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Traumatic brain injury:

Relationships between brain structural abnormalities and cognitive function

Kirsi M. Kinnunen

Goldsmiths, University of London

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Statement of Contributions

Whilst the overall research project that the studies reported in this thesis are part of was an effort by the entire Traumatic Brain Injury Team at Imperial College London, all material presented here is based on my cental role in all aspects of the studies: their design, participant recruitment, the general running of the studies, data collection and analysis, and the interpretation of results and their preparation for publication in journals and presentation at scientific meetings and conferences. As regards data collection, at the same time as I was carrying out neuropsychological testing, other members of our team, including team leader Dr David Sharp, PhD students Ms Valerie Bonnelle and Dr Tim Ham and visiting researcher Dr Xavier De Boissezon, with the help of Ms Emer Hughes and Ms Amy McGuinness, assumed primary responsibility over the collection of the neuroimaging data. It was my responsibility to collect all patient neuropsychological data as well as to oversee the collection of healthy volunteer data, including the training of MSc students Ms Shezmin Kassam and Mr Peter Hawkins of Goldsmiths to carry out the healthy volunteer assessments.

Signed

Kirsi Kinnunen Date

Abstract

Traumatic brain injury (TBI) is the leading cause of disability in young adults and a major public health problem. Persistent cognitive impairments are common, and constitute a significant source of long-term disability. The specific pathophysiological mechanisms underlying these impairments remain poorly understood. As it disconnects brain networks, white matter damage can be a key determinant of cognitive impairment after TBI. Neuroimaging and neuropsychological methods were employed to explore the relationships between indices of brain structure and cognitive function. The participants were 40 TBI patients and 40 healthy controls. First, relationships between focal lesions and cognitive performance were investigated using structural magnetic resonance imaging (MRI) and a battery of neuropsychological tests. The results demonstrated that lesion location and load are not good indices of the cognitive deficits - probably because diffuse axonal injury is poorly assessed by standard MRI. By contrast, diffusion tensor imaging (DTI) can be used to quantify the microstructure of white matter. A 'whole-brain' technique, tract-based spatial statistics (TBSS), was used to flexibly analyse the structure of white matter tracts. Despite only small amounts of focal damage observed using standard MRI, TBSS revealed widespread white matter abnormalities after TBI. White matter damage was found in patients with no evidence of focal damage, and in patients classified as 'mild' clinically. Relationships between white matter tract structure and specific cognitive functions were then explored. The structure of the fornix, an important white matter pathway of the hippocampus, correlated with verbal associative memory across the patient and control groups. By contrast, structure of frontal lobe connections showed distinct relationships with executive function in these two groups. The results emphasise the importance of white matter pathology after TBI and suggest that disruption to specific white matter tracts is associated with particular patterns of cognitive impairment, but also highlight the complexity of these relationships.

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TBI	traumatic brain injury	4
MRI	magnetic resonance imaging	4
DTI	diffusion tensor imaging	4
TBSS	tract-based spatial statistics	4
CT	computed tomography	17
DAI	diffuse axonal injury	17
PTA	post-traumatic amnesia	18
RTA	road traffic accident	21
CSF	cerebrospinal fluid	23
GCS	Glasgow Coma Scale	27
PCS	post-concussive syndrome	31
NICE	National Institute for Health and Clinical Excellence	33
FLAIR	fluid attenuated inversion recovery	35
SPECT	single photon emission computed tomography	36
PET	positron emission tomography	36
MRS	MR spectroscopy	36
fMRI	functional MRI	36
FA	fractional anisotropy	38
MD	mean diffusivity	38
D_{ax}	axial diffusivity	38
D_{rad}	radial diffusivity	38
PFC	prefrontal cortex	42
DMN	default mode network	43
TMT	Trail Making Test	46
VBM	voxel-based morphometry	47
WTAR	Wechsler Test of Adult Reading	47
WAIS-III	Wechsler Adult Intelligence Scale- Third Edition	48
WASI	Wechsler Abbreviated Scale of Intelligence	48
ROI	region of interest	51

LM	Logical Memory	68
DS	Digit Span	69
D-KEFS	Delis-Kaplan Executive Function System	70
CRT	choice-reaction task	72
T	Tesla	73
SNR	signal-to-noise ratio	74
T1	spin-lattice relaxation time	74
T2	spin-spin relaxation time	75
T2*	T2 star relaxation	75
FT	Fourier Transform	75
FMRIB	Oxford Centre for the Functional Magnetic Resonance Imaging of the Brain	74
TR	repetition time	74
TE	echo time	74
FOV	field of view	74
FSL	FMRIB's Software Library	78
FLIRT	FMRBI's Linear Image Registration Tool	79
BET	Brain Extraction Tool	79
FDT	FSL's Diffusion Toolbox	79
SPSS	Statistical Package for the Social Sciences	83
VLSM	Voxel-based lesion-symptom mapping	84
FNIRT	FMRBI's Nonlinear Image Registration Tool	84
GLM	General Linear Model/Modelling	87
EV	explanatory variable	87
TFCE	threshold-free cluster enhancement	88
MNI	Montréal Neurological Institute	88
GOS	Glasgow Outcome Scale	96
MARS	Microbleed Anatomical Rating Scale	102
FDR	False Discovery Rate	104
MB	traumatic microbleeds	135

CHAPTER 1: Introduction

1.1 Motivation for the Research

Traumatic brain injury (TBI) is a major health and socioeconomic problem, accounting for the majority of deaths due to trauma and for a large proportion of lifelong disability globally (Azouvi et al., 2011; Dikmen, Machamer, Powell, & Temkin, 2003). Those who survive a TBI frequently experience marked cognitive impairment that significantly contributes to the disability (e.g. Berg, Tagliaferri, & Servadei, 2005; Green, Colella et al., 2008; van Velzen, van Bennekom, Edelaar, Sluiter, & Frings-Dresen, 2009; Whitnall, McMillan, Murray, & Teasdale, 2006). Traumatic brain injury thus places a substantial burden on public health and social care resources (Berg et al., 2005; Thurman, Alverson, Dunn, Guerrero, & Sniezek, 1999). In many cases, continued community care and rehabilitation is required subsequent to acute treatment (National Collaborating Centre for Acute Care, 2007). Furthermore, most survivors of TBI are young, meaning that some of the problems can be very long-term, and that important developmental processes may be interrupted, including obtaining qualifications, establishing a vocation, attaining financial independence and forming social networks (Fleminger & Ponsford, 2005; Thornhill et al., 2000). Thus, in addition to direct medical and non-medical costs, TBI is associated with significant indirect costs relating to lost productivity due to reduced/lost employability and personal costs including diminished quality of life (Berg et al., 2005). The burden of TBI on public health and social care is therefore substantial (Thurman et al., 1999).

Persistent functional limitations and failure to return to employment after TBI are particularly associated with cognitive impairments (Green, Colella et al., 2008; van Velzen et al., 2009; Whitnall et al., 2006). Amongst the most commonly affected domains of cognitive function are verbal learning and memory, executive function, and information processing speed (Draper & Ponsford, 2008; Levin et al., 1990; Ponsford & Kinsella, 1992; Salmond, Chatfield, Menon, Pickard, & Sahakian, 2005). Although many patients show improvement of cognitive function following their TBI, for a significant number of patients certain impairments persist in the long-term (Dikmen et al., 2009; Draper & Ponsford, 2008; Ruttan, Martin, Liu, Colella, & Green, 2008; Salmond, Menon, Chatfield, Pickard, & Sahakian, 2006). Given the scale of the problem and the costs associated with TBI, further research to investigate brain-behaviour relationships in TBI is highly motivated, and yet such research remains markedly under-resourced

(Lowenstein, 2009; Maas, Stocchetti, & Bullock, 2008). Understanding of the neural underpinnings of the cognitive impairments frequently observed after TBI therefore remains limited. Their identification is further complicated by the heterogeneity of TBI, and, together, these issues contribute to the difficulty in developing effective treatments. This is why further cross-disciplinary work in this field is needed to elucidate the pathophysiologal mechanisms associated with cognitive dysfunction after TBI. The present programme of research assesses a sample of TBI patients using a combination of neuroimaging and cognitive measures and explores their interrelationships.

Currently, the primary investigation of choice for the detection of clinically important brain injury is **computed tomography** (CT) imaging. Brain imaging is used in emergency departments to assess the acute neural consequences of head injury and to identify those patients who are likely to go on to develop serious clinical sequelae (National Collaborating Centre for Acute Care, 2007).

Standard brain imaging, however, cannot detect the diffuse axonal injury (DAI) that may cause subtle disruption of the structural integrity of white matter tissue (Symms, Jäger, Schmierer, & Yousry, 2004). Evidence is beginning to accumulate that individual differences in the structure of white matter may have behavioural relevance (see Johansen-Berg, 2010, for a review) and that loss of white matter integrity may contribute to cognitive impairment in neurological conditions (see Chapter 5, for further discussion). Diffusion tensor imaging (DTI; Basser, Matiello, & Le Bihan, 1994; see section 1.6, pp. 36-40) is an advanced **magnetic resonance imaging** (MRI) technique that holds the promise of substantially improving detection of white matter damage following TBI (Kou et al., 2010; see Chapter 4). The research programme discussed in the subsequent chapters investigates the relationships between the neural and cognitive sequelae of TBI using structural MRI, including DTI, and neuropsychological assessment. Specifically, the principal aim of the research is to identify, using DTI, the white matter correlates of the most prominent cognitive deficits in a group of TBI patients. Having the ability to accurately map after brain injury the functionally relevant changes in white matter structure could inform and considerably improve TBI assessment, clinical management, and outcome prediction.

The following sections provide brief overviews of the definition, epidemiology and aetiology, injury mechanisms and neuropathology, severity classification, and the radiological

investigation of TBI, in particular the potential of DTI in the investigation of white matter injury. The topic of cognitive impairment associated with TBI is then introduced, and the cognitive functions commonly found impaired briefly discussed. A particular emphasis will be on how DTI can be used to investigate the relationship between white matter injury and cognitive impairment following TBI. Finally, a summary of the overall aims, main research questions and hypotheses will conclude this Introduction.

1.2 What Is 'Traumatic Brain Injury'?

1.2.1 Definition. Traumatic brain injury (TBI) is indicated by the combination of a head trauma and a subsequent constellation of neurological, cognitive and behavioural/emotional sequelae, resulting from a cascade of events triggered by the trauma (Kibby & Long, 1996). Traumatic brain injury is one type of an acquired brain injury; other types including, for example, stroke and anoxic/hypoxic injury. The occurrence of a head trauma does not always imply TBI, especially if there are no neurological signs (Maas et al., 2008). Furthermore, subjective symptoms or cognitive impairments can arise following a head trauma, some of which are related to other reasons (e.g. secondary to stress or mood disturbance), which may not in themselves be definitively diagnostic of TBI.

In general, however, a TBI can be diagnosed where there is objective neuroimaging or clinical evidence of neuropathological changes and/or post-injury signs and symptoms consistent with TBI. These typically include: 1) loss of consciousness, 2) confusion or disorientation, 3) post-traumatic amnesia (PTA), i.e. confused or absent memory for a period of time following the trauma, and 4) focal neurological signs such as decreased sensation or perceptual abilities, loss of balance, general weakness, difficulty walking, abnormal reflexes, persistent headache, seizures, and language problems (Carroll et al., 2004; National Collaborating Centre for Acute Care, 2007).

The Mayo Classification System for Traumatic Brain Injury Severity (Malec et al., 2007) was used in the current research to classify TBI as moderate/severe (definite), mild (probable) or symptomatic (possible), depending on the presence and degree of the above criteria. For further detail on the bases of these classifications and how the Mayo criteria were applied here,

the reader is referred to Chapter 2. Section 1.4.5 (pp. 27-29) of this chapter will discuss issues around TBI severity in general.

1.2.2 Characteristic sequelae. Typical physical, somatic, and sensory effects of TBI include seizures, loss of coordination, partial paralysis, sleep disturbance, fatigue, dizziness, headaches, nausea, visual disturbances, sensitivity to light and sound, and loss of hearing or sense of smell (e.g. Riggio & Wong, 2009). Common hormonal effects include single or multiple pituitary-target neuroendocrine disruption (Rothman, Arciniegas, Filley, & Wierman, 2007). The focus here, however, will be on the characteristic range of cognitive impairments following TBI, discussed below (section 1.7, pp. 40-48), as well as in empirical chapters 3 and 5.

Apart from the above, TBI can result in a variety of behavioural and emotional sequelae that amongst others can include personality changes, agitation and aggression, disinhibition, apathy, and motivational impairment (see e.g. Reeves & Panguluri, 2011). Mood disturbance is common after TBI, with clinically significant anxiety and depression frequently reported (Bowen Neumann, Conners, Tennant, & Chamberlain, 1998; Jorge & Starkstein, 2005). Some of these sequelae are not direct effects of the brain trauma, but rather psychogenic effects relating to factors such as psychological reaction to the consequences of TBI, psychosocial stressors, the individual's coping strategies, or medicolegal issues. The degree of each of the sequelae may vary both according to TBI severity and as a function of time since the injury.

Associated with these diverse consequences (other than those which are of psychogenic origin) is damage to the physical integrity of nerve cells resulting from TBI, which is often widespread rather than locally restricted. A severe head injury can damage the brain in several ways, and this can lead to a variety of complications. Some types of brain damage are temporary, whilst others can result in permanent damage. Section 1.4 (pp. 22-32) discusses these injury mechanisms and TBI neuropathology.

1.3 Epidemiology and Aetiology of Traumatic Brain Injury

Epidemiological and aetiological data on TBI are currently rather patchy. With this in mind, Tagliaferri, Compagnone, Korsic, Servadei and Kraus (2006) aimed to compile European

data on brain injury epidemiology through a meta-analytic review of 23 national and regional studies in 13 countries. This task proved challenging, as prevalence of TBI (i.e. the total number of cases in a given population at a specific time), a key piece of epidemiological information, is rarely reported in the literature. Although it would be possible to derive an estimate of TBI prevalence based on its incidence (i.e. the total number of new cases during a specific period of time) multiplied by the length of lifespan post-TBI, it is difficult to derive an estimate of lifetime duration of TBI sequelae, partly as a result of the lack of clear and commonly agreed-upon definition for TBI (see Menon, Schwab, Wright, & Maas, 2010). Thus, no reliable data are available on TBI prevalence in Europe. However, using Tagliaferri and colleagues' (2006) conservative estimate of an average of 10 years of TBI-related disability during a survivor's lifetime, it is possible that in 2010 as many as 11,775,850 individuals were living within the European Union (population of 501.1 million; Eurostat, 2010) who were coping with the effects of TBI.

Incidence of TBI in developed countries, including the UK, has been estimated to be in the region of 200-300 new cases per 100,000 annually (Torner, Schootman, Rizzo, & Tranel, 1996). The incidence has a bimodal age distribution in that it peaks both in late adolescence/early adulthood and again after the age of 70 years (Marquez de la Plata et al., 2008). Advancing age has been found in several studies to affect outcome so that older TBI survivors in general have worse outcomes (e.g. Flanagan, Hibbard, & Gordon, 2005; Katz & Alexander, 1994). The reasons for this may include the brain's limited plasticity and capacity for compensation as well as age-related progressive cognitive decline that would disproportionately affect the older survivors. Older individuals (≥65 years) are also more likely than the young to be hospitalised following their TBI (Rutland-Brown, Langlois, Thomas, & Xi, 2006). According to the National Collaborating Centre for Acute Care (2007), 70-88 per cent of all people in the UK who sustain a head injury are male, and overall, males are reported to experience a TBI about twice as often as females (Langlois, Rutland-Brown, & Thomas, 2004).

The TBI-related fatality rate has been reported to be two to three times lower in the UK than in several other developed countries, including France, Spain, Australia and the United States (Jennett, 1996). Tagliaferri et al. (2006) reported an aggregate European rate of 235 fatal and hospitalised TBIs in a population of 100,000 as well as an average mortality rate of 15 TBI-related deaths per a population of 100,000. The UK incidence of hospital admissions due to TBI

is estimated at 253 per 100,000 (Tagliaferri et al., 2006), with over 15,000 intensive care unit beds occupied by TBI survivors annually (National Collaborating Centre for Acute Care, 2007). Morbidity following TBI is disproportionately increased in the younger age groups, and long-term morbidity overall is increased in TBI survivors compared with the general population (Cameron, Purdie, Kliewer, & McClure, 2008).

Thirteen of the studies reviewed by Tagliaferri et al. (2006) provided data on aetiology. Across these studies, road traffic accidents (RTA) were found to be the most common cause of injury, falls the second, and violent assaults the third most common. However, the variation between studies carried out in different regions and countries was considerable. For instance, a comparison between three of the studies, one from the Glasgow region of the UK (Thornhill et al., 2000), another one from the Aquitaine region of Italy (Masson et al., 2001), and the third one from Finland (Alaranta, Koskinen, Leppänen, & Palomäki, 2000), reveals quite different proportions of the three most consistently reported causes, as shown in Table 1-1.

Whilst the top cause of TBI in the Thornhill et al. (2000) study as well as in the Alaranta et al. (2000) study was falls, it was RTAs in the Masson et al. (2001) study. Strikingly, violent assaults were reported to have caused a considerably larger proportion of TBIs in the Glasgow study than either in the Aquitaine or Finnish study. By contrast, the proportion of TBIs resulting from RTAs was much smaller in Glasgow than in Aquitaine, and also smaller than their proportion in Finland. Finally, TBIs caused by falls appear to be clearly more common in Finland than in either Glasgow or Aquitaine.

Table 1-1

Traumatic Brain Injuries Caused by Each Main Cause in Three European Regions

Region (Study)	Road traffic accidents (RTAs)	Falls	Violent assaults	Other causes
Glasgow, Scotland (Thornhill et al., 2000)	11%	46%	28%	15%
Aquitaine, Italy (Masson et al., 2001)	48%	42%	3%	7%
Finland (Alaranta et al., 2000)	26%	61%	5%	8%

There is further variation in how these causes relate to TBI severity (see section 1.4.5, pp. 27-29). For example, in the UK overall, falls are estimated to cause 22-43 per cent, assaults

30-50 per cent, and RTAs approximately 25 per cent of mild head injuries. The proportion of RTA-related head injuries classified as moderate/severe is considerably greater (National Collaborating Centre for Acute Care, 2007).

These summary data highlight the difficulty of obtaining consistent estimates of TBI aetiology across studies even as it relates to the most commonly reported causes in developed European countries. Amalgamating data from several studies carried out in different regions is an extremely challenging task, plagued by problems of marked variation in aetiology as well as inconsistencies of TBI case definition, assessment, management and outcome evaluation (Menon et al., 2010). Furthermore, accurate epidemiological data cannot be derived from non-population-based studies. As noted by Cameron et al. (2008), conclusions drawn from the literature are limited by the design and methodological shortcomings of most TBI outcome studies. Apart from non-population-based sampling, these include small sample size, injury severity-specific sampling, lack of appropriate control groups, not controlling for potential confounders such as pre-TBI health status, inadequate length of follow-up and considerable loss of participants to follow-up that can bias findings. Thus, although the figures presented here do give some idea of the scale and nature of the problem, they should be considered as rough estimates only of the true extent of the burden from TBI.

1.4 Injury Mechanisms, Neuropathology and Severity of Injury

1.4.1 Physical mechanisms. The principal physical injury mechanism in TBI is *impact loading* (collision of the head with a solid object at speed) (Goldsmith, 1966; in Halliday, 1999), associated with a combination of contact and inertial forces. *Contact force* occurs at the time of impact if the head is prevented from moving, whereas *inertial force* occurs as the head is set in motion by the impact and rapidly accelerates. These forces can injure the brain if the tissue is compressed (i.e. compressive strain) or stretched (i.e. tensile strain) beyond its structural tolerance (Gennarelli & Meaney, 1996). A third type of tissue strain is shear strain, the least well tolerated type of tissue deformation that occurs as one tissue slides against another (Halliday, 1999). According to Goldsmith (1966), other types of physical injury mechanisms include impulsive loading that without significant physical impact results in sudden motion of the head

as well as static/quasistatic loading in which the speed of the occurrence is not critical (e.g. when the head is trapped between slowly moving rigid structures). These are much less common as causes of TBI than impact loading, however (Halliday, 1999).

The mechanical forces and the ensuing tissue strain can result in complex anatomic and physiologic abnormalities. Properties of the cranium and the intracranial components, including mechanically important features of the interior surface of the skull, subarachnoid space, cerebrospinal fluid (CSF), and intracranial soft tissue, predispose the brain to particular types of injury (Halliday, 1999).

1.4.2 Primary and secondary injury. Direct or indirect impact to the brain triggers a cascade of pathophysiological events. Some of these emerge within hours of TBI and resolve over time, whilst others can persist or gradually develop over long periods of time (Beretta, Gemma, Anzalone, 2008; Kou et al., 2010). Types of brain injury giving rise to the neuropathological changes in TBI are often classified as primary or secondary (delayed) injury, depending on at which point following the initial impact they occur. Where the impact causes the head to bend about the centre of angular acceleration, located in the lower or middle cervical spine, both contact and inertial forces are involved (Halliday, 1999). First, as the brain undergoes acceleration, the soft brain tissue is set in motion inside the skull, and contact and inertial forces cause focal injuries such as 'coup' contusions and intracerebral and subdural haematomas (see Figure 1-1A). The mechanical disruption of brain tissue due to the angular acceleration and its associated inertial forces can produce any type of injury apart from skull fractures and epidural haematomas, caused by laceration of veins and arteries by fractured bone edges (Halliday, 1999). Patients who sustain a skull fracture, caused by contact forces producing strain that exceeds the skull's tolerance, are also at a high risk for intracranial injury (Borg et al., 2004; National Collaborating Centre for Acute Care, 2007).

Some hours following TBI, a process known as secondary injury that leads to cellular damage begins to develop due to factors such as hypoxia, oedema or raised intracranial pressure, and can still be ongoing several months, even years, later (Graham, Adams, Nicoll, Maxwell, & Gennarelli, 1995). For example, in a related study reported elsewhere (Ramlackhansingh et al., 2011), our group found evidence in chronic TBI patients of increased microglial activation in subcortical structures, most likely reflecting an ongoing inflammatory

response to TBI that was found up to 17 years post-injury. Brain microglial cells are sensitive to the neural insult in TBI and rapidly become activated, beginning to secrete cytokines (i.e. small protein molecules that act as cellular regulators), and act to prevent further damage to the neural tissue (see Gehrmann, 1996; Gehrmann, Matsumoto, & Kreutzberg, 1995, for reviews). These recent *in vivo* results corroborate previous post-mortem findings in humans, demonstrating the presence of a long-term inflammatory response following TBI (Gentleman et al., 2004), and suggest that instead of being a single event, TBI actually triggers a cascade of neuropathological events, some of which persist in the long-term.

In general, secondary injury refers to various neurochemical and molecular alterations that cause damage by disrupting cerebral blood flow, ion homeostasis and metabolism, or by having direct neurotoxic effects on brain cells (e.g. Raghupathi, Graham, & McIntosh, 2000). These processes may also contribute to overall brain tissue atrophy that can increase over time post-injury (see Bigler, 2001a, for a review), although there is also evidence to suggest that overall brain tissue density remains relatively stable after head injury (Salmond, Menon, Chatfield, Pickard et al., 2006).

1.4.3 'Focal' injury. As Bigler (2001a) points out, use of the term 'lesion' when referring to brain damage resulting from a TBI can be misleading. What may seem like an isolated lesion on standard clinical neuroimaging of TBI (see section 1.5, pp. 33-36) can always involve more extensive damage to the brain. For example, the tissue and vascular disruption as part of primary injury often results in **brain contusions** that primarily cause damage to cerebral grey matter, but that can also extend into the white matter. So-called gliding contusions that affect subcortical structures, and are seen especially at grey-white matter junctions, can also result from the angular acceleration of the impact and cause displacement of brain structures (Halliday, 1999). 'Coup' contusions, often found under the site of the initial impact, are likely to arise from a situation in which a negative pressure is created by the deformation of the skull at the time of impact that is followed by a rapid return to its usual shape (Gennarelli & Meaney, 1996; Halliday, 1999). 'Contrecoup' contusions that resemble coup contusions pathologically affect a site remote from the original point of impact (often, but not necessarily, opposite to it), and are likely to arise from inertial loading that develops from the slight lag between the acceleration of the hard skull and dura and the soft brain (Halliday, 1999). The exact

mechanisms of cerebral contusion formation remain under debate, but their typical pattern following TBI is predominantly fronto-temporal, with regions most frequently affected including the anterior and inferior frontal and temporal regions and the medial temporal region/limbic system (Adams, Graham, Scott, Parker, & Doyle, 1980; Halliday, 1999; Povlishock & Katz, 2005; Ratnaike, Hastie, Gregson, & Mitchell, 2011).

Apart from contusions, the other main 'focal-appearing' neuropathological sequelae of closed-head TBI are subdural, epidural, and intracerebral haematomas. Figure 1-1A illustrates how these lesions can alter the original anatomical location of other brain structures, and may cause the midline of the brain to shift. In the case of haemorrhages that include subarachnoid, intraventricular, and tissue tear types, a simple division between focal and diffuse injury may not hold, and the same applies for cerebral swelling and ischemia. Axonal injury associated with structural disconnection, the neuropathological event of primary interest here, is characterised by its widespread distribution (Halliday, 1999; Saatman et al., 2008; see the next section). Chapter 3 will provide further detail regarding 'focal' injury following TBI, in particular the occurrence of brain contusions and **microbleeds** as observed in the current research sample.

1.4.4 Diffuse axonal injury (DAI). Microbleeds are petechial haemorrhages that are associated with DAI following TBI (De Coene et al., 1992; Scheid, Preul, Gruber, Wiggins, & von Cramon, 2003). However, there is no one-to-one correspondence between the number of microbleeds and the degree of severity of DAI (Scheid, Walther, Guthke, Preul, & von Cramon, 2006). Figure 1-1B shows a histopathology slide from a patient who has sustained a severe TBI (Meythaler, Peduzzi, Eleftheriou, & Novack, 2001), illustrating microbleeds and DAI as observed in the corpus callosum of the patient.

White matter structures located in different parts of the brain have different tissue densities and, thus, also distinct mechanical response characteristics. These characteristics determine the way that the injured structure responds to the application of mechanical energy (Meythaler et al., 2001). In a high-speed RTA inertial forces are generated due to the rotational acceleration of the brain, which can result in disarray of intracellular contents, also at sites distant from the original site of impact. However, although DAI can lead to widespread disruption of the structural integrity of white matter and is the predominant injury mechanism in a large proportion of TBIs, axonal fibres in certain regions are particularly susceptible to DAI

(Meythaler et al., 2001). White matter structures at a high risk include the corpus callosum, parasagittal and superior frontal white matter, frontal and temporal grey-white matter junctions, and the brain stem (Lux, 2007; Meythaler et al., 2001; Scheid et al., 2003).

Despite axons' viscoelastic nature and normally substantial tolerance to stretching, an impact loading TBI can severely damage them (Tang-Schomer, Patel, Baas, & Smith, 2010). The diffuse pattern of injury that results from the shearing and tearing forces during rotational acceleration of the brain can destruct small cytoskeletal components of axons known as neurofilaments and microtubules (see Chapter 4). The localised mechanical damage causes primary axonal swelling, which is followed by disruption of the intracellular components, with potential to ultimately lead to axonal degeneration (Büki & Povlishock, 2006). This secondary degeneration is considered to be the critical mechanism of axonal disconnection for the majority of injured axons (see Li et al., 2010, for a review). The evolution of this process is illustrated in Figure 1-1C (Smith & Meaney, 2000).

