QUANTITATIVE MAGNETIC RESONANCE IMAGING IN TRAUMATIC BRAIN INJURY

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Abstract

Mild traumatic brain injury (TBI) may be complicated by long term cognitive and affective symptoms. Conventional imaging findings often do not correlate with the clinical picture in these patients, and frequently underestimate the extent of damage. Quantitative MR imaging techniques are sensitive to microstructural damage in brain grey matter (GM) and white matter (WM) which appear uninjured on conventional MRI. Previous work has predominantly evaluated their use in acute TBI in moderate and severely injured patients, or in chronic TBI across the severity spectrum.

This thesis explored the application of quantitative T1 (qT1) and quantitative T2 (qT2) relaxometry and diffusion tensor imaging (DTI) in the *acute* evaluation of 44 mild and 9 moderate TBI patients in whom neuropsychological assessment had been performed, and compared the results to those of 30 matched control subjects. By combining the scan data with results from the cognitive testing, this work sought to identify correlations between regions of detectable microstructural damage and the neurocognitive functions related to them.

Differences between groups were observed in whole brain normal appearing GM in qT1, and in frontal lobe normal appearing GM and WM in qT1 and DTI measures. Differences were also observed in memory performance and executive function between patients and control subjects which correlated with injury severity. Significant negative correlations were revealed between whole brain WM qT1 time and executive function and negative correlations were shown between frontal and left temporal GM qT1 time and both memory performance and phonemic fluency. Also demonstrated were a positive correlation between frontal GM MD and phonemic fluency, and a negative correlation between frontal GM FA and both memory and executive function. Lastly, increases in WM FA in the corpus callosum, corona radiata, superior longitudinal fasciculus and cingulum were shown to negatively correlate with all components of verbal fluency.

This work has demonstrated, using quantitative MR imaging, acute differences at a microstructural level between TBI patients and matched control subjects, in tissue appearing normal on conventional imaging. Furthermore, it has shown that these changes correlate with post-concussive cognitive deficits. It is likely that these changes represent damage as a result of traumatic brain injury in the regions responsible for the cognitive functions found to be impaired.

To my wife Emma,

and our beautiful boy, Thomas

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List of Abbreviations

2D	two dimensional
3D	three dimensional
3T	3 tesla
AAN	American Academy of Neurology
AC-PC line	a line between the anterior and posterior commissure
ADC	apparent diffusion coefficient
AMIPB	Adult Memory and Information Processing Battery
APP	amyloid precursor protein
ASL	arterial spin labelling
BET	brain extraction tool
BIRT	Brain Injury Rehabilitation Trust
BMIPB	BIRT Memory and Information Processing Battery
D-KEFS	Delis-Kaplan Executive Function System
DSPAN back	digit span backwards
CDC	Centers for Disease Control
CLEAR	coupled and linked equations algorithm
CNS	central nervous system
CSF	cerebro-spinal fluid
CT	computed tomography
DAI	diffuse axonal injury
DTI	diffusion tensor imaging
DTIFIT	FMRIB's diffusion tensor model fitting tool
DVI	diffuse vascular injury
DWI	diffusion weighted imaging
EPI	echo planar image
FA	fractional anisotropy
FLAIR	fluid attenuated inversion recovery
FLIRT	FMRIB's linear image registration tool
fMRI	functional magnetic resonance imaging
FMRIB	Oxford Centre for Functional MRI of the Brain
FUGUE	FMRIB's utility for geometrically unwarping EPIs
GCS	Glasgow Coma Scale
GCSE	General certificate of secondary education

GM	grey matter
GOS	Glasgow Outcome Scale
GP	General Practitioner
GRASE	gradient spin echo
GRE	gradient recalled echo
HES	hospital episode statistics
ICD-10	International classification of diseases (10^{th} edition)
ICP	intra-cranial pressure
IQ	intelligence quotient
IR	inversion recovery
LOC	loss of consciousness
MD	mean diffusivity
MPRAGE	magnetisation prepared rapid gradient echo
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
mTBI	mild traumatic brain injury
MVA	motor vehicle accident
NART	national adult reading test
NICE	National Institute for Clinical Excellence
PASAT	paced auditory serial addition test
PCS	post-concussive symptom(s)
PGSE	pulsed gradient spin echo
PRELUDE	FMRIB's phase region expanding labeller for unwrapping discrete estimates
PTA	post-traumatic amnesia
qT1	quantitative T1
qT2	quantitative T2
RANDOMISE	FMRIB's permutation-based nonparametric inference tool
RAVLT	Rey Auditory Verbal Learning Test
RCFT	Rey Complex Figure Test
ROI	region of interest
RTA	road traffic accident
SD	standard deviation
SENSE factor	sensitivity encoding

SPECT	single photon emission computed tomography
SoIP	speed of information processing
TBI	traumatic brain injury
TBSS	tract based spatial statistics
TE	echo time
TI	inversion time
TR	repetition time
UK	United Kingdom
US	United States
WAIS	Wechsler Adult Intelligence Scale
WHO	World Health Organisation
WM	white matter
WNV	Wechsler Non-Verbal scale of ability

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Chapter 1. Traumatic Brain Injury

1.1 Background

Head injury is an important cause of death and disability in adults. Each year in the UK more than 112,000 people are admitted from accident and emergency departments with a primary diagnosis of head injury (NICE, 2007). It is ranked fourth as a cause of death in the developed world, and the number of people sustaining head injuries increases yearly. Around 90% of admissions for head injury are classed as mild (or minor), with the remainder divided equally between moderate and severe injuries. The majority of patients with mild head injury are discharged quickly and return to work within weeks or months. However, a considerable number continue to have persisting difficulties related to their injury, including problems with concentration and memory, which affect not only their ability to return to work, but also their personal life (Nolin and Heroux, 2006). It is important to identify those patients who are vulnerable to persisting neuropsychological problems, and it would be helpful to be able to predict, using brain imaging, which patients will develop them. This would allow the patients and their relatives to appreciate the difficulties they may face, and allow early input from a multidisciplinary rehabilitation team with a long term view of improving final outcome.

1.2 Definition of Head Injury

The term head injury is used widely, both by clinicians and lay people, and a search of the literature shows there is no agreed definition. Taken literally it ought to describe an injury to any part of the anatomy above the neck, and so include scalp injuries, facial injuries, injuries to the eyes, teeth etc. However, it has generally been used to mean an injury to the brain *only*, and maxillofacial injuries have been considered separately. Studies published on head injury have defined it in different ways. Inclusion criteria have included: patients with a blow to the head; patients with altered consciousness following a relevant injury; patients with a scalp laceration; patients who underwent a skull x-ray; patients with any injury resulting in skull fracture or unconsciousness; patients with amnesia, seizure or neurological deficit following injury (Jennett and Teasdale, 1977, Klauber et al., 1981, Lagares et al., 2009). More recently the term ‘Traumatic Brain Injury’ (TBI) has been adopted, both to prevent ambiguity and to

indicate that the injury has the potential to cause neurological deficit. TBI has become recognised as a form of brain injury that differs from ischaemic and other causes of brain injury. So, although the words "traumatic" and "injury" are duplication, they serve to focus on trauma. Traumatic brain injury is defined as **an insult *to the brain* caused by an external mechanical force, and one which is not the result of congenital or degenerative damage**.

1.3 Incidence of Head Injury

In 2001 a review paper on head injury in the United Kingdom was published (Kay and Teasdale), stating that over 1 million patients are estimated to attend accident and emergency departments in the UK with a 'head injury' each year. Of these, the authors comment that approximately 20% are thought to require admission, with only 5% ultimately requiring neurosurgical care. However, the 2003 NICE guidelines on head injury state that there were 112,978 admissions to hospitals in England with a primary diagnosis of head injury recorded in the 2000/2001 annual dataset (NICE, 2007). If, as stated by Kay and Teasdale, 20% of hospital attendees require admission, then even taking into account admissions in Wales, Scotland and Northern Ireland, 1 million is a slight overestimation of the incidence of head injury, and the figure would be more likely to be approximately 600,000 or 700,000.

The figure of 112,978 admissions quoted by NICE was obtained from hospital episode statistics (HES) by collating total admission numbers for those with a primary diagnosis code of S00 – S09 using the ICD-10 classification of the World Health Organization (WHO). However, these primary diagnosis codes encompass a number of other injuries; including those to the scalp, ligaments, face, eyes, ears and teeth, and none of these indicate a brain injury per se. Analysis of the HES for 2009/2010 (The Health and Social Care Information Centre, 2011) using specifically the primary diagnosis codes considered most likely to indicate brain injury rather than simply including all those with injury above the neck, reveals a figure of **19,633** for admissions with traumatic brain injury. This was calculated using the codes below (Table 1.1).

ICD-10 code	Primary diagnosis	code + suffix	Description	Admissions:
S06	Intracranial injury	S060	Concussion	2,383
		S061	Traumatic cerebral oedema	110
		S062	Diffuse brain injury	1,826
		S063	Focal brain injury	1,152
		S064	Epidural haemorrhage	1,052
		S065	Traumatic subdural haemorrhage	5,887
		S066	Traumatic subarachnoid haemorrhage	1,776
		S067	Intracranial injury with prolonged coma	0
		S068	Other intracranial injuries	1,289
		S069	Intracranial injury, unspecified	469
S02	Fracture of skull and facial bones	S020	Fracture of vault of skull	1,150
		S021	Fracture of base of skull	1,644
		S027	Multiple fractures involving skull & facial bones	433
		S029	Fracture of skull & facial bones unspecified	361
S04	Injury of cranial nerves			88
S07	Crushing injury of head			13
			Total:	**19,633**

Table 1.1: World Health Organisation ICD-10 codes most likely to indicate brain injury and the numbers of those admitted with that code in England in the year 2009/2010. Source: *HES Online 2010* (re-used with the permission of The Health and Social Care Information Centre.)

Another recent review of traumatic brain injury epidemiology (Bruns and Hauser, 2003) comments that incidence specifically of *traumatic brain injury* is quoted as being approximately 200 in every 100,000 people in developed countries, although the authors do note that this estimate is typically based only upon admissions figures. Extrapolating this to the current population of 62 million would make the UK incidence of TBI 124,000 per year, which is similar to the figure quoted by NICE, but as shown above this may be a slight overestimate.

As can be seen from the evidence above, incidence of brain injury is difficult to quantify, and this is due to a number of factors. In epidemiological studies, data on total numbers of head injuries is obtained from different sources: death certificates, coded hospital admission records and GP records. Data from one of these sources alone may underestimate the total number: if the injury is mild, the sufferer may not present to an accident and emergency department at all. In cases which *are* admitted to hospital where the brain injury is in conjunction with other injuries, the hospital coding on discharge may not record the 'minor' head injury and only reflect the other injuries sustained. Death certificates may also omit a reference to brain injury in cases where the patient dies as a result of other injuries. If studies combine data from multiple sources they risk overestimating the total number of head injuries. Brain injuries referred to a

hospital by a general practitioner may be counted twice if both GP and hospital records are being used to obtain data (Evans, 2006).

1.4 Age

Incidence of TBI evaluated by age typically shows a trimodal distribution, with peaks in young children, in adolescents/young adults and also in the elderly (Bruns and Hauser, 2003). The peak in the elderly is accounted for by the high rate of falls in this population sub-group (Rickels et al., 2010), whilst the peak in adolescents/young adults (predominantly male) is explained by the large percentage of this group that are involved in sports and recreational activities, and the high rate of RTAs (both motor vehicle and bicycle accidents) involving people this age (Crowe et al., 2010). TBI in young children is due in the majority to falls before the age of 4 and RTAs above the age of 4, but in contrast to adolescents, the sufferers are usually pedestrians or bicyclists, rather than vehicle occupants (Durkin et al., 1998).

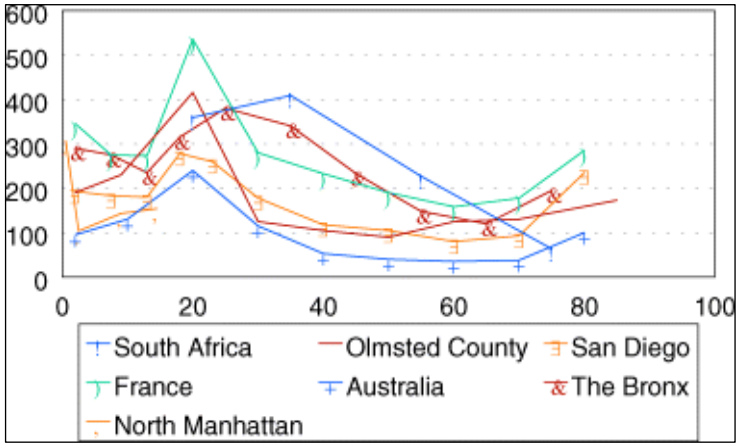

Figure 1.1: Plot of age specific TBI incidence data from seven TBI studies included in a recent review. The vertical axis represents incidence per 100,000 and age is shown on the horizontal axis (Bruns and Hauser, 2003).

1.5 Sex

Head injury is more common in men than in women, with 72% of the quoted 112,978 admissions in England in the year 2000/2001 being male (NICE, 2007). A recent analysis of death certificate data from 1997-2007 from the Centers for Disease Control (CDC) in the US, shows that the rate of TBI deaths was three times higher in males than

in females, with males making 28.8 per 100,000, compared to 9.1 females per 100,000 (Coronado et al., 2011). Interestingly, when that data is stratified by age, the preponderance of TBI in males increases to greater than four times that of females in the 20-24 age range.

1.6 Prevalence of Head Injury

In 2005 there were approximately 3.5 million people surviving a head injury with a disability in the US (Reilly and Bullock, 2005). Estimating the prevalence of TBI is also complicated. Depending upon the severity of the injury, the resulting symptoms and sequelae can last for a few hours, or for a lifetime. As such, calculation of prevalence must take into account not only patients in hospital, but those in rehabilitation units, nursing homes and their own homes. These patients may have a wide variety of symptoms and disabilities, ranging from those in a persistent vegetative state following survival of a severe injury to those with features of 'concussion' in mild TBI. In patients with mild TBI, the symptoms are typically short-lived, but can last for months and even years. Recent studies (Thornhill et al., 2000) have shown that more patients (>50%) suffer long term problems following minor head injury than had previously been thought (Table 1.2).

Glasgow Outcome Scale	Mild TBI (n=333)	%
Severe disability	71	21
Moderate disability	100	30
Good recovery	162	49

Percentage of patients with specific problems		%
Activities of daily living	In home	22
	Outside	34
Physical		58
Mental	Cognitive	43
	Mood	47

Table 1.2: Outcome for patients with mild TBI at 1 year and problems reported. Activities of daily living were: in home - eating, dressing, telephoning, housework; outside - shopping, transport, leisure; physical - vision, hearing, fits, sleep, tiredness, balance, headache, speech; cognitive - decision making, memory, concentration; mood - anxiety, pressure, depression, irritability, temper (Thornhill et al., 2000).

1.7 Classification

The most common method of classifying traumatic brain injury in the clinical setting is by severity. This is usually performed using the Glasgow coma scale (GCS) which was devised by Teasdale and Jennett (1974). Assessments are made, typically 30 minutes post-injury, of the best scores from verbal, eye and motor assessments of the head injured patient, and a total is calculated between 3 and 15 (Table 1.3). The brain injury is then classed as mild (GCS 13-15), moderate (GCS 9-12) or severe (GCS 3-8). The GCS provides an objective assessment that is reproducible, it allows for easy monitoring of deterioration or improvement and it is a useful standard when describing the patient's neurological state.

The Glasgow Coma Scale		Score
Eye opening	Spontaneous eye opening	4
	Eye opening to voice	3
	Eye opening to pain	2
	No eye opening	1
Verbal response	Normal speech, orientated	5
	Normal speech, disorientated	4
	Abnormal speech	3
	Incomprehensible sounds	2
	No verbal response	1
Motor response	Obeys commands	6
	Localises to pain	5
	Flexion withdrawal to pain	4
	Abnormal flexion to pain / Decorticate posturing	3
	Extension to pain / Decerebrate posturing	2
	No response	1

Table 1.3: The Glasgow Coma Scale (Teasdale and Jennett, 1974).

1.8 Definition and Classification of Mild TBI

As described above, the standard definition of mild traumatic brain injury (mTBI) uses the Glasgow coma scale, and includes those having a score between 13 and 15. However, some published work considers those with a GCS score of 13 as having had a *moderate* TBI and classifies mild TBI as having a score of 14 or 15 only. The standard definition was used in this work. There are many other definitions and diagnostic criteria for mild TBI in use, and these have been drawn up by various groups with an interest in mild TBI. The latest definition by the WHO includes a GCS from 13-15 and one or more of confusion or disorientation, loss of consciousness (LOC) for 30 minutes or less, post-traumatic amnesia (PTA) for less than 24 hours and transient neurological abnormalities (Carroll et al., 2004, Fayol et al., 2009).

Some professional bodies have attempted to further classify those with mTBI into subgroups. The American Academy of Neurology (AAN) has devised a 'concussion grading system' (Table 1.4) which consists of 3 grades into which mTBI patients are allocated according to the presence of LOC or transient confusion and the length of symptoms (e.g. headache or dizziness) and mental status changes (e.g. post-traumatic amnesia) (Kelly et al., 1997, Ruff and Jurica, 1999). These sequelae of TBI will be discussed in detail later in this chapter.

Grade 1	Grade 2	Grade 3
Transient confusion	Transient confusion	Loss of consciousness, brief (seconds) or prolonged (minutes)
No loss of consciousness	No loss of consciousness	
Concussion symptoms or mental status abnormalities on examination lasting *less* than 15 minutes	Concussion symptoms or mental status abnormalities on examination lasting *longer* than 15 minutes	

Table 1.4: The American Academy of Neurology concussion grading system (Kelly et al., 1997).

1.9 Aetiology

The causes of brain injury are many and varied. Causes differ in populations according to age, sex, social grouping, and geography. Globally, considering head injuries of all severity and in all ages, they occur most commonly as a result of transport related incidents, the majority being a motor vehicle accident (MVA) (Reilly and Bullock, 2005), although this is not the case in the UK, where they are the second most common cause, with falls being the most common (Kay and Teasdale, 2001). It must be noted that injuries to cyclists and to pedestrians are also recorded as MVAs, and these groups are at particular risk of more severe injuries as a result of the fact that they are more exposed than vehicle occupants. In the UK in 2001, MVAs accounted for 58% of deaths from brain injury, and one third of those admitted to neurosurgical care (Kay and Teasdale, 2001). The death rate following MVA has been found to be higher where alcohol is involved (Kaasik et al., 2007, Lin and Kraus, 2009, Tsui et al.).

Injuries as a result of falls are the second highest cause of head injury in most populations. When the cause of injury is grouped according to age, this becomes the most significant cause of head injury in the elderly (Evans, 2006).

Other frequent causes of head injury include sports and recreational activities and assaults. This includes gunshot wounds to the head, both in areas at war and in those with high levels of gun-crime, such as the US, where they are much more prevalent than in the UK (NICE, 2007).

While head injuries caused by sports and recreational activities are a relatively small group, the numbers attributed to them are increasing as high energy sports such as riding, skiing and snowboarding become more accessible to a greater percentage of the population (Wasden et al., 2009, Loder, 2008, Ball et al., 2007). However, the recent increase in the use of helmets while taking part in alpine sports has been found to reduce the severity of head injury (Greve et al., 2009, Benson et al., 2009).

When head injury causes are grouped according to age, in the UK falls are the most common cause in children and the elderly, whereas, in the 15-24 age group, the most common cause is motor vehicle accidents, the majority of sufferers being male.

1.10 Pathology: Primary Brain Injury

Head injury can be classified according to whether the damage to the brain has resulted from the mechanical forces at the time of injury, or whether it has resulted from the continuing pathological changes within the brain that occur after injury. These two types of injury are termed primary and secondary traumatic brain damage respectively.

Primary traumatic brain damage can occur in a focal or diffuse (multifocal) pattern. Damage may occur to any of the tissues within and around the brain. The skull, meninges and vasculature are often involved, and on a microscopic level the glial cells and neurons themselves are at risk of damage.

1.10.1 *Focal TBI*

Focal brain damage as a result of head injury includes vascular damage, contusions in the brain parenchyma and lacerations. As a result of vascular injury, haemorrhage can occur in the brain itself, termed intracerebral haemorrhage, and between all of the meningeal layers covering it, causing one or a combination of subdural, extradural and

subarachnoid haemorrhage, although usually they expand secondarily and are therefore also classified as causes of secondary brain damage.

1.10.2 *Diffuse injury*

Diffuse injury is a term used to describe areas of damage where there are no specific lesions, such as haematomas or fractures, but where the damage has taken place at a microscopic level. Diffuse and focal injury can co-exist in the same patient after TBI, for instance, there may be an overlying subdural haematoma, with underlying diffuse axonal injury (section 1.10.3). Patients with diffuse injury alone may have significant damage at a microstructural or cellular level, without any easily identifiable lesions seen on brain imaging or macroscopically at post-mortem. Two types of diffuse injury are described; diffuse axonal injury and diffuse vascular injury. Diffuse injury is also used to describe diffuse brain swelling and diffuse hypoxic-ischaemic damage, both of which are features of *secondary* traumatic brain injury (section 1.11).

1.10.3 *Diffuse axonal injury*

Axons are particularly susceptible to shearing or rotational forces acting upon the brain, as a result of their long fixed paths, and can be damaged even in mild TBI. Diffuse damage to axons following trauma has been studied widely, as observers have come to realise that it may account for a number of the sequelae seen in the whole spectrum of TBI; from the subtle neuropsychological changes in patients with mild injury, to the persistent vegetative state following on from severe TBI (Povlishock and Christman, 1995, Adams et al., 1999). This widespread type of axonal damage has been termed diffuse axonal injury or DAI.

In DAI the pathological processes that occur are a result of shearing forces applied to the brain when an angular acceleration/deceleration, or rotation type injury, takes place. The shearing forces have greatest impact at the areas where there is a difference in density of the tissue, such as at the subcortical interface between grey and white matter, and also in midline structures such as the corpus callosum, and the dorsolateral parts of the upper brainstem (Smith et al., 2003). Axonal damage immediately following shear injury may not be complete, although those axons which have remained structurally intact may show evidence of functional disturbance for some time after the injury, and microscopic damage to the axoplasmic membrane may cause impairment of intracellular transport.

An example of this functional disturbance is illustrated by the measurement of amyloid precursor protein (APP). APP is normally transported along axons in immeasurable quantities in healthy neural tissue, but collects in damaged axonal fibres and can be detected immunocytochemically with antibodies 2 hours after the initial injury (McKenzie et al., 1996). The same is true of neurofilament proteins which have also been shown to act as markers for axonal injury (Reilly and Bullock, 2005, cited Maxwell et al., 1997).

Axons sustaining incomplete damage either undergo cytoskeleton remodelling or progress to secondary delayed axotomy through axoplasmic swelling, division and the formation of a retraction ball or axonal bulb, which occurs 8-24 hours post-injury (Povlishock et al., 1992). These axons then undergo Wallerian degeneration, a process that takes place in the end of the axon distal to the area of trauma. After the breakdown of the axoplasm and axolemma, degradation of the myelin sheath and subsequent invasion of macrophages occurs (Beirowski et al., 2005, cited Stoll et al., 2002). These macrophages, together with Schwann cells, phagocytose the debris. Time from injury to the initiation of Wallerian degeneration depends upon a number of factors, including the age of the patient, the type of nerve lesion (axotomy or crush injury) and on the type of nerve fibre, whether thick or thin, myelinated or unmyelinated (Beirowski et al., 2005). The process also occurs more slowly in the CNS than in peripheral nerves and may take up to several days in humans (Vargas and Barres, 2007).

DAI has been classified into three grades according to severity (Adams et al., 1989). The least severe, grade 1, describes axonal injury in the corpus callosum, brainstem and white matter of the cerebral hemispheres. Grade 2 DAI describes the same axonal damage as in grade 1 with additional haemorrhages in the corpus callosum, and grade 3, the most severe, includes the presence of haemorrhages in the rostral brainstem.

1.10.4 *Diffuse vascular injury*

Diffuse vascular injury (DVI) describes multiple small haemorrhages throughout the brain that are a direct result of the shearing forces acting upon small capillaries and venules. It is typically observed post-mortem in patients who die soon after brain injury, as DVI is often incompatible with life. It is associated with severe DAI (grades 2 and 3), and is likely to occur as a result of the same mechanism, with DVI accompanying DAI after injuries with the greatest degrees of head acceleration/deceleration, such as high speed RTAs (Pittella and Gusmao, 2003).

1.10.5 *Pathophysiology of acute TBI*

There are extensive effects of trauma upon the brain at a cellular and neurochemical level, as well as those at a structural level. Data from measurements in human brain injury populations, using microdialysis, MR spectroscopy and positron emission tomography (PET), coupled with data from animal models, has demonstrated extracellular and intracellular metabolic changes as a result of the primary injury alone. The processes outlined below are the principle effects of brain trauma at a cellular level.

1.10.6 *Pathophysiology: glucose metabolism*

Brain function relies predominantly upon aerobic metabolism, with oxygen and glucose the necessary substrates. In TBI, the delivery of these substrates may be affected by direct disruption of the intracranial vasculature or by a reduction in cerebral perfusion as a result of a localised mass lesion or a global increase in intracranial pressure due to brain swelling. Through these different mechanisms, brain injury reduces the delivery of oxygen and glucose substrates to the parenchyma, resulting in metabolic disturbance. Glucose is used, through aerobic metabolism, to generate adenosine triphosphate (ATP) within neuronal tissues. The physical forces in TBI cause membrane depolarisation and loss of the resting potential, which in turn causes release of neurotransmitters from the synaptic endplates. Reuptake of the neurotransmitters then occurs through ion pumping, an energy dependent step that uses ATP. This increased demand for ATP leads to a measurable decrease in extra cellular glucose, as it used up in ATP synthesis.

1.10.7 *Pathophysiology: lactate metabolism*

Astrocytes and glial cells have been shown to use coupled lactate metabolism, as well as glucose metabolism, to meet their energy requirements. Coupled lactate metabolism describes the process where lactate is anaerobically metabolised from glucose by astrocytes and then released into the extra cellular space. There it is taken up by the neurons and used to synthesise ATP *aerobically*. As the delivery of oxygen to neural tissue is impaired in TBI, this aerobic metabolism is reduced, and as a result there is an extracellular build up of lactate. Using this hypothesis of coupled lactate metabolism, it follows that a decrease in neuronal function after TBI may be represented by increasing cerebral lactate levels (Reilly and Bullock, 2005).

1.10.8 *Pathophysiology: calcium influx and glutamate release*

Intracellular calcium (as Ca^{2+} ion) is involved in a number of neuronal functions, including synaptic neurotransmitter release, protein phosphorylation, and gene expression (McIntosh et al., 1997). In animal models, intracellular free calcium ions have been shown to be increased after TBI, both acutely and up to 1 month after injury (Sun et al., 2008). This influx of calcium occurs within minutes of the administered injury (Nilsson et al., 1993). The principle mechanisms for this massive movement of calcium into the neurons are both a direct and indirect result of membrane depolarisation initiated by shearing forces acting on axons. Membrane depolarisation causes activation of voltage dependent membrane channels and leads to direct calcium uptake. The same membrane depolarisation also triggers glutamate release from the presynaptic membrane, which in turn causes overstimulation of glutamate receptors in the postsynaptic membrane, leading to the opening of glutamate receptor gated ion channels, and calcium influx. The massive resultant increase in intracellular calcium then leads to cell swelling, necrosis and eventually programmed cell death (McIntosh et al., 1997).

1.10.9 *Pathophysiology: production of reactive oxygen species*

Reactive oxygen species (ROS) refers to any free radical (an atom with at least one unpaired electron) with an oxygen centre. Oxygen centred free radicals contain two unpaired electrons in the outer shell, and have an extremely high chemical reactivity. They are formed in vivo as a result of aerobic metabolism in mitochondria, and studies have shown increases in levels of these ROS after TBI. Increased levels of ROS give rise to oxidative stress, a state where antioxidant cell defences are depleted with consequent irreversible modification of biologically important macromolecules (Wilson and Gelb, 2002). Signoretti et al. (2010) demonstrated in a rat model, that cerebral ROS production occurs directly as a result of the shearing force of TBI, in the absence of any ischaemic or hypoxic state, and Smith et al. (1994) confirmed these results showing increased levels of hydroxyl radicals (the most oxidising and reactive ROS) within 5 minutes of unilateral cortical impact.

1.10.10 *Pathophysiology: N-acetyl aspartate synthesis*

N-acetyl aspartate (NAA) is an amino acid synthesised specifically by neuronal mitochondria, implicated in osmoregulation where it may be involved in removing intracellular water from myelinated neurons (Baslow, 2003). The formation of NAA is

an energy dependent process, directly coupled to glucose metabolism, and relies on the presence of pyruvate as a source of acetyl coenzyme-A for its production (Baslow, 2003, Signoretti et al., 2010). Its use as a indicator for neuronal damage is based firstly upon the finding that NAA is reduced in conditions causing neuronal degeneration, such as dementia, stroke, multiple sclerosis and tumours, and secondly upon the findings in Canavan disease, a hereditary genetic condition where an error in NAA metabolism results in water imbalance and loss of oligodendrocyte myelin covering in neurons (Baslow, 2003, De Stefano et al., 1995, Lee et al., 2000, Signoretti et al., 2010). NAA is one of the most easily detected compounds in MR spectroscopy of the brain (Signoretti et al., 2010). Numerous papers have studied the effect of TBI on NAA, observing the difference in levels according to the time from injury, and the severity of the injury (Belli et al., 2006, Cohen et al., 2007, Garnett et al., 2000a, Garnett et al., 2000b, Mamere et al., 2009, Marino et al., 2007). NAA has been shown to be reduced in both the acute and subacute phase of traumatic brain injury, in patients with both mild and severe injuries (Belli et al., 2006, Blamire et al., 2002, Cohen et al., 2007, Garnett et al., 2000b). It has also been found to be decreased particularly in areas known to be susceptible to the shearing forces which lead to DAI, such as corpus callosum and midbrain, indicating a link between DAI and reduced NAA (Cecil et al., 1998).

1.10.11 *Pathophysiology: choline and acetylcholine synthesis*

Choline is an amine present in cell membranes and used in the synthesis of the neurotransmitter acetylcholine. It has been shown to be elevated during disease states with increased membrane turnover (e.g. tumours), and following tissue breakdown and inflammation (Brooks et al., 2001, Zauner et al., 2002). Studies have reported elevated choline after TBI, and propose that the increased levels are due to membrane breakdown and neuronal injury (Brooks et al., 2000, Garnett et al., 2000b).

Choline levels may also be increased as a result of a reduction in choline acetyltransferase activity which has been observed post-mortem in TBI patients (Dewar and Graham, 1996, Murdoch et al., 1998). Choline acetyltransferase is an enzyme present within neurons, which synthesises acetylcholine from choline and acetyl coenzyme A. As cholinergic neurones are involved in learning, sleep, memory and arousal, disruption to this network, due to TBI induced reduction in the synthesis of acetylcholine, may be responsible in part for the affective and cognitive symptoms seen in these patients (Oda, 1999, Reilly and Bullock, 2005).

1.11 Pathology: Secondary Brain Injury

Secondary brain injury occurs as a result of multiple ongoing pathological processes which may complicate primary brain damage. It can be a combination of ischaemia leading to hypoxic injury and oedema, raised intracerebral pressure (ICP) as a result of oedema, or due to the mass effect of a large haematoma. Secondary brain injury can be further complicated by other post-injury sequelae, such as infection, hypothermia and seizures.

The emphasis put upon swift management of secondary brain trauma is as a result of the fact that some of the processes are preventable and reversible. Little can be done in terms of preventing damage from primary brain injury, other than taking preventative steps to reduce the incidence of that injury type within the community. However, speedy recognition of processes which result in secondary brain injury, and rapid intervention with the appropriate management and treatment, can dramatically improve clinical outcomes. Guidelines have been developed to direct imaging, monitoring and treatment of secondary brain injury (Bratton et al., 2007a, Cushman et al., 2001, NICE, 2007).

1.11.1 *Ischaemia*

Ischaemia can occur as a result of primary vascular injury which produces focal ischaemia, or as a sequelae of brain swelling and increase in ICP which results in reduced cerebral perfusion pressure and a global reduction in cerebral blood flow. The reduction in cerebral blood flow impacts brain tissue oxygen availability and there is an increase in tissue lactate which has been shown to correlate with unfavourable outcomes (Reilly and Bullock, 2005, Robertson et al., 1999). An ability to monitor cerebral blood flow is therefore of importance, and current guidelines recommend further development of minimally invasive techniques to do this (Bratton et al., 2007a).

1.11.2 *Oedema*

Cerebral oedema post-TBI can be as a result of both vasogenic oedema and/or cytotoxic oedema. Vasogenic oedema describes a process whereby damage to the blood brain barrier results in an increase of protein in the extracellular space, which creates an osmotic gradient and consequently localised swelling, as excess water is transported into tissue from vessels and is subsequently not cleared. Cytotoxic oedema describes the swelling that occurs after hypoxia, where disturbance of the ionic gradients causes water

redistribution from the extracellular into the intracellular compartment, resulting in ischaemic necrosis of cells (Reilly and Bullock, 2005, cited Miller, 1993). Focal oedema and diffuse whole brain swelling can, if of sufficient severity, cause an increase in the ICP, leading again to reduced cerebral perfusion pressure, and poor outcomes post-TBI (Bratton et al., 2007b).

1.12 Symptoms in TBI

In traumatic brain injury, symptoms vary greatly according to the severity of the injury sustained. As discussed above, a depressed conscious level (measured using the GCS) is a symptom of altered brain function after head injury. The degree and length of loss of consciousness both give an indication to the extent of underlying cerebral damage. In the most severe non-fatal head injuries the patient may take months or years to regain consciousness, or the outcome may be one of permanent vegetative state. In contrast, patients sustaining a very mild TBI may present with no symptoms at all. In between these two extremes, TBI patients can suffer with a broad spectrum of signs and symptoms, which may be temporary (either acute or chronic) or permanent (Table 1.5).

Headaches	
Cranial nerve symptoms	Dizziness Vertigo Tinnitus Hearing loss Blurred vision Diplopia Convergence insufficiency Light and noise hypersensitivity Diminished taste and smell
Affective symptoms	Irritability Anxiety Depression Personality change Post-traumatic stress disorder Fatigue Sleep disturbance Decreased libido Decreased appetite Nausea or vomiting
Cognitive symptoms	Memory dysfunction Impaired concentration and attention Slowing of reaction time Slowing of information processing speed
Rare symptoms	Seizures Transient global amnesia Tremor Dystonia

Table 1.5: Sequelae of mild TBI (adapted from Evans, 2006).

Post-concussion symptoms occur in over 50% of patients with mild TBI (Thornhill et al., 2000), and the common symptoms are a combination of physical, cognitive and affective problems.

1.12.1 *Headaches*

Headache after traumatic brain injury can be due to many causes. Raised intracranial pressure, as a result of cerebral contusions, traumatic subarachnoid haemorrhage or an extra-axial haematoma will cause not only headache, but also nausea, vomiting and in severe cases coma. Irritation of the meninges as a result of an extra-axial haematoma or depressed skull fracture will also give rise to headache, which can be accompanied by signs of meningism (e.g. photophobia, neck stiffness). Headaches may also be due to injuries concurrent with the brain injury, such as cervical pathology (e.g. radicular pain from cervical disc damage, occipital neuralgia, carotid or vertebral artery dissection) or facial pathology (e.g. temporomandibular joint injury, myofascial injury, supra- or infra-orbital neuralgia) (Evans, 2006 p100-102). TBI may also stimulate the onset of migraine, cluster or tension headache, and the treatment of TBI with analgesia can also induce analgesic rebound headache. 'Headache' may even be used incorrectly by the TBI patient to describe localised pain as a result of lacerations or contusions of the scalp.

1.12.2 *Cranial nerve symptoms*

Cranial nerve damage, whether temporary or permanent, may be associated with TBI even in mild cases (Coello et al., 2010). The incidence of TBI associated cranial nerve injury reported in the literature varies greatly depending upon the severity of the injury. Jin et al. (2010) examined 3417 patients of varying injury severity and identified 312 (9%) patients with a cranial nerve injury, the majority of whom fell into the moderate and severe groups. However, a study looking specifically at fatal cases of TBI post-mortem, reported much higher rates of cranial nerve injury (Mariak et al., 1997). Symptoms are directly related to the nerve or pathways injured. Head injury is the leading cause of anosmia; olfactory nerve damage presents not only with a reduced or absent sense of smell, but also affects the ability to taste (Costanzo and Miwa, 2006). Injury to the optic nerve and the visual pathways may result in a reduction in visual acuity, blurred vision or even blindness. Diplopia occurs when one or more of the oculomotor, trochlear and abducens nerves are damaged. Dizziness, vertigo, tinnitus and hearing loss can all occur with injury to the vestibulocochlear nerve. Hearing loss and

noise hypersensitivity may also be a result of damage to the external or internal auditory apparatus. Injuries to the *lower* cranial nerves are more likely to be associated with a fatal injury (Jin et al., 2010).

1.12.3 *Affective symptoms*

Irritability, anxiety, depression and sleep disturbance, along with reduced libido and appetite, are common features after head injury, and have been shown to persist in mild TBI longer than had previously been thought. At 1 year post-injury, Thornhill et al. (2000) found that 14% of 333 patients with mild TBI were still suffering with one or more of these symptoms. Fatigue is a persistent post-concussion problem, occurring acutely in almost 75% of 263 adults with mild TBI studied by Norrie et al. (2010) which found that fatigue was exacerbated by depression and that it diminished in the first 3 months and then stabilised. It was found to be persistent in 34% of their patient group at 6 months.

1.12.4 *Cognitive symptoms*

Cognitive symptoms include problems with concentration, memory loss and reduced speed of information processing. These symptoms are detectable, and to an extent measurable with neuropsychological testing, and have been the subject of numerous studies examining TBI patients of all severities, both acutely and in the chronic phase (Hartikainen et al., 2010, O'Jile et al., 2006, Ashton et al., 2005, Draper and Ponsford, 2008, Hughes et al., 2004). A detailed discussion of the symptoms themselves, along with the tests used to identify and quantify them is presented in Chapter 3 of this thesis.

1.13 Mortality

In the UK, the actual number of deaths from head injury is relatively small (6-10 per 100,000 population, 0.2% of all hospital admissions with a head injury) (NICE, 2007). This low mortality rate is due to the fact that the majority (around 90%) of head injury admissions are classed as mild, and fatalities are more likely to be an outcome following moderate and severe head injury (Swann and Teasdale, 1999).

1.14 Summary

Traumatic brain injury (TBI) is a complex condition affecting a large percentage of the global population every day. The term TBI encompasses a variety of intracranial pathologies, which may each be caused by differing degrees of force in any number of different modes and mechanisms. If left unchecked, this heterogeneous group of primary injuries may then lead on to secondary brain injury, which has the potential to cause more harm. Even in its most mild form, TBI has been shown to have an effect on those that suffer it, causing physical, cognitive and mental symptoms that persist well beyond the time of injury, and can affect performance at work, relationships at home and every aspect of daily life. Early detection of damage caused by TBI allows for the input of timely management strategies, and can lessen the impact of any persistent symptoms. The following chapter outlines how current MRI techniques are utilised towards this end, and introduces advanced techniques, which are currently being investigated in the TBI setting.

Chapter 2. Imaging in Head Injury

2.1 Computed Tomography Scanning

Before discussing magnetic resonance imaging, which is the modality on which this work is based, it is first necessary to briefly discuss the role of computed tomography scanning, as this is still the mainstay of imaging in the traumatic brain injury population, and the reader should be aware of this. Typically on admission, TBI patients will undergo computed tomography (CT) scanning first, due to the fact that it has a number of benefits as a primary investigation, when compared to magnetic resonance imaging (MRI). TBI pathology requiring urgent surgical intervention, such as intracranial haematoma, whether intra- or extra-axial, depressed skull fracture, intracranial foreign body or raised intracranial pressure are all easily displayed on CT, and therefore as a first line imaging modality, it is still most useful in aiding further management decisions in the brain trauma patient (Figure 2.1).

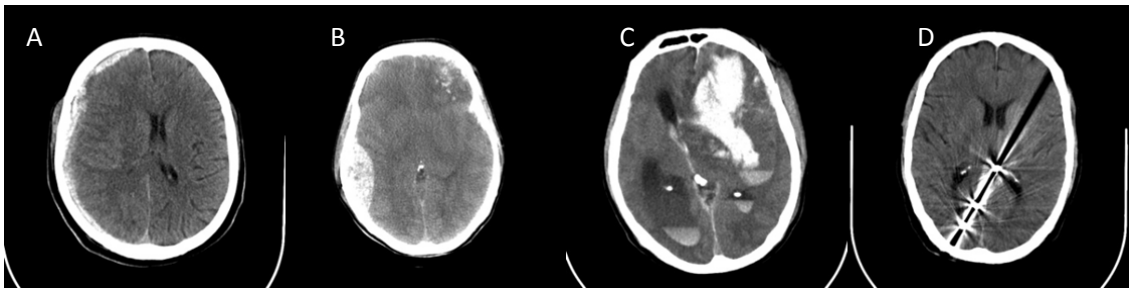

Figure 2.1: Examples of TBI on axial CT scans: (A) Right sided acute subdural haematoma with mass effect and midline shift; (B) Right parietal extradural haematoma, left frontal contusions and generalised brain swelling; (C) Large left frontal intracerebral haematoma with mass effect, midline shift and associated intraventricular haemorrhage with hydrocephalus; (D) Three metal intracranial foreign bodies. *Author's own images.*

With the advent of modern multi-slice CT scanners, imaging time has been dramatically reduced and the patient can be scanned very quickly, allowing early assessment of agitated or unstable trauma patients. In comparison, MRI scanning takes much longer to obtain the same number of images. Furthermore, due to the strong magnetic field, ventilated patients need to be transferred onto MRI compatible equipment, which although now readily available, takes more time, and may not be possible in the unstable trauma patient.

However, there are disadvantages to CT scanning, such as inaccuracies due to partial volume effects. This phenomenon occurs when an area of damage in the region being examined is smaller than the scanning resolution, and as a result does not appear on the image produced by the scanner software. This is specifically a problem when the area of damage is small and because of its location is of great significance, for instance in the brain stem. In these injuries MRI scanning is invaluable as the smaller lesions are much more likely to be detected.

2.2 MRI Basic Principles

Magnetic resonance imaging is a well established imaging technique which relies upon the principles of nuclear magnetic resonance. It takes advantage of the magnetic properties of nuclei (typically hydrogen nuclei) and their interactions in a magnetic field with applied radiofrequency pulses.

The fundamentals of MRI are complex and rooted in the branch of physics known as quantum mechanics. A simple summary of the basic principles can however be presented based on classical analogy. When placed in an MRI scanner, nuclei spinning within all body tissues act as magnets and align themselves longitudinally along the lines of magnetic flux. They either align themselves with the field, in a low energy state, or against the field, in a high energy state (Figure 2.2). Slightly more nuclei exist in the low energy state than in the high energy state, and the result is a net magnetization aligned with the externally applied field.

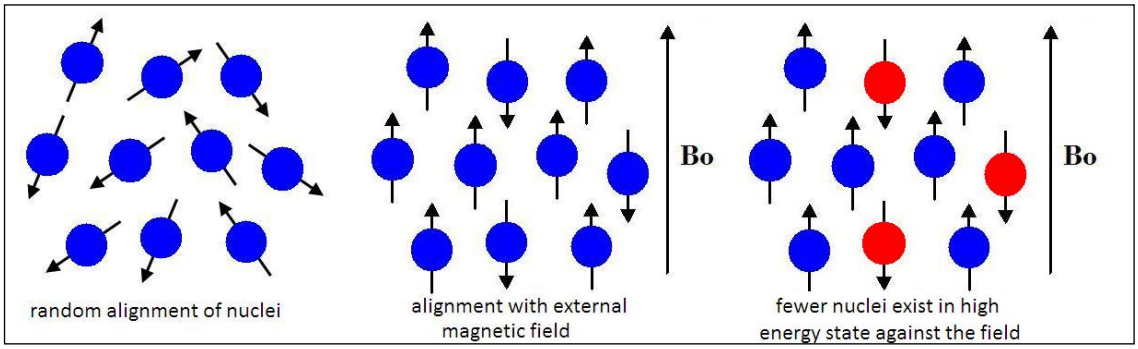

Figure 2.2: Behaviour of nuclei within a magnetic field (Bo) (adapted from Fox, 2011).

When an electromagnetic pulse is applied at a specific and well defined frequency, energy is imparted to the nuclei causing them to 'flip' from the low energy state to the high energy state, altering the net magnetization. The duration of the pulse determines

the degree to which the net magnetization alters from the external field to being diametrically opposite. A pulse only half the length of time needed to completely 'flip' the nuclei will result in the net magnetization vector being at right angles to the field, whereas a pulse length double that time will 'flip' the nuclei the full 180 degrees (Figure 2.3).

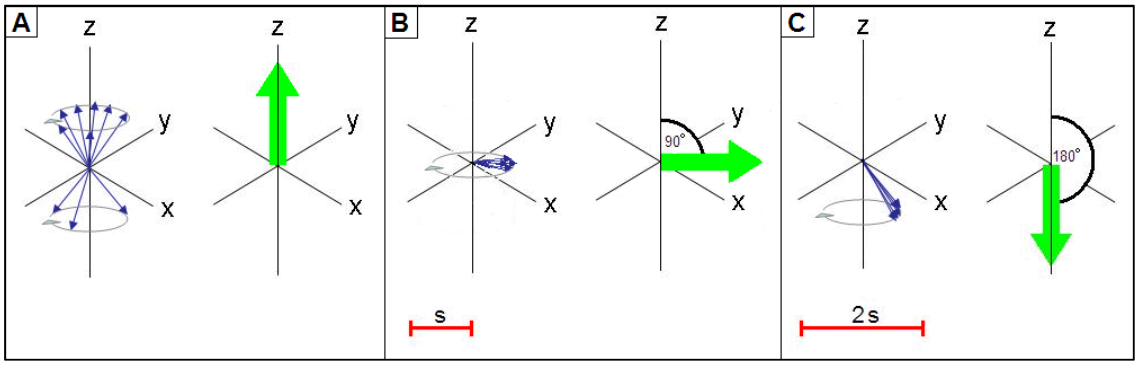

Figure 2.3: Behaviour of nuclei after an applied radiofrequency pulse within a magnetic field 'z': (A) Before radiofrequency pulse; (B) After radiofrequency pulse of duration 's', sufficient to 'flip' nuclei 90˚; (C) After radiofrequency pulse of twice duration 's', sufficient to flip nuclei 180˚. The net magnetization vector is indicated by the green arrow (adapted from Fox, 2011).

When the electromagnetic pulse is turned off, the nuclei realign themselves with the external magnetic field. During the periods when the nuclei are aligned at 90 degrees to the external field, a current is created by electromagnetic induction in detection coils placed around the part of the body that is of interest and this gives the MR signal. The time taken for the nuclei to then realign with the magnetic field is characterised by a time constant called the T1 value of the tissue.

A second effect occurs in terms of the natural spinning of the nuclei, while the electromagnetic pulse is applied, and they all change to spin in phase with each other. When the pulse is turned off, they each return to their out of phase spin. After spinning in phase during the electromagnetic pulse, the nuclei return to their original random spinning patterns and the time taken for them all to return to being out of phase is characterised by a second time constant called the T2 value of the tissue.

Differences between T1 and T2 in different tissue or pathology change the behaviour of the MRI signal from that region leading to contrast in the MR image.

2.3 MRI in Head Injury

Numerous imaging techniques provide information into the sequelae of brain injury, but magnetic resonance imaging (MRI) in particular is useful in assessing the extent of mild brain injury and its recovery, both in the subacute and chronic injury stages (Lagares et al., 2009). Over the past 30 years advances in MRI scanning techniques have allowed detailed examination of the microscopic changes that take place following TBI and have enabled their progression to be evaluated over time. MRI sequences have been developed to allow multiple quantitative factors to be assessed in brain injury, such as cerebral blood flow and blood volume, water diffusion patterns, integrity of white matter tracts, the amounts of certain biochemical compounds present and the activity of targeted areas of brain (Garnett et al., 2001a, Garnett et al., 2001b, Ge et al., 2009, Hergan et al., 2002).

Despite having been shown to be more sensitive in the detection of acute traumatic lesions caused by head injury (Firsching et al., 2001, Paterakis et al., 2000), MRI scanning does not typically play a major role in diagnosis or clinical management of these patients. The long image acquisition times of MRI and the need to transfer the patient onto non-ferrous support equipment render it unsuitable in the emergency management of acute injury, when the patient may be clinically unstable (e.g. on ventilator support). In most cases CT scanning, which is fast and easy to acquire, provides images which are more than adequate to allow initial assessment and management planning in acute TBI (Manolakaki et al., 2009). CT is therefore entirely satisfactory for the diagnosis of acute sub-dural haematomas and extra-dural haematomas for example. However, the more detailed images provided by MRI are useful in the acute stage of injury if the CT findings do not correlate with the clinical neurological picture.

MRI is the preferred mode of scanning in the subacute and chronic phases of head injury, due to its enhanced resolution and level of detail when compared to CT scanning (Le and Gean, 2009). There are a number of different sequences available, each of which provides valuable information in the head injured patient.

2.4 Structural MRI

2.4.1 *T1 and T2 anatomical sequences*

The definitions of T1 value and T2 value are described in section 2.2 above. T1 and T2 'anatomical' sequences are the conventional MRI images with which most clinicians are familiar. They are created by detecting the MR signal produced by the nuclei realigning themselves with the external magnetic field, using a specific inter-scan delay (the repetition time, TR) between subsequent radiofrequency pulses that allows for the best contrast between the tissue types under scrutiny (Figure 2.4). It is therefore a qualitative 'snapshot' of the relaxing nuclei, rather than a true measure of the T1 and T2 values for each tissue visible on the scan.

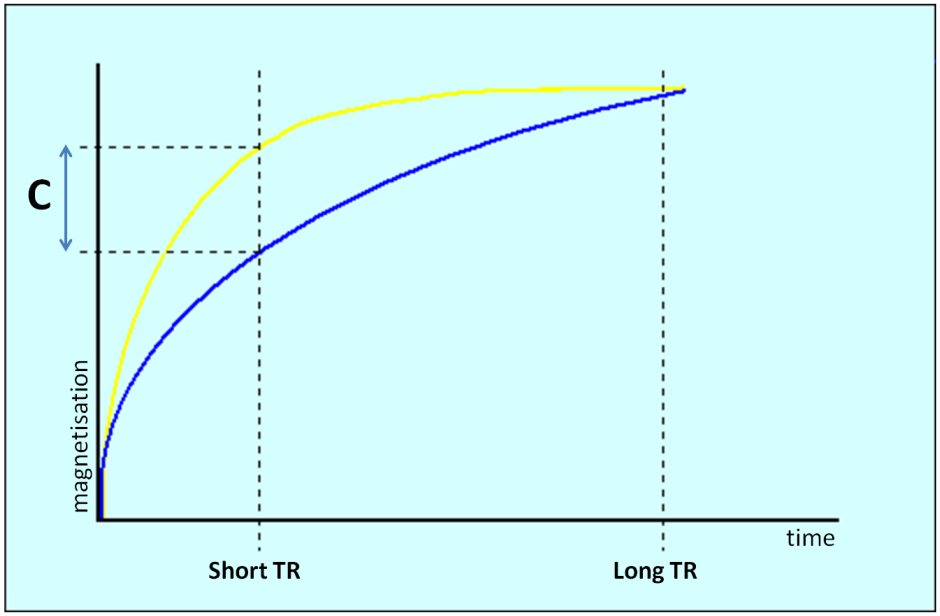

Figure 2.4: Graph showing the T1 relaxation curves for fat (yellow) and water (blue). In T1 *weighted* imaging the delay between radiofrequency pulse and signal detection (the repetition time or TR) is deliberately short, to allow the greatest contrast (C) to be obtained between tissue types. A long TR would give no contrast between tissue types (adapted from Westbrook et al., 2005).

The diagnostic potential of 'anatomical' MRI images relies upon the skills of the observer to detect often subtle evidence of head injury. Soon after the introduction of MRI, T1 and T2 sequences were shown to be inferior to conventional CT scanning in the identification of certain types of intracranial pathology, such as subarachnoid and small intraparenchymal haemorrhages (Metting et al., 2007). Advances in MRI techniques have addressed this problem, and these haemorrhages are now identified by MRI with the same frequency as CT, using T2* weighted gradient recalled echo

sequences (section 2.4.3) (Kidwell et al., 2004). Intracerebral, intraventricular, subdural, and extradural haemorrhages are all easily identified on T1 and T2 images (Figure 2.5).

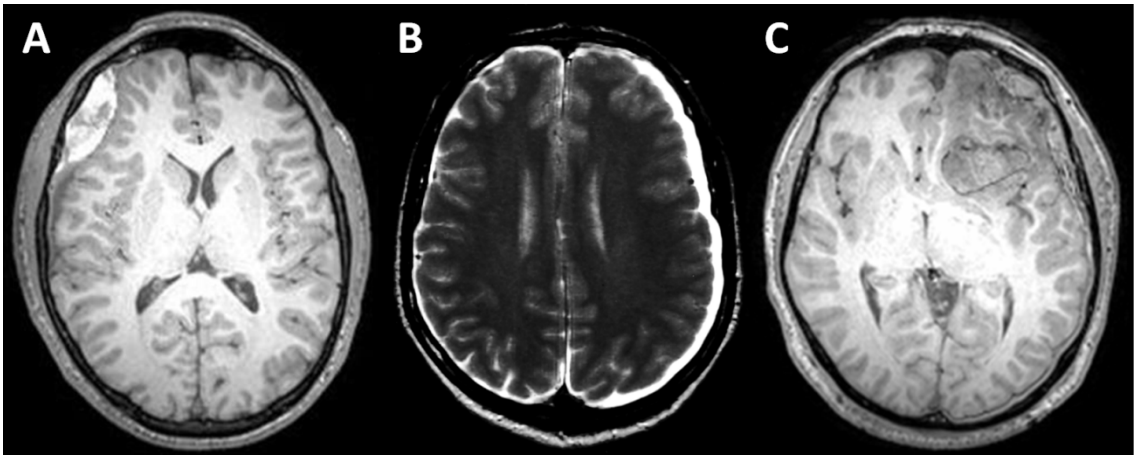

Figure 2.5: TBI on conventional anatomical MRI scans: T1 weighted axial image (A) showing a right frontal acute extradural haematoma with mass effect. T2 weighted axial image (B) showing a left subacute subdural haematoma and T1 weighted axial image (C) showing a left frontal acute intraparenchymal haemorrhage. *Images (A) and (C): author's own; Image (B): (de Noronha et al., 2003).*

However, the appearance of each type of haemorrhage differs not only in its anatomical position, but also with regard to the degree of red blood cell lysis within the haemorrhage over time. The sequence of catabolism of haemoglobin in blood causes it to change from oxyhaemoglobin into deoxyhaemoglobin and then methaemoglobin, before it is metabolised into ferritin and haemosiderin. Each of these forms influences the T1 and T2 relaxation times of the surrounding water in the haemorrhage, leading to altered and characteristic image contrast (Bradley, 1993). Therefore the appearance of blood at different stages post-injury changes both on T1 weighted images, and on T2 weighted images (Table 2.1).

Time from injury (approx. values)	Stage in catabolism	Haemorrhage on: T1 weighted image	Haemorrhage on: T2 weighted image
Hyperacute (1 - 12 hours)	Oxyhaemoglobin	Dark	Bright
Acute (1 – 5 days)	Deoxyhaemoglobin	Isointense	Dark
Subacute (5-15 days)	Methaemoglobin	Bright	Early: Dark Late: Bright
Chronic (15 days +)	Ferritin & Haemosiderin	Isointense	Dark

Table 2.1: Appearance of haemorrhage on T1 and T2 weighted scans (Reilly and Bullock, 2005 p180, cited Gean, 1994 p178).

2.4.2 *FLAIR*

Fluid attenuated inversion recovery (FLAIR) describes an MRI technique which is used to suppress the usually high signal from cerebrospinal fluid (CSF) on T2 weighted images. This scan sequence is particularly useful in TBI as it helps accurately delineate the borders of lesions close to the CSF spaces within the brain from the CSF itself, whether periventricular tissue lesions and oedema or traumatic subarachnoid haemorrhage in the basal cisterns (Figure 2.6). Ashikaga et al. (1997) performed T2 and FLAIR sequences on 56 TBI patients, and found that FLAIR outperformed T2 weighted imaging when detecting post-traumatic sequelae in all. They also noted that FLAIR detected lesions in 9 patients who had no lesion visible on their T2 scan. FLAIR is also useful in the detection of the white matter shearing injuries of DAI in the corpus callosum and in the fornix, when not associated with haemorrhagic petechiae (Le and Gean, 2009).

