due to the small body of research conducted on the topic and the heterogeneity of results reported” but it did conclude that “DTI abnormalities correlate with PCS incidence and symptom severity” (138).

A systematic review by Dodd et al. (146) in 2014 evaluated 31 studies for the diffusion findings but also looked at clinical and methodological factors that could influence results in semi-acute mTBI patients (146). While 13 studies showed reduced FA, 11 reported increased FA, two reported bidirectional finding and five reported no significant change in FA. The pertinent outcome from that systematic review is that chi squared analyses showed the “total number of diffusion images was significantly associated with findings of either increased (DW>30) or decreased (DW<25) anisotropic diffusion” (146). Year of publication and mTBI sample size were not associated with an increase or decrease in FA (p=0.10) (146).

4.2.2 Variability in the literature
The large variability in the findings of advanced imaging in mTBI in the literature may be due to several factors. The lack of a unifying single definition of mTBI or PCS has resulted in considerable heterogeneity of patient samples, making the identification of relevant imaging markers more difficult. Other differences in baseline demographics such as age, sex, and education level has also influenced findings. The different mechanisms of injury potentially introduced confounding into studies as pathophysiology has been presumed to be the same despite different mechanisms. Study sample sizes have also typically been small, leading to limitations in generalizing from these results. Differences in MRI scanners, e.g. 1.5T vs 3T, image acquisition, and also the time from injury to the time of scan are other important variables that lead to heterogeneity in the literature (113)(123)(146).

Timing of the MRI scan in relation to the time from the injury is important as the pathophysiology is different across the time period. For example, in the acute phase in the days after injury, there is disruption to neuronal architecture, cytotoxic oedema, and neuronal swelling, which subsides over time. Increased FA initially may represent these changes whereas increased
MD values months after mTBI could represent neuronal cell loss (42)(116)(144). The differences in timing of the MRI scan in the disease process can also vary between studies and make direct comparisons difficult. The control group used for comparison can also introduce potential confounds; appropriate control groups are vital to move TBI research in a positive direction. In many cases, controls may not have been exposed to trauma itself and therefore brain changes could be because of the stress of the trauma, rather than the TBI itself (113)(14). It is expected that findings will vary depending on whether TBI patients are compared to perfectly healthy controls, controls who have experienced a TBI but who have recovered well, controls who have experienced some sort of non-cranial injury, or those who have some other condition (e.g. chronic pain).
5 Methods

5.1 Introduction

The work presented within this thesis is part of a larger mixed methods exploratory study into mTBI, with emphasis on chronic PCS symptoms and early identification of at risk individuals. The long-term aim of the broader study is to identify methods capable of selecting patients at risk of developing PCS in order to implement early intervention strategies to improve outcomes after mTBI.

Ethical approval has been given by the Upper South A Regional Ethics Committee reference URA/12/05/015/AM10 (see Appendix).

5.2 Aims/hypotheses

The global aim of this thesis is to explore structural and diffusion MRI techniques in relation to mTBI and PCS. Specifically, I will ascertain whether structural and DTI brain measurements differ in the PCS group compared to the non-PCS and control groups. In this way this thesis may provide relevant information on methods capable of predicting development of PCS, which may ultimately lead to improved management of resources and services for those at risk of developing PCS.

I hypothesize that there will be structural and DTI changes in the PCS group compared to non-PCS and controls. Hence the null hypothesis is that there will be no MRI differences between the PCS group compared to non-PCS and controls.

As a secondary analysis I will review the mTBI group, both PCS and nonPCS, as one group to investigate the relationship between MRI and outcome measures; GCS, PTA, HADS A, HADS D, RPQ, RBANS total score, CWIT III and IV.
5.3 Subjects

Data previously collected from 138 participants were used for this study. This section describes the recruitment sources for each of the four study groups. The two mTBI and chronic pain participant groups were part of a Health Research Council funded study conducted at Burwood Hospital, Christchurch, New Zealand. mTBI was defined using the New Zealand Guidelines Group criteria set (2) and participants with mTBI were recruited from the Concussion Clinic at Burwood Hospital. Forty-two subjects met ICD-10 criteria for PCS and comprised the PCS group. Thirty subjects deemed fully recovered from mTBI comprised the non-PCS group. There were two control groups used for comparison against the PCS and non-PCS groups. The first comprised a group of 25 subjects recruited from the Pain Management Service at Burwood Hospital. Chronic pain (Chronic Pain Syndrome) was diagnosed by each participant’s treating physician. In addition for the MRI analysis a group of 41 healthy control participants were recruited from other ongoing studies at the New Zealand Brain Research Institute (NZBRI)- these participants were not part of the original mTBI study additional ethics approval was obtained (see Appendix). The PCS, non-PCS and CP groups were all matched for age, gender, education, injury, severity and pre-injury work status. The healthy control group was matched only for mean age and sex distribution as education and work status were not acquired (as they were not part of the original study) and there was no injury. Group demographics are displayed in Table 5.1.

Sample size in our study (PCS=42 and Non-PCS=30), and $\alpha=0.05$, there was 80% power to detect a difference in means of $d=0.67$ (two-tailed). The original study was PCS and non PCS and CP group and this was studied on the PCS vs non-PCS group.

In the mTBI groups (PCS and non-PCS), Glasgow Coma Scale (GCS) was acquired at the time of first acute presentation to medical services, i.e. at Emergency Department presentation; similarly, the time spent in Post Traumatic Amnesia (PTA) was also recorded (in days). The mechanism of injury was not collected. The time from injury to the time of the MRI scan (in months) was also collected for the mTBI groups, and where applicable, this
was also obtained for the chronic pain group. The PCS, non-PCS and CP groups all underwent neurocognitive testing including: the Repeatable Battery Assessment of Neuropsychological Status (RBANS), which tests across five different cognitive domains and then provides an overall total score (147). This test is administered over 20-30 minutes by a trained psychologist (148); the Colour Word Interference Test (CWIT) from the D-KEFS Battery (149), which measures ability to inhibit a dominant automatic verbal response, speeded cognitive processing and cognitive flexibility. Four scaled scores can be calculated; the Rivermead Post-Concussion Symptom Questionnaire (RPQ), which is written self-assessment tool used for diagnosis and to assess severity of mTBI symptoms (150); Hospital Anxiety and Depression scale (HADS A and HADS D), another written self-assessment is used to assess severity of anxiety and depressive symptoms (151). The healthy control group did not undergo neurocognitive testing and were only included for the MRI analyses.

Differences in the clinical data, symptoms and neurocognitive scores across the two mTBI groups were assessed using one way ANOVAs, with post-hoc pairwise comparisons; p<0.05 was considered significant.

| Table 5-1  Study sample demographics |
|----------------|----------------|-------------|-----------|
|                | PCS            | Non-PCS     | CP        | Controls  |
| Number         | 42             | 30          | 25        | 41        |
| Mean age (years) | 47.3 (SD 14.47) | 43.9 (SD 13.73) | 47.4 (SD 13.68) | 46.2 (SD19.2) |
| Gender (%female) | 40             | 50          | 70        | 70        |
| Time of injury to scan (months) | 30.9 (SD) | 37.6 (SD) | NA        | NA        |

5.4 MRI acquisition

All imaging was performed at Hagley Radiology on a 3T General Electric HDxt scanner (GE Healthcare, Waukesha, WI) with an 8-channel head coil. Subjects
were given ear plugs and/or headphones and asked to remain as still as possible during the examination.

Structural MRI images were acquired using a high resolution volumetric T1-weighted sequence and DTI images, with a 2D diffusion-weighted, spin echo, echo planar imaging sequence, with diffusion-weighting in 64 uniformly distributed directions, and 8 images without diffusion-weighting. Details of the image acquisition are detailed below in Table 5.2.

