Combining Advanced MR Techniques to Investigate Mild Traumatic Brain Injury and its Cognitive Consequences

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Thesis Abstract

Mild Traumatic Brain Injury (TBI) is a major health concern due to its high incidence and lasting cognitive consequences for the patient. Diffuse Axonal Injury (DAI); diffuse damage to axons caused by inertial forces during the TBI, is hypothesised to be a leading cause for these impairments. MRI-based research which has examined mild, acute cases has produced findings which indicate that the mechanisms and progression of mild TBI may be unique and not simply a less pronounced version of severe TBI. This thesis describes a set of experiments which used a variety of advanced MRI techniques to examine the physiological presentation and progression of mild TBI, and how this related to cognitive outcome.

Forty-four mild and 9 moderate TBI patients were recruited an average of 6 days post-injury, scanned using a variety MRI techniques and administered a neuropsychological test battery. Twenty-three of these patients (18 mild and 5 moderate) returned one year later and repeated all testing. Thirty-three matched controls were also recruited and given the same set of tests.

At the acute time-point patients underperformed on a variety of cognitive tests, although performance at the chronic time-point had normalised compared to controls. The distribution of visible lesions (as identified on quantitative $T_1$/ $T_2$ and $T_1$W scans) was found to be similar to patterns previously reported in severe patients. Results also indicated more lesions to be related to greater acute cognitive deficit. Diffusion Tensor Imaging experimentation revealed a number of unexpected, cognitively-relevant metric changes at the acute and chronic time-points. Magnetic Resonance Spectroscopy investigation also showed a number of unusual metabolite concentration changes and also found these to relate to cognitive functioning. Novel hypotheses were formed from these findings.

This work has demonstrated a number of cognitively-relevant physiological changes following TBI which appear unique to mild injury.
Declaration

All of the work in this thesis is my own, aside from initial data collection and some data pre-processing steps. These were carried out by;

Professor Andrew Blamire and Dr. Benjamin Aribisala (Development of in-house data pre-processing tools)
Mr. Christopher Cowie (Participant recruitment and data collection)
Josh Wood and Anna Peel (Administration of psychometric tests)
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The Sir Jules Thorn Charitable Trust, for funding the project and my living costs while I worked through the PhD.

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<th>Definition</th>
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<tbody>
<tr>
<td>3D</td>
<td>three dimensional</td>
</tr>
<tr>
<td>3T</td>
<td>three tesla</td>
</tr>
<tr>
<td>AC-PC line</td>
<td>an line that runs between the anterior and posterior commissure</td>
</tr>
<tr>
<td>AD</td>
<td>axial diffusivity</td>
</tr>
<tr>
<td>ADC</td>
<td>apparent diffusion coefficient</td>
</tr>
<tr>
<td>AFu</td>
<td>acute data of patients who <em>did</em> return for follow-up testing</td>
</tr>
<tr>
<td>AL</td>
<td>acute data of patients who <em>did not</em> return for follow-up testing</td>
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<tr>
<td>BIRT</td>
<td>Brain Injury Rehabilitation Tool</td>
</tr>
<tr>
<td>BMIPB</td>
<td>BIRT Memory and Information Processing Battery</td>
</tr>
<tr>
<td>CBF</td>
<td>cerebral blood flow</td>
</tr>
<tr>
<td>Cho</td>
<td>choline</td>
</tr>
<tr>
<td>CLEAR</td>
<td>coupled and linked equations algorithm</td>
</tr>
<tr>
<td>Cre</td>
<td>creatine</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebro-spinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>CWIT</td>
<td>Colour-Word Interference Test</td>
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<tr>
<td>D-KEFS</td>
<td>Delis-Kaplan Executive Function System</td>
</tr>
<tr>
<td>DAI</td>
<td>diffuse axonal injury</td>
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<tr>
<td>DTI</td>
<td>diffusion tensor imaging</td>
</tr>
<tr>
<td>EPI</td>
<td>echo planar image</td>
</tr>
<tr>
<td>FA</td>
<td>fractional anisotropy</td>
</tr>
<tr>
<td>FLAIR</td>
<td>fluid attenuated inversion recovery</td>
</tr>
<tr>
<td>FLIRT</td>
<td>FMRIB’s linear registration tool</td>
</tr>
<tr>
<td>fMRI</td>
<td>function magnetic resonance imaging</td>
</tr>
<tr>
<td>FUGUE</td>
<td>FMRIB’s set of tools for EPI distortion correction</td>
</tr>
<tr>
<td>GCS</td>
<td>Glasgow Coma Scale</td>
</tr>
<tr>
<td>Glx</td>
<td>glutamine and glutamate</td>
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<td>GOS</td>
<td>Glasgow Outcome Scale</td>
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<td>IQ</td>
<td>intelligence quotient</td>
</tr>
<tr>
<td>Lac</td>
<td>lactate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>LoC</td>
<td>loss of consciousness</td>
</tr>
<tr>
<td>MD</td>
<td>mean diffusivity</td>
</tr>
<tr>
<td>mI</td>
<td>Myo-inositol</td>
</tr>
<tr>
<td>MR</td>
<td>magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>NAA</td>
<td>N-Acetylaspartate</td>
</tr>
<tr>
<td>NART</td>
<td>National Adult Reading Test</td>
</tr>
<tr>
<td>NICE</td>
<td>National Institute for Clinical Excellence</td>
</tr>
<tr>
<td>PASAT</td>
<td>Paced Auditory and Serial Addition Test</td>
</tr>
<tr>
<td>PCC</td>
<td>Posterior Cingulate Cortex</td>
</tr>
<tr>
<td>PD</td>
<td>proton density</td>
</tr>
<tr>
<td>PRELUDE</td>
<td>FMRIB’s phase region expanding labeller for unwrapping discrete estimates</td>
</tr>
<tr>
<td>PTA</td>
<td>post traumatic amnesia</td>
</tr>
<tr>
<td>RANDOMISE</td>
<td>FMRIB’s permutation-based nonparametric inference tool</td>
</tr>
<tr>
<td>RD</td>
<td>radial diffusivity</td>
</tr>
<tr>
<td>ROI</td>
<td>region of interest</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SENSE</td>
<td>sensitivity encoding</td>
</tr>
<tr>
<td>SoIP</td>
<td>Speed of Information Processing</td>
</tr>
<tr>
<td>SWM</td>
<td>superior white matter</td>
</tr>
<tr>
<td>TBI</td>
<td>traumatic brain injury</td>
</tr>
<tr>
<td>TBSS</td>
<td>Tract-Based Spatial Statistics</td>
</tr>
<tr>
<td>T1W</td>
<td>T1 Weighted</td>
</tr>
<tr>
<td>T2W</td>
<td>T2 Weighted</td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>VLF</td>
<td>Verbal Letter Fluency</td>
</tr>
</tbody>
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Chapter 1. Traumatic Brain Injury

1.1 Traumatic Brain Injury

A Traumatic Brain Injury (TBI) refers to any physically-induced head injury which results in altered neural physiology (Menon et al., 2010). TBI has been reported to be the leading cause of death from all trauma admissions to hospitals (Caldwell and McGovern, 1993). A TBI can result from closed or penetrating head injuries, the most commonly reported being road traffic accidents with falls being second in frequency (Tagliaferri et al., 2006), and assault also being relatively common (Thornhill et al., 2000). In America firearms are cited as a major cause of TBI (Sosin et al., 1995), although the mechanisms and impact of a bullet-injury differ from other TBI’s which are typically characterised by a forceful, yet blunt impact to the head. Likewise, blast-injuries are also frequently investigated under the umbrella term of TBI (DePalma et al., 2005), although as these are characterised by various factors such as shockwaves from the explosion and burns, they are also not directly comparable. As this thesis focuses on the more traditional type of injury, it should therefore be considered that any following discussion of the mechanisms and implications of TBI may not always be relevant to firearm or blast injury.

A systematic review of European-based literature (Tagliaferri et al., 2006) estimates that (within Europe) 775,500 people sustain a TBI every year, leading to an average hospitalisation of 235 per 100,000. UK-based research has reported approximately 150 per 100,000 annual admissions (Thornhill et al., 2000). The number of deaths as a direct result from a TBI is estimated at 11 per 100 cases although this drops to 3 in 100 when considering only those who survive long enough to be admitted to hospital. Patients are usually male and within the “young adult” age bracket (variably defined, although usually around 16-25 years of age (Bruns and Hauser, 2003)). Combining the high incidence of acquired disability with the youth of the population means TBI is a major healthcare burden, warranting research to improve our understanding of the mechanisms involved so that patient treatment may become more effective.
1.2 Defining the Severity of a TBI

Healthcare systems and research frequently label individual patients with an injury severity rating based on a variety of factors. The most common method of doing this is through use of Glasgow Coma Scale (GCS (Teasdale and Jennett, 1974), Table 1.1); an assessment of responsiveness administered at the scene of injury. In this assessment patients are given a separate score for their eye opening, and verbal and motor responses, with a higher number indicating a greater degree of responsiveness. These individual scores are then added together for a maximum possible score of 15 (essentially indicating full awareness and ability) and a minimum of 3 (indicating a complete lack of any response). Using this total score, patients may then be classified as “mild” (typically a score of 13-15), “moderate” (9-12) or “severe” (8 or below; this scoring system is common throughout research, as will be discussed over the following pages).
Table 1.1. The Glasgow Coma Scale. At the scene of injury, the patient is given a score for each category based on their responsiveness.

<table>
<thead>
<tr>
<th>Eye Opening</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Spontaneous</td>
<td>4</td>
</tr>
<tr>
<td>To verbal stimuli</td>
<td>3</td>
</tr>
<tr>
<td>To pain</td>
<td>2</td>
</tr>
<tr>
<td>No response</td>
<td>1</td>
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<table>
<thead>
<tr>
<th>Verbal Response</th>
<th></th>
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<tbody>
<tr>
<td>Oriented to time, place and person; uses appropriate words and phrases</td>
<td>5</td>
</tr>
<tr>
<td>Confused</td>
<td>4</td>
</tr>
<tr>
<td>Inappropriate words or verbal response</td>
<td>3</td>
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<tr>
<td>Incomprehensible words</td>
<td>2</td>
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<tr>
<td>No response</td>
<td>1</td>
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<table>
<thead>
<tr>
<th>Motor Response</th>
<th></th>
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<tr>
<td>Spontaneous movement</td>
<td>6</td>
</tr>
<tr>
<td>Withdraws to touch</td>
<td>5</td>
</tr>
<tr>
<td>Withdraws to pain</td>
<td>4</td>
</tr>
<tr>
<td>Abnormal flexion</td>
<td>3</td>
</tr>
<tr>
<td>Abnormal extension</td>
<td>2</td>
</tr>
<tr>
<td>No response</td>
<td>1</td>
</tr>
</tbody>
</table>

(Add the score from each category to get the total. The maximum score is 15, indicating the best level of neurologic functioning. The minimum is 3, indicating total neurologic unresponsiveness)

While the UK NICE guidelines (2014) and a substantial amount of research uses GCS score alone to grade patient severity (e.g. (Arfanakis et al., 2002, Chu et al., 2010, Sidaros et al., 2008, Rutgers et al., 2008)) other scales and means of defining severity exist. The Mayo classification system (Malec et al., 2007) is a scale which uses only “mild” and “severe” labels, and incorporates imaging findings together with the GCS wherein any patient who would otherwise be defined as “mild” (i.e. a GCS of 13-15) becomes “moderate” if there is also visible injury on standard anatomical scans. This scale is sometimes used within research (e.g. (Sharp et al., 2011, Kinnunen et al., 2011)). Information regarding if the patient experienced loss of consciousness (LoC) and / or post-traumatic amnesia (PTA), and if so for how long, is also sometimes utilised (Rao and Lyketsos, 2000). LoC describes a period of time immediately after the injury where the patient is completely unconscious while PTA describes a period of
time after injury where a patient is conscious but is unable to store memories and therefore does not later recall (Rao and Lyketsos, 2000, Russell and Smith, 1961). These are each described in more detail in 1.8.

Thus, LoC and PTA may be used as further indicators of the patient’s state of health (as is recommended in the NICE guidelines (2014) and variably found across previous studies (Asikainen et al., 1998, Sherer et al., 2008)) or, within research, may be combined with the GCS as additional factors which the authors use to define the severity label which a patient is given. This is often done in cases of defining mild injury, in line with the Mild Traumatic Brain Injury Interdisciplinary Special Interest Group of the American Congress of Rehabilitation Medicine guidelines (Esselman and Uomoto, 1995), (e.g. (Inglese et al., 2005, Messe et al., 2011, Miles et al., 2008)). This variably classifies mild injury as either involving a LoC of less than 30 minutes and a GCS of 13-15 (regardless of PTA) or simply PTA of less than 24 hours (regardless of LoC or GCS). It also defines head injuries as mild in cases where the patient is simply dazed / confused / in a transient mental state, but without any information regarding GCS, PTA or LoC. Other research may not consider GCS at all in the definition of injury severity, such as one investigation which instead used a combination of LoC, PTA and imaging findings to define "mild" injury (Little et al., 2010).

As outlined above, “mild” head injury does not have a single working definition within research, although most means of using the term still principally revolve around the idea of the patient needing a GCS of between 13 and 15. As such, further references to “mild”, “moderate” and “severe” injury will refer only to the standard GCS definitions (i.e. a GCS of 13-15 for mild, 8-13 for moderate and 3-7 for severe). Estimates for the incidence of mild TBI claim it accounts for approximately 80% of all TBI cases, with moderate and severe injury then accounting for around 10% each (Tagliaferri et al., 2006). Although mild injury is often clinically presented without any imaging changes, thus increasing the likelihood of being discharged from hospital early, a substantial proportion of these patients suffer on-going symptoms. Although the severity of this disability is typically greater in “severe” patients (Tagliaferri et al., 2006), it is reported (Thornhill et al., 2000) that 83% of TBI cases are discharged within 48 hours despite approximately half of each severity group going on to experience
some form of chronic disability. Within the literature there is a relative paucity of studies which focus on mild TBI patients compared to those examining severe TBI. These considerations present a need for a shift in focus within the research to the nature and outcome of mild TBI.

1.3 Predicting a Patient's Outcome

In order to examine the outcome of a patient, it is helpful to first have a set method of defining how “well” a patient is at a given point in time. The Glasgow Outcome Scale (GOS (Jennett and Bond, 1975)) is widely used as a means of doing this. Using this scale, patients may be categorised into five groups, starting with “Death” and moving through “Persistent Vegetative State”, “Severe Disability (conscious but disabled)”, “Moderate Disability (disabled but independent)”, and finally “Good recovery”, which as the authors state implies a resumption of normal life albeit with some potential minor psychological complications. The scale may be used at any point following injury, although the authors highlight the strong possibility of a patient’s condition significantly improving or worsening during the first year of recovery.

Severity of injury is arguably the single strongest predictor of patient outcome with research showing that lower GCS score is associated with a worse GOS score (Mosenthal et al., 2002) and higher mortality (Chi et al., 2006). Greater severity of injury (as measured by the GCS and a number of other scales) has also been shown to predict worse re-integration into the community (Wagner et al., 2000). However there are numerous other measurable factors which have been shown to affect and / or relate the patient’s prognosis. While some of the following factors are closely tied to severity definitions they have also been shown to hold strong predictive power regarding prognosis when considered on their own.

Younger age predicts both increased chance of survival upon hospital admission (Klauber et al., 1989) and also better outcome as measured both by functional status (Oh et al., 2006) and also the GOS (Mushkudiani et al., 2007). Higher levels of education also predict better functional status (i.e. the degree of acquired disability, (Wagner et al., 2000)) and GOS (Mushkudiani et al., 2007). TBI’s obtained through a motor vehicle accident (Morrison et al., 2004) and the existence of a prior TBI (Wagner et al., 2000) are both associated with worse functional status.
Systolic blood pressure on admission greater than or equal to 135mmHG is a strong predictor of surviving the first 3 days post-injury (White et al., 2001) while low systolic pressure and heart rate are also associated with worse functional status (Oh et al., 2006). Greater length of coma is related to worse GOS (Temkin et al., 1995). Both midline shift (where the brain is shifted left or right in the skull) and the presence of intracranial lesions on CT scan are also associated with increased mortality and worse functional status (Schreiber et al., 2002, Maas et al., 2007, Bahloul et al., 2004, Oh et al., 2006). Unsurprisingly the presence of identifiable pathology following an injury also typically predicts worse GOS (Bahloul et al., 2004, Fabbri et al., 2008, Lee et al., 1998, Maas et al., 2007).

"Prognostic calculators" exist which attempt to bring together factors such as those outlined above so that an accurate prediction of a patient’s outcome may be made at the acute time of injury. Made in collaboration with one another, the CRASH (Perel et al., 2008) and IMPACT (Steyerberg et al., 2008) calculators are the most commonly used of these. The CRASH calculator can be used on any patient with a GCS of 14 or lower, and takes into account the location (by country) of the injury as well as the age of the patient, their GCS, if their pupils react to light and if there is a "major extra-cranial injury" present (i.e. one which "requires hospital admission within its own right"). Using these it predicts the probability of death within 14 days of injury as well as the risk of an unfavourable outcome at 6 months post injury (defined as "dead, vegetative state or severe disability as measured by the GOS"). By contrast the IMPACT calculator allows for the use of a more detailed set of information, taking into account factors such as the patients motor score, imaging findings by CT, if hypoxia or hypotension are present, as well as glucose and haemoglobin levels. This calculator predicts the probability of mortality and unfavourable outcome (again, defined by dead, vegetative state or severe disability as measured by the GOS) at 6 months but can only be used on moderate or severe TBI patients (GCS of 12 or less). Subsequent studies which have investigated these calculators have found them to have a relatively high degree of accuracy and lend support to the use of them in a clinical setting (Roozenbeek et al., 2012, Honeybul et al., 2014, Panczykowski et al., 2012).
1.4 A Note on the Physiological effects of TBI on the Brain

While TBI is a complex injury, the physiological impact it has on the brain can be broadly thought of to fit into two categories. The first of these is characterised by the *focal damage at the site of injury and the impact that this has on brain functioning*. The second of these is *diffuse damage throughout the brain caused by inertial forces and the impact that this has on brain functioning* (Werner and Engelhard, 2007).

1.5 Focal damage

Focal damage is defined as damage to the brain which occurs at the site of (and as a *direct* consequence of) a blow to the head. There are a number of different forms of injury which can result from this, such as cerebral lacerations / lesions, contusions and hematomas in the local area (Andriessen et al., 2010, Maas et al., 2008). A contusion describes what is essentially a bruise on the brain where a number of small blood vessels rupture that, in the case of a TBI, is often caused by the brain striking the skull. Hematomas result from a haemorrhage of local blood vessels and describe the accumulation of blood in an enclosed space. These phenomena share a clear close relationship and often occur alongside one another. Other literature, particularly that which is focused on surgical intervention, may also refer to these under the umbrella term of “mass lesion” (Compagnone et al., 2005). In addition to often occurring at the time of injury, contusions / hematomas may also develop in a delayed manner, which has been shown to predict worse patient outcome (Statham et al., 1989, Yadav et al., 2006).

As these injuries often contribute to raised intracranial pressure, surgical intervention is recommended if these are present regardless of if the patient appears to be in good health otherwise (Bullock and Povlishock, 2007), but only if the mass exceeds a certain size (Mathiesen et al., 1995). Surgical intervention usually takes the form of a mass lesion evacuation (if there is tissue / fluid that can be removed) or a decompressive craniectomy to relieve pressure (Compagnone et al., 2005). Incidence of contusion and hematoma in all TBI patients has been estimated to be 6.3% and 2.2% respectively (Kalsbeek et al., 1980, Kraus, 1980), while the proportion of severe patients who require surgical intervention is approximately 40% (Miller et al., 1981). Regarding mild patients, it is estimated that surgical intervention is required in 3% of
patients who have sustained a severe enough injury to cause a contusion (Dacey et al., 1986). Minor hematomas and contusions resolve without intervention. While a number of other secondary effects such as oedema, inflammation, excitotoxicity etc. are likely to follow focal injuries, they are also shared by diffuse injury and so are described in more detail in the shared section 1.7.

A “coup and contrecoup” injury may also occur, which may best be described as a form of secondary focal injury. As the head is struck the brain may move in the skull; if it is moved enough by the force of the injury it may impact upon the side of the skull opposite to where the head was hit initially, thus creating further focal-type damage. The “coup” injury is the main focal damage at the site of the blow while the “contrecoup” injury is this second impaction. This often results in additional contusion / hematoma at the contrecoup location (Drew and Drew, 2004) and raises the possibility of the brain sustaining what is essentially two focal injuries at opposing sides of the brain. As an example, primary frontal lobe damage may therefore also be accompanied by secondary trauma to the occipital lobe.

The severity of a focal injury is dependent on the force and nature of the impact. Based on the assumptions of the lesion model (Moses and Stiles, 2002), as a focal injury only affects a contained area long term physical and cognitive problems as a result of it are underpinned by the damaged location becoming unable to perform its function. Damage associated with focal injuries is often visible on anatomical scans (Figure. 1.1) and is therefore relatively easy to diagnose and examine. Research has shown that severity of injury (as measured by GCS score) is a predictor of abnormal CT findings such as these (Gomez et al., 1996), adding further evidence to the statement that anatomical scans of mild TBI's may often be "normal appearing".
1.6 Diffuse Axonal Injury

As stated, many patients who have suffered a mild TBI may be deemed fit for discharge from hospital care within a relatively short space of time due in-part to factors such as normal-appearing anatomical scans. However chronic impairment is often still seen in people with unremarkable scans, suggesting that neuronal damage that is beyond the diagnostic reach of techniques such as Computed Tomography (CT) and standard Magnetic Resonance Imaging (MRI) still exists. Diffuse microscopic damage, referred to as Diffuse Axonal Injury (DAI (Adams et al., 1982)), is the commonly accepted form of injury which causes this impairment, and also represents the other major physiological impact a TBI can have on the brain beyond focal trauma.

An incident which results in a TBI often involves rapid movements of the head, causing inertial effects (accelerational, de-accelerational and rotational forces) to act on the brain. As the brain is not a homogenous structure, instead being composed of

![Figure 1.1. A T₁ weighted MRI scan of a TBI patient. Focal damage, appearing as a white area of grey matter, is visible at the anterior of the left frontal lobe (due to anatomical spacing right and left are flipped in this image).](image)
numerous different tissues in different orientations, these physical forces stretch and strain layers of axons causing damage in a relatively diffuse manner. DAI is what results from this pattern of injury. As such, while a contact force such as an object striking the head may in itself result in DAI through a secondary shockwave effect, it is incorrect to consider such blunt trauma as being a direct cause. DAI is viewed as a largely global type of injury, although because of the vulnerable nature of specific neural regions; primarily white matter tracts such as the corpus callosum (CC) and the corticospinal tract (Adams et al., 1989), it has been suggested that it would be more accurately termed a “multi-focal injury”.

In order to most accurately examine both the presence of, and mechanisms involved in DAI, histopathological examination is required. Therefore much of the experimental information concerning its mechanisms comes from animal studies or experiments on cellular models. DAI is characterised both by the initial, mechanical disruption of the neurofilament / cytoskeleton of the axon as well as biochemical cascades which follow this (Povlishock and Katz, 2005). Although the initial forces involved in very severe DAI can tear an axon in two (“axotomy”), this is rare and it is usually secondary effects which are most problematic.

Assuming that axotomy does not occur at the site of damage, axons initially undergo a stretch injury where they can elongate by up to 60% of their original length and take on an undulating (Smith et al., 1999) and distended (Smith and Meaney, 2000) appearance (Figure 1.2, part 2). A “delayed elastic” process may then occur over the course of approximately 45 minutes where the axon returns to its original, normal appearing form (Figure 1.2, parts 3-5). Axoplasmic transport becomes impeded at the site of this primary damage, leading to axonal swelling and the formation of a “bulb” at the terminal end of the axon (also sometimes referred to as a “retraction ball”), which may be seen forming by 2 hours following injury (Figure 1.2, part 6). The composition of this swelling is thought to involve an accumulation of transport materials, organelles (Smith and Meaney, 2000) and remnants of the damaged cytoskeleton, such as neurofilament sidearms (Chen et al., 1999). This often becomes more severe over the course of days / weeks, eventually leading to the axon breaking into two and undergoing Wallerian degeneration, the process of which may be ongoing for months (Povlishock and Katz, 2005), via this secondary axotomy. While this description can be
taken as the textbook explanation of stretch injury, it should be noted that the nature of axonal swelling does not necessarily always follow this discrete pattern of a single, easily identifiable bulb. Depending on the location and load of the inertial forces, an axon may become swollen down almost its entire length (Chen et al., 1999), hypothetically as a consequence of a more uniform stretch rather than one with a focus at a specific point.

Figure 1.2: A series of immunofluorescent microscope images demonstrating an axon undergoing stretch injury. Following initial damage, the axon first takes on an undulating appearance, before returning to its initial appearance via a delayed elastic process. Finally, an axonal bulb is seen developing. Time progression is indicated by numbers. The time between the first and last image is approximately 2 hours. Figure adapted with permission from Smith & Meaney, 2000.

Astrocyte invasion to DAI affected locations is seen at 1 day and myelin is observed to visibly disintegrate and “clump” around 7 days post-TBI alongside phagocytic cell invasion (Mac Donald et al., 2007). Findings from studies which use techniques such as Diffusion Tensor Imaging (discussed further in section 2.3) have
also suggested that by the chronic time point the myelin sheath may completely disintegrate in damaged areas (Song et al., 2003). This further implies that DAI may be severe enough to cause damage to the myelin, but mild enough to spare the axon.

1.7 Secondary Physiological Complications from DAI and Focal Damage

While the exact cause of damage in focal injury and DAI is different from one another, and the immediate physical injury is distinguishable, it should be noted that each injury may lead to the same delayed responses. These represent a collection of symptoms and changes which are more generally associated with any insult to the brain. TBI patients may therefore also present with the phenomena described in this section.

Alterations in cerebral blood flow are common and most often take the form of hypoperfusion (decreased blood flow) leading to possible ischemia (Bouma et al., 1992) which may last for days following the injury; one study reports that 37% of severely injured patients suffered low brain tissue oxygenation levels at 2 hours post injury, decreasing to 18% after 2 days of treatment (Narotam et al., 2009). Ischemia is associated with very poor outcome for the patient and is therefore most frequently seen in cases of more severe injury (Inoue et al., 2005). Hyperperfusion (increased blood flow) may also been seen, albeit to a lesser extent (Werner and Engelhard, 2007). Some research has indicated that hyperperfusion is associated with less severe injury and acute injury symptoms (Sakas et al., 1995). Other research has shown that while it also predicts a good outcome in many patients, it may also lead to severe complications in others due to associated vasoparalysis (Kelly et al., 1997). In these cases it is hypothesised that hyperperfusion may therefore variably represent either an appropriate functional response to injury in good outcome patients, or a dangerous disconnect between blood flow and metabolic need in poor outcome patients (Kelly et al., 1997).

Metabolic functioning is sometimes seen to be impaired leading to a reduction in oxygen and glucose consumption, and a reduction in local concentrations of ATP (Glenn et al., 2003). This appears to occur as a result of mitochondrial dysfunction (Verweij et al., 2000). Hyperglycolysis (increased metabolic uptake of glucose) has also been found in locations close to focal injury (Bergsneider et al., 1997). This is thought
to occur as a consequence of local ionic inbalances (Bergsneider et al., 1997), and may cause further damage to axons if cerebral blood flow does not adequately increase to match this increased energetic demand (Chen et al., 2004).

Excitotoxicity, where an overabundance of excitatory neurotransmitters such as glutamate cause neuronal damage through overstimulation (Rothman and Olney, 1987), is also known to occur following a TBI (Bullock et al., 1998). Here, the increased levels of neurotransmitters leads to increased catabolism of them, which in turn causes cellular damage as neurons allow toxic amounts of calcium ions to enter them, leading to cell death (Yi and Hazell, 2006). A further influx of calcium and sodium due to ion exchange disruption following damage to the sodium channels may also be seen (Wolf et al., 2001), while caspase-3 mediated apoptosis has been observed to be initiated by 1 day post-TBI (Yakovlev et al., 1997). As apoptosis is an energetic process (Liu et al., 1996), if the damaged location does not have access to sufficient ATP then necrosis of cells occurs (Andriessen et al., 2010). Interestingly, necrosis has been seen to increase in the presence of increased extracellular glutamate (Ankarcrona et al., 1995) while intracellular calcium may have a mediating role between which of necrosis or apoptosis occurs wherein relatively low levels of the ion favour apoptosis and relatively high levels favour necrosis (Zipfel et al., 2000).

Oedema (intracranial swelling due to increased fluid) is also common. This may take the form of vasogenic (Dewitt and Prough, 2003) or cytotoxic (Unterberg et al., 2004) oedema. Vasogenic oedema is characterised by an increase in fluid, ion and protein transfer from the endothelial junctions of the blood-brain barrier to the extracellular space within brain tissue. Conversely, cytotoxic oedema is characterised by increased water concentration in the intracellular space of brain cells such as neurons and astrocytes which occurs as a consequence of increased cell membrane permeability and a failure in ATP production (as a possible consequence of hypoxia (Heo et al., 2005)) meaning the cell lacks the energy requirement to adequately pump ions out into the extracellular space (Liang et al., 2007).

Finally, inflammation may also occur. This is initiated first by an increase in the release of various pro-inflammatory cytokines in response to tissue injury, which in turn recruit and activate a variety of other molecules and cells (chemokines,
macrophages, astrocytes and other “immune cells”), resulting in inflammation (Rothwell and Luheshi, 2000, Lucas et al., 2006). These cells and molecules may be endogenous to the brain (e.g. astrocytes (Pekny and Nilsson, 2005)) or exogenous such that they are part of the body’s broader immune response (e.g. neutrophils (Clark et al., 1994)). It is due to these inflammatory processes that damaged tissue is destroyed within days. Of particular note here is astrogliosis; the migration of reactive astrocytes (a form of glial cell) to the site of damage (Pekny and Nilsson, 2005). In many cases these cells remain, effectively causing a lasting scar which is widely acknowledged as being a barrier to effective chronic recovery as it impedes reinnervation (Lucas et al., 2006, Rudge and Silver, 1990, Frisen et al., 1995). However research studying transgenic mice has also shown astrogliosis to have important therapeutic effects as the absence of it leads to a considerably worsened physiological outcome in terms of demyelination, cell death and blood-brain barrier disruption (Faulkner et al., 2004).

1.8 The Effects of TBI on the Patient

Outcome following TBI is very variable both in terms of on-going symptoms and their severity. Patients may experience long term cognitive and / or physical disability that may in turn impact further on their mental wellbeing (e.g. depression may follow) and their social life. There is increased risk of suicide, unemployment, divorce and substance abuse following TBI (Ragnarsson et al., 1999). As it is possible for any area of the brain to be damaged, it is possible for a wide range of neurologically-based symptom to develop. However, as alluded to, focal and diffuse damage contribute to deficits in different ways.

Because of its focus in major white matter tracts, DAI is hypothesised to contribute to cognitive dysfunction through disruption of cortical networks, interfering with communication between functionally-dedicated neural regions coined “nodes” (Mesulam, 1998). It should therefore be thought of differently to cognitive dysfunction caused by focal injury, where the driving factor of the impairment is likely to be a specific node becoming unable to perform its function following damage to it. Determining which of these mechanisms is causing dysfunction in TBI patients can be challenging, as both focal and diffuse damage is likely to have occurred. Nevertheless, studies on mild and moderate TBI patients (e.g. (van der Naalt et al., 1999)) and others
conducted on cases of “pure DAI” (e.g. (Scheid et al., 2006)) have frequently found psychological problems, indicating impairment to exist in all injury severities, and supporting the hypothesis of lasting DAI-induced impairment following a TBI, with or without focal damage. While every injury is undoubtedly individual and different, the somewhat predictable nature of DAI in terms of primarily susceptible locations means that aspects of network disruption might share a common pattern between different patients.

As also alluded to previously, short term impairment following injury is commonly seen in the forms of loss of consciousness (LoC) and post-traumatic amnesia (PTA, sometimes referred to as “post-concussive syndrome”, (Annegers et al., 1998)). Although research has put forward the notion that these symptoms should not necessarily be required for a diagnosis of head injury (Servadei et al., 2001), and that clinical attention must still be paid to patients who have no experienced these (Smits et al., 2007), they are still both regularly found even in TBI patients with a GCS of 15 (Batchelor and McGuinness, 2002). As previously discussed in section 1.2, some research considers either of both of these as criteria for grading severity of injury in patients.

The definitions of LoC and PTA are arguably best described with reference to scales which attempt to grade them. The Galveston Orientation and Amnesia Test (Levin et al., 1979) is one of the most widely used of these. By this scale, LoC is essentially a state of coma prior to PTA, while PTA then describes the brain moving from a total lack of consciousness into an altered state of one on the way to being fully aware again. During PTA the patient may be unable to form new memories and therefore may not later recall this time (Rao and Lyketsos, 2000, Russell and Smith, 1961). When the patient regains “normal consciousness” the PTA is deemed to have resolved (ignoring any more permanent cognitive deficits the patient may have sustained). It is unsurprising that LoC and PTA correlate with severity of injury, and are also risk factors for the development of subsequent problems such as seizures (Annegers and Coan, 2000). While the differing ways that LoC and PTA are considered in defining injury severity means that such comparisons are somewhat tenuous, as an approximation LoC rarely lasts longer than 15 minutes while PTA rarely lasts longer than an hour in mild TBI (Smits et al., 2007). However in more severe cases these
durations can extend to weeks and months if the patient falls into a significant coma (Tate et al., 1991), while a patient in a vegetative state may be considered to never recover from them.

Long term issues following a TBI can be broadly separated into two groups; physical problems and cognitive problems. Headaches, pain, dizziness, visual impairment and (more rarely) epilepsy are typical physical issues (Arciniegas et al., 2005). As discussed, cognitive dysfunction is directly attributable to the locations of the brain which have become damaged, be they white matter tracts or focal locations. Despite the implied variability which can occur from this (and indeed, every case is different) certain symptoms are common following TBI. These problems can be most broadly considered to involve issues surrounding executive function; attention, concentration, speed of information processing etc. (Arciniegas and Silver, 2006), although other symptoms such as depression (Silver et al., 2009) personality change, unstable emotions and the acquisition of affective / sleep disorders are also typical (Reeves and Panguluri, 2011). Severity of symptoms typically increases alongside severity of injury (Dikmen et al., 2009).

1.9 Recovery from TBI

Similar to many cases of cognitive disruption, patients often recover a degree of function following a TBI. While many mildly injured patients do suffer from chronic problems, they have a better prognosis than those with a severe injury. Indeed, while some research reports that approximately half of mild patients see a “full recovery” by 1 month post-TBI (Silver et al., 2009), other research which focuses on severe patients typically report much higher levels of chronic disability with two thirds of patients still experiencing significant cognitive, behavioural and emotional problems two years post-injury (Ponsford et al., 1995). Other studies (as previously outlined) estimate that approximately half of TBI patients in each severity bracket (i.e. mild, moderate and severe) experience some form of chronic disability (Thornhill et al., 2000).

While a substantial recovery within weeks following a TBI is likely due to the resolution of temporary factors such as contusion and oedema, the mechanisms underpinning long term recovery are more complex. Pharmacological intervention can be useful to help the patient on a symptom-by-symptom basis (e.g. prescribing SSRI’s
to help with depression (Fann et al., 2009), while both experience of living with their deficits and other rehabilitation techniques can lead to the patients developing effective coping strategies (Cicerone et al., 2006). Examples of these include cognitive exercises to improve specific skills, being trained to use compensatory objects such as a memory book, and psychotherapy to assist with the burden that the patient’s injury might be placing on their own mental wellbeing and family members (Ragnarsson et al., 1999). There is also substantial evidence that the brain physiologically reorganises neural networks to avoid using damaged locations, similar to other neurological conditions who’s pathology causes cognitive impairment (be they the functionally specific nodes as would be implicated in a focal injury, or parts of white matter tracts as would be implicated in DAI, (Castellanos et al., 2011, Buldu et al., 2011, Castellanos et al., 2010)).

1.10 TBI Summary

TBI is a common and debilitating condition. It results in a decreased standard of life for patients at all injury severities, and as such also places a major, long-term burden on healthcare systems worldwide. Even though mild TBI accounts for approximately 80% of all cases, it remains understudied within the literature. It is therefore of paramount importance to better understand the exact means by which injury to the brain is sustained in mild TBI, and how this affects the cognitive outcome of a patient. Doing so will lay the foundation for more effective treatment strategies to be developed.
Chapter 2. Imaging TBI and DAI

2.1 Imaging TBI: The Principles of MRI

Magnetic Resonance Imaging (MRI) is an important imaging technique for both diagnostic and research purposes. Like any imaging methods, the crux of MRI imaging is in generating contrast so that useful images can be produced. MRI exploits how different tissues respond when placed in a magnetic field and subjected to pulses of radio waves. In more depth, the technique works on the theory that when placed in a strong magnet, the magnetic moment of hydrogen atoms (the main component of water) in the body will align along the magnetic field. If energy is given to the atoms in the form of a radio frequency pulse (RF), the atoms will be knocked off of this alignment before gradually returning to the equilibrium state (“relaxing”) when the pulse stops. As different tissues have different compositions some achieve this re-alignment faster than others. At the same time this shift in alignment creates magnetic flux which can be detected via changing voltages in receiver coils. Simple images can be formed by using the differences in relaxation time to modulate the detected signal level, providing contrast between different tissue types. MRI was first used in an attempt to detect cancerous tumours by examining differing magnetic relaxation times in 1971 (Damadian, 1971) before another pioneering experiment in 1973 used this contrast to produce an image (Lauterbur, 1973).