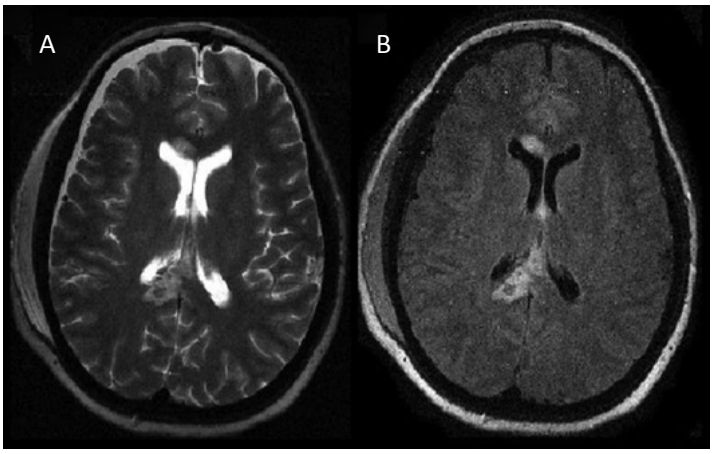

Figure 2.6: T2 (A) and FLAIR (B) axial images showing a right subacute subdural haematoma, right sided superficial scalp swelling and periventricular lesions in the corpus callosum consistent with DAI. The periventricular lesions are more easily identified on the FLAIR image, than the T2 image (Coles, 2007).

2.4.3 *T2* GRE*

T2* Gradient recalled echo (GRE) is an MRI technique which is highly sensitive to small distortions in the scanner's magnetic field within the brain. Such distortions (referred to as magnetic susceptibility effects) arise from differences in the magnetic properties of the water and other components within the tissue such as haemosiderin and ferritin which are the end products of haemolytic breakdown during red cell lysis (see above). The paramagnetic characteristics of these products cause small localised

alterations in the magnetic field, which are represented as areas of hypointensity (Figure 2.7). T2*GRE is therefore of value in detecting haemorrhage, particularly in the subacute phase after injury, or later, as its sensitivity is proportional to the amounts of haemosiderin and ferritin formed as the blood is broken down. Petechial lesions found on T2*GRE in closed head injury are considered to represent haemorrhagic DAI (Mittl et al., 1993). It has been shown to detect traumatic lesions with greater frequency than on T2 sequences (Scheid et al., 2003, Gerber et al., 2004). Gerber et al. also found that T2*GRE findings were better predictors of Glasgow Coma Scale (GCS) and post traumatic amnesia (PTA) than T2 weighted images.

However, T2*GRE does have limitations when imaging inferior fronto-temporal regions, due to the susceptibility to artefact created by the paranasal sinuses and mastoid air cells (Le and Gean, 2009).

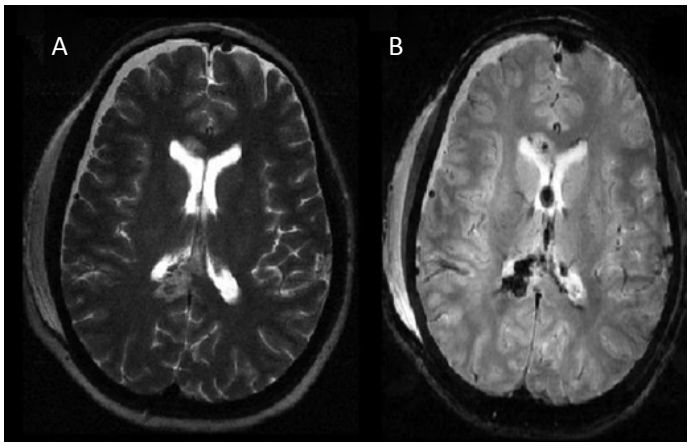

Figure 2.7: T2 (A) and T2*GRE (B) axial images showing a right subacute subdural haematoma, right sided superficial scalp swelling and periventricular lesions consistent with DAI (Coles, 2007). Specific areas of periventricular haemorrhage can be seen in the corpus callosum more easily on the T2*GRE image. These are pathognomonic of grade 2 DAI (see Chapter 1).

2.4.4 *Quantitative T1 and T2 relaxometry*

Diffuse changes associated with TBI may cause global changes which do not produce areas of focal contrast and which are therefore difficult to detect visually. As described in section 2.4.1, conventional T1 and T2 weighted scans are a qualitative 'snapshot' of the relaxing nuclei, rather than a true measure of the brain T1 and T2 times. *Quantitative* T1 (qT1) and T2 (qT2) relaxometry is a technique where a visual map is created where the intensity of the displayed anatomy represents the actual time constant

for decay of that tissue. It can therefore be used to make a quantitative assessment of these values within the tissue, and may reveal more details of underlying pathology than the conventional visual analysis of T1 and T2 *weighted* scans (Clare and Jezzard, 2001).

There has been relatively little examination of TBI using analysis of quantitative T1 and quantitative T2 relaxation times. Naruse et al. (1982) studied the effects of vasogenic and cytotoxic oedema on qT1 and qT2 times in a rat model. They found that in vasogenic oedema there was an increase in qT1 time at 3 hours after injury in grey matter and 6 hours after injury in white matter. The increase persisted for 6 days and peaked at 24 hours. They also observed an increase in qT2 time with vasogenic oedema, but in the white matter only after 2 hours. Again the values peaked at 24 hours, but were faster to return to normal, and did so by 4 days. In cytotoxic oedema, they found that qT1 and qT2 values were again increased, but only in white matter. Interestingly the increase in each took longer to appear, occurring 3 days after injury in both qT1 and qT2. The effects of cytotoxic oedema also lasted for much longer than the effects of vasogenic oedema: they did not return to normal until 49 days after the injury. Sibson et al. (2008) have also demonstrated an increase in quantitative T1 time in the acute phase of injury in their animal model of TBI. They found similar increases in both cases of low-flow ischaemia and in excitotoxicity. The morphologic feature common to both of these pathological processes was the acute activation of astrocytes, and it was this that the authors hypothesised was the cause of the increased qT1.

Clinical studies have also been conducted; Goetz et al. (2004) performed quantitative MRI in 23 TBI patients of mixed severity in the acute phase after their injuries and included measurement of qT1 and qT2 in their scan sequences. They found no significant change in either value when the data from the patient group was compared to data from 13 control subjects. They also found no correlation between injury severity and either qT1 or qT2.

Quantitative T2 relaxation time has also been evaluated in TBI patients in the chronic phase of injury. Mamere et al. (2009) studied 9 moderate and severely injured patients a mean of 3.1 years after trauma, and found an increase in T2 relaxation time in normal appearing brain white matter and specifically in the corpus callosum. They hypothesised that the observed qT2 changes reflected an increase in water concentration secondary to axonal loss.

Further experimental studies measuring T2 relaxation times in rat cortical lesions were conducted in 2009 (Kharatishvili et al., 2009, Immonen et al., 2009). Kharatishvili et al. found not only that T2 values in the brain injured animals increased within 3 days post injury, but that the increase correlated with lesion volume and was able to differentiate between moderate and severe TBI. Immonen et al. observed that qT1 and qT2 increased in the peri-lesional area after 9 and 3 days post-injury respectively. They attributed their finding of raised qT2 time to the increase in extracellular fluid due to vasogenic oedema, and the delayed increase in qT1 time to the accumulation of macromolecules as necrosis and cell death occurs. Zhang et al. (2011) have also studied T2 relaxometry in rats, 5 days after inducing Wallerian degeneration by unilateral cortical ablation (see section 1.10.3). They found no difference in T2 relaxation time between affected cortex and healthy cortex.

2.5 Diffusion Weighted Imaging

2.5.1 *DWI principles*

Magnetic resonance techniques have been developed which allow quantitative evaluation of water diffusion patterns at a microscopic level. The ability to make measurements of molecular self-diffusion by nuclear magnetic resonance has been recognised since 1965, but it was not until 1990 that it was first employed as a clinical tool to identify areas of brain where localised water diffusion had been affected by ischaemic stroke (Stejskal and Tanner, 1965, Neil, 2008).

The diffusion image is obtained by first encoding the location of water molecules within the brain with one magnetic gradient pulse, and then locating them again with a second magnetic gradient pulse after a delay of around 60 milliseconds. Movement of the water molecules between these two pulses affects the MR signal allowing quantitative data about the overall direction and degree of molecular water movement in the tissue being examined to be calculated. This data is the diffusion coefficient, and is a measure of how far, on average, molecules move due to random “Brownian” motion. However, water may not move as far in a structured environment than it may in a uniform one. If there are many structural barriers to its movement, the distance the molecule moves is much lower than it should be due to true diffusion alone. Therefore, the data collected is given the term *apparent* diffusion coefficient (ADC) to acknowledge this.

2.5.2 *DWI in brain injury*

ADC has been studied in relation to brain tissue injury. The overwhelming finding in clinical trials using DWI in acute stroke has been a reduction in localised ADC in areas of ischaemic brain (Kloska et al., 2010, Saur et al., 2003, Schlaug et al., 1997). It is hypothesised that this is due to the presence of cytotoxic oedema in which the net movement of water is from the extracellular space to the intracellular compartment. This is thought to increase cell volume and decrease the net movement of extracellular free water, and this is represented by a reduced ADC. This process happens within 6-12 hours of the onset of stroke, and the ADC subsequently increases slowly over the next 3-6 days, at which point it pseudo-normalises. More recently, in some cases ADC has also been found to be reduced post TBI, and it is suspected that this is due to areas of ischaemic brain tissue secondary to trauma (Ito et al., 1996, Hergan et al., 2002, Blamire et al., 2002). Theories have been proposed to explain the mechanism; firstly that the diffusion coefficient is reduced due to hypoxia and simultaneous cerebral hypotension, and secondly, that diffusion is reduced as a result of the formation of retraction balls following axotomy after shearing injury (Ito et al., 1996, Povlishock and Christman, 1995, Schaefer et al., 2004). A decrease in ADC has also been shown to occur in the presence of inflammatory mediators, and with trauma induced cytotoxic oedema (Blamire et al., 2000, Barzo et al., 1997).

Other studies report an increase in ADC in traumatic brain injury, both in areas susceptible to diffuse axonal injury, and in whole brain white matter analysis (Goetz et al., 2004, Newcombe et al., 2007). Alongside the macroscopic changes that occur in grade 2 and 3 DAI mentioned above, grade 1 DAI describes widespread axonal damage without the focal evidence of haemorrhagic petechiae that allow it to be visualised on T1 and T2 weighted anatomical scans. Given that it is the presence of haemorrhage that is most readily detected in the anatomical T1 and T2 imaging sequences, grade 1 DAI is not well demonstrated by those techniques, and is best identified using diffusion weighted imaging (Huisman et al., 2003).

A number of pathological processes have been shown to be linked to a rise in ADC. Firstly it has been observed in experimental models that ADC increases in areas with vasogenic oedema, after localised disruption of the blood-brain barrier (Barzo et al., 1997). Secondly, it has been noted in rodent models, that a rise in ADC is associated with features of *chronic* ischaemia, and within areas of infarcted brain (Granziera et al., 2007). Thirdly, it has been suggested that the observed increase in ADC is as a result of

a reduction in obstruction to water diffusion, as a consequence of tissue cytostructure damage, as axonal collapse follows on from Wallerian degeneration in the subacute phase of brain injury. Furthermore, a proportional link between degree of increase in ADC and severity of head injury has been reported (Goetz et al., 2004).

2.6 Diffusion Tensor Imaging

2.6.1 *DTI principles*

Diffusion tensor imaging (DTI) is an advanced MRI application which allows quantification of localised water diffusion properties within the brain, and is an extension of DWI making the method more robust.

If we consider a population of water molecules in a beaker of water which are allowed to diffuse freely, there is no restriction to their motion in any direction and their movement over time can be modelled by a perfect sphere. This is termed *isotropic* diffusion. This however is not the case *in vivo* where, in the brain, this diffusion is restricted in one or more planes by both intracellular and extracellular barriers (such as myelin sheaths and axon fibres). In this situation the movement of water molecules can be described by an ellipsoid, and is termed *anisotropic* diffusion (Figure 2.8).

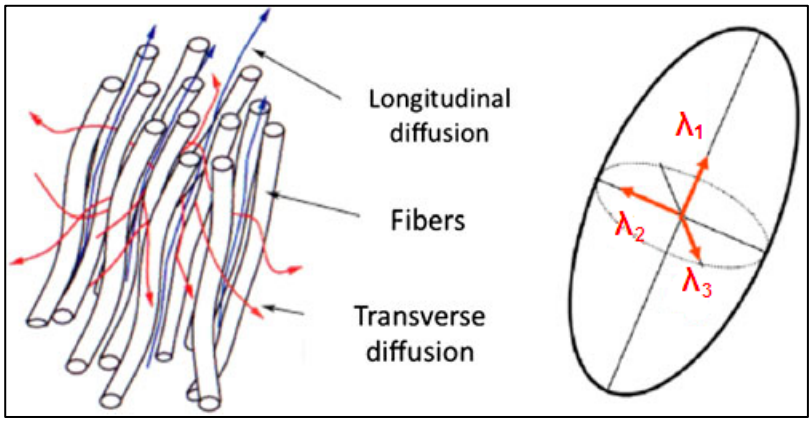

Figure 2.8: A model of anisotropic diffusion of water molecules and its representation by a tensor. *Left*: Diffusion is restricted in the transverse plane by the fibres, and but is less restricted parallel to them in the longitudinal plane. *Right*: Principal axis (λ_1) of the ellipsoid reflects diffusion in the longitudinal plane, the mean of the shorter axes of the ellipsoid (λ_2 and λ_3) equate to radial diffusivity and reflect diffusion in the transverse plane (adapted from Chanraud et al., 2010).

In order to define this ellipsoid mathematically we must make a number of measurements of water diffusion in varying directions and apply a diffusion tensor model to their analysis. This quantifies the magnitude of diffusion in three perpendicular planes (x, y and z) and these magnitudes are termed 'eigenvalues' (λ_1, λ_2 and λ_3). Using this same model we can describe the orientation of the ellipsoid in 3D space using 'eigenvectors' ($\mathbf{v}_1$, $\mathbf{v}_2$ and $\mathbf{v}_3$). In brain white matter, where myelinated axons are running in a particular direction, and water movement across them is restricted, it follows that the greatest eigenvector is parallel to the axon fibres themselves. Using eigenvector values we can calculate rotationally invariant indices of diffusion across the entire brain image. Examples of which would include mean diffusivity (MD) as a marker of isotropy and fractional anisotropy (FA) which quantifies restricted diffusion. FA values range from 0 to 1, with 0 representing total *isotropy*, or free diffusion in all directions, and 1 representing total anisotropy, or diffusion perfectly restricted to one plane only.

It has been proposed that detection of patterns of anisotropy in the brain may provide further information about the underlying tissue micro-architecture. In particular, patterns of local anisotropy have been shown to delineate axon fibre orientation and white matter tract pathways by applying a technique called tractography.

FA has been measured in a number of studies into the use of DTI in head injury. The majority of papers looking at TBI in the acute phase (within 7 days of injury) have shown there to be a decrease in white matter fractional anisotropy post TBI. Arfanakis et al. (2002) show a decrease in FA in normal appearing whole brain white matter, when compared to control groups. Huisman et al. (2004) show a reduced FA in the regions of white matter known to be susceptible to diffuse axonal injury. Newcombe et al. (2007) show reduced FA in both these susceptible regions *and* whole brain white matter when compared to a control group. The fact that the authors of the above papers have been able to show that reduced white matter FA exists in TBI patients in regions known to be most susceptible to DAI has led them to postulate that these FA reductions indicate diffuse axonal injury – the reduction in FA being caused by disruption of axons and their myelin sheaths thereby causing less restriction to water movement across them.

Of the studies that looked both at FA and outcome from TBI, no relationship was found when analysing whole brain white matter, and only the FA in the cerebral peduncle

correlated with the Glasgow outcome scale (GOS) in 30 patients with severe brain injury (Newcombe et al., 2007, Sidaros et al., 2008).

Fractional anisotropy values can be used to map tissue structures in vivo. In the brain, FA values allow mapping of axonal tracts in white matter. As described above, the movement of water molecules within brain white matter is anisotropic, with the largest eigenvector parallel to the axon fibres. Using this information, a map of axonal tracts within the brain can be produced and rendered in 2 and 3 dimensions. This technique of mapping white matter tracts is called tractography. It follows that using tractography, individual patients can be studied to ascertain whether their white matter tracts show evidence of shearing after traumatic brain injury (Gold and Lipton, 2008).

2.6.2 *DTI analysis techniques*

Given that the MD and FA values obtained using DTI describe the micro-movement of water molecules, and these are hypothesised to be different depending upon the tissue type under scrutiny, to simply obtain a mean MD or FA value for the whole brain (grey and white matter values combined in one analysis) would be meaningless. Studies using DTI have therefore used a number of different techniques to obtain the values for the different structures they have been investigating.

The most popular technique employed involves the placement of regions of interest (ROIs) according to anatomical features on a conventional T1 or T2 weighted MR scan co-registered to the DTI data. Mean MD and FA values are then extracted for those regions and compared, either between patient groups, or with values from the same regions in controls (Levin et al., 2008, Matsushita et al., 2011, Newcombe et al., 2011, Niogi et al., 2008a). Other methods of data extraction involve automatically segmenting the data into tissue type (e.g. white matter or grey matter) and obtaining mean data values for each, or using a tractography software program to define tracts of axonal fibres within white matter and extracting MD and FA values from those three dimensional structures (Chu et al., 2009, Goetz et al., 2004, Hong et al., 2009, Newcombe et al., 2008).

Analysis can be performed by translating all available patient and control data into a ‘standard space’, which allows a direct comparison of like for like regions within the brain, or it can be performed in the ‘patient’s space’ or ‘real space’, which some groups feel gives a more accurate representation of the true values being examined in each subject (Aribisala et al., 2011).

Analysis can also be performed at the level of individual voxels, a technique called Voxel Based Morphometry, which is performed by translating the data for each subject into a standard space template, and running the data through a software program which highlights voxels where the data of the subjects being examined differs from that of the population it is being compared against.

2.6.3 *DTI in brain injury*

As DTI is a relatively new application, there have been a large number of recent studies examining its use in many different cerebral diseases and pathological states. The use of DTI in traumatic brain injury has expanded as the technology has become more readily available in the clinical centres that manage this patient group. Analysis of DTI data has for the most part concentrated on white matter regions and the fibre tracts therein. This is of particular interest to the field, as it is hypothesised that damage to those axonal tracts will cause a detectable alteration in both MD and FA, when compared to control subjects without the damage. Initial work has predominantly examined patients in the chronic stages of brain injury, as these patients are typically the least difficult to recruit and to scan. As discussed previously, patients with an acute brain injury are often not stable from a clinical perspective, and therefore are less suitable to undergo scans with the often lengthy acquisition times needed for DTI. That said, a small number of studies have still managed to examine patients in the acute and sub-acute stages of TBI.

2.6.4 *DTI white matter analysis in chronic TBI*

In patients who are months to years from their brain injury, the majority of studies using DTI have described a statistically significant long term increase in the apparent diffusion coefficient (ADC) and in the mean diffusivity (MD) of the white matter areas examined. Popular regions of interest under scrutiny include the corpus callosum, internal and external capsule, inferior and superior longitudinal fasciculus and the fornix, along with less well defined ROIs looking at lobar white matter. These regions have been chosen for study, not only because they contain long axonal fibres known to be disrupted in diffuse axonal injury, but also because these structures are known to be involved in memory, information processing and cognition (see Chapter 3), which are affected in post-concussive syndrome. All of the authors of these studies have postulated a link between the significant findings in their DTI data, where they exist, and the post-concussion symptoms suffered by the subjects they have analysed. In 2005, Inglese et al. (2005) examined 26 mild TBI patients a mean of 5.7 years after their

injury, and compared their DTI findings to those in 29 healthy volunteers. They showed significant increases in mean diffusivity in the splenium of the corpus callosum and in the internal capsule posterior limb in the patient group, when analysed using ROIs selected in normal appearing white matter. Two other studies have demonstrated an increase in apparent diffusion coefficient in similar regions in chronic TBI; Lo et al. (2009) showed an ADC increase in the genu of the corpus callosum in a small number of severe TBI patients examined, and Newcombe et al. (2011) found an increase in anterior and posterior corpus callosum ADC in 68 patients of mixed injury severity when those with a good outcome score were compared to those with a poor outcome.

In the chronic stage after traumatic brain injury, a number of studies measuring fractional anisotropy in these specific white matter tract regions have demonstrated a significant decrease in patients when compared to control groups (Nakayama et al., 2006, Palacios et al., 2011, Xu et al., 2007). In 9 patients with chronic severe TBI Xu et al. (2007) showed a significant decrease in FA in anterior and posterior corpus callosum ROIs, and in the posterior limb of the internal capsule. Nakayama et al. (2006) examined 23 patients who had recovered from coma after severe TBI, and compared them with 23 matched control subjects. They demonstrated a significant reduction in FA within the corpus callosum, not only with anatomically placed ROIs, but also when they performed a voxel-based analysis in standard space. The most recent work in this patient group has been published in 2011 (Palacios et al.) where rather than using ROIs placed according to anatomical landmarks, the authors' analysis used 'tract-based spatial statistics' to compare FA values in the white matter fibre tracts involved with memory, between 15 chronic severe TBI patients and 16 matched controls. Their findings were in keeping with earlier research, and showed a reduction in FA in the corpus callosum, superior and inferior longitudinal fasciculi and in the inferior fronto-occipital fasciculi.

This picture of reduced FA in the fibre tracts of chronic severe TBI patients is also reflected in the mild and moderate brain injury population. The study in 2005 by Inglese et al. showed reduced FA in the corpus callosum, internal capsule and centrum semiovale in 26 mild TBI patients compared to the 29 controls, as well as the increases in MD mentioned above. Kraus et al. (2007) investigated 20 mild TBI patients separately from 17 moderate/severe patients and compared the two groups with 18 control subjects. They not only found a reduction of FA in the moderate/severe patient group in all thirteen regions they analysed, but also demonstrated a significant FA

decrease between the controls and the mild patient group in the cortico-spinal tract, sagittal stratum and superior longitudinal fasciculus. In 2008, a number of studies were performed looking exclusively at mild TBI populations in the chronic phase of injury, and each demonstrated a reduction in FA in the patient groups when compared to control groups (Lipton et al., Niogi et al., Rutgers et al.). Furthermore, in 43 chronic mild TBI patients with persistent symptoms, Niogi et al. (2008a) were able to demonstrate a correlation between the number of examined ROIs showing reduced FA and the mean reaction time in a simple cognitive task, adding weight to the hypothesis that the detectable changes in FA are indicative of underlying tissue damage causing persistent cognitive deficits.

Analysis of lobar or whole brain white matter has shown fewer consistent findings in the chronic stages after brain injury. The lack of significant findings can, in part, be attributed to the dilutional effect of taking mean MD and FA values for all of the white matter voxels in the lobe under scrutiny, or in the case of whole brain examination, the mean values of every white matter voxel in the brain. As we know that some areas of the brain are more susceptible to diffuse axonal injury, it stands to reason that if those areas are grouped together with areas that are less likely to be affected, then the mean values calculated will have a lower likelihood of showing significance. That said, Benson et al. (2007) found a global reduction in whole brain white matter FA in a heterogeneous TBI patient group scanned a mean of 35 months after injury and Lipton et al. (2008) were able to demonstrate a small but significant reduction in whole brain white matter FA in 17 mild TBI patients. Both of these studies used a whole brain histogram analysis and compared the TBI patients to matched control groups.

2.6.5 *DTI white matter analysis in acute and sub-acute TBI*

The majority of papers looking at TBI in the acute phase (within 14 days of injury) have shown an increase in mean diffusivity and a decrease in fractional anisotropy, mirroring the findings in the chronic phase. In 2002, Arfanakis et al. showed a decrease in FA in normal appearing whole brain white matter, in patients with mild TBI less than 24 hours from injury, when compared to control groups. However, the power of their work was reduced by the fact that they only studied 5 patients. Inglese et al. (2005) observed an increase in the MD and a reduction in FA in the splenium of the corpus callosum when performing ROI analysis on 20 mild TBI patients, a mean of 4 days from injury. More recently, Kumar et al. (2009) studied 26 mild and 57 moderate TBI patients within 14 days of injury and again showed a significant increase in MD in the genu of the corpus

callosum and a corresponding decrease in FA, both in the genu and splenium of the corpus callosum. They used ROI analysis and compared the patient groups separately against 33 matched control subjects. In 2011 Ljungqvist et al. published their findings from a longitudinal study in severe TBI, where they examined 8 TBI patients a mean of 7 days from injury, and then again 6 months later. They observed a significant acute reduction in corpus callosum FA which was still present at 6 month follow up, using a tractography-based ROI analysis. They were also able to demonstrate a correlation between the reduction in FA and with TBI outcome, after measuring the extended Glasgow Outcome Scale score at 6 months. These results are being further substantiated by work currently published online and awaiting publication in print: Matsushita et al. (2011) are publishing their findings of a reduction in FA in the splenium of the corpus callosum in 20 patients with a mix of mild and moderate acute TBI, having used an ROI analysis.

However, while the results from most papers follow this pattern of increased MD and reduced FA, a number of studies have demonstrated differing results. In particular, the work of some centres has shown an *increase* in FA in the acute phase of injury (Bazarian et al., 2007, Chu et al., 2009, Hartikainen et al., 2010, Mayer et al., 2010, Wilde et al., 2008). Between 2007 and 2008, two groups published data on acute mild TBI. Bazarian et al. (2007) performed ROI analysis of 6 patients and 6 matched controls within 72 hours of injury, and found an increase in FA in the splenium of the corpus callosum which correlated with post-concussive symptom (PCS) assessment score as well as with neuropsychological test scores in visual motor speed and impulse control. Wilde et al. (2008) also showed an increase in corpus callosum FA along with a reduction in ADC in 10 mTBI patients less than 6 days from injury. They too found a correlation between the increase in FA and PCS severity. More recently Chu et al. (2009) examined 10 mild TBI patients and scanned them at a mean of 2.7 days from injury. Using voxel-based analysis, they demonstrated a number of white matter regions with reduced ADC. An increase in FA was also shown, but the authors note that this finding was in injury-affected regions, rather than in normal appearing white matter remote from any lesion. The increase in FA may therefore not only be due to altered anisotropy as a result of axonal disruption, but may also reflect underlying cytotoxic oedema.

In the sub acute time frame (2-8 weeks) from brain injury, the literature over the last 5 years has shown consistent findings of a reduction in FA in all regions examined, and in

the complete spectrum of TBI severity. Sidaros et al. (2008) and Tollard et al. (2009) both exclusively examined patients with severe TBI within 3 months of injury. Their patient groups numbered 30 and 43 respectively and both studies made comparisons with matched control groups. Sidaros showed a reduction in FA in all white matter regions investigated, which included the corpus callosum, internal capsule, centrum semiovale and cerebral peduncle, and Tollard found a similar reduction in both supratentorial and infratentorial FA, after a mean had been calculated from the FA values for supratentorial ROIs placed in the midbrain, temporal and occipital white matter, internal and external capsule and centrum semiovale, and infratentorial ROIs in the pons. Tollard et al. do note that their results may be affected by partial volume error, as the ROIs in the external capsule and pons contained grey matter values that they were unable to separate out.

Mild and moderate TBI patients have also been studied in the sub-acute injury phase, with similar findings of a decrease in FA, in studies with small patient populations. However, Lange et al. published in 2011 their work on 60 mild TBI patients who were 6-8 weeks post injury, by far the largest mild TBI study cohort yet examined. They found no significant differences in either MD or FA in the corpus callosum, when comparing the entire patient group to 34 control subjects, and no significant differences when they separately analysed the patients in subgroups according to whether or not they were suffering from post-concussive symptoms.

2.6.6 *DTI grey matter analysis in TBI*

Examination of grey matter using DTI has been less widespread, and in the case of cortical grey matter this may be due to difficulties during data processing; the intervolutions of grey and white matter within the gyri and sulci of the cortex make the MR data more susceptible to partial volume effects. It may also be the case that investigators have found it more difficult to hypothesise as to the cause of any identified difference in MD or FA in grey matter regions where there are no long fibre tracts restricting diffusion. However, diffuse axonal injury is not only limited to brain areas containing long axonal tracts, but also occurs at junctions between areas of differing density, such as at the grey-white matter interface (Hou et al., 2007). Here the acceleration/deceleration forces have the greatest impact, as the less dense grey matter moves more rapidly than the denser white matter underlying it. This movement causes focal alteration, leading to delayed disconnection of axons connecting the grey and white matter. Therefore, it can be hypothesised that any observed MD or FA differences

in cortical grey matter may represent these progressive structural and sub-cellular consequences of mechanical deformation (Büki and Povlishock, 2006).

Those studies that have analysed cortical grey matter have shown significant differences in ADC, MD and FA between patients with brain injury and matched controls, both in the acute and chronic phase of injury (Hou et al., 2007, Kim et al., 2011, Newcombe et al., 2011). In a heterogeneous group of acute TBI patients compared to controls Hou et al. found an increase in ADC in deep grey matter structures (along with both deep and cortical white matter), and a *decrease* in ADC within peripheral grey matter. The authors postulate that this reduction in ADC in the peripheral grey matter could reflect differences in diffusional characteristics of contusional versus axonal injury.

2.6.7 *DTI correlation with clinical outcome and neuropsychology performance*

In chronic TBI across the spectrum of severity, a number of studies have shown a correlation between their findings of increased ADC or MD and decreased FA, and clinical outcome. The hypothesis being that these findings indicate underlying structural damage to axonal tracts which causes impairment of higher mental function in those who maintain or regain consciousness after TBI, and may be implicated in the pathological mechanisms in those who do not, resulting in persistent vegetative state or death.

In 2006, Wilde et al. found that a higher FA in the corpus callosum of their 16 children with chronic TBI was related to a better functional outcome measured using the Glasgow Outcome Scale (GOS), while Newcombe et al. published in 2011 their findings of a significant trend of increasing ADC in all regions of interest (grey matter and white matter) with worse outcome, again categorised by the GOS. Newcombe et al. also found that a worse clinical outcome corresponded with significant trends of decreasing FA, but only in white matter ROIs.

In the acute TBI population a correlation was found between both a reduction in ADC, and an increase in FA, and post-concussive symptoms by Chu et al. (2009) using the Rivermead Post-Concussion Symptoms Questionnaire. Perlbarg et al. (2009) scanned 30 patients a mean of 23 days from injury, and then grouped them according to GOS score at 1 year into unfavourable (GOS 1-3) or favourable outcome (GOS 4-5). They found that there was no correlation between ADC and outcome at 1 year, but that FA measured in the inferior longitudinal fasciculus, cerebral peduncle, posterior limb of the internal capsule and posterior corpus callosum was specifically decreased in the

unfavourable outcome group compared to the favourable one. In 2008, Sidaros et al. were able to demonstrate that FA in the cerebral peduncle correlated with 1 year GOS score in 30 patients with severe TBI scanned within 12 weeks of injury.

Many studies have further tried to narrow down a relationship between differences in MD or FA that they have observed, and the specific neuropsychological functions that those brain areas may relate to. In cross-sectional studies, authors have postulated that the differences in diffusion markers indicate structural damage that is causing the ongoing cognitive symptoms, and these findings have prompted longitudinal studies, hypothesising that DTI might have a prognostic role in TBI, by being able to predict who will have these ongoing symptoms, and what those symptoms will be.

In the chronic stages of TBI a reduction in FA in the left posterior cingulate, left hippocampal formation and left frontal, temporal and occipital cortex was found by Salmond et al. (2006) to be correlated with impaired learning and memory. In 14 children with TBI, 6-12 months post-injury, Wozniak et al. (2007) found that FA in the frontal and supracallosal regions was correlated with executive functioning, and that supracallosal FA was also correlated with motor speed, when tested using a number of neurocognitive evaluations. Niogi et al. (2008b) demonstrated a correlation between attentional control and FA in the left hemisphere anterior corona radiata, and a correlation between memory performance and FA in the uncinate fasciculus in 43 patients with chronic mild TBI.

Correlations with cognitive performance have also been found acutely after TBI; Lipton et al. (2009) showed a correlation with lower FA in the dorsolateral pre-frontal cortex and worse executive function in 20 mild TBI patients less than 2 weeks from injury, and a correlation was found by Wu et al. (2010) between FA in the left cingulum bundle and with 30 minute delayed recall in 12 adolescents with mild TBI. Niogi et al. (2008a) found that the number of ROIs with decreased FA compared to controls was significantly correlated with mean reaction time in 34 adults with persistent post-concussive symptoms after mild TBI.

Unsurprisingly, the authors of these papers state that their findings support the hypothesis that diffusion anisotropy measurement can be used as a quantitative biomarker for neurocognitive function and dysfunction, and therefore may contribute additional diagnostic information.

2.7 Summary

MRI has an important role in traumatic brain injury which is increasing in line with current development of MRI techniques. Purely structural MRI imaging techniques have already been shown to be superior to other forms of conventional imaging in the detection of almost all of the heterogeneous features of brain injury (Newcombe et al., 2007, Garnett et al., 2001b, Lagares et al., 2009, Le and Gean, 2009, Lee and Newberg, 2005, Metting et al., 2007). In the clinical setting these are now becoming augmented by sequences such as DTI which generate large quantities of data on the whole brain of head injured patients. It is hoped that with more information about local tissue characteristics at the site of injury it will enable greater insight into the pathophysiological changes caused by traumatic injury. This paves the way, not only for their use in detection of such changes, but also for their role in prognosis and decisions on management in brain injury in the future.

From the review of the literature in each of the MRI techniques above, it can be seen that a large amount of research has been performed on patients who have suffered severe TBI, both in the subacute and chronic stages of their injury (Benson et al., 2007, Blamire et al., 2002, Di et al., 2008, Garnett et al., 2001b, Lagares et al., 2009, Mamere et al., 2009, Newcombe et al., 2007, Robertson et al., 1999, Sanchez-Carrion et al., 2008a, Sidaros et al., 2008, Sidaros et al., 2009, Tollard et al., 2009, Weiss et al., 2008, Belli et al., 2006, Cadoux-Hudson et al., 1990, Carpentier et al., 2006, Hillary et al., 2007). The unstable nature of *acute* severe TBI accounts for the relative lack of research involving this patient group. They are usually too unwell, either as a direct result of their brain injury, or as a result of coexisting injury to other organ systems, to allow safe transfer to the MRI scanner and the long image acquisition times involved.

The use of the different available MRI techniques on patients with mild head injury, has also been well researched, with one paper assessing mild TBI within 24 hours of injury (Arfanakis et al., 2002). However, the numbers of patients recruited into studies on mild TBI tends to be directly related to the time from injury at which they are examined; the largest cohort of mild TBI patients found was 60, in the recent study performed by Lange et al. (2011) where the patients were examined between 6 and 8 weeks post-injury. In contrast the largest cohort found of mild TBI patients studied within 2 weeks of injury was 26, in the study performed by Kumar et al. (2009) into the correlation between neuropsychometric test performance and DTI findings in the corpus callosum.

The largest cohort of mild TBI patients scanned within just 7 days of injury numbered only 10 (Wilde et al., 2008).

While it is understandably important to study patients with severe injuries and the effects that this has upon the brain, it must be noted that patients with an injury this severe make up only 5% of all those who suffer a TBI, and furthermore, those who sustain a severe primary injury, are those least likely to make a meaningful recovery. The author anticipates that by attempting to overcome the barriers to recruitment, and focusing further study on *acute* mild and moderate brain injury, using quantitative MRI techniques, there is the potential to significantly add to the research already performed in this field, and to have greater impact on a larger number of patients.

Chapter 3. Neuropsychology in Head Injury

3.1 Background

The use of neuropsychological testing is well established in the management of head injury. Numerous studies have previously recommended that neuropsychological assessment should be a routine component in the investigation of acute and chronic mild TBI, and as a result various groups, authorities and governing bodies have included this requirement for neuropsychological assessment in published guidelines for the management of concussion (Hunt and Asplund, 2009, Sheedy et al., 2009, Gioia et al., 2009, Tsaousides and Gordon, 2009, Riggio and Wong, 2009, Jaffee et al., 2009, Lovell, 2009, McCrory et al., 2009).

Neuropsychological testing after TBI provides the ability to identify subtle cognitive deficits which may otherwise have been missed by routine clinical assessment and observation. Early identification of these deficits allows for the timely organisation of rehabilitation input, if needed, and can provide information on prognosis in individual cases. Furthermore, information and education about the likely impact that these symptoms will have during their recovery may be passed on to the patient and their relatives.

3.2 Detectable Cognitive Symptoms

As mentioned briefly in Chapter 1, there are a number of cognitive symptoms which may be experienced by patients who have suffered a brain injury. They are more prevalent in those with moderate and severe TBI, but can be present even in those with the mildest injury (Thornhill et al., 2000). Cognitive symptoms associated with concussion include slowed information processing, deficits of learning and memory, problems with attention and concentration and with higher executive functions, such as planning, switching parameters, organising and sequencing (Vanderploeg et al., 2005, Riggio and Wong, 2009). Patients may find that tasks which were routine before their injury have become difficult or impossible to carry out. Even at their most subtle, these symptoms can become evident with focused neuropsychological testing.

The specific cognitive deficits suffered by those with brain injury are related loosely to the location of damage within the brain. These symptoms and the locations they are linked to are outlined below (Table 3.1).

Lesion location	Symptoms displayed
Dorsolateral frontal region	Difficulties in switching parameters or planning, mental inflexibility, slowness in performance, short term memory
Orbito-frontal region	Agitation, disinhibition, poor impulse control
Medial frontal region	Apathy
Temporal region	Memory disturbance and emotional lability

Table 3.1: Cognitive symptoms associated with brain lesion locations (adapted from Riggio and Wong, 2009).

3.2.1 *Attention/concentration*

Attention or concentration can be defined as the ability to focus on a specific task for the necessary period of time. Deficits of attention result in distractibility and increased vulnerability to interference, and problems with attention may result in disorientation in time and place (Hodges, 1994). The functions of attention and concentration rely on specific brain regions: the brainstem, thalamus and cortical areas (dorsolateral pre-frontal, posterior parietal and ventral temporal cortices), along with the white matter tracts that connect them, such as the anterior corona radiata (Niogi et al., 2008b). These regions and the tracts connecting them are the reticular activating system. Lesions or diffuse damage affecting any of its components may result in a concentration or attention deficit, and this can be tested with a number of different neuropsychology tests. The attention/concentration tests specific to this study are the DSPAN back and spatial span back tests (which also test working memory), the colour-word interference test (as well as testing cognitive flexibility) and the PASAT (which tests working memory and information processing speed as well). These tests and their role in TBI will be explained in detail below.

3.2.2 *Memory*

Memory has been shown to be affected by TBI in a number of ways. It is very common for memory of the injury itself to be absent, and also for patients to suffer from a degree

of post-traumatic amnesia: an inability to remember events for a period after the injury, which may last hours or days. However, these memory problems are short lived and closely related to the time of injury. The most debilitating memory symptom reported after TBI is an impairment of short term and working memory function long after the injury. This affects the patient's ability to recover by interfering with every facet of life, from relationships to performance at work and all activities of daily living in the home.

It is theorised that distinct separate types of memory exist, but there is some controversy as to the number of these memory types and where their definitions lie. It is generally agreed that long-term memory exists, and does so separately from other types of memory; it would be hard to deny that each person has a vast store of knowledge and a record of prior events, that in normal circumstances can be accessed and reiterated without difficulty (Cowan, 2008). Long-term memory is also referred to as semantic memory, and can be further subdivided into declarative memory (e.g.: memory for events and factual knowledge) and procedural memory (memory about how an object is used or how a task is performed). The ability to lay down long term memories is dependent on the hippocampus, and storage of long term memory is thought to be associated with the temporal neocortex, predominantly in the left (dominant) hemisphere (Eichenbaum, 2000).

Current thinking is that aside from long-term memory, two other memory types exist: short-term memory and working memory. Scientific opinion differs as to whether these are actually separate from each other, or to what degree there is crossover between the two. Short-term memory is commonly defined as the facet of memory that holds a small amount of information in an easily available state for a limited period of time, and is associated with the frontal lobes, in particular the dorsolateral prefrontal cortex. Working memory however, is defined by different authors in different ways. Some use it to refer to the part of memory used to plan and carry out behaviour, others to refer only to the aspects of short-term memory related to attention. Generally speaking however, working memory is taken to be the combination of multiple components of memory working together (Cowan, 2008). Working memory can be likened to the clipboard facility on a personal computer, where information can be 'copied to' and 'pasted from' to allow for problem solving. An example would be remembering locations to pass and turns to take when given directions by a stranger, and then using the information to follow the instructions to the correct location. The executive component of working memory is associated with bilateral frontal lobe function, while

the component responsible for repetition of words and numbers is located specifically in the posterior inferior frontal gyrus in the dominant hemisphere (Broca's area). Damage to these areas therefore may cause deficiencies in working memory. This is tested for verbally using the list learning test and the DSPAN back test, and non-verbally using the design learning test and spatial span back test.

3.2.3 *Executive function*

Executive function broadly refers to the control of aspects of attention and future planning and decision making. It is involved in decision making processes when those decisions are not automatic responses based on learnt behaviours and previous experience. It encompasses a number of functions: adaptive behaviour, abstract conceptual ability, mental flexibility, problem solving, planning, initiation, sequencing of behaviour and aspects of personality involved in drive, motivation and inhibition. Executive function is associated almost exclusively with the frontal lobes, more specifically with the prefrontal cortex, although in order to orchestrate higher intellectual function, it has abundant connections to almost all other cortical and subcortical structures (Hodges, 1994). Tests for assessing a participant's executive function include the Colour-Word interference test and parts of the verbal fluency test.

3.3 Neuropsychological Tests used in TBI

A vast range of neuropsychological tests exist, some are applicable for use in a variety of conditions, including TBI, while others have been adapted or designed specifically for use in head injury. The neuropsychology test battery used in this work was compiled from established tests selected after discussion with two consultant neuropsychologists in the centre where the research was to be performed. The areas of function we wanted to test were attention, concentration, short term memory and executive function, all of which have been shown to be affected by mild head injury (Smits et al., 2009, Hall et al., 2005). The tests used are examined in the context of head injury below.

Performance in neuropsychological tests is affected by the education level and intelligence of the participant taking the test. In some cases it might affect their ability to understand the test instructions, or if the test involves thinking of words starting with a particular letter, such as in the verbal fluency test (section 3.3.9), the extent of the participant's vocabulary will have a direct effect on the result. It is important to take this

into account when assumptions about the effects of brain injury are being based on the results of the tests. When comparing results between a patient and control subject it is necessary to obtain an indication of the patient's premorbid IQ and education level, and to match the control subject accordingly, to allow for a fair comparison.

3.3.1 *National adult reading test (NART)*

The national adult reading test allows assessment of the participant's intelligence quotient (IQ). The test consists of 50 words which do not conform to normal grammatical rules. This ensures that when they are read aloud by the participant, the correct pronunciation depends upon them having been seen and heard previously. There exists a high correlation between reading ability and intelligence in the normal population, and as such the NART has been shown to be a good measure of pre-injury IQ (Crawford et al., 1989). Performance is linked to the level of education and to social class. Performance is not affected by age, gender and ethnicity. Previous work has shown that NART scores represent premorbid intelligence and are generally resistant to neurological insults such as mild head injury (Crawford et al., 2001, Crawford et al., 1988). However, other studies testing patients within 12 months of injury have shown that the results can be affected by severe TBI, and in such cases using the NART may significantly underestimate the premorbid IQ (Riley and Simmonds, 2003).

In order to calculate the participant's IQ the NART error score is used along with a published table. The mean IQ for each group was compared to ensure similarity between groups.

3.3.2 *The BIRT memory and information processing battery (BMIPB)*

This test battery, published by the Brain Injury Rehabilitation Trust (BIRT), is widely used in the assessment of patients with traumatic brain injury. Compiled in 2007 by Coughlan, Oddy, & Crawford, it is based upon the adult memory and information processing battery (AMIPB) which has been in use since its development in 1985. It is described as a 'revision and extension' of the tests therein, and has been designed specifically for use with the brain injured patient. While large numbers of studies refer to the AMIPB, relatively few have referenced the BMIPB. This is probably due to the fact that it was only published 3 years ago. Three of the BMIPB tests were utilised in this study, speed of information processing, design learning and list learning.

3.3.3 *Speed of information processing*

The first of the BMIPB tests used, the speed of information processing (SoIP) test consists of multiple rows of five double digit numbers. The participant must identify the second highest number in each row, and cross it out. This is a timed test with four minutes allowed. In order to correct for intra-participant differences in motor speed, a second shorter task, consisting of having to cross out as many number elevens in 25 seconds as possible, is then administered, and the overall SoIP calculated from the two scores. This test was first described in the AMIPB, and the authors demonstrated that it was able to differentiate those with cerebral damage from control subjects, although the sample population tested had mixed aetiology (Coughlan and Hollows, 1985). Using the SoIP test, Radford et al. (2004) found significant differences in injury severity between two groups of brain injured patients, when classed by their ability to drive safely. In a longitudinal study into 62 patients with mild TBI, Powell et al. (1996) showed a significant difference between SoIP performance within 48hours of the injury and after recovery at 3 months. Similar speed of cognitive information-processing tests have been shown to be correlated with intelligence test scores, indicating that IQ must be taken into account when evaluating the results of this test (Vernon, 1983).

3.3.4 *Design learning*

In this test, participants are shown a diagram consisting of a 4x4 array of dots with nine straight lines connecting some of the dots in a specific pattern (Figure 3.1). They are shown the design for 10 seconds, and then asked to recreate it from memory on a similar blank 4x4 array of dots. The participant has five attempts to correctly copy the design from memory, but if they correctly copy the design three consecutive times, they need not use all five. The participant is then shown a second different design (again for 10 seconds) and is asked to recreate this second design from memory. Lastly the participant is asked to draw the first design again, without being shown it a further time.

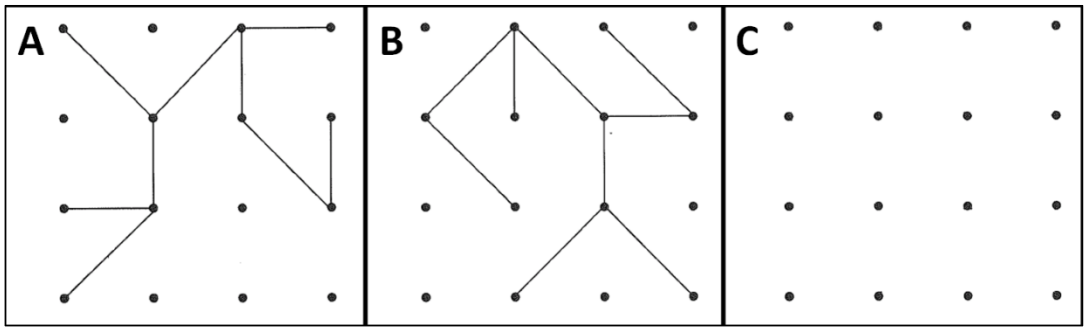

Figure 3.1: The BIRT design learning test: the first design shown to the participant (A), the second (interference) design shown to the participant (B), and the blank array the participant is given to recreate the designs from memory (C) (Coughlan et al., 2007).

The design learning test has been shown to be sensitive to cerebral dysfunction in a comparison study between 42 patients with a history of acquired brain injury and 169 controls, matched for occupational classification and educational level (Coughlan et al., 2007). Further validation of this test in the head injury population may be inferred from examining the use of a comparable test, the Rey Complex Figure test (RCFT). In 100 patients with TBI, examined at a mean interval of 86.46 days post injury, Ashton et al. (2005) demonstrated that performance on the RCFT showed a significant relationship with the presence of a visible diffuse injury on neuroimaging. Schwarz et al. (2009) tested 78 patients with acute moderate and severe TBI and found that 94% scored within the impaired range on the RCFT.

However, although both the design learning test and the RCFT are very similar, it should be noted that the RCFT may be administered without indicating to the candidate that they will need to remember the first drawing, thereby testing incidental learning, rather than effortful learning which is under test when using the BMIPB version.

Both the BMIPB design learning test and the RCFT present the participant with a diagram consisting of connected straight lines in a specific pattern, however, the Rey-Osterrieth Complex Figure (as its name suggests) is very complex, and in our experience, TBI patients and control subjects consistently perform poorly. It is known that performance on the test is affected by age and premorbid IQ (Gallagher and Burke, 2007). Based on the likely demographics of our study population (lower premorbid IQ and limited education), it was felt that a simpler test would be more likely to yield results that allowed differentiation between the patients and controls.

3.3.5 *List learning*

Similar in structure to the design learning test above, the participants have to try and remember as many of a list of 15 words as possible (list 'A') after hearing them read aloud. They have five attempts, but if they remember all of the words on three consecutive attempts, they do not need to use all five. They then hear a second list of a different 15 words (list 'B') and have to recall this new list. Lastly, they must remember as many as they can from the first list (list 'A') without hearing it again. This test was also demonstrated to show a significant difference between those with brain injury and controls (Coughlan et al., 2007).

As with the design learning test above, there are a large number of similarities between the BMIPB list learning test and other established tests. In particular, the Rey Auditory Verbal Learning Test (RAVLT) demonstrates an almost complete overlap with the BMIPB list learning test. However, the BMIPB test was specifically selected for this study as it has numerous parallel test forms. Many of the other existing memory tests have only one test form, and in studies where the participant is to be assessed on a number of occasions, the tests have to be repeated using the same form each time. Repeating a memory test several times may bias the results as participants become familiar with the material and the test results could imply that memory has improved when it has not done so.

There are very few references in the literature of studies using the BMIPB list learning test, presumably as a result of the fact that the BMIPB battery has only been in print for the last 3 years. However, the RAVLT has been used extensively in head injury, and has been shown to be sensitive in both in adults and children (Strauss et al., 2006). Guilmette and Rasile (1995) found that RAVLT scores in 16 mild TBI patients were significantly lower than in 16 matched controls. A significant correlation has also been demonstrated between the RAVLT and 1 year Functional Independence Measure score in severe TBI, and it has been used to identify even longer term cognitive impairment, 10 years after severe TBI (Ryu et al., Draper and Ponsford, 2008). Recall in the list learning test tends to be better at higher IQ levels (Strauss et al., 2006).

3.3.6 *Paced auditory serial addition test (PASAT)*

The paced auditory serial addition test or PASAT was designed in 1977 (Gronwall) specifically to monitor the recovery of patients who had sustained mild head injuries. Spoken numbers are presented to the participant at three second intervals. The

participant is required both to maintain attention when listening to the numbers, and perform mental calculations involving the digits. Participants must add each new digit to the one immediately prior to it and speak the result of their calculation aloud. The participants therefore do not give a running total, but rather the sum of the last two numbers presented to them. After performing the test with the numbers presented at three second intervals, the second part of the test presents the numbers every two seconds. This tests their divided and sustained attention, information processing speed, flexibility and working memory.

The test result is the number of correct sums given out of a possible 60. This is then converted into a percentage as some authors argue that this is a more accurate measure of the information processing capability (Dyche and Johnson, 1991). It has been used extensively in TBI, and has been shown to differentiate between those with both mild and severe TBI and matched controls (Bate et al., 2001, O'Jile et al., 2006, Cicerone and Azulay, 2002).

This test is susceptible to practice effects, and performance is adversely affected by increasing age, a lower IQ and poor arithmetic ability (Tombaugh, 2006). The PASAT is a highly significant, but non-specific test, implying that a low score may not necessarily be due to brain injury and other factors (IQ, age, mathematical ability) must be taken into account (Tombaugh, 2006).

As the PASAT is a difficult test, requiring attention, working memory and information processing, participants can become frustrated, resulting in a proportion not completing the test. This can introduce bias, as the results will then be skewed towards those of high performing participants. As a result of this, some authors have advocated the use of the discontinuation rate of the test as a measure of attention (Vanderploeg et al., 2005). The test is discontinued if the participant cannot get at least two answers correct, whether consecutive or not, in an initial practice sequence presented before the test itself, or if the participant is unable to get at least one answer correct on the three second test, they do not progress to the two second test and are considered unable to perform the test.

The PASAT has been shown to be sensitive to features of concussion (Gronwall and Wrightson, 1974). However, specific analysis of the PASAT score and measures of head injury severity (LOC and PTA) have shown no correlation (O'Shaughnessy et al., 1984, Strauss et al., 2006). Persistent or late post-concussion symptoms are well

identified using the PASAT when compared to other tests, which may be due to the fact that the PASAT requires *both* working memory and processing speed, as opposed to one or the other (Strauss et al., 2006).

The use of the PASAT in brain injury has shown poorer performance in patients when compared to controls. Christodoulou et al. (2001) examined 9 moderate and severe TBI patients with functional MRI (fMRI) while performing a modified PASAT, a mean of 51 months from injury. Compared to 7 control subjects, the TBI patients made significantly more errors. Cerebral activation was found in areas of frontal, parietal and temporal lobes known to be activated in working memory tests. Hattori et al. (2009) reported on their findings using single photon emission computed tomography (SPECT) scanning combined with testing using the PASAT in 15 mild TBI patients in the chronic stage. They found that PASAT results showed a tendency toward lower scores in mild TBI patients when compared to those of 15 controls. In particular, the score of the first trial of PASAT was significantly lower in patients with mild TBI who complained of cognitive fatigue. Interestingly, they also demonstrated that activation in the cerebellar cortex correlated significantly with PASAT performance, decreasing in the mild TBI patients who scored poorly, but that in those same patients there was a significant correlation with activation of inferior frontal and superior temporal cortices instead, suggesting a compensatory mechanism. They suggest that these findings may explain cognitive impairment and cognitive fatigue in the chronic recovery phase of mild TBI. A review of TBI studies using the PASAT was performed in 2006 (Tombaugh) and notes that the PASAT is sensitive to the cognitive effects of concussion if the tests are administered within the first day post-injury, and in chronic TBI patients with persistent post-concussive symptoms the PASAT has also repeatedly shown a relatively high degree of sensitivity.

3.3.7 *Digit span backwards*

The digit span backwards (DSPAN back) is a test from the Wechsler Adult Intelligence Scale (WAIS) battery. Originally developed in 1955, the current version is the 4th edition (WAIS-IV) and was released in 2008. The DSPAN back test involves listening to digits read out by the tester and repeating them out loud in reverse order. The test begins with a sequence of two numbers e.g. ‘3, 4’. The correct response would then be ‘4, 3’. The sequence is then increased by one digit every second trial. This results in two trials of each sequence length. The test is discontinued if the participant repeats the digits incorrectly on both trials in a particular sequence length. Historically the digit

span backwards has been employed as a measure of working memory, as the participant has to remember the numbers *and* reverse the sequence before relaying them back to the tester (Oberauer et al., 2000). Recent work by St Clair-Thompson et al. suggests that in adults it is more likely to be testing short-term memory.

In patients who sustained mild head injuries of different severity, the digit span backwards has been shown to be sensitive in identifying these differences between patients grouped as Grade I, II or III using the American Academy of Neurology guidelines, although the study numbers were small (Mrazik et al., 2000). Hughes et al. (2004) showed that 80 patients with mild TBI performed significantly worse when compared to 80 matched controls in a battery of five 'memory tests' including the DSPAN back, but did not report the individual significance of the tests employed. A study into severe TBI by Kersel et al. (2001) used the DSPAN back to assess 65 patients at both 6 months and one year from injury, and found a significant difference in performance on the test between the two time periods, indicating late recovery of memory function. As the DSPAN back is a test from the WAIS battery, it is expected to be affected by the subject's IQ.

3.3.8 *Spatial span backwards*

The spatial span backwards is a test from the Wechsler Non-Verbal scale of ability (WNV). Similar to the DSPAN back above, it is designed to test working memory, but also tests short-term memory to a degree. The test uses a board with ten randomly orientated blocks attached to it. The block sides facing the examiner show the numbers 1 to 10, the sides facing the candidate are blank (Figure 3.2).

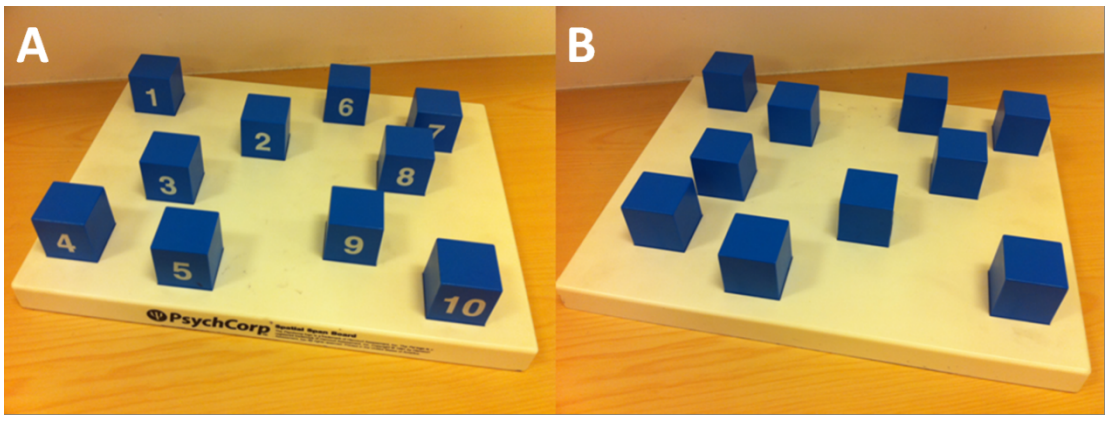

Figure 3.2: Images showing the Psychological Corporation testing board for the 'spatial span backwards' test, showing (A) the side facing the examiner with numbers visible, and (B) the side facing the test participant.