Table 5-2  MRI image acquisition parameters

<table>
<thead>
<tr>
<th></th>
<th>T1-weighted image</th>
<th>Diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse name</td>
<td>SPGR</td>
<td>Spin echo EPI</td>
</tr>
<tr>
<td>Imaging mode</td>
<td>3D</td>
<td>2D</td>
</tr>
<tr>
<td>Imaging plane</td>
<td>Axial</td>
<td>Axial</td>
</tr>
<tr>
<td>Repetition time (TR)</td>
<td>6.7ms</td>
<td>86.4ms</td>
</tr>
<tr>
<td>Echo time (TE)</td>
<td>2.8ms</td>
<td>13,000ms</td>
</tr>
<tr>
<td>Inversion time (TI)</td>
<td>400ms</td>
<td>NA</td>
</tr>
<tr>
<td>Flip angle</td>
<td>15°</td>
<td>90°</td>
</tr>
<tr>
<td>Field of view</td>
<td>250mm</td>
<td>240mm</td>
</tr>
<tr>
<td>Slice thickness</td>
<td>1mm</td>
<td>3mm</td>
</tr>
<tr>
<td>Gap (between slices)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Matrix</td>
<td>256×256</td>
<td>128×128</td>
</tr>
<tr>
<td>Voxel size</td>
<td>0.98×0.98×1mm³</td>
<td>1.88×1.88×3mm³</td>
</tr>
<tr>
<td>Number of slices</td>
<td>170</td>
<td>48</td>
</tr>
<tr>
<td>Coverage</td>
<td>Whole brain</td>
<td>Whole brain</td>
</tr>
<tr>
<td>Parallel imaging</td>
<td>ASSET</td>
<td>-</td>
</tr>
<tr>
<td>Acceleration factor</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>Scan time (minutes)</td>
<td>5:07</td>
<td>7:09</td>
</tr>
<tr>
<td>Diffusion directions</td>
<td>NA</td>
<td>64 uniformly distributed directions</td>
</tr>
<tr>
<td>Acquisitions without diffusion weighting (b value= 0)</td>
<td>NA</td>
<td>8</td>
</tr>
<tr>
<td>b value</td>
<td>NA</td>
<td>1000s/mm³</td>
</tr>
</tbody>
</table>
5.5 MRI pre-processing

5.5.1 Structural

5.5.1.1 Grey matter volume

Image segmentation was performed using SPM12 (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, The Institute of Neurology, London, UK) in Matlab 2015a (Mathworks, Massachusetts, USA 2015). SPM is a software package designed for the analysis of brain imaging data, providing a common framework for image processing and analysis. SPM implements a general linear model and uses a voxel-based approach to analyze the entire brain.

The T1 weighted structural image for each individual was segmented into grey matter (GM), white matter (WM), and cerebral spinal fluid (CSF). We then ran DARTEL (existing template) using the template provided with the VBM8 toolbox (in MNI space, http://dbm.neuro.uni-jena.de/vbm/). GM segments were then normalized using the DARTEL flow fields, modulated, and smoothed (8 mm FWHM). Lastly, a GM mask was created by averaging all smoothed normalized, modulated GM segments, thresholding at 0.15.

A Matlab script was created to calculate the volume of GM, WM and CSF segments for each subject (in subject space). Total intra cranial volume (ICV) was calculated as the sum of the GM, WM and CSF segments; ICV was used as a covariate in later analyses.

5.5.1.2 Cortical thickness

Automated cortical thickness measurements were performed using Freesurfer (version 5.3; available at: http://surfer.nmr.harvard) (111).

Pre-processing of T1 images included: removal of tissue outside the brain from the images, transforming to automated Talairach space, intensity normalization, defining the grey/white matter boundary, correction of topology and placement of the grey/white matter and grey matter/CSF boundaries. Each step was performed independently for each subject. Cortical thickness is calculated as being the distance between grey/white matter and
grey matter/CSF boundaries. Next all surface models in each individual subject were manually inspected to ensure accurate tracing had been performed. The subject template was loaded and opened in Freeview (an imaging viewer programme) and then each of the left and right pial surfaces and left and right white matter outlines were overlaid and inspected for each of the 138 subjects. There were no significant anomalies in the tracing and hence none required manual correction. Cortical maps were smoothed using a circularly symmetric Gaussian kernel across the surface with a full width at half maximum (FWHM) of 10 mm.

5.5.2 DTI
MR image pre-processing and statistical analyses were performed using tract-based spatial statistics (TBSS) in FSL 5.0 (www.fmrib.ox.ac.uk/fsl). Correction of motion- and eddy current distortion on the DWI images was performed. Using DTIFIT the diffusion tensor was then calculated at each voxel, producing fractional anisotropy (FA) and mean diffusivity (MD) images. Then the brain extracted using BET. The FA images were matched to a common space (FMRIB58 FA template) using the nonlinear registration tool FNIRT. The mean FA image was thinned (FA 0.2) to create a mean FA skeleton that represented the middle of all tracts common to the group. Each subject’s aligned FA image was then overlaid onto this common skeleton image. This step minimizes misalignment that is prevalent in standard DTI registration procedures. To create a separate skeleton representing MD values, the nonlinear warps and skeleton projection were then applied to MD images.

5.6 Statistical analyses

5.6.1 Demographic analyses
One way ANOVAs, implemented in R (version 3.3.1), running from R studio (Boston, MA, USA), were used to test for statistically significant differences in demographics, clinical measures (GCS, PTA), and neurocognitive measures (HADS, RPQ) across the groups (PCS, Non-PCS, CP and Controls). Significant ANOVAs were followed by pairwise Tukey’s tests.
5.6.2 Structural imaging analyses
5.6.2.1 Grey matter volume
Smooth, modulated and normalized grey matter images were entered into an ANCOVA model in SPM to assess differences in grey matter volume across the PCS, Non-PCS, CP and Control groups, with age, sex and ICV as covariates. Contrasts allowing investigation of pairwise comparisons between all four groups were tested (Table 5.3).

In a sub group analysis restricted to participants that had experienced a mTBI (i.e. the PCS and non-PCS groups), a series of multiple regression models were performed to investigate the relationship between grey matter volumes and (1) GCS, (2) PTA, (3) HADS A, (4) HADS D, (5) RPQ, (6) RBANS TS, (7) CWIT III and (8) CWIT IV with age, sex, and ICV as covariates.

5.6.2.1.1 Multiple Comparisons
With MRI data, each subject volume contains on the order of a few 100,000 voxels. As separate hypothesis tests are performed at each voxel, high false positive (α error) rates can lead to inaccurate statistical results. For example, using a voxel-wise α value of 0.05, where you would expect 5 false positive for every 100 tests, suggests a total of 5,000 false positive results, which are indistinguishable from true positives. To limit the likelihood of obtaining these high false positive results, we can correct for multiple comparisons by one of two ways: Family-wise error rate (FWE), which is the likelihood of obtaining at least one false positive in a family of tests; or False discovery rate (FDR), which is the proportion of false positive results among all the significant results. At the level 0.05, we know 95% of results are likely to be true and no more than 5% of the voxels above the threshold will be false positives. However again, we still won’t be able to determine which are the truly correct and which are the false positives (152). Hence, cluster-wise FDR will be used to correct for multiple comparisons associated with the grey matter volumetric comparison (FDRc-corrected $p<0.05$).
Table 5-3 Pairwise comparisons tested

<table>
<thead>
<tr>
<th></th>
<th>PCS &gt; non-PCS</th>
<th>PCS &lt; non-PCS</th>
<th>PCS &gt; CP</th>
<th>PCS &lt; CP</th>
<th>PCS &gt; controls</th>
<th>PCS &lt; controls</th>
<th>Non-PCS &gt; CP</th>
<th>Non-PCS &lt; CP</th>
<th>Non-PCS &gt; controls</th>
<th>Non-PCS &lt; controls</th>
<th>CP &gt; controls</th>
<th>CP &lt; controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0</td>
<td>-1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>1</td>
<td>-1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>-1</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>-1</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0</td>
<td>-1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 5-1 Example of design matrix
5.6.2.2  **Cortical thickness**
The same pairwise group comparisons were investigated for cortical thickness as were for grey matter volume. That is, each group was compared to every other group; PCS > Non-PCS, PCS > CP, PCS > controls, Non-PCS > CP, Non-PCS > Controls and CP > Controls, in both the left and right hemispheres. Again age, sex, and ICV were included as covariates.