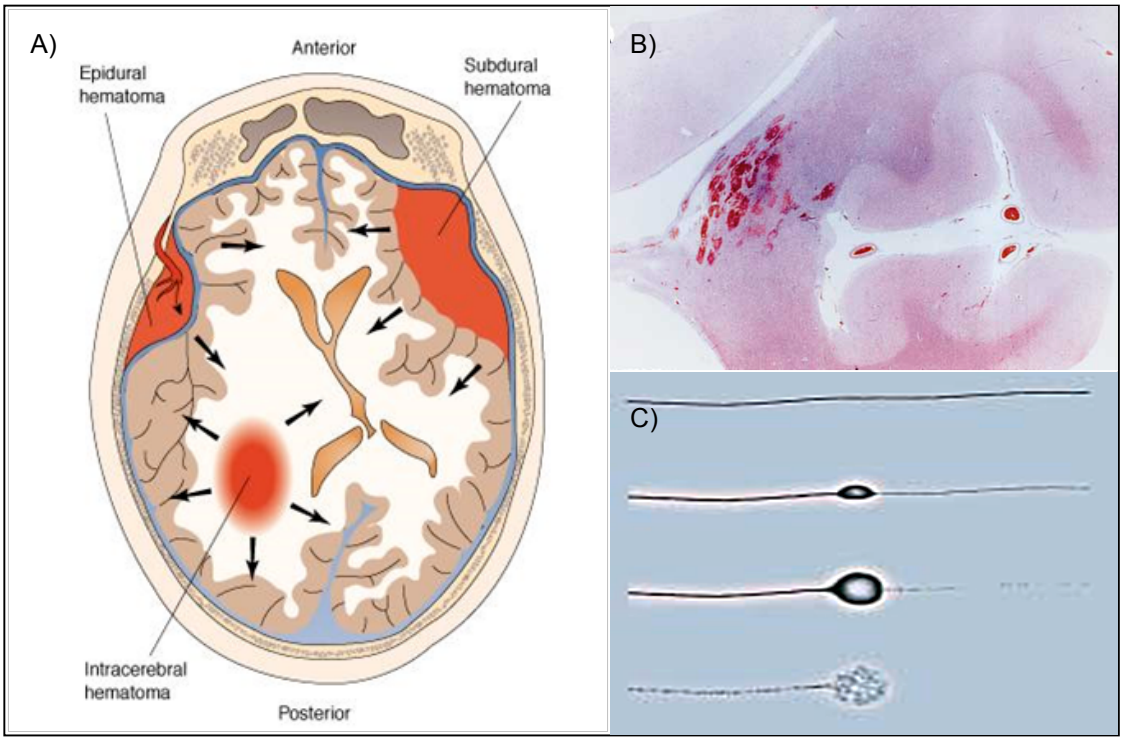

Figure 1-1. 'Lesions' in traumatic brain injury. A) Types of haematoma. Retrieved March 17, 2011, from: https://doctor2008.wordpress.com/tag/epidural-hematoma. B) Diffuse axonal injury and microbleeds in the corpus callosum. Reprinted from Archives of Physical Medicine and Rehabilitation October 2001, 82/10, Meythaler, J. M., Peduzzi, J. D., Eleftheriou, E., & Novack, T. A., Current concepts: Diffuse axonal injury-associated traumatic brain injury, 1461-1471, Copyright (2011), with permission from Elsevier. C) The process of axonal disconnection. Reprinted from The Neuroscientist December 2000, 6/6, Smith, D. H., & Meaney, D. F., Axonal damage in traumatic brain injury, 483-495, Copyright (2011), with permission from Sage Publications.

As the mechanical forces cause the axons to stretch and tear and the usually linear arrangement of the axonal cytoskeleton is lost, the normal flow of water molecules within the axons also becomes interrupted (Büki & Povlishock, 2006). These microscopic characteristics of axonal injury can now be probed using diffusion tensor imaging (DTI), an MRI technique applied to study the diffusion of water molecules in brain tissue (see section 1.6, pp. 36-40). This technique can detect abnormalities in damaged white matter that on standard clinical neuroimaging is deemed to appear normal (e.g. Rugg-Gunn, Symms, Barker, Greenwood, & Duncan, 2001).

Considering the potential of DAI to cause widespread damage to brain tissue, it may also critically contribute to cognitive impairment following TBI (Lux, 2007; Scheid et al., 2006; Sugiyama et al., 2007). A possible mechanism for this would be that DAI interrupts the structural integrity of white matter tracts interconnecting large-scale neural networks that support complex cognitive functions (Mesulam, 1998). For example, impairments of memory and executive function, which have been observed in TBI patients with DAI (Scheid et al., 2006), could relate to the widespread disruption caused to the axonal cytoskeleton by TBI and its associated mechanical forces (Meythaler et al., 2001). Using DTI, it has become possible to investigate the effects of brain injury on the structure of white matter tracts as well as to explore the behavioural relevance of these brain structural abnormalities. For a more detailed discussion of the use of DTI in the assessment of axonal injury following TBI and relationships with cognitive function the reader is referred to chapters 4 and 5.

1.4.5 Classification of injury severity. As mentioned above, inconsistency in the definition of TBI and discrepancy in research methods have made TBI epidemiology difficult to describe accurately. A further complication is the ongoing inconsistency in the classification of TBI severity.

Most typically, TBI severity is classified as mild, moderate or severe based on a patient's score on the Glasgow Coma Scale (GCS; Teasdale & Jennett, 1974), recorded within 48 hours of injury. Based on the patient's verbal responses, physical reflexes and ease of eye opening, GCS score ranging from 3 to 15 is assigned, with higher scores indicating less severe injuries. As highlighted by Zuercher et al. (2009), though, in a critical review of the use of the GCS in TBI assessment, inconsistency in the timing of the initial assessment and in the order in

which each item of the scale is administered, as well as the presence of various confounders, diminishes the reliability of the score in clinical and research settings. Moreover, while the GCS score is easy to record, and as such practical in the early clinical management of TBI, it does not provide information about the specific pathophysiologic mechanisms that underlie the observable neurological deficits (Saatman et al., 2008). Thus, the severity of injury in patients recruited for the present research was estimated using the Mayo Classification System for Traumatic Brain Injury Severity (Malec et al., 2007), a system that integrates information about the lowest recorded Glasgow Coma Scale score in the first 24 hours, length of PTA, duration of loss of consciousness, and initial neuroimaging results. Chapter 2 includes the details regarding the basis of determining the presence of TBI in the current patient sample and how the Mayo system was used for classifying injury severity.

Overall, mild TBIs are by far the most prevalent, with the percentage of moderate to severe injuries out of all hospitalised head injury cases being in the range of 10-30 per cent (Cassidy et al., 2004; Tagliaferri et al., 2006). However, because a significant proportion of patients with mild TBIs do not receive hospital treatment, TBI overall is likely to be a considerably larger problem than suggested by official records. Moreover, some of the patients who do receive medical attention initially are discharged from the emergency department without receiving further care and sometimes also without detailed documentation of their injury.

These issues make it difficult to collect accurate data on TBI severity, also acting to bias the epidemiological data. Fewer than half of the European studies reviewed by Tagliaferri et al. (2006) provided information regarding TBI severity. As expected, patients with TBIs classified as mild were the largest severity group in the majority of these studies. However, it is difficult to compare severity data between studies when the classification systems used for severity stratification vary as they did in the reviewed studies. This inconsistency and the potential inaccuracy in TBI diagnosis and severity classification are compounded by the inherent heterogeneity of TBI that further complicates outcome prediction.

Similar clinical characteristics and standard post-injury care can result in very different outcomes, and this significant individual variability includes long-term outcomes (Wagner, 2010). While most patients show at least some functional improvement, and some show dramatic improvement, during the first one to five years post-injury, others remain the same and a small minority seem to decline (Hammond et al., 2004). This presents a major challenge for

both treatment planning and outcome prediction and underlines the need for a valid and reliable multidimensional classification system that could be used to identify specific patterns of injury after TBI and match these with targeted treatments. This could then, potentially, lead to improved outcomes through more individualised interventions (Saatman et al., 2008). It is worth noting, though, that a range of other factors including diverse rehabilitative interventions, social circumstances, and psychiatric conditions also contribute to TBI outcome, which is why predicting it remains a challenge, even with better indices of injury severity.

However, a particular area where there is room for improvement in current TBI stratification systems is that they do not incorporate measures of the degree of white matter injury. The identification of relevant biomarkers that could be used to more fully characterise patterns of brain injury associated with the different degrees of TBI severity would be a step forward. Diffusion tensor imaging, in particular, could prove useful in providing such biomarkers, although its use in clinical diagnosis of TBI remains to be further validated (Huisman et al., 2004; Lee et al., 2008; see Chapter 4, for further discussion). Such research could answer important clinical questions about the mechanisms, prognosis, and treatment of TBI. For example, identifying a specific 'signature' of white matter damage after TBI associated with different types and degrees of cognitive impairment could have important implications for early outcome prediction and the planning of maximally effective TBI management and rehabilitation practices.

1.4.6 Relationships between injury severity, neuropathology and outcome. Brain injury is capable of altering the physical status of brain cells and vasculature, and can result from any degree of stretching, twisting or compression, but moderate to severe strain is required for more severe degrees of injury (Bigler, 2001b; Gennarelli & Meaney, 1996). Thus, the extent and severity of damage that ensues is related to the strength of the mechanical force applied during the impact (Kibby & Long, 1996). For example, different strain loads produced by by different injuries also mean varying degrees of damage to the cytoarchitecture of axons and, as a result of this, different degrees of severity of axonal damage (Bigler, 2001b; see Chapter 4). Furthermore, there is recent experimental evidence from an animal model of closed-head injury that the direction of head motion caused by rotational inertial forces plays a role in determining the severity of the neuropathological response, with motion along the sagittal plane

being particularly involved in more severe degrees of injury (Eucker, Smith, Ralston, Friess, & Margulies, 2011). This thesis primarily deals with the effects of TBI on the structure of axonal fibres, which is why the role of vascular disruption in the pathophysiology of TBI, associated with cerebral ischemia and infarction, is not discussed here in any more detail, but the interested reader is instead referred to DeWitt and Prough (2003) for a comprehensive overview of this topic.

Despite the problems relating to its classification, injury severity is widely regarded to be among the most significant predictors of TBI outcome (Mushkudiani et al., 2008; Schönberger, Ponsford, Reutens, Beare, & O'Sullivan, 2009). Schönberger et al. (2009) were the first to investigate whether injury severity together with age, another known predictor of TBI outcome, may also relate to the extent of residual brain injury observed in the chronic stages of TBI. Thus, they studied the relationships between age, injury severity (as indexed by GCS scores and PTA duration) and the extent of residual brain damage (degree of brain atrophy and lesion volumes) in 98 TBI patients 2.3 years post-injury on average. Controlling for gender, TBI aetiology, time since injury, and total brain tissue volume, their regression analyses revealed that older age at injury and longer PTA together were associated with larger grey and white matter lesion volumes in a range of brain regions. In addition, longer duration of PTA predicted smaller residual white matter volumes. They also found that older age predicted smaller 'lesion-free' grey matter volumes in several regions, particularly in all neocortical regions, and in the frontal lobe specifically. Surprisingly, though, in the light of a number of studies that have recently demonstrated a relationship between advancing age and breakdown of white matter integrity (see Madden, Bennett, & Song, 2009, for a review), Schönberger et al. (2009) did not find age to be associated with volumes of lesion-free white-matter. This may be partly explained by their use of conventional MRI, though: standard structural MRI is not capable of detecting subtle changes in white matter structure. These findings, as well as emphasising the associations in TBI between initial indirect (clinical) indices of injury severity and subsequent neural pathology, suggest that age may modulate these relationships.

The majority of patients with a mild TBI appear to show a good outcome, and are in general free of residual symptoms after the first year post-injury (Carroll et al., 2004). However, a proportion of such patients experience persistent 'post-concussive symptoms'. A concussion is indicated by transient confusion following a head trauma, with full recovery normally expected

within two to three weeks (Anderson, Heitger, & Macleod, 2006). As there is no common agreement, the terms concussion and mild TBI are often not clearly distinguished in the literature, which complicates the interpretation of research findings (see Bigler, 2008, for a review). Here, concussion and mild TBI are viewed as follows: concussion refers to a symptom of head injury that has not resulted in loss of consciousness or PTA and in the Mayo system (Malec et al., 2007; see Chapter 2) corresponds to symptomatic (possible) TBI, whilst head injuries associated with at least momentary loss of consciousness (<30 min) or PTA (<24 h) are classified as mild (probable) TBIs.

'Post-concussive syndrome' (PCS) is characterised by a collection of markedly non-specific somatic, cognitive and behavioural/emotional symptoms including fatigue, headaches, dizziness, sleep problems, lowered tolerance for alcohol, problems with memory and concentration, irritability, and mood disturbance (Bigler, 2008; De Kruijk, Twijnstra, & Leffers, 2001). Whilst these complaints typically decline within the first few months post-injury, some patients experience subtle symptoms for longer (Williams, Potter, & Ryland, 2010). Post-concussive syndrome is aetiologically ambiguous, however, and many clinicians believe that persistent symptoms (even in cases involving momentary changes in consciousness) reflect psychological factors rather than organic damage. In other words, although mild head trauma is sometimes followed by ongoing problems in daily activities with memory and concentration, it remains controversial whether it can result in persisting sequelae other than those that can follow any traumatic experiences. In some patients there are also pre-injury factors (e.g. a psychiatric condition, alcohol or drug abuse or psychosocial problems) that may contribute to post-injury symptoms (e.g. Mathias & Coats, 1999). Mood disturbance is a common psychological reaction to traumatic events or to their practical consequences, and can also give rise to the kind of non-specific symptoms that fall under the umbrella of PCS, or that may be associated with brain injury.

Thus for instance Ponsford, Cameron, Fitzgerald, Grant and Mikocka-Walus (2011) assessed symptoms that characterise PCS and cognitive function in 123 patients with a suspected mild TBI, compared with 100 matched controls who had sustained traumatic injuries other than TBI. All participants were initially assessed in the emergency department and then at follow-ups first one week and then three months after the injury. Patients with suspected TBI showed significantly more severe post-concussive symptoms than non-TBI controls both initially

and one week post-injury. However, despite being more likely than controls to report ongoing cognitive problems in everyday life at the two follow-ups, the TBI group's performance was impaired on only one test tapping visual memory. Considering the large number of outcome measures used in the study, it is possible that the visual memory result does not reflect a true deficit in the TBI group. These findings highlight the issue that although some patients report cognitive sequelae or other symptoms after mild head trauma, their correspondence with objectively or clinically identifiable brain injury can be poor. Although mild head injury can result in brain damage, in many such cases the results of standard brain imaging and neurological examination are normal. Such observations have contributed to the controversy regarding whether the lasting problems sometimes observed after mild TBI stem from organic sources (Kibby & Long, 1996; Bigler, 2008; Bigler & Bazarian, 2010). In some cases this issue is further complicated by personal injury litigation (see Belanger, Curtiss, Demery, Lebowitz, & Vanderploeg, 2005, for a review).

It is not clear to what extent the symptoms of PCS correspond to the cognitive impairments often observed following more severe TBI, including deficits of memory, executive function, and information processing speed (see section 1.7, pp. 40-48), and further research is needed to explore the factors underlying persistent cognitive dysfunction across the spectrum of TBI severity.

It is possible that current problems with identifying the relationships between the cognitive sequelae of TBI and the presence of brain injury are partly due to the lack of sufficiently accurate diagnostic methods (Bazarian et al., 2007). The degree of neuropathology in mild TBI especially remains a particular challenge to accurately assess using the standard structural neuroimaging techniques (Niogi & Mukherjee, 2010; see the next section). As stretch-induced DAI is a key injury mechanism in TBI, and may be involved in the development of cognitive impairments and other symptoms, a diagnostic technique able to detect such damage would be a substantial step forward (Bazarian et al., 2007). One promising neuroimaging technique for the assessment of more subtle brain structural abnormalities following TBI is DTI. Section 1.8.3 (pp. 51-54) gives a brief overview of how DTI has been so far used to investigate the structure of white matter after TBI.

1.5 Structural Neuroimaging of Traumatic Brain Injury

1.5.1 Investigation of clinically important brain injuries. Neuroimaging is an important element in the clinical assessment and management of patients with brain injury. When brain injury following a head trauma is confirmed (or suspected), the specific location, nature and degree of neuropathological changes can be visualised using neuroimaging techniques, most often CT, but also MRI. This not only informs clinical diagnosis of TBI, its acute management, and outcome prediction, but is also valuable for characterising and classifying types of injury sustained by patients who are enrolled in clinical trials or other research (Borg et al., 2004; Coles, 2007; Duhaime et al., 2010). According to the National Institute for Health and Clinical Excellence (NICE) guidelines for head injury triage, assessment, investigation and early management, a 'clinically important brain injury' is "any acute condition that has been identified by imaging or by assessment of risk factors" (National Collaborating Centre for Acute Care, 2007, p. 5).

Neuroimaging, predominantly CT, in the acute stage (during the first hours and days post-injury) normally includes the identification of haematomas, diffuse cerebral swelling, ischemia and infarction, whilst further brain imaging during the subacute stage (when the patient is medically stable) can focus on assessing the presence of abnormalities that may include cortical contusions and DAI. During the post-acute/chronic phase, here defined as the time subsequent to the first month post-injury, a variety of clinical as well as experimental neuroimaging techniques can be applied to assess different structural and functional brain abnormalities (Gasparetto, Rueda Lopes, Domingues, & Domingues, 2011).

1.5.2 A historical perspective. In the UK, standard identification and characterisation of brain injury to determine the presence of skull fractures and estimate a patient's risk for further complications has seen a shift from predominant use of skull radiography in the 1980's and 1990's to the current convention of CT imaging (Gallagher, Hutchinson, & Pickard, 2007; National Collaborating Centre for Acute Care, 2007). Prior emphasis on skull fracture as a risk factor for intracranial complications as part of the first UK head injury assessment guidelines by a Working Party of Neurosurgeons resulted in the use of radiography. Apart from this, the identification of high-risk patients was based on clinical factors, particularly a patient's level of

consciousness (National Collaborating Centre for Acute Care, 2007). Subsequent modifications to these guidelines, along with technological advances and the increased availability of CT scanners within emergency departments, led to a considerable increase in CT imaging of head injury. For example, between 2002 and 2004 the number of CT requests more than doubled in hospitals (Hassan et al., 2005; in National Collaborating Centre for Acute Care, 2007). The belief that early imaging can improve outcome is also likely to have contributed to this considerable increase. However, what dramatically changed the use of CT to image head injury was the adoption of new evidence-based guidelines developed by the NICE (Gallagher et al., 2007). In addition, the European Brain Injury Consortium (Maas et al., 1997) developed consensus-based guidelines with the aim of harmonising procedures across brain injury research centres. The following section outlines current practice that reflects these guidelines.

1.5.3 Standard neuroimaging of TBI. In the acute stage following a head injury neuroimaging is performed to identify brain damage that requires urgent surgical intervention (Gallagher et al., 2007). The current NICE guidelines for the assessment of adult patients recommend immediate CT imaging if any of the following risk factors are present: GCS below 15, memory loss for events more than 30 minutes before the impact, suspected skull fracture, post-traumatic seizure, focal neurological deficits, or vomiting (National Collaborating Centre for Acute Care, 2007). Modern multi-detector high-resolution CT scanners allow rapid assessment of brain pathology and the need for neurosurgical intervention after acute head injury; they can reveal bony injury and damage associated with intracranial pathology, including skull fracture, blood clots, oedema and ischemia, haemorrhages, ventricular abnormality, and midline shift (Coles, 2007). It can be used even with patients who are severely injured and unstable, and because of the ease with which scans can be conducted, if there is degradation of images due to motion artefact, repeat scans can readily be performed. Once the patients are medically stable, MRI (or repeat CT) may be used to further assess the extent of brain injury (Gallagher et al., 2007). This is normally the case if the GCS has not risen to 15 by 24 hours post-injury (National Collaborating Centre for Acute Care, 2007).

Typical limitations of CT imaging include partial volume errors (i.e. averaging of properties of different substances within a volume) and partial obscurement of posterior fossa or parts of the temporal and frontal lobes, meaning that some pathological changes may remain

undetected (Coles, 2007). This can be particularly problematic considering that these regions are common injury sites following TBI. Computed tomography is also poor at visualising subcortical tissue within the corpus callosum, at grey-white matter junctions, periventicular zone, medial temporal and deep white matter, and the brain stem. Diffuse axonal injury is likely to affect these regions, and is better detected by MRI (Coles, 2007). Despite these limitations some CT findings (e.g. the presence of subarachnoid haemorrhage) and the Marshall score (Marshall et al., 1992) for categorising CT findings have been found to predict outcome in multivariate models of TBI (Murray et al., 2007).

Despite the routine use of CT, MR imaging provides substantially better spatial resolution and can provide important additional information about brain pathology. Issues relating to safety, availability, and logistics however contribute to MRI not currently being the primary recommended imaging technique for the investigation of clinically significant brain injury. The sensitivity of MRI to motion artefact makes it unsuitable for the assessment of some very severely injured and agitated patients. In the UK, patients rarely undergo acute MRI, but as the technology continues to develop and new equipment such as integrated physiological monitoring and anaesthetic equipment become more widely available, MRI may become more suitable for the assessment of unstable patients and thus more widely used. Metallic devices, implants or other foreign bodies, however, remain clear contraindications for MRI (Coles, 2007; Gallagher et al., 2007). Where MRI can be used, it is superior to CT for the identification of cortical and subcortical damage, particularly if the standard high-resolution sequence is complemented by gradient-echo or fluid attenuated inversion recovery (FLAIR) sequences.

Future developments hold promise for further increasing the understanding of the pathophysiology of TBI and exploring how the structural and physiological abnormalities observed may relate to functional outcome after TBI. This could also inform the development of new (and the enhancement of existing) therapeutic agents and interventions aimed at preventing further neuronal complications and thus improving outcome (Coles, 2007).

1.5.4 Towards new advanced imaging methods. Over the past decade it has become increasingly apparent that the standard imaging techniques are not capable of accurately detecting the full extent of white matter damage in TBI (Arfanakis et al., 2002; Rugg-Gunn et al., 2001). A more detailed clinical MRI investigation of TBI can include the use of gradient-echo

T2*-weighted or FLAIR pulse sequences that are sensitive to signal abnormality associated with traumatic microbleeds. Although these MRI techniques can detect white matter damage, techniques such as DTI are required to detect more subtle markers of axonal injury. Several experimental studies have to date demonstrated the capacity of DTI to identify such white matter damage following TBI (see FitzGerald & Crosson, 2011 or Niogi & Mukherjee, 2010, for a review). This could be especially useful for the detection of mild TBI, as currently some patients who have normal standard imaging results after mild TBI go on to experience persistent cognitive problems. If DTI were to reveal subtle abnormalities in white matter structure, and if these abnormalities were shown to consistently predict functional outcome, then their early detection on clinical neuroimaging would be of prognostic value.

There are a range of other brain imaging modalities, including functional MRI (fMRI), magnetoencephalography and electroencephalography, Xenon-enhanced CT, CT perfusion, single photon emission computed tomography (SPECT), positron emission tomography (PET) and MR spectroscopy (MRS). These have been used experimentally in brain injury research to image neural activation and different aspects of brain physiology (Coles, 2007). Although this diversity of assessment methods has the potential to provide important additional information, it also adds to the complexity within this field of research. Work is under way, however, to identify the techniques and indices which are most useful in assessing, classifying, and evaluating outcomes after TBI (see e.g. Duhaime et al., 2010; Maas et al., 2010; Saatman et al., 2008).

1.6 Diffusion Tensor Imaging (DTI)

1.6.1 Diffusion tensor imaging of brain white matter. Compared with conventional CT and MR imaging, diffusion-weighted MRI (Le Bihan & Breton, 1985) measures a fundamentally different physiological parameter in that the image contrast reflects the rate of diffusion (displacement) of water molecules in brain tissue. Investigation of the 3D process of diffusion-driven displacements of water molecules allows probing the structure of brain tissue at a fine scale, that is, to infer properties of tissue *microstructure,* beyond the conventional MR image resolution (Le Bihan et al., 2001). Therefore, diffusion MRI can differentiate between regions with reduced and elevated rates of diffusion and may reveal neuropathological changes

in cases where standard brain imaging cannot. **Diffusion tensor imaging** (Basser et al., 1994) is one application of diffusion MRI and has shown promise in the assessment of neuropathology of TBI, as it can be used to study the structure of white matter tracts that are susceptible to damage by TBI. Specifically, DTI can be used to measure the degree and spatial distribution of anisotropic diffusion within these tracts. The tracts run in three principal directions (superior-inferior, anterior-posterior, and left-right) and are visualised by reconstructing the diffusion-weighted imaging data (Huisman, Sorensen, Hergan, Gonzalez, & Schaefer, 2003).

1.6.2 The diffusion tensor model. The diffusing water molecules probe the local cellular environment of the axonal fibres, reflecting their microstructural characteristics. Diffusion-weighted imaging, by being sensitive to characteristics of the cellular barriers to diffusion, identifies the dispersion patterns of water molecules and, thus, acts as a unique probe of white matter structure (Alexander et al., 2010; Beaulieu, 2002) that can provide useful information about abnormalities following brain injury. Whilst some of this information is apparent from the diffusion-weighted MR images as they are, post-processing of the data is necessary in order to extract more detailed information and carry out statistical analysis (Parker, 2004).

The diffusion signal is often visualised in the form of a 3D vector field, known as the **diffusion tensor**. The tensor model is applied at each imaging voxel to determine the three mutually perpendicular eigenvalues (λ1, λ2, and λ3) that represent the magnitude of the diffusivity of water molecules in each of the three principal directions. The associated eigenvectors (V1, V2, and V3), one per each of the principal directions, can then be derived, as well as a number of additional DTI metrics that characterise the various properties of water diffusion within the axons (Basser and Pierpaoli, 1998). In this way, the tensor model can be used to infer from the imaging data the kind of tissue microstructure that appears to have given rise to the observed pattern of diffusion.

Chapter 2 has more detail on DTI as a technique and its application in the current research programme.

1.6.3 The biological basis of diffusion. Unlike in pure water where diffusion is based on a random *unhindered* pattern and the rate of diffusion is similar in all possible directions (i.e.

diffusion is *isotropic*), in healthy brain tissue cellular structures *restrict* the displacement of water molecules. The microarchitecture of brain tissue that includes membranes and cell walls thus determines the molecular displacement pattern (Alexander et al., 2010). In brain white matter water molecules diffuse more freely along the principal direction of the white matter tracts than they do perpendicular to the tracts. This preference has been labelled diffusion *anisotropy*, numerically approximated by a scalar measure called **fractional anisotropy** (FA), often used to index the degree of structural integrity of white matter. Normally, FA values range between 0 and 1.0, representing the normalised variance between the three diffusivity eigenvalues (as shown in section 2.8.4, p. 80). Greater anisotropy, indicated by a higher FA value is believed to reflect more coherent tissue structure (Arfanakis et al., 2002).

Regional variability in white matter structure and FA is likely to be based on differences in fibre myelination, fibre diameter, and fibre directionality (Bigler & Bazarian, 2010). In damaged white matter, where diffusion is more isotropic, diffusivity is increased perpendicular to the principal direction of the axons. This tendency can be approximated by **mean diffusivity** (MD), the average diffusivity in all directions, based on the three eigenvalues. Two further DTI metrics that are increasingly used in research are **axial diffusivity** (D_{ax}; diffusivity parallel to the main axis) and **radial diffusivity** (D_{rad}; diffusivity perpendicular to the main axis). Previous studies have suggested that anisotropy of diffusion in white matter is likely to primarily depend on intact axonal membranes, whilst changes in radial diffusivity potentially index structural changes in the myelin layer of axons that may act to modulate diffusion anisotropy (see Beaulieu, 2002, 2010). Mouse models of axonal damage and demyelination in particular have implicated axial and radial diffusivity as potential biomarkers of axonal and myelin loss, respectively (Budde et al., 2008; Budde, Xie, Cross, & Song, 2009; Song et al., 2002; Song et al., 2003; Song et al., 2005).

1.6.4 Limits of the tensor model. The diffusion tensor is a helpful way to describe the Gaussian distribution of water molecule displacements in each imaging voxel. Metrics derived from DTI, particularly FA and MD, have become popular as indices of white matter 'integrity' and damage, respectively, and have to date been shown in several studies to have relevance in terms of behaviour and outcome following neuronal injury or illness. However, because changes in these markers do not relate directly to abnormalities in specific features of tissue

microstructure and can be influenced by a variety of properties of the cellular environment, their biological determinants cannot be inferred directly from the diffusion tensor (Alexander et al., 2010).

Moreover, the size of DTI imaging voxels is at a scale of cubic millimetres (e.g. 1.75 x 1.75 x 2 mm), whilst the average size of an axon of the central nervous system is approximately 1μm in diameter. It follows that each voxel contains multiple axonal fibres, which in some cases may have distinct principal orientations. This could, through affecting the DTI metrics extracted from a given voxel, distort the FA value so that it appears excessively low (Tuch, Reese, Wiegell, & Wedeen, 2003). This issue is more relevant in those white matter regions that are characterised by fibre crossings than in voxels contained within white matter tracts that have a clear principal orientation, such as those containing the interhemispheric fibres of the corpus callosum (Jbabdi, Behrens, & Smith, 2010; see Chapter 4 for further description and discussion). The signal decay in diffusion MRI is also more complicated than can be acquired and analysed using DTI. Although *restricted diffusion* is the most apparent type of diffusion in neuronal tissue, mostly due to the directionally restricted water molecule displacement within axons, the entire signal decay also includes *free* and *hindered* diffusion. Alternative diffusion-weighted image acquisition and analysis frameworks include q-ball imaging (Tuch et al., 2003; Tuch, 2004) and the composite hindered and restricted model of diffusion (CHARMED; Assaf, Freidlin, Rohde, & Basser, 2004). Depending on the particular research question, these techniques can be applied, for example, to extract information specific to intra- and extra-axonal compartments and to model complex patterns of fibre orientation within an imaging voxel, or to estimate axonal diameter (Assaf & Cohen, 2009).

It is also important to recognise that the relationships between DTI metrics and underlying injury mechanisms can be complex and vary with time after injury. For example, an inflammatory response such as axonal swelling or cytotoxic oedema can lead to elevated FA in the early stages following a TBI (Bazarian et al., 2007; Mayer et al., 2010). Thus, highly anisotropic white matter does not necessarily imply the absence of neuropathology. Decreased FA, likely to reflect axonal damage, has been observed following both mild and moderate/severe TBI in various white matter tracts in a number of recent studies (e.g. Kraus et al., 2007; Sidaros et al., 2008; see Niogi & Mukherjee, 2010, for a review). There is a need for more longitudinal research to elucidate how changes in DTI indices of white matter structure

may reflect different neural responses to TBI at different stages post-injury, as well as cross-sectional studies to investigate the relationships between DTI findings and specific clinical/cognitive sequelae. Here, the main interest is in exploring how DTI-identified white matter abnormalities may relate to cognitive outcome in the post-acute/chronic phase following TBI.