Starting with a sequence of two, the blocks are tapped in a specific order. For example, the examiner would touch the block with the number '3' on it and then touch the block with the number '4' on it. The test participant observes the examiner tapping the blocks in turn, but does not see the numbers written on the blocks. The participant is then asked to tap the blocks in reverse order from memory. As with the DSPAN back, the sequence is then increased by one block every second trial. This results in two trials of each sequence length. The test is discontinued if the participant touches the blocks in the incorrect order on both trials in a particular sequence length.

The spatial span back test is a useful test of memory in combination with the DSPAN back test. It does not rely on any numeracy or vocabulary skills, as there is no verbal content, and therefore its results are even less likely to be affected by bias. In 46 patients in the chronic phase after severe TBI, Ferri et al. (2004) found that 36 (85.6%) showed a deficit in their working memory as tested using the spatial span back test as part of the Wechsler adult intelligence scale test battery.

3.3.9 *Verbal fluency (letter and category)*

The verbal fluency test has been in use in various forms for the last 20 years, and examines the participant's ability to 'word-find' under restricted search conditions, testing executive function. The most common version of this test, and the one used in this study, uses phonemic and semantic cues. The phonemic cues are the letters 'F', 'A' and 'S'; the participant is asked to produce as many words beginning with the letter given in 60 seconds, without using proper nouns, numbers or multiple words with a common stem. The next section of the verbal fluency test uses semantic cues. The participant is restricted not by initial letter, but by category. The participant is required firstly to name as many animals as possible within 60 seconds, and secondly to produce as many boys' names as possible within 60 seconds. The last section of the test uses a 'switching format': asking the participant to name alternately a piece of fruit and then a furniture object, again as many times as possible within 60 seconds. This last section further increases the demands on the participant's executive function.

The performance of the participant can be assessed not only by recording how many appropriate words are supplied within the 60 second period, but also by observing the types of errors that are made by the participant, and also by examining the order of words produced. Effective verbal fluency relies upon the existence of an intact semantic store for supplying a knowledge base of related words, and also upon an effective

search process to access and retrieve the information (Strauss et al., 2006). Troyer et al. (1997) investigated the cognitive processes behind the components of verbal fluency, and their findings suggest that performance involves clustering and switching, the participant generates a cluster of words within a subcategory that comes to mind, and when that subcategory is exhausted, the participant switches to a new subcategory. They found that clustering is predominantly an automatic process, related to temporal lobe disturbance, and that switching to another subcategory demands a degree of effort and is related to frontal lobe functioning. As this test has a substantial verbal component, it is not surprising to find that there is a correlation between the phonemic fluency component and verbal IQ (Strauss et al., 2006).

Numerous studies of TBI patients using the verbal fluency test have been performed. Jurado et al. (2000) studied 13 TBI patients matched with 26 control subjects. They found that the patients with frontal lobe injuries produced fewer words than the controls in the *phonemic* fluency categories and also found a correlation between lesion size and *semantic* fluency. They noted that not all of their patients were impaired, even in the presence of large bi-frontal lesions. A meta-analysis of verbal fluency performance in brain injured patients was performed in 2004 (Henry and Crawford). It found that the patients had a deficit on tests of both phonemic and semantic fluency, when compared to controls. In particular, phonemic fluency was more strongly related to frontal lobe lesions, whereas temporal lobe damage had a lesser effect on phonemic fluency, although still significant, and a greater effect on semantic fluency. Studies have found that phonemic fluency is most sensitive to left frontal lesions, although the right frontal lobe may be implicated as well (Baldo et al., 2001).

Verbal fluency has been investigated using fMRI by Abrahams et al. (2003), who showed activation in the middle frontal gyrus, anterior cingulate gyrus and inferior frontal gyrus was associated with verbal fluency. They state that verbal fluency strongly engages executive processes, is particularly disrupted by lesions to the frontal lobes, and is sensitive to traumatic brain injury.

3.3.10 *D-KEFS colour-word interference test*

The colour-word interference test was first published in 1935 (Stroop). It consists of pairs of conflicting stimuli, a name of one colour printed in the ink of another colour, presented simultaneously. The version of the test published in the Delis-Kaplan Executive Function System (D-KEFS) is based on Stroop's original test, and includes

an additional test requiring the participant to switch back and forth between naming the ink colour and reading the conflicting words (Delis et al., 2001).

When presented to the candidate, the test consists of four parts. Part one assesses basic naming skills by measuring the speed at which the candidate is able to say aloud the colour of printed blocks as quickly as possible. Part two assesses the participant's ability to read repeating words at speed (the names of colours printed in black ink). In part three the candidate is shown the names of colours, where the ink is a different colour from that spelled by the word, and they must say the colour of the ink, and not read the word. In part four they must switch between reading the word, and saying the colour of the ink, depending upon whether the word is surrounded by a black box (Figure 3.3).

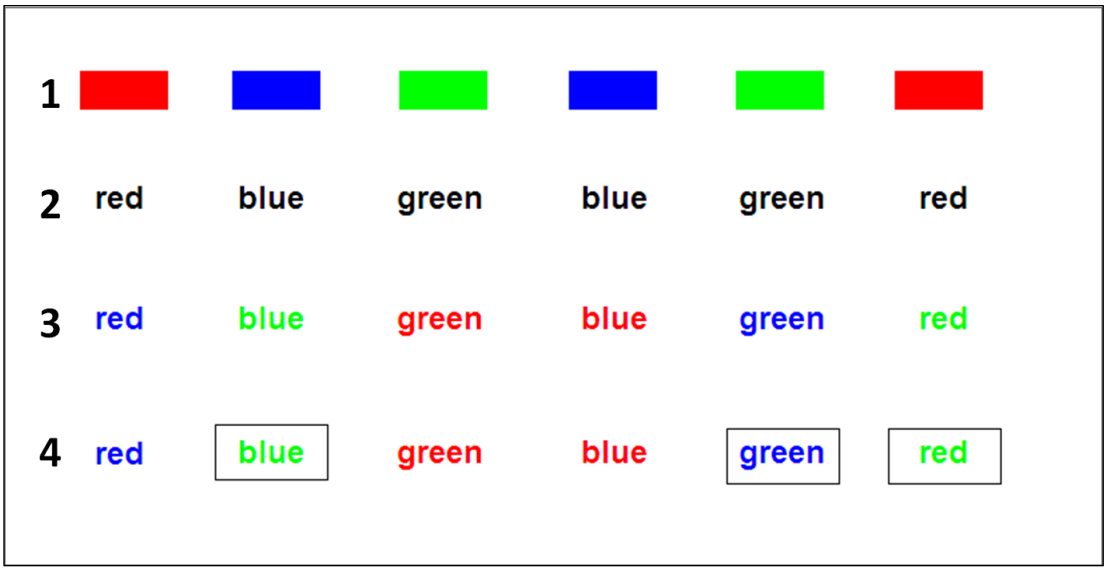

Figure 3.3: Lines 1-4 are shortened examples of the individual parts of the colour-word interference test. In part 1 the participant says the colour of each block aloud; in part 2 the participant reads the words; in part 3 the participant says the colour of the ink each word is written in and does not read the words themselves; in part 4 the participant reads aloud the words in the boxes, but says the ink colour of the words not in boxes.

The time taken for the participant to complete each part of the test is recorded in seconds. The difference in time taken to read the words printed in colours and the same words printed in black is the measure of the interference of colour stimuli upon reading words. The difference in time for naming the colours in which the words are printed and the same colours printed in squares is the measure of interference of conflicting word stimuli upon naming colours (Stroop, 1935).

The first two parts of the colour-word interference test measure basic functional skills, and parts three and four assess executive function: verbal inhibition in part three, and both verbal inhibition and cognitive flexibility in part four. There is a correlation between the test and IQ: the higher an individual's intelligence score, the less likely they are to experience interference (Strauss et al., 2006). Both Stroop's original version and the D-KEFS version of the test have been used by studies into TBI. Bohnen et al. (1992) compared performance on the Stroop test between 10 mild TBI patients with post-concussive symptoms (PCS) at 3 months, and 10 mild TBI patients who were asymptomatic at 3 months. They found that patients with persistent PCS at 3 months performed worse than asymptomatic patients, supporting their hypothesis that cognitive deficits in mild TBI patients are most evident when cognitive demands are high due to dealing with complex tasks. Batchelor et al. (1995) compared 35 patients with mild TBI to controls matched for age, education and IQ, and showed that mild head-injured patients performed more poorly on the original, modified, and interference conditions of the Stroop Test. They state that their findings support the hypothesis that mild head injury results in an identifiable impairment of focused attention. In 1996 (Lacroix and Bailey), another study examined 50 moderate and severe TBI patients with the D-KEFS version of the test, and found that as expected, the patients performed poorly on the additional fourth subtask, and that performance was related to the time from injury in their patient group. Neuroimaging studies have demonstrated an association between performance on the Stroop test and with specific brain regions: with the anterior cingulate cortex, the insular cortex, and other frontal and parietal areas (Carter et al., 1995, Goethals et al., 2004, Pardo et al., 1990, Taylor et al., 1994). A study in 2001 (Stuss et al.) examined 51 patients with single focal brain lesions in frontal and non-frontal brain regions, and compared their results on the Stroop test with 26 healthy control subjects. Their results showed that damage to the left dorsolateral frontal lobe resulted in increased errors and slowness in response speed for colour naming, and that exaggeration of the Stroop interference effect was observed in patients with superior medial frontal lesions (the supplementary motor area), not with cingulate lesions, contrary to the results of previous studies.

3.4 Summary

Neuropsychology has an important role to play in the detection and measurement of cognitive dysfunction in TBI patients. It guides rehabilitation input, provides information on prognosis and educates patients and their relatives about the impact of

their TBI. However, it is also a useful adjunct to any imaging study assessing TBI. In the following methodological chapter, the techniques by which the data was analysed according to the neuropsychological test results are outlined, and in Chapter 6 the group findings of the neuropsychology results are presented and their impact on the scan data analysis discussed.

Chapter 4. Investigation of Structural MRI in Mild and Moderate Traumatic Brain Injury: Methodology

4.1 Rationale for the Study

Around 113,000 patients with head injury are admitted from accident and emergency departments in England each year (NICE, 2007). Mild traumatic brain injury (TBI) accounts for approximately 90% of these attendees and recovery may be complicated by long term cognitive and affective symptoms (Kay and Teasdale, 2001). Thornhill et al. (2000) have shown that the incidence of these sequelae in patients admitted with mild injury is higher than had previously been recognised. Conventional computed tomography (CT) and magnetic resonance (MR) imaging findings often do not correlate well with the clinical picture in these patients, and often underestimate the extent of damage (Lee et al., 2008). Quantitative MR imaging is a term encompassing a number of established MR techniques which have been used previously in patients with TBI. The techniques are sensitive to microstructural damage in areas of brain white matter which appear uninjured on conventional MRI (Goetz et al., 2004). However, studies have predominantly evaluated its use in acute TBI in moderate and severely injured patients, or in chronic TBI across the severity spectrum. It has been little used in the *acute* evaluation of mild TBI. Furthermore, studies that have used quantitative imaging in this patient group have suffered from limited patient numbers, and have often been restricted to one or two quantitative measures (Benson et al., 2007, Goetz et al., 2004, Hou et al., 2007, Inglese et al., 2005, Kumar et al., 2009).

4.2 Study Aims

The aims of this study were therefore:

To ascertain whether damage exists at a microstructural level and is detectable acutely after injury using advanced MR quantitative imaging by examining the mild and moderate TBI population.

To ascertain whether any such observable changes in tissue microstructure are proportional to injury severity, as assessed by the Glasgow Coma Scale.

If damage exists, to determine whether it correlates with post-concussive cognitive symptoms, detectable on neuropsychological assessment, and hence ascertain whether these MR techniques have a potential role in the clinical evaluation of such patients.

4.3 Study Hypotheses

1. Acute differences exist at a microstructural level between patients with traumatic brain injury and matched control subjects that are detectable using quantitative MR scanning, in tissue appearing normal on conventional anatomical MR imaging.

2. Detectable microstructural changes in the TBI population are proportional to the severity of the brain injury sustained, as defined by GCS.

3. Detectable microstructural changes in the TBI population correlate with post-concussive cognitive deficits when assessed using the appropriate neuropsychological tests.

4.4 Study Type

A prospective cross sectional study comparing a patient population with a matched control group.

4.5 Study Design

Patients were recruited according to the protocols outlined below, and were scanned using a 3T MRI scanner. A number of different scan sequences were performed, obtaining data on microstructural changes within the brain as described in detail in section 4.10. Patients were also subjected to a battery of neuropsychological tests, selected specifically to examine the executive functions known to be affected by brain injury. Control subjects were subsequently recruited matched to the age, sex and level of education of the patient cohort. They underwent the same scan protocol and neuropsychological test battery, and the results were then collated and compared between the two groups.

4.6 Patient Recruitment Strategy

Patients were selected for recruitment from a single UK centre: Newcastle General Hospital in Newcastle upon Tyne. Patients were recruited from the accident & emergency and the neurosurgery departments. The initial target numbers for recruitment were set at 35 patients with mild TBI (GCS 13-15) and 35 patients with moderate TBI (GCS 9-12) after advice regarding the expected number of head injury admissions through the centre had been taken from two consultant neurosurgeons. It was suspected that this number of patients was slightly lower than the ideal sample size needed to adequately statistically power the study.

Ideally, in order to minimise the possibility of a type two statistical error (false negative) a power calculation can be made to ensure that the study results are likely to represent the findings in the general population. An assumption would have had to have been made of the expected 'effect size': the actual difference between the control group and the TBI group of the MR quantitative variables being measured. After specifying the power desired from the study and the p value which is to be taken to be statistically significant, the effect size can then be used in a calculation to determine the sample size needed (Figure 4.1).

$$N = \frac{4\sigma^2(z_{\text{crit}} + z_{\text{pwr}})^2}{D^2}$$

Figure 4.1: Formula to determine the sample size needed for a study comparing the means of two groups, where N is the total sample size (patient group and control group), σ is the standard deviation of the groups (which is assumed to be the same for each), D is the smallest expected detectable difference between the means of the two groups, Z_{crit} is the standard normal deviate corresponding to the required significance level and Z_{pwr} is the standard normal deviate corresponding to the required power (Eng, 2003).

For example, in order to detect a mean difference of 25 ms between the mean grey matter qT1 of the control group and the mild TBI group, assuming a standard deviation of 50 ms in the control group, the study would have needed to recruit 86 patients and 86 control subjects for it to have been powered to 90%, with a p value assumed to be

significant at 0.05 (Eng, 2003). However, in view of the time frame available to recruit to this study, and after comparison with other published work in this field it was felt that the study's target numbers were a suitable compromise and would yield relevant data, albeit at a slightly lower power.

Patients were notified to the study investigators in a number of ways. They were either referred by accident & emergency staff, by neurosurgery staff and by the head injury specialist nurse, or they were identified by the study's clinical research associate (the author) from direct contact with the ward staff, or by scrutinising the online neurosurgery on-call referral log.

Patients were approached in person, while they were still inpatients, to allow the study information to be given to them and to determine their suitability as a participant by application of the inclusion criteria (section 4.7). They were provided with the study information sheet and left to read through it for a minimum of 24 hours. They were then re-contacted the following day, and if they had agreed to participate, informed consent was taken. Full ethics and research and development approval was in place, and the 24 hour period between being given the information and being asked whether they wished to participate was a requirement of the research ethics committee. This delay period allowed the patients sufficient time to read the information, reflect on the content and if necessary discuss it with relatives before making a decision as to whether to participate.

A scan appointment was obtained and the details given to the patient. In some cases, they were still inpatients in the hospital when the scan appointment occurred, and these patients were then transported to the scanner by a member of the study team. Those that had been discharged after agreeing to participate but before their scan appointment, were provided with transport to and from the site to enable them to take part.

Wherever possible, the neuropsychology tests were performed before the MR scan, to ensure that any feelings of disorientation, drowsiness and dizziness, which some patients reported after their scan, did not affect the outcome of the neuropsychology tests. In those cases where this was not possible a short break was provided after the MRI scan, before the neuropsychology testing.

4.7 Inclusion Criteria

A number of inclusion criteria were drawn up to ensure the patients would be safe in the MR scanner, to ensure they could get to the scanner without difficulty, and to ensure they themselves were able to provide the study with the necessary data it would need to test the hypotheses without bias. The specific criteria are listed below (Table 4.1). The discussion that follows outlines the reasons for their implementation.

Aged between 16 and 65 years
GCS 9 or above (mild or moderate)
Less than 14 days post injury
No previous history of serious head injury
No previous history of neurological/neurosurgical disorder
No previous psychiatric history (excluding depression treated by GP alone)
No history of drug or alcohol abuse
No coexisting injury limiting transfer to MR Centre
No difficulties understanding the English language
No visual and/or auditory difficulties
No contraindications to MR scanning (e.g.: pacemaker, aneurysm clip etc)

Table 4.1: Study inclusion criteria.

4.7.1 *Age*

Patients aged younger than 16 or older than 65 were excluded. Issues with ethical approval around consent in children determined the lower age range cut off point. The higher range limit was selected to try and reduce the impact of age related brain changes. It is known that brain white matter undergoes degenerative changes with normal aging, the frontal lobes having been recently shown to be particularly susceptible (Salat et al., 2005). While the impact of these changes has not been examined to a great extent in qT1 and qT2 relaxometry, studies have used DTI to assess the effects of age on mean diffusivity and fractional anisotropy, and have found both to

be affected (Zhang et al., 2008). In order to avoid this effect introducing bias into the results, the higher age limit of 65 was selected. However, as this upper age limit was a relatively arbitrary figure, during the progression of the study one patient over the limit was recruited (67 years old) as it was felt that their injuries and cognitive symptoms fitted into the study remit.

4.7.2 *GCS*

Head injured patients were selected initially by severity, using the GCS score obtained at approximately 30 minutes after injury. This slight delay between injury and assessment ensured that the GCS accurately represented the severity of the brain injury, as an assessment of the GCS too soon after injury can lead to an overestimation of the severity due to a transient loss of consciousness. Our interest in the mild/moderate end of the injury spectrum, as discussed in chapter 1, led us to restrict recruitment specifically to those patients with a GCS between 9 and 15. As the study progressed it became clear that the recruitment targets were not being met. At this stage it was decided that in order to recruit sufficient patients, the GCS criteria would be relaxed, and instead *any* patient with a head injury who was fit to participate in the study within the allotted time period would be included. In fact, in spite of this alteration to the inclusion criteria, by the time recruitment was halted, only one patient with a GCS of lower than 9 had been included in the study.

4.7.3 *Time from injury to scan*

Initially the time from injury to scan was set at 6 days. Previous research into localised changes in brain parenchyma following head injury, as well as studies observing ischaemic change after stroke, demonstrate that DWI can show very low apparent diffusion coefficient (ADC) values within the first 2-3 days, which then rebound through normal to elevated values. We aimed not to scan very acutely in case such effects were detected and confounded our observations. Furthermore, the earliest time points at which patients with mild and moderate TBI can safely be scanned are by definition different; the moderate patients being more likely to take longer to be clinically stable enough to undergo scanning as a result of their having the more serious injury. After considering these two factors, we set the window from injury to scan at 3-7 days. As recruitment started it soon became apparent that this was overly ambitious due to a number of limitations, some of which were true of studying the brain injured

population generally, while others were specifically related to the local organisation in our research building. Limitations from a logistical point of view, in terms of the scanner location being away from the immediate clinical area, coupled with the lack of medical support and equipment in its vicinity, meant that patients with a moderate injury were more difficult to recruit in the initial 3-7 day injury-to-scan window than we had anticipated. In order to allow for these moderately injured patients to become more stable, and therefore to be fit enough to be transferred to the scanner and back to the ward, the maximum time allowed from injury to scan was increased, firstly to 10 days, and latterly to 14 days. All patients scanned were within 14 days of their injury.

4.7.4 *Previous medical history*

In order to reduce the impact of existing conditions on the study results, patients were screened with regard to their past medical history. Neurological conditions, previous serious head injury, previous neurosurgery and drug or alcohol abuse all increase the possibility of having an abnormal brain scan, and these patients were excluded to ensure that bias was not introduced into the scan data. Similarly, those with a past history of psychiatric disease were excluded in case this caused a negative impact on their performance in the neuropsychological testing.

4.7.5 *Coexisting injuries*

It is not unusual for head injured patients to sustain other injuries concurrently, depending upon their mechanism of injury. In each case patients were assessed to ensure that they could be safely transferred to the MR scanner without subjecting them to unnecessary discomfort or danger.

4.7.6 *Language barriers*

Patients without adequate understanding of the English language, and patients with visual or auditory difficulties were also excluded, to ensure no bias was introduced into the neuropsychology results.

4.7.7 *Contraindications to MR scanning*

All patients were screened by the study radiographers, to ensure that they would be safe within the scanner.

4.8 Control Subject Recruitment

A number of strategies were used to recruit control subjects for the study. Posters advertising for healthy volunteers were distributed around the local community in public libraries, sports centres, newsagents, supermarkets etc. Flyers were also left at suitable locations. Specific groups of people were approached, such as a local young mothers group, workers on building sites, and pupils at a sixth form college (via their physics teacher). Members of university staff within the offices where the study was run were also asked whether they knew people who were suitably matched and who may be interested in participating.

Control subjects were matched as closely as possible to patients who had already been recruited into the head injury group. Age, sex and level of education were matched as closely as possible (section 4.9). An assessment of educational level was performed to ensure that no bias was introduced into the neuropsychology results. Patients were asked what age they left school, and the highest qualification level they had attained to date. Education level was divided into four groups (Table 4.2).

Group 1	No qualifications
Group 2	GCSE or equivalent
Group 3	A level / Diploma
Group 4	Degree / Higher degree

Table 4.2: Groups defined by level of educational achievement used to allow matching of patients with control subjects.

4.9 Data Collected

Simple demographic data for each patient and control subject was recorded: age at time of scan, sex, employment status/occupation and educational level.

Each patient was asked whether they were currently taking any medication. This data was used to help ensure each participant met the inclusion criteria, for example: a patient would have been excluded if they were taking medication for a psychiatric disease, even if they had not disclosed having such an illness.

Specific data related to the injury was also collected for each patient: the circumstances and mechanism of the injury, the Glasgow Coma Scale score on admission, whether there had been any loss of consciousness (LOC) or post-traumatic amnesia (PTA) and for how long each had lasted, and whether the patient had headache, had been vomiting or had had a seizure. Any other injuries besides their head injury were recorded. Lastly, it was noted whether the patient had undergone a CT scan as part of their clinical management, and if one had been performed the findings were also documented.

4.10 Scan Data Acquisition

MR scans were performed on a 3T whole body Philips Achieva System (Philips Medical Systems, Best, Netherlands) using an 8 channel head coil as receiver and body coil as transmitter. The anatomical images were positioned parallel to the AC-PC line, with the entire brain covered. All the MR scans were co-centred and squared with the anatomical images. The resolution and matrix sizes for each scan sequence were selected to be multiples of one another. They were also each aligned to a common set of angles and origin. The sequences were set up in this way to simplify comparison of the data between scans. Scanning was performed by four senior radiographers employed by the research centre, and the scans were downloaded from the scanner to the computers required for processing by a clinical research associate (the author). The total scan time for this investigation was 25 minutes.

4.10.1 *Anatomical images*

Standard clinical T_1 weighted anatomical images were acquired using 3D MPRAGE sequence, with Echo Time (TE) of 4.6 ms and Repetition Time (TR) of 9.6 ms. The volume was scanned in sagittal orientation with 1 mm isotropic resolution and a matrix size of 240 (anterior-posterior) x 240 (superior-inferior) x 180 (right-left). The scan was accelerated with a SENSE factor of 2 in the right-left direction and the standard image intensity correction was applied using the CLEAR reconstruction algorithm.

4.10.2 *T_1 mapping*

Quantitative T_1 maps were acquired using a rapid inversion recovery (IR) sequence with segmented inversion slab method and multi-slice single shot EPI readout. The image volumes were scanned in transverse orientation with 2 mm isotropic resolution and a matrix size of 128 (anterior-posterior) x 128 (right-left) x 72 (superior-inferior). The 72 slices were divided into 6 segments with corresponding adiabatic inversion slab width

of 60 mm, allowing 12 slices in each segment to be imaged at 12 inversion times (TI) from 0.25 to 2.5 s in even steps. The order of slices was varied during 12 repetition cycles to form the complete relaxation curve (Clare and Jezzard, 2001). The scan was accelerated with a SENSE factor of 2 in right-left\phase direction, with TE of 24 ms and TR of 15 s.

4.10.3 *T_2 mapping*

Quantitative T_2 maps were collected using a GRASE sequence (Oshio and Feinberg, 1991) with TR of 4.7 s. The image volumes were scanned in transverse orientation with 2 mm isotropic resolution and a matrix size of 128 (anterior-posterior) x 128 (right-left) x 72 (superior-inferior). Each slice was imaged at 8 echo times (TEs) starting at 20 ms and increasing with 20 ms increment. Each image was collected in segments of 5 k-space lines.

4.10.4 *Diffusion tensor imaging (DTI)*

DTI images were acquired using a Pulsed Gradient Spin Echo (PGSE) sequence and multi-slice single shot EPI readout, with TE of 71 ms and TR of 2524 ms. The image volumes were scanned in transverse orientation with 2 mm in-plane resolution, 6 mm slice thickness and a matrix size of 128 (anterior-posterior) x 128 (right-left) x 24 (superior-inferior). The scan was accelerated with a SENSE factor of 2 in anterior-posterior\phase direction, and reconstructed with the CLEAR algorithm. Diffusion weighting was achieved by applying a diffusion sensitive gradient pulse in 16 directions with b values of 0 and 1000 smm^{-2}.

4.10.5 *B_0 field mapping*

All imaging acquisitions (except the anatomical images) were based on echo planar imaging (EPI), one of the fastest acquisition methods in MRI. EPI readout suffers substantial spatial distortion in areas of non-uniform magnetic fields (e.g. in the areas around the skull air sinuses). To allow for software correction of this distortion a map of the non-uniformities in B_0 was required. B_0 field maps were acquired using a dual echo 3D GRE sequence with TE_1 of 2.5 ms TE_2 of 5.8 ms and TR of 27 ms. The image volumes were scanned in transverse orientation with 2 mm isotropic resolution and a matrix size of 128 (anterior-posterior) x 128 (right-left) x 72 (superior-inferior). The scan was accelerated with a SENSE factor of 1.5 in right-left\phase direction.

4.11 Scan Data Processing

In order for quantitative MR scan data to be compared between one subject and another, a number of processes must first be applied to each subject's dataset. As all subjects differ from one another in terms of brain size, anatomical variance and position within the scanner, no one scan set is the same as any other.

4.11.1 *Format conversion*

All data was saved from the scanner in Philips' own data format (.PAR and .REC file extensions). These were first converted to the more standard 'Analyse' format (.hdr and .img file extensions) to enable the data to be processed by a range of standard and locally developed tools.

4.11.2 *Brain extraction*

The first step in processing the data was to remove the brain tissue of interest from the encompassing skull bone and cerebrospinal fluid (Figure 4.2). This process is referred to as "brain extraction" and was performed using a brain extraction tool (BET) available as part of the FMRIB Software Library from the University of Oxford (Smith, 2002). The BET uses a surface model approach to accurately segment the data into brain and non-brain classes.

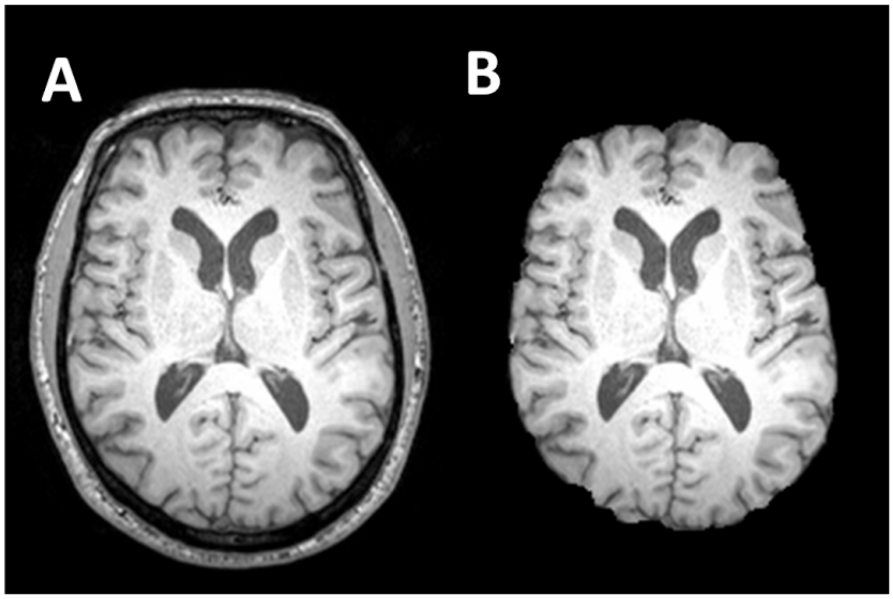

Figure 4.2: An axial T1 weighted MR scan as it is acquired (A), and after brain extraction (B).

4.11.3 *Movement correction*

Each set of scan data was acquired through a number of separate sequences acquired over a period of time. Small movements of the patients during this time, even if only as a result of breathing, would alter their head position within the scanner, and result in each diffusion volume occupying a different space from one another. In order for them to be analysed correctly, this movement was corrected for by registering each image to the first. This process was performed using a linear image registration tool (FLIRT) available as part of the FMRIB Software Library from the University of Oxford (Jenkinson et al., 2002). This tool uses 12 parameter registration: translation, rotation, stretch/compress and skew in each of the 3 axes, and results in each scan being registered to one another. This then allows the application of the same ROI to each of the scan sequences in the 'patient's space'.

4.11.4 *Fitting*

This process fits data from each data type (qT1, qT2 & DTI) to the expected known distribution curves for that specific data type. Raw data was subjected to fitting routines to extract the quantitative parameters. In the qT1 and qT2 data, this was performed by using in house software developed by a research associate. This software found the line of best fit to the data using relevant standard equations for T1 and T2 relaxation. In the DTI data this process was performed by using a fitting tool (DTIFIT) available as part of the FMRIB Software Library from the University of Oxford (Basser et al., 1994).

4.11.5 *Unwarping*

Unwarping was performed to correct distortion due to inhomogeneity in the magnetic field. As mentioned above (section 4.10.5) the quantitative image acquisitions were based on EPI, which suffers substantial spatial distortion in areas of non-uniform magnetic fields (e.g. in the areas around the skull air sinuses). The acquired fieldmap was used to create a map of the field distortion and this was then used to correct the quantitative data. This process used two tools from the FMRIB Software Library from the University of Oxford: the first (PRELUDE) performed 3D phase unwrapping of the field map (Jenkinson, 2003) and the second (FUGUE) used the fieldmap data to unwarp the EPI acquired quantitative data (Jenkinson, 2004).

4.11.6 *Registration*

To allow a region of interest to be defined and applied to each of the datasets, it was necessary to ensure that all scans were aligned with each other. It should be noted that as the scans were acquired in one sitting, they had already been acquired in the same space, assuming there had been no subject movement between each sequence. This registration step was a safeguard to ensure that if there had been any movement, it was accounted for. To achieve this the quantitative T1map was taken as the reference image and each dataset was registered to that image, resulting in all the datasets in the same 'real space'.

4.11.7 *Visible lesion removal*

As outlined above, the aim of this study was to assess tissue which appeared as normal on conventional anatomical MR imaging after TBI. To ensure that this was the case, we first had to individually examine each patient's scan for evidence of any haemorrhagic lesion or associated oedema. In a number of patients no visible lesion could be found. However, in the majority of those recruited, there was evidence of one or more discrete lesions either within the brain parenchyma (contusions, intra-cerebral haemorrhage or oedema) or extra-axial haematoma between the skull and the underlying brain tissue (sub-dural, sub arachnoid or extradural haematomas). As well as examining the T1 weighted anatomical image for each patient, every quantitative dataset was also scrutinised, and any gross, focal abnormalities from any of the scans were targeted for removal. These areas of visible lesion were removed from the data by first manually drawing a binary mask over them in the MRIcro software (Centre for Advanced Brain Imaging, Atlanta GA), and saving that mask as a reversed region of interest, giving the mask over the lesion itself a value of '0' and the mask over the surrounding brain a value of '1'. Each dataset was then multiplied by this mask, resulting in the area where the lesion had been having a value of zero, and the rest of the data retaining its original value (Figure 4.3). This process was applied on every slice where lesions were seen.

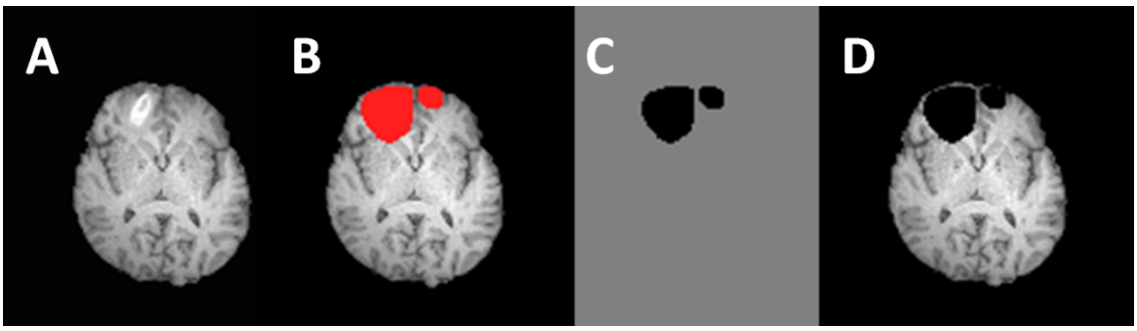

Figure 4.3: Lesion masking. Shown is a T1 weighted anatomical image (A), with right frontal contusion and surrounding bi-frontal oedema. The lesion mask was drawn using MRIcro software (B). The reversed ROI (C) is then multiplied to each dataset resulting in the data with the lesion removed (D).

4.11.8 *Tissue segmentation*

After applying the ROIs described later in sections 4.12.1 and 4.12.2 to the datasets, the data was lastly segmented into tissue type (Figure 4.4). Analysing the data from each region as a whole would have been meaningless, as each ROI contained differing amounts of grey matter, white matter and cerebro-spinal fluid (CSF). We were predominantly interested in the data from the white matter regions in the brain, as these contain the long fibre tracts known to be damaged in TBI, but we also extracted the grey matter data as shearing damage is also known to occur at the grey matter/white matter interface near the brain cortex. The MR signal from the CSF was of no interest, and this data was discarded. Tissue segmentation was performed using an automated segmentation tool (FAST) (Zhang et al., 2001). This tool used a supervised classification method which segmented a 3D image of the brain into the different tissue types using voxel intensity information and nearest neighbour clustering to produce the white matter and grey matter data separated from each other.

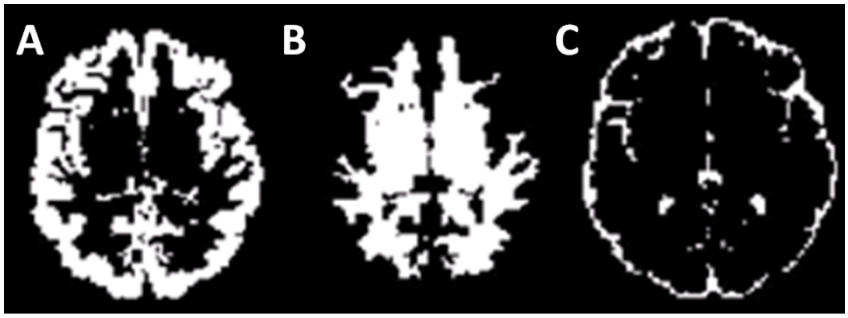

Figure 4.4: Grey matter (A), white matter (B) and CSF (C) tissue segmentation maps.

4.12 Scan Data Analysis

In order to analyse the large amount of data that comes from a single scan, methods must be employed to allow comparison between subjects. As described above, the scans must first be processed to allow registered maps of the data to be created. These maps must then be compared between one subject and another, and between one group of subjects and another group. One method of addressing this problem is to define standard regions within each subject's brain that we wish to compare (regions of interest or ROIs). The ROIs used must be able to be reproduced from one subject to the next, and using this technique, mean values of all the voxels within each ROI can be compared, both between patients and between patient groups. Another method would be to compare every recorded value for every voxel between one patient and another, or between one patient and the mean value in that specific voxel for a group of patients or controls. Methods such as voxel-based morphometry (see Chapter 2) and tract-based spatial statistics (section 4.12.4) employ this technique.

This study utilised two methods of ROI selection for analysis of each quantitative dataset, as well as an additional two methods employed to further analyse the DTI data. The first method, and one which has been used extensively in other published work in this area, involves defining the regions of interest by hand in areas where specific injury (e.g. DAI) is expected to be observed. This type of analysis can be useful as it is hypothesis led and allows the targeting of precise areas. However, the limitation of this type of ROI selection is that some regions where microstructural change exists could be missed if they were not inside the pre-specified target ROIs. Furthermore, this type of analysis is hugely time consuming and potentially prone to bias from user definition of each individual ROI. In order to address these limitations, the second method used was more generic and involved the creation of a template dividing the *whole* brain volume into 14 distinct regions (7 per hemisphere), and the application of this template automatically to each of the patient's datasets. The third technique employed was to define ROIs based upon white matter communications within the brain. For this task specialised software was used to delineate axonal fibre tracts within the brain (tractography), based upon the data acquired by diffusion tensor imaging. The fourth method was a voxel-wise statistical analysis called 'tract-based spatial statistics', and was again performed with specialised software, using the DTI data. This last technique compared the mean fractional anisotropy of white matter tracts common to all subjects

with the individual patient's values. The four methods are described below in greater detail.

4.12.1 *Method 1: Five regions of interest technique*

Of the four analysis techniques, this used the most basic ROIs in order to ascertain whether there was a gross diffuse difference in qT1, qT2, MD and FA values between the patient group and the control group. As a consequence, it was expected that it would only detect large differences between the groups. The first and most obvious ROI to compare between groups, given that DAI is observed predominantly in areas where there are long axonal fibre tracts, was the white matter of the whole brain, and this was selected as the first ROI. Whole brain grey matter was also analysed, as previous work in the field had identified changes in mean diffusivity in patients with TBI. The design of the remaining ROIs was based upon whether changes could be detected remote from any visible lesion on the anatomical scan, and therefore the brain was divided into its hemispheres and each of these was used as an ROI. As a result, comparisons could be made between data ipsilateral and contralateral to any given visible injury. Similarly the next two ROIs were the left and right frontal lobe, more specific than the whole hemisphere, and chosen due to the relationship with the frontal lobe white matter and executive function known to be disrupted after TBI. Using these two ROIs a comparison could again be made between areas of normal appearing white matter ipsilateral and contralateral to any observed visible injury on the anatomical T1 image.

The whole brain and left and right hemisphere ROIs were drawn on the 'standard brain' T1 weighted image (MNI_T1.hdr) that is supplied with the WFU_Pickatlas software (Maldjian et al., 2004, Maldjian et al., 2003), after it had been brain extracted using the brain extraction tool in the FMRIB Software Library mentioned above (Figure 4.5). The left and right frontal lobe ROIs were adapted from the frontal lobe ROIs supplied with the WFU_Pickatlas software (Lancaster et al., 1997, Lancaster et al., 2000). The ROIs were drawn onto axial images on each slice, and then combined to form ROIs in 3D (Figure 4.6).

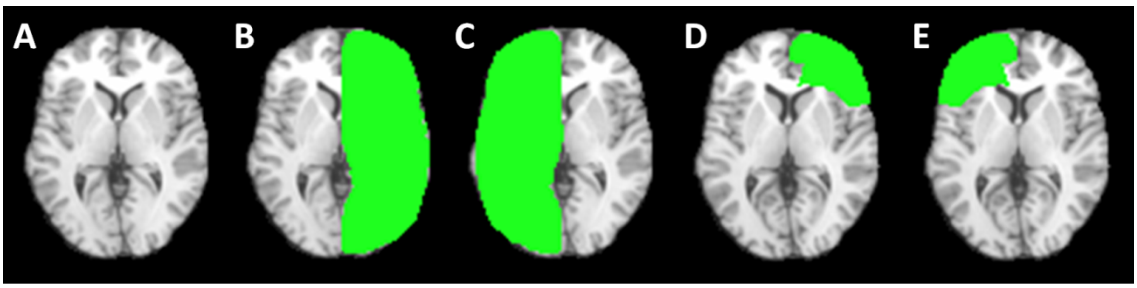

Figure 4.5: ROIs from the 5 ROI method: Five images showing (A) the T1 'standard brain' supplied with WFU_Pickatalas software, and the same axial slice overlaid with: left hemisphere ROI (B), right hemisphere ROI (C), left frontal ROI (D) and right frontal ROI (E). The whole brain ROI was a combination of the left and right hemisphere ROIs.

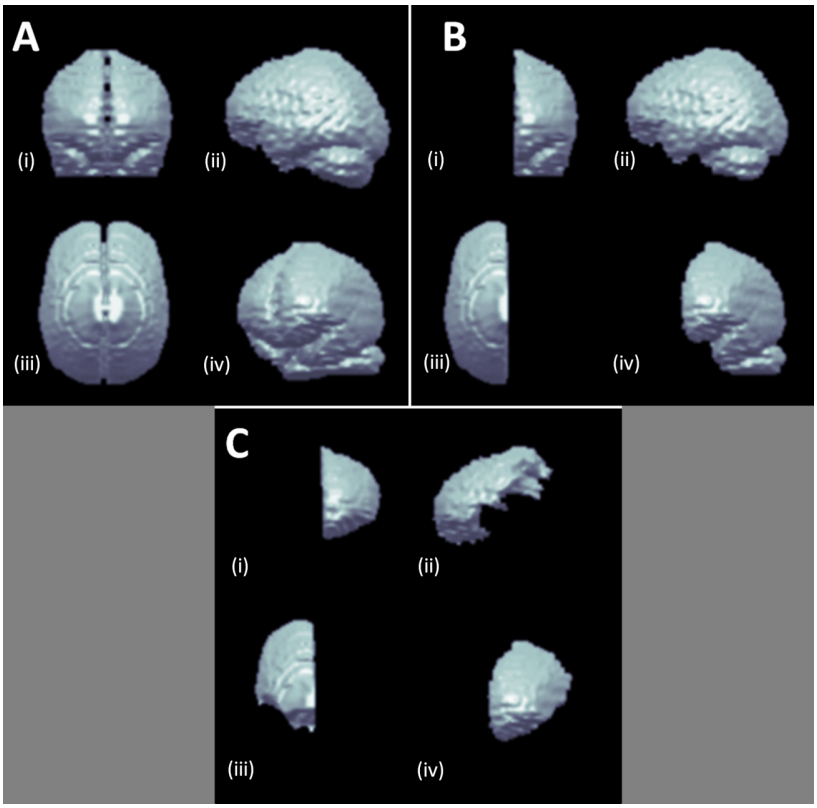

Figure 4.6: 3D renders of the ROIs from the 5 ROI method: whole brain (A), right hemisphere (B) and right frontal (C) regions of interest, each viewed (i) anteriorly, (ii) laterally, (iii) superiorly and (iv) antero-laterally.

In order to apply these ROIs to the patient data in 'real space', the standard pick atlas T1 weighted image was registered to the patient's T1 weighted image and the transformation matrix was obtained. This transformation matrix was then applied to the ROIs, registering them with the patient's 'real space'. The registered ROIs were then multiplied by the datasets in turn, resulting in a 3D volume containing data only from

the ROI that it was a product of (Figure 4.7). Lastly the data was segmented into tissue classes (section 4.11.8).

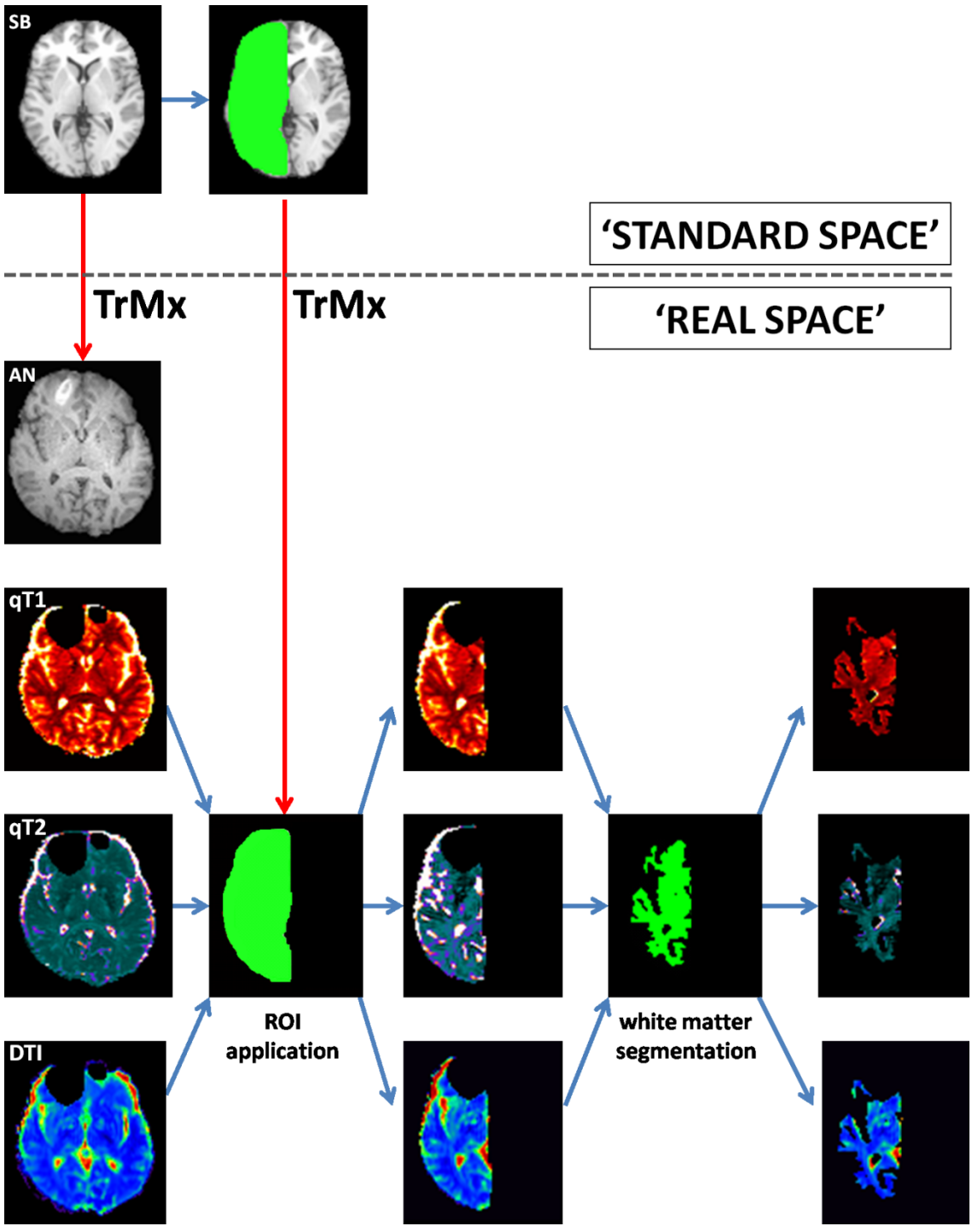

Figure 4.7: Diagram outlining the data extraction process in the 5 ROI method. The 'standard brain' T1 weighted image (SB) supplied with the WFU_Pickatlas software was registered to the subject's T1 weighted anatomical image (AN) and a transformation matrix (TrMx) from 'standard space' to 'real space' was obtained. The ROI drawn on the 'standard brain' in 'standard space' was then converted into 'real space' by applying the same transformation matrix. The ROI in 'real space' was applied to the lesion extracted datasets: quantitative T1 (qT1), quantitative T2 (qT2) and diffusion data (DTI). The resultant data then underwent tissue segmentation to extract the data from the white matter only.

4.12.2 *Method 2: Fourteen automated regions of interest*

The second method of defining regions of interest used a locally developed automated method, defined by a research fellow on the study team (Aribisala et al., 2008). The remit was to design a software program that could automatically divide the data acquired from the scans into regions within the subject's own space. This method utilised a template of 14 pre-defined regions of interest, together making up the entire brain volume (Table 4.3). The 14 regions were devised by combining Brodmann areas and projecting these amalgamated cortical regions inwardly across the white matter to the brain midline, as described in a study published previously by our centre (Aribisala et al., 2011). The template was created in standard space having been drawn on the standard anatomical T1 weighted brain extracted image (ch2bet.hdr) supplied with the MRIcro software (Figure 4.8).

Region	Brodmann areas
Temporal	13, 14, 15, 16, 20, 21, 22, 28, 34, 35, 36, 38, 41, 42
Temporo-occipital	37, 39
Parietal	1, 2, 3, 5, 7, 23, 26, 27, 29, 30, 31, 40
Temporo-parietal encompassing deep structures	48
Inferior frontal	9, 10, 11, 12, 24, 25, 32, 33, 43, 44, 45, 46
Superior frontal	4, 6, 8
Occipital	17, 18, 19

Table 4.3: The Brodmann areas combined to create the regions of interest used in the 14 automated ROI method (Aribisala et al., 2011).

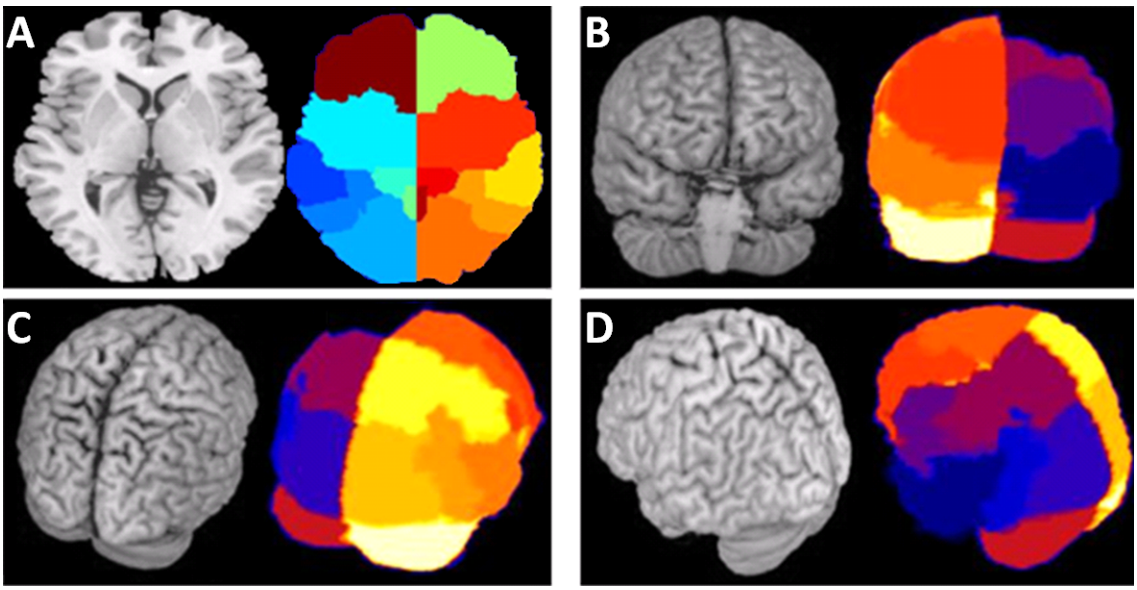

Figure 4.8: Diagram showing the 14 ROI template alongside the standard T1 weighted anatomical image supplied with the MRIcro software. The first image (A) in 2D is in the axial plane. The rendered images in 3D demonstrate how the template appears from the anterior (B), posterior/superior (C) and left lateral (D) aspects.

The first step in this method was to register the subject's high resolution anatomical T1 weighted image, which was acquired in 1 mm slices, to the lower resolution quantitative T1 map data (T1MAP). This is called 'down sampling' and resulted in a T1 weighted image at the lower resolution of the T1MAP (2 mm slices), which was also the same as the resolution of the T2MAP. The DTI data (MD, FA and primary eigenvalues) were acquired at an even lower resolution (6 mm slices), and were interpolated to make them the same resolution. The standard anatomical T1 weighted image supplied with MRIcro was then registered to our 'down sampled' subject's T1 weighted image to give the transformation matrix for that particular subject which converted the standard brain in standard space into the real coordinates of the specific patient. This transformation matrix was then applied to the 14 region template giving us the same template in low resolution in the subject's space. Next the 'down sampled' T1 weighted image was segmented into tissue classes (section 4.11.8), and multiplied by the lower resolution 14 region template in the subject's space, giving 14 grey matter ROIs and 14 white matter ROIS, which were lastly applied to each dataset (Figure 4.9).

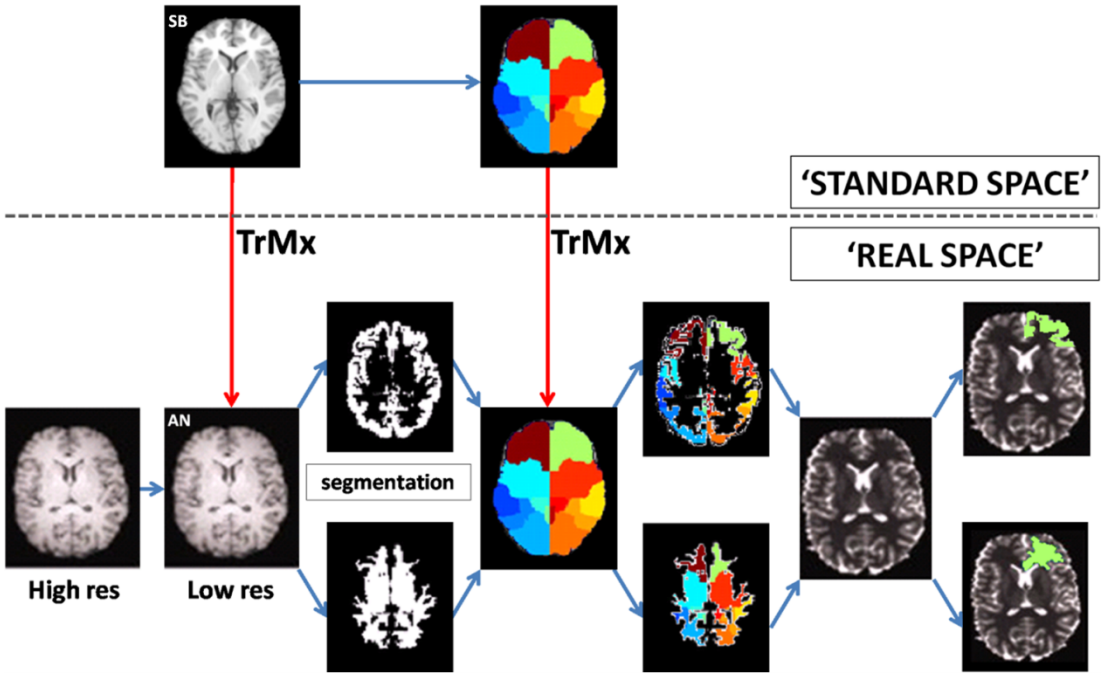

Figure 4.9: Diagram outlining the data extraction process in the automated 14 ROI method. The low resolution 'standard brain' T1 weighted image (SB) supplied with the MRIcro software was registered to the subject's T1 weighted anatomical image (AN) after it had been 'down sampled' from high resolution to low resolution. From this process the transformation matrix (TrMx) from 'standard space' to 'real space' was obtained. This matrix was then applied to the 14 region whole brain ROI template which had been drawn on the 'standard brain' in 'standard space' to convert it into 'real space'. The 'down sampled' low resolution T1 weighted image was then segmented into tissue classes and multiplied by the lower resolution 14 region template. The resultant 14 grey matter ROIs and 14 white matter ROIs were then applied to each dataset in turn.

4.12.3 *Method 3: Tractography based regions of interest*

The third method employed to define regions of interest used the software 'DTIStudio' (Jiang et al., 2006). This software package allows the user to load diffusion tensor imaging data in an unprocessed form; in the case of this study, in the PHILIPS scanner format (.PAR and .REC file extensions). Using the data from these files, combined with the b vector values for each subject (strength and direction of the diffusion encoding), the program calculates image maps for MD, FA and the principle eigenvectors and eigenvalues. From the FA and eigenvector maps it is possible to compute the fibre tracts for each subject, after inputting a threshold value of the minimum FA to track, and the cut-off angle below which the tract should be disregarded. In this work the minimum FA threshold was set at 0.2, and the cut-off angle at 41˚, these values were used in previously published work by Wakana et al. (2007) and have been shown to produce fibre tract ROIs which correspond to known anatomical tracts previously defined from pathological specimens.