The MR signal has three main properties which can be manipulated in order to gain different types of information about the subject being scanned. The first of these is proton density (PD), which refers to the density of water molecules (more water means more hydrogen atoms to generate a stronger signal). The relaxation times $T_1$ and $T_2$ refer to the rate at which the protons return to alignment along the magnetic field. $T_1$ refers simply to the time for the atoms to return to full alignment along the magnet field, while $T_2$ instead defines the rate at which the signal “dephases” due to interactions between different atoms; these interactions between them mean that they start to precess at different rates which affects the net magnetisation.

Each of these factors can be used to produce image contrast. This is achieved by manipulating scanner variables to enhance the signal from the factor of interest and supress signal from the other two. The main scanner variables we can control to do
this are; the length of time between consecutive RF pulses (called $TR$), the length of time we wait from an RF pulse to when we take a signal measurement (called $TE$) and the strength / timing / direction of different magnetic gradients that are applied to the subject during scanning (called the $b$ value). Thus, a PD image would show greater intensity in areas of higher water content. A $T_1$ weighted ($T_1$W) scan is adept at viewing basic anatomy of the brain and showing areas of fatty tissue. A $T_2$ weighted ($T_2$W) scan is adept at showing areas of pathology in the brain such as white matter lesions and oedema. While these scan types represent some of the most basic forms of MRI images, manipulation of other factors can be used in more advanced means to create “advanced MRI images”, as described below.

2.2 Advanced MRI and DAI

The identification and diagnosis of DAI following TBI is difficult due to its microscopic nature. While CT remains the standard scan for assessment of TBI patients (NICE guidelines (2014)) and is effective in detecting focal damage, it is not well equipped to detect DAI compared to some other scan techniques. These often attempt to use biomarkers as a means of identifying pathology (Bazarian et al., 2007). One of the most popular of these alternative methods is the advanced MRI technique of Diffusion Tensor Imaging.

2.3 Diffusion Tensor Imaging

Diffusion tensor imaging (DTI (Basser et al., 1994)) is an MRI technique that uses water molecule movement to provide quantitative information regarding the microstructure of the brain in vivo. DTI uses the diffusion coefficient of the MR signal to produce image contrast, unlike more conventional MRI scan types which (as described) typically use relaxation times instead. In simpler terms this means that the diffusion-behaviour of water molecules in the brain is quantified and used to produce images which visualise the structural orientation of different brain tissues. This is most often used to "image" and analyse the brain's white matter tracts.

As stated DTI works by using the diffusion coefficient of MR scans for image contrast. As the MR signal is primarily produced from water molecules, the amount that water diffuses during a scan time through random thermal motion (or "Brownian"
motion; approximately 10μm with the timeframe of a typical MR measurement (10-100ms, (Mori and Zhang, 2006)) is another aspect of the MR signal. While this impedes standard anatomical MR scans by fundamentally limiting the resolution which can be achieved (effectively introducing blurring beyond a certain, extremely-high resolution point), DTI instead exploits this motion by creating an image of this diffusion. This one image is achieved by creating two images. The first of these is of the subject (a brain) under a strong, linear magnetic gradient, creating a phase difference as atoms resonate at different frequencies depending on their position along this gradient. This differing rate of precession can be calculated as the “Larmor frequency” using the Larmor equation (Equation 1, below), by knowing the scanner bore strength and the gyromagnetic ratio of different elements (the frequency of a specific element in a magnetic field strength of 1T). A second image is then acquired when an opposite gradient of the same strength is applied for the same period of time.

\[ \omega = \gamma B_0 \] [1]

**Equation 1.** The Larmor equation; the relationship between the rate of precession of a proton (\(\omega\)), the gyromagnetic ratio (\(\gamma\)) and the magnetic field strength (\(B_0\)).

In a hypothetical situation where all atoms in the brain remained static, this opposite gradient would cancel out the phase difference from the first, rephasing all the atoms perfectly. However, as water diffuses, atoms of different initial phases become "out of place". This makes the rephasing of the second gradient imperfect, which in turn leads to a signal loss in the second image compared to the first. Therefore, by subtracting one of these images from the other, diffusion-based contrast is produced. While doing this once only gives information regarding one linear direction, it can be repeated using multiple different gradient alignments, gaining diffusional information of the brain in a three dimensional space. With a minimum of six directions (although ideally many more would be used), this data may then fitted to the "diffusion ellipsoid" (Figure 2.1, (Basser et al., 1994)); a mathematical model which allows the direction and extent of water diffusion to be visualised in a three dimensional space. Each voxel from a DTI image is essentially one of these ellipsoids.
As the diffusion of a water molecule will be determined by its surrounding structure (it will not be able to diffuse far into the wall of an axon, but will diffuse relatively unimpeded down the length of one) this data is capable of producing images of the brains microstructure. Further, as comparisons between scans of the brain \textit{in vivo} and \textit{in vitro} produce similar results, it can also be concluded that DTI predominantly detects fixed microstructure and not physiological processes (Sun et al., 2005). Basic images of the brain produced by this method therefore show tissue \textit{directionality} as indicated by the principle direction which water diffuses in (or the water's "anisotropy"). An example of this is given below in Figure 2.2, where a computer program has produced a 3 dimensional representation of the brain’s fibres based on the underlying water diffusion.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{diffusion_ellipsoid.png}
\caption{The diffusion ellipsoid. This shape visualises the nature of water diffusion in a voxel and is what DTI data is transformed into. The shape is defined by three directions (the arrows in the figure) which describe the direction and extent of water diffusion in a three dimensional space. The directions are referred to as \textit{eigenvectors} while the magnitudes of diffusion in these directions are \textit{eigenvalues} (or $\lambda_1$, $\lambda_2$ and $\lambda_3$). $\lambda_1$ always describes the largest magnitude, while $\lambda_2$ and $\lambda_3$ are placed perpendicular to $\lambda_1$.}
\end{figure}
This method is called tractography and is primarily used to visualise the brain’s white matter tracts. Methodologically this is achieved by locating adjacent voxels where the anisotropic magnitude is sufficiently large to indicate a surrounding structure and the principle directions of anisotropic are aligned, indicating a continuous structure, i.e. a nerve fibre tract. Researchers have used this technique for projects such as creating atlases of the brain’s major white matter tracts (Wakana et al., 2004).

Quantitative measurements can be extracted from this method allowing for statistical analysis. These are referred to as metrics. One widely used example of these is Mean Diffusivity (MD). This is the average of the diagonal terms of the diffusion tensor; describing the diffusion characteristics of the white ignoring direction. Theoretically, this would be a low figure in locations which contain a large amount of structure and would increase as this structure is lost. The Apparent Diffusion
Coefficient (ADC) is sometimes used as an alternative and is very similar to MD, being instead calculated as the average of 3 single orthogonal measured directions. Thus, MD and ADC results are the same in purpose and largely identical in methodology. They are considered comparative and are both referred to as “MD” following this point.

Further metrics are more directly based on the ellipsoid shape. As $\lambda_1$ describes the direction where water diffuses to the greatest extent, this eigenvalue is often interpreted as being indicative of a water molecule diffusing down the cylinder of an axon (whereas $\lambda_2$ and $\lambda_3$ would then describe water diffusing into the sides of the axon). $\lambda_1$ can be therefore referred to as the Axial Diffusivity (AD), while $\lambda_2$ and $\lambda_3$ can be averaged to describe diffusion in a radial direction, referred to as Radial Diffusivity (RD, see Figure 2.3). When examining diffusion in an axon AD is relatively unrestricted compared to RD, as diffusion in the radial direction is impeded by the myelin sheath (Song et al., 2003). Measures of anisotropy can then be derived from the Eigen values, the most commonly used being Fractional Anisotropy (FA). FA is a number between 0 and 1 which describes how unidirectional water diffusion is (higher numbers indicated higher anisotropy). These principles are outlined in Figure 2.3, overleaf.
2.4 DTI in TBI

Though DAI can be detected with T2-weighted MRI (Scheid et al., 2003), it is not easily imaged by standard MRI and CT due to its microscopic nature and is often overlooked by such scans (Smith et al., 1995). DTI has been proposed as a useful, in-vivo method of investigating DAI following TBI (Huisman et al., 2004), using changes in DTI metrics to assess the extent of axonal injury. In this view, a “healthy” axon is deemed to have a self-containing structure and purposeful direction, giving it high anisotropy. However a DAI-damaged axon will have either experienced acute trauma, or will be undergoing Wallerian degeneration in the more chronic time-frame. Such
events hypothetically increase its MD and lowering its FA as events such as axonal disconnection and demyelination occur and affect AD and RD.

Broadly speaking, there are two main approaches to analysing DTI data. In one case software can be used to place a Region of Interest (ROI) on a DTI scan to then extract information about the fibres which run through the specified area (e.g. (Bigler and Bazarian, 2010)). Alternatively, the brain can be analysed as a whole by spatially normalising the scans of the participants to remove intra-subject variation in brain size and shape and then comparing data voxel-to-voxel (e.g. (Kinnunen et al., 2011)). The ROI approach represents a focal investigation and has seen the most experimental use. It provides high statistical sensitivity within the ROI, although lacks the analytic scope of a global technique and needs an a-priori hypothesis regarding a specific location to be used effectively. The voxelwise approach represents this global alternative, being advantageous in that it can statistically analyse the whole brain, making it effective for exploratory analyses. However the additional normalising processing which takes place between participant scans means the data in the shared standard space may become slightly warped (although this is a common methodological caveat in MR research). Ultimately the best method to use will likely depend on the research question.

The past 13 years have seen an increasing amount of research being conducted in this area (Hulkower et al., 2013), leading to recent research claiming that DTI has become "the most sensitive and predictive MRI biomarker in mild TBI research" (Bigler, 2013). Most overlooked however is the inclusion of patients in the acute phase of injury (<2 weeks, although this is understandable due to the complications of recruiting patients within this timeframe), and examination of mild patients in the chronic phase of injury. Longitudinal research is also relatively lacking within the field, meaning that assumptions regarding the behaviour of DTI metrics at different time-points are primarily formed by piecing together work from different experiments / patient groups. Further issues include areas of change in the brain following TBI may often be missed due to most reports using a ROI approach and also a relative lack of data concerning AD / RD alterations in favour of reporting FA alone. With these methodological drawbacks, it is best to consider the changes caused by TBI by collating results from the literature rather than focusing on individual studies.
Acutely, MD does not present a clear picture of change. Reports can be found of increasing (e.g. (Inglese et al., 2005, Miles et al., 2008, Lipton et al., 2009)), and decreasing (e.g. (Wilde et al., 2008, Naganawa et al., 2004)) MD. Chronically however, there are a striking number of papers which demonstrate a MD increase (e.g. (Rugg-Gunn et al., 2001, Rutgers et al., 2008, Newcombe et al., 2010)) to suggest that elevated MD is the expected long term change from TBI. There is a trend that decreased MD is a result primarily limited to studies in acute, mild patients (e.g. (Wilde et al., 2008, Chu et al., 2010)). MD primarily increasing supports the hypothesis that DAI-affected locations generally lose integrity and structure. As cases in which MD decreases are mostly acute and mild, the decrease could be driven by phenomena such as astrogliosis (Budde et al., 2011), or the formation of oedema which is thought to decrease MD acutely (Barzo et al., 1997). Ischemia has also been shown to decrease MD acutely which could contribute to this in some cases (Vangelder et al., 1994).

FA is the most frequently investigated metric, and is very reliably found to decrease following TBI (e.g. (Kumar et al., 2009, Lipton et al., 2009, Matsushita et al., 2011)). However there are some cases which contradict this by reporting an elevation in FA, which are often associated with the same patients who exhibit a decrease in MD (e.g. (Bazarian et al., 2007, Wilde et al., 2008)). These cases are again in primarily acute, mild patients. It has also been shown that following an acute increase, FA may normalise over a period of 3-5 months (Mayer et al., 2010).

RD is seen to reliably increase following injury (e.g. (Sidaros et al., 2008, Arfanakis et al., 2002, Newcombe et al., 2007)) while unexpected decreases have occurred only in the same papers which demonstrated an unexpected increase in FA. Papers which have examined AD primarily report it as being unchanged (e.g. (Newcombe et al., 2007, Ljungqvist et al., 2011, Ewing-Cobbs et al., 2008)) while increases (Kraus et al., 2007) and decreases (Arfanakis et al., 2002) are reported in fairly similar quantities. As with other metrics however, a more reliable pattern is seen in chronic cases where AD convincingly increases (e.g. (Sidaros et al., 2008, Caeyenberghs et al., 2010, Newcombe et al., 2010)) in a majority of cases.

To summarise these anisotropy results, the reliable finding of an FA decrease after TBI supports the notion of general disruption of axons, reducing directionality.
This appears primarily driven by a fairly reliable increase in RD, likely itself caused by the disintegration of the myelin sheath. Cases in which FA increases are the same as those where RD decreases and appear to occur in acute, mild TBI subjects. One of these papers (Wilde et al., 2008) shows FA to decrease in only 2 of 10 mild (GCS 15) subjects, but notes that these were the patients examined at the furthest point post-TBI (4 and 6 days). This suggests that an FA increase is perhaps a phenomenon of the mild, very acute TBI patient. Conclusions regarding what drives this change are difficult to form. One hypothesis (Chu et al., 2010) is that the formation of oedema can drive FA up in parallel with the MD decrease. This is hypothesised to be due to water moving from the extra-cellular space to the intra-cellular space; as extra-cellular water is primarily detected in DTI the decreased amount of this leads to decreased MD (Barzo et al., 1997). Alternatively it is possible the injury and current after-effects are so slight that axons are compressed by the accident rather than strained (Wilde et al., 2008). AD also contributes to the FA change with its more variable nature representative of the greater number of events which can occur axially; bulb formation, disconnection and direct shearing (Polvishock & Katz, 2005) in more severe cases could all imply different outcomes for the measure. Indeed, a further hypothesis regarding acute increases in FA states this it could be a result of astrogliosis, whereby the long processes of invading astrocytes confer increased AD and thus increased FA to the area (Budde et al., 2011). Ultimately though, it appears that when the brain stabilises chronically AD becomes predictably increased.

It is cases that both acute and mild which generally introduce the most variability to these patterns. This is likely due to both the multitude of initial immune responses and their proportionate relationship to injury severity. Chronically, patients settle into a predictable pattern of increased MD and decreased FA, which is driven primarily by increased RD and AD. It should be noted that mildly injured patients still show more variability than severely injured patients in the chronic phase of injury, although tend to conform to the pattern described. This is suggestive of neuroanatomy with decreased structure due to neuronal death and demyelination. These changes are summarised in Table 2.1, below.
Attempts to link DTI findings to cognitive outcome have also yielded results, suggesting that these metrics can be valid markers of damage. One paper (Salmond et al., 2006) found positive correlations in chronically injured patients of mixed injury severity between MD and measures of learning and memory in the posterior cingulate, hippocampus and temporal, frontal and occipital cortices. Also using chronically injured patients of mixed injury severity, another paper (Kraus et al., 2007) employed a unique technique termed “white matter load”; the number of their 13 ROI’s with reduced FA compared to controls. This was found to correlate to cognitive domain scores (summarised scores of multiple individual tests which examine the same, more general cognitive function) of executive function and memory, while individual ROI’s also showed a large variety of correlations with executive, memory and attention domain scores. Further research into mildly injured patients (Miles et al., 2008) examined if acute DTI metrics held any power in predicting psychological outcome at 6 months. One correlation was found between averaged acute FA values from the centrum semiovale / CC / posterior internal capsule and the “Prioritization form B” test (a test of an individual’s ability to prioritise).

Other work (Little et al., 2010) using another “cognitive domain” approach in patients of varying injury severity and time since injury found positive correlations between FA in the thalamic tracts and cognitive test scores. While these papers thus far used ROI methodology, voxelwise investigations into links between DTI metrics and cognitive impairment have more recently surfaced. One of these (Kinnunen et al.,

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Table 2.1. A Table to demonstrate the changes in DTI metrics following TBI, separated by time since injury and injury severity. An arrow indicates that an increase/decrease as a result of TBI in that metric is the most frequently reported result, a flat line indicates that no change is the most frequently reported result and “Variable” indicates that there is no clear pattern of change reported.
2011) used a chronic patient cohort of mixed injury severity and found a correlation between FA in the fornix and associative memory in addition to discovering that performance on the set-shifting task (a test of executive function) was correlated to MD and RD. The directions of all the relationships highlighted here, as well as others which have been reported (e.g. (Arenth et al., 2014), are suggestive of greater injury as measured by DTI (i.e. decreased FA / increased MD) to underlie greater cognitive dysfunction.

2.5 Magnetic Resonance Spectroscopy

Magnetic Resonance Spectroscopy (MRS) is another magnetic resonance technique that can be used to investigate TBI by quantifying changes in neural metabolite levels. MRS exploits the principle that atoms resonate at slightly different frequencies depending on their local (chemical) environment. This phenomenon can be manipulated to create plots of signal frequency or “chemical shift” mapped against signal intensity - the MR spectrum. Chemical shift essentially represents a range of frequencies, the value of which are dependent on both the differing atomic nuclei which they originate from and also the B₀ strength of the scanner. By referencing the shift to the Larmor Frequency of the scanner a new value is created known as "parts per million" (ppm), allowing for different atoms to be distinguished from one another. The presence of a metabolite can therefore be detected in MRS by the identification of a resonance peak at the metabolites known ppm. The size of the peak can also be used to assess the concentration of the metabolite (via the area underneath the peak; the “peak integral”) or can be expressed as a ratio in comparison to the peak integral of other metabolites.

MRS methodology typically employs voxels of interest (VOI’s) in order to avoid saturation from very strong lipid signals which are present in the skull. Alternatively post-scan manipulations such as spatial encoding and lipid saturation may be applied to address this. Regardless of which of these techniques are applied, analysis of MRS data usually then involves investigating metabolite concentrations in pre-defined VOI’s. As the concentration of water in brain (>50 molar) is many orders of magnitude larger than the concentration of metabolites (millimolar quantities), large areas of tissue must be sampled in order to gain a reliable metabolite signal. MRS therefore has
a relatively large spatial resolution with sampled volumes usually being at least 1cm$^3$, and often more. MRS studies can be divided by use of single voxel or MR spectroscopic imaging (MRSI) methodology. The single voxel approach acquires its spectrum from one relatively large voxel (2-3cm$^3$), while MRSI instead employs imaging gradients to localise the signal to a matrix grid, increasing its resolution but requiring more time to complete (Marino et al., 2011). Repositioning a large VOI in a longitudinal investigation can present difficulties also as the brain is likely to have changed in structure over time. However this is primarily problematic for studies obtaining spectra from the site of the injury as partial volume of normal tissue is likely to change; many studies use the same location (e.g. frontal white matter) as a VOI across all patients.

2.6 MRS in TBI

MRS is popular in TBI research due to its uses in quantifying metabolites relevant to neural functioning / damage in vivo. The common objective of doing this is to investigate TBI using metabolite concentration as a biomarker; analysing the concentration of specific metabolites between different experimental groups (e.g. controls vs. patients) or against other values of interest (e.g. psychometric test scores) to gain insight into way that TBI affects brain functioning. The most commonly studied of these are N-acetylaspartate (NAA), Choline (Cho), Creatine plus Phosphocreatine (Cr) and Lactate (Lac). Myo-inositol (mI) and glutamate/glutamine (Glx, see Lin et al., 2005) have also been studied, albeit to a lesser extent. The appearance of a typical MRS spectra as well as the locations of these peaks is demonstrated in Figure 2.4, below.
Reported directions of changes in metabolites are relatively consistent following TBI. Of the metabolites described, N-acetylaspartate (NAA) arguably attracts the most focus despite its exact purpose in the mature brain being one of intense debate. It is known to have a direct relationship with the mitochondrial synthesis of ATP and is found almost exclusively in neurons of the CNS (Bates et al., 1996). The combination of these facts means it is variably viewed as a marker of cell viability or of general neuronal density (Moffett et al., 2007). Acutely, NAA has the most predictable alteration with a multitude of studies reporting a decrease following TBI (e.g.)
(Nakabayashi et al., 2007, Tollard et al., 2009, Vagnozzi et al., 2010, Garnett et al., 2000b)). NAA levels appear related to time since injury, frequently demonstrating a “recovery”; after being significantly decreased compared to controls at 3 days, NAA was demonstrated to return to control levels by 30 days post injury in a mild patient cohort (Vagnozzi et al., 2010). In a severe patient cohort, NAA levels were shown to decrease over time to become significantly lowered compared to controls at 10 days post injury, before they recovered to control levels in good outcome patients by 40 days (Signoretti et al., 2008). Such recoveries are more common in milder injury. Cases where NAA remains low over time (e.g. (Garnett et al., 2000a)) tend to have a more severe patient cohort and it has also been shown that patients who died following injury had significantly lower NAA than patients who lived (Belli et al., 2006). Other research such as a case study at 21 months post-TBI (Danielsen et al., 2003) is suggestive that even with severe injury the recovery may still occur if enough time is given. It is not a reasonable assumption however that this recovery represents an increase in the number of neurons to control levels. It is instead hypothesised that the drop in NAA is due to suppressed mitochondrial activity while the recovery is able to occur after the brain establishes a normal output of ATP molecules (Vagnozzi et al., 2010).

The metabolites found at the Choline peak (Cho, primarily phosphorylcholine and glycerophosphocholine (Miller et al., 1996)) are known for their membrane-related roles, such as being released as a product of inflammation (Brenner et al., 1993). Cho is reported to increase following injury, although this is not as predictable a change as the decrease of NAA. While this increase is often reported (e.g. (Govindaraju et al., 2004, Marino et al., 2007, Sarmento et al., 2009)), results in which Cho levels do not change appear almost as frequently (e.g. (Vagnozzi et al., 2010, Signoretti et al., 2008, Kirov et al., 2007)). Chronic and longitudinal investigations have suggested that if Cho does increase, it is not subject to the normalisation that NAA demonstrates. One study examined patients at an average of 7 days and then a year later, finding Cho levels to be increased at both time-points (Holshouser et al., 2006). Longitudinal investigations have also supported the idea that a Cho increase can be relatively delayed; Cho in TBI patients compared between acute (up to 35 days) and chronic (up to 10.6 months) time points was found to increase between the conditions (Garnett et
Considering injury severity, while there appears to be a slight trend for Cho to be elevated more frequently in studies with severe patients, there is sufficient variability in this explanation from conflicting papers (e.g. severe injury but no Cho increase (Wild et al., 1999), or mild injury with a Cho increase (Govindaraju et al., 2004)) which complicated interpretation and understanding of the role of Cho as a biomarker for cellular injury. It therefore appears that the factors determining an elevation in Cho may not be strictly related to severity (i.e. “severity” at least as measured by the GCS), or that the specific form of damage caused by DAI may not reliably induce a change in Cho.

The metabolites found at the Creatine peak (Cre, creatine and phosphocreatine (Ross and Sachdev, 2004)) function as parts of a transport network to link energy-intensive sites with areas of ATP production (Wallimann et al., 1992). In TBI research, the Cre peak is used primarily as a stable reference point for ratio comparison with other metabolites, as it is broadly assumed to be stable due to an insensitivity to injury (Ross et al., 1998). Therefore, while it is measured as frequently as NAA and Cho, it is often not considered for statistical analysis. Despite this, research has demonstrated that its levels can vary with injury. A recent study (Yeo et al., 2011) focused on this point, finding Cre levels to be increased acutely before partially normalising chronically in a mild patient group. This is supported by other research which has found elevated Cre acutely in a mild group while also observing Cre to be correlated with cognitive function and emotional distress (Gasparovic et al., 2009). Cre has also been shown to be positively correlated with GCS in the chronic phase of injury, further indicating that the metabolite’s levels hold a relationship with TBI (Walz et al., 2008). The authors of one of these studies suggested that elevated acute Cre could indicate increased energy availability and expenditure for immune response and repair processes such as membrane pump upregulation (Yeo et al., 2011), basing this hypothesis on previous work which has shown that dietary supplements of Cre may have various (physical and cognitive) therapeutic effects following a TBI (Sakellaris et al., 2006, Sakellaris et al., 2008, Sullivan et al., 2000). Results such as these are suggestive that Cre should be examined experimentally and that the use of Cre as a stable reference point in ratio analysis should be conducted with caution.
Lactate (Lac) is a product of anaerobic respiration; the presence of a Lac peak is considered a marker of hypoxic (or ischaemic) injury with research indicating it to be a product of altered glucose metabolism acutely (Hillary et al., 2007) and to primarily proliferate from macrophages chronically (Petroff et al., 1992) (N.B. this study was conducted in stroke patients), although compromised long term metabolism could also continue to produce Lac. Lac holds the same ppm as lipid and so these can be confused unless appropriate spatial saturation is used to eliminate extra-neural lipid signals during the acquisition process. The healthy brain does contain low levels of Lac, although these are not usually at sufficient concentrations to be detected by MRS (Lin et al., 2005). Thus, the analysis of Lac is different to that of other metabolites as investigations often look primarily for the presence of a Lac peak, opposed to a change in its quantity. Lac has been detected in a number of studies (e.g. (Marino et al., 2007, Makoroff et al., 2005, Holshouser et al., 2006)) and most frequently in ones using severely injured participants. This relationship to severity is also supported by its detection being a strong predictor of a poor outcome in the patient (e.g. (Ashwal et al., 2000, Holshouser et al., 2000, Brenner et al., 2003)). However there is evidence it is not a suitable marker of DAI, as one investigation (Holshouser et al., 2005) found it to only be present when the VOI was near a visible injury. Taken together, these previous studies suggest that Lac may only be detectable in the most damaged tissue.

Myo-Inositol (mI) is known to function as a CNS osmolyte (Fisher et al., 2002). It is one of the more difficult metabolites to examine using MRS as it requires a short echo time in order to be detected. It is found in high concentrations in astrocytes and so an increase in the mI peak has been proposed as a marker of astrogliosis (Hattingen et al., 2008). Supportive of this, studies have found mI to increase following injury at acute and chronic stages (Garnett et al., 2000a, Danielsen et al., 2003, Ashwal et al., 2004, Yoon et al., 2005). One study suggested it to be the most elevated in poorer outcome patients; a possible indication of gliosis (Ashwal et al., 2004). A negative correlation between mI and IQ following TBI in a chronic patient cohort has also been reported (Babikian et al., 2006). To date, no studies have reported mI levels in mild TBI patients, meaning that a relationship between mI and severity cannot yet be commented on. The mixed time course (i.e. time from injury to scan) of these investigations suggests a mI increase is a stable change following injury. This further
indicates a lasting alteration in the composition of injury sites following TBI to one which increasingly favours glial cells compared to neuronal ones, i.e. gliosis.

Like ml, Glx also requires a short echo time to be detected. The Glx peak is a combination of the amino acids glutamine and glutamate, the latter of which is the major excitatory neurotransmitter in the brain. Due to the signal being a mix of these, and the small number of studies which have reported Glx in a TBI population, there are only few hypotheses regarding what any concentration changes mean. After finding that raised Glx is associated with poorer outcome in TBI patients, one study suggested that a raised Glx peak may reflect heightened extracellular levels of these due to a neural membrane breakdown (Shutter et al., 2004); an environment hypothesised to be damaging to neurons via excitotoxicity (Arundine and Tymianski, 2004). It’s also worth considering if increased Glx may instead reflect increased glutamate-based communication between synapses if the local cell population is not too damaged to function in this way. Alternatively, a diminished peak could reflect that the local cell population is too damaged to function in this matter and instead represent reduced glutamate-based communication between synapses. Some recent work (Yeo et al., 2011) found that Glx becomes increased in white matter but reduced in grey matter in acute, mild TBI before finding Glx to normalise chronically. Similar findings were also reported in a prior study (Gasparovic et al., 2009), albeit with only a trend for increased white matter Glx. However, other work has found Glx to be increased in white and grey matter in an acute patient group (Shutter et al., 2004). Due to the variability of these results, and the aforementioned issues surrounding a relative lack of studies which have reported Glx, there is a lack of a satisfying explanation for either direction in change. While Glx is clearly affected by TBI, it is the least well understood of the metabolites investigated by MRS and so future work should approach the study of it with relatively open hypotheses.

MRS measures have also produced relationships with cognition. One study (Friedman et al., 1998) used a patient cohort of mixed injury severity and time since injury and found white and grey matter NAA and Cr to positively correlate with psychological domain scores. Another (Ariza et al., 2004) used a chronic patient cohort of mixed injury severity and found the ratio of NAA/Cho in the basal ganglia to be positively correlated with tests of attention, and for NAA/Cho in the hippocampus to
be positively correlated with performance on verbal learning and facial recognition tests. Other longitudinal work in mildly injured patients (Yeo et al., 2011) found that premorbid intelligence was related to the speed that Glx and Cr (white matter only) normalized over time with higher intelligence predicting a faster recovery.

One consideration to hold in mind when interpreting MRS data is that the poor spatial resolution of MRS means that many investigations are unable to create pure grey / white matter voxels or test a very specific neural location. This is problematic as results have suggested the metabolic reaction to TBI may differ between grey and white matter, such as cases where differing NAA and Cho results have been found between the types of tissue (Friedman et al., 1998).

2.7 Limitations to Cognitive Research

Linking biomarkers to cognitive deficit is a powerful technique. Obtaining such a finding indicates that the marker is measuring something neuro-psychologically valid while also allowing us to examine how and where neurophysiology influences facets of cognition. However there are considerations that should be made both when conducting this type of work and examining past literature on the topic. As the number of combinations of specific neural regions and cognitive tests are great, direct comparisons between reports are difficult to make. It is wise also to refrain from making overly-specific conclusions regarding the exact cognitive faculty that a single psychometric test measures and the exact anatomical feature it appears to correlate with. The popularity of methodology which tests cognition in TBI on a less specific level supports this; domain scores which examine “executive functioning” opposed to one of the myriad of sub-functions that a particular test is designed to measure, or tractography methods such as “white matter load” (Kraus et al., 2007) which uses averaged markers of damage from multiple ROI’s instead of one. Although more approximate, comparisons between measures such as these often prove highly effective in yielding results as they operate more on the level of the neural network than a simpler cause / effect relationship, while also eliminating some of the heterogeneity between individual TBI’s with regards to both neurophysiological and psychological phenomenon.
An interesting effect is present within this field with regards to healthy participants. Repeating a correlation between a biomarker and a cognitive test score in a sample of TBI patients and healthy controls would indicate that that relationship is not due to injury, but instead is actually a normal aspect of brain functioning which had not been affected by injury. Research often fails to consider controls for their own analysis, despite this holding the potential to further validate the DTI / MRS metrics. When controls are studied in this way, cognitively-relevant results are sometimes found (Little et al., 2010) and sometimes not (Ariza et al., 2004). Examined as a whole however, relationships with psychological functioning are not as forthcoming as they are with patient groups alone which does support the notion that at least most of these findings are caused by injury. One notable case (Kinnunen et al., 2011) found controls to contribute to correlations between FA and associative memory when analysed with patients, although when analysed as a single group no significant results were found. This suggests that control cognitive functioning may vary with measures such as FA but that the healthy FA range is so compact that there is not sufficient spread to produce a correlation when analysed on its own.

2.8 Combining Modalities

While DTI and MRS each hold considerable use individually to investigate TBI, investigations have been conducted which proves them to more powerful when used together. Correlations have been noted between MRS metabolites and DTI metrics (e.g. (Babikian et al., 2010)) and combining FA with NAA levels has also proved to hold more power in predicting the patients outcome than use of either measure alone (Tollard et al., 2009). These findings suggest that MRS and DTI metrics measure different aspects of DAI but remain related to one another. Aside from the increased information which using more than one technique brings, the use of them in a single study would be advantageous in producing metabolic and tractographic data for the same patient cohort, making comparisons considerably more valid. Multi-modal methods such as these undoubtedly give access to a new level of analysis, however research that has been conducted in this way is sparse. These eclectic studies represent a vastly underused approach which holds significantly increased power for studying the effects of TBI.
2.9 Conclusions on Past Research and Thesis Aims

MRS and DTI have both proved to be excellent techniques to investigate TBI. The ability to quantify axonal damage and neural metabolite levels \textit{in vivo} held many promises when the techniques were both first used in the late 90’s. These promises have been fulfilled to an extent by an increase in knowledge; assessment of TBI with DTI and MRS metrics has revealed many insights regarding TBI’s effects on the brain at a neuronal level, with particular focus on severe and chronic injury. However acute and mild patients remain understudied when considering both the potential of data at this time point to drive therapy to affect outcome, and majority demographic which mild TBI patients represent. In addition the field is hampered by a relative lack of longitudinal data making the comparison time points post-TBI problematic, and work which combines MRI modalities is largely absent despite the its tremendous potential. Further, despite a large increase in knowledge on the topic, work thus far has failed to reach any clinically relevant conclusions. Focus should be increasingly placed on examining if the literature which has been developed on the topic can be applied to give patients better outcomes.

This thesis therefore aims to principally examine if the physiological changes observed following mild TBI separate to those expected following a severe injury. In order to do this, advanced and standard MRI techniques were combined with cognitive test data to characterise the neurophysiological and cognitive profile of a predominantly mild TBI patient group at both the acute (6 days) and chronic (1 year) time-points of injury. Links between the physiology of the brain and cognitive outcome will also be sought. It was broadly hypothesised that the mild nature of our patient population would lead to novel microstructural and metabolic findings (via DTI and MRS investigation), as evidence that the presentation of mild TBI is not simply a less severe version of severe injury, but the result of a different underlying set of injury and response processes. Specifically;

1. (Chapter 4) The relationship between focal lesion load and cognitive outcome will be examined. It is hypothesised here that lesion load will predict cognitive deficit via correlational analysis, although will not be wholly effective in doing this (expecting that DAI will also contribute to cognitive outcome).
2. (Chapter 5) Longitudinal changes in DTI metrics will be examined using TBSS, with relationships between DTI metrics and cognitive outcome also being tested. Due to the longitudinal / mild nature of our data, it is hypothesised here that acute findings will replicate previous research where increases in FA are a marker of cognitively relevant damage. As these more novel acute findings are often attributed to transient mechanisms which are expected to resolve over time, it is then hypothesised that at the chronic time-point the metric alterations will more closely follow the more typical pattern of expected changes (i.e. decreased FA) with relationships with cognitive outcome also supporting decreased FA as being indicative of damage.

3. (Chapter 6) Longitudinal changes in metabolite concentrations will be assessed in an MRS investigation, with relationships between metabolic concentrations and cognitive outcome also being tested. Metabolites will be ratioed to water concentration instead of Cre. It is hypothesised that increases in Cre following injury will be observed and relationships with cognitive data will support this change as being a marker of damage. Changes in NAA and Cho, and relationships between these and cognitive data are also expected, although these findings are hypothesised to follow the expected pattern of decreased NAA / increased Cho being markers of damage.
Chapter 3. Study Overview: Participant Recruitment, Data Collection and Preliminary Psychometric Testing

3.1 Declaration

The experiments in this thesis use participant data which was collected as part of a large multi-disciplinary research programme. Obtaining participant data; participant recruitment, matching of controls to patients, performing scans / obtaining psychometric test results etc. was conducted by Mr. Christopher Cowie and occasionally additional research assistants. Some of the data was subsequently analysed and presented for another thesis authored by Mr. Cowie.

The current thesis focuses on unused data from that sample. However, for completeness some of the more basic aspects of the data covered in this chapter share some overlap with the prior work; Mr. Cowie subjected acute data to tests which compared psychometric test performance between patients and controls and provided further commentary on these findings. Although data in section 3.6 is similar to this, the analyses and interpretations presented here were conducted and formed by the current author.

3.2 Recruitment Overview

3.2.1 Participant Recruitment: Patients were recruited from the accident and emergency and neurosurgery departments at Newcastle General Hospital. Patients were initially approached with information about the study while they were still inpatients. This approach was made in person by one of; the A&E staff, neurosurgery staff, the head injury specialist nurse or by Mr. Cowie following identification he made through discussions with hospital staff / examining the hospitals referral log. During this initial contact, effort was made to determine if the patient met the study’s inclusion criteria. If the criteria were met and the patient was interested they were then given the participant information sheet and a minimum of 24 hours, as per the ethics requirements for the experiment, to consider participating. Contact was re-made with them 24 hours later to follow up on their decision and informed consent was then taken if they had decided to take part. A scan booking at the Magnetic Resonance Centre (MR Centre) was made at the earliest opportunity. If this took place while the participant was still an inpatient they were accompanied by a member of the
study team to the Centre. If they had been discharged then transport was arranged for them.

Patients were also made aware at the time of recruitment that they would be asked to return for a follow-up set of testing in 1 year. Patients were duly contacted by the study team 1 year later and asked if they would return. If they agreed to this, transportation was again arranged to the MR Centre and the exact same testing procedures were repeated. This marked the end of the patient’s involvement in the study.