For the cortical thickness comparisons, I used a cluster-wise correction for multiple comparisons employing the precomputed Z Monte Carlo simulations, with an initial voxel-wise, cluster forming threshold of $z=3$ ($p=0.001$), to provide cluster-wise correction for multiple comparisons. Results were thresholded at a corrected $p<0.05$ ($Z>1.3$). We accounted for testing in both hemispheres. In 2016, Eklund et al. (153) published a mass empirical analysis of 3 million random group analyses from various software packages, such as FSL and SPM, to calculate empirical familywise error rates. Based on this work, in this study, I used an initial voxelwise / cluster-forming threshold of $z=3$ ($p<0.001$) in order to avoid inflated false positives when correcting for multiple comparisons.

5.6.3  **DTI**
Voxel-wise statistics on the skeletonized images (first FA, then MD) were performed using a permutation-based inference tool in FSL for nonparametric statistical thresholding (“randomise”). The same group differences were then assessed (PCS, Non-PCS, CP and Control) with age, sex, and relative motion during the DTI scan as covariates. As before, I specifically looked at pairwise comparisons between all individual groups. For each contrast, the null distribution was generated over 5,000 permutations and the $\alpha$ level set at $p < 0.05$, corrected for multiple comparisons (family-wise error correction using threshold-free cluster-enhancement (TFCE)) (154).
6 Results
6.1 Demographic and Clinical Characteristics

One way ANOVA for age showed no statistically significant differences across the four groups. Pearson’s Chi-squared test for gender was performed and no relationship was found between groups ($p=0.09$). See table 1.

Within the TBI groups, GCS and PTA scores were not significantly different between the PCS and non-PCS groups. However, the PCS group had significantly higher scores on RPQ ($T=9.1$), HADS Anxiety ($T=2.4$), and HADS Depression ($T=2.9$), than the non-PCS group ($p<0.05$).

Post hoc Tukey’s HSD tests revealed that the non-PCS group had statistically significant lower scores in RPQ, HADS A and HADS D compared to the CP groups ($p<0.05$). The PCS group and the CP group had no statistically significant difference between HADS A or HADS D. With respect to RPQ scores, the PCS groups scored slightly higher but comparison between the PCS and CP groups neared but did not reach significance with $p=0.058$. Analysis of neurocognitive tests found CWIT IV time for completion was higher in PCS compared to Non-PCS ($p<0.05$). RBANS and CWIT III showed no significant difference between PCS and non-PCS groups. The time interval between injury and scan was not statistically significant $T=0.56$ and $p=0.58$ (Tables 6.1 and 6.2).

<table>
<thead>
<tr>
<th></th>
<th>PCS</th>
<th>Non-PCS</th>
<th>CP</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>42</td>
<td>30</td>
<td>25</td>
<td>41</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>47.3</td>
<td>43.9</td>
<td>47.4</td>
<td>46.2</td>
</tr>
<tr>
<td></td>
<td>(SD 14.47)</td>
<td>(SD 13.73)</td>
<td>(SD 13.68)</td>
<td>(SD 19.21)</td>
</tr>
<tr>
<td>Female (%female)</td>
<td>18</td>
<td>15</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>(43%)</td>
<td>(50%)</td>
<td>(64%)</td>
<td>(66%)</td>
</tr>
<tr>
<td>Time of injury to scan (months)</td>
<td>30.9</td>
<td>37.6</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>(SD 33.7)</td>
<td>(SD 70.76)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 6-2 Injury severity and neuropsychological tests

<table>
<thead>
<tr>
<th></th>
<th>PCS</th>
<th>Non-PCS</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCS</td>
<td>14.7 (SD 0.60)</td>
<td>14.7 (SD 0.53)</td>
<td>NA</td>
</tr>
<tr>
<td>PTA</td>
<td>2.4 (SD 0.74)</td>
<td>2.1 (SD 0.91)</td>
<td>NA</td>
</tr>
<tr>
<td>HADS.A</td>
<td>8.0 (SD 3.4)</td>
<td>5.7* (SD 4.35)</td>
<td>9.2 (SD 4.92)</td>
</tr>
<tr>
<td>HADS.D</td>
<td>6.7 (SD 6.71)</td>
<td>2.9* (SD 2.93)</td>
<td>7.5 (SD 7.48)</td>
</tr>
<tr>
<td>RPQ</td>
<td>31.3 (SD 9.04)</td>
<td>12.7* (SD 6.91)</td>
<td>26.4^ (SD 13.98)</td>
</tr>
<tr>
<td>RBANS TS</td>
<td>105 (SD 13.77)</td>
<td>110 (SD 12.77)</td>
<td>NA</td>
</tr>
<tr>
<td>CWIT III</td>
<td>68.37 (SD 31.0)</td>
<td>55.6 * (SD 13.0)</td>
<td>NA</td>
</tr>
<tr>
<td>CWIT IV</td>
<td>75.71 (SD 29.71)</td>
<td>61.63 (SD 12.06)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Glasgow Coma Score (GCS), Post traumatic amnesia (PTA), Hospital Anxiety and Depression Scale- anxiety (HADS A: higher score= more symptoms) and depression (HADS D: higher score= more symptoms), Rivermead Post Concussion Symptom Questionnaire (RPQ: higher score= more symptoms), Repeatable Battery for the Assessment of Neuropsychological Status total score (RBANS TS: standardized age corrected score) and Colour Word Interference Test (CWIT III and IV; scores are in seconds to completion (higher score= poorer performance)). *shows ANOVA significant difference between PCS and Non-PCS p<0.05, ^ PCS and CP groups nearing but not reach significance with p=0.058

#### 6.2 Structural Results

Table 6.3 presents summary MRI metrics, including total grey matter volume, total white matter volume, total CSF volume, total intracranial volume, and absolute and relative motion during the diffusion acquisition. Absolute motion is the movement of the head in an image compared to the first image and relative motion is the movement to the direct preceding image.
Table 6-3 Summary MRI measurements.

<table>
<thead>
<tr>
<th></th>
<th>PCS</th>
<th>Non-PCS</th>
<th>CP</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey matter volume(cm)</td>
<td>707.1 (SD 94.82)</td>
<td>700.9 (SD 101.86)</td>
<td>674.0 (SD 63.15)</td>
<td>682.6 (SD 81.99)</td>
</tr>
<tr>
<td>White matter volume(cm)</td>
<td>475.1 (SD 64.55)</td>
<td>460.3 (SD 57.79)</td>
<td>473.8 (SD 55.44)</td>
<td>452.7 (SD 56.5)</td>
</tr>
<tr>
<td>ICV(cm)</td>
<td>1551.7 (SD 176.92)</td>
<td>1499.1 (SD 154.52)</td>
<td>1513.2 (SD 160.45)</td>
<td>1497.7 (SD 147.27)</td>
</tr>
<tr>
<td>Absolute Motion(mm)</td>
<td>1.9 (SD 0.37)</td>
<td>1.8 (SD 0.25)</td>
<td>1.8 (SD 0.21)</td>
<td>1.9 (SD 0.31)</td>
</tr>
<tr>
<td>Relative Motion(mm)</td>
<td>0.6 (SD 0.17)</td>
<td>0.6 (SD 0.15)</td>
<td>0.6 (SD 0.17)</td>
<td>0.5 (SD 0.18)</td>
</tr>
</tbody>
</table>

One way ANOVA showed no statistically significant difference between groups in grey matter, white matter, CSF or ICV. Age and sex were covariates.