Once these fibre tracts have been defined, DTIStudio allows the user to select specific bundles of fibres in a given user-defined region. The regions are defined by placing two ROIs onto the 2D axial, coronal or sagittal diffusion images, and by selecting the fibres that run between those two ROIs (Figure 4.11). Wakana et al. utilised this technique and showed it to have both high intra-rater and inter-rater reproducibility. Once these user defined bundles have been selected out, they in turn can be saved as a region of interest. This study defined three separate ROIs in this way: the genu, body and splenium of the corpus callosum (Figure 4.10).

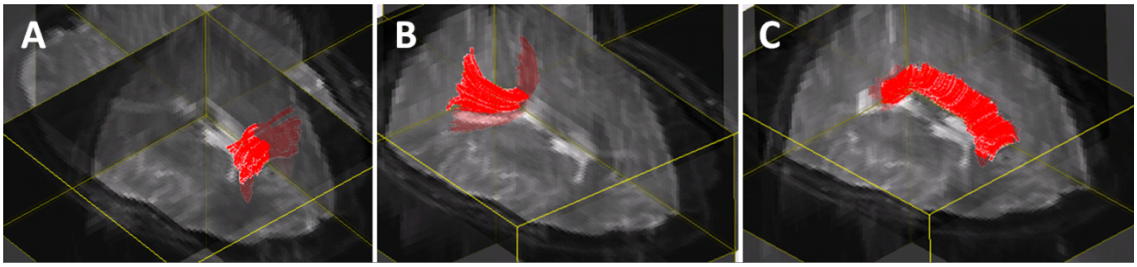

Figure 4.10: Examples of the tract-based ROIs in the genu (A), splenium (B) and body (C) of the corpus callosum created using DTIstudio software.

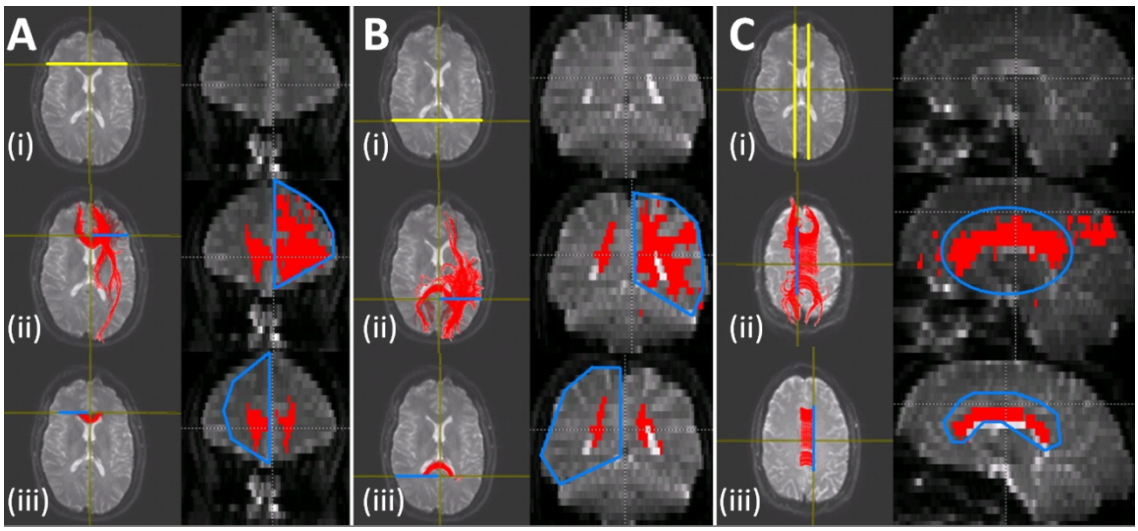

Figure 4.11: Illustration showing how tractography based ROIs in the genu (A), splenium (B) and body (C) of the corpus callosum were created using the DTIstudio software. Yellow lines on the axial images (i) indicate the position of the coronal or sagittal sections on which the tract-capture outlines (displayed in blue) were drawn. After drawing the first outline (ii), all fibres passing through it were displayed (in red). Drawing the second outline (iii) with the 'CUT' command selected only those fibres that passed through each of the outlines, but fibre ends protruding beyond the outlines were not included.

The genu fibre bundles were created by placing the two ROIs on the coronal slice of the diffusion weighted image, at a specific point in the frontal lobe. This point was defined by first choosing the axial image containing the most anterior projection of the lateral ventricles. On this image, the coronal slice would then be placed at the most posterior point of the grey matter in the anterior central sulcus. One ROI would be drawn around each cerebral hemisphere on this coronal image using the 'CUT' command, which resulted in the fibres within the genu of the corpus callosum being selected out.

The splenium fibre tracts were selected out using a similar method. The same axial slice used in the creation of the genu ROI was used, but with the coronal slice placed at the most posterior point of the posterior horns of the lateral ventricles. As above, one ROI would be drawn around each side of the cerebral hemispheres on this coronal image using the 'CUT' command, which resulted in the fibres within the splenium of the corpus callosum being selected out.

The corpus callosum body ROI was designed by first selecting the DWI axial slice with the basal ganglia visible. The sagittal image was placed at the medial border of the basal ganglia on the right side. The first ROI was then drawn around the corpus callosum on the sagittal image, before returning to the axial image to place the sagittal slice at the medial border of the basal ganglia on the left. The second ROI was drawn around the corpus callosum on this second image. The 'CUT' feature was again used, resulting in the fibres of the body of the corpus callosum being selected.

The final step in this method is to utilise these fibre bundle ROIs by applying them to the original data, and extracting the mean values for the MD and FA from each.

A limitation of the DTIstudio software was that only the diffusion data in its original form could be loaded into the program, and therefore the ROIs designed using it could only be applied to this original unregistered data. In its original state this data had not undergone visible lesion removal. In order to ensure that the MD and FA data extracted using this technique came only from normal appearing brain, the DTI maps were examined at the time of ROI creation for any visible lesion, and tract selection in such regions was avoided.

4.12.4 *Method 4: Tract based spatial statistics (TBSS)*

The last analysis technique used was tract-based spatial statistics or TBSS (Smith et al., 2006), which is a voxel-wise statistical analysis method. TBSS analysis is performed in

'standard space', **not** the 'patient's space', and uses FA images calculated internally from the *raw* scanner diffusion data, without having had any visible lesions masked and removed. Firstly all the FA data from all of the subjects was registered by aligning each dataset into a common space. Next, a mean FA image was created and thinned to create a mean FA skeleton which represented the centres of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton. Voxel-wise cross-subject statistics were then calculated on the resultant data using a permutation-based nonparametric inference tool (RANDOMISE) available as part of the FMRIB Software Library from the University of Oxford (Bullmore et al., 1999, Hayasaka and Nichols, 2003). This method uses 5000 iterations and is designed for use when the distribution of the data is not known. The result was displayed as an overlay of the tracts common to all the subjects in the group with regions of those tracts that significantly differ ($p<0.05$) from the mean highlighted in red if they were higher, and blue if they were lower (Figure 4.12).

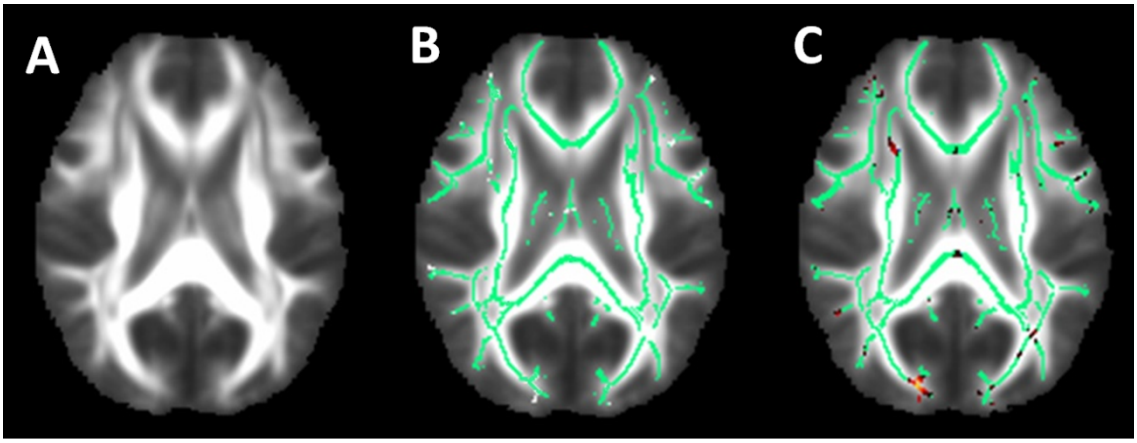

Figure 4.12: TBSS images showing the mean FA image created from a group of subjects (A) and the same image with the overlay of the mean FA skeleton of tracts common to the group in green (B). Image (C) shows the image and mean FA skeleton with areas highlighted in red where the mean FA is significantly higher (right frontal and occipital lobe tracts in this case).

4.13 Statistical Analysis of Scan Data

Scan data was entered into a spreadsheet along with the injury characteristics of the patient group and the demographics of both the patients and controls. Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago IL). For the first three of the data analysis methods described above, the same basic statistical tests were applied. The data was tested to assess whether it was normally distributed using the Kolmogorov-Smirnov test. Whole group analysis, to assess the differences between the mean values of qT1,

qT2, MD and FA in each tissue type between the mild and moderate patient groups and the controls, was performed using either the one-way ANOVA or the Kruskal-Wallis test, depending upon whether the data was parametric or non-parametric. The t test or the Mann-Whitney U test was then used to determine if there were significant differences between the individual groups (between the mild TBI and moderate TBI groups, the mild TBI and control groups and the moderate TBI and control groups). A p-value of less than 0.05 was taken to be significant.

Further sub-group analysis was performed on the 5 ROI data after grouping the mild TBI patients into their respective grade (I, II or III), when classified using the American Academy of Neurology (AAN) concussion grading system (see Chapter 1, Section 1.8). As above, the first analysis aimed to establish whether there were significant differences between the 3 grades and the control group, and used either the one-way ANOVA or the Kruskal-Wallis test. Any that were found to be significant were then analysed further using the t test or Mann-Whitney U test.

In the first analysis method using 5 ROIs, sub-group analysis was also performed on the patients with a visible lesion, assessing whether differences could be detected between the patient groups and the control group in normal appearing tissue both adjacent to and remote from the lesion. This was done by grouping the sided ROIs according to whether they were ipsilateral or contralateral to any visible lesion on the T1 weighted anatomical scan, and then the data was analysed using the same statistical tests as the preceding analyses outlined above.

Bivariate correlation between the admitting GCS and the measured scan variables was assessed using either the parametric Pearson correlation coefficient or the non-parametric Spearman's rank correlation coefficient, depending upon whether or not the data was normally distributed. The same coefficient was then used to assess correlation between the mean scan data values and the individual features of concussion (loss of consciousness in seconds and PTA length in minutes).

4.14 Neuropsychological Testing

As described in Chapter 3, the neuropsychology test battery was compiled from established tests, some of which were designed specifically to detect abnormalities *after brain injury*. The tests used were selected after discussion with two consultant

neuropsychologists in the centre where the research was to be performed. The areas of function we wanted to test were attention, concentration, memory and executive function all of which have been shown to be affected by mild head injury. Below is a list of the tests used and the cognitive domain examined by each (Table 4.4).

The tests were initially conducted by the study clinical research associate (the author) after training in their administration from one of the consultant neuropsychologists. As the study progressed, two neuropsychology research assistants were employed, and took over the testing of the study subjects after sitting in with the author while testing patients on three occasions. This made sure the tests were administered in exactly the same manner with each study subject.

Test Battery	Assessed Functions
NART	Premorbid intelligence
SoIP	Executive function (information processing)
Design learning	Visuospatial learning, attention, concentration, short-term and working memory
List learning	Verbal learning, attention, concentration, short-term and working memory
PASAT	Executive function (information processing), attention, concentration and working memory
DSPAN back	Short-term and working memory
Spatial span back	Short-term and working memory
Verbal fluency	Executive function (clustering and switching)
Colour-word interference	Basic functional skills (naming and attention) and executive function (verbal inhibition and cognitive flexibility)

Table 4.4: Neuropsychological tests used in the study, and the cognitive functions tested by each.

The test battery took approximately 1 hour and 15 minutes to administer to the subjects. Results were initially recorded on paper and subsequently transcribed onto a computer

database on the same day, to enable statistical analysis. At the start of the study this was performed by the author and in the latter part by the neuropsychology research assistants. The scoring and totalling of the test results was also performed by one of the neuropsychology research assistants.

4.15 Statistical Analysis of Neuropsychology Data

The neuropsychological data collected was examined using the Kolmogorov-Smirnov test for normality. As a consequence, the analysis was performed using either non-parametric or parametric statistical tests, depending upon whether they were shown to be normally distributed or not. For a comparison of the means of each test result in each group (mild TBI, moderate TBI and controls), the ANOVA or Kruskal-Wallis test was used. The test means that showed a significant difference between groups were then further analysed using the independent samples t test or the bivariate Mann-Whitney U test to ascertain where that significance lay; whether between the mild TBI group and the controls, the moderate TBI group and the controls, or between the mild and moderate TBI groups. A p-value of less than 0.05 was taken to be significant.

4.16 Analysis of Scan Data with Reference to the Neuropsychological Data

The last set of statistical analysis performed evaluated the scan data with reference to the neuropsychology test results. Correlations were assessed between quantitative data (qT1, qT2, MD and FA) obtained using the 5 ROI and 14 ROI methods and the neuropsychology test results, using either the Pearson correlation coefficient or the Spearman's rho coefficient, depending upon whether the scan data had previously been shown to be normally distributed. The same method was also used to assess any correlation between the diffusion data (MD, FA and eigen values) obtained using the tract-based and TBSS methods and the neuropsychology test results. The p-values taken to be significant differed in each analysis, as Bonferroni corrections were made for multiple comparisons (see section 6.2).

Chapter 5. Results: Demographics and Quantitative MR Imaging Findings

5.1 Demographics

5.1.1 *Recruitment*

The study actively recruited for 24 months, from 17th December 2008 until 16th December 2010. A total of 44 patients with mild TBI and 9 patients with moderate TBI were admitted to the study, making a total number of 53 patients. During the same period, a total of 30 control subjects were recruited, matched to the age and educational level of the patient group.

During recruitment, the enrolment rate of entrants into the study was approximately 30% of those identified to the study team. The 70% of patients who were not entered into the study were divided almost equally between those who did not meet the inclusion criteria after a more detailed review, and those who would have been suitable, but declined to participate.

5.1.2 *Patient group demographics*

The age range of the TBI patients recruited was between 16 years and 67 years old. The mean age of the patients was 34 years (SD 14.6 years). The age distribution reflected that of the TBI population in the developed world (see Chapter 1) with the greatest peak occurring in the adolescent/young adult age bracket (Figure 5.1).

Sex of the patient group was predominantly male, again a reflection of the pattern seen worldwide, with 44 (83%) men and 9 (17%) women recruited. A complete description of all the patient details is provided in tables 5.2 and 5.3. Some of the important features of the group are summarised here.

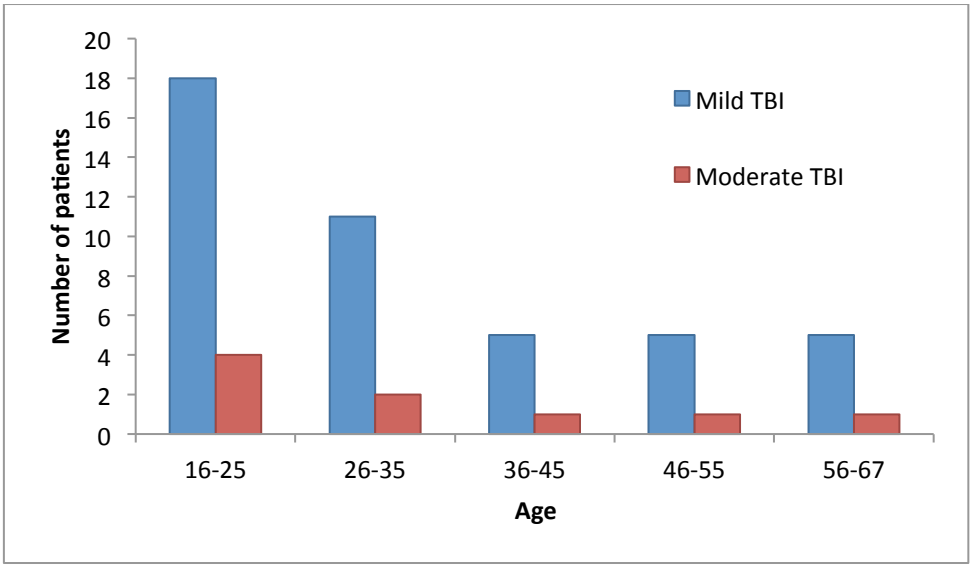

Figure 5.1: Graph showing age distribution of recruited TBI patients.

The cause of TBI in the patients recruited was again similar to that reported in the literature, although more in common with the pattern in the US, assaults were the most common cause in the study group. As expected, the other major causes were falls, traffic accidents and sports related injuries (Figure 5.2). Interestingly, when the injury causes were grouped according to whether the patient had had a mild or moderate TBI, a greater proportion of the moderate TBI group had sustained assaults and none had suffered a fall.

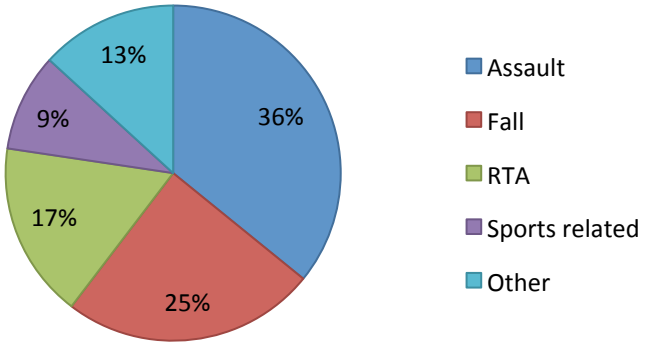

Figure 5.2: Chart showing injury causes in the TBI group (mild and moderate).

5.1.3 *Injury characteristics*

All of the mild TBI patients were symptomatic at the time of recruitment (Table 5.2). The majority suffered with headache (95%). Other symptoms included loss of consciousness (61%), antegrade amnesia (61%), vomiting (57%), post-traumatic amnesia (55%) and seizure (2%). Only 4 patients did not undergo a computed tomography (CT) scan as part of their clinical management. Of those that had a CT scan (n=40), 88% showed positive findings of varying pathology. To characterise the patients more completely, and in order to perform a sub-group a priori analysis, the mild TBI patient group was stratified using the American Academy of Neurology concussion grading system (Table 5.1).

AAN grade	Number of patients
Grade 1	7
Grade 2	10
Grade 3	27

Table 5.1: Mild TBI patients grouped by AAN grade.

In the moderate injury group (Table 5.3) all patients were again symptomatic, and notably, all suffered with post-traumatic amnesia. Only 2 of the moderate TBI patients were able to recollect the injury itself. Other symptoms were headache (89%), loss of consciousness (78%) and vomiting (66%).

	Sex	Age	GCS	AAN	TBI cause	Head-ache	Vomit	Seizure	LOC (mins)	Antegrade amnesia	PTA (hours)	CT scan and T1W MRI scan findings
1	Male	47	14	3	Fall	+	-	-	1	-	-	Contusions
2	Male	64	14	3	Fall	+	-	-	5	+	2	Subdural haematoma
3	Male	17	14	3	Other	+	-	-	5	+	-	Extradural haematoma, base of skull fracture
4	Male	21	15	1	RTA	+	+	-	-	+	-	Intracerebral haematoma, contusions and vault fracture
5	Male	63	14	3	Fall	+	+	-	10	+	-	Contusions
6	Female	39	14	1	Fall	+	-	-	-	+	2	Normal scan
7	Male	46	15	3	Assault	+	-	-	3	-	-	Normal scan
8	Female	25	15	1	Other	+	-	-	-	-	-	Normal scan
9	Male	25	15	3	Other	+	-	-	2	+	3	Subdural haematoma, sub-arachnoid haemorrhage and base of skull fracture
10	Male	67	14	3	Other	+	-	-	2	+	0.5	Contusions and sub-arachnoid haemorrhage
11	Male	35	14	3	Assault	+	+	-	5	+	-	Contusions
12	Female	40	14	3	Fall	+	-	+	10	+	-	Sub-arachnoid haemorrhage
13	Male	23	15	3	Assault	-	+	+	1	-	-	Normal scan
14	Male	17	15	3	RTA	+	+	-	2	-	1	Normal scan
15	Male	17	15	3	Fall	+	+	+	5	-	-	Contusions
16	Female	34	13	3	RTA	+	-	-	5	+	30	Vault fracture
17	Male	44	14	2	Assault	+	-	-	-	+	4	Normal scan
18	Female	32	15	3	Fall	+	+	-	1	-	0.5	Contusions, sub-arachnoid haemorrhage and base of skull fracture
19	Male	21	14	2	RTA	+	-	-	-	+	1	Intracerebral haematoma, vault and base of skull fractures
20	Male	65	15	2	RTA	+	-	-	-	-	-	Vault fracture
21	Male	45	15	3	Other	+	+	-	1	-	-	Normal scan
22	Female	51	15	2	Assault	+	+	-	-	-	-	Vault fracture
23	Male	27	15	1	Fall	+	+	-	-	-	-	Contusions

Table 5.2: Demographic data and injury characteristics for the mild TBI patient group, including GCS score, AAN grade, presenting symptoms and CT scan findings. (Continued on subsequent page)

Number	Sex	Age	GCS	AAN	TBI cause	Head-ache	Vomit	Seizure	LOC (mins)	Antegrade amnesia	PTA (hours)	CT scan and T1W MRI scan findings
24	Male	23	14	3	Sports	+	-	-	2	+	12	Normal scan
25	Male	33	15	3	Assault	+	+	-	1	-	-	Subdural haematoma, contusions and vault fracture
26	Male	28	15	1	Assault	+	-	-	-	-	-	Intracerebral haematoma and vault fracture
27	Male	35	15	2	Assault	-	+	-	-	+	-	Vault fracture
28	Female	28	14	3	Assault	+	-	-	1	+	2	Subdural haematoma, contusions and vault fracture
29	Male	25	14	2	Assault	+	+	-	-	+	7	Contusions
30	Male	48	15	1	Fall	+	+	-	-	-	-	Extradural haematoma
31	Male	57	14	3	Fall	+	+	-	1	+	1	Contusions and vault fracture
32	Male	20	15	1	Other	+	+	-	-	-	-	Contusions
33	Male	22	13	2	Fall	+	+	-	-	+	4	Contusions
34	Male	26	13	2	Fall	+	-	-	-	+	12	Intracerebral haematoma and contusions
35	Male	20	15	3	Sports	+	+	-	1	+	1	Contusions
36	Male	28	14	3	Sports	+	+	-	0.5	+	6	Contusions and sub-arachnoid haemorrhage
37	Male	51	14	3	Fall	+	+	+	10	+	2	Normal scan
38	Male	28	14	3	RTA	+	-	-	15	-	-	Subdural haematoma, contusions and base of skull fracture
39	Male	21	14	2	Assault	+	-	-	-	+	3	Sub-arachnoid haemorrhage and vault fracture
40	Male	19	15	3	Assault	+	+	-	20	+	6	Subdural haematoma, contusions and vault fracture
41	Male	19	13	3	Assault	+	+	-	1	+	2	Subdural haematoma, contusions, sub-arachnoid haemorrhage and vault fracture
42	Male	44	15	2	RTA	+	+	-	-	-	0.5	Extradural haematoma and vault fracture
43	Male	25	14	3	Assault	+	+	-	1	+	5	Contusions, sub-arachnoid haemorrhage and vault fracture
44	Male	16	15	3	Assault	+	+	-	2	+	18	Contusions and vault fracture

Number	Sex	Age	GCS	TBI cause	Headache	Vomited	Seizure	LOC (minutes)	Antegrade amnesia	PTA (hours)	CT scan and T1W MRI scan findings
1	Male	23	10	Other	+	+	-	1	+	1	Subdural haematoma and vault fracture
2	Male	23	10	Assault	+	+	-	1	+	7	Subdural haematoma, contusions and vault fracture
3	Male	29	12	Assault	-	+	-	-	-	2	Contusions and base of skull fracture
4	Male	52	12	Assault	+	+	-	15	+	4	Subdural haematoma, contusions and vault fracture
5	Male	25	12	RTA	+	+	-	10	+	6	Contusions and vault fracture
6	Female	22	12	Sports	+	-	-	-	-	0.5	Contusions
7	Male	37	12	Sports	+	+	-	3	+	336	Contusions and base of skull fracture
8	Male	27	7	Assault	+	-	-	5	+	1	Extradural haematomas and base of skull fracture
9	Female	64	9	RTA	+	-	-	10	+	240	Contusions and sub-arachnoid haemorrhage

Table 5.3: Demographic data and injury characteristics for the moderate TBI patient group.

5.1.4 *Control group matching*

Thirty control subjects were recruited, matched as closely as possible to the patient group in terms of age and educational level in an attempt to prevent bias in the neuropsychological test results due to educational attainment or differences in IQ. Comparisons of key measures between the patient groups and controls are summarised in Table 5.4. The mean age of the control group was slightly higher than that of the patient groups, although this difference was not significant. Although the groups were well matched in terms of educational level, a greater percentage of the control group were better educated, and this difference was found to be significant after assessing the predicted IQ scores between groups using the ANOVA test ($p<0.001$).

		Mild patient group	Moderate patient group	Control group	Inter-group difference assessed with ANOVA
Number (M, F)		44 (37, 7)	9 (7, 2)	30 (22, 8)	
Mean age (range, SD)		34 (16-67, 14.7)	34 (22–64, 14.8)	40 (19-66, 15.6)	p=0.071
Educational level	No qualifications	6 (14%)	3 (33%)	5 (17%)	
	GCSE or equivalent	22 (50%)	1 (11%)	6 (20%)	
	A-level or equivalent	5 (11%)	3 (33%)	5 (17%)	
	Degree or above	11 (25%)	2 (22%)	14 (46%)	
Mean predicted IQ derived from NART score (range, SD)		99.6 (76-127, 13.4)	102.3 (81-122, 12.2)	112.3 (86-128, 9.2)	**p<0.001**

Table 5.4: Comparison of patient group and control group characteristics.

This mismatch was due to the fact that the majority of the patient group were young and had a relatively low level of education, whereas those who responded to the advertisements for control subjects tended to be older (most who enquired were of retirement age) and the majority were well educated. Numerous steps were made to attempt to recruit more suitably matched control subjects, but the voluntary nature of the study made it difficult to find willing recruits.

5.1.5 *Time from injury to scan*

All patients were scanned and underwent neuropsychological testing within 14 days of their injury (Figure 5.3). The mean time between injury and imaging assessment was 6 days (range 1-14 days, SD 3.2 days). There was no significant difference between the injury to scan time for the mild TBI group (mean 6 days, SD 2.9 days, range 1-14 days) and the moderate TBI group (mean 7 days, SD 4.2 days, range 2-13 days) when assessed using a t test (p=0.28). The majority of mild TBI patients were scanned between 2 and 7 days of their injury. Eleven patients did not undergo neuropsychological testing on the same day as being scanned, and this was predominantly due to the fact that both the neuropsychology test battery and the scan acquisition time were lengthy; the more symptomatic patients were unable to tolerate both at the same sitting. The mean delay between neuropsychological assessment and scan in these eleven patients was 2.6 days (range 1-7 days).

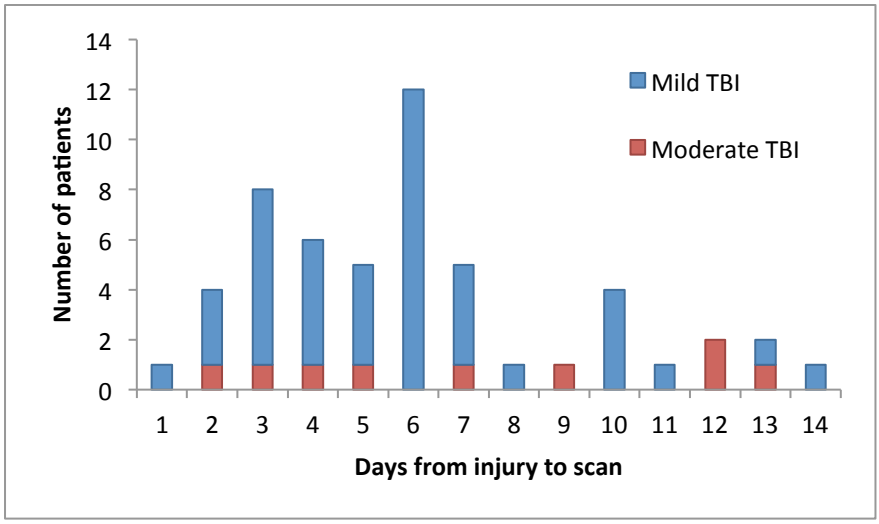

Figure 5.3: Graph showing the difference in time between sustaining the TBI and being scanned.

5.1.6 *Scan data obtained*

Each scan sequence was obtained in all of the 53 patients and 30 control subjects who took part in the study, resulting in complete datasets of quantitative T1 (qT1), quantitative T2 (qT2) and diffusion tensor data (DTI) for analysis, as well as T1 weighted anatomical scans for each patient. Examples of the scan sequences obtained are shown in figure 5.4. The parameters analysed from the DTI data were the mean diffusivity (MD) and the fractional anisotropy (FA). As outlined in Chapter 4, the T1 weighted images and the quantitative

maps were visually inspected for visible lesions, before these lesions were extracted from the data using the method previously described. Figure 5.5 shows the T1 weighted anatomical scan of a mild TBI patient with a typical visible lesion (a right frontal contusion) and how that lesion was masked for the extraction process described in section 4.11.7.

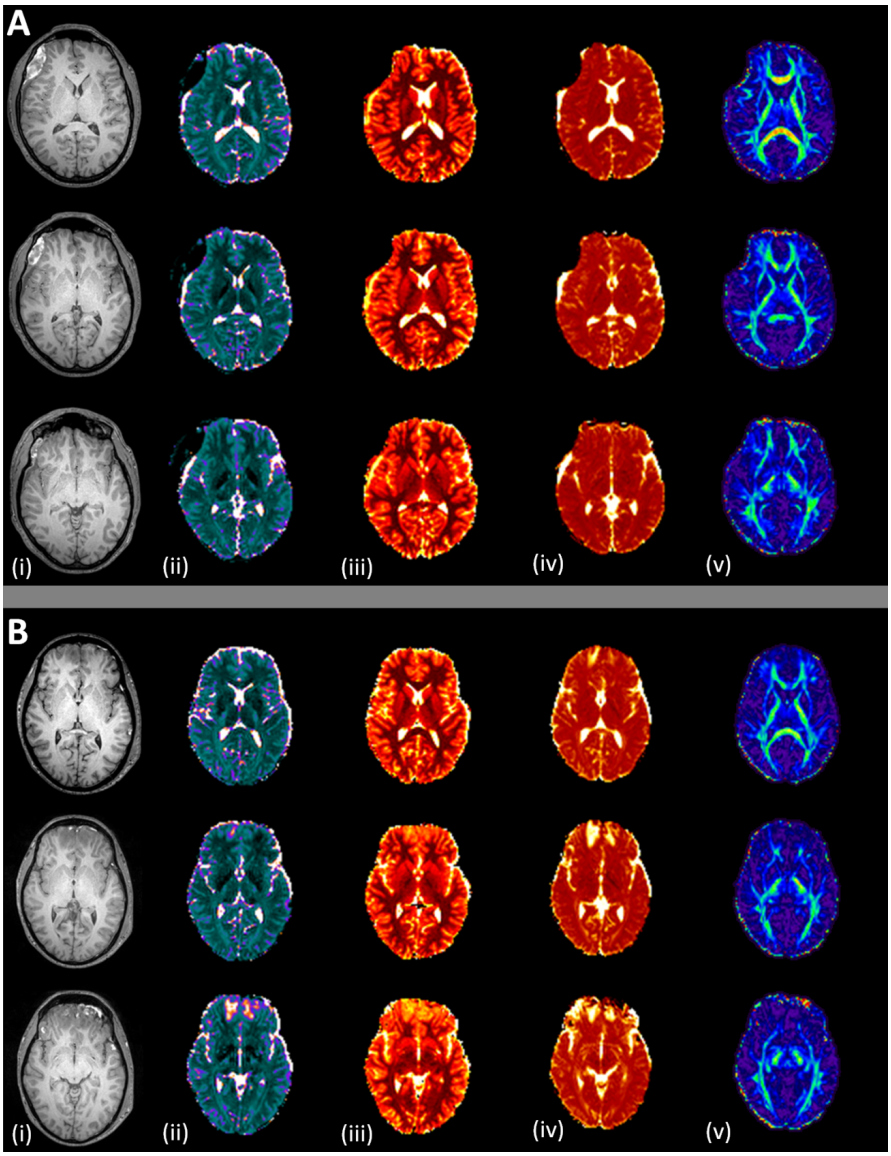

Figure 5.4: Examples of the scan sequences obtained in each patient. (i) The T1 weighted anatomical scan before brain extraction, (ii) quantitative T1 map, (iii) quantitative T2 map, (iv) mean diffusivity map and (v) fractional anisotropy map, in a mild TBI patient with a right frontal extra-dural haematoma (A), and in a moderate TBI patient with bi-frontal and left temporal contusions and associated oedema (B). Note that with the exception of the T1 weighted anatomical scan, the images are simply a visual representation of the quantitative data collected, and subsequently analysed.

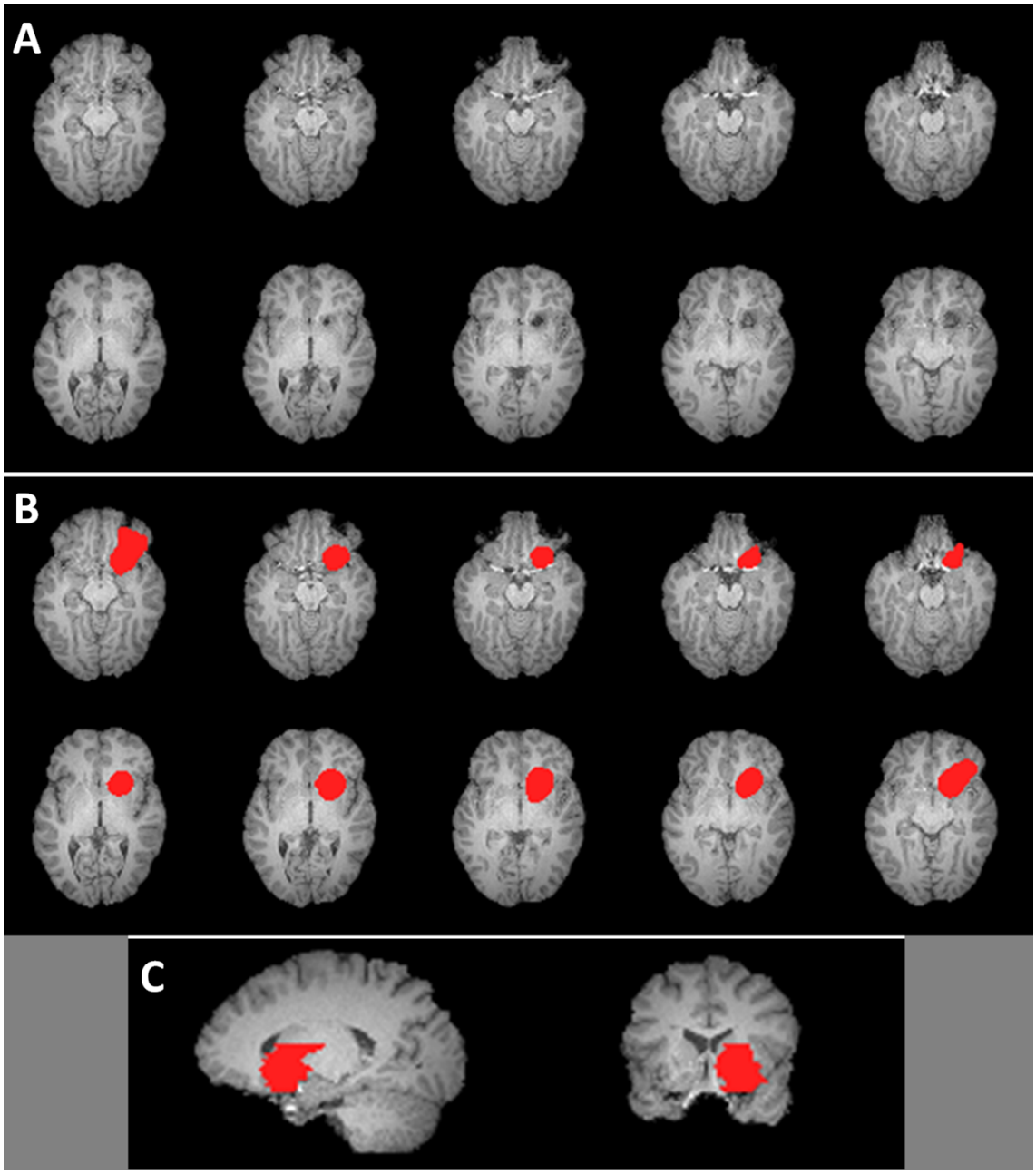

Figure 5.5: Axial T1 weighted anatomical images showing a mild TBI patient with a left frontal contusion (A). The contusion was masked by hand on each axial image (B), and when combined this resulted in a 3D masked volume, also shown in the sagittal and coronal planes (C).

Visible lesions (extradural, subdural and intracerebral haematomas, contusions, sub arachnoid haemorrhage and oedema) were present in 34 of the 44 mild TBI patients (77%) and in all 9 of the moderate TBI patients (100%). Data on the type of lesions found in each patient is included in the demographic data and injury characteristics tables above (Tables 5.2 and 5.3). Lesion location was recorded by laterality (Table 5.5) and by lobar location (Table 5.6).

	No lesion visible	Unilateral lesion		Bilateral lesions
		Right sided	Left sided	
Mild TBI	10	11	10	13
Moderate TBI	-	1	4	4

Table 5.5: Laterality of visible lesions present in the patient groups that were identified on the MR data.

	Mild TBI	Moderate TBI
Frontal	15	-
Fronto - temporal	6	4
Fronto-parietal	1	-
Temporal	6	-
Temporo-parietal	2	2
Parietal	3	-
Fronto-temporo-parietal	1	3
No visible lesion	10	-

Table 5.6: Lobar location of visible lesions present in the patient groups that were identified on the MR data.

5.2 Method 1: Five Regions of Interest Technique

A basic analysis (described in section 4.12.1) was performed on the data using 5 extended regions of interest to address two questions:

i) Can diffuse injury throughout the brain be detected in large areas (whole brain grey matter, whole brain white matter)?

ii) Can diffuse damage be detected remote from and close to existing lesions after data from those lesions had been extracted (using contralateral and ipsilateral hemisphere and frontal lobe regions of interest).

5.2.1 *Test for normality*

Data using the 5 ROI analysis technique were available in all subjects for all measures (Appendix A). Prior to statistical analysis, a test for normality was performed using the Kolmogorov-Smirnov test on all the whole brain datasets in both grey and white matter, in the control subjects only. (Patient data was not subjected to this analysis as it was expected that damage in the patient groups may have had an effect on the distribution of the data). This test was used, rather than the Shapiro-Wilk's test, as the sample size was greater than 50. All of the scan data was found to be normally distributed (Table 5.7).

5ROI Data Type	Kolmogorov-Smirnov Significance p-value
Whole brain white matter qT1	0.807
Whole brain white matter qT2	0.984
Whole brain white matter MD	0.950
Whole brain white matter FA	0.986
Whole brain grey matter qT1	0.548
Whole brain grey matter qT2	0.932
Whole brain grey matter MD	0.893
Whole brain grey matter FA	0.901

Table 5.7: 5 ROI data tested for normality using the Kolmogorov-Smirnov test. A non-significant p-value ($p > 0.05$) indicates normally distributed data.

All the other ROIs used in this technique (left and right hemisphere, left and right frontal lobe) were assessed in the same manner using the Kolmogorov-Smirnov test and all were shown to be normally distributed. As a result, parametric tests were used for their subsequent analysis.

5.2.2 *Group comparison – whole brain grey and white matter data*

The first analysis performed was a three way group comparison (one-way ANOVA) between the mild TBI, moderate TBI and control groups for each the whole brain qT1, qT2, MD and FA data values. There were no significant differences demonstrated between the 3 groups for any of the four white matter datasets evaluated (Figure 5.6). However, the ANOVA test did reveal a significant inter-group difference (p=0.009) in the grey matter qT1 dataset, although no significance was found between groups for the grey matter qT2, MD or FA datasets (Figure 5.7).

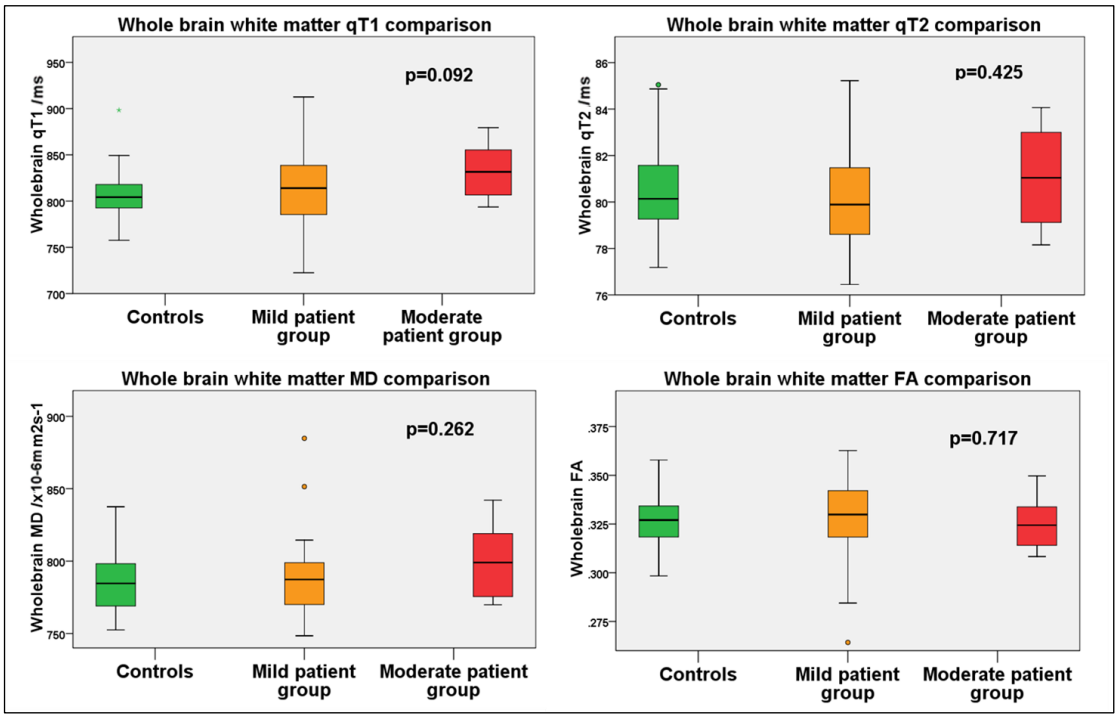

Figure 5.6: Box plots showing distribution of whole brain white matter qT1, qT2, MD and FA means between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). All the ANOVA test p-values were greater than 0.05 indicating no significant difference between the groups.

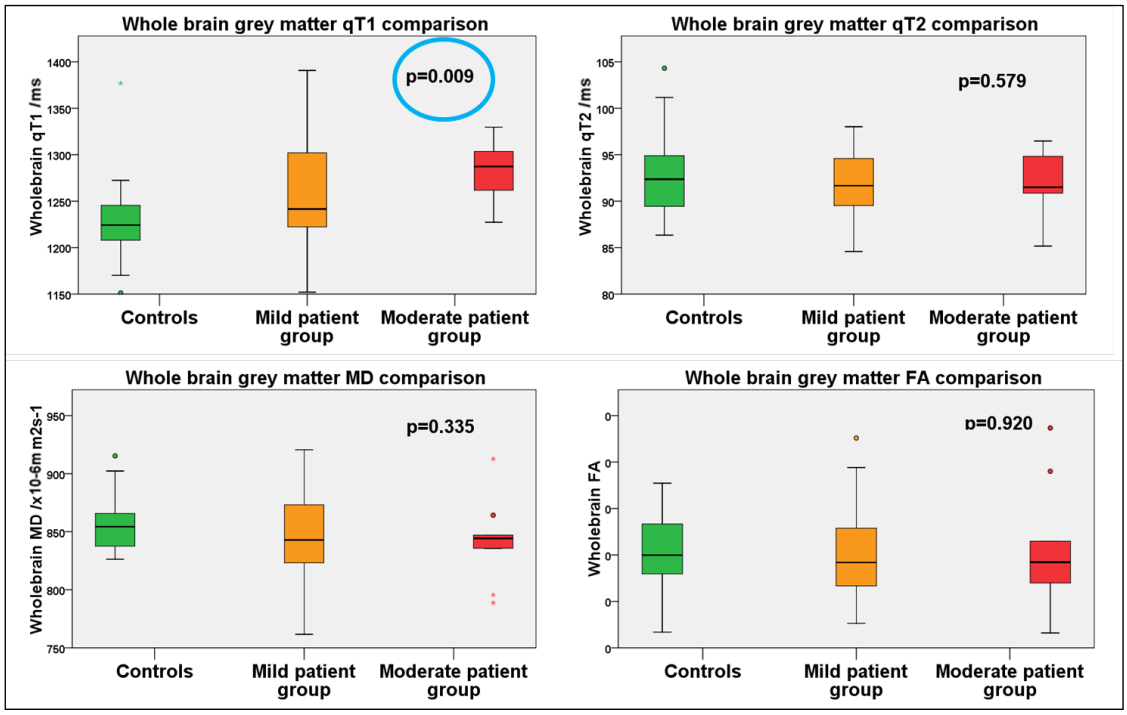

Figure 5.7: Box plots showing distribution of whole brain grey matter qT1, qT2, MD and FA means between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-value of 0.009 for the qT1 data indicates a significant difference between the groups for that dataset only.

To analyse the significant difference found in the whole group comparison, *post hoc* t test analysis was performed on the whole brain grey matter qT1 between each pair of groups; mild TBI and controls, moderate TBI and controls, and mild TBI and moderate TBI. This was performed using the t test for independent samples showed a significant increase in the mean whole brain grey matter qT1 value between the mild TBI group and the controls (p=0.018) and between the moderate TBI group and controls (p=0.001), but there was no significant difference found between the mild TBI group and the moderate TBI group (Figure 5.8).

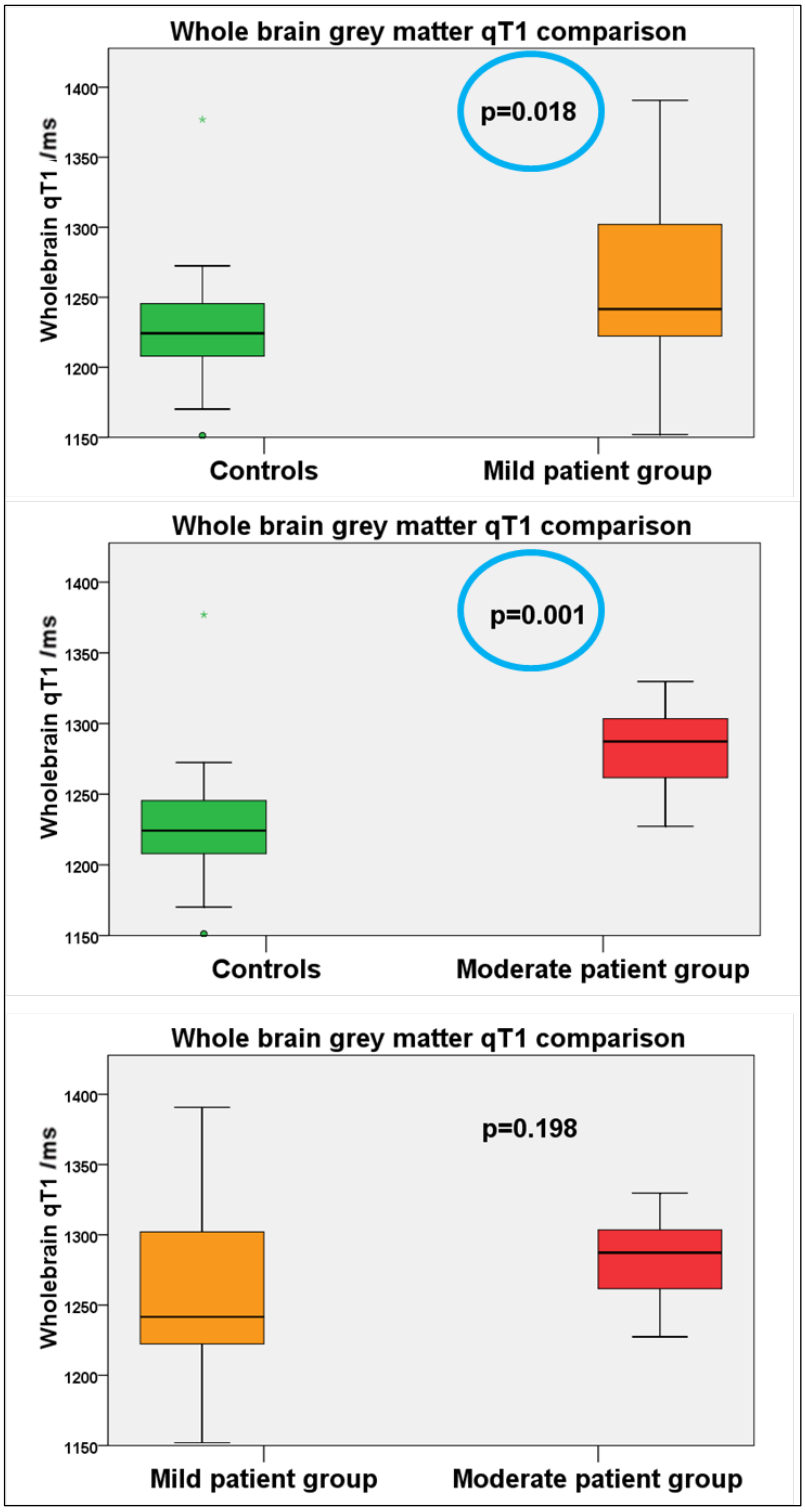

Figure 5.8: Box plots showing distributions of whole brain grey matter qT1 means for the mild TBI group and controls, the moderate TBI group and controls, and for the mild TBI and moderate TBI groups. The t test p-values show significant increases in the mild TBI group and moderate TBI group when compared with the control group, but not between the TBI groups themselves.

5.2.3 *Sub-group comparison – mild TBI assessed by AAN grade*

A sub-group analysis was conducted using the one-way ANOVA test comparing mild TBI patients grouped according to AAN grade (Table 5.1) and the control group. This analysis was performed for whole brain white matter qT1, qT2, MD and FA (Figure 5.9) and for whole brain grey matter qT1, qT2, MD and FA (Figure 5.10). No significant inter-group differences were found, although comparison of the grey matter qT1 group means was approaching significance.

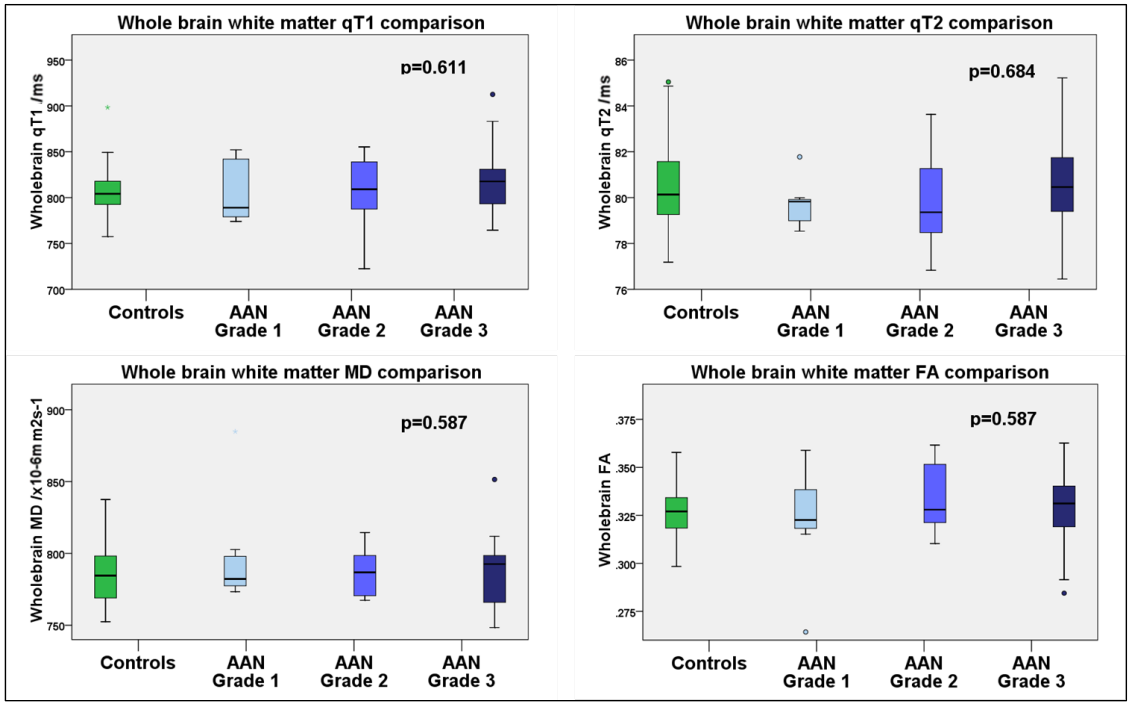

Figure 5.9: Box plots showing distribution of whole brain white matter qT1, qT2, MD and FA means between mild TBI patients grouped according to AAN grade and control subjects. All the ANOVA test p-values were greater than 0.05 indicating no significant difference between the groups.

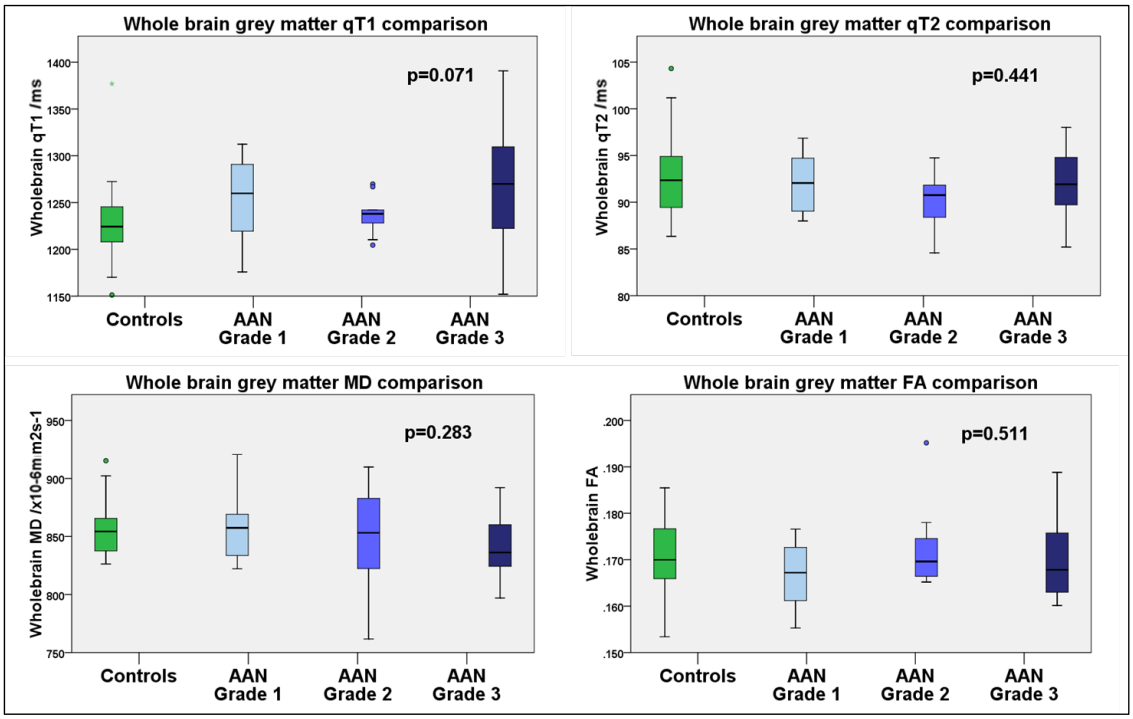

Figure 5.10: Box plots showing distribution of whole brain grey matter qT1, qT2, MD and FA means between mild TBI patients grouped according to AAN grade and control subjects. Note the ANOVA test p-value of the comparison between grey matter qT1 means (p=0.071), which although not significant, is approaching significance.