Control subjects were recruited by poster and flyer advertising throughout the local community. In addition to this certain people / groups of people were approached in person, including a local young mothers group, students at a 6th form college (though their teacher) etc. Members of the study team also asked people who they knew who they believed may be suitable for the study. These more direct methods of control recruitment were made in an effort to gain matched subjects.

3.2.2 Inclusion Criteria: Inclusion criteria were created to ensure both patient safety and high data quality. These criteria are outlined below in Table 3.1. Shaded cells indicate that the criteria is specific to patient participants only, while non-shaded cells apply to patient and control participants. Note that in addition to meeting these criteria, control participants were also matched to patients. More information on the matching criteria is provided in section 3.2.4.
The minimum age limit was applied to avoid consent issues around testing children. Restricting the participant sample to adults is also beneficial in keeping all participants comparable to one another; research has frequently suggested that the response and recovery from TBI differs in children (Tavano et al., 2014, Taylor and Alden, 1997), implying that the study of them should be kept separate. An upper age limit was also selected for the same reason; older TBI patients have an increased likelihood of existing co-morbid conditions which may confound findings, such as deteriorating frontal white matter (Salat et al., 2005), and also have limited functional recovery following compared to younger adults (Moshenthal et al., 2004). It should be noted that one subject over this age (68 years) was recruited on the basis of their presentation otherwise being very suitable.

GCS of no less than 9 was chosen to avoid the recruitment of any severe patients, supportive of the studies broader interests. However this criteria was relaxed during recruitment due to recruitment targets not being met. Due to this, one patient with a GCS of 7 was used in the study. This patient, H08 (Table 3.2), otherwise demonstrated LoC and PTA durations well within the boundaries of moderate patients.
It was therefore deemed acceptable to include this patient within the “moderate” sub-group. It should be noted that testing described throughout Chapters 4-6 considers if the inclusion of this moderate sub-group has affected the validity of researching mild TBI.

In order to capture acute patient data, time from injury to scan was initially set to be no more than 6 days. However this criteria was also relaxed first to 10 days, and finally to 14, after being deemed too ambitious. Problems encountered with the original 6 day goal were reported to include logistical difficulties of the scanner being placed away from a clinical workplace and the lack of immediate medical support which came with this, making the movement and care of more injured patients difficult. All patients were scanned within 14 days of their injury.

Any participants with previous history of any neurological / psychological problems or substance abuse were excluded in order to avoid factors which would confound the study of the TBI in question alone. A neurological / substance abuse history may contribute to an abnormal brain scan, while a psychiatric history could impact upon cognitive performance. Further, participants who were not fluent in English or who had visual / auditory problems were also excluded so that cognitive performance scores would not be impacted by communication issues irrelevant to cognitive function. Finally, the study radiographer team assessed each participant to ensure it was safe to expose them to the MRI scanner.

3.2.3 Demographic Information: Demographic information was collected from each participant. For all participants (patients and controls), this information included:

- Age at time of scan
- Sex
- Employment status / occupation
- Educational level. This was categorised into 4 groups;
  - No qualifications
  - GCSE or equivalent
  - A level / Diploma
  - Degree / Higher Degree
Additional information was collected from patients specifically, listed below:

- Any medications they were currently taking (this was asked as another means of ensuring that they met the inclusion criteria, e.g. a patient taking psychiatric medication would be excluded in case of them having an otherwise undisclosed mental illness)
- Mechanism of injury
- GCS on admission
- Presence / duration of Loss of Consciousness (LoC) and Post Traumatic Amnesia (PTA)
- If a headache was present
- If the patient had vomited
- If the patient had experienced any seizures
- Injuries other than to the head which had been sustained in the accident
- If a CT scan had taken place as part of their routine clinical care; if one had been performed then the findings from this were also taken

3.2.4 Control Matching: In addition to having to meet criteria outlined in Table 3.1, controls were matched to patients on age, gender and highest level of education. Age and gender were used to keep controls as physically matched to patients as possible, while highest level of education was used in an attempt to increase validity in neuropsychological comparisons between controls and patients.

3.3 Final Sample Characteristics

Approximately 30% of participants initially identified agreed to take part. Of the 70% who didn’t, half had not met inclusion criteria while the other half declined their involvement following some discussion. Participant recruitment ran for 24 months from 17/12/2008 until the 16/12/2010, although some follow-up patient testing occurred after this period of time.

3.3.1 Patient Demographics: The final participant sample included 53 patients at the acute time point (44 mild, 9 moderate; including the 1 severe patient), 23 of whom returned at the chronic time point (18 mild and 5 moderate; not including the 1
severe patient). Of the patients who didn’t return for the follow-up testing, 4 actively declined to return while the remaining 26 were either not able to be contacted due to out of date information or did not respond to attempts made to contact them.

At the acute time point patients were aged between 16-68 years (mean=33.9, SD=14.6) and had a male / female ratio of 44:9. Scanning at this point occurred a mean of 6 days post injury (SD=3.2, range=1-14 days). The follow-up group were aged between 18-65 years at the time of their acute scan (mean=39.26, SD=15.27) and had a male / female ratio of 18:5. Follow-up testing occurred a mean of 379 days following the initial scan (SD=22.49 days, range=343-424 days). The main demographic and injury characteristics of these of patients are summarised in Table 3.2.
Table 3.2: A Summary of basic demographic and injury data in the patient group, broken down by injury severity and time-point.

<table>
<thead>
<tr>
<th></th>
<th>All Acute Patients (N=53)</th>
<th>Acute Mild Patients (N=44)</th>
<th>Acute Moderate Patients (N=9)</th>
<th>All Returning Patients (N=23)</th>
<th>Mild returning patients (N=18)</th>
<th>Moderate returning patients (N=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Male,Female)</td>
<td>44,9</td>
<td>37,7</td>
<td>7,2</td>
<td>18,5</td>
<td>15,3</td>
<td>3,2</td>
</tr>
<tr>
<td>Age at time of acute scan (mean±SD)</td>
<td>33.92±14.6</td>
<td>33.91±14.84</td>
<td>34±14.22</td>
<td>39.26±15.72</td>
<td>39.06±15.77</td>
<td>40±17.33</td>
</tr>
<tr>
<td>Time from injury to acute scan in days (mean±SD, min-max)</td>
<td>6.056±3.2 1-14</td>
<td>5.773±2.94 1-14</td>
<td>7.44±4.22 2-13</td>
<td>6.13±3.252 1-13</td>
<td>5.444±2.617 1-10</td>
<td>8.6±4.39 2-13</td>
</tr>
<tr>
<td>GCS (median)</td>
<td>14</td>
<td>14</td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Average duration of Loss of Consciousness in minutes (mean±SD)</td>
<td>3.029±4.585 (19 did not experience LoC)</td>
<td>2.616±4.348 (17 did not experience LoC)</td>
<td>5±5.43 (2 did not experience LoC)</td>
<td>2.935±3.995 (7 did not experience LoC)</td>
<td>2.139±2.785 (6 did not experience LoC)</td>
<td>5.8±6.46 (1 did not experience LoC)</td>
</tr>
<tr>
<td>Average duration of Post-Traumatic Amnesia in hours (mean±SD)</td>
<td>13.9±56.41 (19 did not experience PTA)</td>
<td>2.919±5.704 (19 did not experience PTA)</td>
<td>66.4±127.9 (all experienced PTA)</td>
<td>30±87.4 (10 did not experience PTA)</td>
<td>2.75±7.11 (10 did not experience PTA)</td>
<td>116.3±160.4 (all experienced PTA)</td>
</tr>
<tr>
<td>Time from acute scan to chronic scan in days (mean±SD, min-max)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>378.61±22.49 343-424</td>
<td>380.89±19.93 357-423</td>
<td>370.4±31.4 343-424</td>
</tr>
</tbody>
</table>
**3.3.2 Injury Characteristics of the Acute Patients:** While each injury is clearly individual, after reviewing the causes injuries were split into six categories for ease of classification; “assaults”, “falls”, “road traffic accidents”, “sporting accidents”, “other” and “not known”. In this case “road traffic accidents” account for instances where a collision involving a motorised vehicle occurred (usually a car, although in one case a motorcycle). While there were some injuries which involved falling from a moving bicycle, these were classed simply as “falls” (except for one case which involved a bicycle race, which was categorised as a “sporting accident” instead). “Other” is used in cases of accidents which don’t fit immediately into any other category (e.g. one patient who accidentally hit their head against a metal door), while “not known” is used in cases where the cause was unable to be reported (some patients’ LoC and PTA prevented them from being able to remember how they were injured). The distribution of these is shown in Figure 3.1, below.

![Pie chart showing distribution of causes of injury in the acute patient sample.](image)

**Figure 3.1**: A pie chart to show the distribution of causes of injury in the acute patient sample.

From Figure 3.1 we see that assaults form the main cause of accident, followed relatively closely by falls. This goes against a trend where road traffic accidents are often found to be the most common mechanism of injury (one review found it to be the leading cause in America, Australia and China (Bruns and Hauser, 2003)) but is
supportive of other research based in the UK where falls and assaults were the two leading causes, ahead of road traffic accidents (Thornhill et al., 2000). A total of 12 patients (22.6% of the total group) were under the influence of alcohol at the time of the accident.

Of the acute patient sample, 50 patients (94.3%) experienced a headache, 31 (58.5%) experienced vomiting and 4 (7.5%) experienced a seizure. Abnormal imaging findings were reported in 44 (83%) of the patients. These initial imaging findings were reported from a mix of CT and T1W MRI scans; most of these patients had a CT scan as part of their clinical investigations and those who did not had a T1W MRI scan as part of this project (later described).

**3.3.3 Injury Characteristics of the Returning Patients:** Of the patients who returned, “falls” overtook “assaults” as the main cause of the injury when compared to the acute patient sample as a whole (assaults became the second most common). Sporting injuries also swapped positions with road traffic accidents to become the fourth most common type of injury in this patient sub-group. The distribution of these is shown in Figure 3.2, overleaf.
Of the returning sample, 22 patients (95.6%) had experienced a headache at the acute stage, 14 (60.9%) experienced vomiting at the acute stage and 1 (4.3%) experienced a seizure at the acute stage. Abnormal imaging findings were reported in 19 (82.6%) of these patients. Appendix A describes the injury characteristics of all the patients in more detail.

**3.3.4 Patient Sub-Group Consistency:** Comparing demographic and injury information between the main body of patients (all patients at the acute stage) with subgroups (e.g. acute mild patients, all follow-up patients etc.) shows good consistency. Chi Square test comparison showed that the ratio of male / female patients was not significantly different when testing the full acute patient sample against any sub-group (this test was not able to be performed against returning moderate patients as the sample size was too small). Further, t-test comparison also indicated that there was no significant difference between the age of the main body of patients and any sub-group.
While the most common mechanisms of injury changed slightly between the acute and returning patient sample, it should be considered that due to the returning group having a reduced number, the actual number of patients that these changes represent is small. Indeed, a Chi square test comparison showed no significant difference in the ratio of injury mechanisms between the acute and follow-up patient samples. It was deemed unnecessary to conduct statistical testing to compare the proportions of patients who experienced headache / vomiting / seizure or who had abnormal imaging findings, as these are all extremely similar descriptively.

3.3.5 Control Demographics: The final control sample consisted of 33 participants. Controls were aged between 19 and 66 years (mean=40.94, SD=15.26) and had a male / female ratio of 24:9. Despite attempts to match controls to patients, t-test comparison revealed control subjects to be significantly older than both the acute mild patient group ($p=0.047$), and all acute patients considered together ($p=0.039$). Otherwise there was no significant difference between the age of controls and acute moderate patients or any of the “returning” patient sub-groups, although considering the former findings these non-significant results are possibly due to reduced sample size. Chi Square testing showed no significant difference in the male / female ratio between controls and all patients / any of the patient sub-groups (except moderate returning patients where sample size was too small to test).

The distribution of educational levels appears similar between conditions (Figure 3.3, below), although it should be noted that while GCSE / equivalent is the most common level attained in the acute and returning patient sample with undergraduate or higher degree being second, these positions are reversed in the control group. Similarly, in the returning patients having no qualifications became more common than having an A-Level / Diploma. Despite these descriptive differences, Chi Square testing showed no significant difference in the ratios of educational levels between controls and all patients / any of the patient sub-groups (except moderate returning patients due to sample size being too small to test). Table 3.3, below, shows this control demographic information in comparison to the patient demographic information.
Table 3.3. A summary to compare demographic control data with demographic patient data. Data takes a ratio form when describing gender, and takes the form of “mean±SD” when describing the age at the time of the acute scan.

<table>
<thead>
<tr>
<th></th>
<th>Control (N=33)</th>
<th>All Acute Patients (N=53)</th>
<th>Acute Mild Patients (N=44)</th>
<th>Acute Moderate Patients (N=9)</th>
<th>All Returning Patients (N=23)</th>
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<td>15,3</td>
<td>3,2</td>
</tr>
<tr>
<td>Age at time of acute scan</td>
<td>40.94±15.26</td>
<td>33.92±14.6</td>
<td>33.91±14.84</td>
<td>34±14.22</td>
<td>39.26±15.72</td>
<td>39.06±15.77</td>
<td>40±17.33</td>
</tr>
</tbody>
</table>

Figure 3.3. Pie charts to show the distribution of highest level of education in control and acute / returning patient groups.
3.4 MRI Scanning

3.4.1 MRI Scanning Protocol: Upon arriving at the MR Centre, participants first completed their MRI scan. Scans were performed on a 3T whole body Philips Achieva System with an 8 channel head coil as a receiver and a body coil as a transmitter. The full scanning protocol took 55 minutes to administer and included anatomical scans ($T_1W$) and quantitative data scans (Diffusion Tensor Imaging, quantitative $T_1$ and $T_2$ mapping, Magnetic Resonance Spectroscopy and Cerebral Blood Flow). The anatomical images were positioned parallel with the anterior commissure / posterior commissure line (AC / PC line), with the whole brain covered, and the quantitative data scans co-centred and oriented with these. While resolution of each scan varied, they were designed to be multiples of one another to allow for easy comparison between scan types. Scanning was performed by the radiographer staff working at the MR Centre, while the scan data was downloaded from the scanner to computers for post-processing and analysis. Not all types of scan data are used in this thesis and as a full experimental chapter has essentially been given to each scan type which has been used, the full specification of each protocol is described in the accompanying chapter. However, generic pre-processing steps which were applied to all scan data are described here. These steps were performed by Mr. Benjamin Aribisala, an Image Processing Specialist working on the research program.

3.4.2 Format Conversion: Scan data taken from the scanner was first converted from the scanners native file types (.PAR and .REC extensions) to the “Analyze” format (.hdr and .img extensions) so that they were compatible with a range of scan-processing software.

3.4.3 Movement Correction: Correction was required to account for inevitable small movements made by the participants during scanning, meaning that the same co-ordinate space in any two scans from one participant would not describe the same tissue without this correction. Each participants’ set of images were therefore registered to their $T_1W$ anatomical image. This was done using FLIRT; a linear image registration tool which is part of the FSL software package (Smith et al., 2004). During this process, each image is translated, rotated, stretched / compressed and skewed in each spatial axis to register each image into the same “patient space”.

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3.4.4 Fitting: The data from each quantitative scan type was fitted to the appropriate model for that data type. This was performed by a mixture of in-house software developed by a research associate (for CBF and quantitative T₁ and T₂ data), and by publically available fitting tools available as a part of the FSL software package (DTI). MRS data fitting procedures are described in section 6.2.4.

3.4.5 Unwarping: This was performed in order to correct for data changes influenced by inhomogeneity in the scanner’s magnetic field. For this, a B₀ field map was acquired using a dual echo 3D GRE sequence (TE₁ = 2.5 ms, TE₂ = 5.8 ms, TR = 27ms, transverse orientation, 2mm isotropic resolution, matrix size: 128x128x72, accelerated with a SENSE factor of 1.5 in the right-left / phase direction). This field map described the non-uniformities in the magnetic field, and was therefore used to correct other scan data. For this correction, two more FSL tools were used. PRELUDE first performed 3D phase unwrapping of the field map before FUGUE used the field map to unwarp the other scans.

3.5 Neuropsychological Testing

3.5.1 Neuropsychological Test Battery: The composition of the test battery which was administered to participants was the result of discussions with two local consultant clinical psychologists, and designed to measure a variety of cognitive domains; attention, concentration, memory and executive function. Table 3.4 lists the full test battery / the cognitive functions which each test assesses, while more detail on each task is given after.
These tests are a mixture of select tasks taken from a number of test batteries alongside other common, stand-alone tests. An overview of each task is given below. However, as only a subset of the scores gained from each task were used in analyses, description of how exact scores were gained from these are given later in section 3.6.1.1.

**The National Adult Reading Test (NART) (Nelson, 1982).** This is a common, stand-alone test that is often used in research as a measure of premorbid IQ. The test involves the participant reading aloud 50 printed real words. The words chosen are included in the test for their irregular, non-phonetic spellings, and while words at the start of the list are common they become increasingly unusual as the list progresses. Due to this, it is unlikely a participant would be able to correctly pronounce a word they do not already recognise.

**Speed of Information Processing (SoIP) (Coughlan, 2007).** This test is taken from the BIRT Memory and Information Processing Battery (Coughlan, 2007). SoIP involves presenting the participant with a single sheet of paper which has multiple rows of five, two-digit numbers. The participant is instructed to cross out the second-highest
number on each row as quickly as possible. A total of 4 minutes are allowed for this. The participant also completes a second sheet with a similar layout but with a lot of “11”s. They are instructed this time to simply cross out as many “11”s as possible in order to test their motor speed alone. For this portion of the test, 25 seconds is allowed.

**Design Learning (Coughlan, 2007).** This test is also taken from the BIRT Memory and Information Processing Battery. The participants are shown a grid of dots (4x4 – “Design A”) which are connected by varying patterns of straight lines, producing an overall design. The participant examines the design for 10 seconds and is told to remember it before it is hidden, and is then given a blank grid of 4x4 dots and asked to repeat the design they have remembered onto this. This is repeated a maximum of four more times with the same design, however if the participant produces two consecutive, correct responses then the full 5 attempts are not needed. The participant is then shown a new design (“Design B”) and the same procedure repeated with this. Finally, the participant is asked to once again draw Design A without being shown it again. Examples of the Design Learning Stimuli are given in Figure 3.4, below.

![Design Learning Stimulus A](image1.png) ![Design Learning Stimulus B](image2.png) ![Design Learning Participant Response Grid](image3.png)

**Figure 3.4.** A demonstration of the stimuli used in the Design Learning Task. “Stimulus A” and “Stimulus B” both show examples of what the participant is shown by the examiner. The participant must attempt to copy these onto the “Participant Response Grid”.

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List Learning (Coughlan, 2007). This test is also taken from the BIRT Memory and Information Processing Battery. It is identical to Design Learning in procedure, but uses a different stimuli. Instead of being shown a design that the participant must copy, a list of 15 words (“List A”) is read to them. The participant must attempt to recall the whole list, and is given a maximum of five attempts to do so with the list being re-read to them once for each attempt. After they recall the list three consecutive times, or five attempts have elapsed, a second list of 15 words (“List B”) is read to them which they must then attempt to recall. Only one attempt is allowed for this second list. Finally, the participant must once again attempt to recall List A, without this list being re-read to them.

Paced Auditory and Serial Addition (PASAT) (Gronwall, 1977). This is another common, stand-alone task. This involves the participant listening to a pre-recording of someone giving a list of single digit numbers. The participant is instructed to add the current number to the one before it, and to repeat that total aloud, i.e. not giving a full running total, but the sum of the two most recent numbers. The test is run twice, one with intervals of three seconds between numbers being read out and the other with two second intervals. A total of 61 numbers are presented to the participant in each condition (so that the participant returns a total of 60 answers). The 3 second condition therefore lasts 3:03 minutes while the 2 second condition lasts 2:02 minutes. Shorter practice runs are performed by the participant for each condition before the main one. If a participant fails to give two correct answers during the 3 second practice test, they are not administered the test. Likewise, if a participant fails to give a single correct answer during the main 3 second test, they are not administered the 2 second one.

Backwards Digitspan (Wechsler, 2008). A task currently featured in the 4th edition of the Wechsler Adult Intelligence Scale. This involves the experimenter saying aloud sequences of digits of varying lengths (which the test refers to as “spans”), which the participant must then repeat to the experimenter but in reverse order, e.g. “4 7 6” would become “6 7 4”. Span lengths are performed in blocks of nine (i.e. there are nine three digit numbers, nine four digit numbers, nine five digits numbers etc.). Every time a participant succeeds in giving two consecutive correct answers at one span length, the experimenter switches to the next length up. If a participant gives two
incorrect responses in a row then the test is discontinued. Digits are first read out with a span of 2, and can increase to 10.

**Backwards Spatialspan (Wechsler, 2006).** A task currently featured in the Wechsler Non-Verbal scale of ability. This task is similar to Digitspan in that it tests the “span” of the participant’s memory, but uses physical patterns instead of numbers. A board with ten identical blocks fixed to it in random-appearing locations is presented to the participant. From the participant’s point of view, each of the blocks are blank and so only differentiated from one another by location. However each block has a number printed on the “back” (relative to the participant) of it which the experimenter can see. The experimenter uses the numbers to tap certain blocks in certain orders, which the participant must watch and then repeat in reverse order. Similar to the Digitspan task, the sequences begin with a low number of taps (2) but increase in one tap for each two consecutively correct repetitions the participant achieves (up to 10). The task is again stopped when the participant gives two incorrect responses in a row. A picture of the Spatialspan stimulus is given in Figure 3.5.

**Verbal Fluency; Category and Letter Conditions (Delis et al., 2001).** These tasks are taken from the Delis-Kaplan Executive Function System tasks. The Letter Condition in this task is commonly referred to as “Verbal Letter Fluency” (VLF) while the Category Condition is referred to as “Category Fluency”. These tasks each involve instructing the participant to state as many words as they can think of that either begin with a specific letter (VLF), or to state as many words as they can think of that fit within a specific category (Category Fluency). In the VLF condition, three letters are used as cues; “F”, “A” and “S”, while in the Category Fluency condition, two categories are used as cues; animals and boys names. Participants are given a minute per cue (e.g. a minute for each of “F”, “A” and “S” in the VLF condition). In the VLF condition the participant must not state names, numbers, places or variations of previously used words. Similarly, in the Category Fluency condition, participants must not use variations of previously used words (e.g. “horses” would not be accepted after “horse”).

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Figure 3.5. Picture of the Backwards Spatialspan stimuli, showing the difference between the experimenter’s and participant’s points of view.
**Colour-Word Interference Test (CWIT) (Delis et al., 2001).** This task is also taken from the Delis-Kaplan Executive Function System tasks. It is a derivative of the “STROOP” task, where the participants executive function is tested by having to inhibit one aspect of a stimulus (e.g. a written word) in favour of another to give a correct response (e.g. the colour of ink that the word is printed in). Two baseline conditions are first administered to the participant; naming of patches of colour and reading colour-words which are printed in black ink. For each of these tasks, a page is shown to the participant which contains 60 examples of the stimuli. The participant has 90 seconds to give as many responses as they can. In the third condition, another sheet of 60 stimuli is presented to the participant. These stimuli are all colour-words, but printed in ink who’s colour doesn’t match the word (e.g. “red” or “blue”). The participant is instructed to name the colour of the ink, while ignoring what the word says and is given 180 seconds to do this. In the final condition, another 60 stimuli are given to the participant. These are again colour-words printed in conflicting colour of ink, but half of them now have a black box around them. This time, the participant must name the colour of the ink when the word doesn’t have a box around it, but name the word when it does have a box around it. The participant is again given 180 seconds to complete this.

**3.5.2 Neuropsychological Test Battery Administration:** The administration of the tests was initially conducted by Mr. Cowie, before being taken over after a period of time by two neuropsychology research assistants (Miss. Anna Peel and Mr. Joshua Wood). Mr. Cowie trained these assistants in the administration of the battery to ensure inter-examiner reliability. The full testing procedure took approximately 1 hour and 15 minutes. Participant scores were recorded on paper throughout the testing and later transcribed to a computer. The participant completed the test battery during the same visit to the MR Centre as their scan, or within 7 days if the patient was unable to tolerate both in the same sitting (this was the case for 11 patients who were tested a mean of 2.6 days following their scan). Although the administration of the tests was split between Mr. Cowie and the research assistants, the initial scoring and totalling of the results was conducted only by the research assistants. Further testing of the data described next in this chapter (section 3.6) was performed by the author of this thesis.
3.6 Preliminary Neuropsychological Comparisons

Before experimenting with scan data, basic comparisons between cognitive test scores of patients and controls were made in order to assess the cognitive profile of the patients and to see if this changed over the course of the year.

3.6.1 Methods

3.6.1.1 Test Interpretation: While some of the cognitive tests had clear outcome scores, others had a number of recorded measures which could be used for analysis (e.g. number of errors, number of correct responses, time to complete, baseline conditions etc.). Main scores of interest were therefore identified from the cognitive tests to be used in these analyses. This identification was made on the basis of the instructions given with the specific test to determine the most pertinent outcome measures. These are as follows;

NART: The error score on the NART test (number of mispronounced words) is checked against an accompanying published table to calculate the predicted full IQ of the participant. This IQ score is used.

SoIP: A total score adjusted for motor speed is calculated using the main total (correct) score and the motor speed score. This adjusted score is used.

Design Learning: Three total scores are calculated. The first of these is the total of all of the correct responses given in the first five attempts made at Design A (“A1-A5”). The second is the number of correct responses given on the single attempt at Design B (“B1”), while the final is the number of correct responses given on the re-attempt at Design A (“A6”).

List Learning: Similar to Design Learning, three total scores are calculated. Again, the first of these is the total of all of the correct responses given in the first five attempts made at List A (“A1-A5”). The second is the number of correct responses given on the single attempt at List B (“B1”), while the final is the number of correct responses given on the re-attempt at List A (“A6”).

PASAT: Two total scores were used for this, one for each condition (2 / 3 second intervals). These scores were the percentage of responses the participant gave which were correct in each condition.
**Backwards Digitspan:** As per test instructions, the final score is the participants “span”, i.e. the length of the longest string of digits they successfully recalled twice in a row.

**Backwards Spatialspan:** As per test instructions, the final score here is also the participants “span”, i.e. the length of the longest sequence of blocks they successfully recalled twice in a row.

**Verbal Fluency:** Two scores were used for this, one for each VLF and Category Fluency conditions. In each case, as per test instructions, these scores were the total correct responses that the participant gave across all trials within each condition.

**Colour-Word Interference Test (CWIT):** Two scores were used for this, one for each of the experimental conditions (i.e. stating the colour of ink the word is printed in, “Colour Naming Condition”, and switching between stating the colour of the ink or the word itself, “Switching Condition”). As per test instructions, the time taken to complete each task (including the baseline conditions) is first scaled to correct for age using an accompanying published table. The final corrected score for Condition 3 is then calculated by subtracting the first baseline score (naming colour patches) from the initial Condition 3 score. The final corrected score for Condition 4 is calculated by subtracting the sum of the first two baseline conditions from the initial Condition 4 score.

A high score on all of these tasks indicate good functioning, apart from CWIT data where a low score indicates good functioning.

**3.6.1.2 Data Analysis:** Control scores were first tested for normality by both examining histograms / probability plots of their distribution and administering the Anderson-Darling test. An analysis was then conducted to compare control NART scores against patient NART scores in order to examine for any inherent differences in IQ between groups.

Test performance on all other tasks was then compared between controls, mild and moderate groups using the acute patient data by one-way ANOVA if performance had been shown to be normally distributed, or by the Kruskal-Wallis test if it had been shown that performance was not normally distributed. Post-hoc tests to this ANOVA were conducted; control vs. mild / moderate and mild vs. moderate. As there is a clear
hypothesis of greater injury severity predicting worse outcome, these tests were one-tailed. T-tests were used for these if performance had been shown to be normally distributed, or by the Mann-Whitney U test if performance was not normally distributed.

For testing using the returning patient data it was deemed unsuitable to still consider moderate patients as a single group due to the reduced sample size. Comparison was therefore made by t-test between controls and all returning patients for each cognitive test if performance had been shown to be normally distributed, or by the Mann-Whitney U test if it had been shown that performance was not normally distributed. These tests were two-tailed as it was not deemed a reasonable to expect patients at this time to still underperform compared to controls.

Finally, where any significant findings had been found in a particular cognitive test, post-hoc regression analyses were conducted on that test in order to examine if differences in cognitive test scores were due to being injured or because of the potential of a lower pre-morbid IQ. For this, the test score was used as the response variable with NART as one predictor variable and if the participant had a TBI or not as the other predictor variable. This analysis was conducted once with acute patient data and once with follow-up patient data for each previously-significant cognitive test.

As not all participants (particularly patients) could complete all cognitive tests, the sample size for each comparison described above differed per test. Table 3.5, overleaf, summarises the total N for each test and at each time-point.
3.6.2 Results

3.6.2.1 NART: Testing was conducted to examine if there were any differences in IQ between the two groups. For this, NART scores were used as a measure of IQ to compare controls to what should theoretically be the patient’s pre-morbid IQ (if the patient NART had a different distribution to controls then there would be evidence that pre-morbid intelligence had not been measured). The Anderson-Darling test on NART distribution showed that both Control \( (p=0.119) \) and Patient \( (p=0.355) \) NART performance was normally distributed.

However, t-test comparison found a significant difference in IQ, as measured by NART, wherein control NART (mean=112.03, SD=8.98) was significantly higher than
NART of all patients (mean=100, SD=13.1, t(77)=4.88, \( p<0.001 \)) and some patient subgroups; acute mild patient IQ (mean=99.6, SD=13.4, t(69)=4.74, \( p<0.001 \)), IQ of all returning patients (mean=104.2, SD=13.5, t(35)=2.41, \( p=0.021 \)) and IQ of returning mild patients (mean=104.4, SD=13.7, t(25)=2.13, \( p=0.043 \)). While no significant difference was found between control NART and moderate patient NART either in the acute sample or returning sample, this should be expected due to reduced N in the group testing as the mean of these sub-groups was still descriptively lower than that of controls (102.3 and 103.6 respectively).

Please note the following about how significant findings are portrayed in the Figures over the following pages. An asterisk (“*”) above or below a box plot does not indicate significance, but instead shows outlier values (counted as any values which are 1.5 times (or more) outside of the interquartile range). Significance is instead represented by the presence of an arrow. A dashed arrow which moves from the control boxplot and across the acute patient boxplots (mild and moderate) shows that a significant effect was found between those three groups when the data was compared by ANOVA / Kruskal-Wallis. Alternatively, a solid horizontal bracket which moves between any two group’s box plots indicates a significant difference was found between those groups when the data was compared by t-test / Mann Whitney U analysis in either the main control vs. returning patient analysis, or any post-hoc analyses for the ANOVA.
3.6.2.2 SoIP: The Anderson-Darling test on SoIP distribution showed that control (p = 0.119) SoIP performance was normally distributed. One-way ANOVA showed a significant effect whereby performance appeared decreased in mild (mean=59.39, SD=20.14) and moderate (mean=49.05, SD=13.54) groups compared to controls (mean=74.47, SD=21.66, F(2,80)=7.55, p=0.001). One-tailed post-hoc t-test analysis revealed all possible differences to be significant; controls performed significantly better than both mild (p=0.002) and moderate (p<0.001) patients, while mild patients also performed significantly better than moderate patients (p=0.046). However, t-test comparison indicated that SoIP performance in controls and returning patients (mean=70.4, SD=14.7) was not significantly different (p=0.416). These findings are shown in Figure 3.6.

![Figure 3.6](image.png)

**Figure 3.6.** Box plot to show SoIP performance in Controls, mild patients and moderate patients at the acute time-point, and returning patients. A dashed arrow indicates a significant effect in those groups it covers as found by ANOVA / Kruskal Wallis. A solid bracket between two specific groups indicates a significant difference as found by t-test / Mann Whittney.
3.6.2.3 Design Learning: The Anderson-Darling test on Design Learning distribution showed that Control performance was normally distributed in the first condition (A1-A5, $p=0.119$) but was not normally distributed in either of the second two (B1; $p=0.026$, and A6; $p<0.005$). The histograms and probability plots for these are shown in Figure 3.7.

![Histograms and probability plots for Design Learning scores in B1 (top) and A6 (bottom) conditions.](image)

**Figure 3.7.** Histograms and probability plots to examine for normality in the distribution of Control Design Learning scores in B1 (top) and A6 (bottom) conditions.

In the A1-A5 condition: One-way ANOVA showed a significant effect whereby acute moderate patient performance (mean=29.75, SD=9.24) appeared decreased compared to controls (mean=37, SD=5.86) and mild patients (mean=36.81, SD=7.69, $F(2,79)=3.57$, $p=0.033$); Post-hoc one-tailed t-test comparison showed no significant difference between controls and mild patient performance ($p=0.452$), but demonstrated both controls ($p=0.033$) and acute mild patients ($p=0.039$) to perform significantly better than acute moderate patients. Further, t-test comparison indicated
that Control and returning Patient (mean=39.19, SD=6.58) performance was not longer significantly different ($p=0.223$).

In the B1 condition: Kruskal-Wallis analysis showed no significant difference in performance between controls (median=6, average rank=42.3), and acute mild (median=6, average rank=39.5) and moderate patients (median=6, average rank=48.6, overall $p=0.592$). Post-hoc, one-tailed Mann-Whitney U analysis showed no significant differences between any group comparison; controls vs. mild patients ($p=0.312$), controls vs. moderate patients ($p=0.266$), mild vs. moderate patients ($p=0.161$). Further, Mann-Whitney U analysis between control and returning patient performance (median=6) also showed no significant difference ($p=0.478$).

In the A6 condition: Kruskal-Wallis showed no significant difference in performance between controls (median=9, average rank=42.8), and acute mild (median=9, average rank=41.1) and moderate patients (median=9, average rank=38.3, overall $p=0.856$). Post-hoc, one-tailed Mann-Whitney U analysis showed no significant differences between any group comparison; controls vs. mild patients ($p=0.370$), controls vs. moderate patients ($p=0.316$), mild vs. moderate patients ($p=0.371$). Mann-Whitney U analysis between control and returning patient performance (median=9) also showed no significant difference ($p=0.896$). All of the Design Learning findings are shown in Figure 3.8.
**Figure 3.8.** Box plots to show Design Learning performance in Controls, mild patients and moderate patients at the acute time-point, and returning patients. A1-A5 condition is shown top-right, B1 condition is shown top-left and A6 condition is shown bottom. A dashed arrow indicates a significant effect in those groups it covers as found by ANOVA / Kruskal Wallis. A solid bracket between two specific groups indicates a significant difference as found by t-test / Mann Whittney.
**3.6.2.4 List Learning:** The Anderson-Darling test on List Learning distribution showed that Control performance was normally distributed in the first (A1-A5, p=0.808) and third (A6, p=0.056) conditions but was not normally distributed in second condition (B1, p=0.045). The histogram and probability plot for this is shown in Figure 3.9.

![Histograms and probability plots to examine for normality in the distribution of Control List Learning scores in the B1 condition.](image)

In the A1-A5 condition: One-way ANOVA showed a significant effect whereby acute mild (mean=44.45, SD=12.23) and moderate (mean=34.75, SD=18.34) patient performance appeared decreased compared to controls (mean=57.25, SD=8.3, F(2,81)=17.36, p=<0.001). Post-hoc, one-tailed t-test comparison showed that controls performed significantly better than both mild (p=<0.001) and moderate patients (p=0.006), but that there was no significant difference between acute mild and acute moderate performance (p=0.094). t-test comparison indicated that Control and returning Patient (mean=53.3, SD=10.5) performance was no longer significantly different (p=0.159).

In the B1 condition: Kruskal-Wallis analysis showed no significant difference in performance between controls (median=6, average rank=46.2), and acute mild (median=5.5, average rank=41.3) and moderate (median=6, average rank=34.2) patients (p=0.4). Post-hoc, one-tailed Mann-Whitney U analysis showed no significant differences between any group comparison; controls vs. mild patients (p=0.187), controls vs. moderate patients (p=0.115), mild vs. moderate patients (p=0.217). Mann-Whitney U analysis between controls and returning patient performance (median=8) also showed no significant difference (p=0.304).
In the A6 condition: One-way ANOVA showed a significant effect whereby acute mild (mean=8.73, SD=3.68) and moderate (mean=5.75, SD=5.15) patient performance appeared decreased compared to controls (mean=11.94, SD=2.3, \(F(2,81)=14.19, p<0.001\)). Post-hoc, one-tailed t-test comparison showed that controls performed significantly better than both mild (\(p<0.001\)) and moderate patients (\(p=0.007\)), but that there was no significant difference between acute mild and acute moderate performance (\(p=0.078\)). t-test comparison indicated that Control and returning Patient (mean=11.24, SD=3.25) performance was no longer significantly different (\(p=0.399\)). All of the List Learning Findings are shown in Figure 3.10.