6.2.1 Grey Matter Volume
No significant statistical voxelwise differences were found in grey matter volume between the PCS or the Non-PCS groups compared to controls. The CP group compared individually with all other groups, that is PCS, Non-PCS and controls, showed reduced grey matter volume in the cerebellum (Figures 6.1, 6.2, 6.3). These findings were particularly localized to the inferior cerebellar peduncles, vermis and cerebellar tonsils. The cerebellar grey matter reduction was most extensive and had the largest T values in the CP < control comparison.

![Figure 6-1 PCS>CP comparison showing reduced grey matter volume in CP subjects.](image)

(Left to right-axial (z =43), coronal (y =75) and sagittal (x=92) images), corrected for multiple comparisons using FCRc<0.05.)
Figure 6.2 Non-PCS>CP comparison showing reduced cerebellar volume in CP patients
(Left to right- axial (z=38), coronal (y=76) and sagittal (x=95) view)

Figure 6.3 Controls>CP comparison showing reduced cerebellar volume in CP patients
(Left to right- axial (z=35), coronal (y=76) and sagittal (x=95) view)

Within the whole mTBI group (restricted to only PCS and Non-PCS), no voxels survived correction for multiple comparisons when investigating the association between grey matter volume and any of the following: GCS, PTA, HADS A, HADS D, RPQ or CWIT III and IV. There was positive association between grey matter volume and RBANS total score which can be interpreted as a reduction in grey matter volume in the left cerebellum in the mTBI group with lower RBANS scores (Figure 6.4). There were no differences between the PCS and Non-PCS groups.
6.2.2 Cortical Thickness
There were no statistically significant differences in cortical thickness between any of the groups. The cortical thickness analysis included only supratentorial cortex. Given grey matter volume reduction was observed in the cerebellum in the CP group, an infratentorial finding, the cortical thickness result is consistent with the volumetric results.

6.3 DTI Results
6.3.1 Inspection of Normal White Matter tracts
The mean white matter skeleton mask was overlaid on the study specific mean FA and inspected visually. Its shows normal anatomy of the white matter tracts, which is useful as it gives confidence in the methods and results obtained (Figure 6.5).
6.3.2 mTBI group results
The PCS group and non-PCS group showed no significant differences in FA or MD relative to controls, nor were there differences between the PCS and non-PCS groups in FA or MD.

Analysis within the whole mTBI group (restricted to only PCS and Non-PCS) found a positive association between CWIT IV and FA. That is the higher the CWIT IV score, the higher the FA (TFCE-corrected p<0.05). These findings were evident in the corpus callosum, the internal and external capsule and the cerebral peduncle (Figure 6.6). There were no changes in MD. There were no significant findings with the CWIT III and RBANS total score in FA or MD.
Figure 6-6 FA Comparison of positive association mTBI and CWIT IV

Study specific Mean FA as a base with overlay of mean skeleton mask. The blue represents areas of significantly reduced FA (yellow arrows) in mTBI patients (TFCE corrected p<0.05). Image reference level x=98, y=128, z=81.
**Chronic Pain group results**

The CP group showed statistically significant reduction in FA (TFCE-corrected \( p<0.05 \)) compared to the Control group in bilateral thalami and the body of the right corpus callosum. In the comparisons between CP and the PCS and Non-PCS groups some findings neared significance but did not meet the corrected \( p<0.05 \) threshold. With regard to MD, there was a statistically significant difference (TFCE-corrected \( p<0.05 \)) between the CP and control group, with increased MD in multiple white matter tracts in both cerebral hemispheres and within the brainstem (\( p<0.05 \)).

**6.3.2.1 Anatomy of CP findings**

The significant reduction in FA in the CP vs control group occurred within the left and the right thalamus and within the body of the right corpus callosum (Figures 6.7, 6.8, 6.9).

*Figure 6-7 FA Comparison of CP> controls*

*Study specific Mean FA as a base with overlay of mean skeleton mask. The red represents areas of significantly reduced FA in CP patients (TFCE corrected \( p<0.05 \)). This sagittal image is through the left thalamus.*
Increased MD in the CP group compared to controls occurred in many white matter tracts. Anatomically these areas of increased MD started from the brainstem, included both thalami, internal capsules (anterior and posterior
limbs) and the external capsule, right more than left. The corpus callosum was affected bilaterally, but the right more so than the left (Figure 6.10).

Figure 6-10  MD Comparison CP< controls

Study specific Mean FA as a base with overlay of mean skeleton mask. The red represents areas of increased MD in CP patients (TFCE-corrected p<0.05).
7 Discussion

With the difficulty of studying in vivo human models of mTBI, advances in neuroimaging and software design are emerging areas of brain research. In the absence of macroscopic neural damage, it therefore follows that microstructural changes result in damage to the cell or disruption of physiological function leading to ongoing neurological signs and symptoms after the initial biomechanical force. These are likely multifactorial or a series of interrelated events, whatever the pathophysiology; metabolic, vascular/loss of autoregulation, damage to the blood brain barrier, inflammatory/immune mediated responses and neuronal damage, and have not been visible on traditional imaging techniques of CT or MRI. If such brain changes exist advanced MRI techniques such as the ones used in this study may detect such microscopic changes or their sequelae and this provides another area to direct future research into injury prevention and therapeutic treatment.

In this study, I used structural (T1-weighted) and diffusion tensor imaging (DTI) to investigate grey matter atrophy, cortical thickness, and white matter integrity across four participant groups: PCS, non-PCS, CP, and healthy controls. There were no significant differences in brain volume, cortical thickness, or DTI metrics along the centres of principal white matter tracts between the PCS and non-PCS groups, nor when these two mTBI groups were compared to controls. However, the CP participants exhibited reduced cerebellar grey matter volume relative to all three groups (PCS, non-PCS, and healthy controls) and significant reduction in FA and increased MD in several white matter tracts. There were no cortical thickness differences in any group.

7.1 Demographic and Clinical Characteristics

Groups were well matched for age and gender. This helps reduce the potential impact of age and gender on results.

The PCS group had significantly higher scores on RPQ, HADS Anxiety (HADS A) and HADS Depression (HADS D) than the non-PCS group ($p<0.05$). This is an expected finding. In particular, the PCS groups had higher post concussive
symptom scores than the recovered non-PCS group because self-reported symptom burden was used to determine group membership. This finding supports published research that demonstrates increased risk for depression and anxiety in populations demonstrating chronic health symptoms such as PCS (85). These findings also support the allocation of patients to the correct groups and enhance the strength of comparisons between the two groups.

Analysis of neurocognitive test results found CWIT IV time to completion scores were on average higher in the PCS compared to Non-PCS group ($p < 0.05$) but there were no other significant findings between the groups. This suggests the PCS groups were slower on average to complete the task, and this would be consistent with slower cognitive processing. RBANS total scores and CWIT III scores were not significantly different between the PCS and non-PCS groups. This suggests that while self-reported symptom scores were significantly different between the two groups, there were only small differences between the groups regarding objective cognitive test scores. The CWIT-IV task evaluates higher level attentional control (149) and these findings could be suggestive of differences between the groups in the attention domain. However these findings could also be explained by the overlay of depression and anxiety as demonstrated by HADS scores.

The non-PCS group had significantly lower scores across RPQ, HADS A and HADS D compared to the CP group ($p < 0.05$). The PCS group and the CP groups were similar and had no significant differences in their HADS A or HADS D scores. The PCS group had slightly higher RPQ scores than the CP group though the groups neared but did not reach significance with $p=0.058$. This is useful as it shows significant similarities in the clinical and psychosocial nature of the PCS and CP groups, which might make MRI findings, especially if similar, very interesting. However as discussed in the results section I found no brain changes, structural or DTI, in the PCS group compared to controls, and rather there was a significant difference between the PCS and CP groups in the reduction of cerebellar volume in the CP group compared to the PCS group. This will be discussed in later sections.
7.2 Structural Imaging Discussion

7.2.1 mTBI
No significant statistical voxelwise differences were found in grey matter volume or cortical thickness between the PCS and the Non-PCS groups or compared to the control groups, which is not consistent with the majority of the published literature. While there are a few of studies which have reported atrophy and cortical thinning (134)(135)(133), one significant complication with the mTBI advanced MRI literature findings is that the study sample sizes are generally small. In this study, the sample size is robust compared to other studies, providing more statistical power. In total, 138 subjects were recruited for this study (42 PCS, 30 Non-PCS, 25 CP, and 41 healthy controls).