1.7 Cognitive Impairment Associated with Traumatic Brain Injury

1.7.1 Cognitive impairment after TBI and neuroanatomical correlates. As well as impairments of complex cognitive functions including verbal learning and memory and executive function, deficits following TBI in basic cognitive parameters, particularly information processing speed, have been documented in numerous studies (Ponsford, 1995). It has also been demonstared that post-TBI impairments of memory, executive function and processing speed can persist in the long-term (Draper & Ponsford, 2008). The current research focuses on cognitive function within these three domains, as discussed in the following sections (1.7.2-1.7.4).

Converging evidence from neuropsychological, post-mortem neuropathological, and neuroimaging studies implicates the basal forebrain, medial temporal, and midbrain structures as frequent sites of damage by TBI and as brain regions that support the cognitive functions of interest hrere (e.g. Salmond et al., 2005). Menon (2003) notes that the TBI-associated 'traumatic penumbra' (i.e. brain tissue surrounding the focal injuries) is primarily found in the frontal and temporal lobes. Bigler (2008) adds that given the close proximity of many medial temporal lobe and midbrain structures to each other, these may be equally susceptible to injury via the mechanical impact in TBI. Due to their involvement in supporting cognitive function, structural damage sustained by frontal, temporal and midbrain structures could critically contribute to the frequently reported post-TBI impairments of learning and memory, executive function and information processing speed.

Nevertheless, the location or extent of focal brain injury often does not fully explain a TBI patient's cognitive problems (Bigler, 2001a). In part, this could be explained by much of the brain being at risk for DAI, the type of damage that is unlikely to be spatially restricted to perilesional areas. Furthermore, structural disconnection of key cognitive networks by DAI may

result in impared network function and cognitive impairments similar to those more traditionally attributed to focal injuries (Mesulam, 1998; Miller & D'Esposito, 2005). Disconnection caused by DAI could in fact occur in the absence of any apparent focal damage and yet impair cognitive function (Kolb & Wishaw, 2009).

The following sections briefly consider putative pathophysiological correlates of post-TBI impairments of verbal learning and memory; executive functions of set-shifting, cognitive flexibility and word generation fluency; and information processing speed. The empirical chapters 3 and 5 will provide more detail relating to specific aspects of the hypothesised relationships between brain structural abnormalities after TBI and these cognitive functions.

1.7.2 Neuroanatomical substrates to verbal learning and memory. Verbal learning and memory entail processes and strategies used by an individual for the encoding, storage and retrieval of verbal information. Impairments may reflect any or all of the following problems: encoding difficulty, poor use of organizational strategies, inappropriate retrieval strategies, or failure to retain information. Apart from different memory processes, a distinction has traditionally been made between short-term memory and long-term memory. Early studies of amnesic patients, especially H.M. (Scoville and Milner, 1957) and K.F. (Shallice and Warrington, 1970), who showed a double dissociation between short and long-term memory, provided convincing evidence for these to be anatomically distinct 'systems'. New theoretical models, an increasing interest in the neural basis of memory function, and increasingly sophisticated neuroimaging techniques have, however, shifted the focus to understanding human memory in terms of interrelated rather than separable memory systems.

Traditionally, long-term memory is conceptualised as explicit (or declarative) or implicit (or non-declarative). The content of declarative memory can be semantic or episodic. Whilst semantic memory stores general knowledge, not linked to a specific personal experience, episodic memory refers to memory for specific, time-linked events (episodes) experienced by an individual. Tulving (2002) refers to episodic memory as 'mental time travel', which usually involves recalling not only what happened (item information) but also where and how the events occurred (context information). Semantic and episodic memory systems are believed to support verbal long-term memory: whilst semantic memory refers to general knowledge, episodic memory refers to the learning, storage, and retrieval of information within a spatio-temporal

context (Tulving, 2002). Whether these two systems can truly be distinguished, particularly at the neural level, is questionable as interactions between various episodic, semantic and executive processes involved in long-term memory are likely (Hodges & Graham, 2001; Simons & Spiers, 2003; Squire, 2004). A general consensus from lesion studies of patients with focal brain injury has been that semantic and episodic memory are dissociable systems, whilst more recent functional neuroimaging research has demonstrated interactions between their neural correlates in specific brain regions in (e.g. Prince, Tsukiura, & Cabeza, 2007).

The general approach in neuroimaging research has been to attempt to delineate the neural underpinnings of episodic memory encoding and retrieval at a level of distributed neural networks. Interactions between medial temporal and prefrontal structures in particular have been implicated in these functions (Cabeza, Ciaramelli, Olson, & Moscovitch, 2008; Dickerson & Eicherbaum, 2010; Nyberg, 2008; Simons & Spiers, 2003). Thus, whilst damage to the medial temporal lobe itself almost always results in failures of some aspects of episodic memory function (Spiers, Maguire, & Burgess, 2001), damage to prefrontal structures may impair various 'executive' mnemonic processes, which transform perceptual representations into long-term memories and facilitate their retrieval (Simons & Spiers, 2003). Parietal regions are likewise believed to be involved in the efficiency of episodic memory processing, due to their role in supporting attention (Cabeza, 2008).

1.7.2.1 White matter connections of the hippocampal formation. A critical role in efficient memory encoding and recall has been proposed for an extended hippocampal-diencephalic system (Aggleton and Brown, 1999). This system comprises the hippocampi, the fornix, the mamillary bodies, the anterior thalami and the cingulum bundles, and has important efferent projections to the cingulate and prefrontal cortices.

Due to its location and arch-like shape, the fornix is particularly susceptible to the biomechanics of TBI, and indeed, fornix damage is frequently observed on structural MRI following moderate to severe TBI (Tate & Bigler, 2000). As well as structurally connecting the hippocampi with the diencephalon, the fornix connects the hippocampal formation with parts of the prefrontal cortex (PFC) (e.g. Croxson et al., 2005). Frey and Petrides (2002), for one, have highlighted the involvement of hippocampal-orbitofrontal interactions in episodic memory encoding. White matter connections such as these are particularly vulnerable in TBI, where

deformation of brain structures often occurs due to the mechanical forces and possible rotational movement (Bigler, 2008). Chapter 5 further discusses the role of the fornix in verbal learning and memory and the possible effects of fornix damage on memory function.

1.7.2.2 A link between the human default mode network (DMN) and memory? Since Greicius, Krasnow, Reiss and Menon (2003) conducted their network analysis of the default mode hypothesis – that certain brain regions show a coherent pattern of activity during rest and 'deactivation' during task performance - the *default mode network* (DMN) has been identified in several resting-state functional connectivity MRI studies. The DMN is indeed normally found to show greater activity during rest than during most externally oriented tasks (Damoiseaux & Greicius, 2009). The posterior cingulate cortex/retrosplenial cortex, the medial PFC and the medial temporal lobe are amongst the DMN's key nodes. These regions are also commonly activated during episodic memory retrieval tasks (Greicius, 2008; Greicius, Supekar, Menon, & Dougherty, 2009), and this apparent link between the default mode and regions that normally support episodic memory function has recently received considerable interest (see e.g. Sestieri, Corbetta, Romani, & Shulman, 2011). Although the DMN is not the focus of the current thesis, there is a link between the DMN and the discussion in Chapter 5 of the relevance to cognitive function of white matter tracts interconnecting its nodes, which will be considered in the General discussion (Chapter 6).

To summarise, given that verbal episodic memory is putatively supported by neural networks consisting of medial temporal, prefrontal and parietal regions connected via white matter tracts, and degradation of the structure of these tracts is likely as a consequence of TBI, structural disconnection could be an important mechanism of memory impairment following TBI. In particular, impairments of verbal learning and memory after TBI that are common (e.g. Draper & Ponsford, 2008), but may not be clearly linked with verifiable focal damage, could reflect the effects of such white matter damage disrupting the anatomical connectivity within large-scale functional networks. This hypothesis is explored in the present research.

1.7.3 Neuroanatomical substrates to executive functions. The specific cognitive functions that are considered 'executive' vary between individual theorists as well as the context and purpose of particular research projects. 'Executive function' as a broad construct includes a

diversity of cognitive processes relating to planning, initiating and organizing behaviour, reasoning, problem-solving, and decision-making. Furthermore, relationships between related concepts of 'executive function', 'cognitive control' and 'executive control' have not been clearly defined in the past. A recent collaborative project called Cognitive Atlas (http://www.cognitiveatlas.org/) led by Poldrack suggests that executive function and executive control are synonymous terms which refer to a top-down control system managing other cognitive processes in a goal-oriented manner, whilst cognitive control describes this modulation in a narrower sense. Executive control is required in everyday life for managing and adjusting behaviour according to the current context and goals. Successful control depends on the ability to configure appropriate task sets and direct attention towards relevant stimuli (Dumontheil, Gilbert, Burgess, & Otten, 2010).

1.7.3.1 Executive dysfunction in TBI. The variability in definitions of executive function and its disorders complicates both experimental studies and clinical assessment of executive function. Numerous methods are also used to assess executive impairment following brain injury across research and clinical settings, and they vary in sensitivity and specificity (Godefroy et al., 2010). It follows that a diversity of types of executive dysfunction are possible. In order to tackle these challenges, Godefroy et al. (2010) proposed criteria for defining and streamlining assessment of 'the dysexecutive syndrome'. They carried out a validation study of these criteria in a large sample of 461 patients with various neurological conditions including TBI, stroke and mild cognitive impairment, compared with 461 age- and education-matched controls. The cognitive deficits which emerged as most highly indicative of a dysexecutive syndrome related to the deduction and generation of rules; set maintenance; set-shifting and response inhibition; and generative fluency.

Difficulties with establishing and maintaining a task set and impairments of attention are common executive control deficits following TBI (e.g. Scheibel et al., 2007). Behaviour that is both context-relevant and goal-directed critically depends on these abilities being intact, which is why such impairments can cause daily inconvenience for an individual who has sustained TBI. Although behavioural changes observed in patients with frontal lesions commonly indicate executive dysfunction, including problems with set-maintenance and set-shifting, suppression of prepotent responses, behaviour- and error-monitoring difficulty, and deficits of sustained and

divided attention (Godefroy, 2003; Stuss & Benson, 1984), there is inconsistent evidence regarding the association of these changes with specific focal lesions. This is probably because most executive functions rely on several nodes of distributed brain networks and their large-scale connections rather than a particular functional region. As with verbal learning and memory, executive control can be impaired in TBI by DAI that disrupts a critical network's connectivity.

1.7.3.2 Neural networks and executive function. The conceptualisation of executive control in terms of neural network function has led to the identification of several networks relating to different aspects of executive function. For example, in resting state fMRI studies a number of networks have been identified that, apart from the DMN, include the 'executive control' network consisting of the superior, middle and ventrolateral PFC and the anterior cingulate and paracingulate regions (Beckmann, DeLuca, Devlin, & Smith, 2005), as well as the 'salience network', believed to support conflict and error processing that consists primarily of the anterior cingulate cortex and anterior insula (Seeley et al., 2008). Such 'resting state networks' appear to explain much of the activation patterns seen during task fMRI studies, and potentially are building blocks for the control of cognitive function (see e.g. Dosenbach et al., 2006).

Functional connectivity (i.e. simultaneous activation) patterns of brain regions during task performance have also been analysed to investigate which particular functional nodes are involved in specific control processes. For example, some fMRI studies of TBI patients performing attentionally demanding tasks have shown atypical activation of the medial/posterior parietal (precuneus and posterior cingulate) cortex (Levine et al., 2006; Smits et al., 2009; Rasmussen et al., 2008), which may reflect compensation for prefrontal dysfunction. This suggestion is consistent with an observation by Kim et al. (2009) that whilst TBI patients initially showed more prefrontal activation than healthy controls during an attentionally demanding task, cognitive rehabilitation which improved their task performance was paralleled by a shift to increased activation of the precuneus and anterior cingulate cortices. It is also possible, however, that rather than indicating compensatory processes, the relatively increased activation of more posterior regions after TBI reflects a failure by the patients to regulate activity of the DMN. Such abnormalities in DMN connectivity patterns might underlie the attentional deficits after TBI, and could impede sustained maintenance of cognitive control (see Bonnelle et al.,

2011).

Chapter 5 will discuss the *structural connectivity* patterns believed to support executive function.

1.7.4 Neuroanatomical substrates to information processing speed. In the extensive literature on response speed as an index of cognitive efficiency, 'simple processing speed' is a term used to refer to responses which require only basic perceptual judgements. 'Complex processing speed' refers to tasks involving high-order cognitive processes such as those relating to executive control, and is thus tapped by performance on 'executive' tests such as the Trail Making Test (TMT; Reitan, 1958; Reitan & Wolfson, 1985) or Stroop-based interference tests. The difference between complex and simple processing speed on these tests (e.g. more complex TMT Trails B minus simple Trail A) has been referred to as 'cognitive processing speed', indexing the elements of response times attributable to higher level cognitive processes (see e.g. Chiaravalloti, Christodoulou, Demaree, & DeLuca, 2003; Kochunov et al., 2010; Turken et al., 2008).

1.7.4.1 Slowed information processing speed after TBI. Impairments of information processing speed have been found to occur following both mild (see Frencham, Fox, & Maybery 2005, for a review) and severe TBI (e.g. Felmingham, Baguley, & Green, 2004). It may also be at least transiently observed in patients who, following a mild TBI, go on to fully recover (Crawford, Knight, & Alsop, 2007; De Monte et al., 2005). Felmingham et al. (2004) found that simple processing speed was disproportionately slower in TBI patients with 'predominant DAI' than in those with mixed brain pathology but 'minimal DAI'. However, this was a very small study, consisting of only 10 patients, and awaits replication.

1.7.4.2 Relevant white matter tracts and their degradation by TBI. As will be discussed in more length in Chapter 5, information processing speed is also likely to be dependent on the structural integrity of white matter tracts, such as those that connect functional brain regions involved in perceptual-motor processing. Turken et al. (2008) investigated the relationship between FA (as an index of white matter integrity) and cognitive processing speed on the Digit Symbol test in 39 healthy participants using voxel-based

morphometry (VBM; Ashburner & Friston, 2000) applied to DTI data. They found that processing speed was positively correlated with FA in white matter connecting the parietal, temporal and frontal regions, including the long-coursing superior and inferior longitudinal fasciculi.

A number of DTI studies (e.g. Arfanakis et al., 2002; Huisman et al., 2004; Inglese et al., 2005; Sidaros et al., 2008) have now shown breakdown following TBI of the structural coherence of interhemispheric white matter connections, particularly involving the corpus callosum, as well as pathways containing corticospinal fibres such as the internal capsule. The anatomical location of these connections makes them vulnerable to the biomechanical impact of TBI. Slowed performance may thus be seen after TBI on tasks that depend on intact structural connections between the two hemispheres or between brain regions involved in the integration of perceptual and motor processes (Bigler, 2008).

1.7.5 Estimating the effects of brain injury on specific cognitive functions. When assessing to what extent head injury has affected an individual's level of cognitive functioning, it is necessary to compare current functioning with their estimated pre-injury level. A common approach to estimate premorbid IQ is to measure performance on tests that are believed to be resistant to neurological damage, that is, believed to 'hold' the pre-injury level of general intellectual function. These include tests of reading pronunciation such as the Wechsler Test of Adult Reading (WTAR; Wechsler, 2001; see Chapter 2). The discrepancy observed between performance on these 'hold' measures and current performance on other neuropsychological tests can then be used to quantify the extent of impairment in more specific domains of cognitive function (Green, Melo et al., 2008).

Although word pronunciation is correlated with literacy, and literacy with verbal IQ (Lezak, Howieson, & Loring, 2004), accepting its use as an index of general intellectual ability rests on the assumption that such tests can, at least to an extent, capture this complex ability (Green, Melo et al., 2008). It has also been questioned whether word pronunciation tests are truly resistant to the effects of head injury (Riley & Simmonds, 2003). It is, of course, possible that some patients will have injuries to functional brain regions or important connections that mediate abilities including reading ability and language production, which may distort WTAR-based estimates of premorbid IQ in these cases. Green, Melo et al. (2008) administered the

WTAR to a sample of 24 severely brain-injured patients at two and five months post-injury. Performance remained stable over time, with minimal change observed in individual patients. Estimates of the patients' premorbid intellectual function when these were based on their WTAR scores were also close to those derived based on demographic data. In general it appears that in those patients who speak and read English fluently the WTAR can provide a useful estimate of premorbid IQ to which the level of cognitive functioning following TBI can be compared.

Apart from premorbid IQ, an individual's performance on neuropsychological tests may partly relate to current IQ. In order to determine the extent to which scores relate to the more specific aspects of cognitive function such as verbal learning and memory and executive control, some reference points are needed. To assess a patient's post-injury level of verbal and nonverbal intellectual function, widely used tests include subtests from the Wechsler Adult Intelligence Scale- Third Edition (WAIS-III; Wechsler, 1997) and Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). Whilst these test batteries and their subtests are not intended to localise brain injury, certain profiles in a patient's performance across subtests, or as reflected in scores on aggregate indices, may contribute to hypotheses regarding more specific cognitive deficits.

1.8 Investigating Traumatic Brain Injury and Its Sequelae Using Neuropsychological and Neuroimaging Methods

1.8.1 The role of clinical neuropsychology. The predominant application of clinical neuropsychology as an evolving science traditionally was the assessment of behavioural change for diagnostic purposes, but other applications firmly began to gain ground as research contributed to improved definition of diagnostic categories and the understanding of brain-behaviour relationships continued to increase. Currently, treatment planning and outcome evaluation are an integral part of the neuropsychological approach to the management of head injury, with reasons for referrals to cognitive assessment including identification of treatment needs, evaluation of treatment efficacy, and forensic or medicolegal questions (Lezak et al., 2004). Although neuropsychological assessment is of limited diagnostic value on its own, it is a critical element of research into the neural substrates of behavioural phenomena. The

development of non-invasive neuroimaging techniques that can be used to identify structural abnormalities of brain grey and white matter tissue *in vivo* has provided an increasingly sensitive alternative to neuropsychological assessment for the detection of brain damage. However, it remains the case that standard neuroimaging of TBI may be normal, and yet the patient shows impairments on neuropsychological assessment.

One possible explanation is that neuropsychological assessment may be insensitive to detecting real-world difficulties. Traditional neuropsychological measures and the controlled testing environment correspond poorly with the often novel and unstructured situations that the patients may struggle with in their day-to-day lives (see e.g Burgess, Alderman, Volle, Benoit, & Gilbert, 2009). It is also possible that despite normal performance on cognitive tests, neuropathology exists that can only be verified post-mortem, but that may explain some of the real-world difficulties. The cognitive processes involved in responding effectively to complex everyday situations are likely to partly depend on the integrity of the anatomical connections of functional brain networks (Bigler, 2008; Mesulam, 1998). Furthermore, TBI is a multifactorial condition in which cognitive outcome likely interacts with not only neurological, but also various behavioural factors. Therefore, a patient's psychological reaction to the injury, including possible mood disturbance, may also contribute to the cognitive difficulties (Ponsford, 1995).

1.8.2 The network approach to understanding cognitive impairment. As discussed above, high-order cognitive function is dependent on the integrated and efficient functioning of distributed brain networks, and these can be disrupted by TBI. White matter tracts structurally connect nodes of the functional networks. As structural and functional connectivity are thus related, studying the patterns of white matter damage after TBI could provide important insights into the cognitive impairments.

Recent developments in quantitative analysis of brain structure and connectivity within brain networks have contributed to advancing our understanding of structural and functional network organization. The structural connections, forming the anatomical substrate of the human brain, support the coherent physiological activity that spans spatially distinct nodes of the functional networks, and thus provide the basis for cognitive function (Fries, 2005; Mesulam, 1998; Singer, 1999). The analysis of large, high-quality data sets to investigate patterns of connectivity has led to the realisation that a range of different complex systems can be

described by a set of common parameters used to quantify the key principles of organization. *Graph theoretical* approaches were developed within the cross-disciplinary field of network science. Their usage for the quantitative analysis of patterns of network structural and functional connectivity is based on the following observations: the way in which the brain systems are connected resembles the way other complex networks are built, that is, (a) they consist of interconnected 'hubs' and (b) they are characterised by the principles of small-world topology, modularity, hierarchy and centrality. These features of brain networks have been demonstrated in several connectivity studies of humans and non-human animals (see Bullmore & Sporns, 2009).

When a large-scale brain network is conceptualised as a complex system, graph theory provides a mathematical tool to analyse and describe the interconnections and interactions between its various elements. This approach can also be used to quantify the extent of disconnection, such as might follow brain trauma. First, to characterise brain networks, the nodes of the networks are defined based on (for example) DTI data. Then, a measure of the degree of association between distinct nodes is derived: this might be, for instance, the probability of a white matter tract connecting two brain regions in a group of participants. Next, an association matrix is generated, including all pair-wise associations between the network nodes. Finally, because the behaviour of the interacting elements within a network is characterised by a combination of statistical randomness and regularity, the calculated network parameters of interest are compared to the equivalent parameters derived from a population of random networks (see Bullmore & Sporns, 2009, for further details).

It has been proposed in studies of structural connectivity that regions within the 'structural core' of the human brain include the posterior cingulate cortex, the precuneus, and the inferior parietal cortex. These regions constitute the posterior elements of the human DMN, and may be the connector hubs linking all major structural connectivity nodes (Hagmann et al., 2008; Honey et al., 2009). The white matter tract known as the cingulum bundle interconnects the posterior cingulate and the medial prefrontal cortices (Greicius et al., 2009; van den Heuvel, Mandl, Luigjes, & Pol, 2008). Other structural connections between network nodes may not be direct, but instead mediated by connections that first pass through other regions (Damoiseaux & Greicius, 2009). As previously discussed, damage to critical structural connections that link the functional brain regions involved in supporting complex cognitive functions could underlie some

of the impairments often observed following TBI.

1.8.3 Application of DTI to the investigation of white matter damage and relationships with cognitive deficits. As outlined above (section 1.6, pp. 36-40), DTI is emerging as an increasingly popular neuroimaging technique in both research and clinical practice. Application of DTI data analysis techniques including region of interest (ROI) approaches, whole-brain voxelwise methods and quantitative tractography have contributed to advancing our understanding of how TBI can alter the structure of white matter connections. Overall, DTI research of TBI has shown that associative pathways connecting the temporal and frontal regions are frequently damaged (Niogi & Mukherjee, 2010). There is also evidence that DTI indices of white matter abnormalities can identify mild TBI more sensitively than standard imaging techniques (e.g. Rugg-Gunn et al., 2001; Arfanakis et al., 2002; see Chapter 4), and that the extent of DTI abnormalities may correlate with TBI severity (Benson et al., 2007). Furthermore, the structure of the white matter tracts often found damaged after TBI appears to have behavioural relevance in that some correlations have been found with neuropsychological measures of cognitive function (e.g. Kennedy et al., 2009; Kraus et al., 2007). These topics are reviewed in more detail in Chapters 4 and 5.

***1.8.3.1 Why quantify white matter damage using diffusion tensor imaging*?** White matter abnormalities following TBI have been found in sites where microbleed evidence of DAI is typically observed (e.g. Inglese et al., 2005), but also in areas that extend beyond those where microbleeds are seen. Scheid et al. (2006) showed that persistent impairments of memory and executive function may be present in TBI patients who show only microbleed evidence of DAI in the absence of other traumatic or non-traumatic MRI abnormalities. The number of microbleeds did not, however, correlate with the degree of cognitive impairment shown by these patients. This may suggest that whilst microbleeds are a marker of DAI, the true extent of white matter damage is likely to be greater, and microstructural damage, not detected by standard imaging of TBI, may also have cognitive consequences. Thus, an index of white integrity may also be useful in predicting cognitive and functional outcome following TBI, and in more accurately characterising the extent of white matter damage (Huisman et al., 2004; Lee et al., 2008).

1.8.3.2 Region of interest (ROI) approaches. Diffusion tensor imaging studies employing ROI approaches have demonstrated white matter abnormalities after TBI in several tracts (Kennedy et al., 2009; Kraus et al., 2007; Niogi, Mukherjee, Ghajar, Johnson, Kolster, Sarkar et al., 2008; Sidaros et al., 2008). In a longitudinal DTI investigation of outcome after severe TBI with 30 patients assessed early after injury (average of 8 weeks post-injury) and 23 re-assessed approximately one year post-injury, Sidaros et al. (2008) found that the structural organization of white matter within certain regions, including the corpus callosum and the internal capsule, predicted functional outcome, and explained variance in outcome beyond information provided by clinical assessment and conventional brain imaging. Whilst fractional anisotropy (FA) within the corpus callosum remained low over time, particularly in patients with unfavourable outcomes, FA within the internal capsule returned to normal or supranormal levels in patients with more favourable outcomes. Reduced white matter integrity in TBI has also been found to correlate with long-term cognitive deficits of learning and memory, attention, and executive function (Hartikainen et al., 2010; Kraus et al., 2007; Niogi, Mukherjee, Ghajar, Johnson, Kolster, Lee et al., 2008; Salmond, Menon, Chatfield, Williams et al., 2006; see Chapter 5, for further discussion).

The structure of the corpus callosum after TBI has received particular interest. Researchers focusing on this connection specifically have found evidence that acutely after injury FA appears to be reduced, whilst diffusivity perpendicular to the principal direction is typically elevated (Kumar et al., 2009; Rutgers et al., 2008). Although the corpus callosum frequently sustains damage in TBI and its structural organization in terms of the principal direction of diffusion is well-known, it nevertheless represents only a small fraction of the total white matter which could in principle be affected by TBI. Chapters 4 and 5 will discuss in some detail the potential benefits of whole-brain voxelwise over ROI approaches to investigating the neural and cognitive sequelae of TBI.

1.8.3.3 Whole-brain and voxel-based approaches. The effects of TBI on the integrity of structural connections have recently been investigated using whole-brain methods such as histogram approaches (e.g. Benson et al., 2007, Lipton et al., 2008), a quantile approach (Bazarian et al., 2007), and voxel-based morphometry (e.g. Bazarian et al., 2007; Salmond,

Menon, Chatfield, Williams et al., 2006; Xu, Rasmussen, Lagopoulos, & Håberg, 2007). The quantile approach may be superior to the use of histograms for examining the effects of brain injury on the DTI indices of white matter structure (Bazarian et al., 2007), whilst VBM (Ashburner & Friston, 2000) can be useful in identifying voxel-based differences in brain structure between groups of participants. However, preprocessing steps required prior to VBM analysis that include warping and smoothing in image registration may introduce considerable bias into the data. Tract-based spatial statistics (TBSS; Smith et al., 2006), the voxelwise method of choice in the present research, benefits from a powerful two-stage registration process that reduces such bias.

1.8.3.4 Tract-based spatial statistics (TBSS). In TBSS, information derived from the diffusion tensor about the structural properties of white matter in each voxel of a participant's diffusion image is used to calculate a 'map' (e.g. FA map) that describes the structure and organization of all major white matter tracts (Smith et al., 2006). Chapter 2 (section 2.10.3.1, pp. 84-87) will provide a more detailed description of the various steps in TBSS, but in brief these involve aligning all participants' FA maps to a common target using nonlinear registration, and then creating a 'skeletonised' FA image for carrying out voxelwise statistics to identify the voxels on the white matter skeleton that show between-groups differences or correlations with demographic, clinical or behavioural variables. Similar DTI metric images can be created using non-FA images, such as mean, axial or radial diffusivity maps, which can then be subjected to statistical analysis.

As an automated 'whole-brain' method TBSS attempts to combine the strengths of VBM-type and tractography analyses, and thus aims to resolve possible issues with poor alignment, spatial correspondence of anatomical structures across participants and the requirement to pre-specify tracts of interest. That TBSS calculates group differences in white matter structure based on the centre of the tracts (the 'skeleton' voxels) only, can markedly reduce the likelihood of including voxels in the analysis that are not truly located within brain white matter; a problem associated with VBM analyses of white matter (see Chapter 2, section 2.10.3.1, pp. 84-87, for a more detailed description). A clear advantage of TBSS over ROI approaches is that it provides an automated observer-independent way of analysing DTI data. Furthermore, as the centres of white matter tracts are likely to be more robust to TBI-induced

atrophy than their edges, TBSS seems particularly well-suited for investigating the structure of white matter tracts after brain injury.