**Figure 3.10.** Box plots to show List Learning performance in Controls, mild patients and moderate patients at the acute time-point, and returning patients. A1-A5 condition is shown top-right, B1 condition is shown top-left and A6 condition is shown bottom. A dashed arrow indicates a significant effect in those groups it covers as found by ANOVA / Kruskal Wallis. A solid bracket between two specific groups indicates a significant difference as found by t-test / Mann Whittney.
### 3.6.2.5 PASAT: The Anderson-Darling test on PASAT distribution showed that
Control performance was normally distributed in both conditions (2 second intervals; $p=0.17$, 3 second intervals; $p=0.22$). In the 2 second interval condition: One-way ANOVA showed a significant effect whereby acute mild (mean=39.73, SD=28.17) and moderate (mean=18.5, SD=18.69) patient performance appeared decreased compared to controls (mean=56.43, SD=12.48, $F(2,73)=9.06$, $p=<0.001$). One-tailed post-hoc $t$-test analysis revealed all possible differences to be significant; controls performed significantly better than both mild ($p=<0.001$) and moderate ($p=0.003$) patients, while mild patients also performed significantly better than moderate patients ($p=0.022$). However $t$-test comparison indicated that performance in the Controls and returning Patients (mean=56.4, SD=16.8) was no longer significantly different ($p=0.499$).

In the 3 second interval condition: One-way ANOVA showed a significant effect whereby acute mild (mean=61.45, SD=29.59) and moderate (mean=39.83, SD=28.22) patient performance appeared decreased compared to controls (mean=81.2, SD=13.52, $F(2,73)=9.82$, $p=<0.001$). One-tailed post-hoc $t$-test analysis showed that while controls performed significantly better than both mild ($p<0.001$) and moderate ($p=0.009$) patients, there was no significant difference between mild and moderate patient performance ($p=0.067$). $t$-test comparison also indicated that performance in the Controls and returning Patients (mean=79.7, SD=13.5) was no longer significantly different ($p=0.712$). All of the PASAT findings are shown in Figure 3.11.
Figure 3.11. Box plots to show PASAT performance in Controls, mild patients and moderate patients at the acute time-point, and returning patients. The 2 second interval condition is shown top and the 3 second interval condition is shown bottom. A dashed arrow indicates a significant effect in those groups it covers as found by ANOVA / Kruskal Wallis. A solid bracket between two specific groups indicates a significant difference as found by t-test / Mann Whitney.
3.6.2.6 Backwards Digitspan: The Anderson-Darling test on Backwards Digitspan distribution showed that Control (p<=0.005) performance was not normally distributed. The histogram and probability plot for this and are shown in Figure 3.12.

Kruskal-Wallis analysis showed a significant effect in performance whereby control performance (median=7, average rank=52.3) was higher than acute mild (median=5, average rank=36.9) and moderate (median=5, average rank=28.0) patient performance (H=10.67, p=0.005). One-tailed post-hoc Mann-Whitney U analysis showed that while controls performed significantly better than both mild (p=0.003) and moderate (p=0.004) patients, there was no significant difference between mild and moderate patient performance (p=0.189). Mann-Whitney U analysis between controls and returning patient performance (median=6) also indicated that controls still performed significantly better than returning patients (p=0.037). These findings are shown in Figure 3.13.
**Figure 3.13.** Box plot to show Backwards Digitspan performance in Controls, mild patients and moderate patients at the acute time-point, and returning patients. A *dashed* arrow indicates a significant effect in those groups it covers as found by ANOVA / Kruskal Wallis. A solid bracket between two specific groups indicates a significant difference as found by t-test / Mann Whitney.
3.6.2.7 Backwards Spatialspan: The Anderson-Darling test on Backwards Spatialspan distribution showed that Control (p<0.005) performance was not normally distributed. The histogram and probability plot for this and are shown in Figure 3.14.

![Figure 3.14](image)

Figure 3.14. A histogram and probability plot to examine for normality in the distribution of Control Backwards Spatialspan scores.

Kruskal-Wallis analysis showed a significant effect in performance whereby control performance (median=8, average rank=50.5) was higher than acute mild (median=7, average rank=35.4) and moderate (median=8, average rank=43.1) patient performance (H=7.46, p=0.024). Post-hoc, one-tailed Mann-Whitney U analysis showed that while controls performed significantly better than mild patients (p=0.003) there was no significant difference between control and moderate patient performance (p=0.272) or acute mild and acute moderate patient performance (p=0.999; a two tailed test was also conducted here which also showed no significant difference; 0.486). Mann-Whitney U analysis indicated that performance between controls and returning patient performance (median=7) was no longer significant (p=0.234). These findings are shown in Figure 3.15.
Figure 3.15. Box plot to show Backwards Spatialspan performance in Controls, mild patients and moderate patients at the acute time-point, and returning patients. A dashed arrow indicates a significant effect in those groups it covers as found by ANOVA / Kruskal Wallis. A solid bracket between two specific groups indicates a significant difference as found by t-test / Mann Whitney.
3.6.2.8 Verbal Fluency: The Anderson-Darling test on Verbal Fluency distribution showed that Control performance was normally distributed in both conditions (VLF; \( p=0.957 \), Category Fluency; \( p=0.111 \)). In the VLF condition: One-way ANOVA showed a significant effect whereby acute mild (mean=33.83, SD=12.43) and moderate (mean=18.57, SD=11.15) patient performance appeared decreased compared to controls (mean=41.63, SD=9.38, \( F(2,77)=13.24, p=<0.001 \)). One-tailed post-hoc \( t \)-test analysis revealed all possible differences to be significant; controls performed significantly better than both mild (\( p=0.002 \)) and moderate (\( p<0.001 \)) patients, while mild patients also performed significantly better than moderate patients (\( p=0.006 \)). However \( t \)-test comparison indicated that performance in the Controls and returning Patients (mean=38.8, SD=10) was no longer significantly different (\( p=0.303 \)).

In the Category Fluency condition: One-way ANOVA showed a significant effect whereby acute mild (mean=37.47, SD=9.96) and moderate (mean=25.75, SD=11.71) patient performance appeared decreased compared to controls (mean=44, SD=9.02, \( F(2,79)=12.04, p=<0.001 \)). One-tailed post-hoc \( t \)-test analysis revealed all possible differences to be significant; controls performed significantly better than both mild (\( p=0.004 \)) and moderate (\( p=0.002 \)) patients, while mild patients also performed significantly better than moderate patients (\( p=0.013 \)). However \( t \)-test comparison indicated that performance in the Controls and returning Patients (mean=40.05, SD=7.56) was no longer significantly different (\( p=0.091 \)). All of the Verbal Fluency findings are shown in Figure 3.16.
Figure 3.16. Box plots to show Verbal Fluency performance in Controls, mild patients and moderate patients at the acute time-point, and returning patients. The VLF condition is shown top and the Category Fluency condition is shown bottom. A dashed arrow indicates a significant effect in those groups it covers as found by ANOVA / Kruskal Wallis. A solid bracket between two specific groups indicates a significant difference as found by t-test / Mann Whitney.
3.6.2.9 CWIT: The Anderson-Darling test on CWIT distribution showed that Control performance was normally distributed in both conditions (Colour naming; \( p=0.513 \), Switching; \( p=0.104 \)). In the Colour Naming condition: One-way ANOVA showed no significant effect when comparing control (mean=24.66, SD=9.39), and acute mild (mean=26.88, SD=15.43) and moderate (mean=21.86, SD=20) patient performance \( (p=0.599) \). Post-hoc, one-tailed \( t \)-test comparison showed no significant in any comparison; control vs. mild patient \( (p=0.221) \), control vs. moderate patient \( (p=0.365) \), mild vs. moderate patient \( (p=0.273) \). \( t \)-test comparison indicated that performance in the Controls and returning Patients (mean=28, SD=23.2) was also not significantly different \( (p=0.53) \).

In the Switching condition: One-way ANOVA showed no significant effect when comparing control (mean=10.5, SD=10.56), and acute mild (mean=20.93, SD=19.18) and moderate (mean=26.43, SD=54.44) patient performance \( (p=0.065) \). Post-hoc testing here was conducted as two-way \( t \)-test comparisons after inspection of the means and boxplot (Figure 3.17). It was found that controls performed significant worse than mild patients \( (p=0.004) \), although there was no difference between controls and moderate patients \( (p=0.47) \) or mild and moderate patients \( (p=0.8) \). \( t \)-test comparison indicated that performance in the Controls and returning Patients (mean=55, SD=199; there was one highly anomalous result which skewed the mean up) was also not significantly different \( (p=0.321) \). All of the CWIT findings are shown in Figure 3.17.

All findings are then summarised in Table 3.6, following CWIT Figures.
Figure 3.17. Box plots to show CWIT performance in Controls, mild patients and moderate patients at the acute time-point, and returning patients. The Colour Naming condition is shown top and the Switching condition is shown bottom. The highly anomalous result in the follow-up condition has been removed to display the graph on a sensible scale.
### Table 3.6. A summary of findings from comparing cognitive test scores.

<table>
<thead>
<tr>
<th>Task</th>
<th>Sub-Condition</th>
<th>Acute Mild / Acute Moderate</th>
<th>Post-Hoc Findings</th>
<th>Returning Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>SoIP</td>
<td>-</td>
<td></td>
<td>All comparisons significant</td>
<td></td>
</tr>
<tr>
<td>Design Learning</td>
<td>A1 - A5</td>
<td></td>
<td>Controls outperformed mild and moderate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B1</td>
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<td></td>
<td>A6</td>
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<td>List Learning</td>
<td>A1 – A5</td>
<td></td>
<td>Controls outperformed mild and moderate</td>
<td></td>
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<tr>
<td></td>
<td>B1</td>
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<td></td>
<td>A6</td>
<td></td>
<td>Controls outperformed mild and moderate</td>
<td></td>
</tr>
<tr>
<td>PASAT</td>
<td>2 Second Intervals</td>
<td></td>
<td>All comparisons significant</td>
<td></td>
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<td></td>
<td>3 Second Intervals</td>
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<td>Controls outperformed mild and moderate</td>
<td></td>
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<tr>
<td>Backwards Digitspan</td>
<td>-</td>
<td></td>
<td>Controls outperformed mild and moderate</td>
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<tr>
<td>Backwards Spatialspan</td>
<td>-</td>
<td></td>
<td>Controls outperformed mild</td>
<td></td>
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<tr>
<td>Verbal Fluency</td>
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<td></td>
<td>All comparisons significant</td>
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<td></td>
<td>Category Fluency</td>
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<td>All comparisons significant</td>
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<td>Colour Naming</td>
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<tr>
<td></td>
<td>Switching</td>
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<td><em>Mild patients outperformed controls</em></td>
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</table>

**Key**

- = A significant effect was found when comparing controls / acute mild / acute moderate performance on that task by ANOVA or Krukal-Wallis (in every case of this happening patients underperformed compared to controls).

= A significant difference was found when comparing control performance against returning patient performance on that task by t-test or Mann Whitney U (in every case of this happening returning patients underperformed compared to controls).
3.6.2.10 Regression Analyses: Regression analyses were conducted to examine if these group differences were due to the TBI or a pre-morbid difference in IQ. The findings from these are shown in Table 3.7, overleaf.
Table 3.7. As NART had been found to be significant different between patients and controls, multiple regression was conducted using NART and “Group” (Patient / Control) as predictors of performance in each test which had been shown to be of interest. Individual p values are given for each predictor (highlighted if significant), along with the R² and overall model significance for each analysis.

<table>
<thead>
<tr>
<th>Task</th>
<th>Sub-Condition</th>
<th>Acute Time-point</th>
<th>Follow-up Time-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>SoIP</td>
<td>-</td>
<td>NART = 0.084</td>
<td>NART = 0.293</td>
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<tr>
<td></td>
<td></td>
<td>Group = 0.029</td>
<td>Group = 0.769</td>
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<tr>
<td></td>
<td></td>
<td>R² = 16%</td>
<td>R² = 3.3%</td>
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<tr>
<td></td>
<td></td>
<td>p = 0.001</td>
<td>p = 0.433</td>
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<td>Design Learning</td>
<td>A1 - A5</td>
<td>NART = 0.115</td>
<td>NART = 0.607</td>
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<td></td>
<td>Group = 0.028</td>
<td>Group = 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R² = 6.9%</td>
<td>R² = 3.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = 0.065</td>
<td>p = 0.403</td>
</tr>
<tr>
<td>List Learning</td>
<td>A1 – A5</td>
<td>NART = 0.301</td>
<td>NART = 0.141</td>
</tr>
<tr>
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<td></td>
<td>Group = &lt;0.001</td>
<td>Group = 0.405</td>
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<tr>
<td></td>
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<td>R² = 31.2%</td>
<td>R² = 8.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = &lt;0.001</td>
<td>p = 0.112</td>
</tr>
<tr>
<td></td>
<td>A6</td>
<td>NART = 0.314</td>
<td>NART = 0.044</td>
</tr>
<tr>
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<td>Group = 0.001</td>
<td>Group = 0.932</td>
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<td></td>
<td></td>
<td>R² = 20.4%</td>
<td>R² = 9.3%</td>
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<td>PASAT</td>
<td>2 Second Intervals</td>
<td>NART = 0.004</td>
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<td>R² = 12.3%</td>
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<td>3 Second Intervals</td>
<td>NART = 0.088</td>
<td>NART = 0.018</td>
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<tr>
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<td>Group = 0.014</td>
<td>Group = 0.588</td>
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<td></td>
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<tr>
<td>Backwards Digitspan</td>
<td>-</td>
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<td>NART = 0.008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group = 0.659</td>
<td>Group = 0.249</td>
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<td>R² = 22.4%</td>
<td>R² = 21.4%</td>
</tr>
<tr>
<td></td>
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<td>p = &lt;0.001</td>
<td>p = 0.002</td>
</tr>
<tr>
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<td>-</td>
<td>NART = 0.235</td>
<td>NART = 0.146</td>
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<td>R² = 7.6%</td>
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<tr>
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<td>Verbal Fluency</td>
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<td>NART = 0.01</td>
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<tr>
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<td>Group = 0.989</td>
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<td></td>
<td>R² = 25.1%</td>
<td>R² = 14.4%</td>
</tr>
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<td></td>
<td>p = &lt;0.001</td>
<td>p = 0.021</td>
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<tr>
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<td>NART = 0.024</td>
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<td></td>
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<td>Group = 0.482</td>
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<td></td>
<td></td>
<td>R² = 19.8%</td>
<td>R² = 14.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p = &lt;0.001</td>
<td>p = 0.02</td>
</tr>
</tbody>
</table>
3.6.3 Discussion: At the acute time-point, patients were found to underperform on a number of cognitive tests compared to controls (Table 3.6). By comparing these tests with the cognitive skills they purport to measure (Table 3.4), patients initially appeared to be impaired on various aspects of executive function (information processing, clustering and switching) as well as visuospatial and verbal learning, attention, concentration, short-term memory and working memory. One interesting case comes from mild patients performing significantly better than controls in the switching condition of the CWIT task, although this is unlikely to reflect anything injury-specific and is more possibly an outcome of either / or the CWIT task otherwise appearing very insensitive to detecting cognitive deficit in this patient group and potential multiple comparison problems. Examining the returning patient data indicates that patient cognitive performance improved over the course of a year as there was no longer a significant difference in any cognitive test performance when compared to controls, except for Backwards Digitspan. This indicates a degree of cognitive recovery in most domains (Backwards Digitspan specifically measures short-term and working memory), although simply examining test performance doesn’t allow for conclusions to be made about if this recovery is due to a lack of lasting physiological damage or the development of effective coping strategies.

The cognitive profile of these patients appears in keeping with what is commonly reported in the literature. One paper studying mild TBI (Belanger et al., 2005) found strong deficits in “delayed memory” and verbal fluency in patients when studied less than 3 months post-injury. Another paper focusing on mild TBI highlighted the attentional deficits that are often found (Cicerone, 1996), while another found mild patients to underperform on the PASAT task and a word association test (another broad method of investigating executive function, (Brooks et al., 1999)). The apparent recovery of our patients at 1 year post injury is also supportive of literature which suggests that cognitive function in mild patients improves rapidly during the first few weeks following injury, and often normalises by 3 months (Schretlen and Shapiro, 2003).

As there was evidence of pre-morbid IQ being lower in patients than in controls (section 3.6.2.1) it was necessary to consider if these findings were more influenced by the TBI, or by otherwise lower IQ. Regression analysis was conducted for this. At the
acute time point, it was found that NART (IQ) and Group (Patient / Control) were individually effective in explaining the variance of approximately half of the cognitive test scores each (Table 3.7), indicating that the injury was indeed the cause of significant deficit in SoIP, Design / List Learning and the 3 second interval condition of the PASAT test, but that NART instead contributed to significant deficit in the 2 second interval condition of the PASAT test, Backwards Digitspan and Verbal Fluency. At the follow-up time point only NART was found to be a significant contributor to the variance in some cognitive test scores, although it should be noted again that at this time-point patients only still underperformed in the Backwards Digitspan task (where NART was a significant predictor).

There is debate in the literature as to how effective NART is as a measure of pre-morbid IQ. One study (Riley and Simmonds, 2003) found that patients with a severe TBI performed better on a second NART test taken a year after the first one, implying that on-going recovery from their injury improved their performance on the test. The authors concluded that NART may therefore underestimate pre-morbid IQ. Another recent study also concluded that performance on the NART test within 1 month of injury also underestimated premorbid IQ, and that mild patients were significantly impaired on NART performance within 1 month of their injury compared to 6 months post-injury (Skilbeck et al., 2013). In our data, observing that performance on a number of tests where NART was the significant predictor improved over time (from being significantly different at 2 weeks to not significantly different at 1 year against controls) also supports the hypothesis that recovery, and therefore injury, is still influencing task performance in cases where the regression analysis did not show this; if these acute test differences were only caused by IQ then we would not expect to see a change in them over time.

Differences in mild and moderate patient performance were also examined at the acute time point (this was not repeated for the follow-up data as sample sizes were too small). This found that moderate patients were significantly impaired compared to mild patients in the 2 second interval condition of the PASAT task as well as both conditions of the Verbal Fluency task. Although these were the only significant findings, it was observed that this comparison was approaching significance in other tasks; SoIP and the A1-A5 condition of the Design Learning Task each produced a p
value less than 0.1. With the moderate patient group contributing only 6 - 8 responses on cognitive tests it is likely that further significant findings were missed in these comparisons due to a small sample size. While these analyses lend support to the notion that there are few enough moderate patients in our sample that they don’t necessarily skew the data, they also give some evidence that there may be true differences between the mild and moderate patients. This will be considered when conducting all subsequent analyses.
Chapter 4. Examining the Probability Distribution of Lesions

4.1 Overview

This chapter describes procedures to map the distribution of visible, focal lesions at the acute phase of injury. Images showing lesion distribution are produced for all patients and some relevant patient sub-groups. Further, statistical testing is then conducted to examine relationships between total acute lesion volume for each patient and variables such as severity of injury, LoC, PTA and psychometric test performance. Based on previous research it is hypothesised that lesion distribution would be focused in the frontal lobe. It is also hypothesised that the extent of lesioning would be related to psychometric test performance, although would not be a wholly sensitive predictor of this as some cognitive deficit is expected to be caused by non-visible DAI.

4.2 Methodology

4.2.1 Data Acquisition: A 3D T₁ weighted sequence was used to obtain basic regional anatomy, with scanning taking place in a sagittal orientation with 1mm isotropic resolution (MPRAGE, TR=8.1ms, TE=4.6ms, matrix size 240×216×180) and accelerated with a SENSE factor of 2 in the right-left direction. Image intensity correction was applied with the CLEAR reconstruction algorithm.

The T₁ mapping used a rapid inversion recovery sequence with segmented inversion slab method and multi-slice single shot EPI readout. Images were scanned in a transverse orientation with 2mm isotropic resolution (TR=15s, TE=24ms, matrix size 128x128x72) and accelerated with a SENSE factor of 2 in the right-left phase direction. The 72 slices were split into 6 segments of 60mm width, with each segment being imaged at 12 inversion times from 0.25s to 2.5s in even steps, with the order of slices being varied throughout these to form the complete relaxation curve.

The T₂ mapping images (GRASE, TR=4.7s, TE=20ms – 160ms in 8 even steps [each slice being imaged at 8 different echo times], matrix size 128x128x24) were scanned in a transverse orientation with 2mm isotropic resolution and collected in segments of 5 k-space lines.
All scan data was subjected to movement correction, fitting and unwarping as described in section 3.4.

4.2.2 Lesion Segmentation: Mr. Cowie had previously conducted work which involved removing visible focal lesions from the 3D T₁W and quantitative T₁ and T₂ scans taken at the acute stage, so that normal-appearing brain tissue could be investigated. To do this, the T₁W scan was first inspected, and a binary ROI drawn around any abnormalities using MRIcro software. This ROI was then overlayed in turn onto the quantitative T₁ and T₂ scans and extended to include any further visible lesions which became visible between scan types, resulting in each patient having a single ROI which defined visible damage in all 3 modalities. Lesions were identified in a total of 45 patients; 36 mild patients and all moderate patients. This new image therefore only contained voxels with values of either “0” (areas where lesion had been identified) or “1” (areas of normal appearing tissue). This process is demonstrated in Figure 4.1. While Mr. Cowie then used these as masks to eliminate areas of lesion from scan datasets, these data were recovered here as the first step in producing images to show the most commonly damaged locations in TBI patients.

Figure 4.1. A demonstration of the lesion removal technique. 1. Shows a T₁W scan of a patient with a visible focal lesion in their anterior frontal lobe (the focal damage extends fully to the boundaries of the ROI when also examining the qT₁ and qT₂ scans). 2. Shows the ROI drawn around the affected area. 3. Shows the binary lesion image created by this ROI.
4.2.3 Lesion Data Manipulation and Visualisation: The FSLMaths tool (included in the FSL package) was first used to invert the lesion ROI images, so that areas which contained damaged tissue now had a value of “1” while healthy tissue had a value of “0”. This was achieved by subtracting “1” from each image before multiplying each of these new images by themselves. Each patient’s basic T₁W scan was then individually registered to MNI152 (2mm) standard space using the FLIRT tool of the FSL package. The accompanying lesion ROI images were included as secondary images during these registrations, so that the same registering manipulations which FLIRT applied to the T₁W scan were also applied to the lesion ROI, creating a set of lesion ROI images in MNI152 (2mm) standard space. As this registering process sometimes creates new, non-binary values around the edges of lesions due to blurring from the warping process, FSLMaths was again used to threshold these new images so that all voxels with a value less than “1” once again became “0”.

These steps produced a dataset consisting of comparable lesion ROI’s for each patient. FSLMaths was then used to create averaged images of these so that the most commonly damaged locations could be visualised in standard space (adding the images together and then dividing that image by the total number in the group). This averaged image was then used as an overlay on top of the MNI152 (2mm) standard template. This method was first used on all acute patient data, although an error was found at this stage. One of the lesion ROI images had areas defined outside of the bounds of the brain. In order to correct this, a further binary mask was created based on the MNI152 (2mm) template, where any brain tissue had a value of “1” and anywhere outside of the brain had a value of “0”. This mask was then multiplied with any final, averaged overlays to remove the additional voxels.

Following this correction, MRICron was used to produce final averaged images demonstrating locations of common acute damage within different patient groups; all patients together, mild patients, moderate patients, patients who returned at follow-up (AFu) and patients who did not return at follow-up (AL). Images which combined mild / moderate groups and AFu / AL groups were also made. While the images of only one groups data used a single colour of varying intensity to show lesion distribution,
these combined images used different colours for each base overlay so that the
distribution of lesions between the sub-groups could be directly compared. In these
instances, manipulations were made to the overlays so that the colour shading was
solid (i.e. was not affected by how frequently lesions were found in a given area) for
visual clarity.

4.2.4 Lesion Volume Analyses: The fslstats tool (a part of FSL) was used to
extract the number of non-0 voxels present in each patient’s lesion ROI image (the
images used for this were the final versions of the lesion ROI’s after all manipulations
had been applied, i.e. they had been registered into standard space and then re-
thresholded to remove any voxels which didn’t hold a value of exactly “1”). As these
images only contain “1”’s, for lesion space, and “0”’s, for non-lesion space, this
number therefore describes the total number of lesion voxels per patient, in standard
space and can be considered a measure of total lesion volume per patient.

Patients who did not have any lesions were assigned a value of “0”, before
having this data combined with the total number of lesion-voxels for all the other
patients. These values were then subject to a number of statistical comparisons. The
first set of analyses focused on testing for lesion volume differences between sub-
groups of patients, and seeking to find relationships between lesion volume and non-
psychometric variables (i.e. GCS, PTA and LoC). The distribution of the total lesion voxel
count was first examined for normality using the Anderson-Darling test in the following
groups: all patients, mild patients, moderate patients, AFu patients and AL patients.
The total number of lesion-voxels was then compared between mild / moderate
groups, and AFu and AL groups using t-tests if the data was normally distributed and
the Mann-Whitney U test if the data was not normally distributed. The comparison
between mild and moderate patients was one tailed due to the clear hypothesis that
greater injury severity would be expected to lead to a greater amount of lesions.
Correlational analyses were also conducted. For these, the distribution of GCS, PTA and
LoC scores were first examined by the Anderson-Darling test to assess normality.
Correlations were then sought between the total lesion-voxel count and GCS / PTA /
LoC using Pearsons correlation if data was normally distributed, and Spearmans rank if
data was not normally distributed. Finally, “FSLutils” tools were used to extract the
total number of non-zero voxels present in the MNI152 (2mm) template brain, so that
relevant results which reported “total lesion voxel count” could also be reported as the % of the brain which the lesion occupies.

The second set of analyses focused on testing psychometric data with lesion volume data. For these, the acute scores on all psychometric test where patients had been found to underperform compared to controls (as previously summarised in Table 3.6) were correlated against total lesion-voxel count. Pearsons correlation was conducted in the case of normally distributed data while Spearmans rank correlation was used in the case of non-normally distributed data (while the distributions of GCS, PTA and LoC are reported here, the distributions of psychometric test scores can be found in section 3.6.2). These correlations were conducted considering all patients as a single group. Finally, groupwise analyses were also conducted comparing test scores between patients with visible lesions and patients without visible lesions. The data used here were the acute scores on all psychometric tests where patients had been found to underperform. t-tests were used for normally distributed data and Mann-Whitney U was used for data which was not normally distributed. All testing was conducted using an alpha value of 0.05.
4.3 Results

4.3.1 Lesion Distribution in all Patients: Visual inspection (Figure 4.2) indicates the anterior, inferior and lateral parts of the frontal and temporal lobes to be most frequently damaged, although lesions are also found in many other cortical locations around the brain. The marginally posterior, medial parts of the brain appear the most unaffected.

Figure 4.2. Outputs showing areas of visible damage in all patients at the acute time-point. The variation in shading represents frequency of lesioning in that location; the shade of colour becomes brighter and more intense if more lesions were found in that area.
4.3.2 Lesion Distribution in Mild Patients: Visual inspection (Figure 4.3) again indicates the anterior, inferior and lateral parts of the frontal and temporal lobes to be the most frequently damaged while other damage is more sporadically found around the cortex.

Figure 4.3. Outputs showing areas of visible damage in mild patients. The variation in shading represents frequency of lesioning in that location; the shade of colour becomes brighter and more intense if more lesions were found in that area.
4.3.3 Lesion Distribution in Moderate Patients: Despite the greatly reduced sample size for this patient sub-group, visual inspection (Figure 4.4) again shows a similar pattern of damage to mild patients with lesions most strongly concentrated in the anterior, inferior and lateral parts of the frontal and temporal lobes with other damage more sporadically distributed around the cortex.

**Figure 4.4.** Outputs showing areas of visible damage in moderate patients. The variation in shading represents frequency of lesioning in that location; the shade of colour becomes brighter and more intense if more lesions were found in that area.
4.3.4 Direct Mild / Moderate Comparison of Lesion Distribution: Contrasting the distribution of damage in the mild and moderate patient groups (Figure 4.5) does show a number of locations where damage is only present in one group, particularly in the mild group, although with a similar overall distribution (as indicated by the purple areas).

![Figure 4.5](image_url)

**Figure 4.5.** Outputs showing areas of visible acute damage split by mild and moderate patients. Areas of mild patient damage are represented by blue while areas of moderate damage are represented by red. Locations where damage was only present in a single group is therefore shown in only that colour, while areas of common damage between these sub-groups are mixed, creating a purple shading. Note that shading of base colours is solid (i.e. the same shade regardless of how often lesions were found in that location) for clarity.
4.3.5 Lesion Distribution in the AFu Sub-Group: Visual inspection of the lesion distribution in the AFu patient sub-group (Figure 4.6) again shows a similar pattern as previously reported.

Figure 4.6. Outputs showing areas of visible damage in the AFu patient sub-group. The variation in shading represents frequency of lesioning in that location; the shade of colour becomes brighter and more intense if more lesions were found in that area.
4.3.6 Lesion Distribution in the AL Sub-Group: Visual inspection of the lesion distribution in the AL patient sub-group (Figure 4.7) again shows a similar pattern as previously reported.

*Figure 4.7.* Outputs showing areas of visible damage in the AL patient sub-group. The variation in shading represents frequency of lesioning in that location; the shade of colour becomes brighter and more intense if more lesions were found in that area.
4.3.7 Direct AFu / AL Comparison of Lesion Distribution: Contrasting the distribution of damage in the AFu and AL patient groups (Figure 4.8) does show a number of locations where damage is only present in one group, particularly in the mild group, although again with a similar overall distribution (represented by the turquoise areas).

Figure 4.8. Outputs showing areas of visible acute damage split by patients who did return at follow-up (AFu) and patients who did not return at follow-up (AL). Areas of AFu damage are represented by green while areas of AL damage are represented by blue. Locations where damage was only present in a single group is therefore shown in only that colour, while areas of common damage between these sub-groups are mixed, creating a turquoise shading. Note that shading of base colours is solid (i.e. the same shade regardless of how often lesions were found in that location) for clarity.
4.3.8 Lesion Volume Analyses: Testing for normality of the lesion-voxel counts using the Anderson-Darling test showed data to not be normally distributed in all patient, mild patient, AFu patient and AL patient groups (all \( p \) values <0.005), likely due to the high number of patients with no visible lesions. Only the data in the moderate patient group was shown to be normally distributed (\( p = 0.371 \)). This necessitated using non-parametric statistics for all further groupwise and correlation analyses which involved the lesion-voxel counts (i.e. all analyses except psychometric performance between patients with lesion vs patients without lesions, where the analysis techniques used were governed by the normality of the psychometric data itself).

Regarding the first set of analyses (concerning non-psychometric data), Mann-Whitney U analysis showed a significant difference between the total lesion-voxel count of mild (median=2773.5 (1.21% of total brain volume)) and moderate patients (median=8177 (3.58% of total brain volume), \( p = 0.037 \), one-tailed test) but showed no significant difference between AFu (median=2569 (1.12% of total brain volume)) and AL patients (median=3995.5 (1.75% of total brain volume), \( p = 0.478 \), two-tailed test). No significant correlations were found between total lesion-voxel count and GCS (\( p = 0.059 \)), LoC (\( p = 0.981 \)) or PTA (\( p = 0.391 \)).

Regarding the second set of analyses (concerning psychometric data), correlational analysis showed two significant, negative correlations between total lesion-voxel count and each of the Verbal Fluency conditions at the acute time-point; VLF (\( R = -0.319, p = 0.027 \), Figure 4.9), Category Fluency (\( R = -0.308, p = 0.03 \), Figure 4.10). As the general pattern of these correlations were quite weak it was decided that multiple comparison correction was appropriate. Following bonferoni correction (which revised the alpha value to 0.005) these did not remain significant. The full set of correlational findings are shown overleaf in Table 4.1. No other correlations were significant.
Groupwise analyses on psychometric data showed significant differences on all tests except SOIP and Backwards Digitspan / Spatialspan wherein patients without lesions performed better than patients with lesions. These findings are shown overleaf in Table 4.2.

<table>
<thead>
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<th>R value</th>
<th>p value</th>
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<td>0.232</td>
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<td>PASAT</td>
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<tr>
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<td>Category Fluency</td>
<td>-0.308</td>
<td>0.03</td>
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Table 4.1. Results from Spearmans Rank correlation analyses between the total lesion-voxel count and all performance on psychometric tests where patient had previously found to be impaired.
4.4. Discussion

**4.4.1 Lesion Distribution:** The overall probability distribution of lesions (Figure 4.2) shows evidence that the anterior, inferior frontal cortex is the area of the brain most frequently injured to the extent that lesions can be seen on more basic anatomical scans. Other lesioning was detected more sporadically around the rest of the cortex, albeit to a lesser extent. These findings support previous work which found cortical contusions, particularly present within the anterior, inferior and lateral aspects of the frontal and temporal lobes to account for 84% of detectable, non-DAI damage.
following closed head injury (Gentry et al., 1988). Medial parts of the brain and areas marginally posterior to these (the thalamus, splenium of the corpus callosum etc.) appeared to have been spared of lesions detectable by these types of scans. Examining patient sub-groups showed some areas which were damaged in one sub-group but not in the counterpart sub-group. However the overall distribution remained comparable, supporting that neither the mild / moderate or AFu / AL groups are fundamentally different to one another (Figures 4.3-4.8).

The distribution of these lesions is perhaps unsurprising as most methods of injury (road traffic accidents, assaults etc.) would tend to involve impacts to the front / front sides of the head. Injuries which primarily involve the back of the head are less conceivable. As such the number of lesions which were detected in areas such as the occipital lobe are likely relatively reduced because of this, or may have been caused by contra-coup injury which affected only a proportion of otherwise frontal injuries.

**4.4.2 Lesion Volume Analyses:** Further experimentation extracted the total number of lesion-voxels for each patient as a measure of that individual’s total volume of lesions. This data was first used to again test for differences between mild / moderate and AFu / AL groups. As may be expected, moderate patients had a significantly higher lesion-voxel count compared to mild patients. Supporting this, a correlational analysis comparing lesion-voxel count with GCS was nearly-significant; this would have suffered from a lack of power coming from more severe scores and would likely have been significant with more moderate patients. AFu and AL comparison showed no significant difference supporting that the patients who did and who did not return for follow-up examination had no fundamentally different pattern of focal lesions. Neither LoC and PTA correlated with lesion-voxel count, implying that the duration of unconsciousness or amnesia associated with the injury are not related to the amount of visible lesions.

Lesion volume information was also examined in relation to psychometric performance. Correlations were sought between lesion-voxel count and acute test scores in tasks where patients had previously been shown to underperform. Two of these were initially significant with performance on both conditions of the Verbal Fluency task (VLF and Category fluency) negatively correlating with lesion-voxel count,
implying that higher lesion volume is related to worse performance. However these did not retain significance following bonferroni correction. However, groupwise testing of performance on this set of tasks between patients with lesions and patients without lesions returned many significant results, all indicating worse performance in patients with lesions.

These psychometric findings support previous literature which indicates the areas that have been shown to be most frequently damaged to be relevant to aspects of cognition which our psychometric battery tests. Research has found various areas of the frontal lobe to be involved in a broad range of cognitive functions including executive control, inhibition, attentional selection, working memory (specifically including storage of spatial and object information) and problem solving (Duncan and Owen, 2000, Smith and Jonides, 1999, Aron et al., 2004, Dias et al., 1996). The frontal lobe is in fact so often associated with executive function-style tasks (as most of our cognitive test battery involves) that some research interchangeably uses the term “frontal lobe tasks” with “executive tasks” (Miyake et al., 2000). Areas of the temporal lobe are also known to be involved in functions utilising aspects of memory such as declarative memory and encoding of verbal and non-verbal information (Squire et al., 2004, Kelley et al., 1998).

The reason why correlational analysis returned fewer findings than groupwise analysis is likely because examining total lesion volume is a blunt a method of predicting test scores which measure specific deficits. Mesulams neural node theory (Mesulam, 1998) implies that all that specific cognitive function is dependent on a network of focal nodes involved with that process. Total lesion volume across the brain is therefore irrelevant to a single test score provided that some of the relatively small areas of the brain which govern the cognitive domain measured by the test are damaged. As groupwise analysis between patients with / without lesions ignores total lesion volume (opposed to correlational analysis which depends upon it) this is therefore more accurate predictor of task performance.

However, not all tasks were shown to be related to visible lesions, supporting theories of non-visible damage also affecting cognitive outcome following TBI. As DAI has been shown to occur in mild TBI and to predominantly affect major white matter
tracts it should be considered that some of the cognitive symptoms displayed by these patients could be primarily due to microscopic damage.

4.5 Chapter Summary

Lesion probability mapping has shown our patient cohort to primarily suffer from visible damage to anterior, inferior and lateral aspects of the frontal and temporal lobes. While the acute distribution of lesions does not appear different between mild / moderate patients and patients who did / did not return at follow-up, further statistical testing concerning lesion volume implied that visible lesion volume may increase with injury severity. Groupwise and correlational analysis also suggested that visible lesions predict worse psychometric test performance, although measuring the actual volume of lesioning is not as sensitive a measure of doing this as simply noting if the patient does or does not have visible lesions. Finally, as some test performance remained un-related to the presence of / volume of lesioning there is evidence to suggest that non-visible damage may also be contributing to some cognitive deficit. As such, the specific hypothesis that lesion load would be a reasonable predictor of cognitive deficit is not supported, although the findings do more broadly indicate that focal lesions partially contribute to cognitive outcome.
Chapter 5. Diffusion Tensor Imaging Investigation

5.1 Overview

This chapter describes a DTI-based investigation conducted on the group of participants previously described in Chapter 3. This investigation aimed to expand on previous work in the field by focusing on patient groups and research techniques which have been arguably underused thus far in DTI research pertaining to DAI following TBI and acquired cognitive deficit. We present a comprehensive, longitudinal DTI study in mild and moderate TBI, using TBSS to examine DTI metric changes between acute and chronic time points and explore how this relates to acquired cognitive deficits following TBI. We hypothesised that the cognitive dysfunction widely observed during the acute period post TBI would have a neurobiological correlate in acute DAI detected by diffusion imaging changes. We further hypothesised that this would still be recognisable in the chronic phase. The main aspects of this investigation have been published (Croall et al., 2014) (Appendix C), although additional analyses / details are provided here.