5.2.4 *Sub-group comparison – ROIs remote from any visible lesion*

Further group comparisons were performed looking specifically to determine whether significant differences could be found remote from, and adjacent to, any visible lesion after those lesions had been extracted. This analysis was performed to test those datasets where there had been no evidence of a significant difference between patients and controls in the whole brain analyses, to assess whether diffuse damage could be detected in the somewhat smaller ROIs of the hemisphere and frontal lobe. The frontal lobes were analysed individually for three reasons: in blunt trauma contusions are particularly prevalent in the basi-frontal regions due to the close relationship of the brain surface to the floor and walls of the anterior fossa, the frontal lobes are heavily involved in the cognitive functions known to be disrupted in post-concussive syndrome and because the majority of the study patients with a visible lesion had frontal lobe or combined frontal lobe damage.

The analysis observing remote areas compared ROI data contralateral to any visible lesion with the same ROI in the control subjects. Only the patients with unilateral visible lesions

were analysed in this manner, as patients with bilateral visible lesions by definition did not have a lesion free hemisphere. As outlined in the methods section, both hemisphere and frontal lobe ROIs were applied to the data; the hemisphere ROI was applied to ascertain whether any detectable remote changes were significant enough to be identified within a large ROI, and the more specific frontal lobe ROI was applied to determine whether any changes could be detected in frontal regions known to be commonly affected in TBI.

A one-way ANOVA test was used to compare the contralateral hemisphere grey and white matter mean qT1, qT2, MD and FA in the patient groups, with the averaged value of both the right and left hemisphere ROI mean values of the controls (Table 5.8). Before this averaged value was calculated, the left hemisphere ROI mean values and right hemisphere ROI mean values of the control group were first compared with a t test to determine whether there was any significant difference between them due to their laterality, and as would be expected, they were found not to be significantly different.

Comparison of *hemisphere* data (contralateral to any visible lesion) between TBI patients with unilateral lesions and controls (mild TBI n=21, moderate TBI n=5, control n=30)		ANOVA P-value
White matter	qT1	0.228
	qT2	0.687
	MD	0.197
	FA	0.645
Grey matter	qT1	**0.047**
	qT2	0.854
	MD	0.880
	FA	0.287

Table 5.8: p-values from one-way ANOVA comparison of contralateral hemisphere ROI means between mild TBI patients, moderate TBI patients and controls, for all four datasets (qT1, qT2, MD and FA) in white matter and grey matter. Grey matter qT1 was found to be significantly different between groups (p=0.047).

As with the whole brain data analyses above, significant differences were demonstrated between groups in the contralateral hemisphere grey matter qT1 data (Figure 5.11), but not between groups in any of the other grey matter quantitative values, nor between groups in

any of the white matter datasets. Further *post hoc* analysis between the three groups was performed using the t test between the individual pairs (mild TBI and controls, moderate TBI and controls and mild and moderate TBI) but p-values were all non-significant.

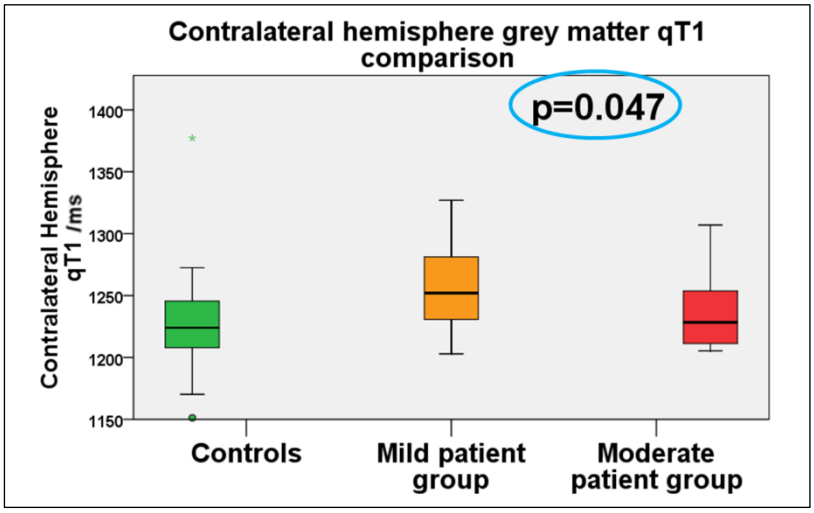

Figure 5.11: Box plot showing distribution of hemisphere grey matter qT1, contralateral to any visible lesion in the mild and moderate TBI groups and mean values for the left and right hemispheres in control subjects. An ANOVA test on the qT1 data indicates a significant difference between the groups (p=0.047).

Analysis of the *frontal lobe* ROI data contralateral to any visible lesion, using the one-way ANOVA test, revealed no significant differences between groups in any of the quantitative data in either white matter or grey matter.

5.2.5 *Sub-group comparison – ROIs adjacent to any visible lesion*

In the same manner, the hemisphere and frontal lobe ROI data was used to determine whether any differences could be detected *adjacent* to any visible lesion seen in the patient groups, when compared to the control group. In this analysis ROI data ipsilateral to visible lesions was compared using the one-way ANOVA test with the averaged data from both left and right ROIs in the controls, as used in section 5.2.4 above.

Analysis of the datasets with respect to the white matter in the hemispheres and frontal lobes containing extracted visible lesions revealed no statistically significant differences between TBI patients and controls. However, a significant difference was shown to exist between TBI groups and controls in the hemisphere ipsilateral to any visible lesion in both

the grey matter qT1 mean (p=0.003) and the grey matter MD mean (p=0.007) *and* in the frontal lobe ipsilateral to any visible lesion in the grey matter MD (P=0.008) (Figure 5.12). There were no significant differences found in the grey matter qT2 or FA in the hemisphere ipsilateral to any visible lesion.

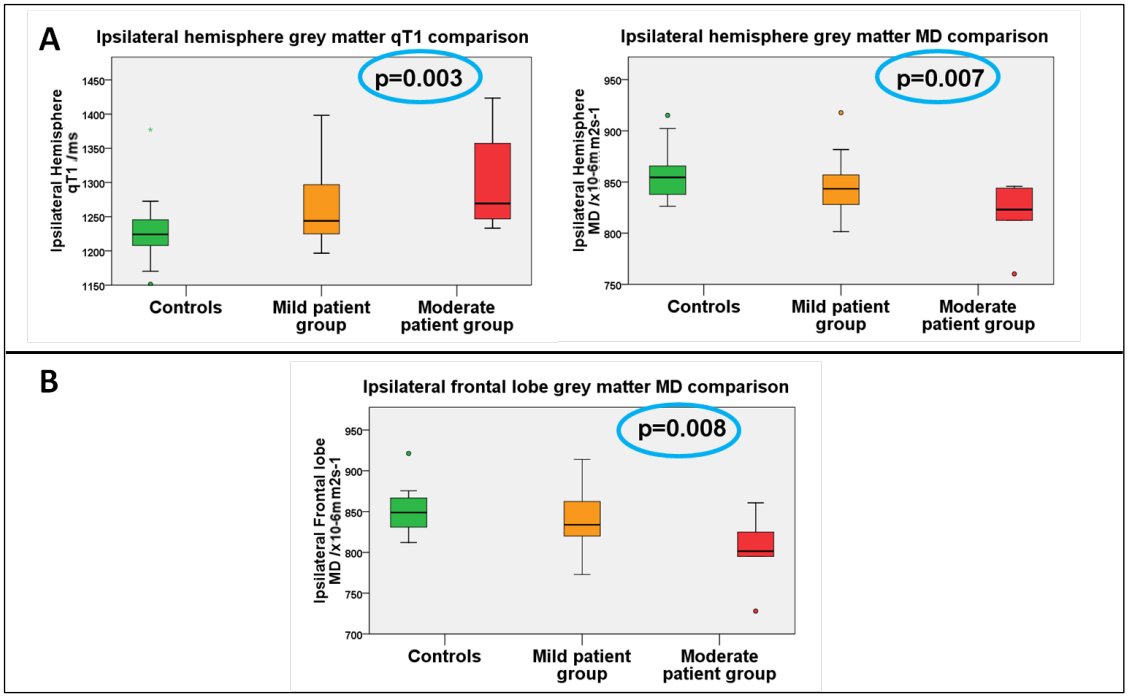

Figure 5.12: Box plots showing distribution of grey matter qT1 and MD means in the *hemisphere* ipsilateral to any visible lesion (A) and grey matter MD means in the *frontal lobe* ipsilateral to any visible lesion (B) for the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-values indicate a significant increase in qT1 time in the patient groups, and a significant decrease in MD in the patient groups, compared to the controls.

To ascertain where these significant differences lay, a t test was used between individual pairs of the three groups. A significant increase in grey matter qT1 relaxation time in the ipsilateral hemisphere was found in the mild TBI group compared to the control group (p=0.015), but not between the moderate TBI group and controls, or between the mild and moderate TBI groups (Figure 5.13).

In the grey matter MD there was a significant reduction in the moderate TBI group compared to the control group in the ipsilateral hemisphere (p=0.002) and ipsilateral frontal lobe (p=0.001), but there was no significant difference between the mild TBI group and either the control group or the moderate TBI group (Figure 5.14).

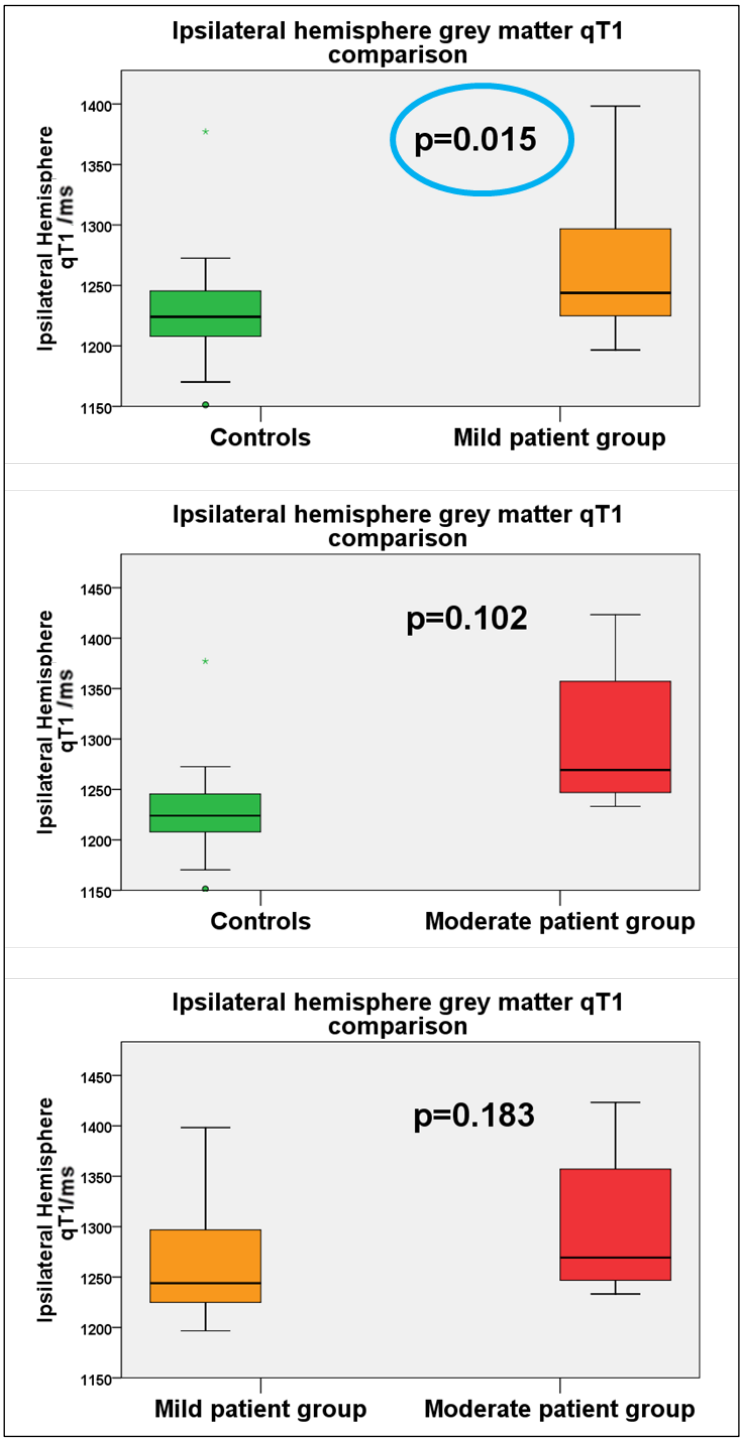

Figure 5.13: Box plots showing distributions of hemisphere grey matter qT1 means ipsilateral to any visible lesion for the mild TBI group and controls, the moderate TBI group and controls, and for the mild TBI and moderate TBI groups. The t test p-values show a significant increase between the mild TBI group and the control group, but not between the moderate TBI group and the controls, or between the TBI groups themselves.

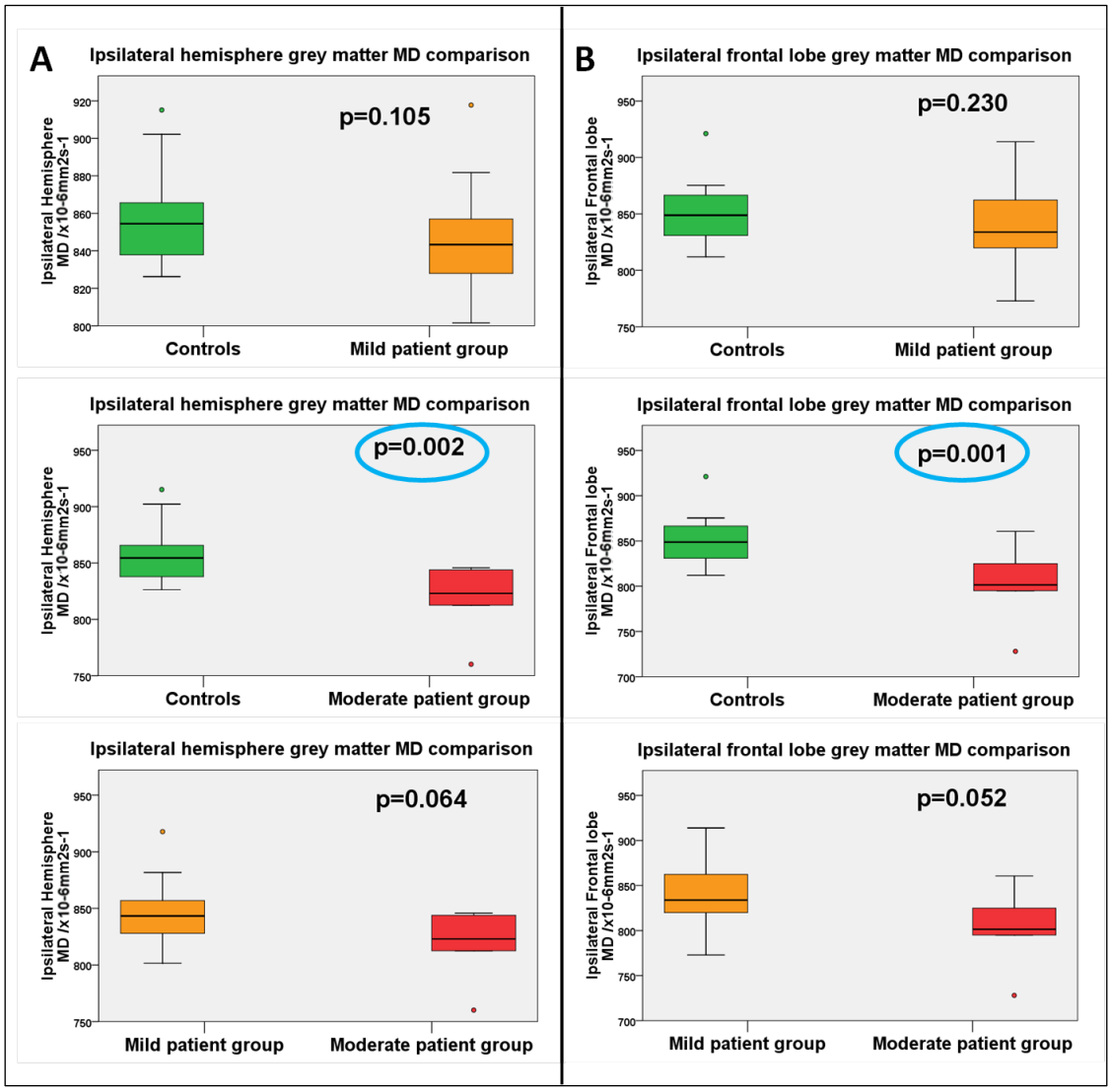

Figure 5.14: Box plots showing distributions of hemisphere (A) and frontal lobe (B) grey matter MD means ipsilateral to any visible lesion for the mild TBI group and controls, the moderate TBI group and controls, and for the mild TBI and moderate TBI groups. The t test p-values show a significant decrease between the moderate TBI group and the control group in both ROIs, but not between the mild TBI group and the controls, or between the TBI groups themselves.

5.2.6 *Further analysis – correlation with GCS, LOC and PTA*

Assessment of correlation between injury characteristics (GCS, LOC and PTA) and the whole brain quantitative dataset mean values in each tissue type was performed using the Pearson correlation coefficient, as the data had been shown in the majority to be normally distributed (Table 5.9). A positive correlation was found between the *white matter* quantitative T1 time (qT1) and the GCS at initial assessment of the entire patient group, comprised of both mild TBI and moderate TBI groups (Figure 5.15). This finding was in

contrast to the results from the individual group comparisons above, in which the ANOVA and t test analyses showed that the difference in the qT1 means was not a significant discriminator between groups. However, there was no correlation between GCS and any of the other whole brain quantitative means (qT2, MD or FA), either in white matter or grey matter.

Whole brain data		Correlation p-value using Pearson coefficient		
		GCS	LOC /minutes	PTA /hours
White matter	qT1	**0.035**	0.303	0.344
	qT2	0.253	0.160	0.067
	MD	0.107	0.184	0.053
	FA	0.383	0.188	0.286
Grey matter	qT1	0.190	0.212	0.321
	qT2	0.401	0.292	0.379
	MD	0.459	0.455	0.378
	FA	0.317	0.201	0.253

Table 5.9: P-values showing a significant correlation between whole brain white matter qT1 mean and GCS (red).

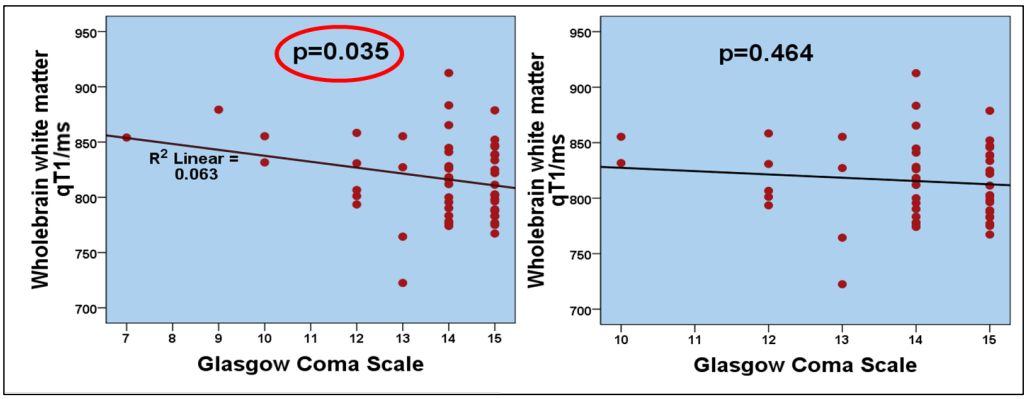

Figure 5.15: Scatter plots to show the relationship between whole brain white matter qT1 and GCS score at initial assessment. The left hand plot shows the significant correlation identified by the initial analysis, although this appears to be driven by outliers with low GCS scores. The right hand plot shows the same analysis repeated after data from those patients was removed, and shows no significant correlation.

Further analysis of the correlation between whole brain white matter qT1 and GCS showed that this significant correlation was driven by outliers: two moderate TBI patients who had a GCS score of 7 and 9 respectively. Their whole brain white matter qT1 values were *within* the range of values demonstrated by those patients with a GCS score of 15, and so were unlikely to be truly significant. Indeed, when data from these patients was excluded from the analysis, no significant correlation was found ($p=0.464$) (Figure 5.15).

Duration of loss of consciousness (LOC) showed no correlation with any of the whole brain quantitative data means in either tissue type, nor was there any correlation between the length of post-traumatic amnesia (PTA) and any of the whole brain quantitative datasets.

5.3 Method 2: Fourteen Automated Regions of Interest

As discussed in Chapter 4, user-defined ROI analysis as performed on the data above is useful as it allows the targeting of specific areas of interest and is hypothesis led: whole brain ROI to test whether diffuse changes could be detected in the *entire* grey or white matter, and lateralised ROIs to test whether changes could be detected remote from or adjacent to extracted visible regions of damage. However, it has a number of disadvantages. When selecting large ROIs, small areas of subtle damage may not be detected, as a result of the diluting effect of the surrounding normal areas contained within the same ROI. Another limitation of this type of ROI selection is that some regions where microstructural change exists could be missed if they were not inside the pre-specified target ROIs and lastly the analysis itself is hugely time consuming and potentially prone to bias from user definition of each individual ROI. In order to address this limitation, an automated method was used which involved the creation of a template dividing the *whole* brain volume into 14 distinct smaller regions, 7 per hemisphere (Table 5.10), and the application of this template automatically to each of the patient's datasets.

Region	Number assigned for analysis	
	Right	Left
Inferior frontal	1	8
Superior frontal	2	9
Temporal	3	10
Parieto-occipital	4	11
Occipital	5	12
Temporo-parietal	6	13
Parietal	7	14

Table 5.10: Number (1-14) assigned to each region, right and left, for purposes of analysis.

5.3.1 *Test for normality*

Data using the 14 automated ROI analysis technique (section 4.12.2) were available in all subjects for all measures (Appendix B and Appendix C). The Kolmogorov-Smirnov test was performed on all the datasets in both white and grey matter for each region in the

control subjects only (Table 5.11). The majority of the quantitative T1 and quantitative T2 scan data was found to be normally distributed, but data from a few of the qT1 and qT2 regions was not. Therefore, as non-parametric tests are more conservative in their calculation of significance, they were used in the analysis of these two datasets. The DTI data (both MD and FA) was found to be normally distributed and for these datasets parametric tests were used.

Tissue type / Region	qT1 data p-value	qT2 data p-value	MD data p-value	FA data p-value
WM 1	0.658	0.581	0.875	0.608
WM 2	0.961	0.100	0.818	0.726
WM 3	0.951	0.331	0.912	0.943
WM 4	0.756	0.420	0.769	0.505
WM 5	0.243	0.605	0.861	0.954
WM 6	0.921	0.232	0.972	0.848
WM 7	0.965	0.544	0.814	0.998
WM 8	0.962	0.265	0.699	0.910
WM 9	0.974	0.116	0.737	0.628
WM 10	0.968	0.543	0.822	0.583
WM 11	0.868	0.238	0.743	0.958
WM 12	0.884	0.420	0.930	0.578
WM 13	0.425	0.204	0.678	0.899
WM 14	0.733	0.721	0.950	0.987
GM 1	0.997	0.205	0.812	0.986
GM 2	0.823	0.341	0.571	0.502
GM 3	**0.021**	0.279	0.994	0.589
GM 4	**0.019**	0.051	0.924	0.660
GM 5	0.089	**0.035**	0.910	0.904
GM 6	0.574	0.466	0.567	0.977
GM 7	0.918	0.702	0.497	0.975
GM 8	0.988	0.240	0.994	0.991
GM 9	0.992	0.790	0.952	0.853
GM 10	**0.015**	0.131	0.932	0.946
GM 11	0.593	0.328	0.812	0.920
GM 12	0.203	**0.025**	0.907	0.479
GM 13	0.772	0.406	0.994	0.731
GM 14	0.596	0.450	0.873	0.696

Table 5.11: 14 ROI data tested for normality using the Kolmogorov-Smirnov test on the control data in each region (1-14) in white matter (WM) & grey matter (GM). Significant values ($p<0.05$) in the qT1 and qT2 sets indicated the data was not normally distributed. Non-significant values in the MD and FA sets showed that this data followed a normal distribution.

5.3.2 *Group comparison by region – qT1 data in white and grey matter*

Analysis of differences between the quantitative T1 values in both the white matter and the grey matter in the 14 regions of interest between mild TBI patients, moderate TBI patients and controls was performed using the non-parametric Kruskal-Wallis test (Table 5.12).

Region	White matter qT1		Grey matter qT1	
	Right	Left	Right	Left
Inferior frontal	0.062	**0.040**	0.135	**0.040**
Superior frontal	0.239	0.155	0.439	0.722
Temporal	**0.004**	**0.013**	**0.044**	**0.019**
Parieto-occipital	**0.042**	**0.016**	0.087	**0.006**
Occipital	0.269	**0.040**	0.079	**0.005**
Temporo-parietal	0.114	0.126	**0.018**	**0.008**
Parietal	0.265	0.268	0.448	0.096

Table 5.12: P-values obtained by performing the Kruskal-Wallis test. A significant difference ($p<0.05$) in the mean quantitative T1 time (qT1) between groups (mild TBI, moderate TBI and controls) was demonstrated in both white and grey matter regions, more regions were significantly different in the left hemisphere.

This revealed significant inter-group differences in the white matter (Figure 5.16) in left inferior frontal (p=0.040), left temporal (p=0.013), right temporal (p=0.004), left parieto-occipital (p=0.016), right parieto-occipital (p=0.042) and left occipital regions (p=0.040); and in the grey matter (Figure 5.17) in left inferior frontal (p=0.040), left temporal (p=0.019), right temporal (p=0.044), left temporo-parietal (p=0.008), right temporo-parietal (p=0.018), left parieto-occipital (p=0.006) and left occipital regions (p=0.005). Both left and right temporal regions showed significant inter-group differences in mean qT1 in white matter and in grey matter.

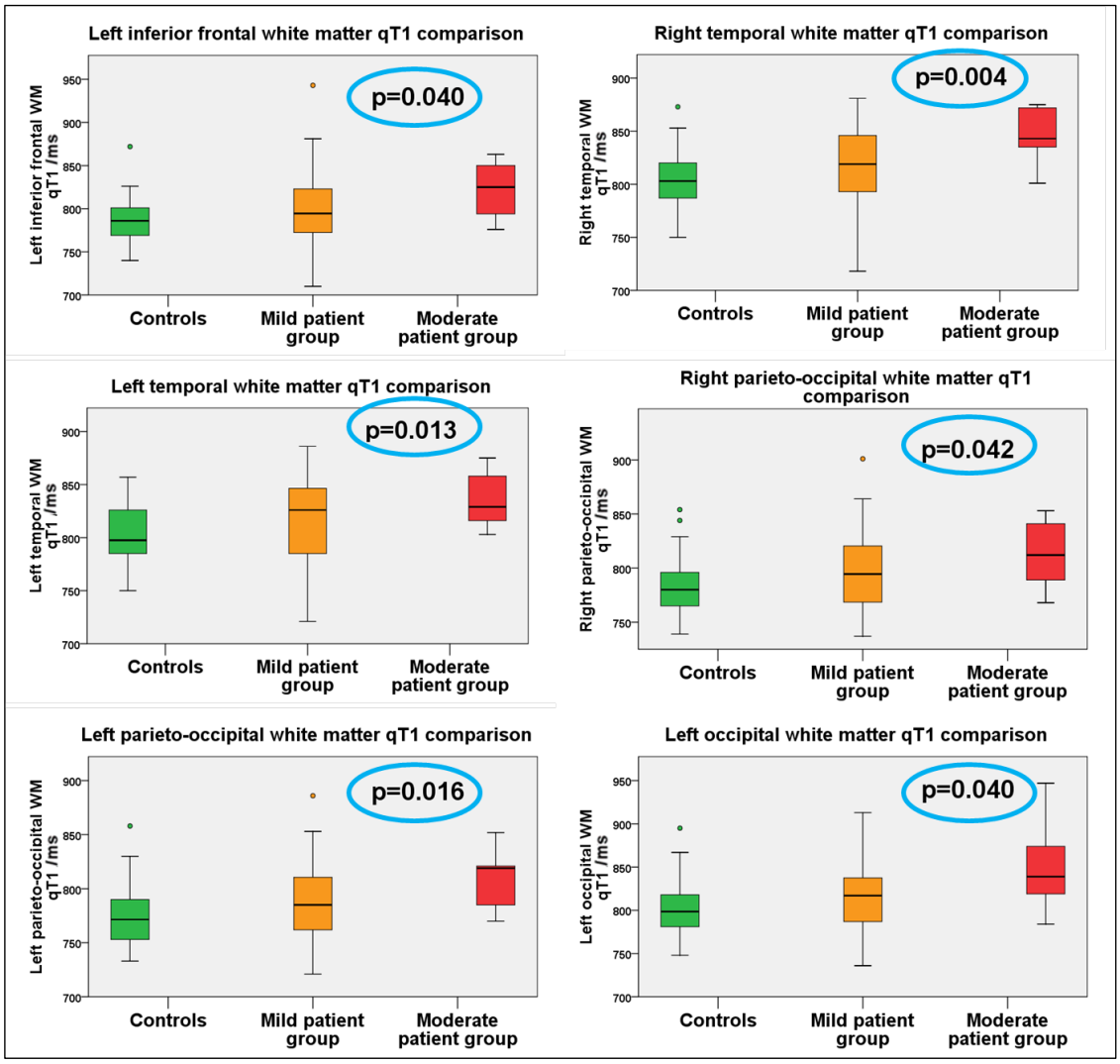

Figure 5.16: Box plots showing distribution of white matter qT1 means in the regions where significant differences were observed between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The Kruskal-Wallis test p-values indicate a significant increase in qT1 time in the patient groups compared to the controls.

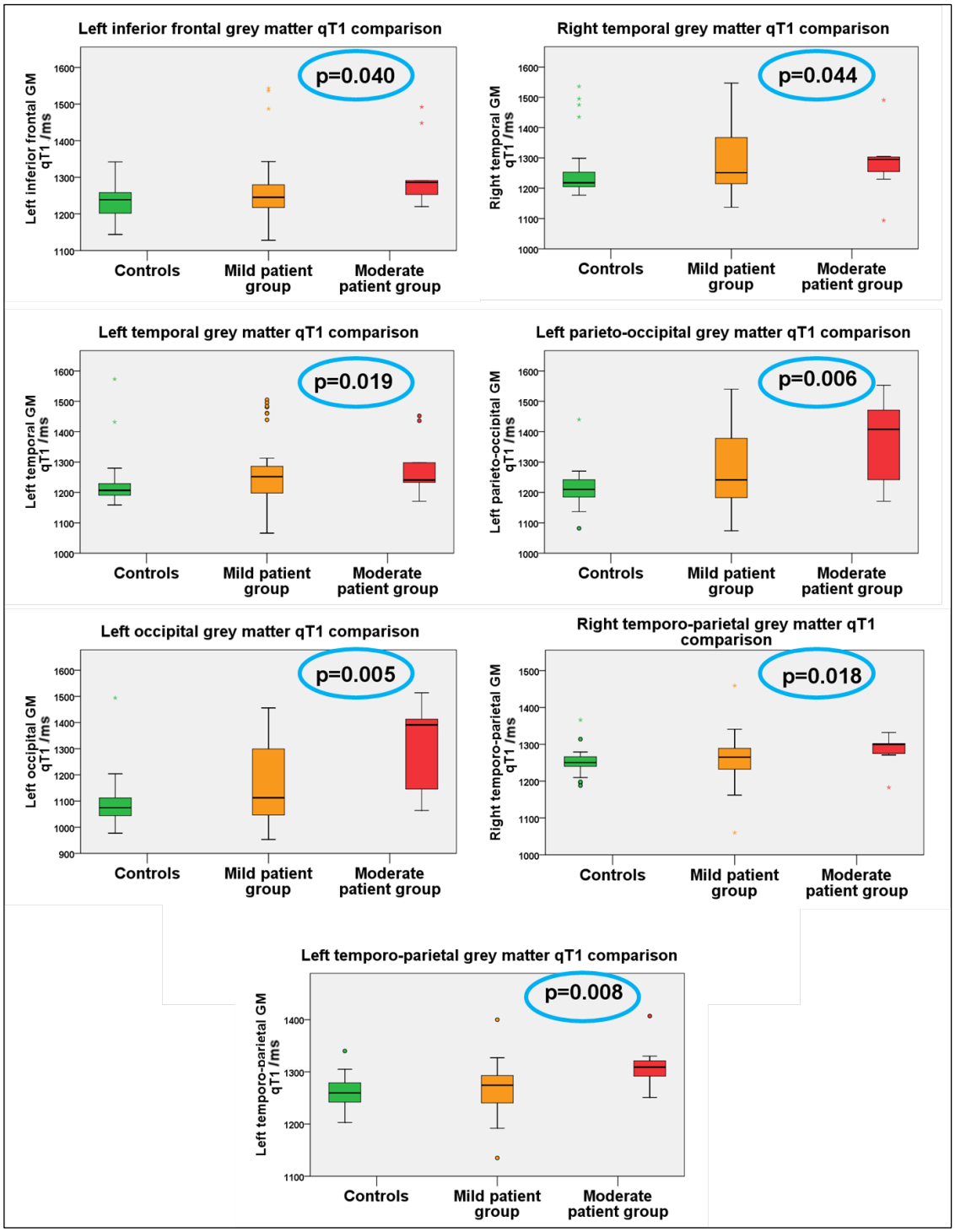

Figure 5.17: Box plots showing distribution of grey matter qT1 means in the regions where significant differences were observed between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The Kruskal-Wallis test p-values indicate a significant increase in qT1 time in the patient groups compared to the controls.

5.3.3 *Group comparison by region – qT2 data in white and grey matter*

Analysis of differences between the quantitative T2 values in both the white matter and the grey matter in the 14 regions of interest between mild TBI patients, moderate TBI patients and controls was performed using the non-parametric Kruskal-Wallis test (Table 5.13).

Region	White matter qT2		Grey matter qT2	
	Right	Left	Right	Left
Inferior frontal	0.279	0.845	0.496	0.615
Superior frontal	0.699	0.409	0.364	0.528
Temporal	0.460	0.225	0.913	0.181
Parieto-occipital	0.272	0.278	0.408	0.247
Occipital	0.936	0.615	0.305	0.082
Temporo-parietal	0.915	0.634	0.414	0.341
Parietal	0.364	0.177	0.360	0.145

Table 5.13: P-values obtained by performing the Kruskal-Wallis test. No significant differences in the mean quantitative T2 time (qT2) between groups (mild TBI, moderate TBI and controls) were demonstrated in either white or grey matter regions.

This revealed no significant inter-group differences in mean quantitative T2 time in either the white matter or grey matter in any of the 14 regions examined.

5.3.4 *Group comparison by region – MD data in white and grey matter*

Analysis of differences between the mean diffusivity values in both the white matter and the grey matter in the 14 regions of interest between mild TBI patients, moderate TBI patients and controls was performed using the parametric one-way ANOVA test (Table 5.14).

Region	White matter MD		Grey matter MD	
	Right	Left	Right	Left
Inferior frontal	0.578	0.477	0.754	0.878
Superior frontal	**0.008**	**0.006**	**0.014**	**0.034**
Temporal	0.082	0.227	0.771	0.969
Parieto-occipital	**0.048**	0.358	0.490	0.995
Occipital	0.087	0.392	0.377	0.864
Temporo-parietal	0.380	0.578	0.140	0.365
Parietal	0.099	0.123	0.123	**0.028**

Table 5.14: P-values obtained by performing the ANOVA test. A significant difference ($p<0.05$) in the mean diffusivity (MD) between group means (mild TBI, moderate TBI and controls) was demonstrated in both white and grey matter regions.

This revealed significant inter-group differences in mean MD values in the white matter (Figure 5.18) in left superior frontal ($p=0.006$), right superior frontal ($p=0.008$) and right parieto-occipital regions ($p=0.048$); and in the grey matter (Figure 5.19) in left superior frontal ($p=0.034$), right superior frontal ($p=0.014$) and left parietal regions ($p=0.028$). Interestingly both left and right superior frontal regions were significantly different between the patient groups and controls in both white *and* grey matter analyses of MD means. MD comparison in the grey matter regions showed a decrease in the MD values of the patient groups when compared with the controls, in contrast to the increase in MD observed in the white matter regions. This correlates with similar findings in the 5 ROI analysis (section 5.2.5).

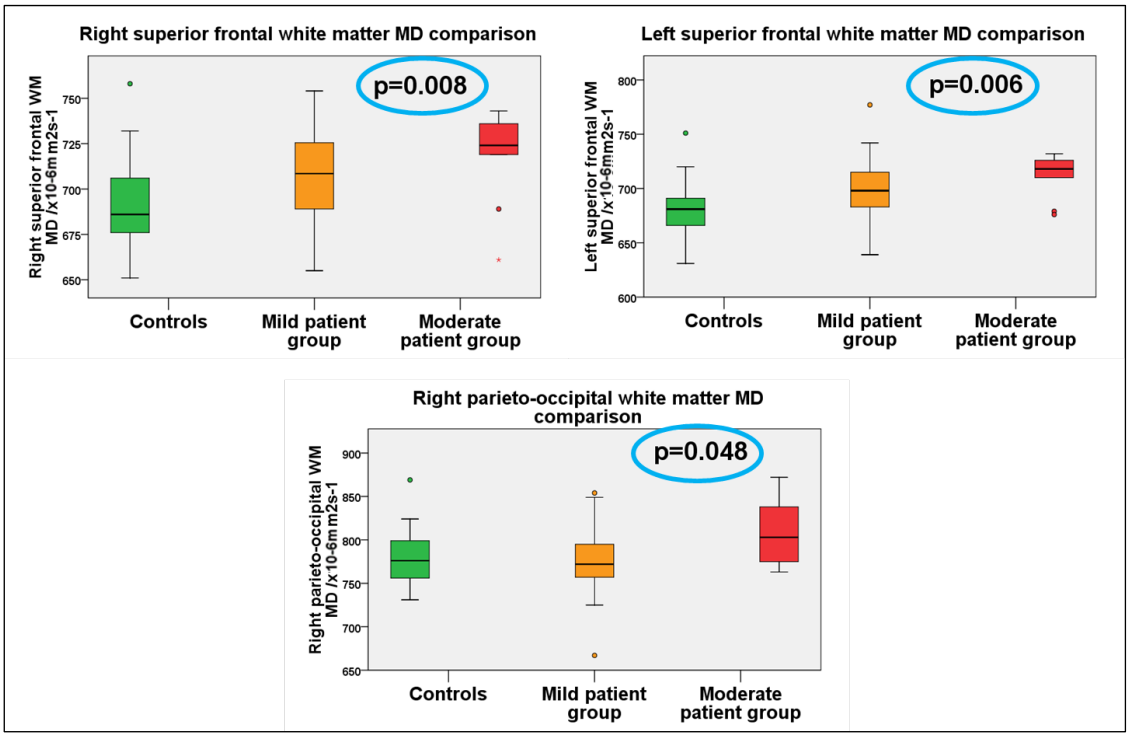

Figure 5.18: Box plots showing distribution of white matter MD means in the regions where significant differences were observed between groups. The ANOVA test p-values indicate a significant increase in mean diffusivity in the patient groups compared to the controls, although the right parieto-occipital white matter MD p-value is only just significant and the box plot shows minimal differences between each dataset.

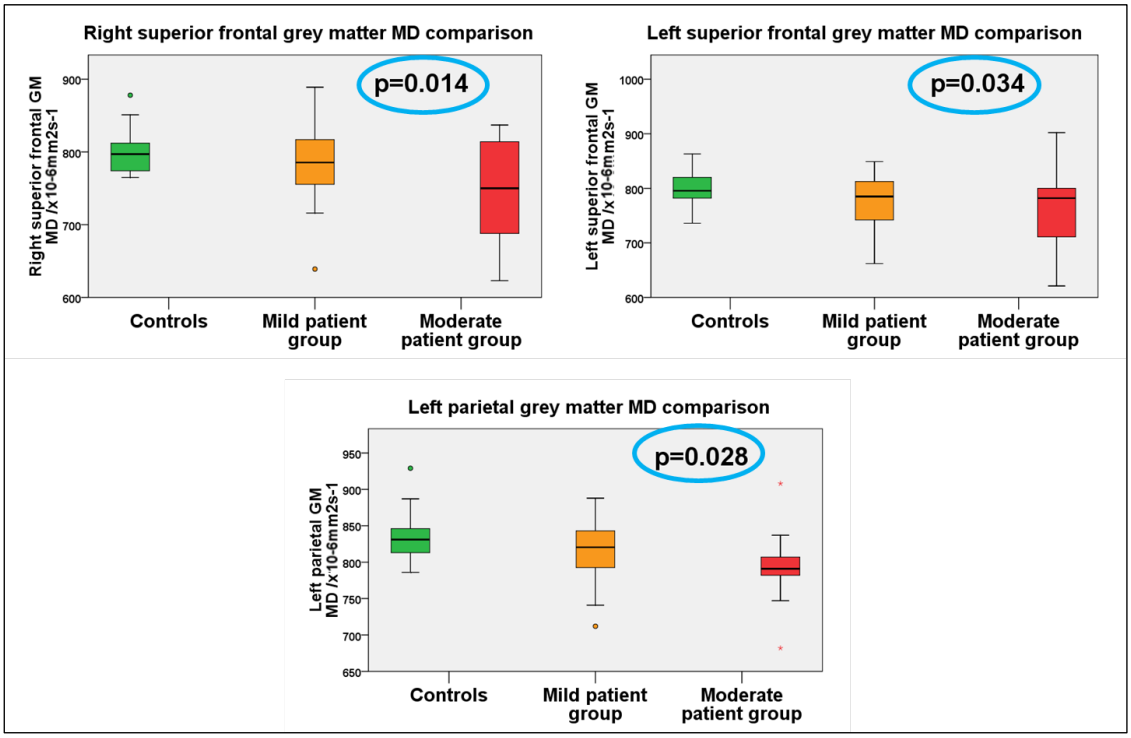

Figure 5.19: Box plots showing distribution of grey matter MD means in the regions where significant differences were observed between groups. The ANOVA test p-values indicate a significant decrease in MD in the patient groups compared to the controls.

5.3.5 *Group comparison by region – FA data in white and grey matter*

Analysis of differences between the fractional anisotropy values in both the white matter and the grey matter in the 14 regions of interest between mild TBI patients, moderate TBI patients and controls was performed using the parametric one-way ANOVA test (Table 5.15).

Region	White matter FA		Grey matter FA	
	Right	Left	Right	Left
Inferior frontal	0.385	0.548	0.213	0.895
Superior frontal	0.458	0.857	0.137	0.399
Temporal	0.112	0.945	0.425	0.967
Parieto-occipital	0.840	0.272	0.455	0.804
Occipital	0.418	0.489	**0.039**	0.513
Temporo-parietal	0.373	0.399	**0.040**	0.307
Parietal	0.765	0.716	0.266	0.395

Table 5.15: P-values obtained by performing the ANOVA test. A significant difference ($p<0.05$) in the fractional anisotropy (FA) between group means (mild TBI, moderate TBI and controls) was demonstrated only in two grey matter regions.

This revealed significant inter-group differences in mean FA in the grey matter (Figure 5.20) in right temporo-parietal ($p=0.040$) and right occipital ($p=0.039$) regions. There were no significant differences in mean FA in any of the 14 white matter regions examined.

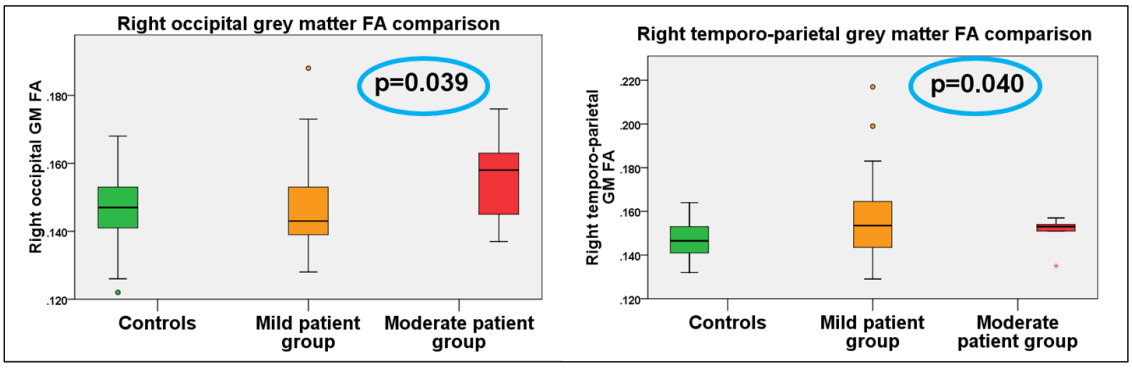

Figure 5.20: Box plots showing distribution of grey matter mean FA in the regions where significant differences were observed between groups. The ANOVA test p-values indicate a significant increase in FA in the patient groups compared to the controls.

5.4 Method 3: Tractography Based Regions of Interest

This technique defined ROIs based upon white matter tracts within the brain. For this analysis specialised software was used to delineate axonal fibre tracts within the brain, based upon the data acquired by diffusion tensor imaging. This technique combined advantages of both the hand-defined and the automated ROI methods. Similar to hand-defined ROI analysis, tractography based regions of interest are hypothesis led and allow the targeting of specific areas of interest, but in common with the automated method, the use of software to aid in the design of the ROIs reduces the time involved in performing the analysis and decreases the likelihood of user-introduced bias in the results. The ROIs designed using this method were the genu, splenium and body of the corpus callosum (Figure 4.9). These areas were selected as they are known to be regions susceptible to diffuse axonal injury, have been shown in previous MRI studies to have altered diffusion properties (see Chapter 2, sections 2.6.4 – 2.6.7), and are involved in cognitive functions known to be affected by TBI.

5.4.1 *Test for normality*

Data using the tractography designed ROI analysis technique (section 4.12.3) were available in all subjects for mean diffusivity (MD), fractional anisotropy (FA) and all three eigenvalues (Appendix D). All the data from the genu, splenium and body of the corpus callosum was found to be normally distributed using the Kolmogorov-Smirnov test (Table 5.16). The data was therefore analysed using parametric tests.

	Corpus Callosum Genu	Corpus Callosum Splenium	Corpus Callosum Body
MD	0.896	0.607	0.461
FA	0.934	0.861	0.185
E1	0.859	0.726	0.475
E2	0.508	0.721	0.122
E3	0.872	0.716	0.212

Table 5.16: Tract-based ROI data tested for normality using a Kolmogorov-Smirnov test. All values have a non-significant p-value ($p > 0.05$), indicating normally distributed data.

5.4.2 *Group comparison – mean diffusivity*

Mean diffusivity data was obtained for each of the three tractography defined ROIs: the genu, splenium and body of the corpus callosum. The mean MD values for each ROI in the mild TBI, moderate TBI and control groups were compared using the ANOVA test (Figure 5.21). No significant difference was observed between the MD means of each group in any of the three ROIs analysed.

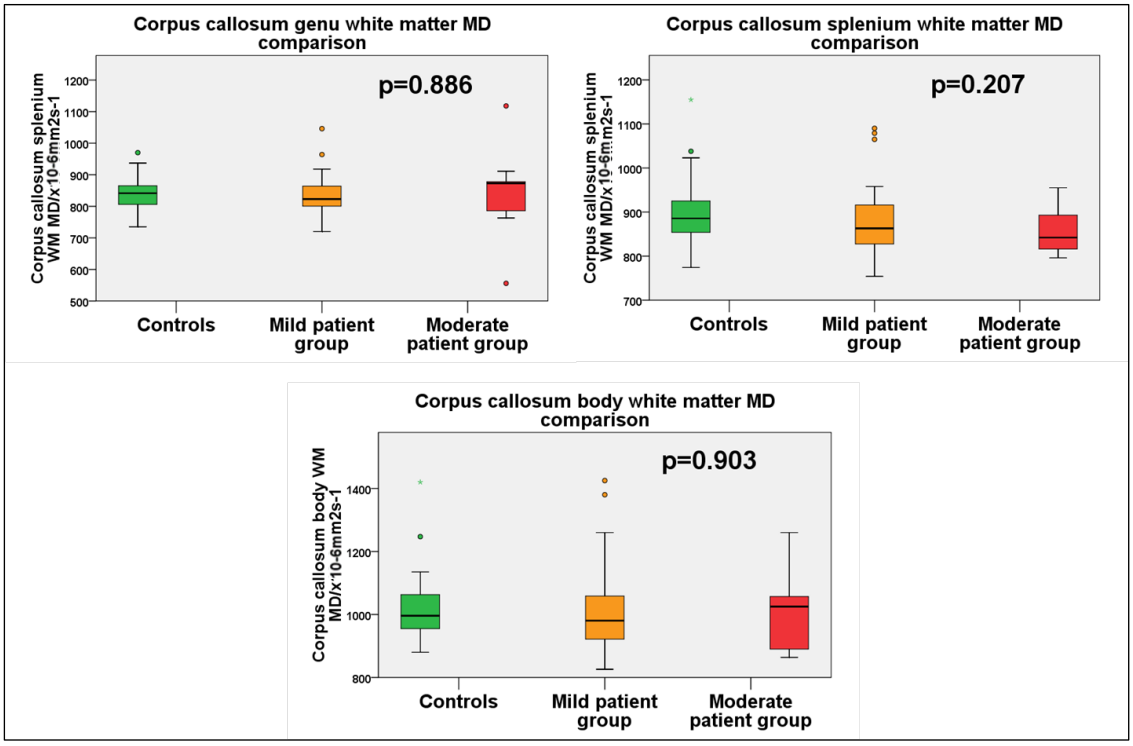

Figure 5.21: Box plots showing distribution of white matter mean MD between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-values indicate no significant differences in MD in the patient groups compared to the controls in any of the three tractography defined ROIs.

5.4.3 *Group comparison – fractional anisotropy and eigenvalues*

Fractional anisotropy data and data for each of the three eigenvalues was obtained for each of the three tractography defined ROIs: the genu, splenium and body of the corpus callosum. The mean FA values for each ROI in the mild TBI, moderate TBI and control groups were compared using the ANOVA test (Figure 5.22). No significant difference was observed between the FA means of each group in any of the three ROIs analysed.

The mean values for each eigenvalue in each ROI in the mild TBI, moderate TBI and control groups were also compared using the ANOVA test and again there were no significant differences found between the groups in any of the three ROIs analysed.

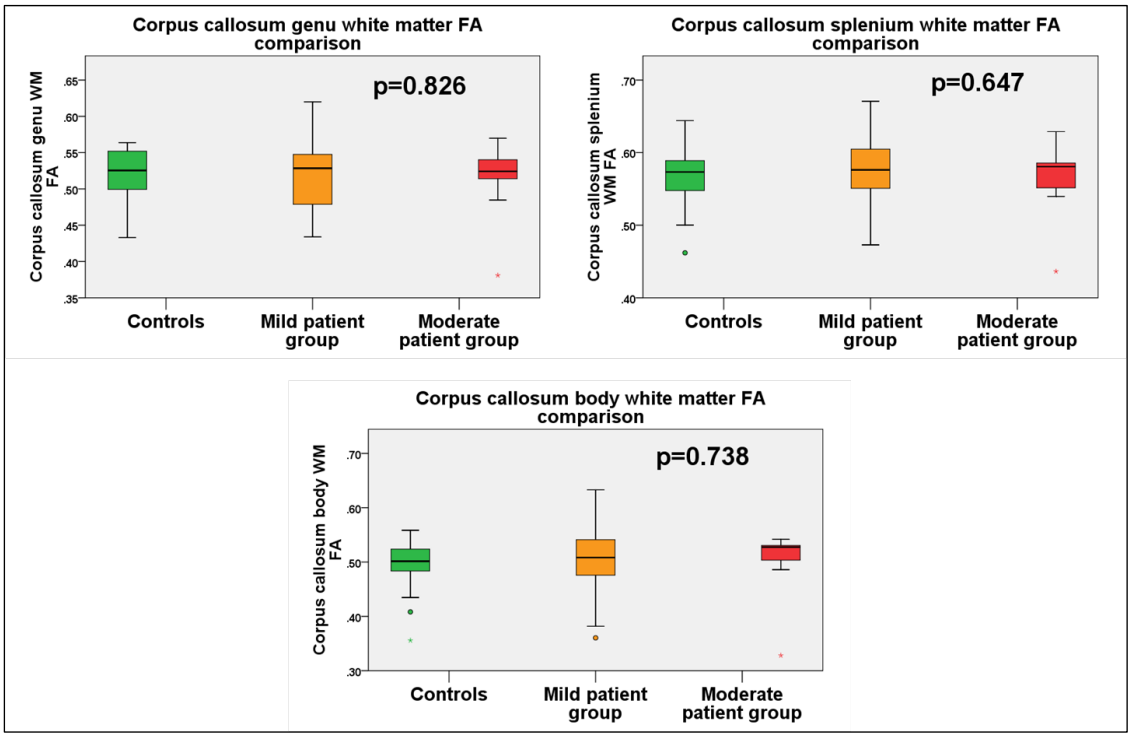

Figure 5.22: Box plots showing distribution of white matter mean FA between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-values indicate no significant differences in FA in the patient groups compared to the controls in any of the three tractography defined ROIs.

5.4.4 *Sub-group comparison – mild TBI assessed by AAN grade*

A sub-group analysis was conducted comparing mild TBI patients grouped according to AAN grade (Table 5.1) and the control group, using the ANOVA test in the MD and FA data and in the eigenvalue data. This analysis was performed for each of the three tractography designed ROIs in white matter. No significant inter-group differences were demonstrated.

5.5 Method 4: Tract Based Spatial Statistics (TBSS)

The fourth method used was a voxel-wise statistical analysis called 'tract-based spatial statistics', and was again performed with specialised software, using the DTI data. This last technique compared the mean fractional anisotropy of white matter tracts common to all subjects with the individual patient's values. TBSS combines the benefits of an automated technique: it encompasses the whole brain white matter reducing the likelihood of missing any areas where there is microstructural change and reduces the time required to analyse the data. To a degree it is also hypothesis led, as TBSS examines white matter tracts, and these commonly show damage as a result of the shearing forces of TBI (Chapter 1, section 1.10.3).

5.5.1 G*roup comparison – white matter tract fractional anisotropy*

TBSS was used to carry out a voxel-wise t test comparison of mean white matter tract fractional anisotropy (FA) values. It was not possible to perform an analysis with three variables (ie: between the mild TBI patients, moderate TBI patients and control group) and therefore this analysis was performed firstly between all TBI patients grouped together and the controls subjects, and secondly between the mild TBI patients and the control subjects. Comparison using the moderate group alone was not performed as the group size was felt to be too small for this style of analysis. T test comparison of white matter tract FA means of the whole TBI patient group with the control subjects revealed significant increases only in small areas in the left superior region of the corona radiata (Figure 5.23). Further analysis using a t test between mild TBI patients only and the control subjects revealed similar significant increases in small areas in the left superior region of the corona radiata (Figure 5.24).

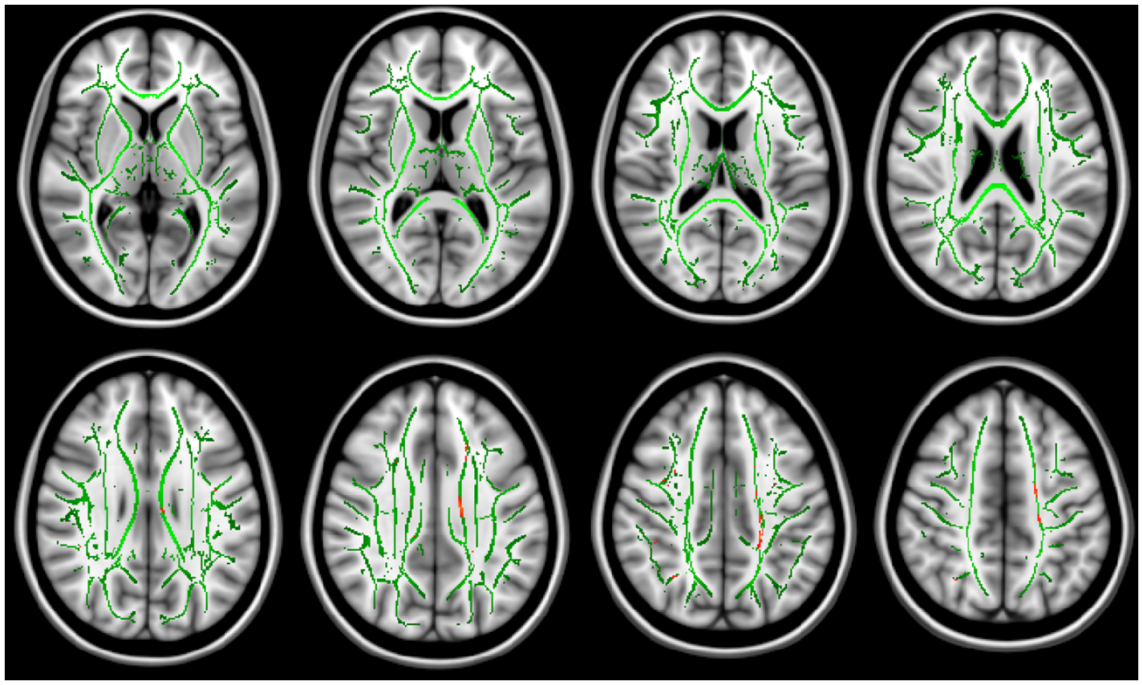

Figure 5.23: Axial images created using TBSS showing the mean FA tract skeleton (green) and voxels in which the t test showed a significant difference ($p<0.05$) between the whole TBI patient group and the control group (red).