No approach is without its limitations, however. Whilst for large white matter tracts TBSS is likely to be superior to VBM in terms of minimising partial voluming relating to the confounding of the DTI metrics by non-white matter tissue, a possible disadvantage relates to partial volume effects that may still exist, particularly for thin tracts whose diameter can be smaller than the size of an imaging voxel. Therefore, when interpreting TBSS-derived group differences or correlations for DTI measures extracted from regions potentially affected by partial voluming (from the neighbouring CSF or from tracts located at grey-white matter junctions), one should carefully check the accuracy of the aligment of each individual's tracts with the standard-space template and the effects observed. In addition, TBSS encounters problems in crossing fibre areas with multiple fibre orientations within an imaging voxel, as its skeleton-based approach cannot resolve these. This results in the skeleton being disconnected at tract junctions. The search strategy used by TBSS for extracting the FA values from each individual's standard-space registred FA image and projecting these to the alignment invariant tract skeleton is such that the analysis is restricted to the high-anisotropy tract centres and values from low anisotropy voxels suppressed. Thus, in patients with pathological changes near or in the white matter tracts, TBSS may at this projection stage exclude some areas from further analysis. Finally, as with other methods, if effects from head motion during data acquisition are not adequately dealt with during the data pre-processing, this can increase image blurring and bias the DTI metrics. Whilst TBSS may have its disadvantages as briefly outlined above, for the purposes of the current research its advantages are considered to outweigh these (see Chapter 2, section 2.10.3.1, pp. 84-87, for further justification).

1.9 Main Objectives, Research Questions and Hypotheses

This programme of research is based on a 'disconnection model' of TBI, proposing that axonal injury leads to disconnection and thereby contributes to the cognitive impairments commonly observed following TBI. The main aims are to investigate (i) the neuropsychological correlates of structural brain abnormalities observed on standard MRI after TBI; (ii) the impact of TBI on the structure of the brain's white matter connections, assessed using DTI; (iii) the

relationships between clinical indices of injury severity and abnormalities of white matter tract structure; and (iv) the relationships of structural properties of white matter tracts with indices of verbal learning and memory, executive function, and information processing speed.

Three studies investigate these issues in a group of 40 patients and a control group of 40 healthy volunteers. Chapter 2 provides an overview of the general methods and materials; the specific subsamples and methods and materials used in each of the three studies are described in Chapters 3, 4, and 5. Chapter 3 explores neuropsychological correlates of whole-brain atrophy and 'focal' injury as indexed by standard MRI. Chapter 4 explores the potential of DTI in identifying the presence and extent of more subtle white matter damage following TBI. Finally, Chapter 5 explores the relationships of DTI indices of white matter structure with measures of verbal learning and memory, executive function and information processing speed. The specific research questions and hypotheses relating to these studies are presented in each chapter.

The general questions and hypotheses addressed by the research programme are:

1) Is neuropsychological performance in the domains of verbal learning and memory, executive function and information processing speed associated with the degree of whole-brain atrophy after TBI?

   Hypothesis: Greater whole-brain atrophy after TBI will be associated with worse performance in these domains of cognitive function.

2) Will performance in the cognitive domains investigated correlate with 'focal' lesions after TBI in specific anatomical locations or with the degree of lesion load (volume/number)?

   Hypothesis: The anatomical location of lesions and lesion load will predict neuropsychological function in the domains studied.

3) Will patients with TBI show abnormalities of white matter tract structure when compared with age-matched healthy controls?

   Hypothesis: Patients with TBI will show widespread abnormalities of white matter structure, compared with healthy controls.

4) Will the presence of brain microbleeds and/or clinical severity of injury after TBI be associated with abnormalities of white matter tract structure?

Hypotheses: Patients with microbleeds will show greater and more extensive abnormalities of white matter tract structure than patients without microbleeds. White matter abnormalities will be found in patients with mild TBI as well as in those with more severe injuries.

5) Will specific cognitive deficits after TBI in the domains investigated be associated with the structure of specific white matter tracts?

Hypothesis: After TBI, deficits in the domains of verbal learning and memory, executive function and information processing speed will be associated with abnormalities of the structure of specific white matter tracts.

CHAPTER 2: Methods and materials

Chapter 2 provides an overview of the research programme described in this thesis, briefly describing and giving the rationale for all the methods, materials, and research procedures. For more detailed descriptions, the reader is referred to the Methods and Materials sections of empirical chapters 3, 4 and 5. However, as the focus of the structural neuroimaging aspect of this thesis is on DTI, which involves various data pre-processing and analysis steps, a detailed description of these methods is provided here, with only a summary included in the relevant empirical chapters (4 and 5).

2.1 Overall Design

All three studies that make up the research programme described in this thesis were cross-sectional in design, comparing patients with TBI and neurologically healthy controls on a range of neuroimaging and neuropsychological measures. Some participants completed all assessments and others just a subset; sample sizes thus vary between studies and analyses. Group membership acted as the between-subjects factor in planned and *post-hoc* contrasts. Units of measurement for the dependent variables ranged from units such as the number of imaging voxels or indices of the magnitude of diffusion of water molecules within these voxels to the number of correct responses or completion time (s/ms) of a neuropsychological test. The research programme was approved by the Hammersmith, Queen Charlotte's and Chelsea Research Ethics Committee and by the Departmental Ethics Committee of the Department of Psychology, Goldsmiths, University of London.

2.2 Recruitment of Participants

All participants were a minimum of 18 years of age and capable of giving written informed consent according to the Declaration of Helsinki (World Medical Association, 2008). Patients were recruited from three locations based in London, United Kingdom: the acute brain injury units of Charing Cross Hospital and the National Hospital for Neurology and Neurosurgery, and from the Royal Free Hospital. Potentially eligible patients had sustained a closed head injury, which had resulted in a definite or probable TBI (see Table 2-1 for details regarding this classification) and had presented to the clinics describing some cognitive impairment. Potential participants were identified by the clinics' clinicians and sent an introductory letter inviting them to participate, as well as an information sheet outlining the study. A telephone or e-mail contact was then made by the researcher to further discuss participation.

The opportunity sample of healthy control participants was recruited via e-mail, telephone, or personal contact. Some controls were recruited after they made contact in response to a poster advertisement for volunteers.

The general exclusion criteria applying to both groups were: (a) significant on-going

neurological problems other than TBI, including the need for anti-epileptic medication, (b) significant on-going psychiatric problems, including the need for anti-depressant medication, (c) a history of significant neurological problems other than TBI, (d) a history of significant psychiatric illness, (e) current or previous drug abuse or excessive use of alcohol, (f) pregnancy or breast feeding, and (g) other contraindication to magnetic resonance imaging, such as presence of metallic devices in the body.

Inclusion criteria for patients only were: (a) a definite or probable TBI as defined using the Mayo Traumatic Brain Injury Severity Classification System (Malec et al., 2007; see Table 2-1) and (b) a minimum of one month elapsed since the head injury.

Table 2-1

Mayo Traumatic Brain Injury Severity Classification System

A. Classify as **Moderate to Severe (Definite) TBI** if one or more of the following criteria apply:	**1**. Death due to this TBI **2.** Loss of consciousness ≥ 30 min **3.** Post-traumatic amnesia ≥ 24 h **4.** Worst Glasgow Coma Scale score within the first 24 h < 13 (unless attributable to e.g. intoxication, sedation or systemic shock) **5.** One or more of the following present: • Intracerebral haematoma • Subdural haematoma • Epidural haematoma • Cerebral contusion • Haemorrhagic contusion • Penetrating TBI (dura penetrated) • Subarachnoid hemorrhage • Brain Stem Injury
B. If none of Criteria A apply, classify as **Mild (Probable) TBI** if one or more of the following criteria apply:	**1.** Loss of consciousness of momentary to less than 30 min **2.** Post-traumatic amnesia of momentary to less than 24 h **3.** Depressed, basilar or linear skull fracture (dura intact)
C. If none of Criteria A or B apply, classify as **Symptomatic (Possible) TBI** if the following criterion applies:	One or more of the following symptoms present: • Blurred vision • Confusion (mental state changes) • Dazed • Dizziness • Focal neurologic symptoms • Headache • Nausea

Patient-specific exclusion criteria were: (a) a history of previous TBI, (b) neurosurgery other than invasive intracranial pressure monitoring, and (c) gross impairment of perceptual or language function. A range of TBI patients with varying levels of chronicity, injury severity,

anatomical pattern of injury and degree of cognitive impairment was deliberately recruited to allow for specific hypotheses to be tested within and between subgroups of patients, as defined in chapters 3 to 5.

The control-specific inclusion criterion was to be in good general health. The control-specific exclusion criteria were (a) a history of TBI and (b) an impairment of perceptual or language function.

Relevant background information, including the patients' clinical details, were obtained through interviewing the participants and reviewing available medical records that were either provided in advance by the recruiting clinician with the patient's permission or by the patient during the research appointments. Apart from demographic information (gender, age, ethnicity, pre-injury occupation, total years of education, the highest level of education), these details included cause of injury, time since injury, presence/duration of loss of consciousness, initial Glasgow Coma Scale (GCS; Teasdale & Jennett, 1974) score, length of post-traumatic amnesia (PTA), and any information on initial clinical neuroimaging findings.

2.3 Designs of Empirical Studies

2.3.1 Study 1 (Chapter 3). A between-groups design was used to investigate the relationships between neuroimaging measures of brain structure from standard MRI and indices of neuropsychological performance in TBI patients and healthy controls. First, 40 patients and 39 controls were compared on whole-brain volume measurements, normalised for each individual's head size. Next, 36 patients were compared with 26 controls on an extensive battery of neuropsychological tests (see section 2.6, pp. 63-73). A subset of these measures were explored in terms of their neuroimaging correlates in the patient group only; these tapped verbal learning and memory, executive function, and information processing speed. These analyses entailed: (a) testing for correlations between whole-brain tissue volumes and neuropsychological performance, (b) contrasting the neuropsychological performance of patient subgroups defined by lesion type profiles, (c) lesion mapping to identify the anatomical location and volume/number of brain contusions and white matter lesions, and identifying their neuropsychological correlates, and (d) testing for correlations between lesion load (total size/number of lesions) and neuropsychological scores.

2.3.2 Study 2 (Chapter 4). A between-groups design compared (a) 28 TBI patients vs. 26 healthy controls and (b) patient subgroups, on four DTI indices of the structure of the brain's white matter tracts: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (D_{ax}) and radial diffusivity (D_{rad}). A whole-brain voxelwise technique, tract-based spatial statistics (TBSS; Smith et al., 2006; see section 2.10.3.1, pp. 84-87), was used, and its results subjected to permutation-based statistical inference. Within the patient group, the effects of microbleeds as biomarkers of diffuse axonal injury (DAI) and injury severity on white matter structure were also investigated using TBSS within the group of patients. A nonlinear statistical technique was used to test for significant differences between groups in white matter structure.

The following groups were compared: (a) patients vs. controls, (b) patients with evidence of DAI vs. controls, (c) patients with evidence of DAI vs. patients without, and (d) patients with mild TBI vs. controls.

2.3.3 Study 3 (Chapter 5). Data from the Study 2 sample of 28 TBI patients and 26 healthy controls were further analysed to explore the relationships between the four DTI indices of white matter structure and cognitive function (verbal learning and memory, executive function and information processing speed). Tract-based spatial statistics, together with a nonlinear permutation-based multivariate regression technique, were employed to investigate possible relationships between the structure of specific white matter tracts and neuropsychological performance in healthy controls and TBI patients separately and across the two groups.

2.4 Participants

A total of 40 patients with definite or probable TBI and 40 healthy controls were recruited.

There were 30 men and 10 women in the patient sample, aged between 18 and 66 years (M ± SD = 39.4 ± 11.7). The majority (57.5%) of patients were of White British ethnic background, whilst 12.5 per cent were of Mixed Race, 7.5 per cent of White European (non-UK) origin, 5 per cent of White American, and another 5 percent of White (other) background. The

remaining (12.5%) identified themselves as belonging to one of Black British, Black African, Somali, Arab, or Indian ethnic groups.

Prior to their injury, patients' occupations included employment in the following sectors: professional (40%), skilled (22.5%), managerial (12.5%), and unskilled (7.5%). In addition, 10 per cent had been students pre-injury and 5 percent had been unemployed. Total years of education in the sample ranged from 11 to 21 years, with a median of 15.5. All patients had attained at least a secondary school level of education, whilst nearly a half of the group (45%) held a university degree.

The top three causes of injury were: assaults (35%) road traffic accidents (RTA) (32.5%), and falls (20%). Three patients (7.5%) had sustained sports-related brain injuries, one patient had been involved in a work-related accident, and in one case the cause of the injury was uncertain. The GCS scores (lowest) recorded at the scene of injury, or at the earliest occasion thereafter, ranged from 3 to 15; PTA duration ranged from <24 hours to 180 days. In a number of cases information regarding the lowest GCS score or the length of PTA was missing, which is common in studies of this type. At the time of participation, all patients were in the post-acute/chronic stage of TBI with a minimum of one month and a median of 13.5 months post-injury (range = 1 to 73 months). Table A1 in Appendix A provides clinical information on individual patients.

Magnetic resonance imaging, performed as part of the research was reviewed by a senior consultant neuroradiologist. Nine patients (23%) had no abnormalities visible on T1- or T2*-weighted MRI. The remaining 31 (77.5%) had focal lesions (brain contusions or white matter lesions) and/or superficial siderosis (i.e. haemosiderin deposits). Atrophy, contusions, superficial siderosis and gliosis at the site of impact and the contrecoup injury were the most common changes reported.

There were 40 healthy control participants (20 men and 20 women), aged between 19 and 60 years (M ± SD = 33.9 ± 10.4 years). All control participants had completed at least a secondary school education. Controls' MRI scans were also reviewed by the neuroradiologist to determine that they were normal.

Comparisons between the main demographic characteristics of subsamples of patients and controls participating in each empirical study are reported in the corresponding chapters.

2.5 Justification of Sample Sizes

Conventional power analyses cannot readily be applied to neuroimaging studies, which entail the analysis of whole-brain voxelwise data, because the standard error varies across the thousands of imaging voxels. Instead, the sample sizes were determined based on a review of those employed in previously published studies employing similar methods. Previous studies employing structural MRI techniques and neuropsychological assessment have demonstrated relationships between structural brain abnormalities and cognitive deficits after TBI in samples of 24 (Warner et al., 2010) and 65 (Tate, Khedraki, Neeley, Ryser, & Bigler, 2011). Abnormalities of white matter structure, as indexed by DTI metrics, have been detected in groups of as few as nine TBI patients, compared with healthy controls (Xu et al., 2007). In general, DTI studies have shown white matter abnormalities after TBI in samples ranging from <10 to 43 patients (see Niogi & Mukherjee, 2010). Benson et al. (2007) reported an effect of TBI severity on white matter structure assessed using DTI in 20 patients. Region-based DTI indices have correlated significantly with neuropsychological scores in a sample of nine TBI patients (Kennedy et al., 2009) as well as in larger samples of between 24 and 43 patients (Kraus et al., 2007; Little et al., 2010; Niogi, Mukherjee, Ghajar, Johnson, Kolster, Lee et al., 2008). Changes in DTI measures of white matter structure over time after TBI have been identified in single cases (Voss et al., 2006) as well as at group level (Sidaros et al., 2008).

The present samples of 28 to 40 patients and 26 to 39 healthy controls used in different studies and analyses compare favourably with the existing literature and stand a plausible chance of detecting effects of similar magnitude to those reported elsewhere. For investigation of clinical features within the TBI group using quantitative analysis, an attempt was made to include at least 10 in each contrasting subgroup. The analyses based on these subgroups are exploratory, and it is accepted that some of the samples may be too small to detect real differences, or that they could, conversely, give rise to Type I errors. Where appropriate, corrections for multiple comparisons have been made to mitigate the latter risk.

2.6 Neuropsychological Assessment

2.6.1 Justification for test selection

Several factors were considered in the process of identifying the specific TBI outcome domains and finally selecting the measures employed in the research. Although measures to

assess other domains of functioning, including emotional and behavioural, were also considered and selected for the overall TBI research programme (see section 2.6.6), the focus of the current thesis was solely on cognitive outcome. This is because cognitive impairment is common and significantly contributes to disability after TBI (e.g. Berg et al., 2005; Green, Colella et al., 2008; van Velzen et al., 2009; Whitnall et al., 2006), as also set out in the aims of the wider research programme, investigating the effects of disconnection after TBI on cognitive control, and how these may impact learning. Following a consultation with the Principal Investigator of the overall research programme, it was determined how the doctoral research discussed in this thesis could help fulfil the aims and be embedded within the programme. The broad areas of cognitive function of primary interest here were then agreed upon, which were learning and memory, executive function and information processing speed. This was based on a comprehensive review of the relevant literature that identified these three as domains that are frequently impaired following TBI, and in which deficits can persist in the long-term (Draper & Ponsford, 2008; also see Chapter 1, section 1.7.1, pp. 40-41). Following this overview, with particular focus on identifying possible long-term cognitive impairments following TBI, test selection then proceeded as follows, constrained by the considerations described below.

Taking into account both the emerging and anticipated collaborations to tap into other aspects of the wider research programme, such as to create a TBI research network and database, the set of measures (and the expertise required for administering these) needed to be available at external centres with whom links were being developed or planned. Different, but related projects were then to be carried out within this collaborative set-up, and the test battery needed to be general enough to be applicable to a variety of related studies, whilst also being able to address the questions of the research discussed in this thesis. In addition, it was agreed for practical reasons that the administration of the full battery of tests should not take longer than two hours.

As discussed in Chapter 1, impairments of verbal learning and memory, executive function and speed of information processing are frequently observed after TBI. The battery of tests used in the current research was therefore designed to focus on the assessment of cognitive function within these domains. Emphasis was given to selecting a limited set of measures that would cover each of the three domains, but that would also comply with the limitations outlined above. The selected measures included (i) valid, robust, and widely

applicable measures with documented utility in TBI, (ii) measures chosen due to the specific aims of the research, and (iii) an experimental measure specifically developed for the project that, although requiring further validation, may have particular utility in assessing processing speed deficits after TBI (see Wilde et al., 2010, for examples of measures in each of these categories). Although other measures tapping different aspects of the three broad domains of function could have been used, including emerging measures or those purported to better measure everyday function (see e.g. Burgess et al., 2006), these were not included for the reasons outlined here. It is acknowledged, however, that newer measures currently near publication, or those still under development or in need of further validation, may have significant potential to be superior to some traditional neuropsychological measures.

Each of the measures selected for inclusion in the final battery of tests used for the research programme is briefly described in the following sections, including, where available, information about their psychometric properties in assessing cognitive outcome after TBI, as well as their predictive validity in terms of other domains of functioning (e.g. global level of function, cognitive and physical activity, or quality of life).

2.6.2 Premorbid intellectual ability: The Wechsler Test of Adult Reading. The Wechsler Test of Adult Reading (WTAR; Wechsler, 2001) is used for estimating an individual's level of intellectual functioning before the onset of injury or illness. It consists of a list of 50 words that represent atypical forms of grapheme-to-phoneme translations, presented in order of increasing difficulty. Use of the WTAR for estimating premorbid IQ is based on the premises that (a) using words with atypical pronunciations minimises the ability of the individual to apply standard pronunciation rules and (b) despite post-injury or -illness (or age-related) cognitive decline in other domains of functioning, reading ability is known to remain relatively stable and correlates fairly strongly with premorbid IQ.

The test is quick to administer and can be used across a wide age range (16 to 89). The examinee is simply asked to pronounce each word aloud, starting with the first and then proceeding until the last one without skipping any items. United Kingdom-specific norms derived from a large standardisation sample are available to convert raw scores to age-scaled standard scores, which allow an individual’s performance to be referenced to his/her age group and individuals to be compared with each other.

In TBI, the use of the WTAR allows estimation of the cognitive impact of head injury by comparing a patient's WTAR-estimated premorbid ability to their current neuropsychological performance. Green et al. (2008) have investigated the psychometric properties of the WTAR in TBI patients. They assessed a group of 24 patients (mostly severely injured) at 2 and 5 months post-injury. Whilst performance on tests of current intellectual ability improved significantly over time, WTAR scores remained stable in the group and in individual patients. Hanks et al. (2008), who examined the predictive validity of the WTAR relative to functional outcome at one year post-TBI (*N* = 174) found the WTAR to be a significant predictor of outcome above and beyond injury severity. Their outcome measures included various rating scales tapping functional ability/disability and quality of life. However, Mathias, Bowden, Bigler and Rosenfeld (2007) urge caution after comparing patients with mild (*N* = 82), moderate (*N* = 73) and severe (*N* = 61) TBI, and orthopaedic injury controls (*N* = 95). Despite good demographic matching of groups, the most severely injured patient group showed significantly worse WTAR performance, both soon after their TBI and six months later. This suggests that the WTAR may underestimate premorbid IQ in some more severely injured TBI patients.

2.6.3 Current reasoning ability.

2.6.3.1 Similarities. This is a subtest of the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) and is an untimed test of verbal abstract reasoning. Pairs of semantically related words are presented (e.g. circle and square), and the examinee asked to express in which way they think that these two words are alike (i.e. to find the similarity between them). There are a total of 26 items, with difficulty level gradually increasing. Scores on each item range from 0 (incorrect) to 2 (correct), with 1 indicating partial accuracy. The test is discontinued after four consecutive incorrect answers. The maximum possible score is 52, and age-scaled scores are computed, as for the WTAR.

Green et al. (2008) used Similarities as an index of current intellectual ability in their assessment of 24 TBI patients at 2 and 5 months post-injury and found that scores on a parallel version of the test improved significantly over time. This finding, although based on a small sample, suggests that Similarities is sensitive to reasoning difficulties after TBI. Bowman (1996) evaluated the predictive validity of several neuropsychological measures relative to activity impairment in everyday life at chronic stages of head injury (on average 5.7 years post-injury) in

a large sample of patients (*N* = 483). They found that Similarities performance, assessed on average 2.2 years prior to outcome measurement, importantly contributed to the equation that best predicted this outcome.

2.6.3.2 Matrix Reasoning. Matrix Reasoning, also from the WASI, is an untimed multiple-choice test of nonverbal reasoning. A series of 35 matrices (32 for ages 45-79) that involve a visual pattern or sequence are presented to the examinee. In each matrix one piece is missing and the examinee's task is to complete it with one of five possible response options. As with Similarities, difficulty increases across trials. Verbal interaction is kept to a minimum, apart from prompting the examinee if they are taking too long to respond. The test is discontinued after four incorrect responses across five consecutive items. As with Similarities, total scores are converted to age-scaled scores.

Donders, Tulsky and Chu (2001), who studied the criterion validity of the Matrix Reasoning in a sample of 100 patients with TBI, found that it did not differentiate between the patients with moderate/severe or mild TBI and demographically matched healthy controls. They thus concluded that the test is robust to the effects of TBI. Similarly, Ryan et al. (2005) found that TBI patients scored more highly on Matrix Reasoning than on all other WASI subtests. However, contrasting evidence exists. For example, Green et al. (2008) observed improvement on the test over time from 2 months to 5 months post-TBI, and that the magnitude of the improvement was comparable to that they observed for Similarities.

2.6.4 Verbal learning and memory.

2.6.4.1 The People Test. This subtest of the Doors and People battery (Baddeley et al., 1994) taps verbal episodic memory and requires the examinee to learn the first and last names of four people (a doctor, a minister, a postman, and a newspaper boy) in response to a question identifying their profession (e.g. "What was the name of the doctor?"). A picture of each person showing their name and profession is first briefly presented to the examinee, whilst these details are also provided orally (i.e. "This is Jim Green. He is a doctor."). Recall is tested immediately after the presentation of all four pictures. Three learning trials are given, with 1 to 3 points per response (1 for first name, 1 for surname, 1 extra point for both names). If the examinee gets them all correct after only one or two repetitions, s/he is awarded full points for the remaining

trial(s). In the current research programme, the total immediate recall score across all trials was used to index verbal (associative) learning and memory.

The People Test has previously been found sensitive to memory impairment following TBI (e.g. Draper & Ponsford, 2008), but despite the test's apparent face validity, its ecological validity remains to be determined, as does its predictive validity relative to other domains of function.

2.6.4.2 Logical Memory. Logical Memory (LM) from the Wechsler Memory Scale (Third Edition) (WMS-III; Wechsler, 1997) is a test of verbal episodic memory. The examinee listens to a story read out loud by the examiner and then recalls it in as much detail as possible, both immediately following presentation (LM I) and after a delay of 25-35 minutes (LM II). There are two stories, A and B, of which Story B is presented twice, with the examinee's memory tested after each presentation. The second presentation of Story B is intended to reduce the effects of momentary lapses of attention during story presentation upon learning and delayed recall (Lezak et al., 2004). The score used here is based on 'units' that the examinee correctly retrieves after each presentation. The total number of units recalled on LM I (Story A + Story B1) provides a measure of immediate recall (LM I first recall total), whilst the total number of units recalled on LM II (Story A + Story B) provides a measure of delayed recall (LM II total). The maximum score for both LM I first recall and LM II total is 50.

Fisher, Ledbetter, Cohen, Marmor and Tulsky (2000) found that 23 mild and 22 moderate/severe TBI patients performed significantly worse than 45 healthy controls on the LM. West, Curtis, Greve and Bianchini (2011) report similar findings, having controlled for level of effort. However, patients with mild TBI did not show impaired performance, possibly reflecting a lack of memory deficit after relatively subtle brain injury. Knight, Harnett and Titov (2005) investigated memory impairment after TBI, as measured using the LM, relative to prospective memory (PM), required in everyday life for the completion of planned tasks (e.g. remembering to attend a meeting). They compared PM performance of a group of 25 patients (all ≥ 15 months post-injury) and 20 demographically matched controls on a video-based task involving a robbery and a list of tasks to be completed in a nearby city centre. Although the patients' estimates of PM success were equivalent to those made by the controls, they performed worse on the task. Moreover, patients with better PM performance also had higher LM scores (the total

raw scores from the LM I and II).

2.6.4.3 Digit Span. The Digit Span (DS), also from the WMS-III, taps concentration and working memory. It is quick and easy to administer, requiring respondents to repeat number sequences in either forwards or reverse order. Sequences progressively increase in length, with two trials of each length until the examinee fails both trials. One point is awarded for each correct span, making the maximum raw points 16 for the 'forward' and 14 for the 'backward' condition (max total = 30 points). In general, forward DS is considered a test of short-term memory, attention and concentration and backward DS a test of working memory (Franzen, 2000; Tulsky, 2004). However, Wilde, Strauss and Tulsky (2004) have reported that clinical groups, including those with TBI, do not - as a rule - perform worse on the backward than the forward condition, even in the presence of working memory deficits as assessed by other means. This is contradicted by other findings, possibly reflecting differences in patients' lesion sites. For example, Weinberg, Diller, Gerstman and Schulman (as cited in Franzen, 2000) found TBI patients with left-sided lesions to be impaired on both forward and backward DS, and those with right-sided lesions to be impaired on backward DS only. As well as a marker of cognitive impairment, the DS has been used to detect improvement of cognitive function over time after TBI (Kersel, Marsh, Havill, & Sleigh, 2001; Millis et al., 2001).

2.6.5 Executive function.

2.6.5.1 Trail Making Test. The Trail Making Test (TMT; Reitan, 1958; Reitan & Wolfson, 1985) is quick and simple to administer and engages a variety of cognitive abilities, including visual search and scanning, attentional capacity, visuomotor processing speed, set maintenance, and mental flexibility in sequencing and set shifting (Lange, Iverson, Zakrzewski, Ethel-Kingc, & Franzen, 2005; Tombaugh, 2004). It consists of two parts: TMT-A that requires the examinee to sequentially connect 25 numbered circles randomly distributed on a sheet of paper; and TMT-B, where the examinee again connects circles in sequence, but alternating between numbers and letters (i.e. 1-A-2-B-3-C-4-D etc.). Time taken to complete each condition is recorded. A 'difference' score, indicating mental flexibility was calculated here by subtracting the TMT-A completion time from the TMT-B completion time (TMT-A − TMT-B = alternating-switch cost).

The TMT has been found to be sensitive to the effects of TBI (e.g. Lange et al., 2005; Reitan, 1958). Burgess, Alderman, Evans, Emslie and Wilson (1998) compared 92 neurological patients (*M*±SD age = 38.5±15.1) of varying etiologies (59% with head injuries) with 216 nonpatient controls (*M*±SD age = 46.1±19.8) and reported the following percentages for patients performing at or below the 5% level of the controls: 32.1 for TMT-A, 38.9 for TMT-B, and 25.9 for TMT-A − TMT-B. Recent evidence indicates that the test is also sensitive to the degree of injury severity; Demery, Larson, Dixit, Bauer and Perlstein (2010) showed that moderate/severe TBI patients performed significantly worse on both TMT-A and TMT-B than either mild TBI patients or healthy controls. In addition, patients with mild TBI performed significantly worse than controls on the more complex TMT-B. Hanks et al. (1999) studied the relationship between TMT-B performance and outcome after 6-months of acute rehabilitation, as measured using scales tapping a variety of domains including TBI-related disability and community integration. In their mixed sample of 90 TBI, orthopedic, and spinal cord injury patients, TMT-B performance was found to be a strong correlate of functional outcome. Other previous studies have reported similar associations between the TMT and TBI outcome (e.g. García-Molina, Tormos, Bernabeu, Junqué, & Roig-Rovira, 2012; Little, Templer, Persel, & Ashley, 1996; Ross, Millis, & Rosenthal, 1997).

2.6.5.2 Color-Word Interference test. The Delis-Kaplan Executive Function System (D-KEFS; Delis, Kaplan, & Kramer, 2001) Color-Word Interference test is based on the famous Stroop (1935) procedure, and is construed as tapping verbal inhibition, set-shifting and cognitive flexibility. Performance is however affected by a variety of additional abilities, including initiation, simultaneous processing, maintaining a set, sustained attention and information processing speed.