Within mTBI research there are several definitions and mechanisms of injury that may affect pathophysiology. While it is difficult to select patients based solely on the mechanism of injury, in this study other group demographics were well matched. The PCS, non-PCS and CP groups were all matched for age, gender, education, injury, severity and pre-injury work status. The healthy control group was matched for mean age and gender distribution. Likewise within the TBI groups, GCS and PTA scores were not significantly different between the PCS and non-PCS groups. This is a strength of the study as it shows that at baseline, or the time of injury, all characteristics were similar between the two groups. Therefore, ‘injury severity’ per se should have a minimal effect on the results of the study. Injury mechanism data was not collected, similar with other literature, but this could influence the pathophysiology of mTBI and result in different outcomes.

Another confounding factor may be the time between injury and MRI. Some studies suggest that a scan too close to the time of injury may miss brain changes that occur much later after injury (133)(146). This was most likely not the case in this study, as the mean time of injury to MRI 30.9 months in the PCS group and 37.6 months in the non-PCS group. Perhaps also the time period from injury to scan was too long in my study- this will be discussed in the DTI section below. A further complication with interpreting the literature is heterogeneity, including both heterogeneity of injury and heterogeneity of patient groups (age, sex, etc.). However, with a good sample size and well-
characterized groups, this study’s lack of significant structural differences between PCS and Non-PCS groups still adds knowledge to the current body of literature on the topic.

The only significant structural finding from the mTBI group came in analysis of the PCS and Non-PCS groups as a single mTBI group. There was a positive association between grey matter volume and RBANS total score which can be interpreted as a reduction in grey matter volume in the left cerebellum in the mTBI group with lower total RBANS total scores. But again, there was no significant difference between the PCS and Non-PCS groups in grey matter volume. RBANS is a neurocognitive test that evaluates performance across five cognitive domains; immediate memory, visuospatial/constructonal, language, attention and delayed memory (147). For this analysis the RBANS total score was used. This is because the total score has been shown to be the most robust of the RBANS indices in TBI samples (155). It is unclear why there are only slight cerebellar changes in the mTBI group. This is unusual as these higher cognitive tasks are supratenotrial in anatomical basis. It is possible this might represent modulation or processing of information in descending tracts. These findings are discussed further in section 7.4.

7.2.2 Structural Imaging in CP Discussion
The CP group compared with all other groups, that is PCS, Non-PCS and controls, showed reduced grey matter volume in the cerebellum. These findings were particularly localized to the inferior cerebellar peduncles, vermis and cerebellar tonsils. The cerebellar grey matter reduction was most extensive and had the largest T values in the CP < control comparison; the PCS group had the least amount of reduction in volume when compared to the CP group. This is a very interesting finding. Looking at other CP research into grey matter volumes, and even in DTI, there are only a handful of papers that have reported reduced grey matter volume in the cerebellum, as most studies focus on cortical changes (87)(89). The first study was Rodriguez-Raecke (2013) who investigated 20 patients with unilateral chronic hip pain due to osteoarthritis. This group monitored structural changes up to a year post hip replacement. Initially they found reduced cerebellar volume pre-operatively. Post operatively, after resolution of pain, the grey matter changes in the cerebellum
increased (87). This either suggests that the changes in chronic pain are not neuronal atrophy or brain damage and that these changes are, to some degree, reversible. But also worth considering in this context is that as a patient becomes more mobile after hip replacement, compared to pre-operative levels, the increased motor function after hip replacement could also contribute to these structural changes. An fMRI study by Zambreanu (2005) used healthy volunteers and showed significantly increased activation in the contralateral brainstem, cerebellum and other supratentorial locations during hyperalgesia. The brainstem activation was localized to the midbrain reticular formation and the periaqueductal grey matter suggesting that these areas are involved in central sensitization or descending control of pain (89). These areas were involved in the reduction of grey matter volume in this study; hence, my results support this earlier published work in describing the role of the cerebellum in processing of chronic pain, specifically as a relay system. Similarly, cerebellar differences are supported by my DTI findings, to be discussed later.

Significant grey matter findings elsewhere such as the cingulate cortex, orbitofrontal cortex, insula and operculum and dorsolateral prefrontal cortex have been found and widely reported in chronic pain (76)(91)(90). These anatomical areas are all part of the supraspinal nociceptive processing. Despite this published evidence of reduced grey matter in the supratentorial anatomical areas listed, I did not find such results in this study. This was repeated in my cortical thickness analysis, where there were no statistically significant differences in supratentorial cortical thickness between any of the groups. Given grey matter volume reduction was observed in the cerebellum in the CP group, the supratentorial cortical thickness result is consistent with the volumetric grey matter volume results. While the volumetric and thickness analyses showed a consistent null result in supratentorial regions in the current study, it is surprising that no other atrophy or thinning was found in the CP group. Perhaps this is due to the small number of chronic pain patients (n=25) in this study, or the cause or type of chronic pain in this study wasn’t defined as rigorously as in other studies.
Given the current cerebellar findings, it would be interesting to investigate whether the CP patients demonstrated any clinical signs of cerebellar dysfunction, such as ataxia. This was not explored in this study, but maybe an area of future work. Comparison of the CP patients and their clinical examination, in terms of cerebellar signs, compared to other groups with reduced grey matter volume in the cerebellum, such as those with stroke or tumour perhaps, would also be an interesting comparison.

7.3 DTI

7.3.1 DTI in mTBI
The only significant finding with the mTBI group and DTI came in the analysis of the whole mTBI group (restricted to only PCS and Non-PCS) which found a positive association between CWIT IV scores and FA in the areas of the corpus callosum, the internal and external capsule and the cerebral peduncle. That is the higher the CWIT IV score, the higher the FA (TFCE-corrected p<0.05). These findings did reach statistical significance, but the reason why they were not found in CWIT III or RBANS is interesting and makes the interpretation of this difficult. There were no association changes in MD relating to CWIT IV and no significant findings with the CWIT III scores or RBANS total score in FA or MD in the mTBI group.

The PCS group and non-PCS group showed no significant differences in FA or MD relative to controls, nor were there differences between the PCS and non-PCS groups. This negative finding does not support the majority of published literature on the topic. A quantitative literature review by Hulkower et al. in 2013 looked at 100 DTI in mTBI articles with a combined patient number of 2337 subjects (113). In 96 out of 100 studies, FA values were reduced in patients with mTBI, with four of the studies finding elevated FA values. Abnormal FA was most commonly found in the corpus callosum with other areas including the internal capsule, the fronto-orbital fasciculus, fornix, cingulum bundle and superior and inferior longitudinal fasciculus. By this comparison, perhaps the lack of statistically significant FA differences in my study are the most surprising. This further adds to the heterogeneity of the literature findings. A surprising finding was in almost all ROI and tractography studies, MD values were abnormally low in mTBI relative to
controls. As mentioned previously, on reviewing some of the referenced literature I suggest this is most likely an error and the findings mislabeled in the Hulkower paper. Looking at several of the referenced studies the reported change in MD should read as increased (140)(141)(142)(143). With regard to whole brain analysis, thirteen studies that used this method, had varying results but with a maximum of only six studies finding increased MD in analyses (113). As mentioned previously, sample size in this study was adequate. With the sample size in our study (PCS=42 and Non-PCS=30), and \( \alpha=0.05 \), there was 80% power to detect a difference in means of \( d=0.67 \) (two-tailed). Comparably sized studies in the literature have found positive results but given this power calculation perhaps this study was under powered and this could explain the lack of identifiable statistically significant results.