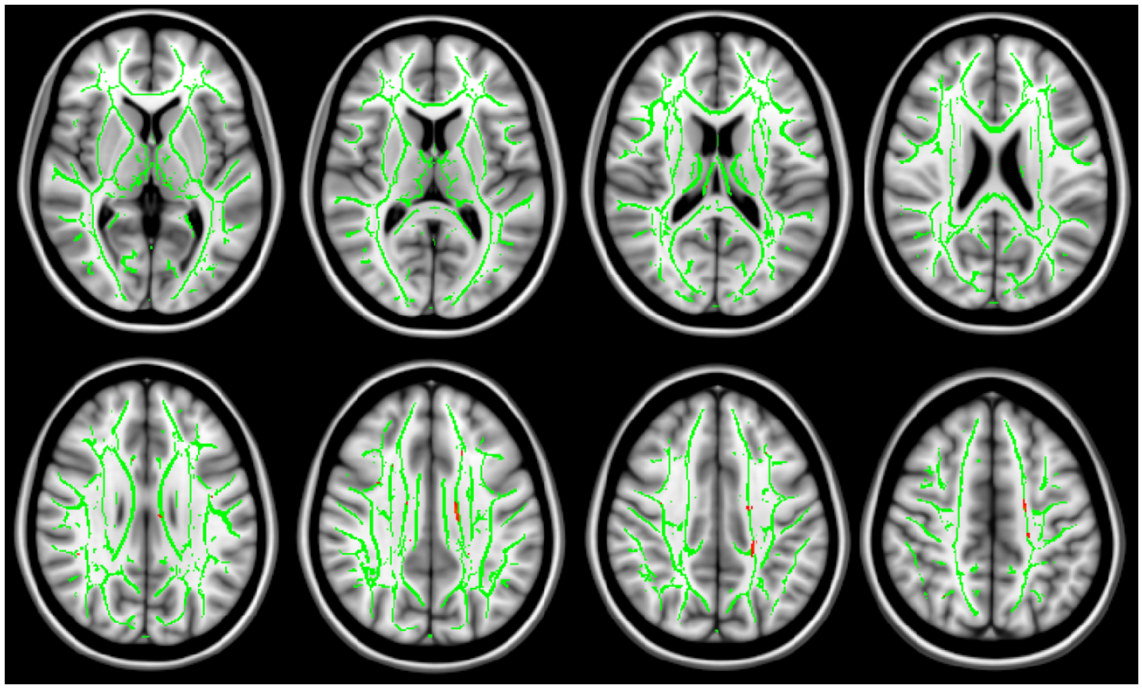

Figure 5.24: Axial images created using TBSS showing the mean FA tract skeleton (green) and voxels in which the t test showed a significant difference ($p<0.05$) between the mild TBI patient group and the control group (red).

5.6 Discussion

Analysis of the quantitative scan data in both grey and white matter revealed significant findings in the TBI patient groups when compared to the control group. This was true of data obtained using both the 5 ROI method and the 14 automated ROI method.

The first analysis method, using the 5 hand defined regions of interest was the most basic of the analysis techniques. Used extensively in published work in this field, it had the benefit of being hypothesis driven, and despite the method being a very time consuming process, the ROIs were straight forward to create as they were simply drawn on to the scan slices using specialised software.

5.6.1 *Whole brain analysis*

The largest and most crude of the 5 ROIs was the whole brain ROI; three-way group comparison of the data obtained from this region showed no differences in the white matter in any of the quantitative datasets being examined: qT1, qT2, MD or FA. For there to have been detectable inter-group differences in these very large ROIs, the majority of the tissue within the brains of the TBI patients would have had to have had differing quantitative values, or the magnitude of existing values would have to have been different to such an extent that the diluting effect of including the entire brain would have been cancelled out. With this in mind, the fact that the 3-way group comparison of whole brain grey matter qT1 data showed a significant inter-group difference implies that there was sufficient change in qT1 time in the patient groups compared with that in the control group to allow a difference to be observed. This was not the case however, in any of the other grey matter quantitative datasets under scrutiny (qT2, MD or FA).

Analysis of this whole brain grey matter qT1 finding to assess where the inter-group significance lay revealed that there was a detectable increase in both patient groups when compared to the control group, but that there was no significant difference *between* the mild and moderate patient groups. This suggests that if the observed qT1 increase was due to the TBI sustained, that the increase may not be proportional to the severity of injury sustained. Of course, other explanations may be either that the moderate TBI group sample size may have been too small for a difference between that group and the mild TBI patients to have been significant, or it may be that the patient group classification method: mild or moderate

according to admitting GCS may not have accurately represented the true severity of microstructural damage sustained.

5.6.2 *Analysis by AAN grade*

Sub-group analysis of the mild TBI patient group after classifying them using the American Academy of Neurology concussion grading scale revealed no significant inter-group differences in whole brain grey matter or white matter in any of the quantitative scan datasets. While it appeared that the increase in grey matter quantitative T1 time in the mild TBI patients compared to the control group was reaching significance ($p=0.071$), the trend of the group means was not consistent: the mean whole brain grey matter qT1 value in the AAN grade II mild TBI patients was lower than that of the mean in the AAN grade I group (Figure 5.10). If the observed increase in qT1 time in the grade I patients compared to the controls was a result of underlying changes in the grey matter due to the sustained TBI, and if as hypothesised those changes were linked to the symptoms experienced by the patients, it would not fit that the grade II patients with longer lasting symptoms had a lower mean qT1 time than grade I patients whose symptoms had all resolved by 15 minutes. This unexpected disparity could not be explained by a difference in group numbers, as the mild TBI patients classed as grade I numbered 7, and those classed as grade II numbered 10. However, this highlights the fact that the numbers in each group were small, and it may be that with greater numbers, the trend would have been of a consistent increase in qT1 time in proportion to AAN grade severity.

5.6.3 *Analysis of regions remote from any visible lesion*

When brain tissue was analysed in ROIs remote from any visible lesions, after those lesions had been extracted from the data, once again it was only the grey matter qT1 mean that showed significant inter-group differences using the 3-way ANOVA analysis. Further examination of the box plot showed that although the mild TBI group mean was greater than that of the control group, the moderate TBI group mean and inter-quartile range were lower than that of the mild TBI group, and almost identical to the mean and inter-quartile range of the control group (Figure 5.10). Interestingly, post hoc paired analysis performed using a t test showed no inter-group differences at all. These results either indicate that no damage existed in brain tissue remote from any visible lesion in these patients or, perhaps

more likely, that the 5 ROI analysis technique was not sensitive enough to identify existing microstructural damage remote from visible lesions.

5.6.4 *Analysis of regions adjacent to any visible lesion*

Unsurprisingly, analysis of areas close to the extracted visible lesions revealed differences in more than one quantitative dataset: both in grey matter qT1 and in grey matter MD. It is reasonable to assume that the most likely *visually normal* regions to have detectable quantitative abnormalities would be those regions adjacent to existing visible damage. These 'ipsilateral to visible damage' ROIs showed an increase in grey matter qT1 time and a decrease in grey matter mean diffusivity, although these differences were not consistently found between the same groups. The hemisphere grey matter qT1 increase was only significant between the mild TBI group and the controls; once again this may be due to the small moderate TBI group sample size, although the difference between the hemisphere grey matter qT1 values between the moderate TBI group and the control group was sufficient for the inter-quartile ranges of each group not to overlap (Figure 5.12). The significant decrease in grey matter mean diffusivity was observed only in the moderate TBI patient group, and although the box plots indicated a reduction in MD values between the mild TBI group and the controls, this did not reach significance (Figure 5.13 A).

5.6.5 *Fourteen automated ROI technique findings*

The 14 automated ROI technique, while still dividing the brain into relatively arbitrary regions, showed a number of benefits. Firstly, as the process was automated, the analysis took less time than when using the 5 ROI technique. Secondly, when the 14 regions were combined, they covered the entire brain volume ensuring that no regions were left unexamined. Lastly, the ROIs themselves were smaller and less crude than those in the 5 ROI analysis. This meant that they were more likely to yield significant findings, where they existed, by reducing the data 'dilution'. However, there was also a theoretical disadvantage to using this method, in that by moving to smaller ROIs, the analysis risked not identifying features common to the whole brain, since local variations near the site of impact would become more prominent. A further disadvantage to using this technique, and one addressed in the chapter reporting the scan data findings in relation to the

neuropsychology test scores, is the issue of multiple analyses. When the number of ROIs under analysis increases, Bonferroni corrections should be made to ensure that any significant findings are not a result of chance alone. If a very large number of ROIs are being analysed, the adjusted significant p-value can become so low, that none of the findings appear significant.

Analysis of the data using the automated 14 ROI method revealed similar findings to the 5 ROI method: significant differences between TBI patients and the control group in ROIs in the qT1 and MD datasets. In addition, there were also significant findings in the grey matter FA in two of the fourteen regions. There were no significant findings in the qT2 data, in either grey matter or white matter, or in the white matter FA.

Contrary to the findings in the 5 ROI analysis, the differences observed in qT1 and MD were present in the white matter regions as well as in the grey matter. Both white and grey matter qT1 datasets showed significant differences in around half of the 14 ROIs, six regions were significant in the white matter qT1 comparison, and seven regions were significant in the grey matter qT1 comparison. Five of these ROIs were the same in each tissue type, ie: they showed significant increases in both grey matter and white matter qT1. The significant ROIs were also predominantly left sided, which correlates with the laterality of the majority of the visible lesions in both the mild and moderate TBI groups, and suggests that at the very least, the 14 automated ROI technique was sufficiently sensitive to detect changes in normal appearing tissue adjacent to lesions detected by visual analysis of the T1 weighted anatomical scans.

The mean diffusivity data showed differences between the TBI patient groups and the control group in left-sided and right-sided superior frontal regions in *both* white and grey matter. The significant grey matter FA findings were in the right occipital and right temporo-parietal regions. These regions together project into the centre of the brain and include the majority of the deep grey matter structures.

The fact that white matter changes were observed between groups in the 14 ROI analysis, and not from data obtained using the larger user-defined 5 ROI method may be due to the fact that the diluting effect of calculating a mean value from all the voxels within larger ROIs would have been reduced by calculating the mean from fewer voxels in the smaller ROIs in the automated method. By employing the automated 14 ROI method which

reduced the size of the ROIs into which the whole brain was divided, smaller regions of microstructural change have been identified where using the 5 ROI technique they had not been evident.

5.6.6 *Findings in qT1 and qT2 data*

The findings of increased grey matter and white matter qT1 values in this study support other work which has found similar increases in both experimental and clinical TBI. Naruse et al. (1982) studied the effects of vasogenic and cytotoxic oedema on qT1 and qT2 times in a rat model and found that in vasogenic oedema there was an increase in qT1 time in grey and white matter, and in cytotoxic oedema an increase in qT1 time in white matter only. The increase in qT1 they observed in vasogenic oedema occurred after 24 hours and was detectable for 6 days. Although the work by Naruse et al. was performed in rats, the findings of this study are similar: an increase in grey matter qT1 within the acute and subacute phase of TBI. Therefore, the observed difference in this work may have been due to the presence of vasogenic oedema, although if such a consistent qT1 increase in both grey and white matter frontal regions *were* due to oedema, one might expect to see visible evidence of this on the T1 weighted anatomical images, and this was not the case; any areas with visible oedema had deliberately been masked off and extracted from the data. It follows that if vasogenic oedema was the cause of the rise in tissue qT1 time in this work, it would have had to have been at a level undetectable by visual T1 image evaluation.

Sibson et al. (2008) put forward the idea that qT1 increases they observed both in low-flow ischaemia and separately in induced excitotoxicity in an experimental animal model, were due to the acute activation of astrocytes, as this process had been found to be common to both pathological processes. Astrocyte activation is known to occur in brain injury within the acute phase, and is therefore likely to be a contributory factor to the observed qT1 increase in this work (Morganti-Kossmann et al., 2007). This finding would support the conclusions in a paper by Garnett et al. (2001b) which examined seven patients in the subacute stage after moderate and severe TBI using MR spectroscopy. The authors found that there was a decrease in *N*-acetylaspartate and an increase in choline compounds in the brain injured patients, and attributed these changes to a reduction in neuronal population and an increase in the astrocyte population respectively, as choline compounds had

previously been shown to be increased in regions with greater numbers of astrocytes (Garnett et al., 2001b, cited McBride et al., 1995).

There were no significant differences in the quantitative T2 time in any of the ROIs evaluated in this work, whether in grey or white matter. This finding was in contrast to the experimental work by Naruse et al. who found an increase in white matter qT2 with induced TBI causing vasogenic and cytotoxic oedema, and also in contrast to work by Mamere et al. (2009) who found an increase in T2 relaxation time in normal appearing brain white matter and in the corpus callosum in patients with moderate and severe TBI. They hypothesised that the observed qT2 changes reflected an increase in water concentration secondary to axonal loss, and claimed that this theory was supported by their associated finding of an increased apparent diffusion coefficient in the same regions. However, it must be noted that they studied moderate and severe TBI patients a mean of 3 years after injury, and it is therefore possible that their observed qT2 values are a feature only of more severe TBI in the chronic phase, and do not occur in mild TBI acutely.

The absence of T2 findings in this work is supported by the study by Goetz et al (2004). They studied 23 patients of mixed TBI severity within an injury to scan time comparable to that in this study (mean 7.6 days), and found no significant increase in qT2 relaxation time in the patient group when compared to 13 controls, and no correlation between qT2 time and injury severity. They explained the lack of qT2 findings on the fact that T2 relaxation changes after diffuse axonal injury were known to be small and inconsistent (Goetz et al., 2004, cited Pierpaoli et al., 2001). If this were the case, positive findings might have been expected in the 14 automated ROI analysis, as the ROIs used in that method were smaller. Given that there were no significant findings, even the ROIs in this method may have been too large to detect qT2 changes.

5.6.7 *Findings in DTI data*

The majority of studies using DTI in TBI have shown an increase (although some observed an initial reduction) in mean diffusivity or apparent diffusion coefficient in white matter post TBI. However, in keeping with the results from this work, two studies extending their analyses to include cortical grey matter have observed a reduction in localised diffusion, in TBI patients with a similar injury to scan time as in this study (Hou et al., 2007, Newcombe et al., 2008). Hou et al. observed a significant decrease in peripheral grey matter apparent

diffusion coefficient (a similar value to mean diffusivity, see Chapter 2, section 2.5) when they compared 35 mild, moderate and severe TBI patients with 35 control subjects, although they showed an increase in all other regions examined. They were uncertain as to the cause of this reduction, but suggested that it may be a result of contusional rather than axonal injury, perhaps implying that localised peri-contusional oedema was responsible for their results. Newcombe et al. also observed a significant decrease in ADC in the cortical grey matter of 22 moderate and severe TBI patients scanned within a mean of 28 hours of injury, when compared to 25 matched controls. They attribute this reduction in diffusion to the presence of cytotoxic oedema which may have increased the volume of intracellular fluid, thereby restricting the diffusion of the extracellular fluid. As extracellular fluid is more easily diffusible than intracellular fluid, this would have resulted in a net reduction in diffusion, and therefore account for the decrease in ADC. It should be noted that the findings of both these studies were in TBI patients with moderate and severe injuries, and despite the fact that the significant reduction in grey matter MD was only present in the moderate TBI patients in this study, the box plots in figure 5.12 show that the mean and inter-quartile range of the *mild* TBI group was also below those of the control group. This may indicate that there are similar, but less severe changes occurring in mild TBI patients.

In the 5 ROI analysis this decrease in grey matter mean diffusivity was only observed in the analysis of ROIs adjacent to any visible lesions, and therefore it may indicate that existing TBI damage extends at a microstructural level beyond the visible boundaries of contusions or oedema seen on conventional imaging, into the surrounding normal appearing tissue. This may signify more extensive tissue disruption than had previously been appreciated in such TBI cases; possibly the debated ‘penumbra’ around an intracerebral haematoma which describes a circumference of disrupted but viable tissue around the visible central area of irrecoverable cell damage (Kirkman, 2011). It has been shown previously in an MRI study of *spontaneous* intracerebral haematoma (ICH), that the apparent diffusion coefficient (ADC) was *not* altered around the ICH compared to normal tissue in the contralateral hemisphere (Schellinger et al., 2003), and although the cause of the ICH in their study group was spontaneous and not traumatic, it is unlikely that this difference in aetiology alone would account for a measurable difference in mean diffusivity in our study group, where none had been shown in their patients.

The 5 ROI analysis method produced no white matter changes of significant magnitude that they were detectable using these large ROIs, and this is in contrast to the expected results, and those published by other authors using similar analysis techniques. Newcombe et al. (2007) studied 33 moderate and severe TBI patients within 1 week of their injury, and compared the scan data to that of 28 matched controls. They used a whole brain white matter ROI and found a significant increase in ADC (the measurement comparable to mean diffusivity) and a reduction in fractional anisotropy (FA). There were no such significant whole brain white matter findings in this work, despite having similar study numbers and scanning within a similar time-frame. This may be due to the less severe injuries sustained by the predominantly mild TBI patient group. However, analysis of the 14 automated ROI data showed that there were significant white matter differences in the patient groups when compared to the control subjects, both in the quantitative T1 values and in the mean diffusivity. As mentioned above, this pointed to the lack of results in the 5ROI data being due simply to the fact that those ROIs had been too large to detect the changes.

In the data analysed using the automated 14 ROI technique, the increase in white matter MD in both superior frontal regions is supported by previously published work in the field, although the lack of any observed change in FA in similar regions, specifically no *decrease* in FA, was unusual. A number of papers have analysed TBI using diffusion tensor imaging, and irrespective of the type of analysis technique employed involved, whether user defined ROIs, whole brain ROIs, or voxel-based morphometry, have found an increase in MD and an associated decrease in FA. Lipton et al. (2009) performed a voxel-wise analysis on 20 mild TBI patients within two weeks of injury, and observed an increased MD and decreased FA in a number of white matter regions, five of which were in the frontal lobes, compared to 20 matched control subjects. Inglese et al. (2005) performed DTI in 20 mild TBI patients a mean of 4 days after injury, and 26 mild TBI patients a mean of 5.7 years after injury, using whole brain histogram analysis and manual ROI placement. While they did not report any significant findings in their whole brain data, they found an increased MD and reduced FA in the corpus callosum and internal capsule in both the acute and chronic TBI patients, and postulated that the findings were due to a loss of axonal structural integrity caused by diffuse axonal injury. Similar findings were published in 2009, where Kumar et al. (2009) reported increased MD and decreased FA in the genu of the corpus callosum in moderate TBI patients analysed a mean of 8.9 days after injury using a semi-

automated analysis technique. The authors also found a positive correlation between genu MD and cognitive performance in both mild TBI and in moderate TBI patients; although they note that their findings were less widespread in the mild TBI patients.

Although some of these papers have included frontal white matter ROIs and whole brain analysis, as can be seen from the descriptions of the findings above, the majority of previous analysis has been targeted on the white mater tracts known to be affected by the shearing forces involved in TBI: the corpus callosum, internal and external capsule. For this reason, the tractography designed ROIs of the genu, splenium and body of the corpus callosum were included in the data analysis in this work. Based on the hypothesis that these areas are particularly susceptible to damage, examination of the ROIs ought to have revealed positive findings, but they did not. MD and FA values in the genu, splenium and body of the corpus callosum showed no difference between the mild and moderate TBI groups and the control subjects. This finding was not altogether unexpected, as diffusion studies in these regions have shown conflicting results in similar patient groups to those in this work. A number of papers have demonstrated a decrease in FA in the genu, body and splenium of the corpus callosum (Huisman et al., 2004, Inglese et al., 2005, Kumar et al., 2009, Lipton et al., 2009, Ljungqvist et al., 2011, Matsushita et al., 2011, Perlbarg et al., 2009) attributed to shearing injuries to the axons causing tract disruption. This FA reduction has been shown to correlate with outcome at discharge (Huisman et al., 2004), outcome at 1 year (Perlbarg et al., 2009) and with executive function (Lipton et al., 2009). Other papers have shown the opposite: an increase in FA in the corpus callosum, attributed to the presence of axonal cytotoxic oedema as a result of TBI (Bazarian et al., 2007, Chu et al., 2009, Mayer et al., 2010, Wilde et al., 2008). One recent paper e-published ahead of print (Lange et al., 2011) reports no significant MD or FA differences in the corpus callosum in 60 mild TBI patients compared with 34 control subjects, and no correlation between corpus callosum diffusion measures and post-concussive symptoms. The work by Lange et al. is perhaps most similar to this work in terms of patient population studied, and supports the findings in this study in the corpus callosum ROIs. It must be noted that there were methodological differences, both regarding the type of data analysis performed and in the time from injury to scan, which was subacute (6 to 8 weeks post-injury).

It is difficult to explain why there was an observed increase in frontal white matter MD, but no associated observed decrease in the FA. It is possible that the MD increase was due to

axonal disruption, but that the disruption occurred in areas where fibres were not rigidly arranged in straight tracts, perhaps in regions where fibre bundles were crossing one another, or turning through larger angles. Shearing of axons in these areas would still give an increase in MD, as water molecules would be free to traverse regions where there had previously been intact myelin sheaths, and MD is a measure of an average change in diffusion, not a directional measure. However, as FA is calculated from eigenvectors which are direction dependant, if there were sufficient crossing fibres then any change in FA in one direction as a result of axonal shearing would be cancelled out by a similar FA change in another direction. The ROIs in which the white matter MD increases were detected included both superior frontal regions, which include not only projection fibres (between the cortex and sub-cortical structures) such as the corticopontine and corticobulbar tracts, the anterior region of the corona radiata, the superior thalamic radiation and the cingulum, but also the commissural fibres (between one hemisphere and another) of the genu of the corpus callosum and short association fibres between adjacent areas of cortex . It is entirely feasible that even if a number of fibres within those tracts were disrupted, causing a small change in FA values, crossing fibres from other tracts may have cancelled out these differences. The findings in the corpus callosum ROIs would support this theory as they showed neither significant MD nor FA findings. As the tracts in those areas are not crossed by others and run a relatively straight course, if there had been an increase in MD observed in these regions without an observed decrease in FA, it would have implied that the lack of FA findings was due to some other reason. The fact that there were no corpus callosum DTI findings in this work indicated that in this predominantly mild TBI patient group, there was not a sufficient degree of damage in these regions to allow detection.

The last section of this discussion concerns the results from the TBSS voxel-wise analysis, which was the most targeted of all the analysis techniques employed. By restricting the regions under observation to fibre skeletons in the centre of the white matter tracts common to all subjects, this method was most likely to identify significant findings if they existed. Inter-group comparison between the entire TBI patient group and the control subjects revealed only a very small significant area of increased FA in the left superior region of the corona radiata. This finding was also observed when only the mild TBI patients were compared to the control group. These observations underline the results from the other analysis techniques employed, and explain why there were no changes observed in FA in

the other ROIs. A significant FA increase in such a small region would have been rendered undetectable by the averaging of the surrounding FA values in all but the smallest and most precisely placed of ROIs. The fact that there *was* a detectable FA increase using this method, implies that in the mild TBI group, even though the injuries sustained were insufficient to severely affect the conscious level of the patients, they still involved sufficient force to cause microstructural change in the corpus callosum. Indeed, it is likely that there were other white matter tract regions affected in individual patients within the group, but that any change in quantitative values was cancelled out by the lack of such detectable change in others.

5.7 Limitations

The main limitations in the scan data analysis section of the study were due to the size of the ROIs, both in the 5 ROI and 14 automated ROI methods. They were large and relatively crude, able only to detect difference in quantitative values sufficient to alter the means in those ROIs by a significant degree. As a result, it may be that more subtle or localised quantitative findings have been missed. This failing would hold true of the qT1 and qT2 data, as these were not obtained in the tract-based ROIs and nor were they available from the voxel-wise analysis. This problem was addressed in part for the MD and FA data, which were analysed using the tract-based ROIs, although again, the MD data was not available from the voxel-wise analysis. The tract-based ROIs, while more targeted, still required user-input and therefore were susceptible to bias during their design.

It must be noted that a greater number of the visible lesions identified either on CT or T1 weighted anatomical MR were left sided, and although all visible lesions were masked out and extracted from the data, this discrepancy may have introduced bias into the results in the 14 automated ROI analysis, as a greater number of significant inter-group differences in the qT1 data were observed in the regions on the left side, although the opposite was in fact true of the FA data, which only showed significant differences in right sided regions, and significant findings in the MD data were divided equally between the left and right hemispheres. It is even less likely to have affected the other analyses, as the 5 ROI data was analysed according to whether it was ipsilateral or contralateral to any extracted visible

lesion, and the tractography designed ROI analysis only involved regions in the centre of the brain.

It is also possible that the data was affected by the difference in injury to scan time in the patient group. As discussed in section 4.7.3, the window from injury to scan was initially set at 3-7 days, but as recruitment started it became apparent that this was overly ambitious, predominantly as a result of the scanner location being away from the immediate clinical area, which meant that patients were often still too unwell to be scanned. In order to allow for these moderately injured patients to become more stable, and therefore to be fit enough to be transferred to the scanner and back to the ward, the maximum time allowed from injury to scan was increased to 14 days. However, all of the patients were scanned within the allowed 14 days, the majority much sooner in fact, and the result was still a patient group scanned within a relevant acute time period where changes ought to have been detectable.

5.8 Conclusions

5.8.1 *Ability of quantitative MR scanning to detect microstructural changes*

This study has revealed an increase in quantitative T1 relaxation time in whole brain normal appearing grey matter in mild and moderate TBI patients within two weeks of injury, when compared to matched control subjects. The ROI analysis findings have also demonstrated frontal lobe changes in normal appearing tissue in the patient groups, specifically an increase in both grey and white matter qT1 time, an increase in white matter mean diffusivity, a decrease in grey matter mean diffusivity, and a small area in the left superior region of the corona radiata that was shown to have an increased FA. There were no observed differences in quantitative T2 relaxation time. These findings support the first of the study hypotheses:

> **"Acute differences exist at a microstructural level between patients with traumatic brain injury and matched control subjects that are detectable using quantitative MR scanning, in tissue appearing normal on conventional anatomical MR imaging."**

Although it cannot be said for definite what these observed changes in the quantitative values represent, based on the discussion of previous work in the field, it is possible that the increase in grey and white matter qT1 time is indicative of ongoing diffuse vasogenic oedema and astrocyte activation as part of the autoimmune response following traumatic brain injury. The reduction in grey matter MD may represent peri-contusional damage, as the majority of visible damage seen on the T1 weighted scans (and then extracted) was cortical, whether as a result of an extra-axial haematoma pressing on the brain surface, or due to contusions in the cortex itself. The reduction in grey matter MD may therefore be due to areas of ongoing ischaemia and cell necrosis adjacent to these cortical lesions, or possibly cytotoxic oedema, where the relative increase in intracellular fluid and reduction in extracellular fluid would cause reduced free diffusion. The increase in *white* matter MD is most likely to be due to axonal disruption causing a reduction in the restriction of micro-

movement of water molecules, although the lack of associated FA findings weakens this theory.

It is likely that if data from each *individual* patient had been analysed with respect to the mean values from the control group, that more significant differences would have been found, particularly with respect to those with mild TBI. By grouping all of the mild TBI patients together, the data from those with less severe injuries may have cancelled out the findings in those with more severe injuries. By performing further analysis, assessing correlations between the scan data and the neuropsychology test results, it was hoped that the data would not be affected by those mild TBI patients whose injury was so mild as to render them asymptomatic.

5.8.2 *Relationship between detectable microstructural changes and brain injury severity*

Post-hoc inter-group analysis using a t test on the data obtained using the 5 ROI analysis method, revealed that different quantitative datasets were able to differentiate between different study groups. For instance, the whole brain grey matter qT1 data showed significant differences between the control group and each of the TBI groups (mild and moderate), but was not able to differentiate between the patient groups themselves. The grey matter qT1 time in the hemisphere ipsilateral to any extracted visible injury showed a significant difference between the mild TBI group and control subjects, but not between the moderate TBI group and controls.

In contrast, the grey matter mean diffusivity in the hemisphere ipsilateral to any extracted visible injury showed no significant difference between the mild TBI group and control subjects, but did show a difference between the moderate TBI group and controls. No data from any of the regions in the 5 ROI analysis was able to differentiate between the mild TBI and moderate TBI groups, presumably due to the small number of moderate TBI patients, and due to the relatively broad range of injury severity seen in the mild TBI group: from those patients who were almost asymptomatic to a number who displayed a large number of post-concussive symptoms.

Therefore, although the trends shown in the data suggest that with a greater number of moderate TBI patients a significant difference between patient groups would be apparent, as a result of the lack of significant differences shown between the quantitative scan data

values in the mild and moderate TBI patient groups, the findings do not support the second of the study hypotheses:

> **"Detectable microstructural changes in the TBI population are proportional to the severity of the brain injury sustained, as defined by GCS."**

It must be noted that this hypothesis is based upon the severity of brain injury according to the GCS score of the patients upon admission, which although easily reproducible and known to correlate with TBI outcome, does not necessarily reflect the degree of post-concussive symptoms suffered by those with mild TBI. By examining the scan data for correlations with scores from the neuropsychology tests, it was anticipated that detected microstructural changes would, in fact, be related to the severity of cognitive function impairment, and this is the topic of the next chapter.

Chapter 6. Results: Neuropsychology Findings and the Relationship Between Impaired Cognitive Function and Imaging Abnormalities

6.1 Neuropsychology Test Result Group Comparisons

Details of the neuropsychological tests, their previous use in TBI and how they are administered are covered in Chapter 3, section 3.3. Neuropsychology data was available in all but one of the patient group (one moderate TBI patient did not tolerate the testing), and in all of the control subjects. A number of both the mild TBI and moderate TBI patients were unable to complete all of the neuropsychological test battery, and as a result the numbers of participants completing each test varied (Table 6.1).

Neuropsychology test	Mild TBI patients	Moderate TBI patients	Control subjects
NART	41	7	30
SoIP	43	8	30
Design learning	42	8	30
List learning	44	8	30
PASAT	40	6	28
DSPAN back	43	8	30
Spatial span back	43	8	30
Verbal Fluency	41	7	30
Colour-word interference	43	8	30

Table 6.1: Numbers of study participants for whom data was available in each neuropsychology test, displayed by group.

6.1.1 *Test for normality*

Prior to statistical analysis a test for normality was performed on the neuropsychology data from the control subjects only using the Kolmogorov-Smirnov test (Table 6.2). The data from all of the neuropsychology test scores were found to be normally distributed, except for the score for the post-interference recall of design 'A', in the design learning test.

Therefore the appropriate parametric tests were used throughout the neuropsychology test score analysis, except for in the analysis of that one design learning test score.

Neuropsychological Test	P-value	Neuropsychological Test	P-value
NART	0.466	DSPAN back	0.253
SoIP adjusted total	0.916	Spatial span backwards	0.318
Design learning A1-5	0.895	Verbal fluency (letter)	0.926
Design learning B	0.278	Verbal fluency (category)	0.563
Design learning A6	<0.001	Category switching total	0.798
List learning A1-5	0.998	Switching accuracy	0.460
List learning B	0.301	Colour-word interference 1	0.174
List learning A6	0.207	Colour-word interference 2	0.532
PASAT 3 second	0.562	Colour-word interference 3	0.873
PASAT 2 second	0.543	Colour-word interference 4	0.782

Table 6.2: P-values obtained by performing a Kolmogorov-Smirnov test on the neuropsychology test scores data. A non-significant p-value ($p > 0.05$) indicates normally distributed data.

6.1.2 *National adult reading test (NART)*

Test results in the NART arranged by group were reported in Table 5.4 above, along with the p-value ($p<0.001$) obtained from group comparison using the one-way ANOVA test. These were discussed in section 5.1.4.

6.1.3 *Speed of information processing*

The speed of information processing test assessed executive function, and resulted in a total score out of 105. From this score, a value was subtracted for motor speed, obtained from the second part of the test, giving an adjusted total for each test candidate taking into account any difficulties associated with the motor performance of the task. Group comparison of the SoIP scores was made using the one-way ANOVA test (Figure 6.1). The result showed that performance in the mild and moderate TBI groups was significantly worse than in the control subjects ($p=0.001$).

In order to determine whether the mild TBI patients performed better than the moderate TBI patients, a t test was used to compare the two groups. The result showed that there was no significant difference in their performance (p=0.147).

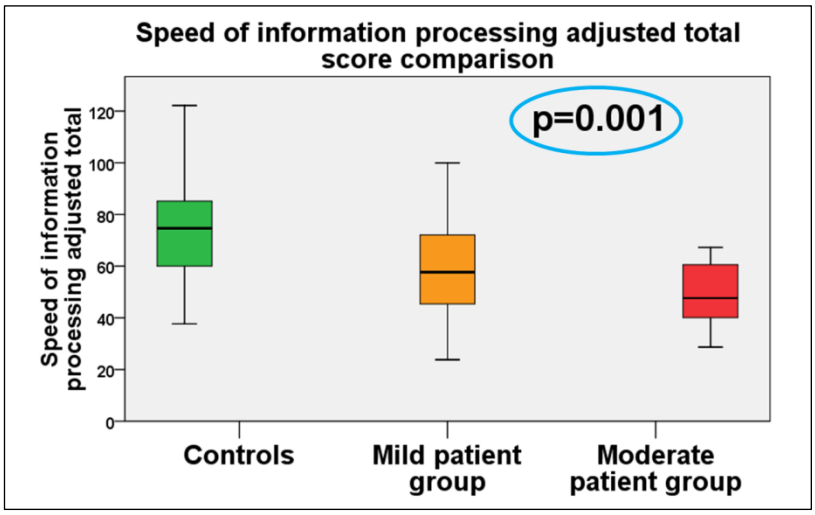

Figure 6.1: Box plot showing the distribution of speed of information processing test scores between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-value indicates a significant reduction in test performance in the patient groups when compared to the control subjects.

6.1.4 *Design learning*

Three scores were recorded for the design learning test which assessed visuo-spatial learning, attention, concentration and short-term and working memory. The first score was comprised of the number of correct reproductions of the lines on the original design in the first 5 attempts. The second score assessed the subject's performance on the interference design, and the third on the recall of the original design. They were scored out of 45, 9 and 9 respectively. Group comparison of the first and second design learning scores recorded for each subject was made using the one-way ANOVA test (Figure 6.2) and group comparisons for the third score was made using the Kruskal-Wallis test. The results showed that there was a significant difference between groups in the first score (the first five attempts at remembering, p=0.025), but there were no significant differences in performance between the patient groups and the control subjects in the second score (interference test, p=0.501) or in the third score (recall after interference, p=0.782). The relationship between each individual pair of groups was assessed using a t test, and found

that the only significant difference lay between the mild TBI patient group and the moderate TBI patient group (p=0.025), indicating a reduction in short-term memory function between the two groups.

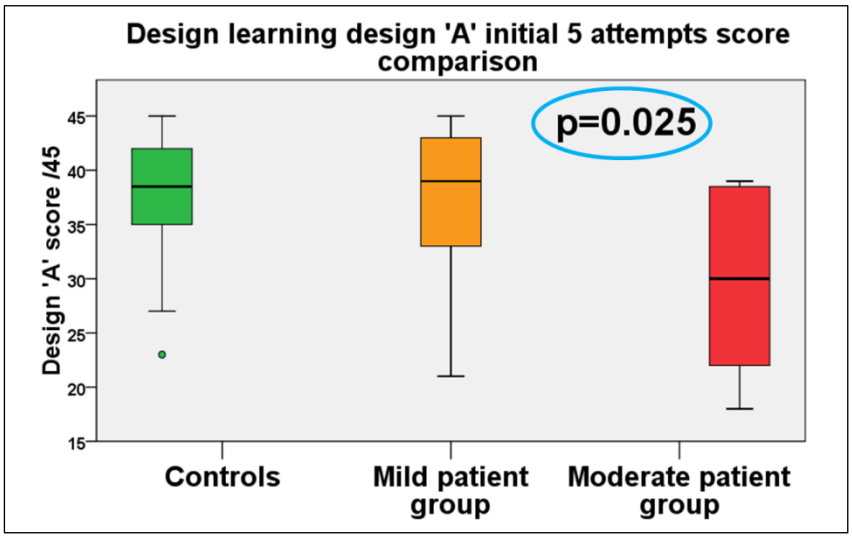

Figure 6.2: Box plot showing the distribution of design learning initial 5 attempts test scores between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-value indicates a significant reduction in test performance in the patient groups when compared to the control subjects. Scrutiny of the box means shows that this significant difference does not lie between the mild TBI patient group and the control group, confirmed by performing a t test between the two groups (p=0.661).

6.1.5 *List learning*

For the list learning test (which assessed verbal learning, attention, concentration and short-term and working memory) three scores were recorded, the first for the number of correct words remembered from list 'A' in attempts 1-5, the second totalled the correct number of words remembered from the interference list 'B', and the third totalled the number of words correctly remembered from list 'A' after the interference. The three were scored out of 75, 15 and 15 respectively. Group comparison of the three list learning scores recorded for each subject was made using the one-way ANOVA test (Figure 6.3). The results showed that performance in the control subjects was significantly better than in the mild and moderate TBI groups in the first score which measured the total number of words recalled from list 'A' in five attempts ($p<0.001$) and in the third score which assessed the subject's recall of

words from list ‘A’ after interference (p<0.001). There was no difference in performance between groups on recall of words in the interference list ‘B’ (p=0.072). However, when the scores shown to be significantly different by ANOVA test were analysed further using a t test, no significant difference between mild TBI and moderate TBI patients was found (p=0.063, p=0.054). This may be due to the small number of moderate TBI patients, as examination of the box plots shows that the mean of the moderate TBI groups is lower still than that of both the control and mild TBI groups, and the p-values are approaching significance.

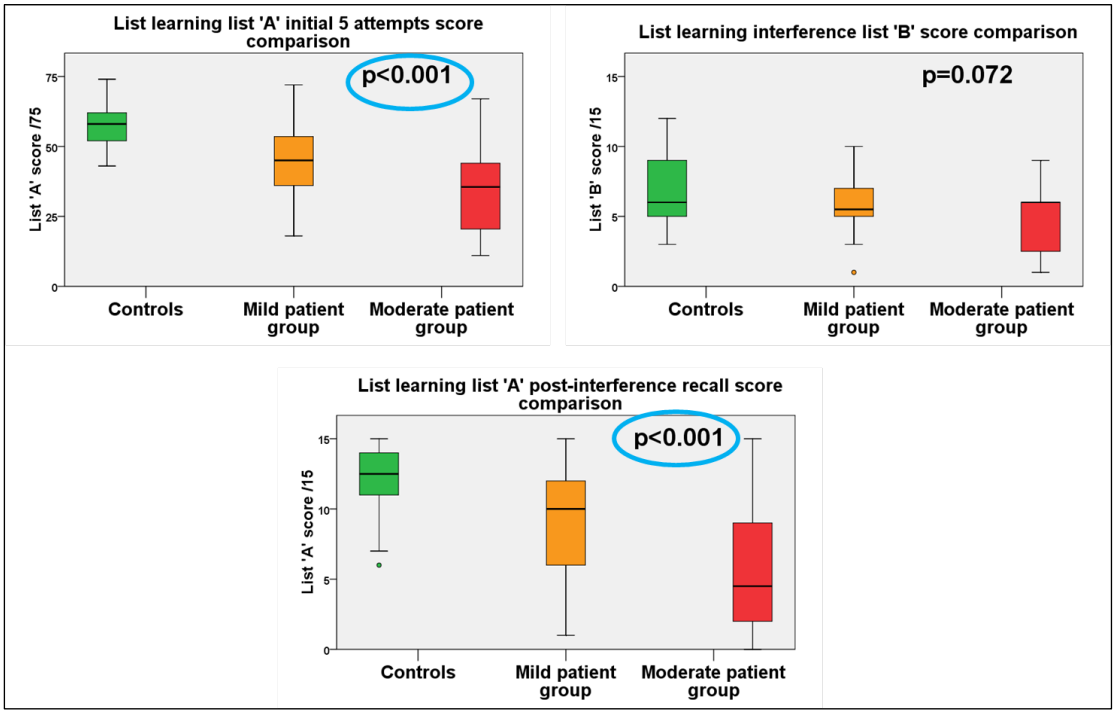

Figure 6.3: Box plots showing the distribution of list learning test scores between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-values indicates a significant difference in the scores between the patient groups and the control subjects in initial recall of list’ A’, and delayed recall of list ‘A’ after interference, but not in the recall of the interference list ‘B’.

6.1.6 *Paced auditory serial addition test (PASAT)*

The PASAT was administered both with 3 second and 2 second intervals between presented numbers and therefore 2 scores were recorded, each out of 60. The PASAT assessed working memory and executive function. Both scores were converted into a percentage before analysis, in compliance with the test instructions (see section 3.3.6).

Group comparison of the two PASAT scores recorded for each subject was made using the ANOVA test (Figure 6.4). The result showed that performance in the control subjects was significantly better than in the mild and moderate TBI groups in both the 3 second PASAT score ($p<0.001$) and the 2 second PASAT score ($p<0.001$). A t test analysis between the individual pairs of groups (control subjects and mild TBI, control subjects and moderate TBI, and mild TBI and moderate TBI) showed significant differences between the mean scores of the control subjects and the mild TBI patients (PASAT3 $p<0.001$, PASAT 2 $p=0.002$) and between the control subjects and the moderate TBI patients (PASAT 3 $p=0.014$, PASAT 2 $p<0.001$) but not between the mild TBI patients and moderate TBI patients (PASAT 3 $p=0.101$, PASAT 2 $p=0.082$).

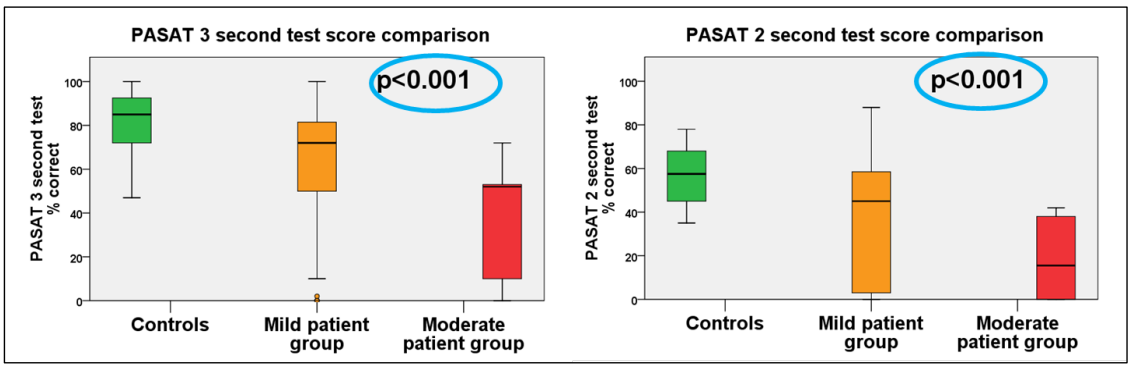

Figure 6.4: Box plots showing the distribution of PASAT scores between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-values indicate a significant difference in the scores between the patient groups and the control subjects in both the 3 second and the 2 second tests.

6.1.7 *Digit span backwards (DSPAN back)*

The DSPAN back test assessed short-term and working memory, and produced a single score (out of 14): a total of the number of correct sequences relayed by the participant. Group comparison of the DSPAN back score recorded for each subject was made using the ANOVA test (Figure 6.5). The result showed that performance in the control subjects was significantly better than in the mild and moderate TBI groups ($p=0.025$).

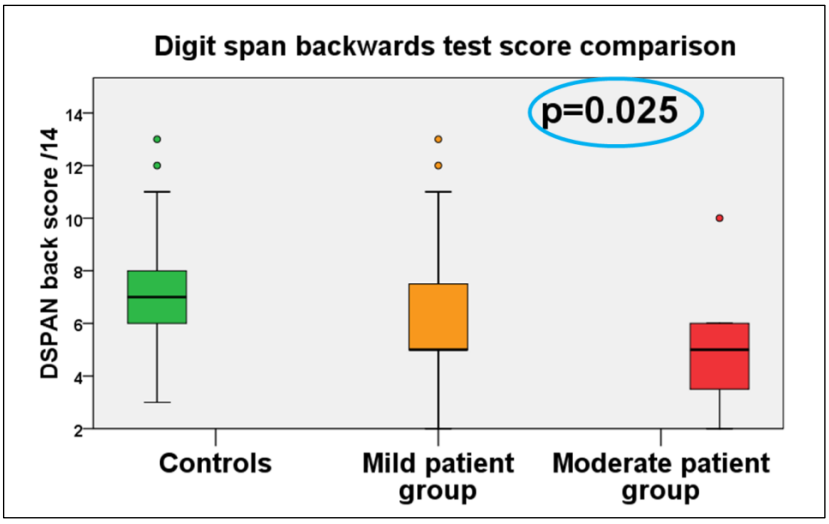

Figure 6.5: Box plot showing the distribution of DSPAN back test scores between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-value indicates a significant difference in the scores between the patient groups and the control subjects.

Individual analysis of the DSPAN back scores, by comparing the pairs of groups using the t test, again demonstrated significant differences between the test scores of the mild TBI patients and control subjects (p=0.035) and between the moderate TBI patients and the control subjects (p=0.016), but not between the mild TBI patients and the moderate TBI patients (p=0.269).

6.1.8 *Spatial span backwards*

The spatial span backwards test was also scored out of 14: a total of the number of correct sequences completed by the participant. It also assessed short-term and working memory. Group comparison of the spatial span backwards test score recorded for each subject was made using the ANOVA test (Figure 6.6). The result showed that there was a significant difference in performance between the patient groups and the control subjects (p=0.022). Examination of the box plot shows that although the mean value of the mild TBI patient group is below that of the control group, the moderate TBI group mean is in fact greater than the control group mean. Further analysis of this relationship by comparing each individual pair of groups using the t test revealed that the only significant difference existed between the mild TBI patients and control subjects (p=0.006), and not between the moderate TBI group and the control subjects (p=0.325), or between the mild TBI group and the moderate TBI group (p=0.427) in contrast with the previous tests. This suggested that

there was a large spread of performance in this particular test, which did not seem to be directly related to TBI severity.

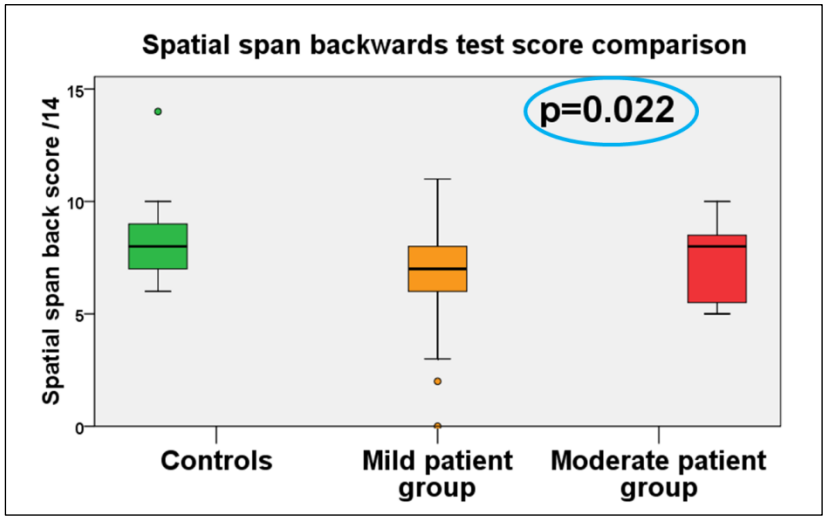

Figure 6.6: Box plot showing the distribution of spatial span backwards test scores between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-value indicates a significant difference in the scores between the patient groups and the control subjects.

6.1.9 *Verbal fluency*

The verbal fluency test assessed executive function: specifically clustering (an automatic process) and switching (a process requiring effort). Four scores were recorded for each subject in the verbal fluency test: letter fluency, category fluency, category switching and total switching accuracy. These were the totals of the correct words the subject was able to produce either starting with the letters given, or from the category provided and scores for the total number of category switches made and their accuracy. All four scores had been shown to be normally distributed (section 6.1.1) and therefore group comparisons were made using the ANOVA test (Figure 6.7). The mean values of the four scores were found to be highly statistically significant between groups (letter fluency $p<0.001$, category fluency $p<0.001$, category switching $p<0.001$ and switching accuracy $p<0.001$).

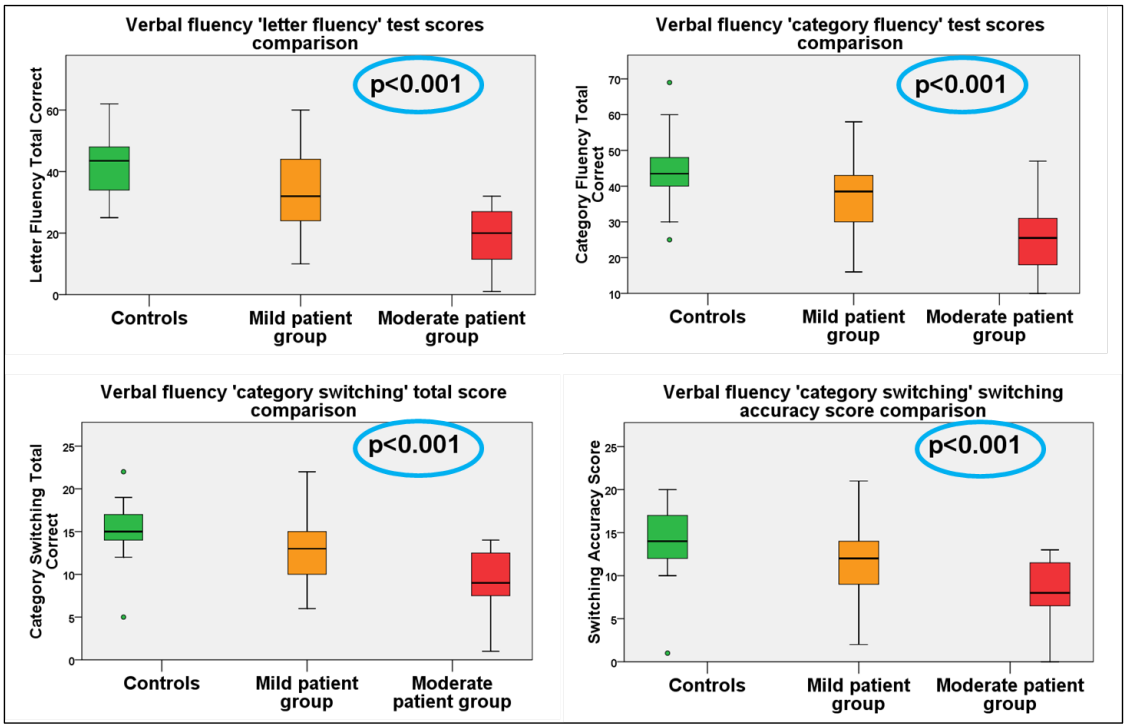

Figure 6.7: Box plots showing the distribution of verbal fluency test scores between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA test p-values indicate a significant difference in the scores between the patient groups and the control subjects in all four sections of the test.

T test comparison of the mean scores of individual pairs of groups showed that there was a significant difference in letter fluency between each pair: mild TBI patients and control subjects ($p=0.001$), moderate TBI patients and control subjects ($p<0.001$) *and* mild TBI and moderate TBI patients ($p=0.004$). The ability of the verbal fluency test to differentiate between each one of the study groups against the others also held true for the category fluency mean scores: a t test showed significant differences between each pair: mild TBI patients and control subjects ($p=0.004$), moderate TBI patients and control subjects ($p<0.001$) and mild TBI and moderate TBI patients ($p=0.005$). Both the category switching and switching accuracy scores also showed a significant difference when tested with a t test between each individual pair of groups: mild TBI patients and controls (category switching $p=0.002$, switching accuracy $p=0.012$), moderate TBI patients and controls (category switching $p<0.001$, switching accuracy $p<0.001$) and mild TBI patients and moderate TBI patients (category switching $p=0.007$, switching accuracy $p=0.016$). These results show that the verbal fluency tests give a clear performance measure which seems to be related to injury severity.

6.1.10 *D-KEFS colour-word interference test (Stroop)*

The colour-word interference test produced four scores for each candidate: the total times needed to complete each part of the test in seconds. Parts 1 and 2 assessed basic functional skills (naming and attention), and parts 3 and 4 assessed executive function (verbal inhibition and cognitive flexibility). Comparison of the four scores between mild TBI patients, moderate TBI patients and the control subjects was made using the ANOVA test (Figure 6.8). The results showed that the tests were completed significantly faster by the control subjects than by the mild and moderate TBI groups in the first part (p=0.010), second part (p=0.040) but not in the third part of the test (p=0.120). In the fourth part of the test, there was a significant difference between groups (p=0.024), but interestingly the mild TBI group mean time was greater than that in the moderate TBI group.

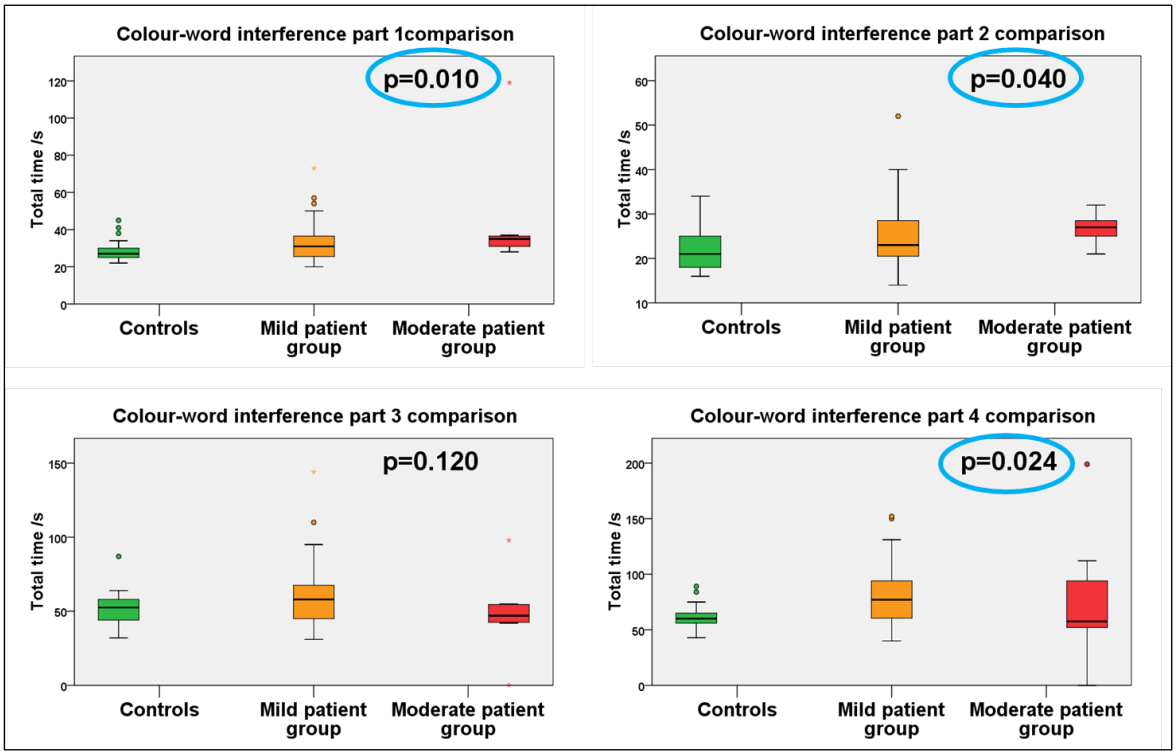

Figure 6.8: Box plots showing a comparison of colour – word interference test times between the mild TBI group (orange), moderate TBI group (red) and control subjects (green). The ANOVA p-values in parts 1 and 2 indicate a significant difference in the times taken to complete the tests between the patient groups and the control subjects. There was no significant difference in performance between groups in part 3 of the test, and in part 4, although the ANOVA p-value was significant, scrutiny of the plot shows that the moderate TBI group mean is below that of the mild TBI group in whom performance was poorest.

6.2 Analysis of Scan Data with Reference to the Neuropsychology Data

In the final set of statistical analysis performed the scan data was evaluated by assessing correlations between neuropsychological test performance and quantitative scan data values in brain regions known to be involved in the functions tested. This correlation analysis was only performed in the neuropsychological tests where a difference had been shown between groups. All of the neuropsychology tests used have been previously shown to be correlated to the premorbid IQ of test subjects to some degree (section 3.3). Therefore all correlations between scan data and neuropsychology test scores were assessed with the predicted IQ from the NART as a covariant of no interest.