Two ‘baseline’ conditions assess the examinee’s ability to perform the basic tasks of naming and reading. The more complex ‘inhibition’ condition presents colour names, which are printed in a conflicting colour (e.g. ‘RED’ printed in green). The examinee has to inhibit the prepotent response of reading out what is written (i.e. the colour name) and to instead name the ink colour. The final ‘inhibition/switching’ condition requires the examinee to alternate between two task sets as cued by the test sheet, that is, to either read the colour name or to name the ink colour. Completion time on each trial is recorded as well as two types of errors: uncorrected

and self-corrected. If the examinee makes three consecutive errors, the examiner provides a prompt to respond as appropriate. This verbal prompt is only provided once during a trial and the stopwatch is kept running throughout. For the purposes of the current research, a contrast score indexing cognitive flexibility was calculated by subtracting the average of the completion times of the two baseline trials (naming and reading) from the time to complete the more complex inhibition/switching trial.

Variants of the Stroop have been widely used in clinical practice to assess the effects of TBI on executive function (e.g. Fork et al., 2005; García-Molina et al., 2012). Goverover, Arango-Lasprilla, Hillary, Chiaravalloti, & DeLuca (2009) compared D-KEFS Color-Word Interference test inhibition/switching performance of 10 patients with TBI (all with positive CT/MRI results or documented loss of consciousness ≥ 24 hours) and 15 demographically matched healthy controls. The patients, all more than a year post-injury, did not show significantly impaired performance relative to the controls. However, it is worth noting that in addition to focusing on only the inhibition/switching condition of the test the sample in the Goverover et al. (2009) study was very small and replication of this finding in a much larger group of TBI patients is needed. The predictive validity of the test relative to outcome after TBI within other domains of function also remains to be determined.

2.6.5.3 Verbal Fluency/letter fluency. Also from the D-KEFS, the Verbal Fluency/letter fluency assesses an individual's word generation fluency. Like the Color-Word Interference test, this test taps into a variety of processes including task initiation, simultaneous processing, maintaining a set, systematic retrieval of responses, and information processing speed. The examinee is required to generate different words that begin with a particular letter (usually F, then A, and finally S), but that are not proper nouns or numbers. The words are generated under time constraints (one minute per each of the three letters). The outcome variable used here was the total words that the examinee generated across the three letters, excluding any words that were repeated or broke the rules specified above.

Reduced verbal productivity after brain injury has been demonstrated in several studies using the letter fluency task (see Lezak et al., 2004). For example, Strong, Tiesma and Donders (2011) found 65 TBI patients of varying levels of injury severity to score significantly lower than 65 demographically matched healthy controls. The sensitivity of the FAS verbal fluency

procedure for neurological impairment screening puroses has been questioned, however (Burgess et al., 1998). In the Burgess et al. (1998) sample of 92 neurological patients of mixed etiologies only 10.8 percent performed at or below the 5% level of the large sample of nonpatient controls. The task has nevertheless shown sensitivity to TBI and to frontal lobe damage (Henry & Crawford, 2004a; Henry & Crawford, 2004b), in particular to left-sided frontal damage (Delis et al., 2001). There is also some evidence from the Burgess et al. (1998) study to support the test's ecological validity relative to carer-reported everyday executive problems on the Dysexecutive Questionnaire (DEX; Wilson, Alderman, Burgess, Emslie, & Evans, 1996), as well as relative to the difference in DEX scores based on other- versus self-ratings. The task has in addition shown predictive validity in terms of the level of functional independence after mild to severe TBI (median/range GCS score = 8/3-15) following discharge from acute rehabilitation (Hanks, Rapport, Millis, & Deshpande, 1999).

2.6.6 Information processing speed: Choice-reaction task. A two-choice reaction task (CRT), developed in-house, provided a measure of information processing speed. Following the presentation of an inital cue (+) at the centre of a computer screen for 1400 ms, each trial then consisted of an initial fixation cross displayed for 350 ms, immediately followed by the presentation of a target, consisting of arrows pointing to the left or the right (<<< or >>>) for a maximum of 1200 ms. The inter-stimuli interval (ISI) was set at 1750 ms.

Stimuli were presented in blocks of 14 trials, within each of which the ratio of left, right, and rest (fixation only) trials was set at 5:5:4 in random order. A practice block was followed by five experimental blocks; the practice and experimental phases were each preceded by four stimuli not used in the analyses. Thus, there were 70 experimental trials altogether.

Stimulus presentation and recording of responses were implemented using Matlab 7.4.0 (R2007a) software on a Windows operated HP laptop computer with a 15.6-inch screen. The fixation cross and the arrows were white, and these were presented against a black background. The size of the target arrows was 100 pixels.

The examinee was informed that white arrows pointing either to the left or the right would begin to appear in the centre of the computer screen and that they should respond as quickly and accurately as possible by pressing the left (C) or right (M) keys, depending on the direction of the arrowheads. These instructions were given verbally as well as presented on the

computer screen. The time taken by the examinee to respond to each target following its presentation (i.e. the behavioural choice-reaction time) was automatically recorded by the computer. Median reaction times for accurate trials were then computed.

Versions of the CRT have been used in numerous studies to investigate processing speed deficits, and have been shown to be sensitive to slowed processing speed after TBI (e.g Niogi, Mukherjee, Ghajar, Johnson, Kolster, Sarkar et al., 2008; Willmott, Ponsford, Hocking, Schönberger, 2009).

2.6.7 Self-report measures. A number of self-report measures were included in the complete battery of psychological measures employed in the wider research programme of which the present studies are part. For completeness, these are listed in Appendix B.

2.7 Structural Neuroimaging: Basic Principles of Methods.

2.7.1 The MR signal. The ability of MRI to produce a detailed image of the anatomy of the brain is based on the use of electromagnetic waves (i.e. radio signals) and a strong magnetic field to quantify hydrogen atoms that are located within the brain as part of its numerous water molecules. An important principle of physics is that when hydrogen atoms are exposed to a magnetic field, produced by an electric charge in motion, their nuclei (containing one proton each) exist in one of two states at any one time: a high-energy state or a low-energy state. Protons have mass and are positively charged. In addition, they spin about their axis, which produces a small magnetic field referred to as the 'magnetic moment'. Essentially, their spin quality causes protons to act like tiny magnets. Normally, protons with spin exist in a random distribution, but when they are placed in a magnetic field, such as the one generated by the MRI scanner, some of the protons will align *against* the external magnetic field, whilst the spin vectors of a small majority will align *with* the field, representing the low-intensity state. The size of the low-intensity majority at any given instant is proportional to the strength (B_0) of the magnetic field. Stronger fields result in a greater difference between the two energy states; that is, a larger majority of protons are aligned with the field (Bear, Connors, & Paradiso, 2007; Westbrook, Roth, & Talbot, 2011). The ability of MRI to produce a high quality image is based

on this excess of protons aligned with the magnetic field. In a magnetic field of 3.0 Tesla (T), as used in the current research, approximately 10 hydrogen protons out of every one million contained within a typical imaging voxel contribute to the MR signal. This results in a relatively high signal-to-noise ratio (SNR) without the need to increase scan time in order to increase the number of signal averages as would be necessitated by the use of lower magnetic field strengths. Apart from allowing for shorter acquisition times, the superior SNR associated with 3.0 T contributes to enhanced image acquisition through providing higher image resolution (Moseley, Liu, Rodriguez, & Brosnan, 2009).

2.7.2 Time constants (T1, T2 and T2*) and image contrast. The key to generating the MR signal is to cause the protons to 'jump' from one energy state to another via the application of energy generated by passing a radiofrequency wave through the head as it is positioned between the poles of the MRI scanner, effectively a large magnet. Protons wobble, or 'precess', about the axis of the external magnetic field, and the frequency of this precession is determined by the strength of the magnetic field. When the signal is set at the right frequency (i.e. the resonance frequency of hydrogen), the protons in the low-energy state absorb the energy from the signal and flip to the high-energy state (Bear et al., 2007).

When the signal is turned off, the absorbed energy is retransmitted at the resonance frequency and the excited protons return to their original low-energy state, realigning with the magnetic field. The time course of the excited spin protons returning to what is called 'thermal equilibrium' is mathematically described by an exponential curve, and the recovery rate is characterised by the time constant *T1*, the *spin-lattice relaxation time*. The uniqueness of rates of T1 recovery to thermal equilibrium between different types of tissue enables MRI to provide a clear contrast between grey and white matter tissues of the brain, as seen on T1-weighted MR images (Goldstein & Price, 2004). Brain contusions following brain injury, for example, can readily be seen on high-resolution T1-weighted images, as these appear darker than healthy brain tissue, thus reflecting the damage.

Another phenomenon is the *dephasing* of initially excited protons that following the radiofrequency pulse are *in phase*, producing the net magnetisation. In effect, this transverse magnetisation associated with dephasing behaves in the same way as net magnetisation; that is, the spins rotate about the axis of the external magnetic field, and a release of energy is

followed by dephasing. Due to cumulative dephasing, the transverse magnetisation decays exponentially as the spin elements in different energy states interact and exchange energy. This causes the decrease in transverse magnetisation or signal decay (Westbrook et al., 2011).

The time constant that describes the return to equilibrium of the transverse magnetisation (i.e. the rate of the signal decay) is known as *T2*, or the *spin-spin relaxation time*. The combined time constant describing the factors that actually contribute to the decay of transverse magnetisation, including molecular interactions and variations in the strength of the magnetic field, is called *T2 star (T2*) relaxation*. This dephasing occurs due to magnetic field inhomogeneity that is a property of all magnets (Goldstein & Price, 2004; Westbrook et al., 2011). This, although susceptible to signal losses near tissue boundaries, provides an increased contrast for certain types of tissue, and is particularly suitable for imaging traumatic microbleeds in the brain's white matter, appearing on T2*-weighted images as small dark signal abnormalities (i.e. white matter hypointensities).

2.7.3 Reconstructing the MR signal: k-space and Fourier Transform. Taking advantage of the fact that the frequency at which protons emit energy is proportional to the size of the magnetic field, the MR signal can be quantified and the amounts of hydrogen measured at a fine spatial scale. Spatial encoding in MRI is based on magnetic field gradients, which allow the re-coding of spatial data as frequency data. A critical property of the MRI scanner here is that its magnetic fields vary from one side to the other, giving a spatial code to the electromagnetic waves emitted by the protons in which high-frequency signals are associated with atoms closer to the strong side and low-frequency signals with atoms closer to the weak side of the magnet. To construct a single high-resolution image representing the distribution of hydrogen atoms throughout the brain, a series of measurements is required, each with the gradients of the magnet oriented at different angles (Bear et al., 2007).

The Fourier Transform (FT) is a frequency-dependent mathematical technique that is used for decomposing the MR signal into its constituent frequencies. Thus, it refers both to the representation of the MR signal in the frequency domain and to the process of transforming the signal. The originally time-dependent MR signal is converted from the time domain to the frequency domain, and into 'k-space' using a FT. An inverse 2D FT is then used to reconstruct the MR image. The k-space is a 2D plot of spatial frequencies, in which every point of the space

contributes to the MR image constructed. Although this space is conceptually infinite, only those frequencies that have been used in the original spatial encoding of the MRI data are critical. Locating the correct frequency spectrum involves complex mathematics, and in practice is performed computationally via Fast Fourier Transform. The inverse 2D FT of the MRI data, collected in k-space, finally results in the familiar 3D-representation of the MR image in 'image-space', and contains the intensity values together with the spatial coordinates x, y, and z (Westbrook et al., 2011).

2.7.4 Diffusion tensor imaging.

2.7.4.1 Diffusion weighting and image acquisition. Diffusion-weighted images are acquired by applying powerful gradients with rapid switching in polarity on either side of a 180° excitation pulse. The characteristically random diffusion motion leads to shifts in the phase and loss of pulse signal, whilst regions where motion is decreased show little or no signal loss and thus appear bright on diffusion-weighted images (Coles, 2007). The sensitivity of the imaging signal to detecting the diffusion of water molecules is dependent on the gradient strength applied at image acquisition, characterised by the *b*-value (Basser & Özarslan, 2009). The *b*-value (expressed in s/mm^2) is a parameter that reflects the strength and duration of the magnetic field gradients applied during the MR pulse sequence (i.e. the level of diffusion weighting). In other words, the *b*-value summarises the attenuating effect on the MR signal of all diffusion and imaging gradients applied in one direction. Images of reasonable quality can be acquired using a *b*-value of 1000, commonly used in recent DTI investigations.

A minimum of six noncollinear directions of measurement are required to visualise white matter tracts and study their microstructural properties, but for more complex analyses that depend on visualisation of individual white matter tracts 64-direction DTI is preferable. A series of diffusion-weighted images is acquired and to summarise the attenuating effect of all gradient waveforms applied in the three (x, y and z) directions, a symmetric (instead of scalar) *b*-matrix is then calculated for each diffusion-weighted image.

2.7.4.2 Diffusion coefficient and diffusion tensor. Diffusion tensor imaging measures the rate and directionality of the diffusion of water molecules in brain tissue.

Fick's first law (Fick, 1855a, 1855b) defines diffusion net flux (J) as:

$J = -D \nabla C$

where J is the net particle flux (vector), C is the particle concentration, and D is the constant of proportionality (the diffusion coefficient).

In general, particles tend to flow from regions of high concentration to regions of low concentration- hence the negative sign for the diffusion coefficient. The rate of flux (J) is proportional to the concentration gradient (C) as well as to the diffusion coefficient (D). The value of D is determined by the size of the diffusing molecules, the temperature, and microstructural features of the environment. This sensitivity of D to the local microstructure enables its use as a probe of properties of brain tissue (Basser & Özarslan, 2009). A symmetric (effective) diffusion tensor is required to characterise diffusion in anisotropic tissue such as brain white matter (Basser & Jones, 2002). The effective diffusion tensor, D, at each imaging voxel can be estimated from the series of diffusion-weighted images acquired. This estimation is based on the relationship between the magnetic field gradients applied during the DTI sequence and the echo attenuation measured in each voxel (Basser & Jones, 2002). A variety of DTI parameters that quantify the structure and organization of brain tissue can then be derived from the tensor. The DTI metrics derived for the purposes of the current research are described below (section 2.8.4). Also see section 2.8.3 for the specific MRI sequence used in the current research to acquire the diffusion-weighted images, as well as section 2.8.4 for the DTI data preprocessing methods.

2.7.4.3 Quantitative DTI. Diffusion tensor imaging comprises not only the MR measurement of the effective diffusion tensor, but also the extraction and the visual display of the information contained in the tensor at each imaging voxel. Numerous studies have now used this technique to derive metrics that reflect the structural properties of white matter, such as fractional anisotropy (FA) or mean diffusivity (MD). Most commonly, these metrics have been measured from a small selection of specific regions or tracts of interest. Here, TBSS (Smith et al., 2006), a ‘whole-brain’ voxel-based method, is applied. Several review articles are now available that provide comprehensive overviews of the basic principles of diffusion tensor MRI data acquisition, processing and quantification (see Le Bihan, 2003, for a comprehensive

introduction to these). The interested reader is in particular encouraged to refer to Le Bihan et al. (2001) for a historical overview of DTI, and Basser and Jones (2002), for the various considerations relating to DTI data acquisition, experimental design and data processing.

2.8 Structural Neuroimaging Sequences

2.8.1 Magnetic resonance imaging apparatus. Structural MRI, including DTI data were obtained using a Philips Achieva 3.0 T MRI scanner (Philips Medical Systems, Netherlands) using Nova Dual gradients, a phased array head coil, and sensitivity encoding (SENSE) with an under-sampling factor of 2. Quadratic shim gradients were used to correct for magnetic field inhomogeneities within the brain.

2.8.2 T1- and T2*-weighted magnetic resonance imaging sequences. High-resolution T1-weighted (MPRAGE) images were acquired with the following acquisition parameters: repetition time (TR) = 9.6 ms, echo time (TE) = 4.5 ms, 150 slices, slice thickness = 1.2 mm, flip angle = 8°, in-plane resolution = 0.94×0.94 mm, matrix size = 208x208 mm. Gradient-echo T2*-weighted images were obtained using the following sequence and parameters: echoplanar imaging with whole-brain coverage, TR = 2,000 ms, TE = 30 ms, 31 ascending slices, slice thickness = 3.25 mm, gap = 0.75 mm, flip angle = 90°, matrix size = 112×87 mm, field of view (FOV) = 280×220×123 mm.

2.8.3 Diffusion tensor imaging sequence. Diffusion-weighted volumes with gradients applied in 64 non-collinear directions were collected (16 directions per each of a total of four DTI runs). The following parameters were used: 73 contiguous slices, slice thickness = 2 mm, FOV = 224 mm, matrix = 128 x 128 (voxel size = 1.75 x 1.75 x 2 mm), *b* value = 1000 s/mm^2, and four images obtained with no diffusion weighting (*b* = 0 s/mm^2), one per each DTI run.

2.8.4 Preprocessing of diffusion tensor imaging data: A summary. Pre-processing of the diffusion data was carried out using DTIStudio (Jiang, van Zijl, Kim, Pearlson, & Mori, 2006), a freely available resource program, and various image processing tools from the Oxford

Centre for the Functional Magnetic Resonance Imaging of the Brain's (FMRIB) Software Library, FSL (Smith et al., 2004; Woolrich et al., 2009). The four pairs of PAR and REC files from the Philips Achieva scanner, each containing 16-direction DTI data, DTI sequence-specific gradient files, and associated bvals and bvecs were used as the starting point. All four DTI runs were first concatenated and the diffusion weighted images registered to the b = 0 s/mm^2 images by affine transformations using the FMRIB's Linear Image Registration Tool (FLIRT; Jenkinson & Smith, 2001; Jenkinson, Bannister, Brady, & Smith, 2002). This corrected the data for motion and eddy-current distortions due to the gradient coils that can compromise image quality by introducing stretches and shears. Brain extraction was then performed on the b = 0 image using the Brain Extraction Tool (BET; Smith, 2002) Version 2.1 to remove the skull and generate an image containing brain tissue only as well as a mask image. Due to individual differences in the relationship between the skull and brain tissue, the extraction factor used varied between individual participants. Each image was checked after the extraction, and where too much or too little had been extracted, as was often the case, the factor was adjusted accordingly until an accurate extraction was achieved (smaller values = larger brain outline).

Finally, dtifit from the FSL's Diffusion Toolbox (FDT; Behrens et al., 2003) was used to perform the three-dimensional fitting of the tensor model on the data. The image input into dtifit was the data image derived as a result of the previous preprocessing steps. The mask image applied was that of the skull-extracted brain. In addition, the bvecs and bvals were input. Voxelwise fractional anisotropy (FA) and mean diffusivity (MD) maps were then generated, as well as images to quantify the three eigenvalues (λ1, λ2, and λ3) that represent the magnitude of diffusivity along each of the principal axes of the diffusion tensor (see Figure 2-1).

A series of 3D images describing various properties of the diffusion tensor thus resulted, including:

- dti_V1 – the first eigenvector
- dti_V2 – the second eigenvector
- dti_V3 – the third eigenvector
- dti_L1 – the first eigenvalue (λ1), i. e. parallel or 'axial diffusivity'
- dti_L2 – the second eigenvalue (λ2)
- dti_L3 – the third eigenvalue (λ3)

- dti_MD – mean diffusivity

  The trace (λ1 + λ2 + + λ3) of the diffusion tensor (D), is an *invariant* (i.e. orientation-independent) index of overall diffusion in a voxel or region. The mean diffusivity (MD) is given by trace(D) ÷ 3.

- dti_FA – fractional anisotropy

  The following combination of the tensor eigenvalues λ1, λ2 and λ3 determines the fractional anisotropy FA of a voxel/region:

$$FA = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda 1 - \lambda 2)^2 + (\lambda 2 - \lambda 3)^2 + (\lambda 3 - \lambda 1)^2}}{\sqrt{\lambda 1^2 + \lambda 2^2 + \lambda 3^2}}$$

  Axial (D_{ax}) and radial (D_{rad}) diffusivity images were also derived from these FDT outputs:

  D_{ax} = dti_L1 (λ1)

  D_{rad} = dti_L2 (λ2) + dti_L3 (λ3) ÷ 2

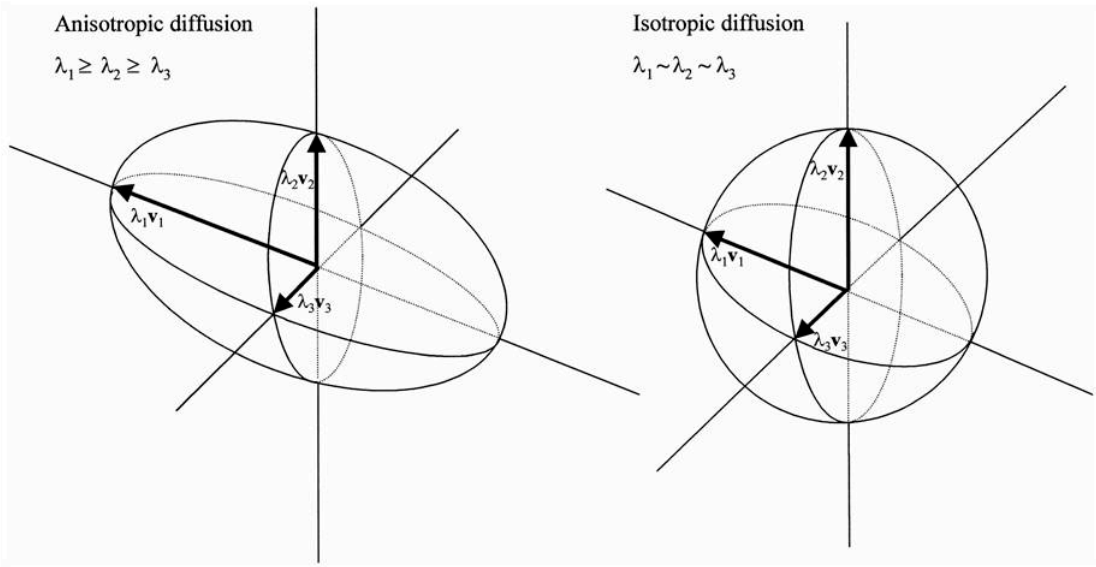

Figure 2-1. Anisotropic and isotropic diffusion. The probability function for displacement of water molecules can be depicted as an ellipsoid when diffusion is anisotropic as opposed to when diffusion is isotropic. The eigenvalues λ1, λ2, and λ3 represent the magnitude of diffusion along the three principal directions and the eigenvectors V1, V2, and V3 the length of each axis. Reprinted from Radiology December 2000, 217, Wiegell, M. R., Larsson, H. B. W., & Wedeen, V. J. Fiber crossing in human brain depicted with diffusion tensor MR imaging, 897-903, Copyright (2011), with permission from the Radiological Society of North America.

Figure 2-2 shows the first eigenvector (V1), corresponding to the orientation of the principal axis of the diffusion tensor, overlaid on the FA map of a healthy control participant.

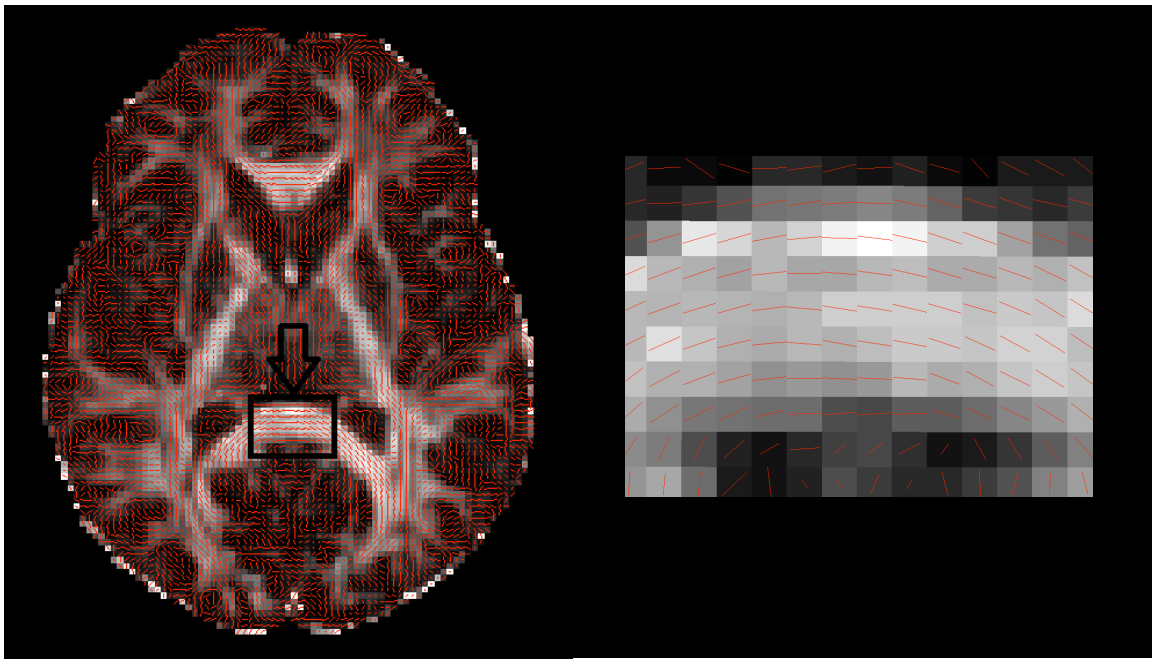

Figure 2-2. Fractional anisotropy (FA) modulated by V1. A single axial slice (MNI Z = 17) of a healthy control participant's FA map with V1 (overlaid) displayed as red lines. On the right, a section of the corpus callosum is shown magnified; in this highly anisotropic white matter the principal diffusion direction (indexed by V1) is aligned with the longest axis of the white matter tract. MNI = Montréal Neurological Institute.

A more detailed description of the DTI data preprocessing steps is provided in Appendix C.

2.9 Procedure

2.9.1 Consenting and interview. Informed consent was sought from all participants. During the initial interview, which immediately followed their consent, demographic information was collected from all participants. Clinical history was additionally collected from the patients, particularly where there were gaps after clinical records had been reviewed, or where those were not available.

2.9.2 Neuropsychological assessment. Neuropsychological assessment of patients was usually split across two approximately one-hour sessions, one prior to each of the two neuroimaging sessions. Neuropsychological assessment of controls was performed within a

single session of approximately an hour and a half, separate from their single neuroimaging session. For a few participants, practical considerations, such as long travel to the research unit, work commitments, or MRI scanning constraints, necessitated either that all assessments were carried out during one day, or that the neuropsychological assessment was carried out during one visit and all neuroimaging during the second visit. The sequence of the individual neuropsychological tests was fixed across participants:

1) WTAR
2) TMT-A and TBT-B
3) The People Test- immediate recall
4) Color Word Interference Test
5) Verbal Fluency/letter fluency
6) The People Test- delayed recall
7) CRT
8) LM I
9) Matrix Reasoning
10) Similarities
11) LM II
12) Digit Span

A stopwatch was used for measuring the completion time of all timed traditional neuropsychological tests. For the computerised choice-reaction task, all response times and errors were automatically recorded by the computer, and the results saved as files.

Patients were given all self-report scales at the end of their first research visit and asked to complete them at home, either returning them to the Investigator during their second visit or within one month via the post using a pre-paid and addressed envelope.

2.9.3 Neuroimaging. Most participants underwent high-resolution T1-weighted MRI and gradient-echo T2*-weighted MRI. A subsample of participants additionally underwent DTI. All neuroimaging was performed using a Philips Achieva 3.0 T scanner based at the Robert Steiner MR Unit (Imperial College London).

The presence of microbleeds (small dot-like haemorrhages) on T2*-weighted MRI was

used to determine the presence of DAI after TBI (see Chapter 4). Structure of the brain's white matter connections was quantified using a variety of metrics derived from DTI (see section 2.8.4, pp. 78-81).

Neuroimaging assessment of patients was divided into two sessions: a 40-minute structural MRI session that included DTI and functional MRI session (the functional MRI assessment was carried out as part of a larger research programme and is not discussed in this thesis). All neuroimaging of controls was performed within a single 1 ½-hour session, with a short break between the structural and functional parts of the scanning. The scan sequences were performed in a fixed order across participants: The T1 and T2*-weighted images were always obtained prior to DTI.

2.10 Statistical Data Analysis Methods

2.10.1 Demographic and neuropsychological data. Statistical Package for the Social Sciences (SPSS) Version 16.0 (SPSS Inc., Chicago, IL) was used to carry out all statistical analysis on the demographic and neuropsychological data in which neuroimaging data were not involved. Independent samples *t*-tests or Chi-square goodness of fit tests were performed as appropriate to compare the patient and control groups or patient subgroups on key demographic and clinical variables (see empirical chapters 3, 4, and 5, for details). To establish the current level of cognitive performance in the patient group compared with healthy controls, independent samples *t*-tests were performed on standardised residuals of neuropsychological test scores; potential confounding variables, on which the groups differed significantly, were regressed out (see chapters 3 and 5, for details). Levene's Test for Equality of Variances was applied before the calculation of any *t*-statistics, as unequal variances increase the risk of Type I errors. Where Levene's Test was significant ($p < .05$), indicating unequal variances, the adjusted *t*-statistic and *p*-value were used.