5.2 Methodology

5.2.1 DTI Data Acquisition: Participants were scanned using a single shot EPI diffusion sequence on a 3T Philips Achieva MRI scanner with an 8 channel SENSE head coil. A 3D T1 weighted sequence was used to obtain basic regional anatomy (MPRAGE, TR=8.1ms, TE=4.6ms, matrix size 150×240×240, isotropic 1mm resolution). DTI scans were collected with the following parameters: TR/TE=2524/71ms, 24 slices, b=0,1000smm⁻², 16 direction, 2x2x6mm³ resolution. A magnetic field map sequence was also acquired for correction of geometric distortion of the DTI scans (dual echo 3D GRE, TR=27ms, TE=2.6/6.1ms, matrix 128x128x72, 2mm resolution).

5.2.2 Main DTI Data Analysis: DTI scans were processed using the FSL toolbox (Smith et al., 2004). Raw scan data were corrected for geometric distortion (FUGUE) and residual eddy current effects were removed by affine registration of each scan to the b=0 s.mm⁻² image. Scans were then fit to the diffusion tensor model to produce maps of mean diffusivity (MD), fractional anisotropy (FA) and principle eigenvalues and eigenvectors. Voxelwise statistical analysis of the data was carried out using the TBSS
(Smith et al., 2006) package. Figure 5.1 describes the steps involved in using TBSS and gives examples of typical outputs. For each subject all diffusion scans were transformed into the MNI152 standard space using non-linear registration (FNIRT) to a target FA template image provided in TBSS. The accuracy of the non-linear transformation was verified by visual inspection of the data. A mean FA skeleton representing the centres of all tracts common to the group was created by averaging the registered FA images and then thresholding the mean FA image at FA>0.2. Each subject's registered FA data was then projected onto the mean FA skeleton, which accounted for residual misalignments between subjects after the initial non-linear registration. Data for MD and the 3 principle eigenvalues were also projected onto the mean skeleton by using non-linear registration and projection vectors from the FA images.

The resulting registered sets were fed into voxelwise cross-subject statistics. All TBSS statistical analyses were run with 5000 permutations other than those ran with 500 permutations, as described in section 5.2.3. Additionally, “threshold free cluster enhancement” (TFCE (Smith and Nichols, 2009)) output images were used for analysis with $p < 0.05$ being treated as significant. TFCE is a non-binary technique where clusters of significant voxels are identified and enhanced in the output image. It achieves this by producing a new value for each voxel which is the weighted sum of the local clustered signal. TBSS was used to examine differences in acute DTI metrics (FA, MD, AD and the constituent parts of RD; $\lambda_2$ and $\lambda_3$) in a between subjects control vs. patient analysis. Follow-up DTI metrics were analysed by between subjects control vs. patient analysis. All patients were considered as a single group when testing for control versus patient metric differences. However some post-hoc analyses focused on examining any differences between mild and moderately injured patients (described in section 5.4.2).
5.2.3 Neuropsychological Data Analysis: Due to strong cognitive deficits being shown between all experimental groups in cognitive test scores (described in Chapter 3), VLF, Category Fluency, PASAT (3 second condition) and SoIP test scores were chosen to be (separately) regressed against FA in TBSS as a pilot investigation (run with 500 permutations). T-stat images were examined from these as a means of looking at the raw findings; only regressing voxels with a T-stat above 3 were counted as “significant”. The number of these significant pixels were counted from each of these images using the histogram function in the software ImageJ. PASAT returned no significantly regressing pixels, while a summary of the other three cognitive test results
is shown in Table 5.1, below; VLF convincingly returned the most significant results and was chosen to be investigated further. To determine whether there was a relationship between DTI changes and cognitive performance TBSS was used to perform regression analysis of VLF scores against FA, MD, AD and the constituent parts of RD; \( \lambda_2 \) and \( \lambda_3 \), while controlling for NART in both acute and follow-up conditions. This analysis was completed for controls and patients separately. \( \lambda_2 \) and \( \lambda_3 \) images were averaged to create a single image for RD. As not every patient was able to complete the psychometric tests due to reasons relating to their injury, sample size for statistical testing which involved this data was reduced compared to testing which involved only DTI metric data (acutely; 7 patients were excluded, chronically; 2 patients were excluded). Additionally, as psychometric data for 1 control participant was absent this also reduced the Control group to 32 for relevant testing.

<table>
<thead>
<tr>
<th>Test (type of regression)</th>
<th># of significant pixels above T-stat 3</th>
<th>Comments on location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Letter Fluency (positive)</td>
<td>55</td>
<td>Brainstem and short fibres between superior parietal / frontal lobes.</td>
</tr>
<tr>
<td>Letter Fluency (negative)</td>
<td>4789</td>
<td>Very diffuse spread.</td>
</tr>
<tr>
<td>Category Fluency (positive)</td>
<td>79</td>
<td>Diffuse areas within brainstem, cerebellum, occipital and frontal lobes.</td>
</tr>
<tr>
<td>Category Fluency (negative)</td>
<td>1618</td>
<td>Very diffuse spread</td>
</tr>
<tr>
<td>SOIP (positive)</td>
<td>53</td>
<td>Very diffuse spread</td>
</tr>
<tr>
<td>SOIP (negative)</td>
<td>165</td>
<td>One focal spot in short fibres between occipital and parietal lobes.</td>
</tr>
</tbody>
</table>

Table 5.1. An overview of how many pixels with a T-stat above 3 were found to regress when comparing FA with VLF, Category Fluency, PASAT (3 second conditions) and SOIP. PASAT is not included here as no regressing pixels were found.
5.3 Results

Please note that the slices used in figures have been chosen for both clarity of results and ease of comparison between analyses and time-points. However due to the widespread nature of reported changes, some tract locations which demonstrated a significant result may be reported in the text but not shown in a figure. The coordinates used in the following figures are in MNI space.

5.3.1 Acute Groupwise Differences in Diffusion Metrics: At the acute time point groupwise control versus patient comparison revealed multiple, widespread locations of increased FA (Figure 5.2), AD (Figure 5.3) and MD (Figure 5.4) in the patient group with a complex spatial relationship to one another. The FA increases were observed bilaterally in a tract later identified as the ascending fibres of the corpus callosum (see section 5.4.1) and also association fibres. Major locations of increased MD were posterior aspects of the ascending fibres of the corpus callosum and also association fibres. These changes in FA and MD were seen to overlap with increased AD. While AD showed extensive increases throughout the white matter tracts, RD did not demonstrate any locations of significant difference.
Figure 5.2. TBSS outputs demonstrating locations of FA change following TBI at the acute time point. All changes are increases (coloured red/yellow) in the Patient group compared to controls. Z co-ordinates are based around the AC/PC line being Z=0. Colour bar values are 1 – p value.
Figure 5.3. TBSS outputs demonstrating locations of AD change following TBI at the acute time point. All changes are increases (coloured red/yellow) in the Patient group compared to controls. Z co-ordinates are based around the AC/PC line being Z=0. Colour bar values are 1 – p value.
Figure 5.4. TBSS outputs demonstrating locations of MD change following TBI at the acute time point. All changes are increases (coloured red/yellow) in the Patient group compared to controls. Z coordinates are based around the AC/PC line being $Z=0$. Colour bar values are $1 – p$ value.
5.3.2 Chronic Groupwise Differences in Diffusion Metrics: Comparison of follow-up patient (12 months post injury) vs. control data showed groupwise differences in the patients but with altered patterns of changes compared to the acute time point (Figures 5.5-5.7). FA was now decreased in the Anterior Forceps but was no longer different to control in the locations where the most widespread differences had been seen acutely (Figure 5.5). In further contrast with the acute data, RD was now increased in areas such as the anterior forceps, thalamic projections and association fibres (Figure 5.6), while AD had returned to normal and did not demonstrate any locations of significant difference in the follow-up patient vs. control analysis; a complete reversal of the pattern observed acutely. MD remained significantly increased in the same locations where it was observed acutely, but had also expanded to include the same part of the Anterior Forceps where FA was decreased (Figure 5.7).
Figure 5.5. TBSS outputs demonstrating locations of FA change following TBI at the chronic time point. All changes are decreases (coloured blue/light blue) in the Patient group compared to controls. Z co-ordinates are based around the AC/PC line being Z=0. Colour bar values are 1 – p value.
Figure 5.6. TBSS outputs demonstrating locations of RD change following TBI at the chronic time point. All changes are increases (coloured red/yellow) in the Patient group compared to controls. Z co-ordinates are based around the AC/PC line being Z=0. Colour bar values are 1 – p value.
Figure 5.7. TBSS outputs demonstrating locations of MD change following TBI at the chronic time point. All changes are increases (coloured red/yellow) in the Patient group compared to controls. Z co-ordinates are based around the AC/PC line being Z=0. Colour bar values are 1 – p value.
5.3.3 Acute Cognitive Testing and TBSS Analysis: While full psychometric test performance differences between patients and controls was covered in detail in Chapter 3, VLF findings are repeated here for clarity. Patients VLF performance ($Mean = 31.6, SD = 13.3$) was significantly worse at the acute time point compared to controls ($Mean = 41.63, SD = 9.38, t(77) = 3.95, p < 0.001$). Widespread locations of negative regression between acute FA and acute VLF scores (controlling for NART) were found in the patient group in the ascending fibres of the corpus callosum, the superior corona radiata and other association fibres (Figure 5.8). Analysis of the other DTI metrics revealed locations of positive regression between acute VLF and RD (Figure 5.9) in the same locations as the FA results, and small locations of positive regression between acute VLF and MD in posterior association fibres (Figure 5.10). Acute VLF and acute AD did not demonstrate any locations of regression. The control group showed no locations of any regression between any metric and VLF.
Figure 5.8. TBSS outputs demonstrating locations of regression between FA and VLF scores (controlling for NART) in the Patient group at the acute time-point. All regressions are negative (coloured blue/light blue). Z co-ordinates are based around the AC/PC line being Z=0. Colour bar values are 1 – p value.
Figure 5.9. TBSS outputs demonstrating locations of regression between RD and VLF scores (controlling for NART) in the Patient group at the acute time-point. All regressions are positive (coloured red/yellow). Z co-ordinates are based around the AC/PC line being Z=0. Colour bar values are 1 – p value.
Figure 5.10. TBSS outputs demonstrating locations of regression between MD and VLF scores (controlling for NART) in the Patient group at the acute time-point. All regressions are positive (coloured red/yellow). Z co-ordinates are based around the AC/PC line being Z=0. Colour bar values are 1 – p value.
5.3.4 Chronic Cognitive Testing and TBSS Analysis: Follow-up cognitive test scores showed no significant difference in patient vs. control comparisons. Follow-up patient VLF was regressed against follow-up DTI metrics. This was conducted despite the lack of a groupwise difference in patient VLF performance in order to see if any evidence of the acute regression remaining chronically. Negative regressions between FA/VLF remained in the fibres of the corpus callosum although were far less widespread than in the acute condition, remaining primarily in the Body of the corpus callosum (Figure 5.11). A very small cluster of negative regression between AD/VLF was also located in the right CC fibres, although this was deemed too small to be a confidently reportable finding. RD/MD did not regress with VLF. Note that the illustrated slices used in Figure 5.11 have been changed from those used previously in order to better show these findings. All of these main findings are further summarised in Table 5.2.
**Figure 5.1** TBSS outputs demonstrating locations of regression between FA and VLF scores (controlling for NART) in the Patient group at the chronic time-point. All regressions are negative (coloured blue/light blue). Z co-ordinates are based around the AC/PC line being Z=0. Colour bar values are 1 – p value.
Table 5.2. A summary of results from main DTI group-wise and regression analyses.

<table>
<thead>
<tr>
<th>Fractional Anisotropy</th>
<th>Mean Diffusivity</th>
<th>Radial Diffusivity</th>
<th>Axial Diffusivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groupwise Diffusion Tensor Imaging findings versus Control Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Acute</strong></td>
<td>↑ Ascending CC and assoc. fibres</td>
<td>↑ Posterior CC (splenium) and assoc. fibres</td>
<td>No difference</td>
</tr>
<tr>
<td><strong>Chronic</strong></td>
<td>↓ Anterior forceps</td>
<td>↑ Posterior CC (splenium), assoc. fibres and anterior forceps</td>
<td>↑ CC, thalamic projections assoc. fibres and anterior forceps</td>
</tr>
</tbody>
</table>

**Diffusion Tensor Imaging vs. Verbal Letter Fluency**

| Acute | ↑ CC, ascending CC fibres and assoc. fibres correlates with **poorer** VLF score | ↑ CC and assoc. fibres correlates with **better** VLF score | ↓ CC, ascending CC fibres and assoc. fibres correlates with **poorer** VLF score | No regression |
| Chronic | ↑ CC, correlates with **poorer** VLF score (although less widespread than acutely) | No regression | No regression | ↑ CC fibres correlates with **poorer** VLF score (very small location) |
5.4 Post-Hoc Analyses

A total of three post-hoc analyses were conducted.

- An analysis to replicate the main TBSS findings in a tractography investigation (5.4.1).
- An analysis to identify any potential differences between mild and moderate patients (5.4.2).
- An analysis to identify any potential differences between those patients who did and did not return for follow-up testing (5.4.3, and Appendix B).

5.4.1 Post-Hoc Tract Analysis

5.4.1.1 Aim: One tract had featured particularly prominently in acute groupwise and regression analysis findings (Figure 5.12). As the damage TBI and DAI causes can affect white matter tracts in a way which impacts upon DTI metrics in ways which may lead to problems such as different tracts being measured between groups (e.g. in cases where there may be selective destruction of crossing fibres following TBI), it was decided that a more detailed, tractographic investigation of this region would be appropriate so that tract structure could be more directly observed. Additionally, as it was unclear which specific tract was exhibiting this increased FA, this analysis also sought to identify it.
Based on the location the tract was deemed most likely to be the ascending fibres from the corpus callosum. Therefore a tractographic, ROI-based analysis was conducted on the acute data using the program DTI Studio (Jiang et al., 2006) which focused on capturing these fibres and extracting FA, AD, RD and MD values from them. Groupwise analysis was then also conducted in an attempt to replicate the acute FA increase in the patient group.

5.4.1.2 Methodology: Philips REC files were subject to automatic image registration before being converted to maps of the diffusion metrics by calculation of the diffusion tensor. A colour FA map was used for visualisation and the sagittal plane was displaced by a small number of slices (usually 4) towards the left hemisphere until

Figure 5.12. A TBSS output with arrows to highlight the unknown tract. This particular output shows acute increases in patient FA at a z-coordinate level of 40.
it sat on the edge of the corpus callosum (Figure 5.13, A). The axial plane was then displaced to a location marginally superior to the top of the corpus callosum, allowing visualisation of the ascending fibres in the axial view (Figure 5.13, B). Using the “cut” function, an oval ROI was then drawn on the sagittal view encompassing the entirety of the CC (Figure 5.13, C). Continuing with the “cut” function, a second rectangular ROI was then drawn on the axial slice encompassing the length of projected fibres (Figure 5.13, D). The 3D view was then inspected and any unwanted fibres which had been included (e.g. from other tracts than the CC, Figure 5.13, E) were excluded by use of the “not” function. Throughout this, the general appearance of tract structure was visually examined with regards to any notable differences between control and patient tract appearance. Metric data was extracted from the fibres and used for comparison with VLF scores. Control metric values were tested for normality with the Anderson-Darling Test, and found to all be normal. Comparisons between patient and control metrics (AD, RD, FA and MD) were therefore made using t-tests while a regression analysis also examined any relationship between patient metrics and VLF performance, controlling for NART.
A: Sagittal plane was moved marginally into the left hemisphere.

B: Axial plane was placed in a location slightly superior to the top of the CC so that the ascending fibres (highlighted) could be seen.

C: Using the “cut” function, an oval ROI was drawn around the CC in the sagittal plane.

D: Using the “cut” function, a rectangular ROI was drawn around the ascending fibres in the axial plane.

E: Any unwanted fibres were visually identified and removed by a “not” function ROI to finish (F).

**Figure 5.13.** Instructions for localising the ascending corpus callosal fibres in DTI Studio. A: Sagittal plane is moved marginally into the left hemisphere. B: Axial plane is risen to a location slightly superior to the top of the CC so that the ascending fibres (highlighted) can be seen. C: Using the “cut” function, an oval ROI is drawn around the CC in the sagittal plane. D: Using the “cut” function, a rectangular ROI is drawn around the ascending fibres in the axial plane. E: Any unwanted fibres were visually identified and removed by a “not” function ROI to finish (F).
5.4.1.3 Results: Control and patient tract shape and structure appeared broadly comparable with one another with no clear repeated differences. Findings from statistical analyses are shown in Table 5.3. From the Table, we see that FA is once again shown to be significantly higher in the patient group than in controls, although in this instance this increase appears to be due to decreased RD rather than increased AD. The negative regression between FA and VLF was also replicated, with a positive underlying regression between RD and VLF being shown to drive this.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Control Values</th>
<th>Patient Values</th>
<th>Control vs. Patient p value</th>
<th>Metric Regression Statistics</th>
<th>NART Regression Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td>Mean=0.3986 SD=0.0405</td>
<td>Mean=0.4309 SD=0.0498</td>
<td>0.002</td>
<td>T Value=-2.36 p=0.023</td>
<td>T Value=1.5 p=0.141</td>
</tr>
<tr>
<td>AD</td>
<td>Mean=0.001321 SD=0.000105</td>
<td>Mean=0.001295 SD=0.000085</td>
<td>0.234</td>
<td>T Value=0.91 p=0.37</td>
<td>T Value=2.83 p=0.007</td>
</tr>
<tr>
<td>RD</td>
<td>Mean=0.000687 SD=0.00009</td>
<td>Mean=0.000631 SD=0.00007</td>
<td>0.005</td>
<td>T Value=2.57 p=0.014</td>
<td>T Value=2.26 p=0.029</td>
</tr>
<tr>
<td>MD</td>
<td>Mean=0.000898 SD=0.00009</td>
<td>Mean=0.000853 SD=0.00007</td>
<td>0.018</td>
<td>T Value=2.15 p=0.037</td>
<td>T Value=0.133 p=0.01</td>
</tr>
</tbody>
</table>

5.4.1.4 Summary: DTI estimates tract structure based on the underlying diffusion tensors. While DTI has been shown to accurately replicate actual white matter tracts in the brain (Wakana et al., 2004), this type of estimation is imperfect as the calculation of the tract can be undermined by various other factors which also affect the diffusion tensor. For example the existence of two fibre bundles of perpendicular orientations in one location would result in a low anisotropy value despite there being strong fibre organisation. Previous research has also found that
the physical stress of the inertial forces involved in DAI is greater on axons which are oriented away from the direction of the main local fibre bundle (Cloots et al., 2011). If axons of one orientation are selectively damaged this would not only increase the local FA value but also mean create the potential of a patient / control difference in the same area being due to measurements from what are essentially two different tracts. Other research has also demonstrated how injuries such as TBI can lead to problems in tractography; Squarcina et al., (2011) showed how the often-expected reduction in FA which might follow TBI leads to tract estimation often failing in areas of high damage. Thus the analysis presented here aimed to resolve such possible confounds by visually localising the same tract in both experimental groups and replicating the metric changes as reported by TBSS.

Tracts appeared visually comparable between patient and control groups. Groupwise testing also replicated the main TBSS analysis for this region by comparing metric values from the same localised tract. Although the groupwise results are slightly inconsistent considering the finding of decreased RD, these findings for the most part repeated those of the main TBSS analysis for this region. Some deviations in the pattern of findings are reasonable considering the likelihood for error in tractographically defining the exact same region that the TBSS analysis had highlighted.

It should also be noted that the positive regression found in both analyses between RD and VLF possibly indicates a degree of decreased RD which may not have been substantial enough to show as a significant result in the TBSS analysis (discussed further in section 5.5.3) but becomes more detectable when more directly examining the tract in question. It should also be considered if a reduction in RD as reported here could be due to perpendicular fibres being destroyed; however on this point such an event would also be expected to lead to increase in AD in the same location which is counter to the findings here where AD is statistically unchanged but also descriptively decreased in patients. This disparity of AD findings between tractography and TBSS analyses suggests that the AD changes for this region in the TBSS analysis may have been confounded by additional changes other than tract structure. On balance it seems most reasonable to conclude that the tract structure of this location was
broadly comparable between patients and controls. Additionally, it can be concluded that the identity of the tract was indeed the ascending fibres of the corpus callosum.

5.4.2 Post-Hoc Examination of Mild and Moderate Patients

5.4.2.1 Aim: As a primary objective of this thesis is the examination of mild TBI, additional testing was conducted to examine if including 9 moderate patients with the 44 mild ones had skewed the data in any way which would undermine this.

5.4.2.2 Methodology: A TBSS analysis was conducted comparing all acute metric data between mild and moderate patients. This analysis was also repeated, but using the Mayo classification system for mild / moderate patients (Malec et al., 2007). In this way, many “mild” patients (as measured by their GCS score alone) were re-classified to “moderate” if they had any clinical imaging findings. This meant that 13 patients remained “mild” while the “moderate” contained the remaining 40. Finally, a further TBSS analysis was conducted to investigate if the main findings of the investigation would have been any different if only the mild patients (as measured by GCS) had been used. To do this, all main analyses were repeated using mild-only patient data.

5.4.2.3 Results: No areas of any significant difference were found in any metric when comparing mild and moderate patients classified by either the GCS or Mayo classification. It should be noted that this was the only analysis where the Mayo classification system was used to define patient severity; from this point on “mild” and “moderate” are once again defined by GCS score alone. Acute mild patient vs. control TBSS analysis revealed a comparable set of results where FA was increased (driven by increased AD) alongside some locations of increased MD (Figure 5.14 compared to the original analysis of the whole group shown in Figures 5.2 – 5.4).
Figure 5.14. Acute, groupwise TBSS outputs using only the mild patient data.
Chronic mild patient vs. control TBSS analysis did not reveal any findings at the p=0.05 level, however did show a comparable set of results where FA was decreased (driven by increased RD) and MD was increased when the significance threshold was reduced to p=0.15, as shown in Figure 5.15. As the group size for mild alone was now only 18 patients (compared to the whole chronic group), the lower significance is most likely due to reduced statistical power associated with the smaller sample size.

Figure 5.15. Chronic, groupwise TBSS outputs using only the mild patient data.
Acute TBSS analysis regressing VLF scores with mild patient metrics revealed a comparable set of results where FA negatively regressed with VLF (driven by a positive VLF / RD regression) and MD positive regressed with VLF (Figure 5.16).

**Figure 5.16.** Acute, TBSS regression outputs using only the mild patient data
Finally, chronic TBSS analysis regressing VLF scores with mild patient metrics also revealed a comparable set of results where a negative regression between FA and VLF remained in the anterior forceps (Figure 5.17).

5.4.2.4 Summary: Comparisons between mild and moderate patients by either GCS or Mayo severity classifications showed no differences, strongly suggesting that there is no underlying physiological difference which would have interfered with the main TBSS results. Additionally, repeating the main analyses using only the mild patients produced the same set of results aside from one (groupwise examination of mild patients at the chronic time-point) which required a reduction in alpha value which may be expected considering the reduced sample size. Including a minority of moderately injured patients has therefore not undermined the validity of investigating mild TBI.

5.4.3 Post-Hoc examination of Patients who did not return at Follow-up: Further analysis was conducted to investigate if there were any difference between the AFu and AL patient subgroups (i.e. the acute data of those patients who did and who did not return for follow-up testing). There were many analyses conducted in
order to do this, and so for the sake of clarity they are reported fully in Appendix B, but summarised here.

Preliminary testing involved conducting further TBSS analyses involving AFu vs. control comparisons (to see if the main findings of the study would remain the same if only those patients who returned at follow-up had been used) and AFu vs. AL comparisons. Initial differences were found wherein AFu vs. control TBSS comparisons did not fully replicate the findings earlier reported (section 5.3.1) when using all patients as a whole group. Further, a difference in FA was found when comparing the AFu group against the AL group, where higher FA was found in a small number of locations in the AL group. It was considered that a reduction in statistical power could have influenced these, and that being able to examine the actual spread of metric values in tract locations of interest (i.e. where FA was found to be increased in the AL group) would be beneficial.

Further analyses were therefore conducted. Average metric values from tract sections were extracted and tested, and a full examination of any differences in psychometric data between AFu and AL groups was conducted. Testing of the metric values indicated that the previously reported differences were likely due to sample size, and examination of the spread of data in the location where increased FA was reported in the AL group showed a very similar spread of data. It was concluded that the AFu and AL groups are likely not inherently different from one another, and that any differences which may exist will not have affected the findings of the study.

5.5 Discussion

This study found patterns of diffusion change following mild and moderate TBI compared to control subjects which varied in location and direction between the acute and chronic time point. FA was found to be raised acutely, but decreased chronically following TBI and with a different spatial distribution at each time, suggesting that diffusion changes evolve over time. The acute increase was largely driven by increased AD, while the chronic fall was driven by increased RD. Negative regressions between FA and VLF were also identified at both acute and follow-up times revealing a biological basis for the cognitive dysfunction. While the acutely increased FA could be
due to immune response factors, the chronic negative FA/VLF regression is suggestive of a lasting type of damage where cognitive deficit is driven by increased FA.

5.5.1 Acute Metric Changes: The observation of areas of acutely increased MD is consistent with the previous literature (e.g. (Inglese et al., 2005, Miles et al., 2008)). Widespread increases in FA were also found. Although this is an unusual finding in the wider neuroimaging literature, similar observations have been reported in a minority of other small scale imaging studies, particularly in acute, mild TBI patients (e.g. (Bazarian et al., 2007, Wilde et al., 2008, Chu et al., 2010, Mayer et al., 2010, Ling et al., 2012, Yallampalli et al., 2013)) and has been hypothesised to be due to the formation of oedema (Ling et al., 2012). However, both in experimental models and clinical studies in TBI, the presence of cytotoxic oedema is also associated with reduced MD (or its alternative metric: apparent diffusion coefficient (Marmarou et al., 2006, Ito et al., 1996)). In the current study MD was either unchanged, or increased. Additionally, our groupwise increases in FA were associated principally with elevated diffusion along the axon (increased AD), while RD was unchanged. In previous studies reporting increased FA, RD was typically reduced where reported (Mayer et al., 2010, Chu et al., 2010, Ling et al., 2012). Cytotoxic oedema therefore does not explain our observations and the lack of such findings is suggestive that it might not be present within this predominantly mild patient cohort.

One further possibility which was considered by post-hoc testing (Section 5.4.1) is selective destruction of specific fibres. For example, greater destruction of fibres of one orientation compared to another may induce an increase in FA. However the analysis presented concluded that tract structure appeared largely comparable between patients and controls, and for the most part also succeeded in repeating the TBSS results. Previous research has also demonstrated larger axons to be subject to greater strain during TBI (Jafari et al., 1997). Large axons would exhibit higher RD and lower FA. Destruction of these through this selective vulnerability could lead to areas populous with large axons seeing an FA rise following TBI as the relative proportion of intact, but smaller, FA-high axons increases. However, while either or both of these phenomenon could contribute towards an increase in FA, as they both focus on neuronal loss neither would be expected to resolve over time. The manner in which
areas which demonstrated an acute increase normalised at the chronic time-point would be troublesome to explain if these explanations were to be principally used.

Another acute factor which might influence an FA change is excitotoxicity. With this, increased excitatory amino acids following injury (predominantly glutamate (Bullock et al., 1998)) causes over-activation of neurons which leads to either necrosis or apoptosis (Zipfel et al., 2000). However such degradation or destruction of neurons would be considered to lower FA as they would cause a marked decrease in its structure, meaning excitotoxicity is also unlikely to be driving this finding. Research showing excitotoxicity to increase with the severity of injury (Palmer et al., 1993) is also suggestive that it might not be a prevalent injury within this mild/moderate patient cohort. Another possible explanation is increased temperature in the brain. The possibility of a temperature increase should also be considered. Higher temperature has been shown to increase reported diffusion levels in DTI metrics (Reischauer et al., 2009) and hyperthermia is a known effect to follow TBI (Jiang et al., 2002). This could cause AD to increase although leave RD relatively unaltered if the myelin sheath were to be intact at this phase. However hyperthermia is mainly found in severely injured patients and also associated with an outcome of moderate or severe disability as measured by the GOS (Geffroy et al., 2004). It is therefore unlikely to have occurred in this predominantly mild patient cohort. Additionally, this previous work shows that hyperthermia typically involves temperature increases in the range of 1-3°C (with higher figures indicating increasingly severe injuries) which is very unlikely to be statistically influential considering that a difference of 5°C is required to produce a discernible effect on metric values (Reischauer et al., 2009).

Another alternative explanation may lie in the findings of other recent work which has suggested astrogliosis as a possible cause of acute FA increases. Following injury, fibrous astrocytes often undergo reactive astrogliosis migrating to the site of injury, locally increasing the density of these cells (Pekny and Nilsson, 2005). It was reported that the organisation of these cells increased the AD within the affected tissue, while RD remained unchanged thus increasing the measured FA (Budde et al., 2011). Although MD was not reported, this increase in AD (without any change in RD) would also increase the reported MD. Considering this hypothesis, it is also possible that the lack of any AD change in the tractography analysis may be due to this process
being more detectable in TBSS as this includes more of the area that surrounds the tract. Our observations are therefore most consistent with astrogliosis and with the known chronology of the immune response following CNS injury (Mac Donald et al., 2007), and therefore we consider astrogliosis to be the most likely underlying cause of our finding of increased acute FA. Future research could expand to using the recent method of Diffusional Kurtosis in conjunction with DTI. Studies have shown this combination brings increased sensitivity in detecting cognitively-relevant physiological change (Grossman et al., 2012) and astrogliosis (Zhuo et al., 2012) compared to DTI alone.

5.5.2 Chronic Metric Changes: Follow-up data supported the wider literature in chronic brain injury with findings of reduced FA, although the affected tract locations were not the same as the locations where FA was seen to be increased acutely. Locations of acutely increased FA (e.g. the ascending fibres of the corpus callosum) had normalised by the follow-up time point and did not show a statistical difference compared to controls. Conversely, other locations (e.g. the anterior forceps) which did not show any significant change acutely progressed to reduced FA chronically. This indicates that though many different white matter tracts may be affected by DAI they are not subject to exactly the same injury / response pattern; locations which appear damaged acutely may appear normal chronically and while areas subject to long term damage may appear normal acutely. This finding emphasises the importance of controlling for time since injury in similar studies on TBI patients.

The FA decrease was driven by an underlying RD increase, while MD was increased extensively and AD was unchanged. Raised RD is evidence that the axonal membrane and myelin sheath have disintegrated (Song et al., 2002). The reductions in FA were observed principally in the anterior forceps, suggesting that this region has experienced the most severe long term damage following TBI. This finding is supported by the fact that anterior callosal fibres are known to be sensitive to DAI and damage to them relates to long term prognosis (Matsukawa et al., 2011). The normalisation of AD values further implies that its acute increase was due to a temporary factor such as mild astrogliosis (chronic scars are sometimes known to form following this, although only in severe cases (Sofroniew, 2009)). It should be noted that at both time-points locations of increased MD always overlapped with underlying increases of AD/RD.
Therefore, while MD is one of the simplest diffusion metrics to measure and may therefore have a role in the general detection of tissue injury, our data demonstrates that interpretation of altered MD requires full assessment of the diffusion eigenvectors.

5.5.3 Acute Cognitive Testing: Patients were found to underperform on the VLF task in the acute phase after injury with their performance strongly and negatively regressing with white matter FA and positively regressing with RD and MD. These regressions were located across widespread areas of the white matter tracts and particularly within the ascending fibres of the corpus callosum in the left hemisphere. This finding supports previous work which has shown the VLF network of healthy individuals to involve frontal, parietal and temporal locations with an emphasis in the left hemisphere (Gaillard et al., 2000, Pihlajamaki et al., 2000).

While the groupwise increase in FA was mainly due to increased AD, the relationship with cognitive performance was driven by a positive regression between RD and VLF which implies the presence of a subtle reduction in RD. The contrasting observation that RD was not significantly different in the acute injury phase between the patient and control groups in groupwise analysis, but reduced RD was associated with differences in functional performance between patients, is likely due to differing statistical power between tests. The variance of RD values explained by regression within the acute patient group is large whereas the difference in mean RD values between the two groups is small, making regression within groups more powerful than between group comparisons.

Within the axon, undamaged neurofilament has been shown to be related to both larger axon diameter and more efficient conduction velocity (Kriz et al., 2000), while DAI is associated with disruption of the neurofilament, involving loss of neurofilament sidearms via trauma-induced proteolysis (Maxwell et al., 1997). We therefore postulate that reduced axonal diameter may contribute to altering both diffusion properties (reducing RD and increasing FA) and to the deficit in VLF performance which becomes more severe as RD decreases.

5.5.4 Chronic Cognitive Testing: Chronically, patient performance on VLF was not significantly different to that of the controls, suggesting a degree of recovery in
terms of cognitive performance. However negative regressions between FA and VLF were still found. These chronic regressions presented primarily in the body of the corpus callosum and the anterior forceps as opposed to the more diffuse pattern seen acutely. This shift of location to connecting fibres between the two hemispheres could be indicative of network reorganisation to increase right hemisphere involvement as a compensatory mechanism, as has been demonstrated previously (Voets et al., 2006). Further future work is needed to investigate whether this is the case.

Examining AD and RD did not provide evidence that either was specifically responsible for the chronic relationship between performance and FA, implying that relatively slight changes in each are contributing to this finding. The finding that decreased FA was associated with better VLF performance in the chronic phase is one which is challenging to explain due to its apparent contradiction to counterpart groupwise findings in the same tract location which indicate FA to decrease as a result of damage, and also its relative novelty with respect to the wider literature. Many events are known to occur in the chronic phase of injury such as a relative increase in the proportion of axons with smaller diameters (Jafari et al., 1997), glial scarring (Stichel and Muller, 1998) and network reorganisation (Voets et al., 2006), all of which may contribute to an effect whereby the patients with the greatest VLF deficit have the highest FA, despite FA being generally reduced compared to controls. Further investigations are required to determine the exact mechanisms behind this finding, but it is highlighted as an avenue for future research.

5.5.5 Limitations and Considerations of Post-Hoc Analyses: The loss of patients to follow-up reduced our statistical power to detect longitudinal change. Patients either declined to return for further testing (4/53) or were lost to further contact (26/53). We attribute these losses to the mild nature of our participant’s injuries (possibly making them less inclined to devote further time to the study), and the relatively long (1 year) gap between assessments. It was considered whether those patients returning at 12 months were either likely to suffer greater on-going symptoms than those who did not return, or be influenced by socio-economic or medical-legal factors (whereby a potential diagnosis of long-term damage or on-going cognitive dysfunction may be seen as advantageous). This type of patient self-selection is difficult to quantify and cannot be ruled out in this cohort. Initial TBSS analyses
comparing the acute data between patients who did and did not return produced findings of FA changes which were suggestive that there may indeed be a physiological difference between these sub-groups (section 5.4.3 and Appendix B). However further analysis utilising ROI’s concluded that this was likely an artefact of diminished statistical power. Evaluation of differences in cognitive performance at baseline between these sub-groups showed a difference in VLF, but not in any other task. This indicating that each of these sub-groups were suffering from broadly comparable levels of cognitive impairment at baseline, and that as the AFu group was found to outperform the AL group in VLF that the main findings would if anything be an underestimation of the changes experienced in mild TBI.

A proportion of our patient group were also unable to complete psychometric testing due to reasons pertaining to their injury (12% acutely and 9% at follow-up), reducing the sample size for the regression analyses. Since our hypothesis was that cognitive dysfunction is secondary to microstructural damage, those patients who were unable to complete the tests would be expected to have the greatest microstructural damage. Excluding such individuals would then bias against detecting an effect, again suggesting that our observations are a lower estimate of the importance of white matter injury on cognitive performance following mild TBI.

Age was also not controlled for in these analyses; FA is known to decrease in a number of areas as people get older (Pfefferbaum et al., 2000). Previous demographic testing revealed controls to be significantly older than the acute mild patient sample, and all acute patients considered together as a group (Section 3.3.5). It should therefore be considered that this could have influenced the finding of increased FA in the acute patient sample compared to controls. However it should also be noted that the returning patient sample was not significantly different in age compared to controls. If age had contributed to FA changes, this would therefore be expected to be seen in comparing the patients who did and who did not return for follow-up testing. As discussed, testing of these groups concluded that they were comparable to one another. It is therefore unlikely that age differences drove the increased FA finding.