Additionally, different methods may help to explain the difference with the literature. In addition to smaller groups, many studies use different definitions of mTBI and PCS, which complicates interpretation across studies. Other studies employed older 1.5T MRI scanners; many found positive results. Perhaps the 3T scanners would improve the significance of findings. The time between injury to time of scan also varies significantly between studies, with some suggestion that FA/MD change in the acute to chronic stages. The mean time from injury to MRI-30.9 months in the PCS group and 37.6 months in the non-PCS group-is at the longer end of the time interval compared to other studies and perhaps this influenced my results. Perhaps I missed acute results or perhaps after two years, for example, the healing has occurred and the changes have resolved or become undetectable. A prospective study following mTBI from acute to chronic state with serial imaging over a long period would be a good future study as it would include potential acute injury changes and also stage any changes over time to help develop a picture of pathophysiology over time.

One study did agree with my negative DTI findings. Lange et al. (2012) studied 60 mTBI patients (21 PCS and 39 non-PCS) and 34 controls and found no difference in DTI measures. However the time from injury to time of scan was only 6-8 weeks, which differs significantly from my study interval (137).
Also, the published literature could represent positive reporting bias, though there is no way to confirm this.

I have employed Diffusion Tensor Imaging (DTI) in this study, which produces a diffusion tensor in each voxel of the brain. The diffusion tensor presumes that there is only one single type of fiber in each voxel, which is not always the case. For example, there can be fibers of different directions or crossing fibers within a single voxel. These factors can mean that white matter tracts could be misrepresented. To combat this new techniques such as High Angular Resolution Diffusion Imaging (HARDI) and diffusion kurtosis imaging have been developed, which may provide more accurate information on the microstructural mTBI changes, if present (125)(156)(157).

Overall the heterogeneity of the literature in terms of group definitions, scan protocols, time intervals and small sample sizes combined with a possible positive reporting bias raises questions about the strength of conclusions that can be drawn from the mTBI literature. Newer techniques such as HARDI and diffusion kurtosis may be the next step in the future of diffusion research in mTBI.

7.3.2 DTI Imaging in CP
The CP group showed significant reduction in FA (TFCE-corrected \( p<0.05 \)) compared to the Control group in bilateral thalami and the body of the right corpus callosum. The CP group also tended toward FA differences when compared to the PCS and Non-PCS groups, but these did not survive correction for multiple comparisons. The reduced FA in the thalami and the right corpus callosum in the CP group compared to controls support the findings in the published literature.

With regard to MD, there was a significant difference (TFCE-corrected \( p<0.05 \)) between the CP and control group, with increased MD in multiple white matter tracts from the brainstem, included both thalami, internal capsules (anterior and posterior limbs) and the external capsule, right more than left. The corpus callosum was affected bilaterally, but the right more so than the left. Again, the changes in these locations support the findings in published
literature. Research into CP using other neuroimaging modalities such as fMRI, PET, MEG and EEG has shown several areas of the brain involved in the pathophysiology of pain. The anterior cingulate cortex, insula, frontal cortices, somatosensory cortices, thalami and amygdala are the supratentorial anatomical areas involved in the processing and modulation of pain. The brainstem, particularly the periaqueductal grey matter and the brainstem reticular formation play a role in the descending regulation of pain (89)(91)(90). It is significant that the FA/MD findings in this study can be explained by the relationship of white matter tracts in some of these areas given their role in the pathophysiology of processing and modulation of pain.

Advanced MRI is becoming much more frequently used to investigate chronic pain, and is changing the way CP syndrome is viewed. Given the significant brain changes in this condition detected using fMRI, PET, MEG and EEG, in addition to structural and DTI techniques (89)(90)(91)(88)(93)(95), it is now thought that CP is an organic disease rather than being purely a psychosocial syndrome. The other side of the CP brain changes debate is what comes first: are the brain changes the cause of the chronic pain or are they adaptive changes that have occurred after the development of the syndrome? This question may now be investigated with brain imaging. For example, reversible brain changes have been reported after hip joint replacement (87)(76)(86). Another prospective study using MRI techniques at multiple points over time may help to answer this question.

7.4 Findings within the mTBI groups and Neurocognitive tests

An interesting outcome of this study is firstly, the structural imaging finding of a positive association between grey matter volume in the mTBI group (combined PCS and Non-PCS groups) and the RBANS total score and secondly, the positive findings in the DTI analysis of CWIT IV and FA. Again, there were no MRI differences between either PCS and Non-PCS and the healthy control groups. While RBANS and CWIT IV assess different neurocognitive elements, they may suggest that after a mTBI event there may be detectable changes in brain function in the mTBI group whether fully recovered or not. This has been found by Kraus et al. (158) with a study of 25 male college athletes who had suffered a concussive event compared to a
control group of 25 male college athletes who had not. They measured neural responses to speech and found that the mTBI group had smaller responses to speech than controls. The authors suggest that this adds weight to evidence that a single concussive event can cause neuronal damage even though there is an absence of PCS symptoms (158). This is important in advancing one step further in our understanding of Chronic Trauma Encephalopathy (CTE). As a disease there is much uncertainty around whether this condition is a result of one or several mTBI events, or even accumulation of sub-concussive events (9). Could the neurocognitive tests used in this study and MRI findings serve as a predictor of this condition also? The next step would be to reanalyze our data with a single mTBI group and compare neurocognitive outcomes and MRI findings with a group of healthy controls who have also undergone neurocognitive testing. Unfortunately, our healthy control group did not undergo the neurocognitive testing and so this next step cannot be performed in this study. To build on the results of this study I suggest a prospective longitudinal study with a mTBI group and normal controls using the neurocognitive controls and serial MRI imaging over time.

7.5 CP as a control for PCS

An important point of difference in this study was comparing the CP group to the PCS group in order to compare findings with another group experiencing symptoms who have not had a mTBI. CP has many similarities with PCS, both with the heterogeneity of definitions and the reliance on clinical factors for diagnosis. The uncertain pathophysiology, and the way in which only a small percentage of acute pain (or mTBI) patients go on and develop CP (or PCS) symptoms. The baseline clinical features, namely time course and psychosocial factors involved in the development of each condition are also comparable. PCS and CP also share symptoms, baseline pre-morbid psychological conditions, and personality traits that can affect the development of chronicity in each condition. Studies with direct comparison between PCS and CP show similar levels of self-reported symptoms across physical, cognitive and somatic domains (96)(63)(61). This is reinforced by the similarities in the demographic characteristics between the PCS group and the CP group, with no statistically significant differences between HADS A or HADS D and the RPQ scores. The PCS and CP groups approached but did not reach significance with $p=0.058$
with the PCS group having marginally higher RPQ scores than the CP group. By carefully matching both groups it has allowed the investigation of brain changes as a consequence of having a mTBI, rather than as a consequence of similar symptomology. Does MRI have a role to investigate the brain changes associated with chronic pain and perhaps compared to PCS to show similarities between the two to aid understanding of the pathophysiology of both conditions? It was for these reasons that CP was used as another control group in this thesis.

The interesting outcome in this study was the significant structural and DTI findings in the CP group compared to all other groups, and no significant findings in the PCS group compared to controls in either modality. Initially the idea was to use CP as an ‘active control group’ to compare against the PCS group, as given the cross-over of symptoms, treatment, etc. it was felt that this may provide a more appropriate model to investigate outcomes in TBI. Interestingly, our results support other literature in that the CP group have evidence of an organic disease process. Perhaps it is fair to say that PCS and CP are not as similar as I initially thought and that the pathophysiology of each is in fact dissimilar. Taken to the extreme, based on the findings here, perhaps PCS is not a disease but a chronic psychosocial condition that develops in individuals with at risk baseline characteristics and no observable microstructural brain changes exist with the technology we have available at this time. Again, with only this study it is not possible to make this assumption, but given the heterogeneity of the mTBI literature, the next stage to further clarify if any brain changes exist is research with the newer techniques such as HARDI and diffusion kurtosis by using a prospective long term study using these imaging techniques in series over time.