2.10.2 Standard magnetic resonance imaging data and cognitive correlates. This set of analyses relates to Chapter 3, where the reader is referred for further information. To summarise, normalised whole-brain volume measurements from patients and healthy controls

were first compared using independent samples *t*-tests in SPSS. Then, within the patient group only, several analyses were carried out to investigate possible association between neuroimaging metrics and neuropsychological measures: (a) relationships between whole-brain tissue volumes and neuropsychological test scores were first tested using nonparametric partial correlations which controlled for potential confounding variables; (b) relationships between neuropsychological performance and anatomical location of brain contusions were tested using voxel-based lesion-symptom mapping (VLSM; Bates et al., 2003); (c) the effect of the anatomical location of white matter lesions (deep or infratentorial vs. lobar) on neuropsychological performance was tested using independent samples *t*-tests in SPSS; and finally (d) correlations between lesion load and neuropsychological test scores were tested using nonparametric partial correlation, after again adjusting for possible confounding variables.

2.10.3 Analysis of DTI data and relationships with neuropsychological variables.

2.10.3.1 Tract-based spatial statistics. Tract-based spatial statistics (TBSS) Version 1.2, from FSL (Smith et al., 2004; Smith et al., 2006) was used to carry out a series of voxelwise analyses of the diffusion data. There is no need in TBSS to specify in advance the white matter tracts or regions of interest, as it operates in a fully automated manner. This approach uses a multi-stage registration protocol that includes 'skeletonisation' of individual participants' FA volumes, which aims to ensure that only those white matter voxels that are consistently present across participants are included in the statistical analysis of the diffusion data. Using TBSS, the whole brain can be investigated for changes in local white matter tract structure and their relationships with other variables.

Briefly, the main TBSS steps include: (a) nonlinear alignment using the FMRIB's Nonlinear Image Registration Tool (FNIRT) of all participants' individual-space FA images into a standard diffusion space; (b) the creation of a mean FA image from the aligned FA images of all participants; (c) 'thinning' of the mean FA image to create a skeletonised version of the mean FA image (i.e., the mean FA skeleton), representing the centres of all major white matter tracts that are common to that group of participants, and in this way minimising partial-volume confounds; (d) thresholding the FA skeleton at FA≥0.2 to create a mask in which areas of extremely low mean FA are suppressed and those with considerable inter-individual variability excluded; and (e) warping each participant's FA data onto the FA skeleton as well as projecting

the pre-aligned FA data onto the spatially invariant white matter tract skeleton to create a 4D image containing the projected and skeletonised FA data from each individual. The aim of the latter projection step is to resolve any residual alignment problems following the initial nonlinear registration. See Figure 2-3 below for examples of the quality of the alignment between white matter tracts on an individual participant's FNIRT-registered FA image and the mean FA skeleton, overlaid.

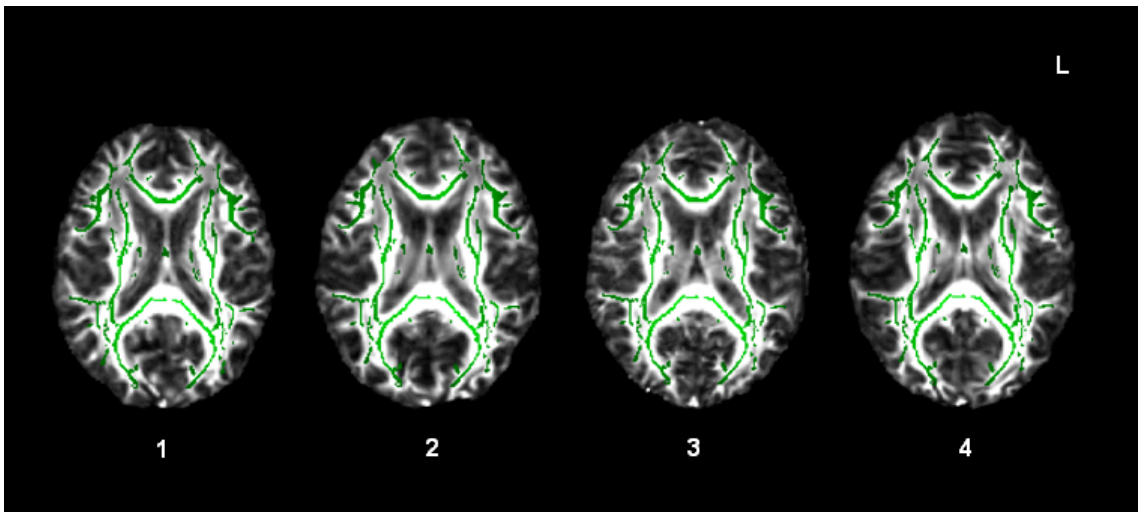

Figure 2-3. Alignment of the mean FA skeleton with individual patients' white matter tracts. The mean FA skeleton (thresholded at mean FA ≥ 0.3, in green) overlaid on four patients' FNIRT-registered FA images: two patients (1 and 2) with normal T1 and T2* scans and two patients (3 and 4) with microbleed evidence of diffuse axonal injury on T2*-weighted MRI. MNI Z = 17 for all four images. L = Left.

Similar warping and projection steps can then be employed on mean, axial, and radial diffusivity data. The resulting skeletonised DTI metric images, containing the projected and aligned data from all participants, can then be fed into voxelwise permutation-based statistical analysis across participants to identify the white matter tracts within the tract skeleton whose local structure correlates with covariates such as group membership or ability on a given task (Smith et al., 2006; Smith et al., 2007).

The primary advantage of TBSS over alternative voxelwise analysis methods, particularly voxel-based morphometry (VBM; Ashburner & Friston, 2000), is its multi-stage registration method, involving initial nonlinear registration of individual participants' images to a common template followed by the projection of each participant's DTI data onto an alignment-invariant representation of all major tracts. As employed here as part of the standard TBSS pipeline, the nonlinear image registration normally achieves a reasonable balance between poor

alignment and too much image deformation/similarity (Smith et al., 2006). However, despite its aims “to improve the sensitivity, objectivity and interpretability of analysis of multi-subject diffusion imaging studies” (Smith et al., 2006, p. 1487), there are potential issues with TBSS whose effects vary between studies. As with any approach, errors can be introduced into the TBSS pipeline at any of the following stages: the study design, sampling, data acquisition and quality control, data processing, statistical modelling, and data analysis. These can then compromise straightforward interpretation of the results obtained. For one, irrespective of the FA projection step in TBSS, the accuracy of the standard-space registration partly determines the accuracy of the alignment of the white matter tracts across multiple participants. A recent study (Keihaninejad et al., 2012) suggests that nonlinear registration of individual scans to a groupwise template prior to the standard-space registration could further improve the accuracy of TBSS analyses. The accuracy of the image processing steps is not only critical to ensure reliability of the results but also partly determines the power of TBSS to detect the effects of interest.

A clear advantage of TBSS relates to its ability to show tract-specific changes in the white matter of the brain. Although VBM can be a useful automated tool to study loss of brain tissue in neurological disease if implemented correctly, it was not specifically designed for studying white matter tracts. There are also particular issues with VBM relating to image segmentation, modulation/image intensity scaling to correct for volume change due to the spatial normalisation, and image smoothing by a user-determined smoothing kernel (i.e. replacing the intensity of each voxel by a weighted average of the neighbouring voxels), which makes the data more closely resemble a Gaussian distribution. Errors introduced due to these steps of the data processing can severely compromise VBM’s ability to accurately detect abnormalities in white matter tract structure, especially due to local registration errors and in those regions where partial volumes (i.e. the contamination of white matter by non-white matter tissue) are likely, such as in periventricular white matter or at grey-white matter junctions. It can also be hard to ascertain whether all observed ‘effects’ are truly due to changes in the density/integrity of brain tissue or some more likely to be due to spatial misalignment. In diffusion data analysis, problems with alignment of brain structures and the subsequent spatial smoothing can mean that a standard space voxel does not contain data from the same part of the same white matter tract of each participant.

Finally, that in TBSS the statistical analysis is carried out on tract centres only arguably makes this method more suitable for the analysis of abnormalities of tract structure in brains affected by atrophy, although (as discussed in Chapter 1, section 1.8.3.4, pp. 53-54), the FA-based tract skeletonisation too has its limitations. An advantage of TBSS compared to region-based analyses such as probabilistic tractography is that it allows one to investigate the whole brain and to do this in a bottom-up, investigator-independent way, with no need to delineate and define the tracts or regions of interest in advance, which could importantly limit the analysis (Smith et al., 2007).

On balance, when potential pitfalls in the pipeline are identified and due care is taken during data pre-processing, co-registration of images and data analysis in order to maximise reliability and interpretability of the results, the advantages of TBSS can be considered to outweigh its disadvantages. In particular, this method appears a useful tool to explore, in a voxelwise manner, white matter tract structure across multiple tracts for the purposes of this thesis.

An additional step-by-step description of the TBSS pipeline is provided in Appendix C.

2.10.3.2 Nonparametric permutation-based data analysis. General Linear Modelling (GLM) within FSL was used to set up a design matrix and fit the data to the model (i.e. what was expected from the data). Each group membership was first modelled as a separate explanatory variable (EV)/column in the matrix. Contrasts were then specified. The empirical chapters 4 and 5 provide information on the specific group contrasts and correlations between variables that were tested according to the hypotheses of each study, but Appendix C contains general examples of how the data were modelled and the contrasts (group differences/correlations) specified.

After setting up the GLM designs and specifying the contrasts, Randomise Version 2.1 was used to carry out the permutation-based nonparametric statistical inference on the variables as specified in the design on a voxel-by-voxel basis. Briefly, Randomise permutes all possible combinations of the data the specified number of times (here 5000) to generate a distribution against which to test the null hypothesis of each contrast or correlation (see Nichols & Holmes, 2002, for further detail). The threshold-free cluster enhancement (TFCE; Smith & Nichols, 2009) method was used to derive cluster-like test statistics. The null distribution of the

test statistic was built up over 5000 Monte Carlo permutations of the data.

One complexity of permutation testing is choosing the appropriate threshold to account for multiple comparisons. Using the TFCE method that produces *t*-statistics with both uncorrected and corrected *p*-values via the permutation testing is a recommended solution for TBSS-based analysis, and avoids the issue of arbitrarily selecting the cluster size thresholds to use for statistical inference (see Smith & Nichols, 2009, for more information).

2.10.3.3 Viewing and interpreting the results. The results were viewed in FSLView by first loading a Montréal Neurological Institute (MNI) template image of the brain (MNI152_1mm_brain), then adding the mean_FA_skeleton image, and finally overlaying both with the *t*-statistic image that showed the corrected *p*-values for each voxel. The threshold for significance was set at .949 in FSLView, which corresponds to $p < .05$, resulting in only those voxels showing that exceed this threshold. Where there was a diffusion metric-related change that was significantly associated with a certain covariate across participants, including group membership, neuropsychological measures and clinical variables (as specified by the design matrix and contrast set-up), the voxels on the white matter skeleton that showed a significant relationship could then be determined from the image display. The identification of individual white matter tracts that showed significant group differences or correlations between variables included in the particular Randomise analyses was based on the atlas tools available in FSLView (JHU White Matter Tractography Atlas, Jülich Histological Atlas, and Harvard-Oxford Subcortical Structural Atlas), as well as the MRI Atlas of Human White Matter (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005).

CHAPTER 3: Standard magnetic resonance imaging of traumatic brain injury and relationships with cognitive function

Many unresolved issues remain in the diagnosis of traumatic brain injury (TBI) and in the planning of targeted treatment and rehabilitation in its post-acute/chronic stages. One complication is that initial clinical neuroimaging findings often correlate poorly with outcome after the acute stages of injury. Cognitive impairments of variable degree are common sequelae of TBI and have a major role in disability that persists in the long-term. Although numerous studies using standard magnetic resonance imaging (MRI) and region/behaviour-of-interest approaches have previously investigated brain structure-cognitive function relationships in the damaged brain, the findings have been inconsistent. Moreover, the concept of 'critical lesion site' for cognitive deficits has recently been questioned. The principal aim of this study was to investigate interrelationships between residual brain damage, assessed using standard MRI, and neuropsychological function in post-acute/chronic TBI. First, the degree of whole-brain atrophy in a sample of 40 patients was estimated by comparing their grey matter, white matter and total brain tissue volumes with those of 39 healthy controls. Neuropsychological correlates of these volumetric measures were then explored within the patient group. Brain contusions and white matter lesions, common after TBI and visible on standard MRI, were then mapped out on each patient's scans and relationships between lesion location and load and neuropsychological function explored. Compared with controls, TBI patients had smaller volumes overall of grey matter, white matter, and total brain tissue. These volumes were not significantly associated with neuropsychological performance. In a voxel-based analysis, brain contusions in the left orbitofrontal and orbitofrontal/insular cortices showed relationships with worse executive function and slowed information processing speed, respectively. Neither the anatomical location of white matter lesions (deep/infratentorial vs. lobar only) nor their number predicted levels of neuropsychological function. Whilst these findings are partly consistent with previous literature in identifying weak relationships between standard MRI-derived indices of brain structure and cognitive function after TBI, they also raise the possibility that more advanced imaging methods may be better suited to exploring the complex dynamics between the often diffuse pattern of structural abnormalities in TBI and cognitive outcome.

3.1 Introduction

Traumatic brain injury (TBI) is a highly heterogenous neurological condition in terms of its aetiology, severity level and sequelae. Although TBI is known to sometimes lead to persistent neurological, cognitive and behavioural sequelae, there are still many unresolved issues, particularly relating to the diagnosis and prognosis of TBI and the neural determinants of outcome in the long term (Scheid & von Cramon, 2010). Cognitive impairments are a frequent consequence of TBI, and where these persist, they contribute to long-term disability after TBI (see e.g. Chen and D'Esposito, 2010; Whitnall et al., 2006).

Traditional approaches to investigating how structural brain damage may relate to cognitive function include the 'lesion-defined' approach (Bates et al., 2003). In this approach, the behaviour of a group of patients with lesions identified in a common brain region (e.g. medial PFC) is compared with that of a control group. Whilst this method can provide valuable information regarding the involvement of particular regions in certain cognitive functions, it provides limited information where a region can in fact be fractionated into several, possibly functionally specialised subregions, and because complex functions can be subserved by a number of anatomically distinct regions.

The second widely used approach is the 'behaviour-defined' approach (e.g. Badre, Hoffman, Cooney, & D'Esposito, 2009). This typically involves grouping patients according to the presence or absence of a specific behavioural deficit, identifying each patient's lesions, and then comparing the two groups' lesion profiles in a standard brain space to locate the commonly injured areas in patients with the deficit. Whilst this approach can be useful in identifying brain regions that appear to be critical for a specific cognitive function, it requires arbitrary cut-offs being applied to continuous data to classify performance as impaired. Apart from the difficulties inherent in determining appropriate cut-offs, important information regarding individual variability in performance can also be lost in such forced binarisation of data (Bates et al., 2003). However, as the current overview will focus on the contributions of the lesion-defined/region of interest (ROI) approach to investigations of brain structure-cognitive function relationships, the reader is referred to alternative sources for a more general discussion of the relative benefits of traditional lesion analysis approaches versus more recent structural and functional neuroimaging methods (see e.g. Rorden & Karnath, 2004; Sutton, Ouyang, Karampinos, &

Miller, 2009).

An important consideration in TBI specifically is that although some injury profiles, and in particular a fronto-temporal pattern of damage, are frequently observed, lesion patterns of individual patients are highly variable. Moreover, the patterns of injury tend to involve diffuse axonal injury (DAI), meaning that focal brain injury is unlikely to fully explain a patient's profile of symptoms. The lack of consistency across patients in which brain regions are damaged complicates investigation of brain-behaviour relationships relying on traditional region-based approaches. The present study applies a selection of lesion mapping, volumetric and voxel-based methods to exploring how structural abnormalities observed on standard MRI of TBI may relate to neuropsychological function in three cognitive domains frequently affected by TBI: verbal learning and memory, executive function and information processing speed.

3.1.1 Common neuropathological effects of TBI. As discussed in more detail in Chapter 1, the neuropathologic effects of TBI include skull fracture, brain contusions, epidural, subdural, subarachnoid and intraparenchymal haemorrhagic lesions, as well as diffuse patterns of injury, consisting of diffuse swelling, ischaemic damage, and DAI (see e.g. Laurer, Lenzlinger, McIntosh, 2000; Saatman et al., 2008). Superficial siderosis, an abnormality developing as a result of subdural or subarachnoid haemorrhage can also occur, secondary to chronic haemosiderin deposition. Some correspondence exists between the physical mechanism of injury and the most prominent type of structural abnormality that results (see Chapter 1), in that the type, magnitude and direction of the loading forces generated at the time of impact partly determine the type, anatomical location and degree of injury that follows (Gennarelli & Thibault, 1985). For example, whereas most focal abnormalities, such as brain contusions, are associated with *impact loading* forces, DAI is caused by *inertial loading* forces. These observations are of limited value in clinical practice, however, as often only partial information is available regarding the particular mechanism of injury and associated loading conditions, and these are in fact often inferred from the type, anatomical distribution, and clinical severity of the brain injury (Saatman et al., 2008).

'Focal' abnormalities are disproportionally more frequently located in the frontal lobes and polar areas of the temporal lobes than other areas of the cerebrum, reflecting these regions' greater susceptibility to the mechanics of TBI (Bigler, 2007; Bigler & Maxwell, 2011;

see Chapter 1). Apart from these focal-appearing abnormalities, cerebral atrophy of variable degree has been observed in numerous studies of TBI (e.g. Bigler, 2001b; Bigler, Ryser, Gandhi, Kimball, & Wilde, 2006; MacKenzie et al., 2002; Sidaros et al., 2009; Warner et al., 2010). This loss of viable brain tissue is believed to occur primarily as a consequence of direct injury to neuronal cell bodies (Povlishock & Katz, 2005). Regional brain atrophy after TBI has most frequently been observed in frontal and temporal poles, the hippocampi, and the corpus callosum (e.g. Ariza et al., 2006; Warner et al., 2010; see Bigler & Maxwell, 2011, for a review). Whole-brain atrophy (a global reduction of brain tissue volume) is not necessarily evident within the first few months after TBI, but may instead develop more gradually over the long-term (e.g. MacKenzie et al., 2002; Sidaros et al., 2009). However, the significance of widespread loss of brain tissue for cognitive outcome in the post-acute/chronic stages after TBI remains unclear.

3.1.2 Standard neuroimaging of traumatic brain injury in the detection of neuropathology. Clinical neuroimaging is an important element of the acute management of TBI. Nevertheless, findings from neuroimaging using standard methods, including CT imaging and more recently structural MRI, often do not correlate with functional outcome after TBI. This is partly due to the lack of more robust tools for the assessment of TBI neuropathology, including more advanced neuroimaging methods that might contribute to enhanced outcome prediction (Irimia et al., 2011).

Computed tomography scans are used very widely for visualising TBI pathoanatomy in acute clinical settings. Reasons include its ability to detect skull fracture, haemorrhages and oedema, but also its relatively low cost, better availability, and ease of use as compared with MRI. More recently, however, despite contraindications for the use of MRI in some cases, and its inappropriateness for the acute assessment of the most severely injured patients, MRI is increasingly used in post-acute management of TBI. It offers several advantages over CT, including superior image resolution to detect more subtle neuropathology (Bigler & Maxwell, 2011; Saatman et al., 2008; see Chapter 1 for further discussion). In particular, it allows different tissue types, physical properties of brain structures and abnormalities to be emphasised and visualised. Quantification of these MRI findings enables the investigation of structural brain correlates of some of the consequences of TBI, such as neuropsychological deficits.

Two types of MRI scan sequences are most often used in such studies: T1- and T2-weighted (see Rorden & Karnath, 2004, for a review). Importantly, T1-weighted MRI offers a good spatial resolution and a clear contrast between grey and white matter tissue and can thus identify structural abnormalities that are missed on CT scans (Laalo, Kurki, Sonninen, & Tenovuo, 2009). In the spectrum of DAI, T2-weighted MRI sequences can be used to image non-haemorrhagic white matter lesions, whilst gradient echo T2*-weighted MRI is sensitive to the petechial haemorrhages (known as 'microbleeds') that primarily affect the white matter tissue and are visible as small round/oval areas of dark (i.e. hypointense) signal abnormality on T2*-weighted images (Gallagher et al., 2007). The majority of TBI patients with moderate to severe injuries, and some patients with mild TBI have abnormalities visible on T1- and/or T2*-weighted images (Saatman et al., 2008). These MRI scan sequences are, however, limited in that they cannot reveal whether an area that appears structurally intact also functions normally. However, they are of demonstrated value in assessing the structural effects of brain injury, and in the detection of brain contusions and microbleeds indicative of DAI.

3.1.3 Neuropsychological correlates of typical MRI findings after TBI.

3.1.3.1 Regional and whole-brain atrophy. Relationships between the extent of brain atrophy after TBI in specific regions and neuropsychological performance have been investigated in previous volumetric MRI studies. Normally, the level of atrophy is inferred from the residual volume of brain tissue, as measured from the MRI scans using some semi-automated technique for tissue class segmentation. This allows for the residual volumes of grey, white and total brain matter to be estimated. Warner et al. (2010), for example, investigated relationships between cortical and subcortical regional brain volumes and cognitive function post-TBI in the same three neuropsychological domains on which the current study focuses. They found that whilst regional volumes of the amygdala and hippocampi were correlated with verbal learning and memory performance, thalamic volume correlated with a composite measure of information processing speed. In regression analyses, a composite of all subcortical brain volumes was found to correlate with both memory performance and processing speed, but not with executive function. By contrast, volumes of several cortical structures (superior frontal and superior parietal cortices in particular) correlated with measures of executive function. Again, however, when all cortical brain volumes were combined in a

regression analysis, this single volumetric index was associated with memory performance and processing speed, but not with executive function.

Other studies have likewise demonstrated a specific relationship between hippocampal atrophy and memory impairment after TBI. For example, Hopkins, Tate and Bigler (2005) compared neuropsychological outcome in relation to brain volume within two groups of brain-injured patients matched for age, gender, and ventricle to brain ratio; one group had TBI, the other anoxic brain injury. Hippocampal tissue loss predicted worse memory performance in both groups. There is also evidence that the relationship between hippocampal atrophy and memory impairment post-TBI is long-lasting: it has been found in patients injured as long as 30 years previously (Himanen et al., 2005).

Findings reported by Warner et al. (2010) suggest that cognitive impairment after TBI is associated not only with focal damage to structures such as the medial temporal structures and the frontal and parietal cortices, but also with more widespread atrophy. This is consistent with the increasingly common view that functions such as verbal memory, executive function and processing speed depend on the intact structure and coherent functioning of large-scale brain networks as well as on individual regions (see Chapter 1). However, few studies have investigated the relationships of whole-brain atrophy with cognitive deficits after TBI, and their findings are inconsistent. Importantly, Hopkins et al. (2005), who found a regional correlation between hippocampal volume and memory performance, noted that the total amount of brain tissue loss was potentially a more critical factor than focal injury to cognitive impairment after TBI. Some support for this suggestion comes from Vannorsdall et al. (2010), who compared the brain volumes and cognitive performance of 14 chronic TBI patients and 28 demographically matched healthy controls. Patients, who performed worse than controls on several of the neuropsychological measures used (including measures of delayed verbal recall, attention and processing speed), likewise showed reduced cerebral volume, mainly attributable to a global reduction of white matter volume. However, Hofman et al. (2001) found only weak correlations between whole-brain volume loss and cognitive sequelae of mild TBI. Tate, Khedraki et al. (2011) recently postulated that the reason why cerebral atrophy is not consistently found to predict cognitive function across studies could lie in methodological differences in estimating the level of atrophy. They compared several ways of estimating atrophy from MRI scans, and concluded that normalised measures of brain volume, corrected for individual variability in total

intracranial volume, were the most robust indices and should be preferred in neuropsychological research.

Schönberger et al. (2009) studied the relationship between age and non-lesioned white and grey matter tissue volume in different brain regions in a sample of 98 mostly young TBI patients who were 2.3 years post-injury on average. They found that older age at the time of injury was associated with smaller residual grey matter volumes in several brain regions. However, because they did not include a control group in their analyses, separate effects of TBI and normal aging on residual brain volumes after TBI could not be evaluated. However, Farias et al. (2011), who studied the relationship between whole-brain volume and cognitive function in a large sample of healthy older adults with a variety of levels of cognitive ability, showed that the total volume of brain tissue significantly predicted cognitive function in a number of domains including episodic memory and executive function. Due to its possible mediating role, it is important to control for age in studies of the effects of brain injury on brain volume and relationships with cognitive function.

In general, studies that have explored whole-brain volume changes in patients with TBI compared with controls have done so using relatively small samples, or have focused on studying select groups of patients, for example those with only mild to moderate TBI (e.g. MacKenzie et al., 2002). As this is poorly representative of the overall TBI population, it remains to be determined whether whole-brain volume loss is evident in the post-acute/chronic stages post-injury across the spectrum clinical severity and various aetiologies.

3.1.3.2 'Focal' lesions and cognitive function. Most brain contusions observed after TBI are of the contrecoup type, affecting primarily the frontal and temporal lobes (Ratnaike et al., 2011). Given the known involvement of these regions in executive function and memory, it is perhaps surprising that non-volumetric MRI studies typically find no or only weak associations between the anatomical location of focal lesions and neuropsychological performance (e.g. Anderson et al., 1995; Lee et al., 2008). Diffuse axonal injury is also a common pathological mechanism in TBI, and lesions indicative of DAI (particularly traumatic microbleeds) are frequently observed in the rostral brain stem, the basal ganglia, the hypothalamus, the superior cerebellar peduncles, the fornices, the corpus callosum, and the frontal and temporal poles. There are currently mixed findings regarding the relationship between microbleeds and

cognitive dysfunction after TBI.

For example, Lee et al. (2008) found that the majority of 36 mild TBI patients, assessed acutely (<2 weeks post-injury) using standard structural neuroimaging (CT and MRI) had some lesions, including axonal injury and brain contusions; however, these were not associated with working memory impairment. In an earlier study, Anderson et al. (1995) found no difference between TBI patients with or without frontal lobe lesions in terms of their scores on widely used tests of executive function including the Trail Making Test (TMT; Reitan, 1958; Reitan & Wolfson, 1985). On the other hand, Felmingham et al. (2004) found that the presence of DAI lesions after TBI was associated with deficits of information processing speed. This study compared TBI patients whose injury was classified as 'predominant DAI' with those deemed to have 'minimal DAI', but as neither of these classifications exclude the presence of other types of injury it is possible that these have also played a role in the results. Scheid et al. (2006) found that the presence of traumatic microbleeds observed on T2*-weighted MRI, in the absence of other brain abnormalities, predicted persistent impairment of verbal learning and memory and executive function. They further confirmed an earlier suggestion (Scheid et al., 2003) that the simple *presence* of traumatic microbleeds was more relevant to outcome than was their *number*. The issue regarding the relationship between the number of microbleeds and cognitive function after TBI will be re-visited in the current study.

Matsukawa et al. (2011) have recently shown that the anatomical location of white matter lesions indicative of DAI is related to clinical outcome at one year post-injury, measured using the Glasgow Outcome Scale (GOS; Jennett, Snoek, Bond, & Brooks, 1981). They retrospectively studied a large sample of TBI patients (N = 261) and identified white matter lesions in lobar regions, the corpus callosum, and the brainstem. Of these locations, the presence of lesions in the corpus callosum most strongly predicted unfavourable outcome after TBI, defined on the GOS in terms of general functioning/disability. As it has not yet been studied, it will also be investigated here whether the anatomical location of white matter lesions relates to the type of neuropsychological impairment seen after TBI. Specifically, the distinction here is made between deep/periventricular and infratentorial (brainstem and cerebellum) versus lobar white matter lesions. This is partly based on DeCarli, Fletcher, Ramey, Harvey and Jagust (2005), who argue that because important white matter pathways traverse deep and periventricular regions, it is not meaningful to distinguish between the impacts on cognitive

function of white matter lesions in these two locations. Thus, neuropsychological function of patients with lesions in lobar regions only is contrasted with that of patients with lesions of deep/periventricular and/or infratentorial white matter (some of these patients in addition having lobar lesions).

3.1.4 Investigating relationships between brain structure and cognitive function. Neuropsychological correlates of brain abnormalities detected after TBI using standard MRI will be investigated here applying a variety of approaches, briefly described in the following sections.

3.1.4.1 Traditional approaches to lesion analysis. Traditional lesion analysis studies of the relationship between focal changes in brain structure and cognitive function start by grouping patients according to the presence of lesions in a pre-selected ROI; they then compare the neuropsychological performance of these patients to that of a control group, consisting of healthy individuals or of patients without lesions in that ROI. An alternative way is to quantify the lesions identified in a specific ROI, or across the brain, and then explore the relationships of their number/volume with neuropsychological measures. Whilst these methods provide useful information about the prevalence and degree of brain damage in certain anatomical locations, selecting an ROI *a priori* means that important structural effects of TBI in other regions, potentially also contributing to the cognitive function of interest, may be missed altogether. Furthermore, a particular problem with manually defining lesions on MR images is that lesions in TBI do not necessarily have clearly defined boundaries, and their impact on the structural integrity of brain tissue can extend beyond the immediately visible site of MR signal abnormality (see Bigler, 2001b).