Finally, our mixed mild and moderate cohort is a potential confound for understanding the effect of mild TBI alone. Comparison of the patient groups (mild
versus moderate) did not show any significant differences in any diffusion metric either by GCS or Mayo severity classification. Further, in comparison to healthy subjects and during regression against VLF performance, exclusion of the moderate patients did not change the direction or distribution of any findings, (other than a reduction in statistical power expected with the smaller group size). Finally, only 2/9 moderate patients had GCS less than 10, so this moderate group is towards the milder end of injury. The findings are therefore consistent with revealing the microstructural changes associated with mild injury severity.

5.6 Chapter Summary

This longitudinal study has produced a comprehensive picture of the diffusion imaging changes following mild/moderate TBI and how these can relate to cognition in a large cohort of patients. Detailed analysis of the complete set of diffusion metrics suggest that gliosis rather than cytotoxic oedema is most consistent with changes in these metrics following acute mild TBI. We have also further quantified acute findings by identifying a proportional relationship with VLF which we have shown persists into the chronic injury phase, at a more subtle but still detectable level. The importance of investigating the component parts of FA is also shown by groupwise / regression findings in the acute phase that would have proved conflicting had they not been shown to be separately driven by AD and RD, respectively. The full potential of DTI to detect different physiological changes resulting from TBI, and to show which among these is affecting cognition, is highlighted while avenues for future research are also indicated. As such, the hypotheses of increased FA indicating cognitive relevant damage at the acute time-point are supported. However, while the hypothesis that metric changes at the chronic time-point would appear more “typical” with respect to the wider literature is also validated, the ongoing negative relationship between FA and VLF at this point is contrary to the expectation that relationships with cognitive outcome would also support the groupwise finding of decreased FA being an indicator of damage.
Chapter 6. Magnetic Resonance Spectroscopy Investigation

6.1 Overview

This chapter describes a, longitudinal, ROI-based MRS investigation on the same participant group introduced in Chapter 3 and studied in Chapters 4 and 5. Here, metabolic changes post-TBI are characterised at both the acute and chronic time-points. Relationships between metabolite concentration and cognitive function are also examined. The ROI’s chosen are a mix of locations selected based either on previous published research, or shown to be of particular relevance in this patient group from findings using DTI in Chapter 5. We hypothesised that metabolite concentration may act as a marker of DAI, and also hold relationships with the observed cognitive deficit described in Chapter 3.

6.2 Methodology

6.2.1 MRS Data Acquisition: This experiment utilised 3D T1W scan data (previously described in 4.2.1) and MRS scan data. The MRS sequence used a slice selective short-TE MRSI technique with a TR of 3450ms, TE of 35ms, 10x10x18mm voxels (24x20 matrix, 5 slices, 18mm thick, 1 average, 1024 samples and a spectral bandwidth of 2500 Hz) with outer volume saturation. The total scan duration for MRS acquisition was 21 minutes.

6.2.2 Regions Investigated: Several thousand voxels were collected in each subject. Due to a lack of standard global investigative techniques for MRS data, this created the need to focus the analysis. For this reason, five regions were chosen to be investigated. These were the ascending fibres of the corpus callosum, the caudate, the putamen, the thalamus and the posterior cingulate cortex. These specific locations were chosen for a variety of reasons. They are all relatively large structures / areas, meaning they are practical in terms of voxel placement. Together, they also form a variety of white matter, and cortical / deep grey matter regions that are mostly normal-appearing by standard imaging techniques in this patient group (section 4.3). This was deemed appropriate as findings from the DTI experiment in Chapter 5 indicate the presence of diffuse damage, which we seek to investigate further. Previous literature has also indicated each of these locations as being of interest following TBI, as explained below.
A region of superior white matter (SWM) was first chosen in an attempt to best capture the ascending fibres of the corpus callosum, based on DTI findings from Chapter 5. These results indicated that this tract holds importance in this patient cohort with respect to both relatively novel metric changes in the acute phase of injury (where patient FA was found to be raised) and cognitive functioning in both the acute and chronic phases of injury (where negative regressions with VLF performance were found). Experimentation here on this region is therefore intended as a natural follow-up to this previous work. The voxel is best placed to capture these fibres, although likely contains a higher proportion of other white matter which is not a part of this tract.

The caudate has been shown to be subject to atrophy following TBI (Kim et al., 2008) and to be related to some cognitive facets regarding risk taking (Newcombe et al., 2011) (although this is not something directly measured by the psychometric test battery used in this project, a proved relationship with cognitive function was deemed worthy of further examination). BOLD signal changes have also been detected in the area in fMRI experiments following mild TBI (Gosselin et al., 2011), further showing the vulnerability of the region to the effects of injury.

The putamen has also been shown to be subject to atrophy following TBI (Warner et al., 2010, Sidaros et al., 2009). Although current literature does not report links with cognitive deficit post-TBI, altered BOLD signals are also found in the putamen after mild TBI (Gosselin et al., 2011), implying a possible link with cognitive outcome.

Cognitively-relevant alterations in the thalamus following TBI have been found (Grossman et al., 2012), with one paper suggesting that the resulting integrity of the thalamic tract plays a key role in any executive dysfunction which may be present (Little et al., 2010). Other work has found the thalamus to be subject to decreased metabolic rates of glucose after injury (Hattori et al., 2003). While one MRS paper has found no significant thalamic metabolic alterations in a mild patient cohort (Kirov et al., 2007), it was still deemed appropriate to include this area as an ROI on the balance of the other previous findings. The current investigation also has a substantially larger group of patients than this previous study (53 compared to 20), giving us a greater sensitivity to detect change.
Finally, the posterior cingulate cortex (PCC) was chosen so that a cortical grey matter structure could also be examined. The PCC has been shown to be vulnerable to structural change following DAI (Kim et al., 2008). The PCC is known to be a central aspect of the brain’s default mode network, and has been previously found to be affected by TBI such that it is not as effectively inhibited during active tasks after injury (Bonnelle et al., 2012). Other work which has produced similar findings has suggested that it may play a central role in cognitive recovery due to reported increased connectivity following TBI which also correlated with faster cognitive response times (Sharp et al., 2011).

6.2.3 Voxel Placement: Voxels were placed bilaterally for each of these regions aside from the PCC which used a single voxel on the brains midline. A total of nine voxels were therefore examined per participant. The locations of the five regions are demonstrated in Figures 6.1 – 6.5. The placement of these was achieved by visual inspection of the participants T$_1$ weighted anatomical scan in MRIcro which was matched to a corresponding grid of MRS voxels. Determining the desired location on the T$_1$W scan therefore also identified the MRS voxel which occupied the same space. However, there was often wasn’t a single corresponding voxel which clearly represented the desired region. In these cases, a choice between possible voxels had to be made based on which gave the best representation by a combination of either / both including a substantial portion of the desired region and containing minimal contamination from neighbouring neural areas.
Defining the region around the ascending fibres of the corpus callosum involved first identifying the centre of the corpus callosum by examining a sagittal slice down the midline of the brain. Maintaining this Y co-ordinate, the points of white matter that were immediately superior and lateral to either side of the body of the corpus callosum were then located as shown. Voxels were taken bilaterally.

**Figure 6.1:** Instructions with visual example pertaining to the placement of the voxels for superior white matter. Image overlay shows ideal voxel locations.
The caudate was located mainly by examination of the axial view of the T1W scan where the margins of the nucleus are most clear. Voxels were taken bilaterally.

**Figure 6.2:** Instructions with visual example pertaining to the placement of the voxels for the caudate. Image overlay shows ideal voxel locations.
The Putamen

The putamen was also located mainly by examination of the axial view of the T₁W scan, although the boundaries can also be seen in the coronal and sagittal views. Voxels were taken bilaterally.

**Figure 6.3**: Instructions with visual example pertaining to the placement of the voxels for the putamen. Image overlay shows ideal voxel locations.
The Thalamus

Being a large structure, voxel placement within the thalamus was relatively uncomplicated. Regions to the posterior of it were often chosen in an effort to avoid contamination from the putamen and caudate. Voxels were taken bilaterally.

**Figure 6.4**: Instructions with visual example pertaining to the placement of the voxels for the thalamus. Image overlay shows ideal voxel locations.
A unilateral grey matter region to the anterior of the occipital lobe was chosen for the final voxel. This was on the same superior / inferior plane as the corpus callosum and as close to the splenium / forceps major as possible without any contamination. As this was on the brains midline, many voxels fell either to one side or the other. In cases where one was not clearly more central then the other, the voxel to the right of the image (left of the brain) was chosen.

**Figure 6.5:** Instructions with visual example pertaining to the placement of the voxels for the posterior cingulate cortex. Image overlay shows ideal voxel location.
**6.2.4 MRS Data Treatment:** Spectroscopic analysis was performed off-line using the automated, standard evaluation software jMRUI. For visual display, data were pre-processed with 5 Hz exponential line broadening, Fourier transformed and manually phase corrected. The residual water peak was filtered using a Hankel Lanczos Singular Values Decomposition Filter (HLSVD). MRS data were then evaluated in the time domain using the QUEST algorithm which was used to fit a weighted combination of metabolite signals directly to the in vivo spectroscopic data. The algorithm uses prior knowledge based on a metabolite basis set of known concentrations measured in vitro. Results were inspected for quality by consideration of factors such as line width and residual signal, and discarded if they were not of a suitable standard. Peak areas of NAA, Cho and Cr were obtained.

It is standard practice in spectroscopic analysis to make inferences about changes in metabolite concentration not by directly examining that metabolite, but by creating a ratio of that metabolite with something else which is assumed to be experimentally stable. This is done to correct for signal decay caused by different parts of the brain being different distances from the head coil. As the Cre peak is assumed to be stable, it is therefore common in TBI research to see NAA and Cho concentrations investigated through NAA/Cre and Cho/Cre ratios (e.g. (Garnett et al., 2000a, Garnett et al., 2000b, Holshouser et al., 2006, Signoretti et al., 2008, Vagnozzi et al., 2010)). However recent evidence has suggested that Cre may be subject to change following TBI, or related to severity or outcome (Walz et al., 2008, Gasparovic et al., 2009, Yeo et al., 2011). Due to this, the current work aimed to avoid using the metabolite as a stable reference point. Similar to one of these previously cited experiments (Gasparovic et al., 2009), water concentration was used instead as a stable reference point. NAA, Cho and Cre peak amplitudes were therefore divided by the water content of the same space (as measured by the proton density of that location) to create comparable ratios.

MRS metabolite levels were normalised to the water signal determined from a proton density image available in each subject. To achieve this, the proton density was first registered into the same space as the corresponding T$_1$W image using FSL. A matlab script was then created which extracted the average of the proton density image voxels which occupied the same area as each MRS voxel. As these values were in the magnitude of hundreds-of-thousands, they were divided by 100,000 in order to...
make the data more reasonable to experiment with. The metabolic peak amplitudes were then divided by these transformed proton density values for the same location. These final figures were used for all subsequent statistical analyses. Although these final values are technically still arbitrary units and not actual metabolite concentration (in order to achieve this, other considerations such as the number of protons in each molecule would need to be dealt with), they are still proportional to concentration and therefore appropriate to conduct analyses with.

**6.2.5 Statistical Analyses:** Final values of NAA, Cho and Cre were subjected to multiple statistical comparisons. Groupwise testing primarily involved comparing patient and control metric values at each time point, but also involved testing with acute patient sub-groups to examine for differences between mild and moderate patients and those patients who did (AFu) and did not return (AL) for follow-up testing.

Testing with psychometric data involved examining correlations with key psychometric test measures in patient (all acute / all follow-up) groups, with each group being correlated with the corresponding performance data (i.e. acute patient metabolic data with acute patient performance / returning patient metabolic data with returning patient performance). The rationale behind these are described in detail in section 6.3.8, prior to the cognitive findings. Post-hoc analyses were also conducted, the methods and rationale of which are described in section 6.4.

At each stage of testing data was subjected to the Anderson-Darling test for normality to determine the types of analyses to be used. For metabolic concentrations, control values were examined for each metabolite in each ROI as an indicator of which test to use for groupwise testing against corresponding acute / returning patient values. Non-parametric correlational analyses for cognitive testing were used if either the corresponding control metabolic data for a given metabolite / region or the control distribution of test scores for the psychometric task being tested against (section 3.6.2) were found to be non-parametric. As before, if data was found to be normally distributed then t-tests were used for groupwise testing while Pearsons R was used for correlations. If data was found to not be normal then the Mann-Whitney U test was used for groupwise testing while Spearmans Rho was used for correlations.
6.3 Results

6.3.1 Typical Spectra and Sample Size Differences due to discarded Spectra:

Typical MRS spectra for patients and controls are shown below in Figure 6.6.

Spectra such as these were considered to be good quality (judging by factors such as fitting, symmetry etc.), and are shown here as an example of the standard which all spectra were measured against. Linewidth was also examined and the data for any metabolite was discarded if the accompanying peak had a linewidth above 40Hz (qualitatively speaking, very few of the spectra which were kept were close to this boundary, and most appeared to be within the range of 20Hz +/- 5Hz). A number of spectra in each ROI did not meet these standards and were discarded. The author noted a pattern wherein a given participant would typically produce acceptable or
Table 6.1: Sample sizes for metabolic data for each participant group. Some data was lost due to spectra being of insufficient quality. Control sample sizes are reduced from a possible total of 33, mild sub-groups are reduced from 44 and 18 (acute and chronic time-points, respectively) while moderate sub-groups are reduced from 9 and 5 (acute and chronic time-points, respectively).

<table>
<thead>
<tr>
<th>Region</th>
<th>Control N</th>
<th>Acute Patient N (mild / moderate)</th>
<th>Returning Patient N (mild / moderate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior White Matter</td>
<td>Left</td>
<td>28</td>
<td>35 / 7</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>28</td>
<td>35 / 7</td>
</tr>
<tr>
<td>Caudate</td>
<td>Left</td>
<td>27</td>
<td>35 / 8</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>27</td>
<td>36 / 9</td>
</tr>
<tr>
<td>Putamen</td>
<td>Left</td>
<td>26</td>
<td>37 / 9</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>26</td>
<td>37 / 9</td>
</tr>
<tr>
<td>Thalamus</td>
<td>Left</td>
<td>24</td>
<td>37 / 9</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>25</td>
<td>38 / 9</td>
</tr>
<tr>
<td>PCC</td>
<td>-</td>
<td>26</td>
<td>38 / 9</td>
</tr>
</tbody>
</table>

Similar to findings shown in Chapter 3, the following pages include a number of box plots. Once again, an asterisk above or below a box-plot does not indicate significant but instead shows outliers in the data (counted as any values which are 1.5 times (or more) outside of the interquartile range). Significance is indicated by arrows which run between the box-plots of the two groups who the significant difference refers to (solid in the case of a sig. difference involving acute patient data, and dashed in the case of a sig. difference involving follow-up patient data). In this case, testing was only conducted using t-test or Mann Whitney U analysis.
6.3.2 Metabolic Findings in the Superior White Matter: Testing for normality on control data showed that all metabolic concentrations were normally distributed except for left hemispheric Cre ($p$<0.005).

At the acute stage of injury, groupwise testing revealed patients to have a number of significantly different metabolic concentrations to controls. NAA and Cre were each increased in patients in each hemisphere of the brain. However this was no longer the case at follow-up, where no differences were found between patients and controls. These groupwise results are shown in Table 6.2, and visualised in Figures 6.7-6.9.

<table>
<thead>
<tr>
<th>Time-point</th>
<th>Metabolite</th>
<th>Left Hemisphere (Control, Patient, $p$ value)</th>
<th>Right Hemisphere (Control, Patient, $p$ value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>NAA</td>
<td>0.1118±0.0217/0.1446±0.0465 (&lt;0.001)</td>
<td>0.117±0.0236/0.1515±0.0394 (&lt;0.001)</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.01317±0.00482/0.01468±0.00532 (0.223)</td>
<td>0.01428±0.00428/0.01563±0.00535 (0.247)</td>
</tr>
<tr>
<td></td>
<td>Cre</td>
<td>Median=0.07989/Median=0.11702 (&lt;0.001)</td>
<td>0.0938±0.0247/0.1175±0.0372 (0.002)</td>
</tr>
<tr>
<td>Chronic</td>
<td>NAA</td>
<td>0.1189±0.0272/0.357</td>
<td>0.1228±0.0304/0.513</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.01298±0.00426/0.893</td>
<td>0.01408±0.00436/0.885</td>
</tr>
<tr>
<td></td>
<td>Cre</td>
<td>Median=0.08174/0.698</td>
<td>0.1002±0.0288/0.462</td>
</tr>
</tbody>
</table>

Table 6.2: Results from groupwise testing comparing metabolic concentrations between controls and acute patients / returning patients in the superior white matter. Metabolite/water ratio values are given as “mean±SD” unless otherwise stated in the cell (this is dependent on the statistical test for that particular comparison being parametric or non-parametric).
**Figure 6.7**: Boxplot showing NAA distributions in the superior white matter in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.

**Figure 6.8**: Boxplot showing Cho distributions in the superior white matter in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.
**Figure 6.9:** Boxplot showing Cre distributions in the superior white matter in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.
6.3.3 Metabolic Findings in the Caudate: Testing for normality on control data showed that all metabolic concentrations were normally distributed except for right hemispheric Cre ($p=0.049$) and left hemispheric NAA ($p=0.033$).

At the acute stage of injury, groupwise testing revealed patients to have a number of significantly different metabolic concentrations to controls. NAA was increased in patients in the right hemisphere, while Cho and Cre were each increased in patients in the left hemisphere. However this was no longer the case at follow-up, where no differences were found between patients and controls. These groupwise results are shown in Table 6.3, and visualised in Figures 6.10-6.12.

<table>
<thead>
<tr>
<th>Time-point</th>
<th>Metabolite</th>
<th>Left Hemisphere (Control, Patient, p value)</th>
<th>Right Hemisphere (Control, Patient, p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>NAA</td>
<td>Median=0.08123, Median=0.08625, 0.317</td>
<td>0.0782±0.0226, 0.915±0.0292, <strong>0.034</strong></td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.01677±0.0035, 0.01927±0.0066, <strong>0.043</strong></td>
<td>0.0166±0.00549, 0.01886±0.00568, 0.101</td>
</tr>
<tr>
<td></td>
<td>Cre</td>
<td>0.0715±0.174, 0.0842±0.0278, <strong>0.021</strong></td>
<td>Median=0.06673, Median=0.07633, 0.091</td>
</tr>
<tr>
<td>Chronic</td>
<td>NAA</td>
<td>Median=0.09307, 0.583</td>
<td>0.0838±0.0228, 0.416</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.0156±0.00609, 0.53</td>
<td>0.0131±0.0061, <strong>0.085</strong></td>
</tr>
<tr>
<td></td>
<td>Cre</td>
<td>0.083±0.0336, 0.266</td>
<td>Median=0.07847, 0.303</td>
</tr>
</tbody>
</table>

Table 6.3: Results from groupwise testing comparing metabolic concentrations between controls and acute patients / returning patients in the caudate. Metabolite/water ratio values are given as “mean±SD” unless otherwise stated in the cell (this is dependent on the statistical test for that particular comparison being parametric or non-parametric).
**Figure 6.10**: Boxplot showing NAA distributions in the caudate in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.

**Figure 6.11**: Boxplot showing Cho distributions in the caudate in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.
Figure 6.12: Boxplot showing Cre distributions in the caudate in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.
6.3.4 Metabolic Findings in the Putamen: Testing for normality on control data showed that only left hemispheric Cho was normally distributed. All other metabolites were non-parametric (left / right NAA p values; 0.006 / <0.005, right Cho p value; <0.005, left / right Cre p values; 0.012, <0.005).

At the acute stage of injury, groupwise testing revealed patients to have a number of significantly different metabolic concentrations to controls. NAA and Cre were each bilaterally increased in patients, while Cho was also increased in the left hemisphere of the brain. At the follow-up time-point, NAA was still increased in the left hemisphere (although it had normalised in the right) while Cre was still increased bilaterally. Additionally, while Cho had normalised in the left hemisphere, it was now decreased in the right hemisphere. These groupwise results are shown in Table 6.4, and visualised in Figures 6.13-6.15.

Table 6.4: Results from groupwise testing comparing metabolic concentrations between controls and acute patients / returning patients in the putamen. Metabolite/water ratio values are given as “mean±SD” unless otherwise stated in the cell (this is dependent on the statistical test for that particular comparison being parametric or non-parametric).

<table>
<thead>
<tr>
<th>Time-point</th>
<th>Metabolite</th>
<th>Left Hemisphere (Control, Patient, p value)</th>
<th>Right Hemisphere (Control, Patient, p value)</th>
</tr>
</thead>
</table>
| Acute      | NAA        | Median=0.08987  
  Median=0.10831  
  **0.014**  
  Median=0.09068  
  Median=0.1186  
  **0.003**  | |
|            | Cho        | 0.01673±0.0041  
  0.02168±0.00574  
  **<0.001**  
  Median=0.01707  
  Median=0.02041  
  **0.052**  | Median=0.01707  
  Median=0.02041  
  **0.052**  |
|            | Cre        | Median=0.07163  
  Median=0.09311  
  **0.005**  | Median=0.07305  
  Median=0.0973  
  **0.026**  |
| Chronic    | NAA        | Median=0.10519  
  **0.019**  | Median=0.09967  
  0.169  |
|            | Cho        | 0.01376±0.0043  
  **0.05**  | Median=0.01351  
  **<0.001**  |
|            | Cre        | Median=0.1014  
  **0.005**  | Median=0.1001  
  **0.021**  |
**Figure 6.13:** Boxplot showing NAA distributions in the putamen in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.

**Figure 6.14:** Boxplot showing Cho distributions in the putamen in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.
Figure 6.15: Boxplot showing Cre distributions in the putamen in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.
6.3.5 Metabolic Findings in the Thalamus: Testing for normality on Control data showed that all metabolic concentrations were normally distributed.

Groupwise testing revealed patients to have a number of significantly different metabolic concentrations to controls. At the acute stage of injury, NAA was increased in patients in the right hemisphere. This was still the case at the follow-up time-point, where left hemispheric NAA was also now found to be increased. These groupwise results are shown in Table 6.5, and visualised in Figures 6.16-6.18.

<table>
<thead>
<tr>
<th>Time-point</th>
<th>Metabolite</th>
<th>Left Hemisphere (Control, Patient, p value)</th>
<th>Right Hemisphere (Control, Patient, p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>NAA</td>
<td>0.0934±0.0291 0.1001±0.0234 0.336</td>
<td>0.0937±0.0255 0.1092±0.0313 0.027</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.01607±0.00538 0.01876±0.00549 0.056</td>
<td>0.01728±0.0056 0.01913±0.00632 0.208</td>
</tr>
<tr>
<td></td>
<td>Cre</td>
<td>0.0645±0.0201 0.0725±0.0195 0.119</td>
<td>0.0714±0.0279 0.0808±0.0243 0.16</td>
</tr>
<tr>
<td>Chronic</td>
<td>NAA</td>
<td>0.133±0.0461 0.005</td>
<td>0.1291±0.051 0.012</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.01672±0.00935 0.797</td>
<td>0.01727±0.00787 0.997</td>
</tr>
<tr>
<td></td>
<td>Cre</td>
<td>0.0869±0.0474 0.081</td>
<td>0.0942±0.0441 0.064</td>
</tr>
</tbody>
</table>

Table 6.5: Results from groupwise testing comparing metabolic concentrations between controls and acute patients / returning patients in the thalamus. Metabolite/water ratio values are given as “mean±SD” unless otherwise stated in the cell (this is dependent on the statistical test for that particular comparison being parametric or non-parametric).
**Figure 6.16**: Boxplot showing NAA distributions in the thalamus in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.

**Figure 6.17**: Boxplot showing Cho distributions in the thalamus in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.
**Figure 6.18**: Boxplot showing Cre distributions in the thalamus in each hemisphere to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.
**6.3.6 Metabolic Testing in the PCC:** Testing for normality on Control data showed that all metabolic concentrations were normally distributed.

Groupwise testing revealed patients to have a number of significantly different metabolic concentrations to controls. At the acute stage of injury, Cho and Cre were increased in patients. While Cre remained increased at the follow-up time-point, Cho became decreased. These groupwise results are shown in Table 6.6, and visualised in Figures 6.19-6.21.

<table>
<thead>
<tr>
<th>Time-point</th>
<th>Metabolite</th>
<th>Control, Patient, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>NAA</td>
<td>0.0639±0.0135, 0.0723±0.0246, 0.065</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.00788±0.00188, 0.00999±0.00374, 0.002</td>
</tr>
<tr>
<td></td>
<td>Cre</td>
<td>0.0456±0.0132, 0.0589±0.0186, 0.001</td>
</tr>
<tr>
<td>Chronic</td>
<td>NAA</td>
<td>0.0696±0.0135, 0.176</td>
</tr>
<tr>
<td></td>
<td>Cho</td>
<td>0.00603±0.00195, 0.004</td>
</tr>
<tr>
<td></td>
<td>Cre</td>
<td>0.059±0.0145, 0.005</td>
</tr>
</tbody>
</table>

*Table 6.6:* Results from groupwise testing comparing metabolic concentrations between controls and acute patients / returning patients in the PCC. Descriptive values are given as “mean±SD” unless otherwise stated in the cell (this is dependent on the statistical test for that particular comparison being parametric or non-parametric).
Figure 6.19: Boxplot showing NAA distributions in the PCC to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.

Figure 6.20: Boxplot showing Cho distributions in the PCC to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.
Figure 6.21: Boxplot showing Cre distributions in the PCC to highlight the differences between patient and control values. Bold arrows indicate when a difference is significant.
6.3.7 Metabolic Findings Summaries: Table 6.7, below, shows the directions of metabolic changes (if any) in each voxel in the patients compared to controls.

<table>
<thead>
<tr>
<th>Voxel</th>
<th>Acute Time-point</th>
<th>Follow-up Time-point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAA</td>
<td>Cho</td>
</tr>
<tr>
<td>Superior White Matter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Left</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Caudate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Left</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>Putamen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Left</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Left</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PCC</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

6.3.8 Note on Multiple Comparisons Problem with Cognitive Data: While the nature of Chapter 5’s DTI investigation made it difficult to investigate more than one cognitive test (due to the complexity of conducting and reporting multiple TBSS regression investigations), examining correlations between cognitive and metabolic data here is far less restrictive and presented an opportunity to create many comparisons. We argue that the nature of DAI means making such broader comparisons is appropriate; as DAI affects many cognitive networks, it also affects the patient’s cognition in a very broad manner. This is evidenced by the many locations of damaged white matter tract in our patient group which were found in the previous chapter (section 5.3), and the number of tests which our patient group were shown to hold an acute deficit in (section 6.3.2).

There was therefore a motivation to examine correlations between numerous cognitive tests and numerous metabolites / ROIs, as focusing on only a specific comparison would likely mean ignoring a great number of other relationships which are equally important and valid. However this would of course create a number of Type 1 errors and there was concern that traditional multiple-comparison correction
methods would simply eliminate all findings due to a very high total number of comparisons. For example, a set of correlations which compared all key data in each main participant group (controls and acute / chronic patients) would lead to 1215 individual $p$-values, bonferroni correction of which would create an unrealistically-achievable new alpha value of 0.00004. In considering how to conduct the cognitive investigation of the MRS data it was therefore necessary to reach a compromise where the comparisons made were focused, but at the same time did not undermine the project’s broader objective of investigating DAI and its cognitive consequences.

It was first decided that only a subset of cognitive tests from the full battery should be used. This subset was chosen so that it focused tests where patients have been shown to hold a deficit, but as a whole still measured a wide variety of cognitive domains. VLF was retained due to the findings of Chapters 3 and 5, which indicated (some) our patients to hold a deficit in it and for it to be of relevance to their neurophysiological changes, respectively. The A1-A5 and A6 conditions of the List Learning task were also retained. This was again partially because patients had been shown to hold a deficit in these (Table 3.6), but also as the task purports to measure a large number of cognitive facets (Table 3.4). List Learning was chosen instead of the comparable Design Learning as testing had shown our patients to hold deficits in more of its conditions (Table 3.6). Finally, PASAT (3 second intervals) was chosen. Patients had been shown to hold a deficit in this task (Table 3.6) and it also measures “information processing” which is a major cognitive facet not specifically examined by either VLF or List Learning (Table 3.4). PASAT (3 second intervals) was chosen instead of other tasks in the battery which measure information processing (PASAT with 2 second intervals and SoIP) as patients were shown to comparably hold the greatest deficit in it. Together, this small subset of tasks measure clustering, switching, verbal learning, attention, concentration, short-term memory, working memory and information processing.

Secondly, consideration was given to metabolites, where it was decided that all 3 should be retained for testing. This decision was made firstly as each metabolite was seen to change in a number of locations in earlier groupwise analysis (Table 6.7), indicating each of them to be experimentally important in these patients. Secondly, there is a unique value to investigating each of NAA, Cho and Cre. The uses and
implications of investigating NAA and Cho concentration data in a TBI setting (neuronal viability/mitochondrial activity and membrane inflammation respectively) are distinct from one another and each infer upon important physiological processes which could indicate / act as a marker for cognitive function. Equally, while the implications of Cre concentration could be considered comparable to NAA (each are hypothetically related to local energetic output), the experimental study of it is relatively novel. As groupwise changes in Cre were also widely observed in it in earlier testing this indicates that it is affected by injury in our patient group and therefore is of interest to study further with regards to cognitive outcome. Thus, with none of the metabolites appearing relatively redundant with regards to cognitive testing it was deemed inappropriate to eliminate any at this stage.

Regarding selecting ROI’s it was also decided that all should be retained. This decision was made based upon our argument that DAI represents a single phenomenon which affects regions in a diffuse pattern. Therefore, the inclusion of multiple ROIs is a valid method of investigating this single variable (particularly in the case here where each ROI was chosen based upon an a-priori hypothesis) and not comparable to a case where the inclusion of multiple regions represents testing of multiple variables. Thus, methods of multiple comparison correction are not appropriate in this context.

As the above methods of selection would still lead to a relatively large number of independent comparisons, an informed approach was used to discard findings as a method of correction. The following method was chosen instead of a standard method of multiple-comparison correction as there was still strong concern that using a technique such as bonferroni would still be too stringent such that Type 1 errors would simply be replaced by Type 2 errors. It was decided that a relationship (where \( p < 0.05 \)) may only remain if it was repeated at least once in a different voxel. E.g. if NAA was found to positively correlate with VLF in acute patients in the left caudate, that finding may only be kept if another positive correlation (\( p < 0.05 \)) between NAA / VLF in the acute patient group was recorded in another voxel. This was based upon the logic that a repeated finding in this manner is strong support that that specific correlation represents a “real” effect; a relationship between a metabolic change and cognitive function as a result of DAI may be expected to be present in various regions (examining
TBSS outputs from Chapter 5, e.g. Figures 5.2-5.4 show this). Conversely there is no reason that a significant-\textit{by-chance} finding would be repeated in this manner. It should be noted that as the chronic TBSS findings (Figure 5.5) suggested that a significant relationship between cognition and physiology may exist in a confined location there is still a reasonable risk of the creation of Type 2 errors from this method. However we argue that on balance this is a reasonable compromise which will allow only for the detection of “real” relationships.

Finally, only the main patient groups (all acute patients and all chronic patients) were subject to the above testing. However, any \textit{specific} correlations which were found to be significant in these groups were repeated in the control group. i.e. the same metabolite / test relationships, in the same ROIs. This was in order to examine if the significant findings were as a result of injury or not. The following bullet points summarise the above paragraphs.

- Only VLF, Letter Fluency (A1-A5 and A6 conditions) and PASAT (3 second conditions) were used for testing.
- All metabolites were investigated as each represents a measurement with \textit{unique} potential implications for cognitive outcome.
- All ROI’s were investigated as we argue they together are more comparable to measuring a single variable (DAI) to measuring multiple variables which are independent of one another.
- Correlations were only be retained if that specific cognitive test / metabolite relationship is found to be significant ($p<0.05$) in at least two of the ROIs.
- These correlations were examined only in the acute and chronic patient groups, although any significant findings (i.e. which are reported at $p<0.05$ in more than one ROI) were repeated in the control group in order to examine if they are a result of injury or not.

Thus, only \textit{repeated} significant ($p=<0.05$) cognitive findings are included in the following pages. All other correlations (either only having found to be significant once,
or non-significant) are not reported or discussed for the sake of clarity and spatial limitations.

6.3.9 Correlations between Metabolite Concentrations and Psychometric Performance: Corroborating psychometric test performance together in the manner described by the previous section revealed that both of the retained conditions of the List Learning Task (A1-A5 and A6) held repeated positive correlations with Cho in the returning patient group. VLF also held repeated negative correlations with NAA in the returning patient group. No correlations were found in the control or acute patient groups by the criteria used here.

The A1-A5 condition of the List Learning task positively correlated with Cho twice. Once in the left hemisphere of the SWM \((r=0.473, p=0.048)\), and once in the right hemisphere of the thalamus \((r=0.504, p=0.033)\). These findings are shown in Figure 6.22.

The A6 condition of the List Learning task also positively correlated with Cho twice. Once in the right hemisphere of the SWM \((r=0.525, p=0.037)\), and once in the left hemisphere of the thalamus \((r=0.645, p=0.004)\). These findings are shown in Figure 6.23. It should be noted that both the A1-A5 and A6 condition findings were reported from the same brain location, albeit in opposite hemispheres.

Finally, VLF negatively correlated with NAA twice. Once in the right hemisphere of the SWM \((r=-0.504, p=0.047)\), and once in the PCC \((r=-0.54, p=0.025)\). These findings are shown in Figure 6.24.
Figure 6.22: Scatterplot (with regression) showing all the significant correlations between Cho and performance on the List Learning (A1-A5) task in the returning Patient group.

Figure 6.23: Scatterplot (with regression) showing all the significant correlations between Cho and performance on the List Learning (A6) task in the returning Patient group.
Figure 6.24: Scatterplot (with regression) showing all the significant correlations between NAA and performance on the VLF task in the returning Patient group.
6.4.1 Post-Hoc Testing with Acute Patient Sub-Groups

6.4.1.1 Aim: Similar to previous chapters, testing was undertaken to ascertain if there were any differences between the acute data of the patients who did return for follow-up testing (AFu) and the acute data of those who did not (AL), and mild / moderate patient subgroups in metabolic concentrations. While AFu / AL testing was undertaken to examine for any methodological problems with comparing data from the acute patient group with data the reduced returning patient group, mild / moderate testing was undertaken to further examine the validity of considering mild and moderate TBI as a single “non-severe” injury.

6.4.1.2 Methodology: Groupwise testing of all metabolic concentrations in all voxels was repeated (as described in 6.2.5) to compare metabolic concentrations between AFu / AL and mild / moderate groups. Control data distribution (as described in sections 6.3.2 – 6.3.6) was used as an indication of if parametric or non-parametric analyses should be used for a given metabolite in a given voxel. As before, due to the reduced sample size of the returning patient group, mild / moderate comparisons were only able to be made with the acute patient data.

6.4.1.3 Results; AFu / AL Comparison: Of testing from all metabolites in all regions, one significant difference was found between AFu and AL patient sub-groups. Cho in the left voxel of the SWM was found to be significantly higher in the AFu group (Table 6.8). In the main groupwise analyses where all patients were compared against controls, Cho showed no significant difference in this region. Following this finding, further testing was considered necessary. Groupwise comparison between AFu / AL and Control Cho in the left voxel of the SWM was conducted. Cho in this region was found to be significantly higher in the AFu sub-group (mean=0.01718, SD=0.00562) than in the control group (mean=0.01317, SD=0.00482, t(32)=-2.5, p=0.018). However no difference was found when testing Cho in this region of the AL sub-group (mean=0.0128, SD=0.00431) against controls (t(49)=0.29, p=0.772). This indicates that Cho may be expected to increase in this region (contrary to the earlier main groupwise findings), but that a characteristic of patients who did not return for follow-up testing in our participant group initially prevented this from being found.
Table 6.8: Groupwise findings from comparing metabolic concentrations of all metabolites in all voxels between AFu and AL patient sub-groups.