Specific ROIs were selected for analysis with reference to each neuropsychology score, according to the anatomical brain regions known to be associated with each particular test (section 3.3). Quantitative data was analysed with the appropriate parametric (Pearson) or non-parametric (Spearman's rho) correlation coefficient according to whether it had been found to be normally or not normally distributed in section 5.2.1 (5 ROI method), section 5.3.1 (14 automated ROI method) or section 5.4.1 (tractography designed ROI method). Quantitative T2 data was not included in this analysis as no significant inter-group differences had been shown in the group comparison in either grey or white matter (section 5.3.3).

All correlations were performed separately in the control subjects and then again in the whole patient group, this was done to ensure that any significant correlations revealed were attributable to changes in the patient group, and not simply representations of a pre-existing relationship in the general population.

Given that in this section, a large number of correlations were to be assessed between each neuropsychology test score and each functionally related ROI from three different analysis techniques, each in 3 different quantitative datasets (qT1, MD and FA) and in both grey and white matter, the likelihood that some of the significant results would be due to chance was high. In order to correct for this, the p-value below which a correlation was deemed to be significant was determined using the Bonferroni correction to adjust for multiple comparisons. It was therefore necessary in each analysis to correct for the total number of ROIs being examined, in both grey and white matter. However, as the qT1, MD and FA values each reported on different aspects of tissue microstructure, they were considered

individually, and accepting this premise, it was not necessary to correct for performing these three measurements.

For example, in the analysis of the speed of information processing (SoIP) adjusted total score, the ROIs examined from the 14 automated ROI method were the left and right superior and inferior frontal regions. Each of these 4 ROIs was examined with respect to grey and white matter, making 8 assessments in total. The Bonferroni correction was made by dividing the existing significant p-value ($p<0.05$) by 8, giving 0.006. Therefore, a significant p-value from this analysis (relating specifically to the SoIP adjusted total score analysed using the data from the 14 automated ROI method) was taken to be $p\leq0.006$.

6.2.1 *Correlation between speed of information processing score and scan data*

The speed of information processing (SoIP) test predominantly assesses executive function. As discussed in section 3.2.3, executive function is associated almost exclusively with the frontal lobes. A correlation analysis was therefore performed between the SoIP adjusted total scores and frontal lobe data from all 4 of the analysis techniques: 5 ROI, 14 automated ROI, tractography based ROI and TBSS.

Executive function is not known to be related to anatomical laterality, and therefore combined frontal lobe data from the 5 ROI technique was assessed, to ascertain whether diffuse frontal lobe changes could be detected and were important in performance of the SoIP task; the four frontal regions from the 14 automated ROI method serving to assess the same area broken down into smaller regions to allow for detection of more focal damage. This combined frontal lobe ROI was created by taking the mean value of the left and right frontal ROIs in each dataset. Correlation between these 'bilateral frontal lobe' ROI datasets and the SoIP adjusted total was then assessed using a significant p-value of $p<0.025$ after performing a Bonferroni correction, taking into account that the same ROI was being assessed in white and grey matter.

In the control group, no correlation was demonstrated between the SoIP adjusted total and qT1, MD or FA in any of the selected regions in either grey or white matter. The only significant correlation seen in the patient group was between frontal lobe grey matter FA and the adjusted SoIP total score ($p=0.004$) (Figure 6.9). No other correlations were demonstrated.

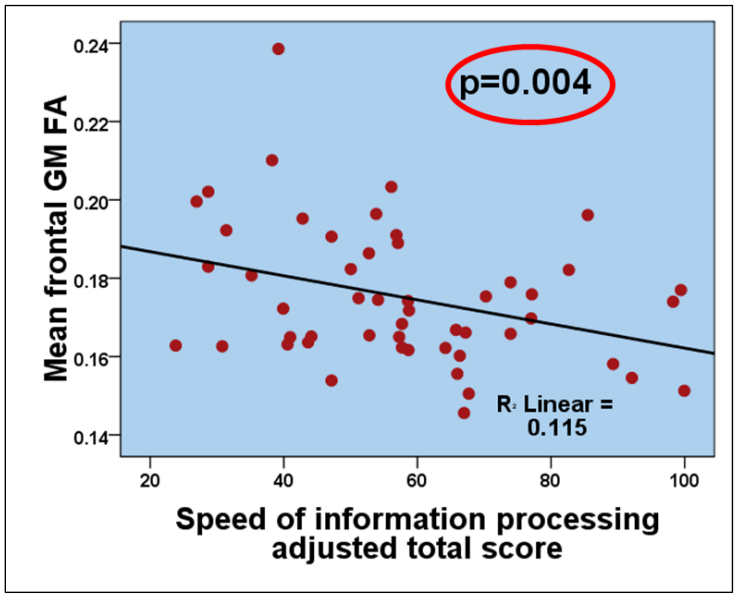

Figure 6.9: Scatter plot to show the significant relationship between frontal lobe grey matter FA and speed of information processing adjusted total score in the combined patient group (mild TBI and moderate TBI).

Frontal lobe data from the 14 automated ROI technique consisted of 4 of the 14 regions: left inferior frontal, left superior frontal, right inferior frontal and right superior frontal. Quantitative datasets from these four regions in both grey and white matter were analysed by assessing a correlation with SoIP adjusted total scores. After a Bonferroni correction, a p-value of $p<0.006$ was taken to be significant. No significant correlations between any of the quantitative datasets and the adjusted SoIP score were demonstrated in either the control group or the patient group, which may have been a result of the more severe Bonferroni correction.

Quantitative scan data obtained from the genu of the corpus callosum using the tractography designed ROI technique was analysed in relation to the SoIP adjusted total but also showed no significant correlation in MD, FA or any of the eigenvalues. A significant p-value was taken as $p<0.05$ in this analysis.

A voxel-wise regression analysis was performed on the TBSS data separately for the control subjects and for the whole patient group. There was no significant relationship between white matter tract FA in either the control group or the patient group and SoIP adjusted total score.

6.2.2 *Correlation between design learning scores and scan data*

The design learning test assesses short-term and working memory. As discussed in section 3.2.2, short-term memory is associated with the frontal lobes and in particular the dorsolateral prefrontal cortex, while the executive component of working memory is associated with frontal lobe function in general. Therefore the first correlation was assessed in the bilateral frontal lobe ROI from the 5 ROI method. As this was analysed in both grey and white matter, the p-value taken to be significant was $p<0.025$.

This analysis demonstrated significant correlations in the patient group between frontal lobe grey matter qT1 means and the interference design 'B' recall score ($p=0.013$) (Figure 6.10). A correlation was also shown between frontal lobe grey matter FA and both the score for the initial 5 attempts at recall of design 'A' ($p=0.022$) and the score for post-interference recall of design 'A' ($p=0.016$) (Figure 6.11). There were no significant correlations in the control group.

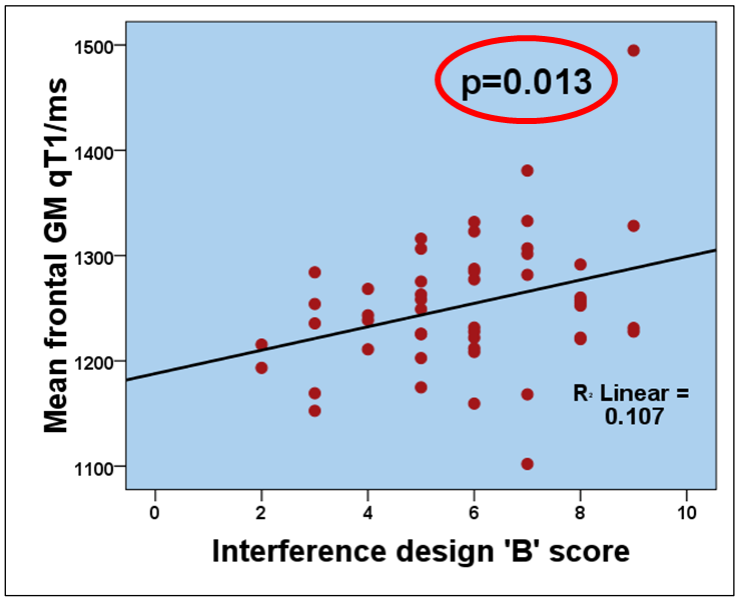

Figure 6.10: Scatter plot showing the significant relationship between the interference design 'B' recall score and frontal grey matter qT1.

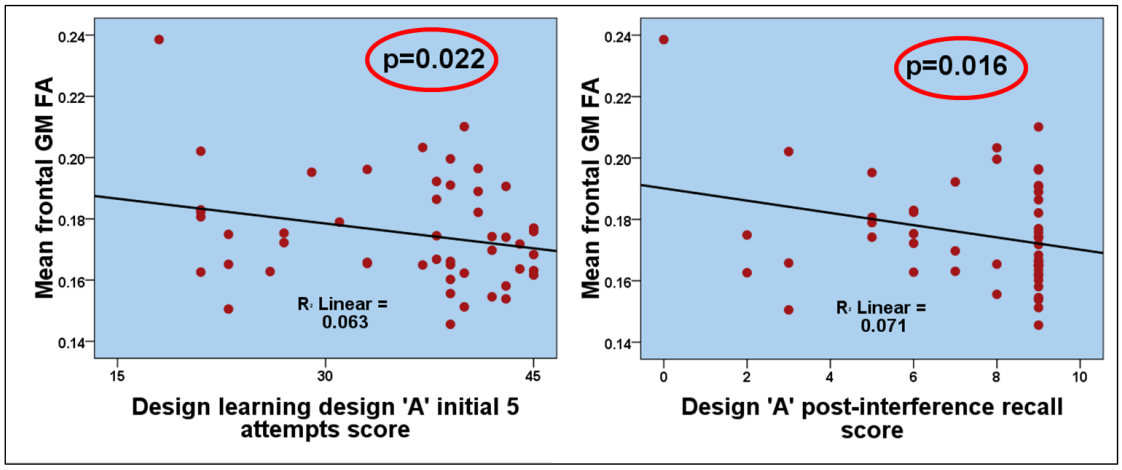

Figure 6.11: Scatter plots showing the significant relationships between frontal grey matter FA and the recall score for the initial 5 attempts at design 'A' (left) and the design 'A' post-interference recall score (right).

As with the speed of information processing test analysis in section 6.2.1, frontal lobe data from the 14 automated ROI technique in both grey and white matter was analysed by assessing a correlation with the design learning test scores. $P<0.006$ was taken as significant as a result of a Bonferroni correction. No significant correlations between any of the quantitative datasets and the design learning scores were demonstrated in either the control group or the patient group.

Quantitative scan data obtained from the genu of the corpus callosum using the tractography designed ROI technique was analysed in relation to the design learning test scores and showed no significant correlation in MD, FA or any of the eigen values using a significant p-value of $p<0.05$.

A voxel-wise regression analysis was performed on the TBSS data separately for the control subjects and for the whole patient group. There was no significant relationship between white matter tract FA in either the control group or the patient group and design learning test scores.

6.2.3 *Correlation between list learning scores and scan data*

The list learning test assesses short-term and working memory, but unlike the design learning test, also assesses the component of working memory responsible for repetition of words. This function is associated with Broca's area, located in the posterior inferior frontal gyrus in the dominant (left) hemisphere, and corresponds to the Brodman areas 44 and 45 in the left hemisphere. These regions were combined with other Brodman areas in the 14 automated ROI method to create the inferior frontal region (section 4.12.2), and this region was therefore of particular interest in the analysis of the scan data with reference to the list learning test scores. The relationship between memory functions and anatomical location in the brain are discussed above in section 6.2.2.

A correlation between the 'bilateral frontal lobe' ROI datasets and the list learning scores was assessed, as was any relationship between *left* frontal ROI datasets and the list learning scores, as this region contained Broca's area. The control group analysis showed no significant correlation, but there were significant findings in the patient group. A significant correlation existed between the left frontal grey matter FA and the list 'A' post-interference recall score (p=0.002). Unsurprisingly this relationship was also evident in the combined bilateral frontal lobe grey matter FA (p=0.003), as this ROI contained the left frontal grey matter ROI already shown to be significant (Figure 6.12).

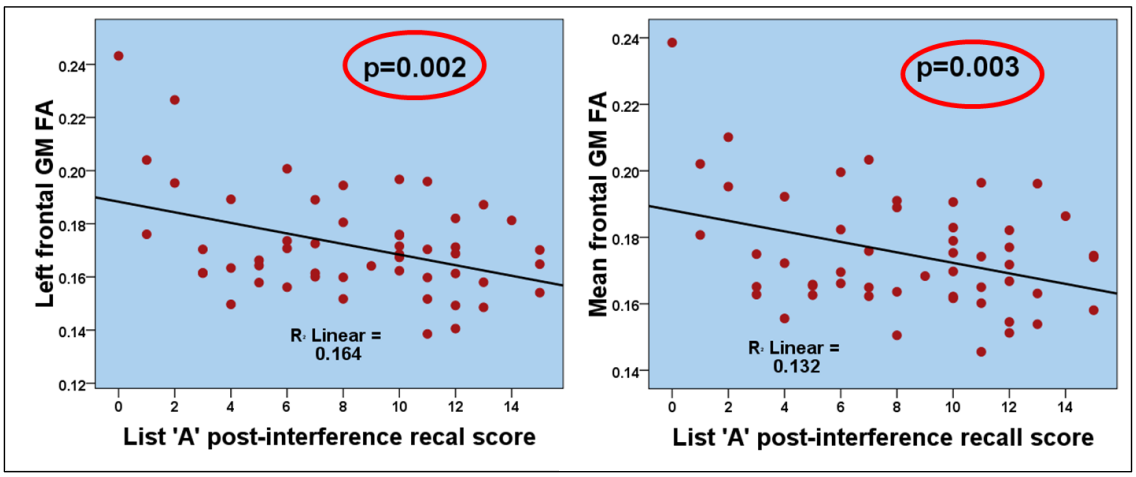

Figure 6.12: Scatter plots showing the significant relationships between left frontal grey matter FA and mean frontal grey matter FA and the design 'A' post-interference recall score.

Data from the 14 automated ROI method was analysed in the same manner. A p-value of $p<0.006$ was taken to be significant. In the patient group a significant correlation was shown to exist between the post-interference design 'A' recall score (testing working memory) and grey matter FA in the left superior frontal region ($p=0.005$) (Figure 6.13). There were no significant correlations in the control group.

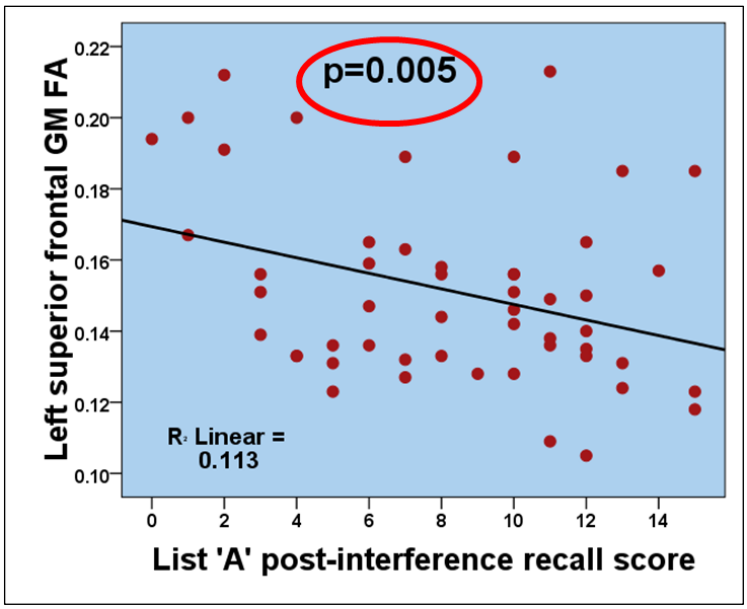

Figure 6.13: Scatter plot showing the significant relationship between left superior frontal lobe grey matter FA and list 'A' post-interference recall score in the patient group using the 14 ROI method.

Quantitative scan data obtained from the genu of the corpus callosum using the tractography designed ROI technique was analysed in relation to the list learning test scores and showed no significant correlation in MD, FA or any of the eigen values.

A voxel-wise regression analysis was performed on the TBSS data separately for the control subjects and for the whole patient group. There was no significant relationship between white matter tract FA in either the control group or the patient group and list learning test scores.

6.2.4 *Correlation between paced auditory serial addition test scores and scan data*

The paced auditory serial addition test (PASAT) evaluates working memory, attention, concentration and executive function (specifically information processing speed). Two PASAT scores were produced for each subject, the percentage correct for both the PASAT three second test and the PASAT two second test. The anatomical brain regions involved with working memory have been discussed already in sections 6.2.2 and 6.2.3 above. As outlined in section 3.2.1, the regions involved in attention and concentration are many: brainstem, thalamus, dorsolateral prefrontal cortex, posterior parietal cortex, ventral temporal cortex as well as the numerous white matter tracts connecting them. Collectively they are termed the reticular activating system. Executive function is associated with the frontal lobes. Due to the many functions tested by the PASAT and the numerous anatomical regions associated with them, evaluation of the scan data with reference to the scores was more complicated than with some of the other neuropsychological tests. A PASAT score correlation analysis was therefore performed not only in the frontal lobe data, but also in whole brain ROIs using the 5 ROI technique, all fourteen regions using the automated ROI technique and all three regions using the tractography designed ROI technique.

Significant correlations were demonstrated between the whole brain white matter qT1 in the patient group data obtained using the 5 ROI method and both the PASAT scores (PASAT 3 second p=0.009, PASAT 2 second (p=0.009) (Figure 6.14), and between mean frontal grey matter FA and PASAT 3 second score (p=0.011) (Figure 6.15).

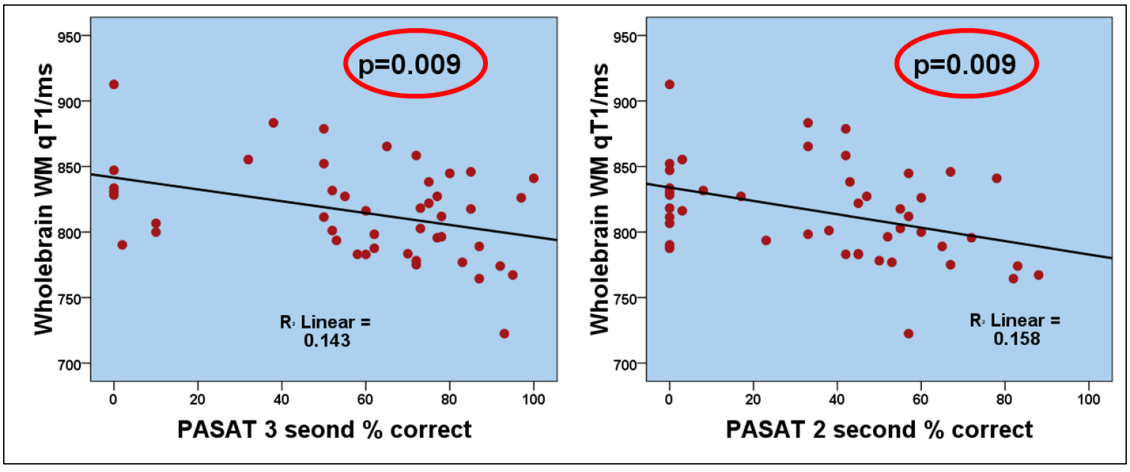

Figure 6.14: Scatter plots showing the significant relationship between whole brain white matter qT1 and PASAT 3 second and PASAT 2 second percentage correct scores.

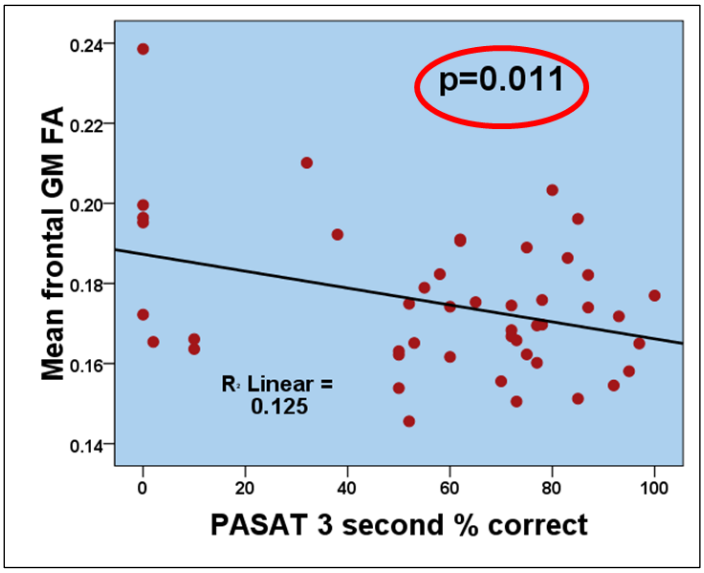

Figure 6.15: Scatter plot showing the significant relationship between frontal grey matter FA and PASAT 3 second percentage correct score.

Data from the 14 automated ROI method revealed no significant correlations in the control group or in the patient group. This may have been due to the large number of correlations assessed in this analysis; after a Bonferroni correction, the p-value taken to be significant was $p<0.0018$.

Analysis of correlations with the PASAT scores and the tractography designed ROIs showed no significant findings in either the control group or the patient group at a significance level of $p<0.017$.

A voxel-wise regression analysis was performed on the TBSS data separately for the control subjects and for the whole patient group. There was no significant relationship between white matter tract FA in either the control group or the patient group and PASAT scores, which is unsurprising given that there were no white matter FA correlations shown in either the 5 ROI or 14 automated ROI analysis.

6.2.5 *Correlation between digit span backwards test scores and scan data*

The digit span backwards test (DSPAN back) produced one score, marked out of a possible 14. The test involved the participant's attention, concentration and short term verbal memory. The brain regions involved in the functions assessed by this test are many, and therefore as with the analysis of the PASAT above (section 6.2.4) correlations were evaluated between the test scores and the frontal lobe data and whole brain ROIs using the 5 ROI technique, all fourteen regions using the automated ROI technique and all three regions using the tractography designed ROI technique.

No significant correlations were demonstrated in any of the datasets in either the control group or the patient group, examining ROIs from the 5 ROI method (significant p-value taken to be $p<0.013$), the 14 automated ROI method (significant p-value taken to be $p<0.0018$), the tractography designed ROI method (significant p-value taken to be $p<0.017$) or the TBSS voxel-wise analysis.

6.2.6 *Correlation between verbal fluency test scores and scan data*

The verbal fluency test is an executive function test that gives four scores per participant. The test consists of two parts, the first tests phonemic or letter fluency and the second tests semantic or category fluency. The first part produces one score, and the second three scores, as it not only assesses the candidate's ability to name objects from within a category, but also their ability to switch between categories. As described in section 3.3.9 phonemic fluency has been shown to be related to frontal lobe function, while semantic fluency is reduced in individuals with temporal lobe lesions. The executive processes involved in semantic fluency are clustering and switching, which are linked to temporal lobe function and frontal lobe function respectively. As a result of these known anatomical relations, the ROIs analysed with respect to the verbal fluency scores were the combined frontal lobe ROI from the 5 ROI method, the left and right temporal lobe ROIs from the 14 automated ROI method, and all three ROIs (genu, splenium and body of the corpus callosum) from the tractography designed ROI technique.

The combined frontal lobe ROI from the 5 ROI technique showed no significant correlations with the verbal fluency scores when assessed in the control group, but did show a number of significant findings in the patient group, after a Bonferroni correction was performed and a p-value of $p<0.025$ was taken to be significant. There were

correlations shown between letter fluency and both frontal lobe grey matter qT1 (p=0.006) and frontal lobe grey matter MD (p=0.014) (Figure 6.16) and between category fluency and both frontal lobe grey matter MD (p=0.010) and frontal lobe grey matter FA (p=0.007) (Figure 6.17). For interest, these scatter plots show the patients grouped by TBI severity according to the admitting GCS score. While the moderate TBI patients (red markers) tend to score poorly in both the letter fluency and category fluency, a number of the mild TBI patients (blue markers) perform equally poorly, indicating a significant reduction in executive function in these mild TBI patients. This highlights the fact that while the admitting GCS is undoubtedly a useful clinical tool for guiding management, it is not a suitable indicator of the severity of cognitive symptoms suffered after brain injury.

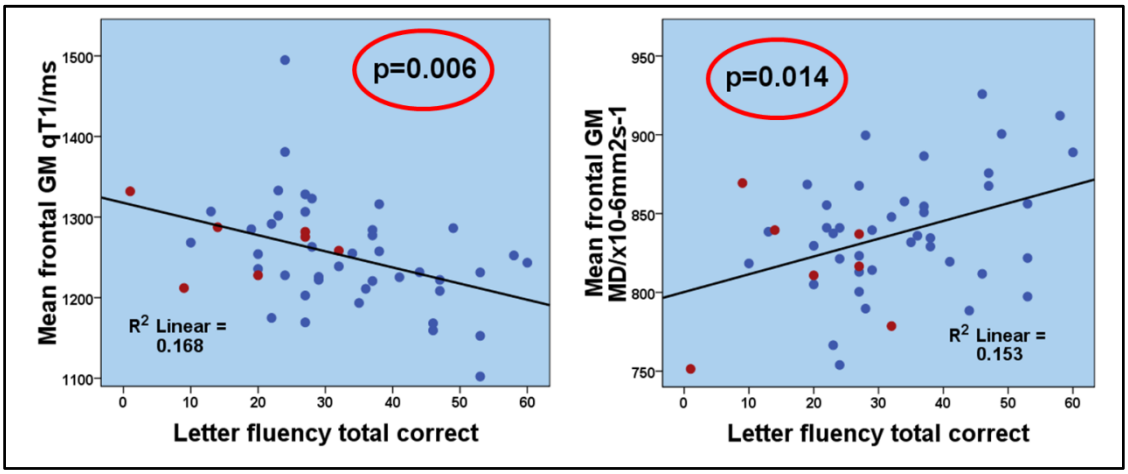

Figure 6.16: Scatter plots showing significant correlations between letter fluency scores and frontal grey matter qT1 and frontal grey matter MD. Patients with mild TBI are represented with a blue marker, and those with a moderate TBI with a red marker.

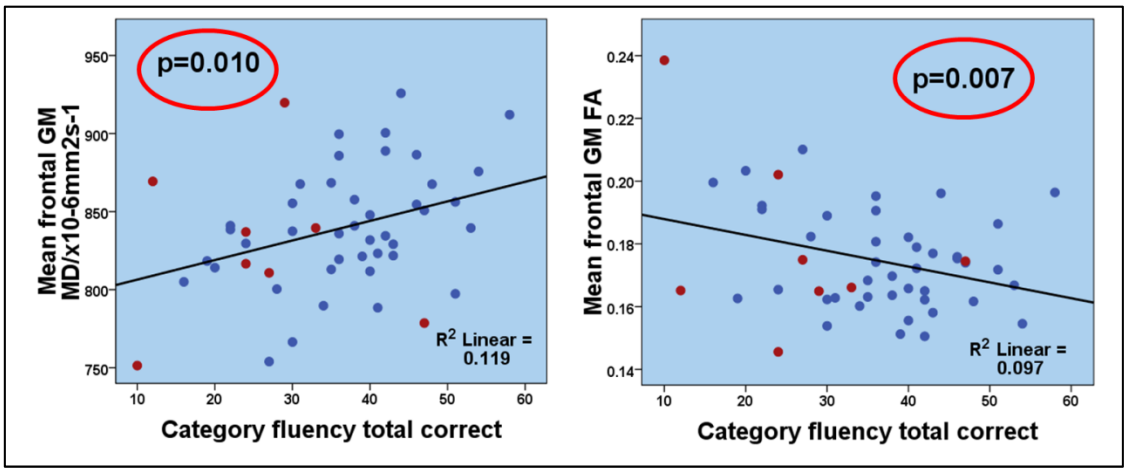

Figure 6.17: Scatter plots showing significant correlations between category fluency scores and frontal grey matter MD and frontal grey matter FA. Patients with mild TBI are represented with a blue marker, and those with a moderate TBI with a red marker.

A significant correlation was also demonstrated between the switching test scores (category switching total correct and total switching accuracy) and the frontal grey matter MD and FA (Figure 6.18). Not only were these significant correlations present in the grey matter diffusion measurements, but white matter FA was also shown to be significantly correlated (Figure 6.19).

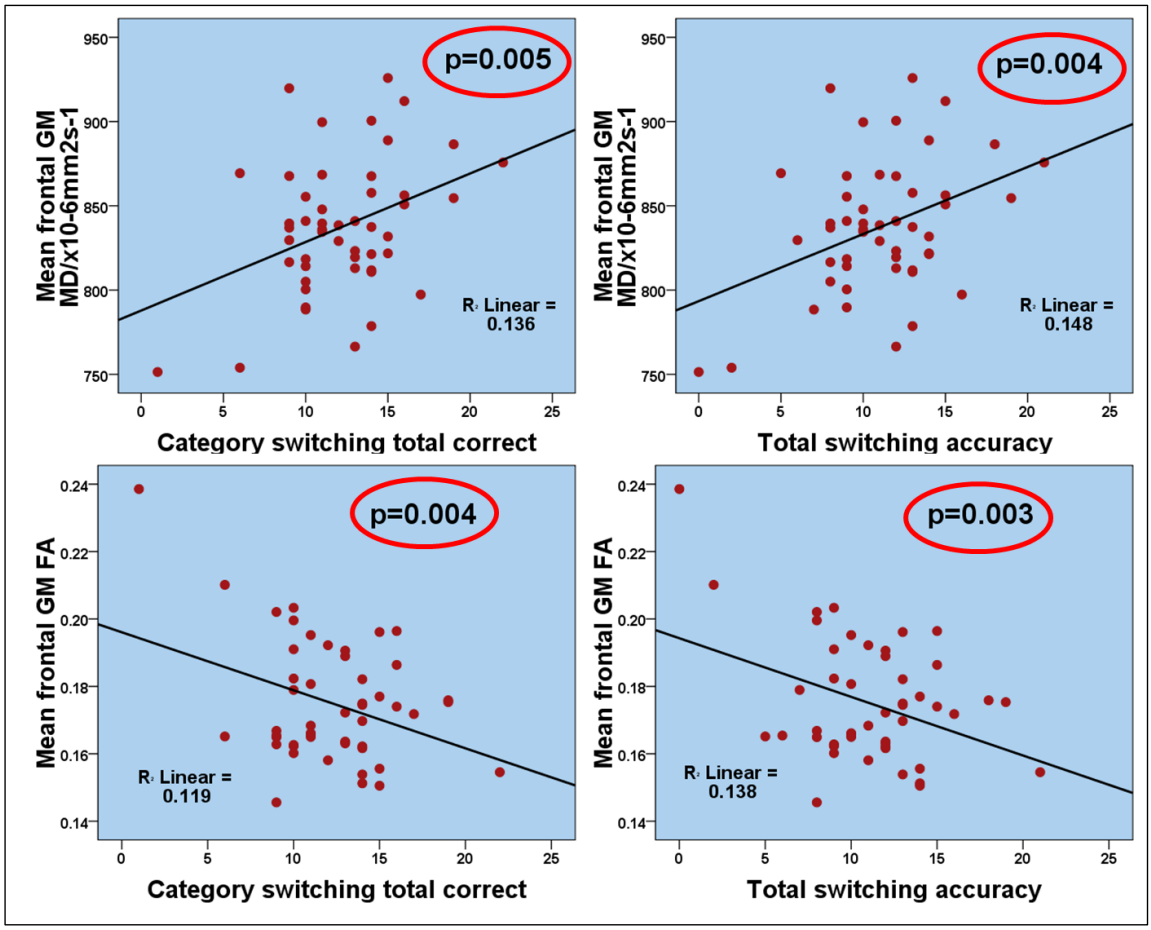

Figure 6.18: Scatter plots showing significant correlations between category switching total correct and total switching accuracy scores and frontal grey matter MD and frontal grey matter FA.

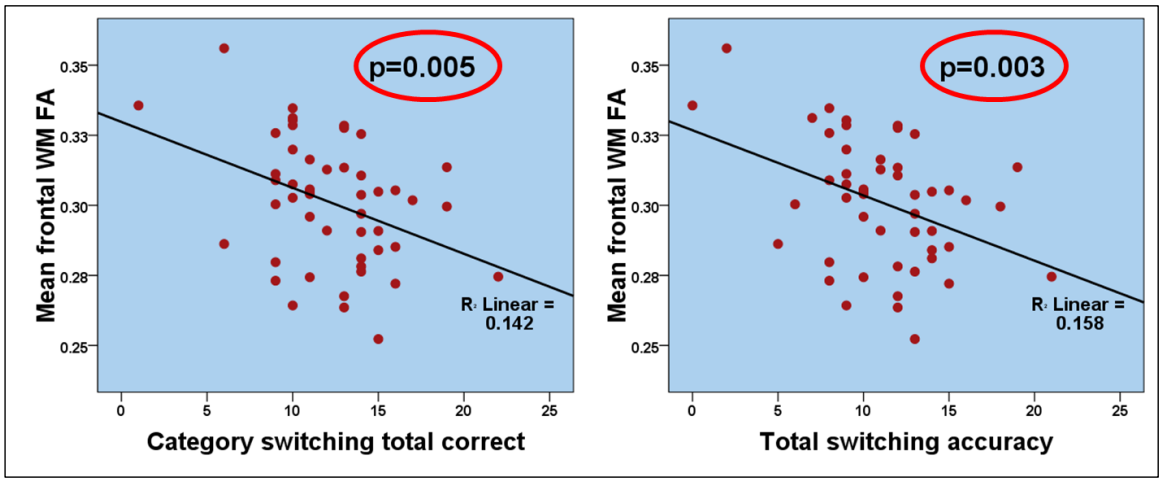

Figure 6.19: Scatter plots showing significant correlations between category switching total correct and total switching accuracy scores and frontal white matter FA.

The 14 automated ROI temporal lobe data in the control group revealed no significant correlations with the verbal fluency test scores after a significant p-value of $p<0.013$ had been determined. However, there was a significant correlation in the patient group between the left temporal region grey matter qT1 and the letter fluency scores ($p=0.006$) (Figure 6.20).

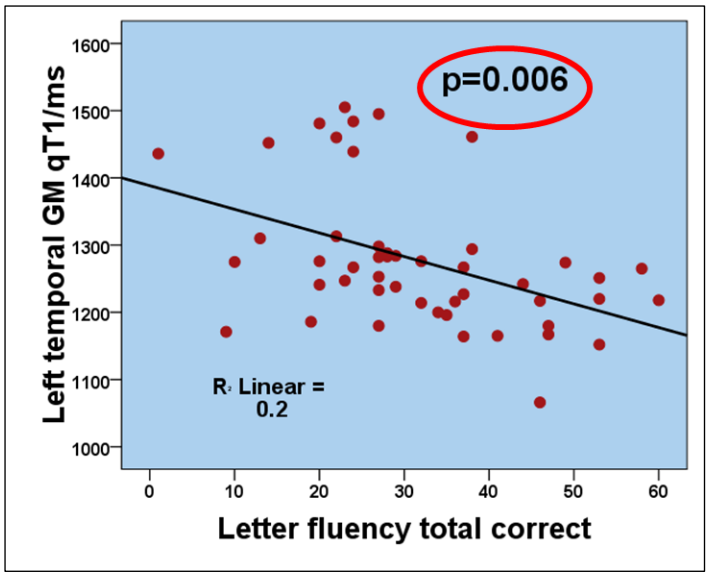

Figure 6.20: Scatter plot showing a significant correlation between left temporal grey matter qT1 and letter fluency score.

Analysis of correlations between the verbal fluency test scores and data from the tractography designed ROIs was performed in the control group and in the patient group. As with all of the previous analyses, predicted IQ was adjusted for by adding it as a covariate of no interest. After a Bonferroni correction had been performed, a p-value of $p<0.017$ was taken to be significant. Correlations were shown in the patient group: between letter fluency and corpus callosum genu white matter FA ($p=0.017$) (Figure 6.21) and between letter fluency corpus callosum splenium white matter MD ($p=0.005$) (Figure 6.22). There were also correlations with the data from the corpus callosum splenium ROI between letter fluency and the second and third eigen values ($p=0.005$, $p=0.005$) (Figure 6.23).

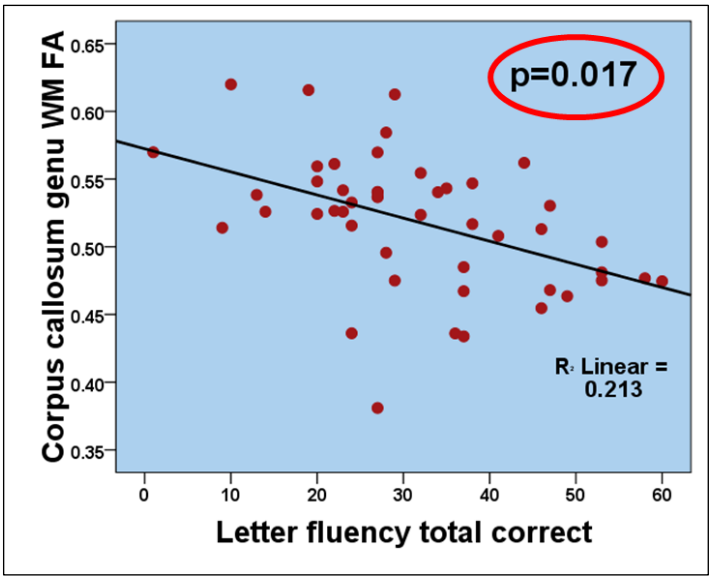

Figure 6.21: Scatter plot showing a significant correlation between corpus callosum genu white matter FA and the letter fluency score from the verbal fluency test.

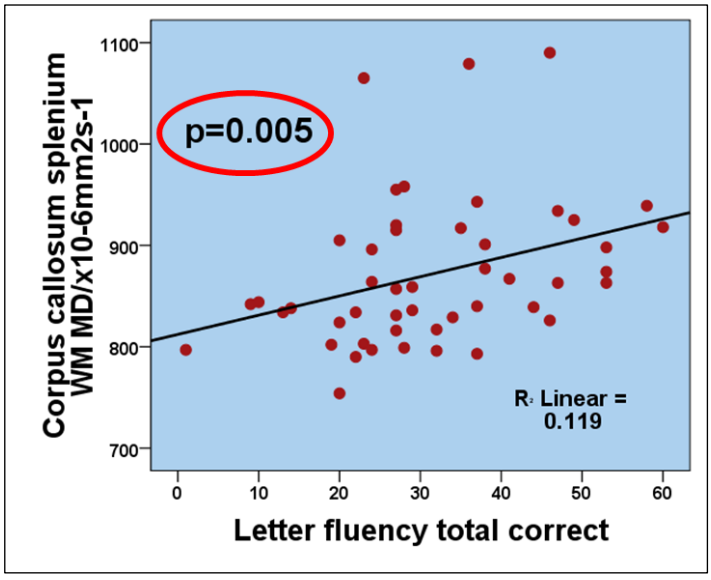

Figure 6.22: Scatter plot showing a significant correlation between letter fluency test scores and corpus callosum splenium white matter MD.

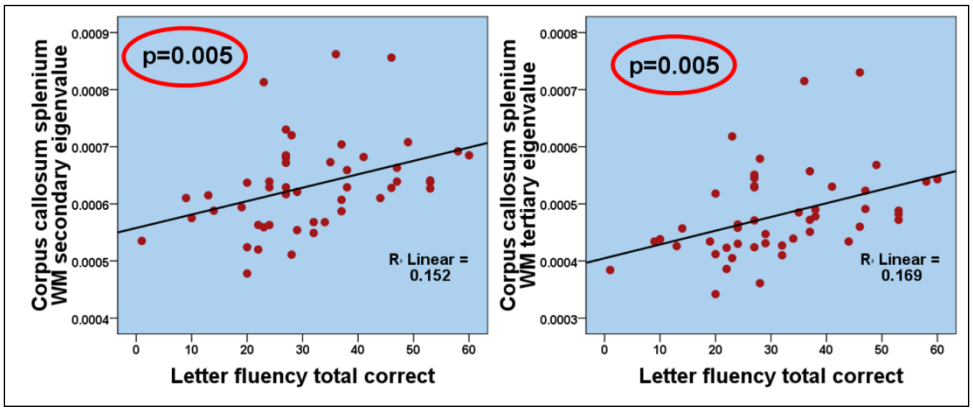

Figure 6.23: Scatter plots showing significant correlations between letter fluency test scores and corpus callosum splenium white matter second (E2) and third (E3) eigen values.

A voxel-wise regression analysis was performed on the TBSS data separately for the control subjects and for the whole patient group. There was no significant relationship in either the control group or the patient group in the category switching or total switching accuracy score, nor were there significant relationships evident in the control group when analysed with respect to the letter fluency and category fluency scores. However, there was a significant regression between the combined patient group white matter tract FA (mild TBI patients and moderate TBI patients) and both letter fluency and category fluency scores ($p=<0.05$). These significant regions were not limited to single voxels, but involved whole tracts or parts thereof.

The areas showing significance after regression analysis with the letter fluency score were predominantly the left and right superior corona radiata, but also fibres in the right genu and right splenium of the corpus callosum, in the right anterior thalamic radiation, left fornix, right and left superior longitudinal fasciculus and right and left cingulum (Figure 6.24). Analysis of the mild TBI group alone, to ascertain whether the observed correlation between increase in FA and decrease in letter fluency score was present in the group with the data from the more severely injured patients removed, revealed correlations in similar regions (Figure 6.25).

Areas showing significance after regression analysis with the category fluency score were fewer in number, but were again primarily located in the left and right superior corona radiata (Figure 6.26) and generally overlapping with those areas found in the letter fluency regression.

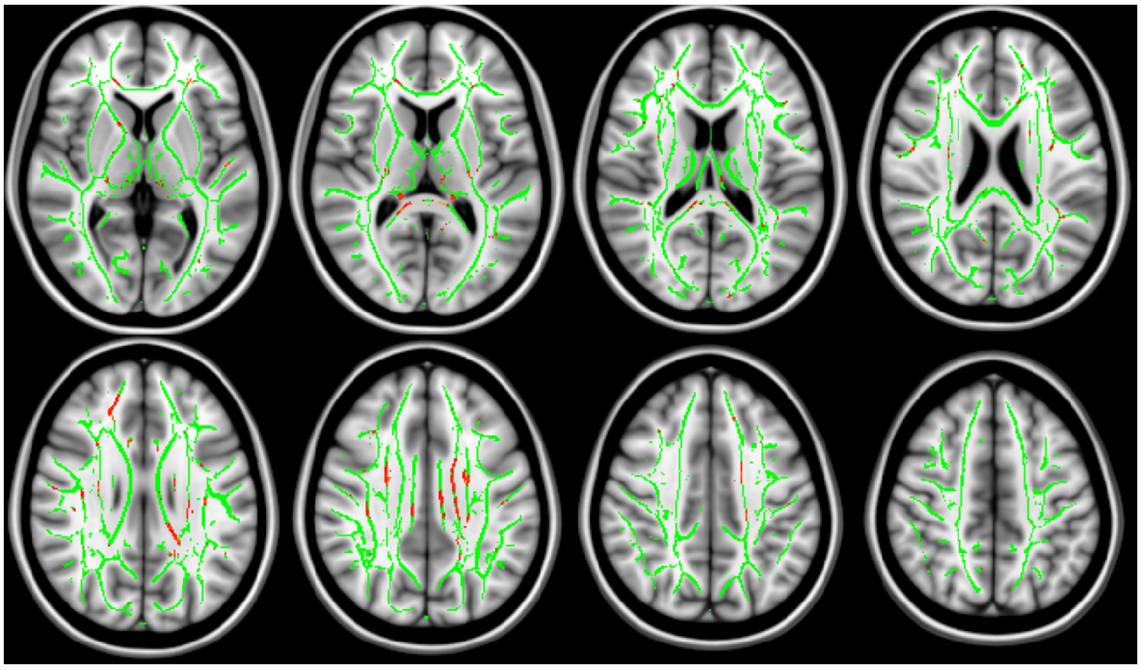

Figure 6.24: Axial images of the regression analysis between letter fluency scores and the whole patient group created using TBSS. The white matter tract skeleton (green) is superimposed on the mean FA map. Voxels marked in red are regions where there was a significant correlation ($p<0.05$) between an increase in FA and decrease in letter fluency scores.

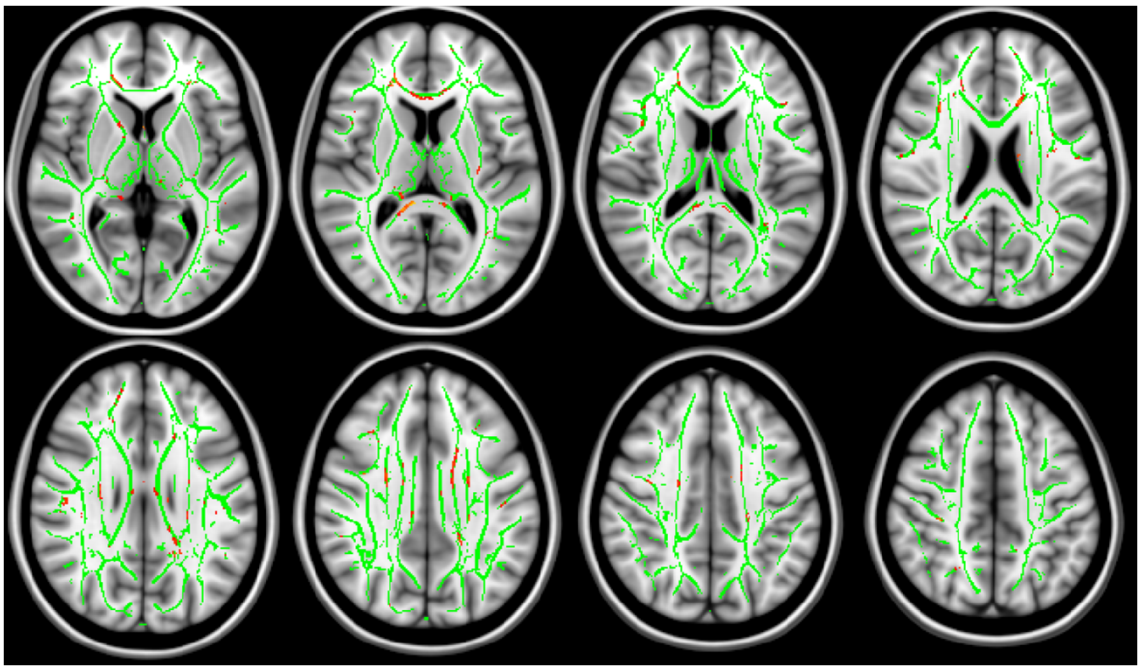

Figure 6.25: Axial images of the regression analysis between letter fluency scores and the mild TBI patient group created using TBSS. The white matter tract skeleton (green) is superimposed on the mean FA map. Voxels marked in red are regions where there was a significant correlation ($p<0.05$) between an increase in FA and decrease in letter fluency scores.

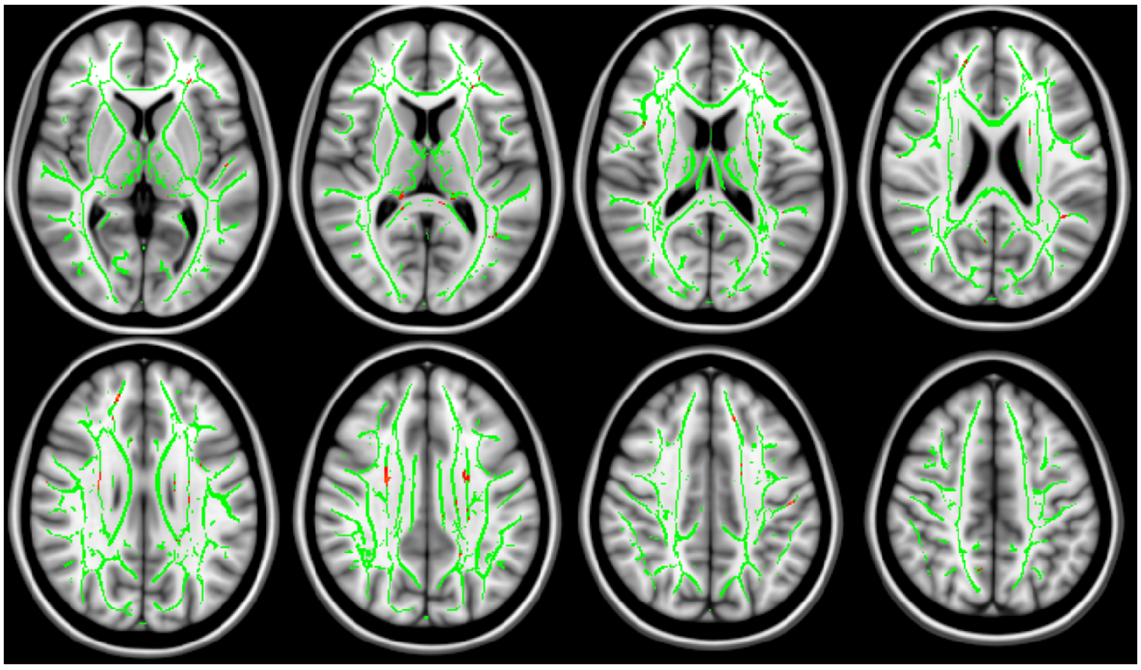

Figure 6.26: Axial images of the regression analysis between category fluency scores and the whole patient group created using TBSS. The white matter tract skeleton (green) is superimposed on the mean FA map. Voxels marked in red are regions where there was a significant correlation ($p<0.05$) between an increase in FA and decrease in letter fluency scores.

6.2.7 *Correlation between colour-word interference test scores and scan data*

The colour-word interference test is an executive function test that also gives four scores per participant. As described in section 3.3.10, the colour-word interference test has been shown to be related to the function of the frontal lobes, anterior cingulate cortices and areas of the parietal lobes. Data was therefore analysed in the frontal and parietal ROIs from the three ROI methods, and a voxel-wise regression analysis was also performed using TBSS.

The 5 ROI data was analysed using a significant p-value of $p<0.025$, and although no significant correlations were evident in the control group, there were correlations demonstrated in the patient group. The frontal region white matter qT1 was shown to correlate with the time taken for part 4 of the colour-word interference test ($p=0.013$) (Figure 6.27), and the frontal grey matter FA showed a correlation with times for both part 3 ($p=0.018$) and part 4 ($p=0.014$) of the test (Figure 6.28).

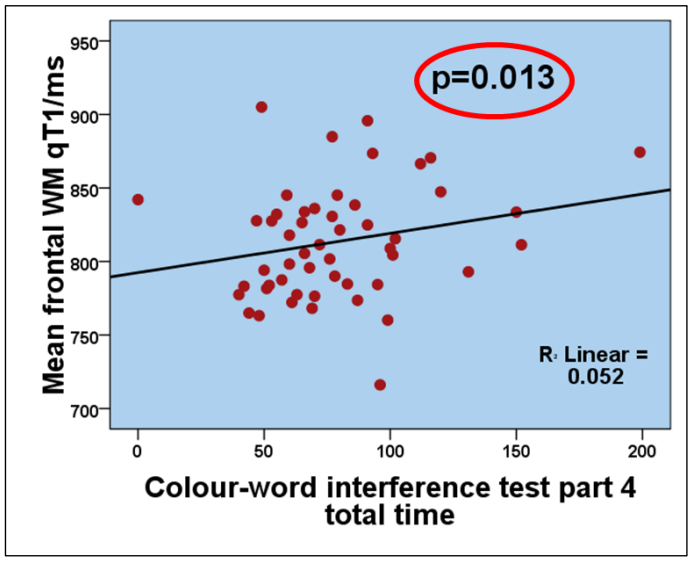

Figure 6.27: Scatter plot showing a significant correlation between colour-word interference test part 4 time and frontal lobe white matter qT1.

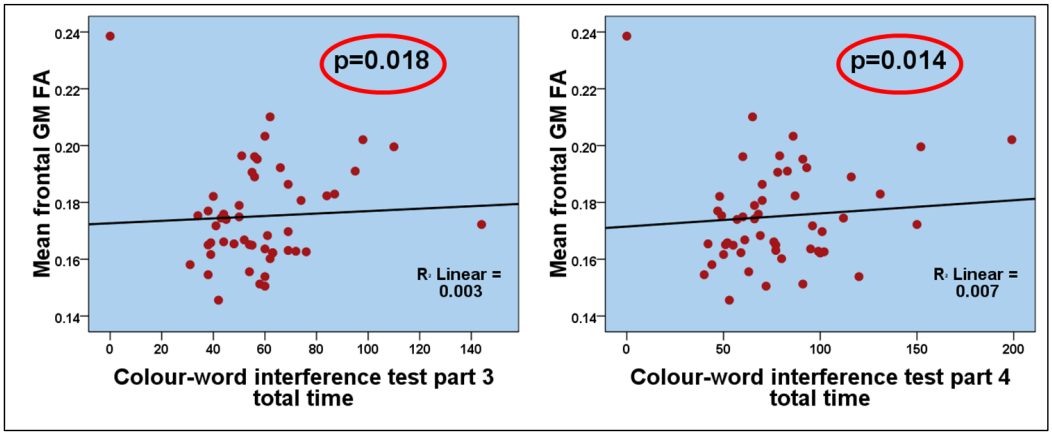

Figure 6.28: Scatter plots showing a significant correlation between colour-word interference test times for parts 3 and 4 and frontal lobe grey matter FA.

A significant p-value of $p<0.004$ was calculated for the 14 automated ROI method analysis, as 6 regions were analysed, each in grey and white matter. Only one dataset in one region showed a significant correlation: left superior frontal white matter qT1 with colour-word interference test part 4 time ($p=0.002$) (Figure 6.29).

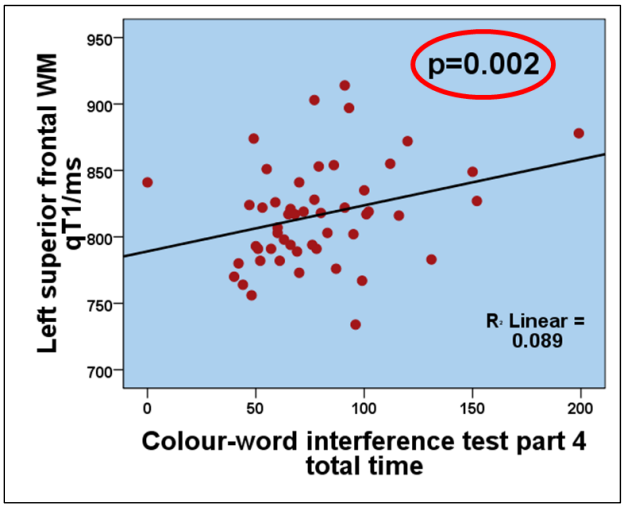

Figure 6.29: Scatter plot showing a significant correlation between colour-word interference test part 4 time and left superior frontal white matter qT1.

There were no significant correlations found in wither the control group or the patient group, when data from the tractography designed ROIs was analysed, after a Bonferroni correction had ascertained a significant p-value to be $p<0.017$.

A voxel-wise regression analysis was performed on the TBSS data separately for the control subjects and for the whole patient group. There was no significant relationship between white matter tract FA in either the control group or the patient group and the colour-word interference test times.

6.3 Discussion

The neuropsychology test results showed that the majority of the test battery used in the study was fit for purpose; each test revealed significant differences between the three groups: mild TBI patients, moderate TBI patients and control subjects. The results highlighted that our study population was representative of the TBI population as a whole, displaying deficits in short-term and working memory, attention, concentration and executive function.

A more detailed examination of the comparison of the test results between groups revealed that some of the tests were only able to differentiate between the control group and the patient groups, but not between the mild and moderate patient groups themselves. Other tests however, such as the verbal fluency test, were clearly related to injury severity and were able to discriminate between the patients who had sustained a mild TBI and those with a moderate TBI.

6.3.1 *Group comparison of predicted IQ*

The first neuropsychology test in the battery was the NART test, which was performed in order to determine the premorbid predicted IQ of the study subjects, and has been shown previously to be generally resistant to mild head injury (Crawford et al., 2001, Crawford et al., 1988). There was a significant difference found between the patient groups and the controls, which indicated that in spite of the author's best efforts at recruiting and matching suitable control subjects by age and educational level, the control group still had a significantly higher predicted IQ than either of the patient groups. This was therefore taken into account in the analysis of correlations between the neuropsychology test scores and the scan data, by including predicted IQ as a covariant of no interest. This ensured that any significant correlations that were uncovered were not biased by the higher IQ of the control group.

6.3.2 *Group comparison of the neuropsychology results by cognitive function*

The remaining tests in the neuropsychology battery could be divided into groups according to the functions tested, although there was a degree of overlap, as participation in most of the tests involved more than one cognitive function. Executive function was tested by the Speed of Information Processing (SoIP) test, the PASAT, the Verbal Fluency test, and the

third and fourth parts of the colour-word interference test. Memory, both short-term and working memory, were tested by the design learning and list learning tests, the DSPAN back and spatial span back tests, and by the PASAT. Attention and concentration were most necessary for the PASAT, list learning and design learning tests, but it could be argued that these cognitive functions were needed for the entire test battery, and as so were measured to a degree by every test.