3.1.4.2 Voxel-based lesion-symptom mapping: A whole-brain approach. Voxel-based lesion-symptom mapping (VLSM; Bates et al., 2003) can be used to test the relationship between lesion location on MR scans and neuropsychological performance, on a whole-brain, voxel-by-voxel basis. This mapping for brain-behaviour relationships does not require patients to be classified according to arbitrary cut-offs defining commonly injured regions or levels of cognitive ability. Instead, VLSM uses continuous data and generates statistical maps at the group level that can reveal patterns of damage associated with impairments of specific

functions. A test statistic is then calculated for each imaging voxel, which describes the strength of the relationship at that voxel.

3.1.5 Aims of the present study. In a group of TBI patients with injuries of varied aetiology and clinical severity, all at least one month post-injury, the current study uses standard MRI methods to detect and quantify whole-brain atrophy and specific abnormalities, including brain contusions and white matter lesions indicative of DAI, and explores interrelationships between these structural abnormalities and cognitive outcome in the domains of verbal learning and memory, executive function and information processing speed. The study addresses the following specific hypotheses:

3.1.6 Hypotheses.

1) Patients in the post-acute/chronic phase after TBI will have smaller grey matter, white matter and total brain volumes than age-matched healthy controls.
2) In the TBI group, level of whole-brain atrophy, indexed by residual volumes of grey matter, white matter or total brain tissue, will be associated with neuropsychological indices.
3) In the TBI group, brain contusions or white matter lesions in particular anatomical locations will correlate with specific neuropsychological indices; specifically (based on the literature previously reviewed) it is hypothesised that:
    a. Medial temporal, prefrontal, and posterior/medial parietal contusions will be associated with worse verbal learning and memory.
    b. Frontal and lateral parietal contusions will be associated with worse executive function.
    c. Contusions affecting the frontal motor areas, the striatum, the insula, or pontocerebellar structures will be associated with slower information processing.
    d. Patients with deep and/or infratentorial white matter lesions will show worse neuropsychological performance than patients with lobar white matter lesions only.

4) Higher lesion loads, indexed by total brain contusion volume or the number of microbleeds, will be associated with more severe cognitive impairment in the domains of verbal learning and memory, executive function and information processing speed.

3.2 Methods and Materials

3.2.1 Design. In this between- and within-groups study patients who were at least one month post-TBI and a group of age-matched healthy controls were assessed using structural MRI (T1- and T2*-weighted) and a neuropsychological test battery.

3.2.2 Participants. Participants were 40 patients with definite or probable TBI and 40 healthy controls. All patients underwent structural MRI and neuropsychological assessment as explained in Chapter 2, and all controls underwent structural MRI. Of the controls, a subgroup of 26 was assessed on the neuropsychological tests. The procedures for the recruitment of participants and the inclusion and exclusion criteria used were also as detailed in Chapter 2. In summary, all participants were a minimum of 18 years of age and capable of giving a written informed consent according to the Declaration of Helsinki (World Medical Association, 2008). The study was approved by the Hammersmith, Queen Charlotte's and Chelsea Research Ethics Committee and the Ethics Committee of the Department of Psychology, Goldsmiths, University of London.

3.2.3 Neuropsychological assessment. The assessment of patients was split between two approximately one-hour sessions with an average of one week's break between the sessions, whereas healthy controls were assessed during a single session lasting approximately one hour and a half. The test battery was designed to tap general intellectual function and three broad domains of cognitive function often impaired after TBI: verbal learning and memory, executive functions, and information processing speed. The following lists the specific indices of cognitive function used in the current study (see Chapter 2 for a more comprehensive description).

- Premorbid and current IQ: The Wechsler Test of Adult Reading (WTAR; Wechsler, 2001) was used to provide an estimate of premorbid intellectual functioning. Current verbal and nonverbal reasoning ability were assessed via the Similarities and Matrix Reasoning subtests from the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999).
- Verbal learning and memory: The People Test immediate recall index (Doors and People battery; Baddeley et al., 1994) was used as a measure of verbal associative learning and recall, and the Logical Memory I first recall total score (Wechsler Memory Scale- Third Edition; WMS-III; Wechsler, 1997) to index immediate recall of structured verbal material.
- Executive function: Set-shifting ability was indexed by the Trail Making Test (TMT; Reitan, 1958; Reitan & Wolfson, 1985), completion time for TMT-B – completion time for TMT-A. Cognitive flexibility/susceptibility to interference was indexed by the Delis-Kaplan Executive Function System (D-KEFS; Delis et al., 2001) Color-Word Interference test inhibition/switching completion time – a baseline (the average completion time across the color naming and word reading conditions). Word generation fluency was indexed by the total correct score on the D-KEFS Verbal Fluency/letter fluency (F, A and S).
- Information processing speed: A computerised two-choice left/right (<<< or >>>) button-press choice-reaction task (CRT; see Chapter 2 for further details) was used to provide a measure of processing speed (median reaction time on correct trials).

Subcomponents of the TMT and D-KEFS Color-Word Interference test also tapped types of processing speed (completion time for TMT-A: visual search, completion time for TMT-B: complex information, and completion times for D-KEFS baseline conditions: naming and reading), but these were not used in the analyses as measures of interest.

3.2.4 Magnetic resonance imaging.

3.2.4.1 Data acquisition and estimation of brain volume. As described in more detail in Chapter 2, participants were scanned on a 3.0-Tesla Philips Achieva machine (Philips Medical Systems, Netherlands) and high-resolution T1- weighted and gradient-echo T2*-

weighted sequences used to acquire the structural brain images.

The brain imaging volumes of interest were volumes of (a) grey matter tissue, (b) white matter tissue, and (c) total brain tissue. These volumes, normalised for head size, were estimated by performing a SIENAX analysis (Smith et al., 2002; Smith et al., 2004) on the native brain-space T1-weighted images. This procedure allows for comparisons of atrophy-free, 'viable' brain volumes across individuals.

The specific steps in the analysis included: (a) separating brain and skull using the Brain Extraction Tool (BET; Smith, 2002), (b) using the FMRIB's Linear Image Registration Tool (FLIRT; Jenkinson & Smith, 2001; Jenkinson et al., 2002) to affine-register the participant's individual-space brain and skull to standard brain space (MNI152_2mm), (c) using a standard-space derived brain tissue mask to remove the brain stem and exclude from the analysis the optic nerve and eye balls, (d) performing tissue type segmentation (Zhang, Brady, & Smith, 2001), (e) deriving a volumetric scaling factor used to normalise an individual's brain tissue volume based on their head size, and (f) calculating estimates of un-normalised and normalised volumes of grey matter, white matter and total brain tissue.

For those patients who had white matter hypointensities identified on the T2*-weighted MR images, the brain volumes were computed with the lesion voxels excluded from the tissue map generation. This was done to avoid the hypointense white matter voxels being misclassified as grey matter. This may occur because the tissue type segmentation technique used here is intensity-based and the intensity of hypointense white matter can be close to that of normal grey matter, whilst the intensity of normal white matter is higher than that of grey matter. The results were checked one by one and relevant parameters modified where necessary.

3.2.4.2 Lesion mapping: Lesion type and anatomical location. Lesion mapping analysis was performed to study the anatomical distribution of brain contusions and white matter lesions after TBI, identified on T1- and T2*-weighted MR images, respectively.

Brain contusions were manually defined on each patient's individual brain-space T1 images using MRICron (http://www.cabiatl.com/mricro/mricron/index.html). The defined lesions were then saved as a single mask and FSLView was used to verify that this mask image was overlapping with the lesions on the individual-space T1-weighted image. The lesion mask was

then affine-registered using FLIRT to a standard MNI152_T1_1mm template within FSL, applying parameters obtained from the normalisation of the individual-space T1 image to standard MNI space, carried out immediately before. The products of this registration, a patient's standard-space T1, and the patient's standard-space lesion mask, were then loaded into FSLView to check that they were well-aligned with the MNI152_T1_1mm template. The standard-space lesion mask was then thresholded at 0.5 using fslmaths with the aim of keeping the lesions close to size of the original lesions, and finally the masks were binarised. This produced a 3D standard-space contusion map for each patient.

White matter lesions were also manually identified, on each patient's T2*-weighted images. MR signal abnormalities identified as microbleeds were classified as 'definite' or 'possible' based on the Microbleed Anatomical Rating Scale (MARS; Gregoire et al., 2009), which distinguishes these petechial haemorrhages from various 'microbleed mimics'. The normalisation procedure for white matter lesions was similar to that used for the normalisation of brain contusions: Lesion masks were saved, their alignment with lesions visible on the original T2*-weighted image was checked, and then they were translated to standard brain space (MNI152_T1_1mm_brain), applying the parameters obtained from the prior FLIRT-normalisation of the individual-space T2* image to standard MNI brain space. The quality of the alignment of each patient's lesion mask with the MNI template was then examined in FSLView.

To obtain 3D lesion probability maps that show the percentage of patients out of the total sample of 40 who had a lesion at each anatomical location, lesion overlap images were then created using fslmaths in FSL. First, the spatially normalised and thresholded binary lesion masks from all patients were added together. The resulting image was then divided by the total number of patients (N = 40) and multiplied by 100 to derive the lesion probability (%) maps as shown in Figure 3-1.

3.2.4.3 Lesion analysis: Lesion load. The total volume of brain contusions was computed as follows. First, fslmaths was used to normalise the volume of these lesions, defined in each individual's brain space, with the SIENAX-derived VSCALING factor. Intra-cranial volumes were made consistent (i.e. normalised) across patients by multiplying the total lesion volume by the VSCALING factor. In addition, the total number of microbleeds across the whole brain was calculated in SPSS for patients with these white matter lesions observed on T2*-

weighted MR images.

3.2.5 Statistical analyses.

3.2.5.1 Brain volume: Patients versus controls. Results from the SIENAX brain volume estimation (head size-normalised grey matter, white matter and total brain tissue) were entered into SPSS and the TBI and control groups then compared. Independent samples *t*-tests were used after adjusting for the potential confounding effects of age, previously demonstrated to affect the structural integrity of the brain (e.g. Fotenos, Snyder, Girton, Morris, & Buckner, 2005; Schönberger et al., 2009). The brain volumes were first regressed against age and the standardised residuals saved. These residuals were then used as the dependent variables, and the groups compared using *t*-tests.

3.2.5.2 Brain volume: Relationships with neuropsychological performance. Statistical relationships between each of the three measures of whole-brain volume (grey matter, white matter, and total brain tissue) and the six neuropsychological measures of interest were assessed within the patient group.

Relationships of potential confounds (age, severity of injury (mild: +1, moderate/severe: −1), and time since injury) with neuropsychological test performance were first explored by calculating nonparametric Spearman rank correlation coefficients: (a) age significantly correlated with median reaction times on the CRT (*rho* = .45, $p < .01$), but not with any of the other cognitive measures, (b) severity of injury significantly correlated with TMT-B – TMT-A completion times (*rho* = − .48, $p < .01$) and CRT reaction times (*rho* = − .42, $p < .01$), but not with the other measures, and (c) time since injury/months post-injury similarly correlated with performance on the TMT-B – TMT-A (*rho* = .38, $p < .05$) and CRT (*rho* = .57, $p < .001$), but not with performance on the other measures.

Therefore, whilst relationships between brain volumes and performance were in general investigated using standard Spearman rank correlation coefficients, correlations between brain volumes and TMT and CRT performance were tested using Spearman rank-based partial correlation, controlling for the effects of the confounds identified above. These partial correlations were calculated based on the residuals from the linear regression of the ranks of the variables of interest on the ranks of the variables partialled out.

3.2.5.3 Relationship between anatomical location of brain contusions and cognitive function. Voxel-based Lesion Symptom mapping (VLSM; Bates et al., 2003), part of MRIcron (http://www.cabiatl.com/mricro/mricron/stats.html), was used to test voxel-wise correlations between brain contusions and neuropsychological performance across the whole brain. Separate analyses were carried out investigating the voxel-based lesion correlates of each of the cognitive variables of interest.

First, patients were divided into two groups at each voxel according to the presence or absence of a lesion affecting that voxel, with voxels affected by lesions in fewer than two patients excluded from the analysis. The patients' scores on the neuropsychological measures of interest were then compared between these two groups, each in their separate *t*-test analyses.

Standard *t*-tests were used instead of the nonparametric Brunner-Munzel rank order test (Rorden, Karnath, & Bonilha, 2007), because the Brunner-Munzel has been shown to inflate the probability of a Type I error if used to test voxels where a very small number (<10) of participants are in either the lesion or the no lesions group (Medina, Kimberg, Chatterjee, & Coslett, 2010). Carrying out the *t*-test yielded a single-tailed *p*-value at each voxel, corresponding to how likely a relationship was between the presence of lesion at that voxel and worse neuropsychological performance.

A False Discovery Rate (FDR; Benjamini & Hochberg, 1995) correction for multiple comparisons was implemented to provide reasonable statistical power in this type of whole-brain analysis while guarding against false positives. The threshold to control the FDR was set at $p_{FDR} < .01$, as recommended by Kimberg (2009), meaning that out of every 1000 voxels exceeding the threshold for statistical significance, no more than 10 could be false positives. Whilst this is clearly more lenient than traditional family-wise (or map-wise) error rate, controlled at $p < .05$, it also guards against false negatives, which could be particularly problematic for this type of exploratory data analysis, with high spatial coherence (whereby the lesion status of a given voxel predicts that of the neighbouring voxels) being an inherent property of the lesion maps being analysed (Kimberg, 2009).

Reflecting the convention in VLSM that a higher score indicates better performance, larger voxel-based *t*-statistics normally mean a stronger effect of the presence of a lesion at that

voxel on behaviour (Bates et al., 2003). However, negative *t*-statistics were expected to result from analyses of the following cognitive measures, where a lower score indicates better performance: TMT-B – TMT-A, Color-Word Interference test inhibition/switching − baseline, and CRT median reaction time.

3.2.5.4 Relationship between anatomical location of white matter lesions and cognitive function. The effect of the anatomical location affected by white matter lesions (deep/infratentorial vs. lobar only) on the six cognitive indices was tested using independent samples *t*-tests.

3.2.5.5 Lesion load and cognitive function. Relationships between the six neuropsychological measures and normalised contusion load (total number of voxels across the whole brain) were then explored, as were their relationships with white matter lesion load (total number of microbleeds).

Given the significant correlations between TMT and CRT performance and potential cofound variables (age, severity of injury, and time since injury) the relationships between contusion and white matter lesion load and these two cognitive variables were explored using Spearman rank-based partial correlation, controlling for the relevant confounds. Correlations between lesion load and the remaining four cognitive variables were tested using standard nonparametric correlation.

3.3 Results

3.3.1 Participant demographics. Groups of 40 TBI patients and 39 controls participated in the brain volume analyses. Demographic and clinical characteristics of these groups are summarised in Table 3-1a below. As evident from this table, the gender ratios in the groups used for the brain volume comparison were significantly different, with more men than women in the patient group and about equal numbers in the control group. The average age in the patient group was also significantly higher than in the control group.

Table 3-1a

Demographics for the Groups with Structural MRI Data

CHARACTERISTIC		TBI (*N*=40)	CONTROL (*N*=39)	TBI vs. CONTROL
Gender (M:F)		30:10	19:20	χ^2 = 5.33 *
Age (*M*±SD)		39.4±11.7	34.2±10.3	*t* = 2.07 *
Cause of injury (number (%) of patients)	Assault	14 (35%)	n/a	
	RTA	13 (32.5%)		
	Fall	8 (20%)		
	Sports	3 (7.5%)		
	Other or unknown	2 (5%)		
Severity of TBI (number (%) of patients)	Mild (probable)	15 (37.5%)		
	Moderate/severe (definite)	25 (62.5%)		
Time post-injury/months (Median/range)		13.5/1-73		

Notes: RTA = road traffic accident. * $p < .05$.

Table 3-1b

Demographics for the Groups in the Comparison of Neuropsychological Performance

CHARACTERISTIC	TBI (*N*=36)	CONTROL (*N*=26)	TBI vs. CONTROL
Gender (M:F)	27:9	12:14	χ^2 = 5.38 *
Age (*M*±SD)	40.0±11.3	35.4±11.1	*t* = 1.58 ^{ns}
Premorbid IQ/WTAR age-scaled score (*M*±SD)	106.6±8.7	115.0±9.2	*t* = −3.68 **
Current verbal IQ/WASI Similarities age-adjusted *T*-score (*M*±SD)	56.8±6.6	52.7±8.9	*t* = 2.08 *
Current nonverbal IQ/WASI Matrix Reasoning age-adjusted *T*-score (*M*±SD)	59.5±7.9	55.9±8.4	*t* = 1.70 ^{ns}

Notes: WTAR = Wechsler Test of Adult Reading. WASI = Wechsler Abbreviated Scale of Intelligence. ** $p < .01$. * $p < .05$. ^{ns} not significant.

A subgroup consisting of 25 controls, plus one additional male participant for whom the T1-weighted MRI scan was not available, participated in the comparison of neuropsychological performance. Thirty-six patients were included in these comparisons, because the WTAR could not be reliably used for the assessment of four of the patients whose first language was not English; in addition, one patient did not complete some of the tests. Table 3-1b shows the average demographic characteristics of the 26 controls, compared with the 36 patients.

The gender ratios in the patient and control groups were again significantly different; however the groups were well-matched for age. Table 3-1b also shows the average age-scaled scores of the patient and control groups on the WTAR, used to estimate premorbid intellectual ability: controls scored significantly higher. Patients, however, outperformed controls on current verbal reasoning ability indexed by WASI Similarities age-adjusted scores. The groups did not differ on WASI Matrix Reasoning age-adjusted scores, indexing current nonverbal reasoning ability.

3.3.2 Neuropsychological profile of the patient group. Table 3-2a shows the means and standard deviations for and results of group comparisons on the six cognitive measures of interest, and Table 3-2b shows these for their subcomponents tapping different aspects of information processing speed.

Table 3-2a

Group Means on the Cognitive Measures

COGNITIVE FUNCTIONS/ MEASURES OF INTEREST		TBI (*N*=36)	CONTROL (*N*=26)	TBI vs. CONTROL
		M±SD	*M*±SD	
Memory				
Associative memory	People Test immediate recall	24.3±4.9	28.6±6.0	$t = -1.16^{ns}$
Logical memory/ immediate	WMS-III Logical Memory I 1st recall total	28.6±6.2	27.2±8.5	$t = 1.27^{ns}$
Executive function				
Alternating-switch cost	TMT-B minus TMT-A completion time (s)	43.3±34.6	29.2±31.3	$t = 0.52^{ns}$
Cognitive flexibility	D-KEFS Color-Word inhibition/switching – baseline (CN + WR ÷ 2) (s)	39.9±20.1	31.5±20.9	[a] $t = 0.88^{ns}$
Word generation fluency	D-KEFS Verbal Fluency/ letter fluency F+A+S total	43.4±9.7	47.4±12.4	$t = -0.60^{ns}$
Processing speed				
Choice reaction time	CRT median RT on correct trials (ms)	455±87	393±52	$t = 2.09$ *

Table 3-2b

Group Means on the Subcomponents of Main Measures

SUBCOMPONENTS OF MEASURES OF INTEREST		TBI (*N*=36)	CONTROL (*N*=26)	TBI vs. CONTROL
		M±SD	*M*±SD	
Visual search speed	TMT-A completion time (s)	28.9±10.9	20.5±5.8	$t = 3.06$ **
Complex processing speed	TMT-B completion time (s)	71.2±40.1	49.8±35.8	$t = 1.14^{ns}$
Complex processing speed	D-KEFS Color-Word inhibition/switching (s)	70.0±24.0	47.9±12.6	[a] $t = 3.49$ **
Speed of naming	D-KEFS Color-Word color naming (CN) (s)	34.8±9.7	29.4±31.3	$t = 0.09^{ns}$
Speed of reading	D-KEFS Color-Word word reading (WR) (s)	23.8±5.2	27.9±5.5	$t = -2.63$ *

Notes: All *t*-values reported are based on group means after the effects of individual differences in WTAR performance were regressed out. [a] Statistic calculated based on a sample of 35 patients and 26 controls due to missing value for one of the patients. WMS-III = Wechsler Memory Scale-III. TMT = Trail Making Test. D-KEFS = Delis-Kaplan Executive Function System. CRT = choice-reaction task. All *p*-values reported as uncorrected for multiple comparisons. ** $p < .01$. * $p < .05$. ^{ns} not significant.

Given the group difference in premorbid IQ as indexed by WTAR age-scaled scores,

these were controlled for in comparisons of all other neuropsychological variables. Thus, WTAR scores were regressed out, and the standardised residuals subjected to comparison via independent samples *t*-tests. Adjusted *t*-values are reported where Levene's Test for Equality of Variances was significant. No multiple comparisons correction of the *p*-value was applied here, because the nature of the enquiry was exploratory.

As can be seen from these two tables, relative to controls, the patient group was impaired only in the domain of information processing speed, and here only on three indices: TMT-A completion time, Color-Word inhibition/switching, and CRT median reaction time. Interestingly, the patient group was faster than controls on the simple task of word reading.

3.3.3 Magnetic resonance imaging findings in the patient group. Structural MRI scans were examined by a Senior Consultant Neuroradiologist for the presence of cortical and subcortical MR signal abnormalities indicative of brain injury. Some patients showed no abnormality, whilst others showed signal intensity abnormalities consistent with brain contusions, DAI or haemosiderin deposits/superficial siderosis; of these, some presented with a combination of different types of damage ('mixed abnormalities').

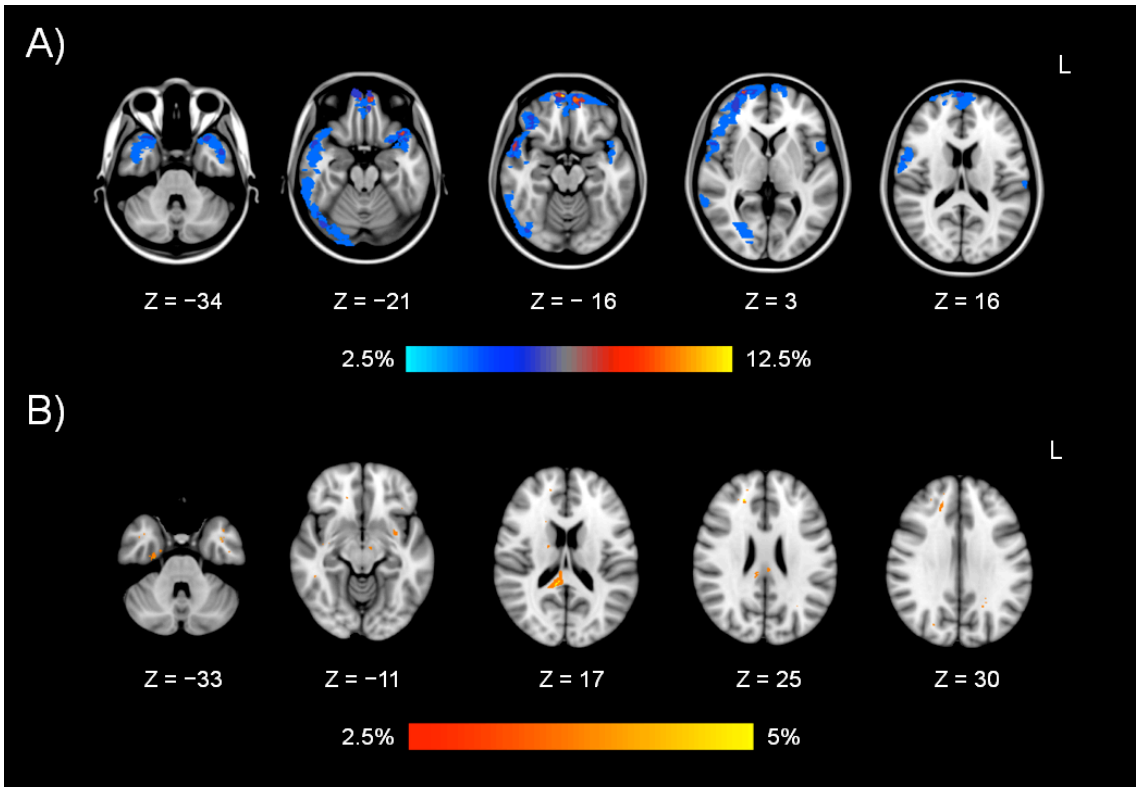

Figure 3-1. Lesion distribution and probability. Percentages of the 40 patients showing MRI evidence of A) brain contusions and B) white matter lesions.

T1-weighted scans showed brain contusions in 17 patients (43% of the sample); T2*-weighted scans showed white matter lesions, including definite and possible microbleeds, in 16 patients (40%), and superficial siderosis in 18 patients (45%). Nine patients (23%) showed no abnormalities on either T1 or T2* scans. Four patients (10%) showed only brain contusions, seven (17.5%) showed only microbleeds, and another four (10%) showed only superficial siderosis.

Brain contusions were mainly located in anterior frontal and temporal regions bilaterally. As seen in Figure 3-1A, the regions with the highest probabilities of contusions were the right frontal pole, the left frontal pole, the left frontal orbital cortex, the right central opercular cortex, the right inferior frontal gyrus, and the left and right temporal poles.

The median number of microbleeds was 10 (range 1 to 39), and they were mainly found in frontal and temporal lobar white matter bilaterally and deep white matter (see Figure 3-1B). The loci with the highest probability of T2* white matter lesions indicative of DAI were right frontal white matter (forceps minor/anterior thalamic radiation) and the splenium of the corpus callosum; in each location two patients had overlapping lesions. In all other white matter regions where lesions were observed, they were small and their distribution scattered, meaning that there was little white matter lesion overlap across patients in general.

Superficial siderosis, as indicated by haemosiderin deposits, was primarily found overlying the frontal cortices bilaterally, the right temporal cortex, and the left pons.

3.3.4 Brain volume.

3.3.4.1 Patients versus controls.

Hypothesis 1: Patients with TBI will have smaller grey matter, white matter and total brain volumes than age-matched healthy controls. As shown in Table 3-3, normalised average volume of viable brain tissue, with possible effects of age regressed out, was significantly lower in the patient group than the control group. This was the case for grey matter, white matter and total brain tissue volumes. To control for the different gender proportions in the patient and control groups, the analyses were repeated between subgroups consisting of just the male participants (30 patients vs. 19 controls). All differences remained significant after Bonferroni correction for multiple comparisons within the family of brain volume measures (grey matter

volume: $t(47) = -3.08$, $p < .01$; white matter volume: $t(47) = -4.63$, $p < .001$; total brain volume: $t(47) = -4.65$, $p < .001$).

Table 3-3

Brain Volume After Traumatic Brain Injury Compared with Healthy Controls

BRAIN VOLUME	TBI (*N*=40)	CONTROL (*N*=39)	TBI vs. CONTROL
Normalised grey matter volume in voxels[a)] Median (range)	782,021 (630,983 to 906,279)	835,367 (747,123 to 909,658)	$t = -3.84$ ***
Normalised white matter volume/voxels Median (range)	737,170 (680,259 to 821,331)	777,700 (706,882 to 857,740)	$t = -3.99$ ***
Normalised total brain volume/voxels Median (range)	1,519,192 (1,371,374 to 1,709,360)	1,613,067 (1,455,698 to 1,765,758)	$t = -4.84$ ***

Notes: [a] Number of imaging voxels. Voxel size = 1 x 1 x 1 mm. *** $p < .001$, Bonferroni-corrected ($p_{adjusted} = .05 \div 3 = .017$).

3.3.4.2 Relationships with neuropsychological performance.

Hypothesis 2: In the TBI group, level of whole-brain atrophy, indexed by residual volumes of grey matter, white matter or total brain tissue, will be associated with neuropsychological indices. Within the 40 patients, nonparametric Spearman rank or rank-based partial correlations (as explained in section 3.2.5.2, p. 103) were conducted between each of the three measures of brain volume and the six main indices of cognitive function. Multiple comparison correction using Bonferroni adjustment of the *p*-value was applied variable-wise for each of the three measures of brain volume ($p_{adjusted} = .05 \div 6 = .008$). None of the total of 18 correlations between the indices of residual brain volume and neuropsychological test performance reached statistical significance.

3.3.5 Lesion mapping results.

3.3.5.1 Anatomical location of lesions. Table 3-4 shows the number and percentage of the 40 patients who were affected by brain contusions (visible on T1-weighted MRI) in each anatomical location and Table 3-5 shows the number and percentage of the 40 patients with lesions indicative of DAI (visible on T2*-weighted MRI) in each white matter region.