<table>
<thead>
<tr>
<th>Voxel</th>
<th>NAA (AFu patient descriptive values, AL patient descriptive values, p value)</th>
<th>Cho (AFu patient descriptive values, AL patient descriptive values, p value)</th>
<th>Cre (AFu patient descriptive values, AL patient descriptive values, p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right Mean/SD=0.1482/0.0412 Mean/SD=0.1543/0.0385 0.625</td>
<td>Mean/SD=0.01675/0.00593 Mean/SD=0.01471/0.00475 0.234</td>
<td>Mean/SD=0.113/0.0355 Mean/SD=0.1213/0.0389 0.478</td>
</tr>
<tr>
<td></td>
<td>Left Mean/SD=0.1494/0.0447 Mean/SD=0.141/0.0484 0.564</td>
<td>Mean/SD=0.01718/0.00562 Mean/SD=0.0128/0.00431 0.01</td>
<td>Median=0.12657 Mean/SD=0.1065 0.055</td>
</tr>
<tr>
<td>Caudate</td>
<td>Right Mean/SD=0.0907/0.0323 Mean/SD=0.0923/0.0266 0.859</td>
<td>Mean/SD=0.01731/0.00552 Mean/SD=0.02034/0.00555 0.073</td>
<td>Median=0.07155 Mean/SD=0.08273 0.233</td>
</tr>
<tr>
<td></td>
<td>Left Median=0.09345 Mean/SD=0.08497 0.268</td>
<td>Mean/SD=0.01876/0.00639 Mean/SD=0.01971/0.00689 0.642</td>
<td>Mean/SD=0.0807/0.0192 Mean/SD=0.0873/0.0337 0.43</td>
</tr>
<tr>
<td></td>
<td>Right Median=0.12829 Mean/SD=0.11421 0.767</td>
<td>Median=0.02067 Mean/SD=0.01931 0.15</td>
<td>Median=0.1006 Mean/SD=0.08859 0.362</td>
</tr>
<tr>
<td></td>
<td>Left Median=0.11126 Mean/SD=0.09945 0.489</td>
<td>Mean/SD=0.02138/0.00381 Mean/SD=0.02196/0.00714 0.727</td>
<td>Median=0.09713 Mean/SD=0.08494 0.397</td>
</tr>
<tr>
<td>Thalamus</td>
<td>Right Mean/SD=0.1072/0.0361 Mean/SD=0.1111/0.027 0.682</td>
<td>Mean/SD=0.01856/0.00562 Mean/SD=0.01963/0.00695 0.564</td>
<td>Mean/SD=0.075/0.0199 Mean/SD=0.0859/0.0269 0.118</td>
</tr>
<tr>
<td></td>
<td>Left Mean/SD=0.0986/0.0239 Mean/SD=0.1013/0.0235 0.696</td>
<td>Mean/SD=0.01842/0.00615 Mean/SD=0.01902/0.00499 0.724</td>
<td>Mean/SD=0.0694/0.0182 Mean/SD=0.075/0.0205 0.332</td>
</tr>
<tr>
<td></td>
<td>PCC Median=0.073/0.0285 Mean/SD=0.0717/0.0212 0.859</td>
<td>Mean/SD=0.00896/0.2 Mean/SD=0.01089/0.0042 0.071</td>
<td>Mean/SD=0.056/0.0158 Mean/SD=0.0615/0.0208 0.305</td>
</tr>
</tbody>
</table>
6.4.1.4 Results; Mild / Moderate Comparison: A number of significant differences were found between metabolic concentrations of acute mild and moderate patient groups. Mild NAA was significantly higher than moderate NAA in each hemisphere of the SWM, and in the PCC. Additionally, mild Cho was significantly higher than moderate Cho in the right putamen and the PCC. All findings are shown in Table 6.9.
**Table 6.9**: Groupwise findings from comparing metabolic concentrations of all metabolites in all voxels between acute mild and moderate patient sub-groups.

<table>
<thead>
<tr>
<th>Voxel</th>
<th>NAA (mild patient descriptive values, moderate patient descriptive values, p value)</th>
<th>Cho (mild patient descriptive values, moderate patient descriptive values, p value)</th>
<th>Cre (mild patient descriptive values, moderate patient descriptive values, p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean/SD=0.1605/0.0348 Mean/SD=0.1063/0.03</td>
<td>Mean/SD=0.01623/0.00543 Mean/SD=0.01264/0.00402</td>
<td>Mean/SD=0.1223/0.0369 Mean/SD=0.0937/0.0308</td>
</tr>
<tr>
<td>SWM</td>
<td>0.002</td>
<td>0.071</td>
<td>0.059</td>
</tr>
<tr>
<td>Left</td>
<td>Mean/SD=0.1529/0.0439 Mean/SD=0.1032/0.0384 0.014</td>
<td>Mean/SD=0.01525/0.00527 Mean/SD=0.01182/0.00497 0.138</td>
<td>Mean/SD=0.1189 Mean/SD=0.08866 0.28</td>
</tr>
<tr>
<td>Caudate</td>
<td>Mean/SD=0.0951/0.0291 Mean/SD=0.0772/0.0266 0.1</td>
<td>Mean/SD=0.01932/0.00604 Mean/SD=0.01703/0.00364 0.162</td>
<td>Mean/SD=0.08205 Mean/SD=0.06858 0.356</td>
</tr>
<tr>
<td>Left</td>
<td>Median=0.08987 Median=0.07456 0.206</td>
<td>Mean/SD=0.01949/0.00703 Mean/SD=0.0183/0.00444 0.553</td>
<td>Mean/SD=0.0857/0.0287 Mean/SD=0.0779/0.0243 0.443</td>
</tr>
<tr>
<td>Putamen</td>
<td>Median=0.12707 Median=0.09919 0.524</td>
<td>Median=0.02078 Median=0.01523 0.022</td>
<td>Median=0.09551 Median=0.09776 0.698</td>
</tr>
<tr>
<td>Left</td>
<td>Median=0.11082 Median=0.10285 0.825</td>
<td>Mean/SD=0.02245/0.00559 Mean/SD=0.01855/0.00557 0.084</td>
<td>Mean/SD=0.09576 Mean/SD=0.08511 0.58</td>
</tr>
<tr>
<td>Thalamus</td>
<td>Mean/SD=0.1073/0.0258 Mean/SD=0.1165/0.0477 0.569</td>
<td>Mean/SD=0.01947/0.00595 Mean/SD=0.01786/0.00776 0.553</td>
<td>Mean/SD=0.0781/0.0236 Mean/SD=0.0908/0.0254 0.178</td>
</tr>
<tr>
<td>Left</td>
<td>Mean/SD=0.0998/0.0246 Mean/SD=0.1011/0.0196 0.867</td>
<td>Mean/SD=0.01859/0.00558 Mean/SD=0.01931/0.00542 0.716</td>
<td>Mean/SD=0.0704/0.0195 Mean/SD=0.0797/0.0187 0.188</td>
</tr>
<tr>
<td>PCC</td>
<td>Mean/SD=0.0779/0.0229 Mean/SD=0.0487/0.0171 0.001</td>
<td>Mean/SD=0.01048/0.00375 Mean/SD=0.0079/0.0031 0.05</td>
<td>Mean/SD=0.0613/0.0186 Mean/SD=0.0488/0.0158 0.06</td>
</tr>
</tbody>
</table>
**6.4.1.5 Summary:** The significant difference between Cho of the AFu and AL patient sub-groups in the left SWM is suggestive that Cho is higher in this region in those patients who did return at follow-up. Further testing which showed Cho to be significantly higher in this region in the AFu sub-group compared to controls, but not in the AL sub-group, indicated that Cho might be expected to increase in this region following injury, but possibly only in a subset of patients. It should be considered if this could have affected the main results in any way.

In the main groupwise findings where all patient metabolic data was compared against controls (sections 6.3.2-6.3.6), Cho in this region was not found to be significantly different between patients and controls at the acute time-point. However the further finding of Cho being significantly higher in the AFu sub-group but not in the AL sub-group (compared to controls) indicates that this region might be susceptible to an acute increase in Cho following a TBI, but only in specific patients. As increased Cho following injury has been previously hypothesised to indicate cell membrane inflammation (Brenner et al., 1993), it could be that a trend exists in those patients who did return whereby there were experiencing more difficulty with their injury and thus were more motivated to attend to it further. In this way, increased Cho in this region at the acute time-point could be predictive of long-term difficulty. Indeed, although Cho was not found to be increased in this voxel at the follow-up time-point (where, by definition, only the AFu patient group was tested), it did show positive correlations with two of the psychometric tests (Figures 6.22 and 6.23). This finding is therefore suggestive that a physiological difference may exist between those patients who did and those patients who did not return, with ongoing severity of injury potentially being the dividing factor. However as only one difference was found in all the voxels, it is not considered problematic to more broadly compare the full acute patient group with the returning patient group. Indeed, the uniqueness of this finding could also indicate that it is a Type 1 error.

As the mild / moderate comparisons clearly showed a strong effect between these sub-groups, discussion of this is included with the rest of the main summary for this chapter in the next section (6.5.3).
6.4.2 Post-Hoc Testing with NAA/Cre Ratios

6.4.2.1 Aim: The primary groupwise findings in this Chapter show a considerably different pattern of metabolic changes, with particular regard to NAA increasing following TBI. However as a relatively novel method of using water concentration as a stable reference point was used in place of Cre, additional analyses presented here aimed to conduct groupwise testing using the more traditional approach of using NAA/Cre values in order to see how comparable our data is to the wider literature findings.

6.4.2.2 Methods: Raw peak values of NAA (i.e. not corrected to water concentration) were divided by raw values of Cre to create NAA/Cre ratios for all subjects in all voxels. As per previous analyses, these were then compared between controls vs. acute / returning patient data for each voxel by t-test / Mann-Whitney U depending on if the control NAA/Cre distribution for that location was shown to be normal by the Anderson-Darling test.

6.4.2.3 Results: Anderson-Darling normality testing revealed control values of NAA/Cre in the following voxels to be not normally distributed: left SWM (p=0.045), left Caudate (p<0.005), right Putamen (p<0.005), PCC (p<0.005). Groupwise analysis showed that NAA/Cre was significantly lower in patients compared to controls at both time-points in the PCC voxel. No other comparisons were significant. Full results are shown overleaf in Table 6.10, with the findings from the PCC voxel then shown in Figure 6.25.
**Table 6.10:** Groupwise findings from comparing the NAA/Cre ratio values between controls and patients.

<table>
<thead>
<tr>
<th>Voxel</th>
<th>Acute NAA/Cre Comparison (Control, Patient, p value)</th>
<th>Chronic NAA/Cre Comparison (Patient, p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean/SD=1.311±0.341 Mean/SD=1.361±0.385 0.574</td>
<td>Mean/SD=1.266±0.301 0.652</td>
</tr>
<tr>
<td>SWM</td>
<td>Right</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median=1.251 Median=1.258 0.569</td>
<td>Median=1.362 0.285</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean/SD=1.13±0.221 Mean/SD=1.17±0.254 0.48</td>
<td>Mean/SD=1.059±0.199 0.305</td>
</tr>
<tr>
<td>Caudate</td>
<td>Right</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median=1.195 Median=1.18 0.571</td>
<td>Median=1.046 0.525</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean/SD=1.23 Median=1.258 0.865</td>
<td>Mean/SD=1.02 0.067</td>
</tr>
<tr>
<td>Putamen</td>
<td>Right</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median=1.223±0.326 Mean/SD=1.194±0.308 0.713</td>
<td>Mean/SD=1.103±0.288 0.252</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean/SD=1.43±0.453 Mean/SD=1.419±0.411 0.923</td>
<td>Mean/SD=1.87±1.67 0.293</td>
</tr>
<tr>
<td>Thalamus</td>
<td>Right</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean/SD=1.483±0.478 Mean/SD=1.432±0.337 0.585</td>
<td>Mean/SD=1.93±1.16 0.141</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median=1.330 Median=1.197 0.015</td>
<td>Median=1.23 0.019</td>
</tr>
<tr>
<td>PCC</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
6.4.2.4 **Summary:** As previously discussed, decreased NAA/Cre values are often found following TBI (e.g. (Nakabayashi et al., 2007, Tollard et al., 2009, Vagnozzi et al., 2010, Garnett et al., 2000b)), meaning the current replication of this in the PCC voxel is not surprising. What is more interesting is that lower NAA/Cre values were still reported in this patient group where NAA and Cre (referenced to water) had otherwise been found to increase. Comparing Table 6.10 to Table 6.7 indicates that, when examining water-corrected data, the PCC voxel exhibited increased Cre but unchanged NAA at each time-point. Combining these observations suggests that some previous reports of decreased NAA/Cre may be due to increased Cre but unchanged NAA. The lack of other significant “ratio” findings in the current dataset is likely due to the general pattern of increases in NAA and Cre often occurring at the same time, which also suggests that using Cre as a stable reference point in ratio analysis may lead to a loss of power in detecting change in both NAA and Cre should increases in each happen at the same time.

![Figure 6.25: Boxplots to visualise NAA/Cre ratios between controls and acute / returning patients in the PCC voxel. Both patient groups are significantly lower than controls.](image)
6.5 Discussion

This investigation presents results of increased NAA, Cho and Cre at the acute phase of injury, and increased NAA and Cre but decreased Cho at the chronic phase of injury. Additionally, while no relationships were found between metabolic concentrations and psychometric performance acutely, a number of correlations were found using the follow-up data indicating that the changes in the metabolite levels of NAA and Cho relate to cognitive outcome. However, none of these correlations occurred in a location with an accompanying groupwise change. I.e. all instances where patient Cho and NAA was found to significantly correlate with psychometric test performance occurred in regions where Cho and NAA concentration were normal compared to controls. This implies that a more complex view may be needed to explain what exactly a “healthy” metabolic concentration is following a TBI. Finally, further examinations of patient sub-groups suggested that while these findings are for the most part common to both mild and moderate patients, in some instances the reported acute groupwise changes are more prominent in the mild sub-group.

6.5.1 Groupwise Findings and the use of Ratios in MRS Investigations:

Increased acute NAA following injury is arguably among the most prominent of our findings, both by how frequently it was recorded as a response in our patients but also in its novelty with respect to the wider literature. These acute increases often normalised by the chronic time-point, but in some instances remained raised while in another became raised after being acutely normal. If it is subject to any change, NAA is very predictably found to decrease regardless of injury severity; this change has been reported in acute / sub-acute, mild / non-severe injury (Sivak et al., 2014, Kirov et al., 2013, Johnson et al., 2012). While it is sometimes found to normalise over a short period time (Vagnozzi et al., 2010), or normalise in patients with a good outcome (Signoretti et al., 2008) (lending support to the theory that a change in its concentration may be short-lived / indicative of more severe damage), it is often also seen to remained decreased (Tollard et al., 2009, Garnett et al., 2000a). To the author’s knowledge, no study has ever reported an increase in NAA at any time-point or in any patient severity following TBI. The reason for decreased NAA which is most often cited is it being due to the neuronal loss / impaired neuronal function incurred in DAI. Detecting an increase following injury in a number of locations and at both acute
and chronic time-points is therefore contradictory to this established view and suggestive that the broad use of lower NAA as a marker of axonal damage is oversimplified, at least when applied to patients with acute, mild TBI. The fact that these patients may show different hallmarks of injury to other injury groups is also supported by the clear and significant changes in white matter diffusion properties presented in Chapter 5, which are also contradictory to findings in more injured patients.

It should also be noted that most previous literature reports metabolites data as ratios to the Cre or Cho peaks rather than to a water reference signal, making direct comparison to our data more complex. Statements made in the previous paragraph of “decreased NAA” more accurately refer to “decreased NAA / Cre”, although are almost always interpreted in the former, more straightforward manner. This is done under the assumption that Cre is stable following TBI, and therefore using it as a statistical reference point in this manner benefits the analyses by eliminating compromising effects such as variations in coil signal intensity. However more recent research has started to consider the idea that Cre may also be affected by TBI, with some studies finding it to either increase in patients (Yeo et al., 2011) or correlate with other measures such as cognitive test scores (Gasparovic et al., 2009) and GCS (Walz et al., 2008) in a direction which supports increased Cre as being a marker of damage. It was because of these previous findings that the statistics in this chapter were conducted using absolute metabolic values which were instead ratioed against water concentration as a means of correction.

Our work also lends support to Cre being subject to change following injury. Like NAA, Cre was also found to be acutely increased in six of the nine possible voxels, and remained raised in three of these at the chronic time-point, strongly indicating that it can be subject to a change in concentration following injury. If Cre is expected to change in this manner, previous literature findings must be reconsidered in light of this. A finding of decreased NAA / Cre may not be due to an actual decrease in NAA, but rather an increase in Cre. This was further evidenced by the post-hoc investigation here which found NAA/Cre ratio values to be decreased in patients in voxels where increased water-corrected Cre but unchanged NAA had been found. Alternatively, an increase of both in concentration may not be detected if one is divided by the other, as
also appeared to be the case in the same post-hoc investigation. We present compelling evidence that the use of Cre as a stable reference point is subject to error and that doing so can lead to misinterpretations when considering which metabolite concentrations have changed and in what direction. While some previous papers have shown spectra which demonstrate that (un-ratioed) patient NAA is lower than control NAA (Garnett et al., 2000b), it seems feasible that increases in Cre could contribute to the repeated finding of decreased NAA / Cre ratios, and also to a lack of reported increased NAA / Cre ratios. As such our primary finding of increased NAA post TBI, though novel, may not necessarily be as unusual as they appear.

In explaining previous decreases in NAA, hypotheses such as a loss of neuronal density or a dysfunction in mitochondrial activity have been given. These are based on work which shows NAA to firstly only be found in neurons (Bates et al., 1996), and secondly to hold a strong relationship with mitochondrial energy production whereby NAA levels linearly indicate mitochondrial activity (Moffett et al., 2007). By this logic, a decrease in NAA indicates a deficit in the neurophysiology either by loss of cells or loss of function. In considering what increased NAA could indicate, it is possible that a combination of local events such as the hypothesised astrogliosis and weakening of neuronal-axonal membranes via damage to neurofilament (as described in section 5.5.3) could have caused compaction of neurons, locally increasing the concentration of NAA. However when considering the relatively large sizes of the regions sampled in this analysis it does not seem entirely reasonable that enough “new” neurons from surrounding areas would be forced into the boundary of a MRS voxel without a similar number of others also being forced out. On balance, the second hypothesis remains more feasible in terms of creating detectably-large effect.

While mitochondrial activity could be expected to be compromised in severe injury, it is possible that non-severe injury would not be sufficiently destructive to disrupt cell energy processes. Responding to the consequences of TBI at a cellular level is a very energetic process. As one example, past research has indicated that the brain experiences an acute phase of raised glucose metabolism at a similar time-point since injury to our acute patients (Bergsneider et al., 1997) as an attempt to correct an ionic imbalance that often occurs following TBI so that an appropriate resting membrane potential can be restored (Reinert et al., 2000). Undamaged neuronal mitochondria
could therefore increase in their activity as a means of achieving this. As NAA levels are known to hold a linear relationship with mitochondrial activity (Bates et al., 1996), a mirrored increase in NAA would also be expected.

Similar explanations may be provided in explaining the increase in Cre; previous work (Yeo et al., 2011) has also suggested that a Cre increase is due to a higher local energy demand as a consequence of the immune response and on-going cellular repair processes. Our results can offer further support to this hypothesis, especially when considering that the SWM voxels were chosen having formed the hypothesis in Chapter 5 that extensive immune-response processes were ongoing in that location at the acute point, although not at the chronic point. Metabolic findings from this location showed both NAA and Cre to be increased bilaterally at the acute point, with no differences being found at the chronic point, repeating the pattern of DTI findings.

While this hypothesis fits with the known physiological processes which occur in the acute timeframe, it is more difficult to apply it to the chronic findings. As stated, most locations where NAA and Cre were acutely elevated returned to normal at the follow-up time-point, although these metabolites did remain raised in a minority of locations (or, in the case of NAA, become raised in a voxel where it was acutely normal). Descriptively observing the pattern of this, it should be noted that for each NAA and Cre, two of the three voxels where chronic increases were recorded were left / right aspects of the same location (the thalamus for NAA and the putamen for Cre), possibly indicating these locations to be selectively vulnerable to this. Reasons why mitochondria would still be overactive at this point are not forthcoming. Considering the time-point, it is likely that these increases are indicative of more permanent / severe damage. It could be that cell energy production is still increased as a means for compensating other local deficits caused by this long term injury. However, without further data it is difficult to satisfactorily conclude on this point, and so it is highlighted as a potential avenue for further research.

Differences in Cho concentration were also recorded. Similar to NAA and Cre, Cho was found to be increased in patients compared to controls in a number of locations acutely, but was found to be reduced in some voxels chronically. These changes were less widespread than NAA and Cre, with the acute significant findings
being recorded in three voxels and chronic findings only in two. Of these locations, the PCC voxel held both an acute increase and a chronic decrease.

Cho has long been speculated to being subject to increases following TBI. It is interesting when considering the potential difficult of reconciling ratio and non-ratio MRS findings with one another that our non-ratio data still shows a trend for acute Cho to increase following injury. The mechanism behind previous Cho increases is primarily hypothesised to reside in Cho’s function as a metabolite connected to the turnover of cellular membranes, with this in turn being supported by findings showing Cho to increase as a response to cellular inflammation (Brenner et al., 1993). Our data still supports this hypothesis. Cellular inflammation would be expected to the smallest degree in the case of mild / moderate injury, meaning we would only detect Cho increases in a minority of locations. Further, while we have hypothesised Cre increases to be a phenomenon of non-severe injury, Cho would be expected to increase more with severity. In this view, Cho / Cre ratios would still show increases in the case of severe injury where Cho is more increased and Cre is less increased. This allows our current findings to comfortably with those of the previous literature, regardless of if such past papers gave spectra which demonstrated non-ratioed Cho concentration to be increased in patients or not.

However the chronic Cho findings are again more troublesome to explain. While sustained increases are sometimes found (Holshouser et al., 2006), chronic decreases in Cho have not, to the authors knowledge, been previously reported. As previous research has shown that the MRS Cho signal primarily represents both free Cho and cellular density (Miller et al., 1996) it is appropriate to consider these findings as being potentially indicative of either or both of these. While a long term loss of cells may seem reasonable following a TBI this is an unlikely explanation for the Cho concentration decrease here, as we would also then expect other metabolite concentrations to decrease in a similar manner. In each of the voxels where Cho was found to be decreased at the chronic time-point, Cre (which is present in all cells) was also found to be increased.

As with many of our more novel findings, it is instead likely that this chronic Cho concentration decrease is due to the non-severe nature of our participant group and represents a form on long-term damage which is not found in more severe cases.
of TBI. The lack of any chronic Cho increases indicates that cellular inflammation no longer remains. Although an increase in Cho is an indicator of damage, relatively high concentrations of the metabolite are still needed in order to maintain healthy functioning in the normal brain. Its release due to inflammation is linked to its more primary function of being an essential aspect of maintaining healthy cell membranes, while it is also a precursor of the neurotransmitter acetylcholine (Blusztajn and Wurtman, 1983). Reservoirs of choline are created for these purposes following a synthesis process involving the catabolism of phosphatidylcholine and the degradation of lysophosphatidylcholine (Lockman and Allen, 2002). A sustained decrease in Cho concentration may therefore reflect deficits in these processes as a consequence of long term local damage.

**6.5.2 Cognitive Findings:** A number of relationships were found between metabolic concentrations and cognitive performance. However, these were found in the returning patient data and not replicated in the controls, indicating these relationships are due to injury.

No correlations were established between metabolic concentration and cognitive functioning in the patients at the acute time-point. This perhaps indicates that, despite metabolic concentration clearly being affected by injury, these alterations are a pure physiological response at the cellular level which do not impact upon cognitive processes (at least in predominantly mild patient cohort). However it should also not be ruled out that associations do exist in this timeframe. It is possible that the stringent criteria for a correlation to be significant resulted in type 2 errors, with the same possibility also being true for control data. It is suggested that more research be conducted before a full conclusion is reached here.

A number of correlations were found with the follow-up patient data. At 1 year post-injury, NAA was found to negatively correlate with VLF performance. Cho was also found to positively correlate with List Learning (A1-A5 and A6 conditions) performance. However all of these metabolite / psychometric test relationships appeared in areas where the relevant metabolite was not significantly different in concentration compared to controls.
While the directions of these correlations superficially support the implications of the groupwise findings (that increased NAA / decreased Cho are chronic markers of damage at the chronic time-point), this also shows that “healthy” concentrations of metabolites may frequently be related to injury-specific cognitive functioning. This could either be due to cognitive performance being more delicately linked to local metabolic concentrations, so as that a concentration change which is not great enough to cause a significant groupwise difference may still affect psychological functioning. In this case it may simply remain true that increased NAA / decreased Cho are indeed generic markers of chronic damage; it is worth noting on this point that mean NAA / Cho values were still descriptively increased / decreased (respectively) in all locations where correlations involving that metabolite were found. Alternatively, effective cognitive function may form different dependencies with the brains physiology following a TBI, which is then reflected by novel relationships with metabolic concentrations. This is an interesting idea, but would require more research to adequately comment on beyond pure speculation.

The disparity between the lack of / presence of cognitive findings in the acute / chronic conditions may be a reflection of acute metabolic changes representing immune-response processes (e.g. increased NAA as a marker of increased mitochondrial activity / increased Cho due to membrane inflammation) in contrast to chronic metabolic changes representing lasting cellular damage (e.g. decreased Cho as a marker of acquired deficit in acetylcholine production or membrane upkeep).

### 6.5.3 Considering Mild / Moderate Differences:

While post-hoc analysis of AFu vs. AL patient sub-groups was once again largely supportive of the whole acute patient sample being representative of the returning patient sample, comparison of acute mild and moderate patient sub-groups indicated considering all acute patients as a single “non-severe” severity may be problematic. A number of significant findings whereby metabolic concentrations of NAA and Cho were increased in the mild patients compared to the moderate patients were found in a variety of regions. Combining the high number of these findings and the consistency of the direction of change indicates that this is a strong effect. This is in contrast with Chapter 5 where mild and moderate data presented similar findings, possibly indicating that a disparity between mild and
moderate injury may be more pronounced in the physiological changes which MRS, but not DTI, detects.

Comparing Table 6.9 with Table 6.7 shows that three of the five significant mild / moderate differences (bilateral NAA in the SWM and Cho in the PCC lobe) had a corresponding patient vs. control difference wherein patient metabolic concentration was higher. It is therefore likely that this pattern of increased NAA / Cho in the mild group drove these findings. This, and other instances such as increased PCC NAA in the mild group, introduces further evidence that many of the novel reported findings may be a response more typical of a patient with a GCS of 13 or higher. Testing with the returning patients was not able to be conducted due to reduced sample size, so it should also not be ruled out that similar effects may persist over time. Similarly, as the acute moderate group is substantially fewer than the acute mild group, there may be many other cases where a lack of power prevented further mild vs. moderate differences from being found.

However, by considering the abundance of (all) acute patient vs. control differences which do not have a corresponding mild vs. moderate difference there is also evidence that for a majority of the time both mild and moderate patients contribute to the broader reported patient metabolic changes to a comparable degree. Indeed, even if the least significant mild vs. moderate comparisons are selected, corresponding (all) acute patient vs. control differences are often still found (e.g. NAA in the left Putamen, NAA in the right Thalamus, Cho in the left Caudate etc.). This suggests that while both mild and moderate patients exhibit these novel groupwise changes, the mild group perhaps has an increased propensity to do so.

6.5.4 Limitations: This work has some limitations. As with Chapter 5, one of the main issues is a substantial loss of the patient group between the acute and chronic time points. Little more commentary on this issue can be given here than has already been covered in section 5.5.5, although it should be noted that appropriate post-hoc tests were also conducted using this data (section 6.4). The results from this also conformed to the notion that the acute patient sample is representative of the returning patient sample, and so making comparisons between the two is not flawed. On a similar note, the problem of not all patients being able to complete cognitive testing again applies to this testing. However the case still remains that those patients
who were unable to partake in cognitive testing would hold the greatest deficits. Losing these patients from testing would bias against detecting experimental effects, supporting the cognitive findings here as being if anything a reduced example of the extent of neuropsychological problems encountered following mild / moderate TBI.

One additional consideration surrounds the use of water as a stable reference point opposed to Cre. Some of the physiological responses to TBI are known to increase water concentration, e.g. oedema (Unterberg et al., 2004), or as a result of a mass lesion having developed (Go, 1997). Conversely, other than pharmacological interventions (which a discussion with Mr. Cowie confirmed was extremely unlikely in any of our patient group), a mechanism where water concentration would decrease following a TBI would not be as reasonably expected. The possibility of water concentration increasing means that diving metabolite values by this may also be an imperfect method of correction. However, if water concentration had increased, this would lead to lower estimations of metabolic concentrations after referencing to water. As the majority of our findings show increased concentrations of a metabolite in patients compared to controls this argues against the idea that water concentration changes may have impacted upon our results.

Another potential criticism is that proton density of a location is also an estimation rather than a direct measure of water concentration. Deriving water concentration by examining the proton density of a location is a common technique, as the vast majority of protons in a given location are known to be present in water (Tofts, 2003). Therefore, hypothetical error in this would be introduced by physiological changes unrelated to water leading to a different proton density value. However it is very unlikely that any such events could cause a meaningful change relative to the amount of signal which water contributes. Finally, some recent research has demonstrated methods that can be used to correct for systematic error in proton density mapping by estimating types of error present through examination of other MR scan modalities taken at the same time as the proton density map (Volz et al., 2012). Following these corrections proton density values were shown to be very close estimates of literature values of water across various brain regions. Future research could consider adopting this approach when estimating water concentration.
6.6 Chapter Summary

This longitudinal work presents evidence of both acute and chronic metabolic change following TBI, with the chronic changes also being relevant to cognitive outcome. NAA increases were reported in patients both acutely and chronically. Though a novel finding, this should not necessarily be considered at odds with previous literature due to the use of Cre as a ratio-reference point in past analyses; Cre was also found to be subject to increases in this work, supporting other recent research. We also therefore present evidence that the use of Cre as a stable reference point for MRS analyses is flawed. Further investigation using the mild and moderate patients as separate groups also indicates that many of these findings may be due to the predominantly mild nature of the tested patient group, offering further support that the neurophysiological response to TBI may present in a contradictory manner between mild and severe cases. As such, the hypothesis that Cre would increase following damage is supported, however cognitive data was not found to hold any relationships with this. Additionally, the hypothesis that other metric changes would appear as “typical” is only partially supported; while Cho increases were expected decreased NAA is a very novel finding.
Chapter 7. Thesis Summary

7.1 Retrospect of Thesis Aims

MRI techniques have been increasingly used to study TBI in the past two decades. “Standard” methods such as T1W imaging are competent in showing areas of the visible, focal damage which often occurs. Conversely, “advanced” techniques may be used to shed light on microscopic DAI, which previously could only be diagnosed via histopathology. Popular among these advanced methods, DTI may be used as a method of studying microstructural changes resulting from damage, while MRS is capable of characterising changes to the metabolic profile of neural regions. Data which is derived from any of these techniques may also be used in seeking relationships with the patient’s cognitive outcome. Such findings provide evidence that the physiological changes identified by these methods are cognitively relevant.

The sum of this past research has arguably produced a consensus on the changes expected after someone sustains a TBI. It appears clear that focal injury is most often sustained in the anterior aspects of the cortex; the anterior frontal and temporal lobes. DTI studies have indicated that neuronal microstructure becomes disorganised and torn; something most characteristically implicated by reduced FA and increased MD, with relationships to cognitive outcome often supporting that these changes mark damage. MRS studies have indicated that in affected areas NAA concentration may decrease, while Cho concentrations may increase; suggestive of neuronal loss / mitochondrial dysfunction and cellular inflammation, respectively. These hypotheses all appear reasonable considering expected outcomes from a TBI, but they are based on data which is heavily biased towards studying severe and chronic head injury.

Though accounting for 80-90% of cases, TBI-research has frequently ignored mild injury. This is also despite early signs indicating that many patients with mild TBI still suffer from lasting and affecting cognitive deficit. Some of the research which has examined a mild patient population reported findings of metric and metabolic changes contrary to the expected directions (as described above) which began to indicate that the conclusions gleaned from severe-focused research may not be applicable to mild cases. It also became apparent that the temporal aspect of recovery affected findings,
reflecting the differing physiological processes which happen over time. A push to isolate mild and acute injury within research began; an approach which this thesis is grounded in. Thus, the broader question(s) asked by this project was “Are the physiological changes observed following mild TBI separate to those expected following a severe injury, how do these change over time, and how do these contribute to the patient’s resulting cognitive profile?”.

To address this question, longitudinal MRI and cognitive data was obtained from a predominantly-mild (and non-severe) patient cohort and matched control subjects. Cognitive deficit in patients was first assessed (Chapter 3), areas of focal damage were identified (Chapter 4), DTI was used to conduct a global investigation into microstructural changes and how these affect cognitive deficit (Chapter 5) and finally MRS was used to characterise metabolic changes in a number of ROI’s and how these affect cognitive deficit (Chapter 6). Findings broadly supported the notion that different physiological responses should be expected following a mild injury (compared to injury of greater severity) and a number of novel conclusions were made.

7.2 Characterising the Neurophysiology of Mild TBI

Chapter 4’s experimentation was perhaps the only instance here where the findings broadly agree with severe-based literature. Images were produced to show the probability distribution of visible lesions in our predominantly mild patient cohort. These qualitatively demonstrated that visible damage in the brain was most frequently found in the anterior, inferior and lateral aspects of the frontal and temporal lobes, while a minority of other lesions were isolated in a non-specific pattern around the cortex. This a similar pattern to that which is reported in studies focusing on severe injury, and this is perhaps not surprising. As primarily focal damage is caused directly by sites of impact to the skull, this would not be expected to differ between mild and severe injury as the main difference between these is instead the force of the impact. The finding that total lesion volume increases with GCS further supported this.

Although the spatial distribution of focal damage in mild patients appeared to conform to the same pattern as in severe patients, DTI and MRS data indicated that the presentation of DAI did not. On this point, it should first be made clear that the repeated finding of significant metric or metabolic differences in normal-appearing
areas of brain shows strong fundamental support of the existence of DAI in non-severe TBI. At a glance, the direction and nature of these changes often appeared as a complete opposite to what is frequently found when examining severe patients. Most notably, DTI and MRS experimentation respectively showed FA and NAA to *increase* in patients. As such alterations first appear almost nonsensical when considered in the context of what these markers are purported to indicate (microstructural organisation for FA and neuronal density for NAA). Considering them in the context of lesser severity and time since injury was required in order to make sense of them.

Crucially, while findings from past research generally pointed towards changes in biomarkers as being direct evidence of physiological damage (e.g. decreased FA to indicate a disrupted and disorganised axonal structure), our conclusions were often hinged on the principle that while DAI does often result from mild injury, it may not be disruptive enough to cause immediate and clear physiological damage. Our data supported the hypothesis that an increased survival of neurons and glial cells would mean the destruction-based hallmarks of severe injury were not present, while at the same time relatively spared cellular functioning would hypothetically result in a greater degree of an immune response and repair processes which a more damaged brain would not be as capable of. The combination of these creates a substantial disparity between the physiology of severe and mild injury. While neuronal microstructure may predominantly suffer from tearing, shearing and cell death in severe injury (leading to decreased FA), it may remain relatively intact in milder injury. This would mean that a response of astrogliosis would instead become the *predominant* physical change, leading instead to increased FA as the processes from astrocytes confer greater directionality to local diffusion. As another example, while a loss of neuronal density may lead to a drop in NAA in severe injury, the relative sparing of neurons in milder injury may leave neurons more capable of engaging in the energetic response to damage, leading instead to an increase in NAA.

The analyses in this thesis may thus be combined to present a picture of the focal, microstructural and metabolic changes expected following non-severe TBI. Focal lesions may still be found, in similar patterns to damage following a severe injury albeit to a lesser extent. DAI affects many of the brains white matter tracts. At the acute stage of injury (approx. 6 days), this causes multi-focal injury to a number of major
neuronal pathways which is sufficient to initiate responses such as astrogliosis but not severe enough so as to cause cellular destruction. The invasion of astrocytes may create an increase in FA when examined by DTI, driven by an increase in AD but unchanged RD. Chronically (approx. 1 year), this process has abated and locations of lasting damage may now be identified by DTI; the genu of the corpus callosum may exhibit decreased FA which is driven by increased RD, now indicative of myelin sheath disintegration.

Metabolically, acute increases in NAA and Cre may be observed in a number of locations. These are hypothesised to be due to increased local energy expenditure as a response to injury. Concurrently, Cho may also increase as a response to cell membrane inflammation. Locations which have suffered chronic damage may also be characterised by still-increased NAA and Cre at 1 year post-injury, although reasonable hypotheses for these sustained increases cannot currently be made and require more research. Conversely, Cho may no longer be expected to be increased but may instead now be decreased; hypothesised to be indicative of a departure of healthy functioning which seeks to maintain an adequate supply of Cho for various cellular processes.

7.3 Characterising the Cognitive Profile of Mild TBI

Cognitive testing indicated that our cohort of non-severe patients experienced a wide range of cognitive deficit at the acute stage of injury. This presented as underperformance on psychometric tasks designed to measure aspects of executive function, visuospatial and verbal learning, attention, concentration, short-term memory and working memory. However by the chronic time point patients were not found to perform significantly differently (in groupwise analyses) on any task compared to controls, indicating a degree of recovery over time.

Examining our data for relationships between cognitive test performance and data pertaining to neurophysiological damage revealed a number of relationships at both the acute and chronic time-points, allowing inferences about the biological basis of these deficits to be made. Focal damage was shown to be related to scores on a number of psychometric tasks which patients had been shown to hold a deficit in. The manner in which these relationships were established was interesting; total lesion volume was an insensitive predictor of this (in terms of correlations with psychometric
performance) and it was instead found that patients who had visible lesions underperformed on cognitive tasks (in groupwise analyses) compared to those who did not. This finding also gave support to the Mayo classification system of head injury severity, suggesting it is superior to the GCS with regards to predicting cognitive outcome. In this way it is unclear if the lesions themselves contributed to deficit, or if the presence of visible damage was instead an indicator of greater overall severity of injury which may lead to more cognitive-relevant neurophysiological changes elsewhere. As a small number of correlations were found when examining total lesion volume, it seems feasible that there is a combination of each of these factors.