The results from the 3-way group comparisons of the tests assessing executive function showed there to be a significant reduction in executive function performance in the patient groups compared to the control subjects. This was true of the SoIP test, PASAT and verbal fluency test, but did not hold true for the third and fourth parts of the colour-word interference test. This latter finding was in contrast to previous use of the colour-word interference test in TBI studies (Batchelor et al., 1995, Bohnen et al., 1992, Lacroix and Bailey, 1996) where performance on parts 3 and 4 has been shown to be worse in symptomatic mild TBI patients compared to those who were asymptomatic, and performance in part 4 has been shown to be significantly poorer in moderate and severe TBI patients when compared to controls. In this study, the ANOVA analysis of the colour-word interference test showed no significant inter-group differences in the third part of the test, and although there was a significant inter-group difference in the fourth part, analysis of the box plot revealed that the mean time taken by the moderate TBI patient group was actually lower than that of the mild TBI patient group. This would have indicated that executive function showed an *improvement* in the more severely injured patients, which was unlikely to have been the case, as it was not supported by the findings in the other three tests used to assess executive function. Each of these other tests showed a significant *reduction* in executive function between the moderate TBI group and the control subjects, but more importantly, a significantly *poorer* performance in the moderate TBI group than in the mild TBI group in the case of the verbal fluency test. This ability of the verbal fluency test to differentiate not only between patients and control subjects, but also between groups classified according to injury severity has also been shown by other studies (Levin et al., 2001, Wilson et al., 1991), although both assessed TBI patients in the chronic stages of injury. While the SoIP and PASAT tests did not show a significant difference in performance between the mild TBI and moderate TBI group when assessed with a t test, the box plots revealed that the mean score and inter-quartile range of the moderate group was

below that of the mild TBI group in both cases, indicating a trend towards poorer executive function as measured by these tests as well.

A reduction in memory function in the patient groups compared to the controls was also evident, albeit less so than the differences in executive function discussed above. The design learning test showed a significant difference in memory function on the first part of the test (5 attempts at copying a design from memory) between the mild TBI and moderate TBI groups. This demonstrated a difference in short-term memory performance, related to injury severity, although there was no significant difference shown between either of the patient groups and the control subjects. The list learning test however, *was* able to differentiate between the control group and the two patient groups, but not between the patient groups themselves. This was true not only of the first part of the test which assessed short-term memory, but was also true in the post-interference recall score which specifically tested working memory. Likewise, the score from the digit span backwards test showed a significant difference between each of the patient groups and the control subjects, but it also did not demonstrate any difference in memory performance related to the severity of injury. These results contrast with findings in the literature, where studies have demonstrated a correlation between injury severity as measured by GCS and severity of memory deficits observed, both in the acute phase and chronic stages after TBI (Perlstein et al., 2004, Ponsford et al., 2004). The results of the spatial span backwards test revealed a large degree of overlap between the three groups, with very similar inter-quartile ranges and means in each group. This suggested that there was a large spread of performance in this particular test, and the results did not seem to be directly related to TBI severity. The PASAT, besides assessing executive function, also relied heavily on working memory, and therefore the results from this test were relevant in determining whether memory function was affected by TBI in this study. As described above, the PASAT results showed significant differences between the two patient groups and the control subjects, but not between the patient groups themselves. This inability of the PASAT test to differentiate between less severely and more severely injured patients is consistent with previous findings in the literature: while studies have shown a difference in PASAT scores between TBI patients and control subjects, they have not been able to show a correlation with measures of injury severity (Bate et al., 2001, O'Jile et al., 2006, O'Shaughnessy et al., 1984, Strauss et al., 2006). Overall, it seems that these tests consistently showed a reduction

in memory performance in the patient groups compared to the control subjects, but the observed reduction in performance was not shown to be related to injury severity, which may again have been due to the very small numbers in the moderate TBI group.

As attention and concentration are cognitive functions required for all of the tests in the neuropsychology battery, and as all of the tests showed significant differences in at least one of their scores, attention and concentration could therefore be assumed to have been reduced in the patient groups compared to the control subjects. However, it is impossible to tease out the reason for the differences in test results and categorically state that it was solely due to a deficit in attention or concentration, when in fact it may have been poorer performance in memory or executive function that was the reason for the significant differences observed. It must be noted that fewer of the TBI patients than the control subjects were able to complete all of the tests in the battery, and this in itself may be an indication of a reduced ability to concentrate or remain attentive in those particular cases.

6.3.3 *Analysis of the scan data with reference to cognitive function*

The third aim of the study was to determine whether existing damage detectable by quantitative MR scanning correlated with any post-concussive cognitive symptoms that had been demonstrated on neuropsychological assessment. In the section above, it has been shown that differences in cognitive function were evident between the patient groups and control subjects, and in the previous chapter such inter-group differences were also shown to have been detectable in the quantitative MR data. This section discusses correlations between the scan findings and the neuropsychology findings.

Before analysis of any correlations could be made, three factors needed to be taken into account. The first was the fact that while the majority of patients completed the neuropsychology test battery, the patient numbers completing the tests in the moderate TBI group were so small (between 6 and 8 in each test) that separate analysis of this group, by examining correlations with the scan data, would have been meaningless. The moderate TBI group was therefore combined with the mild TBI group, and served to strengthen any significant correlations demonstrated in the analysis. The second factor was the impact of the difference in premorbid predicted IQ between the control subjects and the patient groups. Premorbid IQ is known to be correlated with almost all of the neuropsychology tests used in this study, and therefore as mentioned in section 6.3.1, the predicted IQ was

used as a covariant of no interest, so as to take into account any bias which may have been introduced by this difference. The third factor was that scan data from *all* of the analysis methods was analysed with respect to the neuropsychology test scores, and as this involved a large number of correlations, a Bonferroni correction was performed to ensure that any significant results observed were less likely to be a result of chance, and more likely to be representative of a true correlation.

Analysis of correlations between the MR values and the neuropsychology test scores revealed significant differences in data from each of the four analysis methods used. The greatest number of significant correlations existed in the 5 ROI data, in both the whole brain and the frontal lobe regions. One reason for this is likely to be the Bonferroni corrections made; the increased number of regions analysed when data from the 14 automated ROI method was examined led to a very low p-value having to be taken as significant, and there is no doubt that if this strict correction had not been made, more significant correlations would have become apparent. However, such strict Bonferroni corrections were not necessary in the tractography designed ROI datasets, as there were only 3 regions in that method, analysed with respect to only one tissue type: white matter, and although the Bonferroni corrections in this analysis did not reduce the significance level of the p-value at all in some cases (where only one region was examined) there were still very few significant correlations between data from the tractography ROIs and the neuropsychology test scores.

6.3.4 *Neuropsychology test score correlations with the qT1 data*

Interestingly, although the results discussed in Chapter 5 outlined that the only whole brain quantitative dataset to show differences in the group comparison was the grey matter qT1 data, it was the whole brain *white* matter qT1 time that demonstrated significant correlations with the PASAT 3 second and 2 second scores (PASAT 3 R^2= 0.143, PASAT 2 R^2= 0.158). The PASAT requires attention, working memory and information processing, and the score is therefore reduced when any of these functions is affected. The fact that the group comparison of whole brain white matter qT1 time did not show significant differences could be due to the fact that injury may have caused a wide spread of differences in white matter qT1 values, making the group variance (the standard deviation)

higher in the patients and confounding the group statistics, whereas the correlation analysis uses that very variance to explain the individual performance of each patient.

The only other neuropsychology test score found to correlate with white matter qT1, in the frontal lobes in the 5 ROI data, and in the superior left frontal region in the 14 ROI data, was the time taken for the fourth part of the colour-word interference test, although the R^2 value for the correlations with these two ROIs were only 0.052 and 0.089 respectively. This part of the colour-word interference test assesses both verbal inhibition and cognitive flexibility, and has been shown previously to differentiate between symptomatic and asymptomatic mild TBI patients (Bohnen et al., 1992). When assessed purely by group comparison in section 6.1.10, the results, although significant, showed the unexpected finding of poorer performance in the mild TBI group compared to the moderate TBI group. This subsequent finding of a significant correlation between the time taken for the fourth part of the colour-word interference test and white matter qT1 values underlines the importance of evaluating the scan findings in the context of cognitive function, as it appears that by stratifying the patients according to their performance, a relationship with possible microstructural damage has become evident.

Significant correlations were also observed between frontal lobe *grey* matter qT1 time and both the recall score for the interference design in the design learning test (R^2= 0.107), which assesses visuospatial learning and short-term and working memory, and the letter fluency score in the verbal fluency test (R^2= 0.168), which assesses the phonemic fluency component of executive function. The letter fluency score also showed a significant correlation with grey matter qT1 in the left temporal region data from the 14 automated ROI analysis. Of these correlations, the most interesting relationship lay between the temporal lobe grey matter qT1 and the letter fluency score; the R^2 value of 0.2 indicating a stronger correlation than in other regions. Although temporal lobe damage has been shown to most strongly affect *semantic* fluency (Henry and Crawford, 2004), the effect of temporal lobe damage on *phonemic* fluency has still previously been shown to be significant, and this is therefore backed up by the findings of this study: a significant correlation between increase in temporal lobe grey matter qT1 and decrease in letter fluency.

6.3.5 *Neuropsychology test score correlations with the DTI data*

As with the stand-alone analysis of the scan data, the majority of significant findings when correlated with the neuropsychology test scores were found to be in the grey matter DTI datasets. Frontal lobe grey matter MD was found to correlate with all of the separate scores from the verbal fluency test: specifically a *reduction* in MD correlated with a poorer verbal fluency test performance. This indicated a relationship between the diffusion properties of water molecules within the frontal lobe cortices and both the clustering and switching components of executive function.

However, the most striking finding in all of this section of the data analysis was the consistent finding of significant correlations between frontal lobe grey matter FA and the neuropsychology test scores. Significant correlations were revealed with both the initial 5 recall attempts and post-interference recall design learning scores, the initial 5 attempts list learning score and the PASAT 3 second score, all indicating a link between an *increase* in frontal grey matter FA and a reduction in memory performance.

There were also significant correlations shown between frontal grey matter FA and the speed of information processing adjusted total, the category fluency and both switching scores from the verbal fluency test, the colour-word interference test (both part 3 time and part 4 time) and as mentioned above, the PASAT 3 second score. These correlations all signify an association between an *increase* in frontal grey matter FA and a deficit in a number of components of executive function.

In the data from the tractography designed corpus callosum ROIs significant correlations were shown between the letter fluency score from the verbal fluency test and the genu FA and splenium MD. An increase in the genu white matter FA was found to correlate with a decrease in letter fluency score, and a *decrease* in splenium white matter MD was also found to correlate with a decreased letter fluency score. There were significant correlations between the letter fluency score and the secondary and tertiary eigenvalues, the mean of which gives a value for radial diffusivity, perpendicular to the primary eigenvalue, which indicates axial diffusivity (section 2.6.1, Figure 2.8). A decrease in the radial diffusivity in the splenium of the corpus callosum correlated with a reduction in letter fluency.

The last set of correlations to outline were those revealed by the TBSS analysis, which showed a correlation between a poorer score in the verbal fluency test, and increased FA in the left and right superior corona radiata, and to a lesser extent in fibres in the right genu and right splenium of the corpus callosum, in the right anterior thalamic radiation, left fornix, right and left superior longitudinal fasciculus and right and left cingulum. This correlation was evident not only in the whole patient group, but also in the mild TBI group when it was analysed independently. In the TBSS analysis, there was also a correlation demonstrated between a reduced category fluency score and an increase in FA, and although this was present in fewer voxels than the correlation with letter fluency, the significant regions were again primarily located in the left and right superior corona radiata and generally overlapped with the areas where the correlation with letter fluency had been shown.

6.3.6 *Analysis of significant correlations*

Not only have the inter-group comparisons of the neuropsychology test scores (section 6.1) shown that the patient groups in this study suffered with memory and executive function deficits, but the analysis of the scan data with reference to the neuropsychological data (section 6.2) has demonstrated that those deficits correlate with quantitative MR values, both in grey matter and in white matter in a number of regions within the brain. These correlations been shown to exist in the frontal lobes and white matter tracts, and have also been observed in the temporal lobe data and in the whole brain analysis.

The cognitive functions shown to correlate with the scan findings in sections 6.3.4 and 6.3.5 above, are known to be dependent on frontal lobe function, and have been shown previously in studies into TBI to be affected by damage to the frontal lobes (Hartikainen et al., 2010, Kersel et al., 2001, Kim et al., 2009, Kim et al., 2010, Kinnunen et al., 2011, Lipton et al., 2009, McDowell et al., 1997, Sanchez-Carrion et al., 2008b). Likewise, the quantitative scan findings in this study, reported in chapter 5, support findings in previous MR studies of TBI, where similar changes have been thought to represent microstructural damage in tissue appearing normal on conventional CT or MRI (Hou et al., 2007, Naruse et al., 1982, Newcombe et al., 2008, Sibson et al., 2008). The fact that the same cognitive deficits shown in the TBI patient group in this study correlate with differences in the quantitative scan data, makes it likely that the these differences in the data represent

damage in the areas responsible for the cognitive functions seen to be affected, or in networks connecting regions implicit in their function.

In this study, an increase in whole brain white matter qT1 time was found to negatively correlate with executive function, while an increase in frontal and left temporal grey matter qT1 time was found to correlate with both poorer short-term and working memory and a deficit in the phonemic fluency component of executive function. As discussed in section 5.6.6, an increase in grey and white matter qT1 time may be indicative of ongoing diffuse vasogenic oedema and astrocyte activation as part of the autoimmune response following traumatic brain injury (Morganti-Kossmann et al., 2007, Naruse et al., 1982, Sibson et al., 2008). These correlations therefore make it possible that the observed disruptions in cognitive function are caused by these pathophysiological responses to injury. Reasons why this correlation was not demonstrated in comparisons with other neuropsychological tests which examined the same cognitive functions may be that the other tests were less sensitive, or that they utilised different interconnecting pathways and brain networks which had remained undamaged (Kinnunen et al., 2011). No studies have been identified correlating quantitative T1 relaxometry with post-concussive deficits in cognitive function and as a result no comment can be made as to whether the observed correlations can be supported by previous work in the field.

The findings observed in the frontal lobe grey matter DTI data were a correlation between a reduction in frontal lobe MD and a deficit in the phonemic fluency component of executive function, and a negative correlation between FA and both memory performance and a number of components of executive function, namely: clustering (with respect to semantic fluency), switching, information processing, verbal inhibition and cognitive flexibility. As outlined in section 5.6.7, previous studies have observed a reduction in localised grey matter diffusion in TBI patients with a similar injury to scan time as in this study (Hou et al., 2007, Newcombe et al., 2008), and attribute the decrease in grey matter MD to the presence of peri-contusional cytotoxic oedema. In this hypothesis, the increased volume of intracellular fluid as a result of the cytotoxic oedema is thought to limit localised extracellular space, thereby restricting the diffusion of extracellular fluid. As extracellular fluid is more easily diffusible than intracellular fluid, this would result in a net reduction in diffusion, and therefore account for a reduction in grey matter MD.

It is however, difficult to explain the correlations between the poorer neuropsychology test scores and the increase in grey matter FA. Fractional anisotropy is a measure thought to be most relevant in brain white matter, as the movement of water molecules is restricted by the myelin sheaths of the axons within the fibre bundles of this tissue type. FA has therefore been used predominantly as a measure of white matter tract integrity, and significant alterations in FA have been hypothesised to indicate white matter damage. The author has not been able to identify any DTI studies that have looked specifically at FA changes in grey matter after TBI, which may be due to the fact that as there are no myelinated axons within grey matter it has not been considered useful or practical to analyse, nor would there be a simple hypothesis to explain any observed changes. We do know from a study examining grey matter FA in acute stroke, that the microstructure of normal grey matter, although not as restrictive as that of white matter, still has an FA value twice that of the almost completely isotropic CSF spaces, although the grey matter under scrutiny was in the deep grey structures and not in the cortex (Sorensen et al., 1999). In that paper however, the authors found there was no significant difference between the FA measurements in ischaemic grey matter and normal grey matter, and it is therefore unlikely that the finding of increased grey matter FA in this study is due to underlying ischaemia. Another study which observed grey matter FA as part of a combined tissue FA histogram analysis in multiple sclerosis (Cercignani et al., 2001), found that there was a difference in the histogram between patients and matched controls, but attributed this change to differences in white matter FA only.

A possible explanation for the increase seen in grey matter FA draws on findings made by previous studies in white matter DTI measurements. A number of papers have reported on a relationship between increased white matter FA and decreased white matter MD, hypothesising that anisotropy increases due to axonal swelling as a result of cytotoxic oedema which further restricts free movement of water molecules in the extracellular space, in turn *decreasing* the mean diffusivity as a result (Chu et al., 2009, Hartikainen et al., 2010, Wilde et al., 2008). If the same relationship exists between MD and FA in grey matter, then the possible explanation of the reduction in grey matter MD being due to the presence of cytotoxic oedema reducing the extracellular water diffusion may also be the explanation behind the increase seen in grey matter FA. We know that fractional anisotropy increases as diffusion becomes more restricted. Therefore as the extracellular spaces are

compressed by the cellular swelling that occurs in cytotoxic oedema, movement of water molecules may become much more restricted in some directions, and have a predilection for others, mimicking the environment within brain white matter fibre tracts within each grey matter voxel. If this hypothesis were true, then an increase in frontal grey matter FA could indicate cortical damage in the frontal lobes, and as the frontal cortex and especially the prefrontal cortex are implicated in short-term memory function and executive function, damage in this region would explain the multiple correlations evident between frontal grey matter FA and the features of PCS seen in the patient group. The author is not aware of any studies that have looked at correlations between grey matter diffusion properties and post-TBI deficits in cognitive function, and therefore it is not possible to say whether these correlations support or contrast previous findings.

Lastly, it is necessary to discuss the correlations between white matter FA and MD, and the neuropsychology test results. Increases in the FA in a number of white matter tracts and in frontal lobar white matter were shown to correlate with all components of executive function measured by the verbal fluency test, while a decrease in white matter MD in the splenium of the corpus callosum correlated specifically with poorer phonemic fluency. These findings support those reported by Hartikainen et al. (2010) who examined 18 patients 3 weeks after injury and divided them into symptomatic and asymptomatic groups. They found that the symptomatic group had a significantly poorer performance on executive function tests, and also showed a significantly increased FA and decreased ADC in the midbrain. These correlations are perhaps the most easily explained, as executive function is heavily associated with the frontal lobes, and in order to orchestrate higher intellectual function, also relies upon abundant connections to almost all other cortical and subcortical structures (Hodges, 1994). It is known that a reduction in radial diffusivity (perpendicular to the axonal diffusivity which is in line with the axons) can cause an increase in FA, and in the splenium of the corpus callosum there was an observed correlation between a reduction in phonemic fluency and the secondary and tertiary eigenvalues, the mean of which gives the radial diffusivity. Therefore, the pattern of increased FA and reduced MD in these regions so necessary for executive function is likely to represent underlying axonal swelling due to cytotoxic oedema, which in turn has caused the observed deficit in cognitive function.

An interesting observation to be made regarding the analysis of the scan data with the neuropsychology test scores is the difference between the quantitative measures found to have significant correlations and the quantitative measures shown to be significant in simple inter-group analysis. For instance, in the inter-group analysis there were no significant differences in white matter FA, and the only significant differences observed between groups in the grey matter FA were in the right occipital and right temporo-parietal regions. However, by assessing the correlations between white and grey matter FA and the neuropsychology test scores, it became immediately apparent that frontal grey matter FA was significantly correlated with poorer memory performance and multiple deficits in executive function in the patients suffering with PCS. The lack of significant findings when the group comparisons were made may be explained by the fact that the mild TBI group contained patients with a range of PCS, at one end of the spectrum there were patients who by the time they were scanned and tested were asymptomatic, with no visible abnormalities on their CT or T1 weighted anatomical scan, and at the opposite end there were those with ongoing headache, dizziness and nausea, who had a history of LOC at the time of injury and PTA for a period of time afterwards, and whose CT and T1 weighted anatomical MRI scans had shown positive findings. It is likely that the patients with the most mild injury would have had quantitative scan data values very similar to those in the control group, and by grouping all of the mild patients together, it is possible that any significant differences in the more symptomatic patients were diluted to a non-significant level. It could be argued that if this explanation were true, the analysis of the quantitative data after grouping the mild TBI patients by AAN grade would have allowed those results to become evident, but by dividing the mild TBI group into three subgroups, the numbers being compared were then so small that a meaningful analysis demonstrating significant results was unlikely.

6.4 Limitations

The first and most important limitation to mention with regard to the neuropsychology results is the fact that there was a significant difference in the premorbid predicted IQ of the patient groups, compared to that of the control subjects. During the course of recruitment to the study, every effort was made to attempt to match each of the TBI patients to a suitable control subject. However, it would seem that the demographic of those who are willing and

have the time to volunteer (unpaid) as control subjects in an MRI study, is not the same as the demographic of those who are typically brought in to the accident and emergency department in the small hours of the morning with a head injury. While this is obviously in part a generalisation, the TBI patients and control subjects were unfortunately both self-selecting groups with little overlap. This limitation was addressed in the section on analysis of correlations between the neuropsychology test scores and the scan data, by adjusting for predicted IQ as measured by the NART test, but was still potentially a source of bias, not only due to the fact that the neuropsychology test battery was known to be sensitive to IQ, but in some cases the lower predicted IQ of the patient group may even have led to an inability to correctly understand the test instructions.

Another limitation in the analysis of the correlations in this chapter is the variability of the fit of the points on the scatter plots with the line of best fit. While all of the correlations examined in the discussion section were significant, even after Bonferroni corrections had been made, scrutiny of the plots themselves, along with their R^2 values, showed that some of the correlations were stronger than others. It may well be the case that some of the correlations were driven by two or three outliers, particularly in those observed in the colour-word interference test, and were the data from these patients to have been removed, there may not have been a significant correlation. Larger group sizes, specifically a greater number of moderate TBI patients would have strengthened any significant correlations shown.

As in the limitations of the quantitative scan data analysis in section 5.7, the variability in time from injury to neuropsychology testing may have introduced bias into the results. Ideally patients would have all been tested within a shorter time frame from their injury, but as explained previously, a number of factors, such as the clinical state of the TBI patients and the fact that the MR scanner and facility for neuropsychology testing were in a separate non-clinical area, meant that this was not possible.

6.5 Conclusions

The neuropsychology test results showed that each test within the battery revealed significant differences between the three groups: mild TBI patients, moderate TBI patients and control subjects. The results highlighted that our study population was representative of the TBI population as a whole, displaying deficits in short-term and working memory, attention, concentration and executive function. Some of the tests were only able to differentiate between the control group and the patient groups, but not between the mild and moderate patient groups themselves. Other tests however, such as the verbal fluency test, were related to injury severity and discriminated between the patients who had sustained a mild TBI and those with a moderate TBI.

The results from the 3-way group comparisons of the tests assessing executive function (Speed of Information Processing (SoIP) test, the PASAT, the Verbal Fluency test, and the third and fourth parts of the colour-word interference test) showed there to be a significant reduction in executive function performance in the patient groups compared to the control subjects, and in the case of the verbal fluency test, as mentioned above, there was a significant correlation between test performance and injury severity.

A reduction in memory function in the patient groups compared to the controls was also evident, albeit less so than the differences in executive function discussed above. Short-term and working memory function was tested by the design learning and list learning tests, the DSPAN back and spatial span back tests, and by the PASAT. Overall, it seems that these tests consistently showed a reduction in memory performance in the patient groups compared to the control subjects, but the observed reduction in performance was not shown to be related to injury severity, which may have been due to the very small numbers in the moderate TBI group.

The neuropsychology results were then used in the analysis of the scan data, and revealed correlations between post-concussive deficits in cognitive function measured and microstructural changes detected using quantitative MR scan techniques in patients with mild and moderate traumatic brain injury. Significant correlations were shown between an increase in both white matter and grey matter quantitative T1 time and a reduction in performance in working memory and executive function. Similar cognitive deficits in memory and executive function were also shown to correlate with a decrease in frontal grey

matter MD and an increase in frontal grey matter FA. Lastly, white matter FA increases in the genu and splenium of the corpus callosum, corona radiata, superior longitudinal fasciculus and cingulum were observed to negatively correlate with all components of verbal fluency. These findings support the third of the study hypotheses:

> **"Detectable microstructural changes in the TBI population correlate with post-concussive cognitive deficits when assessed using the appropriate neuropsychological tests."**

The observed correlations between the neuropsychology test scores and the differences in quantitative MR values indicate that the cognitive deficits seen in mild and moderate TBI patients are related to detectable grey and white matter micro-structural changes in brain tissue that appear normal on conventional anatomical imaging. It is likely that these changes represent damage as a result of traumatic brain injury in the regions responsible for the cognitive functions seen to be affected.

Chapter 7. Summary

7.1 Main Findings

This thesis explored the use of quantitative MR scanning techniques in mild and moderate traumatic brain injury. These techniques allowed the measurement of a number of parameters: quantitative T1 (qT1) and quantitative T2 (qT2) relaxation times and the mean diffusivity (MD) and fractional anisotropy (FA), the latter two of which were calculated from diffusion tensor imaging (DTI) data. The qT1 and qT2 have previously been shown to reflect underlying pathophysiological changes in brain injury, and MD and FA are known to represent the diffusion properties of water molecules, and have also been found to be altered in TBI. These quantitative measurements were made using four different analysis techniques: a basic 5 regions of interest (ROI) method, an automated 14 ROI method, a tractography-designed ROI method and a voxel-wise comparison: tract-based spatial statistics (TBSS). The first three of these analysis techniques were performed in the 'patient's space' and the latter in 'standard space'. In addition to performing the analysis on the whole brain (5 ROI method) and the divided whole brain volume (14 automated ROI method), specific regions were analysed that had previously been shown to be susceptible to diffuse damage in TBI, namely the frontal lobes, corpus callosum and white matter tracts.

The main aims of this work were to ascertain whether detectable damage existed at a microstructural level after acute mild and moderate TBI and if such damage existed, whether it was proportional to injury severity as assessed by the Glasgow Coma Scale, and whether it correlated with post-concussive cognitive symptoms detected by neuropsychological assessment.

7.1.1 *Quantitative MR scan findings*

This study has revealed an increase in quantitative T1 relaxation time in whole brain normal appearing grey matter in mild and moderate TBI patients within two weeks of injury, when compared to matched control subjects. The ROI analysis findings have also demonstrated frontal lobe changes in normal appearing tissue in both patient groups when compared to the controls; specifically an increase in both grey and white matter qT1 time,

an increase in white matter mean diffusivity, a decrease in grey matter mean diffusivity, and a small area in the left superior region of the corona radiata that was shown to have an increased FA. All of these findings were in normal appearing brain tissue, after any visible lesions had been masked off and extracted from the data. There were no observed differences in quantitative T2 relaxation time in any of the datasets in grey or white matter regions. These findings supported the first study hypothesis: that acute differences exist at a microstructural level between patients with traumatic brain injury and matched control subjects that are detectable using quantitative MR scanning, in tissue appearing normal on conventional anatomical MR imaging.

What these observed changes in the quantitative values represent is still not fully known, but previous work in the field has demonstrated an increased grey and white matter qT1 time in relation to diffuse vasogenic oedema and astrocyte activation as part of the autoimmune response following traumatic brain injury, and it is possible that these pathophysiological responses to TBI were behind the increases in grey and white matter qT1 seen in this study.

Reductions in grey matter MD have been shown in other published work to be related to peri-contusional cortical damage and cytotoxic oedema, and it is possible that the finding of reduced grey matter MD in this work also represents such damage, reflecting ongoing ischaemia and cell necrosis adjacent to cortical lesions, or possibly cytotoxic oedema, where the relative increase in intracellular fluid and reduction in extracellular fluid would cause reduced free diffusion. The increase seen in *white* matter MD was most likely to be due to axonal disruption causing a reduction in the restriction of micro-movement of water molecules.

Inter-group paired analysis of the data revealed that although different quantitative datasets were able to differentiate between different study groups, no data from any of the regions in the 5 ROI analysis was able to differentiate between the mild TBI and moderate TBI groups, presumably due to the small number of moderate TBI patients, and due to the relatively broad range of injury severity seen in the mild TBI group: a spread between patients who were almost asymptomatic to those with large numbers of post-concussive symptoms. This lack of significant differences shown between the quantitative scan data values in the mild and moderate TBI patient groups demonstrated that the findings in the

scan data were not proportional to injury severity as assessed by the Glasgow Coma Scale. This finding did not support the second study hypothesis: that detectable microstructural changes in the TBI population are proportional to the severity of the brain injury sustained.

However, by examining the scan data for correlations with scores from the neuropsychology tests, it was anticipated that detected microstructural changes would, in fact, be related to the severity of cognitive function impairment, a better measure of mild TBI severity than the GCS score on admission.

7.1.2 *Neuropsychology findings*

The neuropsychology test results showed that each test within the battery revealed significant differences between the three groups: mild TBI patients, moderate TBI patients and control subjects. The results highlighted that our study population was representative of the general TBI population, displaying deficits in short-term and working memory, attention, concentration and executive function. Some of the tests were only able to differentiate between the control group and the patient groups, but not between the mild and moderate patient groups themselves. Other tests however, such as the verbal fluency test, were related to injury severity and discriminated between the patients who had sustained a mild TBI and those with a moderate TBI.

In terms of the individual cognitive functions assessed, the results from the 3-way group comparisons of the tasks assessing executive function (Speed of Information Processing (SoIP) test, the PASAT, the Verbal Fluency test, and the third and fourth parts of the colour-word interference test) showed there to be a significant reduction in executive function performance in the patient groups compared to the control subjects, and in the case of the verbal fluency test, as mentioned above, there was a significant correlation between test performance and injury severity. The components of executive function found to be affected were: information processing, verbal inhibition and cognitive flexibility, and both clustering and switching with regard to phonemic and semantic fluency.

The 3-way group comparison also demonstrated a reduction in memory function in the patient groups compared to the controls, albeit to a lesser degree than the differences in executive function discussed above. Short-term and working memory function was tested by the design learning and list learning tests, the DSPAN back and spatial span back tests,

and by the PASAT. Overall, it seems that these tests consistently showed a reduction in memory performance in the patient groups compared to the control subjects, but the observed reduction in performance was not shown to be related to injury severity, which may have been due to the very small numbers in the moderate TBI group.

Attention and concentration were measured in part by all of the neuropsychology test battery, but were functions specifically required for the list learning and design learning tests, and for the PASAT. The results from the group comparisons showed that it was likely that there was a reduction in attention and concentration in the patient groups compared to the control subjects. However, as mentioned above, it is impossible to tease out the reason for the differences in test results and categorically state that poorer performance in a particular test was solely due to a deficit in attention or concentration. A point to note is that fewer of the TBI patients than the control subjects were able to complete all of the tests in the battery, and this in itself may have been an indication of a reduced ability to concentrate or remain attentive in those particular cases.

7.1.3 *Correlations between quantitative MR data and neuropsychology test scores*

The final part of the study analysis involved assessing the scan data using the neuropsychology test scores. This revealed correlations between the post-concussive deficits in cognitive function and the microstructural changes that had been detected using quantitative MR scan techniques in the normal appearing tissues of patients with mild and moderate TBI. Significant correlations were shown between an increase in both white matter and grey matter quantitative T1 time and a reduction in performance in working memory and executive function. Similar cognitive deficits in memory and executive function were also shown to correlate with a decrease in frontal grey matter MD and an increase in frontal grey matter FA. Lastly, white matter FA increases in the genu and splenium of the corpus callosum, corona radiata, superior longitudinal fasciculus and cingulum were found to negatively correlate with all components of verbal fluency. These findings support the third of the study hypotheses: that detectable microstructural changes in the TBI population correlate with post-concussive cognitive deficits when assessed using the appropriate neuropsychological tests.

In this study, an increase in whole brain white matter qT1 time was found to negatively correlate with executive function, while an increase in frontal and left temporal grey matter

qT1 time was found to correlate with both poorer short-term and working memory and a deficit in the phonemic fluency component of executive function. As discussed previously, a possible explanation for these correlations might be that ongoing diffuse vasogenic oedema and astrocyte activation, both of which are known to cause an increase in grey and white matter qT1, have caused disruption to the function of the frontal lobes and the interconnecting afferent and efferent fibre tracts on which executive function relies.

The findings observed in the frontal lobe grey matter DTI data were: a correlation between a reduction in frontal lobe MD and a deficit in the phonemic fluency component of executive function, and a negative correlation between FA and both memory performance and a number of components of executive function, namely: clustering (with respect to semantic fluency), switching, information processing, verbal inhibition and cognitive flexibility. The observed decrease in grey matter MD may be attributable to the presence of peri-contusional cytotoxic oedema. A possible explanation for the increase seen in the grey matter FA may also be underlying cytotoxic oedema, resulting in axonal swelling with further restriction of free movement of water molecules in the extracellular space. This increase in frontal grey matter FA could therefore have indicated cortical damage in the frontal lobes, and as the frontal cortex and especially the prefrontal cortex are implicated in short-term memory function and executive function, damage in this region would explain the multiple correlations evident between frontal grey matter FA and the features of PCS seen in the patient group.

The final correlations observed were between increases in the FA in a number of white matter tracts and in frontal lobar white matter, and all components of executive function measured by the verbal fluency test. A decrease in white matter MD in the splenium of the corpus callosum also correlated specifically with poorer phonemic fluency. These correlations are perhaps the most easily explained, as executive function is heavily associated with the frontal lobes, and in order to orchestrate higher intellectual function, also relies upon abundant connections to almost all other cortical and subcortical structures It is known that a reduction in radial diffusivity (perpendicular to the axonal diffusivity which is in line with the axons) can cause an increase in FA, and in the splenium of the corpus callosum there was an observed correlation between a reduction in phonemic fluency and the secondary and tertiary eigenvalues, the mean of which gives the radial diffusivity. Therefore, the pattern of increased FA and reduced MD in these regions so

necessary for executive function is likely to represent underlying axonal swelling due to cytotoxic oedema, which in turn has caused the observed deficit in cognitive function.

The observed correlations between the neuropsychology test scores and the differences in quantitative MR values indicate that the cognitive deficits seen in mild and moderate TBI patients are related to detectable grey and white matter micro-structural changes in brain tissue that appears normal on conventional anatomical imaging. It is likely that these changes represent damage as a result of traumatic brain injury in the regions responsible for the cognitive functions seen to be affected or in the white matter pathways and networks that interconnect between those regions.

7.2 Limitations

A number of limitations specific to either the quantitative scan data analysis methods or to the aspects of the study that involved neuropsychology testing are mentioned in detail in sections 5.7 and 6.4 above, but will be briefly reiterated here.

With regard to the quantitative MR data acquisition, pre-processing and subsequent analysis, there were a number of limitations recognised by the author as a source of possible bias. The first of these was the discrepancy in the numbers of patients recruited to the mild TBI and moderate TBI groups. Ideally, in order to be able to more accurately assess correlations with injury severity, a greater number of moderate TBI patients would have been desirable. The fact that the time window from injury to scan had to be increased from that originally outlined, to a period double that length (7 days to 14 days), may have introduced bias into the scan data, as some patients may have been scanned after acute microstructural changes had resolved. However, it must be noted that the time window was still very narrow compared to other published work in the field, and was still likely to allow the majority of acute changes to have been identified, if not all. Also mentioned above are the inherent problems associated with ROI size and selection with the possibility that subtle findings may have been ‘diluted’ to a level that was non-significant or missed altogether, and the fact that there was a slight predominance of left sided visible lesions, although this limitation is discussed in detail previously, and is unlikely to have had an impact on the results.

There are two important factors that may have affected the data which have not previously been discussed. The first of these was the fact that the analysis of the T1 weighted anatomical scans and the quantitative imaging maps were examined for abnormalities only by the author, a senior specialist registrar in neurosurgery. While it was felt by the study team that this was appropriate, given the author's experience in assessing neuroimaging particularly in the setting of neurotrauma, it is possible that very subtle areas of damage may have been missed, and therefore not masked off and extracted from the quantitative datasets. In such cases the services of a second observer to analyse the scans may have been beneficial. With this in mind, a *post hoc* analysis of the visible scan abnormalities was performed by a senior radiographer blinded to the reports created by the author, and their observations were found to match the original findings. It should also be noted, that in those patients in whom CT scans had been performed as part of their clinical management, a formal report was available from a clinical radiologist, and the data from those reports was taken into account when the anatomical and quantitative MR images were being assessed.

The second factor not previously mentioned was the fact that the majority of patients included in the study had visible evidence of TBI on their conventional CT or MR scans. A reason for this may have been that most of the patients had been referred to the neurosurgical department, and would have therefore been identified by the referring doctors as patients whose TBI might necessitate more management than a simple overnight stay for observation in a district general hospital. Although these decisions are based in part on the clinical status of the patient, scan findings also heavily influence such decisions, and therefore more of the patients who are transferred to neurosurgery will have abnormal scans. Given that the aim of the work was to identify microstructural damage in *normal* appearing tissue and correlate it with observed deficits in cognitive function, it could be argued that this high rate of visible scan findings may have undermined the study findings. It is possible that the cognitive deficits in the patient group were caused by the *visible* injuries, and that they were not related to the detected changes in normal appearing tissue at all. In order to address this potential criticism, one only needs to scrutinise the reports of the scan findings in the individual patients in Tables 5.2 and 5.3. This shows that although a large percentage of patients had visible abnormalities, the number of those that displayed *intra-parenchymal* injuries, as opposed to extra-axial haematomas and skull fractures, was

only 31 (58%), and of those, the majority had only very small basi-frontal or temporal contusions which did not explain their cognitive deficits. This made these patients representative of the very TBI population this work had set out to examine, and makes it less likely that any reductions in cognitive function were due to visible brain injuries.

With regard to the neuropsychology results, the first limitation was the fact that there was a significant difference in the premorbid predicted IQ of the patient groups, compared to that of the control subjects. This was addressed by adjusting for predicted IQ, by including it as a covariant of no interest in the analysis of correlations between the scan data and neuropsychology test scores, but was still potentially a source of bias. Another limitation in the analysis of the correlations was the variability of the fit of the points on the scatter plots with the line of best fit; scrutiny of the plots themselves and their R^2 values, showed that some of the correlations were stronger than others. However, all of the correlations examined in the discussion section had been found to be significant, even after Bonferroni corrections had been made. Larger group sizes and a greater number of moderate TBI patients would have strengthened any significant correlations shown. The variability in time from injury to neuropsychology testing may also have introduced bias into the results; ideally patients would have all been tested within a shorter time frame from their injury, but as explained previously, a number of factors, such as the clinical state of the TBI patients and the fact that the MR scanner and facility for neuropsychology testing were in a separate non-clinical area, meant that this was not possible.

Finally, the mismatch between the number of patients recruited and the number of matched control subjects ought to be mentioned, due to the fact that ideally a control subject should have been recruited for each patient. Difficulties recruiting suitable control subjects meant that this was unfortunately not possible, but it was felt that sufficient numbers of control subjects had been recruited to enable robust comparisons.

7.3 Future Work

As discussed in the rationale for the study, there have been very few quantitative MR studies evaluating mild TBI in the acute stage after injury, and therefore the scope for further work in this area is great. The findings from cross-sectional studies, such as the

work presented in this thesis may be better understood in the context of a longitudinal data, and indeed, to address this, a proportion of the patient group whose results are presented in this thesis have agreed to return for a follow up scan and further neuropsychology testing using the same scan protocol and test battery. We hope to present the findings from the analysis of this data in due course.

There are other existing MR scan techniques known to provide even more information in brain injury, particularly on the pathophysiological changes that occur. Arterial spin labelling, or ASL, is a technique that measures perfusion using arterial water flowing into the cerebral vasculature as the contrast agent. This is performed by magnetically labelling the inflowing hydrogen nuclei within the arterial blood, and therefore removes the necessity for an exogenous contrast agent and is completely non-invasive. Magnetic resonance spectroscopy (MRS) is another technique which takes advantage of the properties of nuclei to align with a strong applied magnetic field, and has been developed to allow differentiation between the relative concentrations of chemical compounds contained in those nuclei. MRS is therefore able to give detailed data on the chemical changes after microscopic injury within the brain after TBI. Both ASL and MRS data were also obtained in the TBI patient group and the control subjects whose *quantitative data* was presented in this work, and it is intended that the analysis of this additional data will be combined with the findings from this thesis to provide a complete picture of the non-invasive findings in mild and moderate TBI.

One particular further area of interest to the author, given the number of significant findings in *grey* matter fractional anisotropy in this study, would be the development of non-invasive techniques to better examine this brain region in the context of brain injury. Currently it seems, there is little interest in the field regarding the FA properties of grey matter and what changes in them might indicate, particularly after TBI, but this lack of grey matter FA findings using standard DTI sequences is stimulating research into other scan techniques. A recent paper published in Magnetic Resonance in Medicine (Shemesh and Cohen, 2011) has examined the application of a novel DTI technique to determine whether it can provide information on microscopic anisotropy even when the structure of the tissue being analysed is completely randomly oriented. One of the difficulties of analysing grey matter FA data, as mentioned above, is due to the fact that it consists not of an ordered structure of myelinated axons, but instead of neuronal cell bodies of different shapes, along

with dendrites and axons extending from them in a random manner. Shemesh and Cohen state that their findings show that the technique is able to offer microstructural information which cannot be obtained from conventional DTI sequences and they conclude that the technique shows promise in being able to characterise underlying microstructure in brain grey matter. Such methods would have been extremely useful in the analysis of the data from this study, and if used in future studies, may yet shed light on the underlying reasons for the observed increase in grey matter FA.

7.4 Conclusion

Using quantitative MR imaging techniques, this study has demonstrated that microstructural changes exist and are detectable in normal appearing grey and white matter, in patients with mild and moderate traumatic brain injuries when compared to matched control subjects. Furthermore, while the observed changes did not show a correlation with injury severity as measured by the GCS, there were significant correlations between the quantitative scan data and the severity of deficits in cognitive function as measured using an appropriate battery of neuropsychology tests. There were no such correlations found in the control subjects.

Appendix A

Grey matter qT1 values /ms

		Whole brain	Contralateral hemisphere	Ipsilateral hemisphere	Contralateral frontal lobe	Ipsilateral frontal lobe	Whole brain	Contralateral hemisphere	Ipsilateral hemisphere	Contralateral frontal lobe	Ipsilateral frontal lobe
Mild TBI	Mean	813.94	818.8	816.57	816.14	811.76	1255.42	1256.57	1264.89	1251.76	1253.63
	SD	36.4	37.7	39.2	40.2	39.8	54.1	35.1	55.1	46.1	76.0
Moderate TBI	Mean	834.55	829.27	825.08	826.52	823.07	1279.96	1241.17	1306	1230.29	1301.23
	SD	29.6	34.9	33.0	40.1	34.3	34.1	41.3	81.4	30.3	100.9
Controls	Mean	806.72	806.72 (mean left + right)		802.06 (mean left + right)		1229.08	1229.13 (mean left + right)		1240.06 (mean left + right)	
	SD	27.9	27.9		27.4		39.1	39.2		34.7	

White matter qT2 values /ms

		Whole brain	Contralateral hemisphere	Ipsilateral hemisphere	Contralateral frontal lobe	Ipsilateral frontal lobe	Whole brain	Contralateral hemisphere	Ipsilateral hemisphere	Contralateral frontal lobe	Ipsilateral frontal lobe
Mild TBI	Mean	80.12	80.78	80.59	78.69	78.33	91.62	92.3	91.91	93.83	93.3
	SD	2.0	1.9	2.0	2.6	2.8	3.3	2.9	3.1	4.1	4.3
Moderate TBI	Mean	81.08	80.15	80.62	78.29	78.46	92.23	91.51	91.02	92.93	93.74
	SD	2.3	2.2	2.4	2.3	2.3	3.4	4.2	3.8	5.0	3.3
Controls	Mean	80.35	80.36 (mean left + right)		78.21 (mean left + right)		92.50	92.51 (mean left + right)		95.19 (mean left + right)	
	SD	2.0	2.0		2.8		4.1	4.1		4.8	

White matter MD values /x10^{-6}mm^{2}s^{-1}

		Whole brain	Contralateral hemisphere	Ipsilateral hemisphere	Contralateral frontal lobe	Ipsilateral frontal lobe	Whole brain	Contralateral hemisphere	Ipsilateral hemisphere	Contralateral frontal lobe	Ipsilateral frontal lobe
Mild TBI	Mean	787.93	792.33	787.8	770.88	761.53	846.24	851.57	843.9	846.12	840.06
	SD	25.0	25.2	22.1	31.1	27.0	31.7	30.8	26.0	37.9	34.6
Moderate TBI	Mean	800.59	805.42	785.16	773.2	759.57	841.9	851.25	817.09	832.05	801.96
	SD	25.6	25.7	25.4	35.3	41.3	36.4	25.3	34.8	45.5	48.7
Controls	Mean	785.66	785.71 (mean left + right)		754.80 (mean left + right)		855.01	855.00 (mean left + right)		849.93 (mean left + right)	
	SD	21.9	22.0		18.5		21.8	21.8		23.5	

White matter FA values

		Whole brain	Contralateral hemisphere	Ipsilateral hemisphere	Contralateral frontal lobe	Ipsilateral frontal lobe	Whole brain	Contralateral hemisphere	Ipsilateral hemisphere	Contralateral frontal lobe	Ipsilateral frontal lobe
Mild TBI	Mean	0.3290	0.3270	0.3289	0.2958	0.3013	0.1699	0.1685	0.1689	0.1738	0.1745
	SD	0.021	0.018	0.014	0.021	0.020	0.008	0.009	0.008	0.018	0.015
Moderate TBI	Mean	0.3275	0.3195	0.3378	0.2888	0.3113	0.1709	0.1765	0.1760	0.1842	0.1863
	SD	0.015	0.017	0.023	0.023	0.034	0.014	0.020	0.013	0.036	0.037
Controls	Mean	0.3253	0.3254 (mean left + right)		0.2997 (mean left + right)		0.1707	0.1707 (mean left + right)		0.1761 (mean left + right)	
	SD	0.015	0.015		0.017		0.008	0.009		0.013	

Appendix A: Mean qT1, qT2, MD and FA values for each of the 5 regions in the 5 ROI analysis method, in grey and white matter.

Appendix B

White matter qT1 values /ms

		Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10	Region 11	Region 12	Region 13	Region 14
Mild TBI	Mean	801.25	819.89	818.52	797.36	818.57	799.25	821.68	802.34	813.77	818.45	790.61	817.14	805.93	820.89
	SD	41.6	44.2	35.5	37.1	43.5	35.0	38.0	42.5	38.9	37.8	35.7	40.9	41.3	41.5
Moderate TBI	Mean	824.00	836.00	844.11	812.67	837.78	819.33	835.44	821.78	832.00	837.67	809.44	845.00	831.67	832.11
	SD	36.1	33.7	26.3	29.5	44.5	32.7	28.8	32.0	30.8	27.3	26.6	50.3	45.7	25.6
Controls	Mean	791.17	813.40	803.63	782.40	815.23	794.90	814.40	787.97	809.03	802.37	775.80	803.03	801.20	815.43
	SD	28.7	30.0	28.7	28.1	50.1	29.1	28.7	28.8	28.0	30.6	28.9	33.1	36.3	30.3

Grey matter qT1 values /ms

		Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10	Region 11	Region 12	Region 13	Region 14
Mild TBI	Mean	1251.00	1187.77	1296.11	1274.23	1181.48	1260.93	1216.55	1261.80	1182.52	1269.05	1276.93	1156.68	1269.23	1215.18
	SD	75.3	93.1	112.4	149.2	175.0	56.8	102.8	84.9	89.0	103.9	123.2	159.4	44.6	63.5
Moderate TBI	Mean	1284.89	1152.44	1281.89	1338.78	1166.33	1284.44	1224.67	1309.56	1202.33	1282.89	1365.11	1309.67	1309.22	1242.22
	SD	101.1	30.7	102.4	136.0	188.8	43.0	103.0	94.5	100.0	97.5	131.2	159.5	45.3	79.2
Controls	Mean	1227.83	1162.97	1255.13	1224.57	1082.20	1250.93	1187.43	1234.40	1168.83	1227.87	1213.53	1089.07	1260.67	1190.60
	SD	38.4	51.2	96.9	112.3	127.4	34.6	41.3	42.8	45.1	81.9	60.2	93.3	29.1	40.1

White matter qT2 values /ms

		Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10	Region 11	Region 12	Region 13	Region 14
Mild TBI	Mean	75.77	82.50	78.39	79.50	81.61	79.98	81.84	76.36	82.68	77.89	79.45	81.18	79.91	81.82
	SD	2.9	3.7	2.3	2.6	3.6	2.2	2.7	2.7	3.7	2.0	2.2	2.8	2.1	2.7
Moderate TBI	Mean	77.11	83.00	79.00	80.22	82.33	80.67	83.00	76.89	84.11	79.00	80.78	81.33	81.11	83.33
	SD	2.6	2.2	2.4	3.3	2.8	3.2	2.8	3.0	3.6	2.4	2.1	2.8	3.1	2.5
Controls	Mean	75.63	83.10	77.97	80.23	81.97	80.10	82.77	76.30	82.53	77.77	79.50	81.93	80.13	82.77
	SD	2.5	4.1	2.1	2.4	3.0	1.7	2.0	2.4	3.8	2.0	2.2	2.5	1.9	2.0

Grey matter qT2 values /ms

		Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10	Region 11	Region 12	Region 13	Region 14
Mild TBI	Mean	91.93	90.30	93.39	88.39	85.41	90.30	87.89	92.50	90.30	92.36	87.91	83.80	90.43	86.73
	SD	4.4	5.2	5.2	6.6	6.0	3.6	5.7	5.1	5.2	3.9	6.5	6.3	2.5	4.8
Moderate TBI	Mean	93.89	89.33	93.11	91.44	86.00	90.78	87.89	94.00	90.89	92.00	90.78	88.44	91.33	86.78
	SD	4.4	3.0	5.9	6.5	6.7	2.6	5.8	4.7	3.3	4.6	8.9	7.9	2.8	3.2
Controls	Mean	92.30	91.10	93.63	88.67	84.03	89.90	88.37	92.90	91.63	91.67	85.60	82.20	90.97	88.53
	SD	4.2	4.1	6.3	8.2	6.3	3.4	3.5	4.1	4.8	5.7	4.3	5.4	3.2	4.3

Appendix B: Mean qT1 and qT2 values for each of the 14 regions in the 14 automated ROI analysis method, in grey and white matter.

Appendix C

White matter MD values /x10^{-6}mm^{2}s^{-1}

		Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10	Region 11	Region 12	Region 13	Region 14
Mild TBI	Mean	775.39	706.64	834.91	775.86	770.95	777.55	755.91	774.64	696.34	817.14	783.57	767.55	767.70	742.43
	SD	31.1	24.9	33.9	33.0	32.2	24.8	30.4	35.7	28.0	27.8	31.6	30.1	25.1	28.4
Moderate TBI	Mean	778.44	718.00	859.78	805.33	795.89	789.67	769.22	770.00	712.22	827.22	790.44	778.33	772.56	750.56
	SD	33.4	26.6	43.4	38.9	40.8	30.1	23.6	45.0	20.8	29.6	11.2	28.1	23.0	22.9
Controls	Mean	769.53	691.93	834.60	778.57	770.90	777.23	746.33	765.40	682.13	811.03	776.67	763.90	763.47	732.20
	SD	19.4	24.0	21.5	29.2	27.3	23.2	28.2	19.4	24.4	19.2	24.6	23.3	24.2	25.1

Grey matter MD values /x10^{-6}mm^{2}s^{-1}

		Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10	Region 11	Region 12	Region 13	Region 14
Mild TBI	Mean	822.68	785.82	806.75	810.84	814.59	870.91	827.77	825.80	776.16	804.52	812.14	807.91	869.36	816.32
	SD	58.9	47.8	49.5	27.6	32.8	45.5	37.7	55.0	44.6	44.2	31.1	33.5	49.9	40.0
Moderate TBI	Mean	813.67	746.11	794.56	823.22	814.89	882.78	823.78	815.89	756.22	806.78	811.11	813.56	852.67	794.11
	SD	59.0	75.8	44.8	48.5	38.0	42.9	49.7	75.5	86.5	44.7	21.1	29.0	56.7	61.2
Controls	Mean	828.70	798.37	803.93	814.87	825.47	891.37	844.30	823.60	797.40	806.83	811.87	810.17	876.97	832.80
	SD	46.7	28.1	41.6	23.6	33.8	40.0	30.4	42.0	26.6	36.5	23.6	25.4	32.4	28.9

White matter FA values

		Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10	Region 11	Region 12	Region 13	Region 14
Mild TBI	Mean	0.2611	0.3066	0.2797	0.2743	0.2345	0.3265	0.3190	0.2638	0.3162	0.2876	0.2928	0.2538	0.3333	0.3238
	SD	0.022	0.026	0.026	0.034	0.040	0.028	0.027	0.022	0.026	0.023	0.028	0.037	0.032	0.026
Moderate TBI	Mean	0.2570	0.3080	0.2661	0.2736	0.2230	0.3272	0.3129	0.2728	0.3113	0.2852	0.2913	0.2418	0.3374	0.3220
	SD	0.024	0.024	0.016	0.038	0.031	0.021	0.015	0.040	0.034	0.024	0.030	0.044	0.023	0.033
Controls	Mean	0.2545	0.3136	0.2704	0.2782	0.2239	0.3186	0.3180	0.2646	0.3152	0.2881	0.3020	0.2583	0.3256	0.3280
	SD	0.015	0.019	0.018	0.019	0.033	0.021	0.016	0.015	0.016	0.020	0.020	0.032	0.023	0.019

Grey matter FA values

		Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7	Region 8	Region 9	Region 10	Region 11	Region 12	Region 13	Region 14
Mild TBI	Mean	0.1536	0.1605	0.1557	0.1460	0.1459	0.1553	0.1413	0.1481	0.1519	0.1537	0.1393	0.1452	0.1468	0.1378
	SD	0.014	0.025	0.014	0.011	0.011	0.017	0.013	0.018	0.025	0.010	0.010	0.011	0.017	0.011
Moderate TBI	Mean	0.1527	0.1602	0.1547	0.1443	0.1564	0.1513	0.1481	0.1504	0.1461	0.1534	0.1402	0.1423	0.1520	0.1399
	SD	0.021	0.028	0.019	0.010	0.014	0.006	0.017	0.030	0.032	0.007	0.008	0.014	0.015	0.020
Controls	Mean	0.1478	0.1714	0.1516	0.1430	0.1465	0.1469	0.1450	0.1474	0.1578	0.1531	0.1408	0.1469	0.1438	0.1421
	SD	0.011	0.021	0.010	0.009	0.011	0.008	0.012	0.010	0.023	0.010	0.009	0.010	0.008	0.014

Appendix C: Mean MD and FA values for each of the 14 regions in the 14 automated ROI analysis method, in grey and white matter.

Appendix D

White matter MD values /x10^{-6}mm^{2}s^{-1}

		Corpus callosum genu	Corpus callosum splenium	Corpus callosum body
Mild TBI	Mean	836.09	873.80	1009.32
	SD	60.0	73.9	131.5
Moderate TBI	Mean	844.11	856.33	1010.33
	SD	148.1	53.4	124.2
Controls	Mean	843.70	899.17	1022.20
	SD	51.1	79.4	109.3

White matter FA values

		Corpus callosum genu	Corpus callosum splenium	Corpus callosum body
Mild TBI	Mean	0.5205	0.5748	0.5061
	SD	0.047	0.043	0.057
Moderate TBI	Mean	0.5115	0.5631	0.4995
	SD	0.054	0.054	0.067
Controls	Mean	0.5213	0.5676	0.4964
	SD	0.033	0.036	0.042

White matter primary eigenvalues

		Corpus callosum genu	Corpus callosum splenium	Corpus callosum body
Mild TBI	Mean	0.001387	0.001510	0.001618
	SD	0.000088	0.000086	0.000112
Moderate TBI	Mean	0.001375	0.001469	0.001607
	SD	0.000204	0.000107	0.000115
Controls	Mean	0.001398	0.001537	0.001622
	SD	0.000060	0.000100	0.000100

White matter secondary eigenvalues

		Corpus callosum genu	Corpus callosum splenium	Corpus callosum body
Mild TBI	Mean	0.000634	0.000632	0.000772
	SD	0.000065	0.000081	0.000151
Moderate TBI	Mean	0.000658	0.000627	0.000780
	SD	0.000133	0.000060	0.000147
Controls	Mean	0.000641	0.000651	0.000787
	SD	0.000054	0.000079	0.000124

White matter tertiary eigenvalues

		Corpus callosum genu	Corpus callosum splenium	Corpus callosum body
Mild TBI	Mean	0.000487	0.000479	0.000638
	SD	0.000067	0.000079	0.000143
Moderate TBI	Mean	0.000499	0.000472	0.000644
	SD	0.000135	0.000054	0.000144
Controls	Mean	0.000493	0.000509	0.000658
	SD	0.000055	0.000075	0.000114

Appendix D: Mean MD, FA and eigenvalues for each of the 3 regions in the tractography designed ROI analysis in grey and white matter.

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