Patients with chronic pain are usually on multiple medications. The mTBI groups are unlikely to be on medications long term. Hence it is a good question to consider if the medication the CP group are taking will influence these brain changes. In the CP literature, this question has also not been addressed and maybe an interesting point of future research.
8 Conclusion

In this study, I used both structural (grey matter volume and cortical thickness) and DTI techniques to investigate the brains of participants who had experienced a mTBI. The objective was to identify potential brain differences associated with full or poor recovery post mTBI, to see whether differences existed with a group of participants with chronic pain and a group of healthy controls.

No significant statistical voxelwise differences were found in grey matter volume between the PCS and the Non-PCS groups, or compared to the control groups. There were also no significant differences in supratentorial cortical thickness between any of the groups.

The PCS and non-PCS groups showed no significant differences in FA or MD relative to controls, nor were there differences between the PCS and non-PCS groups in FA or MD. As with the current study, many previous studies report an absence of marked brain differences between mTBI patients and controls, or within mTBI. However, this negative finding does not support the majority of published literature on the topic. This may be explained by the heterogeneity of methodology, mTBI definition and timing of MRI scan relative to time after injury across the literature. Perhaps a more accurate comparison would be between mTBI and another trauma group, such as orthopaedic trauma whom did not suffer mTBI as both groups having experienced the trauma event. This method has been used in other published literature (134).

The CP group compared with all other groups showed reduced grey matter volume in the cerebellum. These findings were particularly localized to the inferior cerebellar peduncles, vermis and cerebellar tonsils, which supports existing literature suggesting the cerebellum has a significant role in integration and modulating pain pathways. There were no differences in cortical thickness in the CP group. The CP group exhibited FA and MD differences in the cerebellar/brainstem changes of reduced grey matter volume also support findings in published literature that show the supratentorial anatomical areas and brainstem involved in the processing and modulation of pain.
Overall the heterogeneity of the literature-including clinical group definitions, scan protocols, time intervals and small sample sizes-help to frame my results within the larger field. Lack of significant differences is not an uncommon finding. In my hands, PCS and non-PCS were indistinguishable on T1-weighted images, as well as DTI. Not reaching an established clinical threshold makes drawing comparisons to my study difficult.

Newer techniques such as HARDI and diffusion kurtosis maybe the next step in future research in this topic. Or a new study looking at MRI changes over time starting in the acute stage, just after time of injury, and continuing with serial imaging over time might also prove useful.
References


44. Accident Compensation Corporation. Recognise the Signs and Symptoms of Concussion [Internet]. Available from: http://www.acc.co.nz/

45. Accident Compensation Corporation. Caring for your child after their head injury Warning signs [Internet]. Available from: http://www.acc.co.nz/

46. Accident Compensation Corporation. Knowing about your Mild Traumatic Brain Injury (TBI) [Internet]. Available from: http://www.acc.co.nz/


7.


72. New Zealand Pain Society [Internet]. Available from: http://www.nzps.org.nz


91. Apkarian AV, Hashmi JA, Baliki MN. Pain and the brain:


110. Laboratory for Computational Neuroimaging. FreeSurfer [Internet]. Athinoula A. Martinos Center for Biomedical Imaging. Available from: http://freesurfer.net/fswiki/FreeSurferWiki

111. Fischl B, Dale AM. Measuring the thickness of the human


118. FMRIB AG. Diffusion MRI Processing and Analysis [Internet]. Available from: http://fsl.fmrib.ox.ac.uk/fslcourse/


121. Huisman TAGM. Diffusion-weighted and diffusion tensor


129. Analysis Group, FMRIB, Oxford U. FMRIB Software Library v5.0 [Internet]. Available from: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL

130. Newman E, Moulton E, Becerra L, Borsook D. Morphological


138. Khong E, Odenwald N, Hashim E, Cusimano MD. Diffusion tensor imaging findings in post-concussion syndrome


155. McKay C, Wertheimer JC, Fichtenberg NL, Casey JE. The repeatable battery for the assessment of neuropsychological


19 September 2016

Dr Debbie Snell
Allan Bean Centre, Burwood Hospital
Entrance 3, Christchurch
Private Bag 4708, Christchurch, 8140
Christchurch 8140

Dear Dr Snell

Re: Ethics ref: URA/12/05/015/AM10


I am pleased to advise that this amendment has been approved by the Southern Health and Disability Ethics Committee. This decision was made through the HDEC Expedited Review pathway.

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Ms Raewyn Idoine
Chairperson
Southern Health and Disability Ethics Committee

Encl: appendix A: documents submitted
      appendix B: statement of compliance and list of members
This Agreement between Sharon Jay ("You") and Massachusetts Medical Society ("Massachusetts Medical Society") consists of your license details and the terms and conditions provided by Massachusetts Medical Society and Copyright Clearance Center.

License Number 4078090166727
License date 
Licensed Content Publisher Massachusetts Medical Society
Licensed Content Publication The New England Journal of Medicine
Licensed Content Title Concussion
Licensed Content Author Allan H. Ropper, Kenneth C. Gorson
Licensed Content Date Jan 11, 2007
Licensed Content Volume 356
Licensed Content Issue 2
Type of Use Manner not listed
Requestor type student
Order reference number 
Requestor Location Sharon Jay
4/116 Rossall ST
Merivale
Christchurch, 8014
New Zealand
Attn: Sharon Jay
Billing Type Invoice
Billing Address Sharon Jay
4/116 Rossall ST
Merivale
Christchurch, New Zealand 8014
Attn: Sharon Jay
Total 0.00 USD

Terms and Conditions

Introduction
The publisher for this copyrighted content is Massachusetts Medical Society ("MMS"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your RightsLink account and that are available at any time at http://myaccount.copyright.com).

Scope of License
1. MMS hereby grants to you a non-exclusive license to reproduce the aforementioned content subject to the terms and conditions indicated within the RightsLink transaction. Licenses are for one-time use only. Web posting is limited to the time period selected within your transaction - beginning on the date of this license.
This Agreement between Sharon Jay ("You") and Oxford University Press ("Oxford University Press") consists of your license details and the terms and conditions provided by Oxford University Press and Copyright Clearance Center.

License Number: 4075040532121
License date: Apr 09, 2017
Licensed content publisher: Oxford University Press
Licensed content publication: Neurosurgery
Licensed content title: The New Neurometabolic Cascade of Concussion
Licensed content author: Giza, Christopher C.; Hovda, David A.
Licensed content date: 2014-10-01
Type of Use: Thesis/Dissertation
Institution name: mTBI and advanced MRI
Publisher of your work: n/a
Expected publication date: Apr 2017
Permissions cost: 0.00 USD
Value added tax: 0.00 USD
Total: 0.00 USD
Requestor Location: Sharon Jay
4/116 Rossall ST
Merivale
Christchurch, 8014
New Zealand
Attn: Sharon Jay
Publisher Tax ID: GB125506730
Billing Type: Invoice
Billing Address: Sharon Jay
4/116 Rossall ST
Merivale
Christchurch, New Zealand 8014
Attn: Sharon Jay
Total: 0.00 USD

STANDARD TERMS AND CONDITIONS FOR REPRODUCTION OF MATERIAL FROM AN OXFORD UNIVERSITY PRESS JOURNAL

1. Use of the material is restricted to the type of use specified in your order details.
2. This permission covers the use of the material in the English language in the following territory: world. If you have requested additional permission to translate this material, the terms and conditions of this reuse will be set out in clause 12.
This Agreement between Sharon Jay ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