Examination of DTI and MRS data indicated more direct relationships between DAI and cognitive outcome. Our data in Chapter 5 supported the hypothesis that subtle damage to axonal neurofilament underpinned deficit in the VLF task by affecting the conduction velocity of the neuron. This would also reduce the RD of the axon, but not sufficiently enough for a groupwise reduction in RD to be observed. This phenomenon was indicated by an RD-driven negative regression between FA and VLF. Chronically, FA was still found to regress negatively with VLF although in a different location (the genu opposed to the ascending fibres of the corpus callosum, as was most strongly implicated in the acute data). This was an interesting point as FA was also shown to be reduced in patients compared to controls, often in overlapping voxels. The seemingly contradictory implication of these findings is that chronic FA reduction (due to myelin sheath disintegration) is indicative of damage, yet patients who have lower FA values also tend to perform better on the VLF task. This is highlighted as an interesting point for further research.

A number of correlations were also found between psychometric task performance and various metabolic concentrations in a number of locations in our patients. Interestingly however, none of these were found at the acute stage of injury. NAA was shown to negatively correlate with VLF and Cho was shown to positively correlate with performance on two different conditions of the List Learning task. Each of these findings were complementary to counterpart groupwise results, together indicating that chronically increased NAA / decreased Cho were indicative of lasting damage. This is in contrast to current literature which generally indicates decreased NAA and increased Cho to indicate damage. However none of the regions these
correlations were found in were the same areas as where that metabolite had also been shown to be significantly different compared to controls in groupwise analysis. This indicated that injury-specific relationships between metabolite concentrations and cognitive functioning may occur in areas where metabolite concentration is normal. More work is therefore required regarding the reasons behind these concentration changes and their influence on cognitive function in order to produce satisfactory conclusions as to the exact physiological mechanisms which are impacting upon cognitive outcome.

The apparent contradiction between the lack of chronic patient task underperformance and the number of findings between physiological data and cognitive performance in the chronic phase should be noted. This implies that even if a patient outwardly portrays normal levels of cognitive function, this may still be underpinned by altered neural physiology. The apparent recovery of cognitive function does not therefore indicate a recovered brain on a physiological level. The patient may still be experiencing cognitive deficit but to an extent which would not be found in a groupwise comparison. It should also be considered that coping mechanisms are likely to have developed over time, meaning that some aspects of the observed normalisation have a behavioural rather than strictly-biological component.

7.4 Future Work

The investigations here highlights a number of potential avenues for further research. Principally, due to the strong evidence that the physiological and cognitive profile of non-severe TBI should be considered differently to severe cases, a broad indication is made that more research should be conducted into this demographic. Specific to the conclusions made here, the mechanisms which underpin VLF performance at the chronic phase of injury are of interest and may shed further light onto the nature of long term cognitive outcome in non-severe TBI. Equally, reasons behind the novel chronic metabolic changes of increased NAA / Cre and decreased Cho (each also shown to impact upon cognitive deficit) also remain unclear and present an opportunity for further work. Methodological aspects of this project which were limited may also be improved upon; higher scan resolution, a greater proportion of returning patients and more strict severity criteria would all be of benefit.
Some of the findings in this study also suggest that the analytical techniques used in future research should be more carefully considered. In DTI research, AD and RD metric values should always be examined. Methods which only consider metrics which may be produced through manipulations of these (such as FA) have lower sensitivity which may impact upon the ability to make accurate conclusions. Many of the novel implications gained from this project’s DTI work would not have been possible if AD and RD had not been used in tandem with FA and MD. Further, Cre is implicated to be vulnerable to change following TBI. This finding, in conjunction with other recent research suggestive of the same thing, means that the technique of interpreting NAA and Cho changes through data ratioed to Cre is flawed. Other means of correcting the data (such as using water content as a stable reference point) should instead be sought so that metabolic changes may more accurately be examined.

An effort should also be made to conduct research now with a more direct aim of producing findings relevant to the treatment of patients with a TBI. Methods which take advantage of the brain’s ability to compensate for damage could be explored. For example, the nature of the focal and diffuse injury sustained in a TBI means that the pattern of neural damage is often similar between patients, which would be expected to also lead to similar patterns of cognitive deficit. It is feasible that long term deficit may therefore be predicted by identification of where acute damage has occurred. On this hypothesis, it could be directly examined if methods such as DTI could be used at the acute phase of injury in a prognostic manner concerning the patient’s cognitive outcome. If this is proved to be the case opportunities are then opened whereby more bespoke rehabilitation techniques may be provided to a patient at an earlier point in their recovery, potentially aiding the speed and efficiency with which they adapt. Related to this would be the use of other study techniques. Combining longitudinal fMRI and DTI data would allow for the examination into if locations of tract injury as identified by DTI analysis influenced network reorganisation as identified by fMRI analysis.

7.5 Concluding Remarks

This project has produced important data which sheds some light onto the neuro-psychological consequences of non-severe TBI. Strong evidence is presented that the physiological outcome of non-severe TBI are not comparable to severe cases.
These differing injury mechanisms may be identified by novel-appearing metric and metabolic changes such as increases in FA, NAA and Cre. Many of these changes also appear to affect the cognitive outcome of the patient. Therefore, future research should rightly consider non-severe injury as unique and investigate it as such.
Appendix A

Detailed Injury Characteristics of our Patient Group

Table A.1 (continued over the next three pages) gives individual injury characteristics for all of the patient group who took part in this study.
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Appendix B

Comparing the DTI Data of those Patients who did and did not return for Follow-up Testing

**Aims:** A further potential confound to the findings is the possibility of how effectively a patient recovered being an influencing factor to them when deciding whether or not to return for follow-up testing. In this way it’s feasible that those patients who did not return were the most mildly injured, therefore introducing bias into the data whereby the follow-up group could not be validly comparable to the acute group. To investigate this bias, the acute patient data was split into two groups defined by if that patient did or did not return for follow-up testing. These acute data were then subject to groupwise statistical comparisons against one another.

**Preliminary Methods:** TBSS was once again used to examine if there were any differences in the acute data between those patients who returned for the follow-up testing (here referred to as AFu, N=23) and those who were lost to follow-up (here referred to as AL, N=30). A comparison was also made between the AFu group and control data in as a means of testing if the main findings of this experiment (section) would be different if only those patients who returned at follow-up were used acutely. The same methods as before for the main analysis were used again for groupwise comparisons.

**Preliminary Results:** TBSS analysis revealed that AFu data is not representative of all patients when considered together. Figure B.1, below, shows AFu metric changes compared to controls. MD and AD still exhibit an increase however in contrast to the results concerning all patients, FA shows no change.
Preliminary Summary: These findings are suggestive that there may be an inherent physiological difference between those patients who did and who did not return for the follow-up examinations whereby the phenomenon of increased FA may be attributed to the AL group but not the AFu group. It is possible however that the
reduction in statistical power from splitting the acute group into two sub-groups has affected this analysis. It would also be beneficial to examine the exact spread of data in these patients groups at these locations. For these reasons, further testing was conducted.

**Follow-up Methods:** Although the TBSS findings show locations of significant change they do not allow for the magnitude of this change or the spread within this data to be examined. Therefore, an additional ROI analysis was conducted on the TBSS images to extract exact metric values from specific tract locations, allowing this information to be more closely examined and further statistical tests to be made. Two regions were chosen for this; the ascending fibres of the corpus callosum in the left hemisphere (as this was the location where the strongest FA changes had been observed at the acute time point in the main analysis), and the anterior forceps (as this was the location where the greatest FA difference between the AFu and AL groups had been observed).

MRicro was used to draw a ROI on each tract (axially) in the “mean_FA_skeleton” file that was produced by TBSS for the acute patient vs. control analysis. This was done at z co-ordinate 36 for the left corpus callosal fibres and z co-ordinate 9 for the anterior forceps after visual inspection ascertained that these slices were the most relevant with respect to the findings. It was decided that it would be advantageous for the ROI’s to also be applicable to the follow-up data so that the chronic data may potentially be investigated in the event of further developments deeming this necessary, although this did not occur. To accommodate this goal, the ROI was then loaded onto the “mean_FA_skeleton” file that was produced by TBSS for the chronic patient vs. control analysis and any redundant voxels, which were part of the underlying tract in the acute sample but outside the tract boundary at follow-up, were removed. This left an ROI of the tract in question which was applicable to both acute and chronic samples. These ROI’s are shown in Figure B.3, below. Average metric values were extracted from acute patient and control scans.
To further examine for differences between the AFu and AL sub-groups, all key cognitive test scores (Table 3.6) were first compared between these two groups to examine any difference in psychometric performance at baseline. These were done either by t-test or Mann-Whitney depending on if the control data for that test had previously found to be normal or not-normal, respectively (sections 3.6.2.1-3.6.2.9).

Acute patient FA for each ROI was then split into these sub-groups and compared against one another, and against control data; comparisons with one another would reveal potential metric differences between these sub-groups, while comparisons with the control group would reveal if the significant findings of the experiment would differ when considering these sub-groups separately. Any discrepancies found in these analyses was be further explored by appropriate groupwise testing with AD and RD values to determine the underlying effect driving the change. For any groupwise test involving a metric, control distribution was examined first by the Anderson-Darling test for normality. If data was normal t-tests were used for these analyses while if it was not then the Mann Whitney test were used instead. Regression analysis with VLF as the dependant variable and FA / NART as predictor variables was also repeated in each ROI for each sub-group, and scatterplots were produced so that the spread of data in each sub-group may be examined.

**Results – Cognitive Test Comparisons:** Groupwise comparison of cognitive performance between AFu and AL sub-groups showed one significant difference in
VLF, whereby the AFu group outperformed the AL group. All findings are shown in Table B.1 while the VLF finding is further visualised in Figure B.4.

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**Table B.1:** Findings from groupwise comparison of psychometric performance between AFu and AL patient sub-groups. Only VLF is significantly different.
In light of this finding, post-hoc analysis which compared AFu / AL VLF performance against Control data was conducted in order to see if both groups remained significantly different compared to controls, or if the initial finding of the acute patient group underperforming on the task was due to a disproportionate effect in only one of these subgroups. t-test analysis showed while the difference between AL VLF (Mean=27.5, SD=10.2) and Control VLF (Mean=41.63, SD=9.38) was significant (t(49)=-5.37, p<0.001), the difference between AFu VLF (Mean=36, SD=15) and Control VLF was not (t(34)=-1.58, p=0.125).

**Results - AFu / AL differences in the left corpus callosal fibres:** Statistical examination of the patient sub-groups by t-test demonstrated that the difference between AFu FA (Mean = 0.42, SD = 0.057) and control FA (Mean = 0.4, SD = 0.04) was not significant (t(36) = 1.47, p = 0.149), although t-test comparison between AL FA (Mean = 0.44, SD = 0.043) and control FA showed that AL FA was significantly increased (t(59) = 3.81, p < 0.001). Further testing indicated that this increase was due to a change in RD, which was found to be significantly decreased in both the AFu (Mean=0.00063, SD=0.00007, t(52)=-2.36, p=0.022) and AL
(Mean=0.00063, SD=0.00007, t(59)=-2.79, p=0.007) sub-groups compared to controls (Mean=0.00068, SD=0.00009). t-test comparison showed that FA in the AFu and AL sub-groups were not significantly different from one another (t(39) = -1.37, p=0.178).

Regression analysis indicated that neither FA of the AFu sub-group (R² = 22.92%, T=-2.02, p = 0.057) or the AL sub-group (R² = 17.52%, T=-0.56, p=0.584) held a significant relationship with VLF scores. These data are visualised in Figure B.5.

**Results - AFu / AL differences in the anterior forceps:** Statistical examination of the patient sub-groups by t-test demonstrated no significant difference between AFu / control data (AFu Mean/SD = 0.606/0.047, Control Mean/SD = 0.625/0.375, t(40)=-1.64, p=0.11), AL / control data (AL Mean/SD = 0.629/0.041, t(58)=0.44, p=0.665) or AFu / AL data (t(43)=1.91, p=0.063).

![Figure B.5](image-url)
Regression analysis also showed that neither FA of the AFu sub-group (R²=12.24, T=-1.07, p=0.297) or of the AL sub-group (R²=16.25, T=-0.04, p=0.97) held a significant relationship with VLF scores. A scatterplot combining this information is included below (Figure B.6) nevertheless so that the spread of the data may be visually inspected.

![Figure B.6](image)

*Figure B.6.* A Scatterplot with regression showing the distribution of FA in the AFu (green) and AL (red) patient groups, and their relation to VLF score. The black line shows the line of best fit for all acute patients as a single group. No regressions here are significant; the graph is included so that the spread of this data can be examined.

**Summary:** The significant difference in task performance on VLF is interesting. As this indicated that the AFu group outperformed the AL group, the direction of this is the opposite of what would be expected by the hypotheses of those patients declining to return being less impaired. The lack of significant differences being found in any other task also suggests that if there is a disparity in cognitive ability between these sub-groups, it does not represent a broad deficit. On balance, the idea that the returning patient sample may hold relatively cognitively superior abilities should not impact upon the findings of the study as a) there is a minority of cognitive facets
affected by this and b) as it is the sub-group which returned for further testing who appear to have superior functioning this implies that any evidence of injury found would be an underestimation of the damage incurred in mild TBI.

The majority of the ROI findings are fully supportive of there being no difference either between the sub-groups themselves, or the findings that comparisons between them and the control data produce. The only inconsistency lies with the AFu / control comparison in the corpus callosal fibres not reaching significance while the corresponding comparison with the AL group showed a true significant difference. However further testing indicated that the driving factor in this change (decreased RD) was similarly altered to a significant extent in both the AFu and AL groups. This implies that the same fundamental effect is present in each sub-group but may simply be more exaggerated in one. Again, as the patients who appear the most affected are those who did not return for further testing it also still stands to reason that the patients retained at the chronic time-point would give an underestimation of the effects of TBI. Finally, the TBSS finding of an FA difference between the AFu and AL groups in the anterior forceps (Figure B.2) was not repeated when examining the ROI data which also showed no difference. Examination of the spread of data between the sub-groups (Figures B.5 and B.6) also show no major differences. Each plot shows a very even distribution of values and similar relationships with VLF.

Concerning the corpus callosal fibre data here, it is interesting to note that AD was not found to be increased but RD was again found to be decreased, in contradiction with the main TBSS findings but in support of the tractography analysis of the same region (section 3.4.1.3). However, unlike that tractography analysis, data used here is directly derived from the values in the main TBSS experiment. As the main TBSS findings utilised the TFCE clustering technique, this disparity perhaps indicates that a very local effect of decreased RD exists in the region of the ascending corpus callosal fibres but that this is eliminated when also taking into account the changes in the surrounding areas.

ROI analysis of locations indicated by TBSS (Figure B.2) to contain differing FA results between AFu / AL therefore does not support that these groups are
significantly different from one another. The notion of reduced sample size causing the inconsistencies is further supported by clusters of MD and AD changes in the AFu group compared to controls (Figure A.1) to be reduced in size and number when compared with all patients. Further, examination of scatterplots does not show any skew in the FA distribution between AFu and AL groups. Finally, the only notable observation from these plots is a differing line of regression between FA and VLF in the AF, although regression analysis between these measures in this location have never formed a statistically significant result throughout the study. This still remains the case even when considering the AFu and AL groups separately. These analyses indicate that while some TBSS analysis suggests the AFu and AL groups are not entirely representative of one another, these non-conformities are most likely due to a lack of statistical power rather than an inherent difference between these patients. As such the validity of comparing the follow-up patient group against the acute patient group is not compromised.
Appendix C

The following pages include a joint first-author publication which was based on the DTI portion of this thesis.
White matter correlates of cognitive dysfunction after mild traumatic brain injury

Jain D. Creall, MPas* Christopher J.A. Cowie, MD* Jiabao He, PhD Anna Peel, MA Joshua Wood, BSc Benjamin S. Arribasala, PhD Patrick Michell, PhD A. David Mendelow, PhD Fiona E. Smith, PhD David Miller, DClinPsy Tom Kelly, PhD Andrew M. Blamire, PhD

Correspondence to Jain D. Creall j.d.creall@nwmc.nhs.uk

ABSTRACT

Objective: To relate neurophysiologic changes after mild/moderate traumatic brain injury to cognitive deficit in a longitudinal diffusion tensor imaging investigation.

Methods: Fifty-three patients were scanned on average of 6 days postinjury (range = 1–14 days). Twenty-three patients were rescanne 1 year later. Thirty-three matched control subjects were recruited. At the time of scanning, participants completed cognitive testing. Tract-Based Spatial Statistics was used to conduct voxel-wise analysis on diffusion changes and to explore regressions between diffusion metrics and cognitive performance.

Results: Acutely, increased axial diffusivity drove a fractional anisotropy (FA) increase, while decreased radial diffusivity drove a negative regression between FA and Verbal Letter Fluency across widespread white matter regions, but particularly in the ascending fibers of the corpus callosum. Raised FA is hypothesized to be caused by astroglisis and compaction of axonal neurofilament, which would also affect cognitive functioning. Chronically, FA was decreased, suggesting myelin sheath disintegration, but still regressed negatively with Verbal Letter Fluency in the anterior forces.

Conclusions: Acute mild/moderate traumatic brain injury is characterized by increased tissue FA, which represents a clear neurobiological link between cognitive dysfunction and white matter injury after mild/moderate injury. Neurology® 2014:83:494–501

GLOSSARY

AD = axial diffusivity, DAI = diffuse axonal injury, DTI = diffusion tensor imaging, FA = fractional anisotropy, GCS = Glasgow Coma Scale, MD = mean diffusivity, NART = National Adult Reading Test, RD = radial diffusivity, TBI = traumatic brain injury, TBS = Tract-Based Spatial Statistics, TE = echo time, TR = repetition time, VLF = Verbal Letter Fluency.

Diffuse axonal injury (DAI) resulting from traumatic brain injury (TBI) has been previously studied using diffusion tensor imaging (DTI). This research has led to a general consensus that after injury, white matter structural damage leads to increased water mobility (increased mean diffusivity [MD]) and decreased directionality (decreased fractional anisotropy [FA]). However, previous work predominantly examined the effect of severe and chronic injury, with disproportionately few studies focusing on patients with mild TBI, despite this patient demographic representing approximately 90% of TBI cases. Although studies focusing on acute, mild TBI often report similar results (e.g., see references 10–12), others have notably reported increased FA and decreased MD, contrary to these main observations in severe injury. Those observations suggest a different pathophysiologic of tissue damage that might follow mild injury. Cytotoxic edema, the presence of which has previously been suggested to lower MD, is the primary hypothesized mechanism for these results, although a recent histopathologic investigation has indicated that astroglisis may also cause acute FA increases in mild TBI. Greater understanding of the effects of mild injury on

*These authors contributed equally to this work.

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Go to Neurology.org for full disclosures. Funding information and disclosures relevant to the authors, if any, are provided in the article.
white matter integrity and its impact on clinical presentation requires longitudinal investigation coupled with assessment of cognitive function.

Herein, we present a comprehensive longitudinal DTI study in a predominantly mild patient population. We hypothesized that poor cognitive performance would be directly related to white matter tract damage and therefore used Tract-Based Spatial Statistics (TBSS) to examine DTI metric changes between acute and chronic time points and to explore how these relate to acquired cognitive deficits after TBI.

METHODS Participants. Fifty-three patients with TBI were recruited from patients attending the Accident and Emergency and Neurosurgery departments of the Newcastle upon Tyne Hospitals Trust (age 16–68 years, mean = 33.9, SD = 14.6, sex: 44 males, 9 females). Of these, 44 had a mild TBI (Glasgow Coma Scale [GCS] score 13–15) and 9 had a moderate TBI (GCS score 8–12). Thirty-three control subjects matched for age, sex, and level of education were recruited for comparison. Patients had no history of neurological/psychological problems post-TBI, no history of substance abuse, and no contraindications pertaining to MRI. Patients were studied as outpatients and underwent MRI a mean of 6 days postinjury (SD = 3.2, range = 1–14 days). Of the initial cohort, 25 patients (18 mild, 7 moderate) returned for a follow-up scan 1 year later (57–424 days after initial scan, mean = 262, SD = 22.2 days), with the remaining patients either declining to return (n = 4) or lost to follow-up (n = 20). Full participant demographic information is shown in table 1.

Standard protocol approvals, registrations, and patient consents. The study was approved by the local research ethics committees and all subjects provided written informed consent.

Data acquisition. Participants were scanned using a 3T Philips Achieva MR scanner with an 8-channel SENSE head coil [Philips Medical Systems, Best, the Netherlands]. A 3-dimensional T1-weighted sequence was used to obtain regional anatomy (magnetization-prepared rapid-acquisition gradient echo, repetition time [TR] = 8.1 milliseconds, echo time [TE] = 4.6 milliseconds, matrix size 240 × 216 × 130, isotropic 1-mm resolution). DTI scans used a single-shot echo-planar imaging diffusion sequence (TR/TE = 2,524/71 milliseconds; 24 diffusion directions; b = 0; 1,000 s/mm^2; 16 diffusion directions; 2 × 2 × 2 mm^3 resolution). A magnetic field map sequence was acquired for correction of geometric distortion from the DTI scans (single-echo gradient recalled echo, TR = 57 milliseconds, TE = 1.661 milliseconds, matrix 128 × 128 × 72, 2-mm resolution). The scan protocol also included measurements of cerebral blood flow, quantitative T1 and T2 mapping, and brain metabolism, which are not reported here. Participants were examined with a comprehensive neuropsychological test battery at each time of scanning, or within 7 days if the patient was unable to tolerate both in the same sitting (this was the case for 11 patients who were tested a mean of 2.6 days after their scan). A full list of tests included is available in [Methods] on the Neurology® Web site at Neurology.org here, only Verbal Fluency (VFL) and the National Adult Reading Test (NART) were considered in detail because these were the most informative under the analysis we conducted.

DTI data analysis. DTI scans were processed using the FSL (FMRIB’s Software Library) toolbox for analysis using TBSS (see [Methods]). TBSS was used to examine differences in acute DTI metrics (FA, MD, axial diffusivity [AD], and radial diffusivity [RD]) in a between-subjects centered t test patient analysis. DTI metrics at follow-up were also compared between patients and control subjects in all TBSS analyses, threshold-free cluster enhancement output images were used with p < 0.05 taken as significant.

Neuropsychological data analysis. Acute and follow-up patient task performance was compared with controls using between-samples t test analysis (Bonferroni-corrected). Multiple regression analysis was then conducted using group (patient/control) and NART (as a measure of premorbid IQ) as predictor variables for each task. TBSS was used to perform regression analysis of VFL task scores against FA, MD, AD, and RD at the acute and follow-up times and in controls and patients separately, controlling for IQ using NART. Seven patients were excluded mostly and 2 patients were excluded chronically from regression analysis because they were unable to complete both VFL and NART tasks for reasons relating to their injury. In addition,

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Participant demographic Information</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 33)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>24/9</td>
</tr>
<tr>
<td>Age at time of acute scan, y, mean (SD)</td>
<td>40.94 (15.56)</td>
</tr>
<tr>
<td>Time from injury to acute scan, d, mean (SD), minimum/maximum</td>
<td>—</td>
</tr>
<tr>
<td>GCS median score</td>
<td>—</td>
</tr>
<tr>
<td>Average duration of LoC, min, mean (SD)</td>
<td>—</td>
</tr>
<tr>
<td>Average duration of PTA, h, mean (SD)</td>
<td>—</td>
</tr>
<tr>
<td>Time from injury to chronic scan, d, mean (SD)</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: GCS = Glasgow Coma Scale; LoC = loss of consciousness; PTA = posttraumatic amnesia.
The analyzed white matter skeleton is shown in green with color overlay of significant differences. All changes are increases (colored red/yellow) in the patient group compared with controls. The z coordinates are based around the anterior commissure/posterior commissure line being z = 0. The RD row shows no differences but is included for comparison with Figure 2. Color bar: values are 1 - p value, AD = axial diffusivity, FA = fractional anisotropy, MD = mean diffusivity, RD = radial diffusivity, TBSS = Tract-Based Spatial Statistics.

The psychometric data for one control participant were absent for all tasks, reducing the control group to 12 for relevant testing.

RESULTS Acute groupwise differences in diffusion metrics. At the acute time point, groupwise patient vs control comparison revealed multiple, widespread locations of increased MD, FA, and AD in the patient group. There were no locations that showed a significant difference in RD (Figure 1, Table 2).

Chronic groupwise differences in diffusion metrics. Comparison of follow-up patients (12 months postinjury) with control subjects again showed groupwise differences but with different imaging characteristics compared with those found at the acute time point. While MD remained significantly increased, patient FA was now decreased. In addition, patient AD showed no significant differences in any location, while RD was now increased (Figure 2, Table 2).

Acute cognitive testing and TBSS analysis. Full results of patient vs control task performance and regression analysis for the acute and chronic time points are available in Table e1. Despite recruiting control subjects matched to the patient population for duration of education, there was a significant difference in NART scores (control mean = 112.03, SD = 8.98, patient mean = 100, SD = 13.1, t = 4.38, p < 0.001). NART was therefore also included with group as predictor variables in multiple regression analysis. At the acute time point, patient VLF score (mean = 31.6, SD = 13.2) was significantly worse compared with controls (mean = 41.63, SD = 9.38, t = 3.95, p < 0.001). Multiple regression analysis indicated that group and NART explained 25.1% of the variance (F = 0.251, F_{2,17} = 12.54, p < 0.001). It was found that NART significantly predicted VLF performance (p = 0.001) while group was approaching significance (p = 0.075).
Table 2  Summary of results from groupwise and regression analysis

<table>
<thead>
<tr>
<th>Fractional anisotropy</th>
<th>Mean diffusivity</th>
<th>Radial diffusivity</th>
<th>Axial diffusivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Groupwise DTI findings</strong>&lt;br&gt;vs control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Acute</strong></td>
<td>↑ Ascending CC and assoc. fibers</td>
<td>↑ Posterior CC (splenium) and assoc. fibers</td>
<td>No difference</td>
</tr>
<tr>
<td><strong>Chronic</strong></td>
<td>↓ Anterior fornix</td>
<td>↑ Posterior CC (splenium), assoc. fibers, and anterior forceps</td>
<td>↑ CC, thalamus projections assoc. fibers, and anterior forceps</td>
</tr>
<tr>
<td><strong>DTI vs VLF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Acute</strong></td>
<td>↑ CC, ascending CC fibers, and assoc. fibers correlates with poorer VLF score</td>
<td>↑ CC and assoc. fibers correlates with better VLF score</td>
<td>↓ CC, ascending CC fibers, and assoc. fibers correlates with poorer VLF score</td>
</tr>
<tr>
<td><strong>Chronic</strong></td>
<td>↑ CC, correlates with poorer VLF score (although less widespread than acutely)</td>
<td>No regression</td>
<td>No regression</td>
</tr>
</tbody>
</table>

Abbreviations: assoc. = associated; CC = corpus callosum; DTI = diffusion tensor imaging; VLF = Verbal Letter Fluency; WM = white matter. Arrows indicate whether the given metric is increased/decreased in patients compared with controls. “Groupwise” results describe all locations found to change while “DTI vs VLF” describes only locations found to regress with VLF.

Despite this, TBSS analysis indicated that VLF scores (controlled for NART) negatively regressed with patient FA (worse performance, lower score, associated with higher FA) in widespread locations. Patient MD and RD were also found to hold positive regressions with VLF. Acute VLF score and patient AD did not demonstrate any locations of regression (figure 2, table 2). The control group did not show any significant regression between any diffusion metric and VLF performance, indicating that the variance in patient VLF score is attributable to their acquired injury.

**Chronic cognitive testing and TBSS analysis.** Follow-up patient VLF performance was not significantly different from control. Multiple regression analysis showed that group and NART now explained 14.4% of the variance (R² = 0.144, F₄,₂₅₉ = 4.19, p = 0.002). In VLF performance, NART significantly predicted VLF performance (p = 0.01) while group did not. Despite this, when controlling for NART, negative regressions between FA/VLF were still found after TBSS analysis. AD was also found to negatively regress with VLF in one small location while RD/MO did not regress with VLF (figure e-1, table 2).

**DISCUSSION** DTI has been used to study the white matter tracts in TBI. Injury and degeneration are generally considered to disrupt tissue structure at the cellular level, leading to increased MD and decreased FA. However, these findings come from a body of research weighted toward severe and chronic patients, while mild injury is by far the most common. Our study in a large cohort of patients with mild and moderate TBI has shown extensive increases in MD but these were accompanied by increased FA at the acute time point. These changes were strongly correlated with underperformance on cognitive testing and show a clear neurological basis for dysfunction postinjury.

Although elevated FA post-TBI is an unusual finding in the wider neuroimaging literature, similar observations have been reported in a minority of other small-scale imaging studies, particularly in patients with acute, mild TBI (e.g., see references 13-15, 21-23) and has been hypothesized to be due to the formation of edema. However, both in experimental models and clinical studies in TBI, the presence of cytotoxic edema is also associated with reduced MD (or its alternative metric apparent diffusion coefficient). In the current study, MD was either unchanged or increased. In addition, our groupwise increases in FA were associated principally with elevated diffusion along the axon (increased AD), while RD was unchanged. In previous studies reporting increased FA, RD was typically reduced where reported. Cytotoxic edema therefore does not explain our observations.

An alternative explanation may lie in the findings of other recent work that has suggested astroglisis as a possible cause of acute FA increases. After injury, fibrous astrocytes often undergo reactive astroglisis migrating to the site of injury, locally increasing the density of these cells. It was reported that the organization of these cells increased the AD within the affected tissue, while RD remained unchanged, thus increasing the measured FA. Although MD was not reported, this increase in AD (without any change in RD) would also increase the reported MD. Our observations are therefore more consistent with astroglisis and with the known chronology of the immune response after CNS injury; and therefore we consider astroglisis to be the most likely underlying cause of...
Figure 2  TBSS outputs demonstrating locations of metric changes after traumatic brain injury at the chronic time point.

Increases in the patient group are shown in red, and decreases in the patient group are shown in blue. The AD row shows no differences but is included for comparison with Figure 1. Color bar values are $1 - p$ value. AD = axial diffusivity; FA = fractional anisotropy; MD = mean diffusivity; RD = radial diffusivity; TBSS = Tract-Based Spatial Statistics.

Our finding of increased acute FA. Future research could expand to using the recent method of diffusional kurtosis in conjunction with DTI. Studies have shown that this combination brings increased sensitivity in detecting cognitively relevant physiologic change and astrogliosis compared with DTI alone.

Patients were found to underperform on the VLF task in the acute phase after injury with their performance strongly and negatively regressing with white matter FA and positively regressing with RD and MD. These regressions were located across widespread areas of the white matter tracts and particularly within the ascending fibers of the corpus callosum in the left hemisphere. This finding supports previous work that has shown the VLF network of healthy individuals to involve frontal, parietal, and temporal locations with an emphasis in the left hemisphere.

While the groupwise increase in FA was mainly caused by increased AD, the relationship with cognitive performance was driven by a positive regression between RD and VLF, which implies the presence of a subtle reduction in RD. The contrasting observation that RD was not significantly different in the acute injury phase between the patient and control groups in groupwise analysis, but reduced RD was associated with differences in functional performance between patients, is likely attributable to differing statistical power between tests. The variance of RD values explained by regression within the acute patient group is large, whereas the difference in mean RD values between the 2 groups is small, making regression within groups more powerful than between-group comparisons.

Within the axon, undamaged neurofilament has been shown to be related to both larger axon diameter and more efficient conduction velocity, while DAI is associated with disruption of the neurofilament, involving loss of neurofilament sidearms via...
trauma-induced proteolysis. We therefore postulate that reduced axonal diameter may contribute to altering both diffusion properties (reducing RD and increasing FA) and to the deficit in VLF performance, which becomes more severe at RD decreases.

Diffusion imaging observations at 12-month follow-up supported the wider literature in chronic brain injury with patterns of an FA decrease driven by an underlying RD increase, while MD was increased extensively and AD was unchanged. Increased RD is evidence that the axonal membrane and myelin sheath have disintegrated. The reductions in FA were observed principally in the anterior forceps, suggesting that this region has experienced the most severe long-term damage after TBI. This finding is supported by the fact that anterior callosal fibers are known to be sensitive to DAI and damage to them relates to long-term prognosis. It should be noted that at both time points, locations of increased MD always overlapped with underlying increases of AD/RD. Therefore, while MD is one of the simplest diffusion metrics to measure and may therefore have a role in the general detection of tissue injury, our data demonstrate that interpretation of altered MD requires full assessment of the diffusion eigenvectors.

Chronically, patient performance on VLF was not significantly different from that of the controls, suggesting a degree of recovery regarding cognitive performance. However, negative regressions between FA and VLF were still found. These chronic regressions presented primarily in the body of the corpus callosum and the anterior forceps as opposed to the more diffuse pattern seen acutely. This shift of location to connecting fibers between the 2 hemispheres could be indicative of network reorganization to increase right hemisphere involvement as a compensatory mechanism, as has been demonstrated previously.

Examining AD and RD did not provide evidence that either was specifically responsible for the chronic relationship between performance and FA, implying that relatively slight changes in each are contributing to this finding. The finding that decreased FA was associated with better VLF performance in the chronic phase is one that is challenging to explain because of its apparent contradiction to counterpart group-wise findings in the same tract location that indicate FA to decrease at a result of damage, and also its relative novelty regarding the wider literature. Many events are known to occur in the chronic phase of injury, such as a relative increase in the proportion of axons with smaller diameters, glial scarring, and network
reorganization, all of which may contribute to an effect whereby the patients with the greatest VLF deficit have the highest FA, despite FA being generally reduced compared with controls. Further investigations are required to determine the exact mechanisms behind this finding, but it is highlighted as an avenue for future research.

Our study has several limitations. First, the loss of patients to follow-up reduced our statistical power to detect longitudinal change. Patients either declined to return for further testing (4/53) or were lost to further contact (26/53). We attribute these losses to the mild nature of our participants’ injuries (possibly making them less inclined to devote further time to the study), and the relatively long (1-year) gap between assessments. It should be considered whether those patients returning at 12 months were either likely to have greater ongoing symptoms than those who did not return, or be influenced by socioeconomic or medical-legal factors (whereby a potential diagnosis of long-term damage or ongoing cognitive dysfunction may be seen as advantageous). This type of patient self-selection is difficult to quantify and cannot be ruled out in this cohort. We evaluated whether there was any difference in injury severity or cognitive deficits at baseline between those subjects who subsequently did or did not return for follow-up. Only verbal fluency performance was significant ($p = 0.04$), but paradoxically, those who did not return had worse performance, contradicting the hypothesis that the returning patients were more severely affected.

A proportion of our patient group was also unable to complete psychometric testing for reasons pertaining to their injury (12% acutely and 9% at follow-up), reducing the sample size for the regression analyses. Because our hypothesis was that cognitive dysfunction is secondary to microstructural damage, those patients who were unable to complete the tests were expected to have the greatest microstructural damage. Excluding such individuals would then bias against detecting an effect, suggesting that our observations are a lower estimate of the importance of white matter injury on cognitive performance after mild TBI.

Finally, our mixed mild and moderate cohort is a potential confound for understanding the effect of mild TBI alone. Comparison of the patient groups (mild vs moderate) did not show any significant differences in any diffusion metric. Furthermore, in comparison to healthy subjects and during regression against VLF performance, exclusion of the moderate patients did not change the direction or distribution of any findings (other than a reduction in statistical power expected with the smaller group size: data using the mild patients only are presented in figures e2 to e5). Finally, only 2 of 9 patients with moderate injury had GCS score less than 10, so this moderate group is toward the milder end of injury. The findings are therefore consistent with revealing the microstructural changes associated with mild injury severity.

This longitudinal study has produced a comprehensive picture of the diffusion imaging changes after mild/moderate TBI and how these can relate to cognition in a large cohort of patients. Detailed analysis of the complete set of diffusion metrics suggests that gliosis rather than cytotoxic edema is most consistent with changes in these metrics after acute, mild TBI. We have also further quantified acute findings by identifying a proportional relationship with verbal fluency, which we have shown persists into the chronic injury phase, at a more subtle but still detectable level. The importance of investigating the component parts of FA is also shown by groupwise regression findings in the acute phase that would have proved conflicting had they not been shown to be separately driven by AD and RD, respectively. The full potential of DTI to detect different physiologic changes resulting from TBI, and to show which among these is affecting cognition, is highlighted while avenues for future research are also indicated.

AUTHOR CONTRIBUTIONS

I.D. Cowell and C.J. A. Cowen: design of study, analysis and interpretation of data, drafting and revising the manuscript. J. He: design of study, interpretation of data, revising the manuscript. A.彭 and J. Wood: analysis and interpretation of data, revising the manuscript. B.S. Arbital: design of study, analysis of data, revising the manuscript. F. Mitchell and A.D. Magnus: design and conceptualization of study, interpretation of data, revising the manuscript. F.E. Smith: interpretation of data, drafting and revising the manuscript. D. Miller and T. Koll: design of study, analysis of data, revising the manuscript. A.M. Blumen: design and conceptualization of study, interpretation of data, drafting and revising the manuscript.

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DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

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