<table>
<thead>
<tr>
<th>License Number</th>
<th>4074540454339</th>
</tr>
</thead>
<tbody>
<tr>
<td>License date</td>
<td></td>
</tr>
<tr>
<td>Licensed Content Publisher</td>
<td>Elsevier</td>
</tr>
<tr>
<td>Licensed Content Publication</td>
<td>Journal of Neuroscience Methods</td>
</tr>
<tr>
<td>Licensed Content Title</td>
<td>Neuroinflammation in animal models of traumatic brain injury</td>
</tr>
<tr>
<td>Licensed Content Author</td>
<td>Chong-Chi Chiu, Yi-En Liao, Ling-Yu Yang, Jing-Ya Wang, David Tweedie, Hanuma K. Karnati, Nigel H. Greig, Jia-Yi Wang</td>
</tr>
<tr>
<td>Licensed Content Date</td>
<td>15 October 2016</td>
</tr>
<tr>
<td>Licensed Content Volume</td>
<td>272</td>
</tr>
<tr>
<td>Licensed Content Issue</td>
<td>n/a</td>
</tr>
<tr>
<td>Licensed Content Pages</td>
<td>12</td>
</tr>
<tr>
<td>Start Page</td>
<td>38</td>
</tr>
<tr>
<td>End Page</td>
<td>49</td>
</tr>
<tr>
<td>Type of Use</td>
<td>reuse in a thesis/dissertation</td>
</tr>
<tr>
<td>Intended publisher of new work</td>
<td>other</td>
</tr>
<tr>
<td>Portion</td>
<td>figures/tables/illustrations</td>
</tr>
<tr>
<td>Number of figures/tables/illustrations</td>
<td>1</td>
</tr>
<tr>
<td>Format</td>
<td>both print and electronic</td>
</tr>
<tr>
<td>Are you the author of this Elsevier article?</td>
<td>No</td>
</tr>
<tr>
<td>Will you be translating?</td>
<td>No</td>
</tr>
<tr>
<td>Order reference number</td>
<td>inflammatory changes</td>
</tr>
<tr>
<td>Original figure numbers</td>
<td>figure 1</td>
</tr>
<tr>
<td>Title of your thesis/dissertation</td>
<td>mTBI and advanced MRI</td>
</tr>
<tr>
<td>Expected completion date</td>
<td>Apr 2017</td>
</tr>
<tr>
<td>Estimated size (number of pages)</td>
<td>40</td>
</tr>
<tr>
<td>Elsevier VAT number</td>
<td>GB 494 6272 12</td>
</tr>
<tr>
<td>Requestor Location</td>
<td>Sharon Jay 4/116 Rossall ST Merivale Christchurch, 8014 New Zealand Attn: Sharon Jay</td>
</tr>
</tbody>
</table>
This Agreement between Sharon Jay ("You") and Springer ("Springer") consists of your license details and the terms and conditions provided by Springer and Copyright Clearance Center.

<table>
<thead>
<tr>
<th>License Number</th>
<th>4074540037531</th>
</tr>
</thead>
<tbody>
<tr>
<td>License date</td>
<td></td>
</tr>
<tr>
<td>Licensed Content Publisher</td>
<td>Springer</td>
</tr>
<tr>
<td>Licensed Content Publication</td>
<td>Neurotherapeutics</td>
</tr>
<tr>
<td>Licensed Content Title</td>
<td>Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury</td>
</tr>
<tr>
<td>Licensed Content Author</td>
<td>Jenna M. Ziebell</td>
</tr>
<tr>
<td>Licensed Content Date</td>
<td>Jan 1, 2010</td>
</tr>
<tr>
<td>Licensed Content Volume</td>
<td>7</td>
</tr>
<tr>
<td>Licensed Content Issue</td>
<td>1</td>
</tr>
<tr>
<td>Type of Use</td>
<td>Thesis/Dissertation</td>
</tr>
<tr>
<td>Portion</td>
<td>Figures/tables/illustrations</td>
</tr>
<tr>
<td>Number of figures/tables/illustrations</td>
<td>1</td>
</tr>
<tr>
<td>Author of this Springer article</td>
<td>No</td>
</tr>
<tr>
<td>Order reference number</td>
<td>BBB</td>
</tr>
<tr>
<td>Original figure numbers</td>
<td>figure 1</td>
</tr>
<tr>
<td>Title of your thesis / dissertation</td>
<td>mTBI and advanced MRI</td>
</tr>
<tr>
<td>Expected completion date</td>
<td>Apr 2017</td>
</tr>
<tr>
<td>Estimated size(pages)</td>
<td>40</td>
</tr>
<tr>
<td>Requestor Location</td>
<td>Sharon Jay</td>
</tr>
<tr>
<td></td>
<td>4/116 Rossall ST</td>
</tr>
<tr>
<td></td>
<td>Merivale</td>
</tr>
<tr>
<td></td>
<td>Christchurch, 8014 New Zealand</td>
</tr>
<tr>
<td></td>
<td>Attn: Sharon Jay</td>
</tr>
<tr>
<td>Billing Type</td>
<td>Invoice</td>
</tr>
<tr>
<td>Billing Address</td>
<td>Sharon Jay</td>
</tr>
<tr>
<td></td>
<td>4/116 Rossall ST</td>
</tr>
<tr>
<td></td>
<td>Merivale</td>
</tr>
<tr>
<td></td>
<td>Christchurch, New Zealand 8014</td>
</tr>
<tr>
<td></td>
<td>Attn: Sharon Jay</td>
</tr>
<tr>
<td>Total</td>
<td>0.00 USD</td>
</tr>
</tbody>
</table>

Terms and Conditions
Dear Colleague,

I am a postgraduate student at the University of Otago in New Zealand, writing my thesis on Mild Traumatic Brain Injury and Advanced MRI for the degree of Masters Medical Science.

I am writing to request your permission to include the following material in my thesis for which I believe you own the copyright.


The flow diagram image displayed below only;

If you do not own the copyright in this material, please notify me, and if possible include contact information for the rights holder(s).

I intend to use the material as follows:

1. The image of the flow diagram will be used in my thesis chapter on Mild Traumatic Brain Injury under Management and Neuroimaging. It will be approximately half a page in size. I do not expect to use this image in future publications other than in this thesis.
2. It will be used to illustrate the decision making process in deciding on who to image in Mild Traumatic Brain Injury.
3. When completed, a print thesis copy will be deposited with the University of Otago Library.
4. An electronic copy will be deposited in OUR Archive, the university’s research repository. The completed thesis will be available for inter-library loan and be fully accessible on the Internet via OUR Archive.

I am seeking a non-exclusive, worldwide, perpetual licence to use this material in the way described above. The work will be fully referenced, including any copyright attribution as specified by you. If you consent, please complete the form below and return a copy to me by email.

Yours sincerely,
Dr Sharon Jay

____________________________________________________________________________

I am the rights holder and grant permission for the use of the material as described above:

By [Name & Title] __________________________________________________________
Date: __________________
Company/Affiliation _______________________________________________________

(Optional) Please attribute as follows, if different from standard academic acknowledgement:

____________________________________________________________________________

____________________________________________________________________________

____________________________________________________________________________
Hi Sharon

Thanks for your request – yes that's fine by us.

Kind regards

Susan Cato-Symonds
Information Specialist and Web Director
Family Information Service and Starship Website
(09 307 4955 | internal ext: 25503 | cellphone: 021 307350  *susancs@adhb.govt.nz
Website: https://www.starship.org.nz

Family Information Service| Level 3 (opposite outpatients) Starship Children's Health | Park Road|Private Bag 92024| Auckland 1142|New Zealand

The information contained in this email and any attachments is confidential and intended for the named recipients only. If you are not the intended recipient, please delete this email and notify the sender immediately. Auckland DHB accepts no responsibility for changes made to this email or to any attachments after it has been sent.

From: Sharon Jay [mailto:sharonmjay@gmail.com]
Sent: Thursday, 09 February 2017 2:35 p.m.
To: SS Family Information Service (ADHB)
Subject: Request to use a diagram from your guidelines

Please see attached request to use a flow diagram from your Head Injury Guidelines in my masters thesis.

Many thanks,

--

Dr Sharon Jay