Table 3-4

Number/Percentage of Patients with T1 Brain Contusions

Contusions by anatomical location	**Right hemisphere**		**Left hemisphere**		**Totals by region**	
	Number	Percentage	Number	Percentage	Number	Percentage
Frontal	11	27.5%	10	25%	**13**	**32.5%**
Temporal	7	17.5%	7	17.5%	**10**	**25%**
Parietal	-	-	-	-	**-**	**-**
Occipital	4	10%	1	2.5%	**4**	**10%**
One or more regions	**15**	**37.5%**	**13**	**32.5%**	**17**	**43%**

Table 3-5

Number/Percentage of Patients with T2 White Matter Lesions*

Evidence of DAI[a] by anatomical location	**Right hemisphere**		**Left hemisphere**		**Totals by WM region**	
	Number	Percentage	Number	Percentage	Number	Percentage
Infratentorial WM/Totals	**1**	**2.5%**	**3**	**7.5%**	**4**	**10%**
Brainstem	-	-	2	5%	2	5%
Cerebellum	1	2.5%	1	2.5%	2	5%
Deep WM/Totals	**6**	**15%**	**3**	**7.5%**	**7**	**17.5%**
Basal Ganglia	-	-	1	2.5%	1	2.5%
Thalamus	-	-	-	-	-	-
Internal Capsule	1	2.5%	1	2.5%	1	2.5%
External Capsule	1	2.5%	1	2.5%	2	5%
Corpus callosum	3	7.5%	1	2.5%	4	10%
Deep/periventricular	2	5%	-	-	2	5%
Lobar WM/Totals	**12**	**30%**	**14**	**35%**	**16**	**40%**
Frontal	12	30%	10	25%	15	37.5%
Temporal	7	7.5%	5	12.5%	8	20%
Parietal	5	12.5%	4	10%	8	20%
Occipital	2	5%	1	2.5%	2	5%
Insula	2	5%	1	2.5%	3	7.5%
One or more WM regions	**12**	**30%**	**14**	**35%**	**16**	**40%**

Notes: [a]Traumatic microbleeds or other white matter lesions observed on gradient-echo T2*-weighted MRI. Identification of microbleeds was based on the Microbleed Anatomical Rating Scale (MARS; Gregoire et al., 2009). DAI = diffuse axonal injury. WM = white matter.

3.3.5.2 Anatomical location of lesions and cognitive function. Interrelationships between the anatomical location of brain contusions and scores on the six indices of verbal

learning and memory, executive function and information processing speed were investigated within the patient group. Three specific hypotheses (3a, 3b, and 3c) were tested. The fourth hypothesis (3d) relates to the anatomical location of white matter lesions and patients' neuropsychological performance.

Hypothesis 3a: Medial temporal, prefrontal, and posterior/medial parietal contusions will be associated with worse verbal learning and memory. Voxelwise relationships between the presence of brain contusions and the two indices of verbal learning and memory were tested using VLSM. For the People Test (immediate recall) the largest voxel-based *t*-statistic observed was 3.46 (one-tailed) in the right frontal pole region; this was not significant after FDR correction to adjust for the number of unique *t*-tests carried out. For Logical Memory I (first recall total) none of the voxel-based *t*-statistics survived FDR correction for multiple comparisons or approached the threshold value of $t \geq 4.78$ at $p_{FDR} < .01$. The largest *t*-statistic of 1.85 resulting from the Logical Memory I first recall total/presence of brain contusions comparsions was observed in a cluster located in the left frontal pole region.

Hypothesis 3b: Frontal and lateral parietal contusions will be associated with worse executive function. Three separate VLSM analyses were carried out to test the voxelwise relationships between brain contusions and each of the three indices of executive function. Negative *t*-statistics were expected for the first two measures (TMT-B − TMT-A completion time and the Color-Word Interference index) and positive *t*-statistics for the third (Verbal Fluency/letter fluency total correct), reflecting the convention of VLSM and the scoring directions for these measures. The only voxel-based *t*-statistic (one-tailed) to reach significance was that between brain contusions and the TMT alternating-switch cost index ($t = -4.64$, $p_{FDR} < .01$). The voxel (MNI −36, 23, −22) showing the peak *t*-statistic was part of a small cluster located in the left orbitofrontal cortex. The largest *t*-statistics for the Color-Word Interference index/brain contusions ($t = -0.66$, *ns*) and Verbal Fluency/letter fluency total correct/brain contusions ($t = 1.67$, ns) comparisons were observed in the left temporal pole region and in the right inferior frontal region, respectively.

Hypothesis 3c: Contusions affecting the frontal motor areas, the striatum, the insula, or

pontocerebellar structures will be associated with slower information processing. The largest voxel-based *t*-statistic between brain contusions and CRT median reaction time was −4.02 (one-tailed); this was marginally significant after $p_{FDR} < .01$ adjustment for multiple comparisons. This small cluster indicating a relationship between the presence of brain contusion and slowed information processing speed was centred at a voxel located in the left orbitofrontal/insular cortex (MNI −28, 13, −21).

Hypothesis 3d: Patients with deep and/or infratentorial white matter lesions will show worse neuropsychological performance than patients with lobar white matter lesions only. For nine patients, lesions in deep/infratentorial white matter regions were observed on T2*-weighted MRI (some also had lobar lesions), whilst for seven patients only lobar white matter lesions were visible. Deep/infratentorial white matter lesions were only present in patients with moderate/severe TBI, whereas lesions restricted to lobar regions were found in four patients with moderate/severe and three patients with mild TBI. There were no significant differences between the deep/infratentorial vs. lobar white matter lesion subgroups in age, premorbid IQ (WTAR age-scaled scores), current intellectual ability (Similarities and Matrix Reasoning *T*-scores) or time since injury. Independent samples *t*-tests found no significant group differences in any of the six neuropsychological indices.

3.3.5.3 Lesion load. The median number of voxels affected by brain contusions (individual intra-cranial volume-normalised) in the 17 patients with any contusions present was 5024; the range was 162 to 21,251. The median number of microbleeds in the 16 patients with white matter lesions was 9.5, ranging from 1 to 39. Thus, both volume of brain tissue affected by brain contusions and total number of microbleeds varied enormously, with over half the sample showing zero loads for each index.

3.3.5.4 Lesion load and cognitive function.

Hypothesis 4: Higher lesion loads, indexed by total brain contusion volume or the number of microbleeds, will be associated with more severe cognitive impairment in the domains of verbal learning and memory, executive function and information processing speed. Nonparametric Spearman rank or rank-based partial correlations (see section 3.2.5.5, p. 105)

were calculated between normalised brain contusion load (number of voxels) and the six neuropsychological indices. Bonferroni adjustment of the *p*-value was applied variable-wise to account for the multiple neuropsychological measues ($p_{adjusted}$ = .05 ÷ 6 = .008).

After controlling for the effects of severity of injury and time since injury, normalised contusion load was significantly correlated with the TMT alternating-switch cost index (TMT-B − TMT-A), but in the reverse direction from that expected, with greater lesion load associated with better mental flexibility ($rho_{partial}$ = − .70, p = .008). No other correlations between contusion load and the neuropsychological measures approached statistical significance.

Nonparametric correlations between the total number of microbleeds and neuropsychological performance were similarly explored, adjusting for the effects of confound variables where these were identified. None were significant.

3.4 Discussion

3.4.1 Profile of cognitive impairments after traumatic brain injury. Based on the extensive previous literature on cognitive sequelae of TBI (see Chapter 1 for an overview), it was expected that patients would perform worse than healthy controls on tests tapping verbal learning and memory, executive function and information processing speed. However, this mixed sample of post-acute/chronic TBI patients of different aetiologies and varying levels of injury severity showed only slower processing speed, when possible effects of individual differences in premorbid IQ were controlled for. The observation of slow information processing in the absence of other cognitive impairments is consistent with some previous findings (e.g. Levin et al., 1990). In a meta-analysis of studies of cognitive outcome after mild TBI, speed of processing measures were found to have the largest effect size of all neuropsychological measures (Frencham et al., 2005).

It is interesting that verbal learning and memory impairments, which often persist chronically (e.g. Levin et al., 1990; Draper & Ponsford, 2008), did not differentiate the present patient sample from controls. Whilst a salient explanation is that the groups were imperfectly matched, another possibility is that this is related to the mixed brain abnormality profiles in the TBI group. Whilst previous research (e.g. Chu et al., 2007) has investigated how memory

impairments relate to the effects of injury severity, as measured using traditional classification systems (e.g. based on length of PTA), few if any studies have compared recovery trajectories of patients with different lesion profiles. It could be that in the current rather heterogeneous sample of patients, inter-individual variability in the severity of brain damage and in lesion types acted to mask memory deficits at group level.

Further research is needed to dissociate the effects of initial clinical indices of injury severity and neuroimaging indices of the type, degree and extent of brain damage on cognitive outcome. It is possible that microbleeds, indicative of diffuse pathology, and more focal patterns of brain contusions, could lead to different neuropsychological outcomes. Consistent with this, Ross, Temkin, Newell and Dikmen (1994) found that injury severity, inferred from coma duration, had stronger relationships than focal abnormalities observed on acute CT imaging with neuropsychological performance at one year post-TBI. Conversely, diffuse neuropathology, not readily detected using standard clinical neuroimaging techniques, may play an important role in determining cognitive outcome after TBI.

3.4.2 Residual brain volume after traumatic brain injury. Brain volume, corrected for individual head size, was found to be significantly lower in the TBI patients than in healthy controls. This was observed across grey matter, white matter and total brain tissues, as expected. The current study took individual variability in head size and thus maximum brain size into account by normalising the results from the brain and lesion volumetric analyses using a volume-scaling factor (i.e. factor required to match an individual's brain with the template brain). This factor was derived from the standard SIENAX pipeline for brain tissue volume estimation (Smith et al., 2001). Atlas-based normalisation of brain volume, such as implemented in SIENAX, equates head size across individuals and makes the scaling factor proportional to total intracranial volume. Whilst normalisation of brain volumes based on total intracranial volume markedly reduces the effect of inter-gender variation in cerebral volumes, it only partially accounts for it (Scahill et al., 2003; Whitwell, Crum, Watt, & Fox, 2001). By contrast, correcting brain volumetric analyses for variation in head size has been previously reported to adequately deal with individual variation due to gender. For example, Buckner et al. (2004) validated their head-size normalisation approach against manual TIV measurement in a sample of 147 participants aged 15–96 (194 females, 141 males; mean age 53.2 years). They found that,

apart from being equivalent to volumetric normalisation by manually delineated TIV and reliable across multiple scans obtained from the same individual, the scaling factor was able to correct for gender-associated head size differences. The sacling factor was also importantly found to be minimally biased in participants with marked brain atrophy. This scaling factor is similar to that calculated by SIENAX, allowing more conficence in the reliability of the current results, despite the significant difference between the TBI and control groups in the gender ratios. To explore the possibility that some residual effects of differences between male and female brains may still have affected these results, the comparisons of normalised grey matter, white matter and total tissue volume were also performed between subgroups consisting of males only. The group differences observed between male patients and male controls remained significant for all volumes.

Despite a recent suggestion that normalised measures of brain volume may better predict neuropsychological performance after TBI than uncorrected measures (Tate, Khedraki et al., 2011), the current study found no significant relationships between normalised brain tissue volumes and cognitive function in the three domains investigated. Whilst these negative results might suggest that whole-brain volume is not an important determinant of cognitive performance after TBI, they might also reflect low statistical power of the study to detect real, but possibly relatively weak relationships.

3.4.3 Type and anatomical distribution of 'focal' lesions. Using T1-weighted MRI, brain contusions were identified in 43 per cent of patients and in 10 per cent these were the only type of abnormality observed. T2*-weighted scans showed white matter lesions in 40 per cent of the patient sample, with 17.5 per cent showing no other abnormalities. Superficial siderosis was the only abnormality in 10 per cent, though was present in 45 per cent overall. Strikingly, nine patients (23%) had no MRI evidence of structural damage.

As expected, brain contusions in this sample were most frequently observed in the frontal and temporal lobes, near the poles in particular. White matter lesions were most frequently observed in lobar, then deep, and least frequently in infratentorial (brainstem and cerebellum) white matter. These findings are broadly compatible with other recent studies in which the spatial distribution of lesions visible on MRI has been investigated (Ratnaike et al., 2011; Schönberger et al., 2009). Out of a number of brain regions that Schönberger et al.

(2009) studied, the region most frequently affected by lesions was the frontal lobes, whilst less than half of the 98 patients they assessed had parietal lesions, and only a small minority had lesions in the brainstem or cerebellum.

In human TBI, both DAI and cerebral damage tend to show an anterior preponderance, possibly because the impact loading forces affecting these regions cause the cerebral damage to become superimposed on the axonal strain injury (Lux, 2007). The generally diffuse pattern of changes that constitutes the secondary injury (see Chapter 1) is part of an array of functional, structural, cellular, and molecular changes that interact with each other in complex ways (Laurer et al., 2000). The pathological processes underlying these changes are nevertheless not well understood (Lowenstein, 2009), nor are their relationships with clinical effects of TBI (Povlishock & Katz, 2005). Any combination of these effects, including changes in energy metabolism, breakdown of the brain's blood-brain barrier, oedema formation, activation and/or release of autodestructive neurochemicals and enzymes, changes in intracranial pressure, and inflammation, can cause varying levels of cell damage and death, which can lead to subsequent cognitive dysfunction (Laurer et al., 2000). Conversely, cognitive deficits after TBI have also been observed in the absence of neuropathology identified using conventional means. This highlights the need to consider the role of factors beyond readily visible abnormalities in contributing to cognitive outcome after TBI (Scheid & von Cramon, 2010).

3.4.4 Type and anatomical location of lesions and cognitive outcome. Existing data linking specific anatomical structures to the cognitive functions often impaired following TBI are inconclusive, as is evidence regarding the impact of injury in different regions on neuropsychological performance. Thus, the VLSM analyses of the voxelwise relationships between brain contusions and neuropsychological performance only partly confirmed the hypothesised relationships between structural brain damage and cognitive function. First, no significant relationships were identified, voxelwise, between the presence of brain contusions and measures of verbal learning and memory. Second, the hypothesised relationship between frontal and lateral parietal contusions and worse executive function was partly confirmed in that a significant relationship was found between the presence of brain contusions in the left oribitofrontal cortex and worse performance on the TMT alternating-switch cost index. However, this relationship was seen in one small cluster only. Third, a significant relationship was

observed, as expected, between the presence of brain contusions affecting the insular cortex and slowed information processing speed, but again this was observed only for one small cluster, and in no other regions. Whilst the small sample size and associated lack of statistical power to detect real relationships may have played a role here, it could also be that the relationships simply do not exist. However, further possible explanations for the absence of positive findings will be discussed below, in the context of methodological limitations and considerations (see section 3.4.6, pp. 119-124).

No significant differences in neuropsychological function were observed between patient groups stratified according to the anatomical location of white matter lesions (deep/infratentorial vs. lobar only). This null result is in principle consistent with the viewpoint of DeCarli, Fletcher et al. (2005) that the anatomical location of identifiable white matter lesions per se may not be of major importance in terms of cognitive function, as important white matter tracts traverse several regions meaning that a lesion anywhere along a critical pathway can lead to dysfunction. The lack of a difference in cognitive function between patients with deep/periventricular lesions, which according to a traditional DAI severity classification system (Gennarelli et al., 1982; see Chapter 4) indicates more severe DAI, and patients with lobar lesions only (and thus less severe DAI), further highlights the point that using such broad categories to classify the anatomical distribution of white matter lesions may not have functional relevance. Moreover, this approach to investigating white matter damage in TBI may not be the best way given the possibility of more subtle damage occurring in widespread locations across the brain. Underlining this, in the present study there was very little overlap across patients in terms of the specific location of white matter lesions. These patients were also high functioning, and did not show clear cognitive deficits apart from slower processing speed. In addition, the number of patients in each subgroup was small, which reduced the statistical power to detect subtle differences between patients in cognitive function even if some did exist.

3.4.5 Lesion load (size or number) and cognitive outcome. A surprising finding was that the normalised brain contusion size correlated significantly with performance on the TMT index of executive function in the opposite direction to that expected. Thus, a higher contusion load was associated with better performance. It is worth noting here that the group of patients included in the contusion size-cognitive function analyses included *all* patients with contusions,

including those who additionally had other structural abnormalities. Some possible explanations for such unexpected findings resulting from traditional brain-behaviour analyses will be further discussed below.

The finding that the number of microbleeds was not predictive of cognitive performance is consistent with findings by other groups. Scheid et al. (2006), for example, note that correlations between focal MRI abnormalities, including microbleeds, and neuropsychological function following brain injury are weak at best. It may also be the case that in TBI, where the pattern of damage is often diffuse and a number of white matter tracts interconnecting important functional brain regions potentially damaged, focal lesions play a lesser role in determining cognitive outcome. Moreover, microbleeds, although being a marker of DAI, are only the 'tip of the iceberg', as they show only where there has been haemorrhage within the white matter, but do not indicate non-haemorrhagic WM damage.

3.4.6 Methodological limitations and related considerations. There are several possible reasons for the partially observed structure-function correspondence between lesions and neuropsychological performance. Rorden and Kranath (2004) have considered a range of possible explanations, as summarised below.

First, lesion studies assume that discrete anatomical modules support specific cognitive functions (i.e. cognitive functions are assumed to be localised). It appears, however, that many brain functions are instead supported by distributed brain networks. Second, brain damage in TBI is not limited to the site of impact nor it is constrained by the boundaries of functional 'nodes'. Third, plasticity of the human brain means that lesions that only partially damage a functional area known to be involved in a specific cognitive function may not have any obvious consequences. This complicates investigation of lesion-function relationships after TBI, as does the differential vulnerability of particular brain regions and the potential for disconnection through DAI. Together, these factors mean that a cognitive function that is primarily subserved by a certain brain region may also be impaired by damage to another area, which may in turn have an effect on the overall function of a large-scale network. Fourth, mapping anatomy-function correlations at group level requires aligning lesions from different individuals into standard brain space for the normalisation of brain size, shape and orientation. This process assumes that functional regions in brains of different individuals are in the same anatomical

locations, whilst, in fact, there is great inter-individual variability in this respect. After TBI, there may be a degree of reconfiguration of functional brain networks, making it even more problematic to infer the 'normal' anatomical correlates of a specific cognitive function. Finally, the predominantly fronto-temporal distribution of lesions in TBI may mean that whilst VLSM-type analyses can confirm relationships of cognitive indices with frontal and temporal nodes of distributed networks, they may ignore the contribution of other regions within these networks where no overt lesions are observed. For example, microstructural white matter injury can exist in the absence of damage visible on standard brain imaging after TBI (e.g. Rugg-Gunn et al., 2001); thus, normal scan results do not exclude the possibility that other types of structural abnormality are present and potentially associated with cognitive sequelae.

3.4.6.1 Distributed functional networks support complex cognitive functions. The network-based approach to brain function, inspired by early connectionist accounts (see the next section), contends that cognitive functions result from the flow of information across large-scale networks made up of distinct cortical regions. It thus follows that cognitive dysfunction can result from damage anywhere within a distributed network, including its connections, and not just from focal damage to discrete anatomical regions (Bartolomeo, 2011; Mesulam, 1998). Conversely, performance on complex cognitive tasks, such as those engaging executive control processes, is unlikely to be supported by a single brain region (Bigler, 2001a).

This approach to understanding cognitive function offers a new perspective to the investigation of brain-behaviour relationships (Bartolomeo, 2011; Catani & ffytche, 2005). For example, the involvement of a fronto-parietal network in executive function has recently received considerable interest, particularly in functional neuroimaging research. Seeley et al. (2007) identified an 'executive-control network' linking the dorsolateral frontal and parietal cortices and found that performance on executive tasks correlated with lateral parietal nodes of the network. The historical reference to executive functions as 'frontal lobe functions' may, thus, be misleading. Frontal lesions may, of course, be associated with executive impairment after TBI, either because the site of damage is itself critical to the cognitive function or because of damage to the structural connections between the frontal regions and other parts of the network. The current investigation found minimal support for a direct relationship between the anatomical location of focal injury and specific impairments of cognitive function. As noted by Bigler (2001a), the specific anatomical location or extent of focal injury after TBI often does not

correspond to a patient's profile of neuropsychological deficits.

3.4.6.2 Brain damage in TBI is not limited to the site of impact. Functional 'nodes' of brain networks are connected by long white matter tracts, which are susceptible to DAI, with potential to result in widespread dysfunction. In recent years, the development of more advanced structural neuroimaging techniques, diffusion tensor imaging (DTI) in particular, has started to increase understanding of both the extent of injury to structural connections (see Chapter 4) and how their integrity may relate to cognitive function (see Chapter 5).

Whilst it is possible that some cognitive sequelae of TBI result from damage to particular cortical or subcortical brain regions, depending on the role of that area in specific cognitive functions, the diffuse trauma may play a greater role. Further, despite certain brain regions appearing to be particularly vulnerable to traumatic injury, including those near the frontal and temporal poles, it is highly unlikely that a single brain region is responsible for the frequently observed impairments of complex cognitive functions (Scheid & von Cramon, 2010).

As noted previously, damage to connections within a distributed network can have widespread effects on cognitive function. Wernicke (1885) was the first to argue against strict localisation of function, suggesting that associative connections underlie higher mental functions. However, the greater interest at the time in the cortical localisation perspective meant that Wernicke's theory fell out of favour until Geschwind revived interest in the study of disconnection through his seminal paper, entitled 'Disconnexion syndromes in animals and man' (1965). Geschwind identified a number of syndromes that he believed to result from disconnection caused primarily by lesions of the association cortex, but also lesions of critical white matter tracts (see Catani and ffytche, 2005, for a review). Although he provided a useful framework for studying the clinical and behavioural correlates of anatomically specific lesions, Geschwind did not theorise about differential effects of cortical versus white matter lesions. More advanced structural imaging techniques, particularly DTI, combined with voxel-based data analysis techniques, might give a more detailed picture of the distribution and degree of axonal injury in TBI, enabling further insights into relationships between structural brain damage and cognitive function.

3.4.6.3 Neuropathology of TBI is heterogeneous. A central issue in the early clinical

monitoring and management of TBI is the lack of efficient, valid and reliable assessment methods for diagnosis and treatment planning that could be used in everyday practice. This concern is compounded by the heterogeneity of neuropathology associated with TBI. With these issues in mind, Irimia et al. (2011) recently proposed a workflow for the multimodal assessment of TBI in clinical settings, to identify and quantify structural MR abnormalities including extra- and intra-cortical haemorrhages, oedema, focal lesions, and DAI, and to allow correlation of these metrics with outcome variables.

The extent to which a certain level of neuropathology affects cognitive function can also differ across individuals. One possible factor contributing to this could be 'reserve', that is, an individual's estimated maximum cognitive or brain-based capacity (e.g. Brickman et al., 2011; Tate, Neeley et al., 2011). Thus individuals with greater ability or physiological resilience may be better able to absorb some loss without major effects on observed functioning. 'Cognitive reserve' has been estimated from years of education or performance on tests of premorbid intellectual function (e.g. Brickman et al., 2011), whilst 'brain reserve' has been quantified as total intracranial volume measured from MRI scans (Tate, Neeley et al., 2011). Although Tate, Neeley et al. (2011) did not find total intracranial volume to directly predict a dementia diagnosis in a group of 194 older adults (>65 years), when low volume/reserve was combined with the presence of a genetic risk factor (ApoE-ε4 allele) and male gender, together these three predicted dementia classification. It would be interesting in future research to study whether, and to what extent, cognitive or brain reserve affect the relationships between neurological, cognitive, and functional sequelae of TBI.

3.4.6.4 Associated methodological considerations. The MRI sequences used here were chosen for their known potential to detect brain contusions and traumatic microbleeds. Other structural neuroimaging techniques, such as FLAIR or DTI, are potentially more sensitive to the presence, location and extent of white matter damage, out of which the role of DTI in TBI will be discussed in detail in the next two empirical chapters.

Although voxelwise methods, such as VLSM, may perform better than simply mapping out visible lesions when the aim is to infer relationships between the anatomical location of brain contusions and cognitive function, they are not without their own problems. Here, the small sample size in particular that was available for the voxelwise analyses reduced statistical power

to detect possible true effects. It was also necessary to use parametric methods (*t*-tests), even though voxelwise lesion data are unlikely to be normally distributed in such a mixed sample of TBI patients. The nonparametric alternative available in MRIcron (the Brunner-Munzel rank order test) was not used as it is not recommended where there are fewer than 10 patients in each group (Medina et al., 2010).

Investigating the relationships between multiple measures of neuropsychological function and brain structure also raises a multiple comparisons issue. In the present study this was dealt with by applying Bonferroni correction within the family of brain volume indices to account for the multiple between-groups comparisons carried out on normalised volumes of grey matter, white matter and total brain tissue. When correlations were tested between the neuroimaging measures (grey matter volume, white matter volume, total brain volume, lesion volume, and number of microbleeds) and the six cognitive variables, Bonferroni correction was again applied, variable-wise, to account for the multiple tests carried out between each neuroimaging measure and the six cognitive variables. This ensured, in each case, that the familywise error rate for the planned comparisons, both between groups on brain volume and between the neuroimaging and neuropsychological measures, was kept at $p < .05$. It is acknowledged here, though, that whilst the Bonferroni correction controls for the increased probability of false positives associated with multiple comparisons, this control may come at the cost of increased probability of false negatives and thus reduced statistical power.

Furthermore, the groups in the various analyses were not perfectly matched. Whereas good matching was achieved for age, gender distributions were unequal (as discussed above). Groups also differed in the indices of general intellectual function: whereas the WTAR indicated higher premorbid IQ for controls, patients outperformed controls on current verbal reasoning ability, indexed by WASI Similarities.

Efforts were made to statistically control for these potential confounds, as relevant to each of the separate analyses carried out. Thus, the possible effects of age were controlled for when investigating the relationship between brain volume and cognitive function, because normal aging has been demonstrated in several studies to be associated with structural brain changes including reduced brain volumes (Fjell & Walhovd, 2010). Advancing age has also been shown to contribute to the degree of cognitive impairment observed during the first one to five years post-TBI (Millis et al., 2001). Moreover, people who sustain a TBI at an advanced age

tend to have longer PTA (Sherer, Struchen, Yablon, & Nick 2008), and appear to be more cognitively impaired than younger survivors of TBI (Himanen et al., 2006). Schönberger et al. (2009) recently demonstrated that advanced age at the time of TBI is associated with larger grey and white matter lesion volumes and lower grey matter volume, independently of other possible factors such as cause of injury and time since injury. There are also well-established gender differences in brain size, females appearing to have smaller brains (e.g. DeCarli, Massaro et al., 2005). For this reason an additional brain volume analysis was performed including the male participants only, which showed similar results.

Injury severity is also likely to be associated with brain atrophy after TBI. For example, longer PTA duration is associated with larger grey and white matter lesion volumes and smaller residual white matter volume (Schönberger et al., 2009), as well as with overall brain tissue atrophy, measured by increased ventricle to brain ratio (Bigler et al., 2006; Wilde et al., 2006). Similarly, longer time since TBI is associated with larger grey and white matter lesion volumes as well as smaller residual grey matter volumes across several brain regions (Schönberger et al., 2009). Both these variables were controlled for in the present analyses were they were identified as potential confounds.

Although structural abnormalities visible on conventional MRI acutely after TBI have previously been shown to predict symptoms and disability at one year post-injury (e.g. Hiekkanen, Kurki, Brandstack, Kairisto, & Tenovuo, 2009), these relationships are not necessarily apparent at later stages. Scheid and von Cramon (2010) retrospectively analysed structural neuroimaging and neuropsychological data from 320 post-acute/chronic TBI patients, and as here, did not find strong relationships between any particular structural neuroimaging indices and cognitive function.

3.4.7 Conclusions. The purpose of the current chapter was three-fold. Its first aim was to characterise the type, degree, and anatomical distribution of residual structural brain damage in this sample of post-acute/chronic TBI patients. The second aim was to apply MRI techniques used in standard clinical management of TBI (structural T1-weighted and gradient echo T2*-weighted) to detect these abnormalities. The final and principal aim was to explore the extent to which the neuroimaging indices are associated with neuropsychological function.

The current findings suggest that the interrelationships between structural brain

abnormalities and cognitive outcome after TBI are more complex than can be identified using traditional neuroimaging, lesion mapping and neuropsychological measures. More advanced neuroimaging techniques such as DTI have the potential to map the effects of TBI at a more detailed strctural scale and, thus, open new avenues for the investigation of how brain damage affects cognitive function.

This sets the scene for the following two empirical chapters, which apply DTI and associated image analysis methods to investigating the effects of TBI on the structure of the brain's white matter connections and exploring neuropsychological correlates of white matter tract structure in the healthy and damaged brain.

CHAPTER 4: Whole-brain analysis of white matter structure after traumatic brain injury using tract-based spatial statistics

Diffuse axonal injury (DAI) is a common pathological mechanism in TBI. Such damage to the white matter can alter the structural connectivity patterns of the brain, with a potential to produce persisting cognitive impairment that frequently results in long-lasting disability. Conventional clinical neuroimaging, consisting of computed tomography and standard magnetic resonance imaging, cannot capture the full extent of white matter damage following brain injury. Use of more advanced imaging sequences, sensitive to microbleeds associated with DAI, has suggested that white matter damage in TBI is more frequent and extensive than previously thought. Recently, diffusion tensor imaging (DTI) has emerged as a sensitive tool to identify structural abnormalities of white matter. Diffusion data are often analysed by applying a tensor model at each imaging voxel to extract quantitative measures such as fractional anisotropy (FA), reflecting the structural integrity of white matter. These data can then be subjected to a voxelwise statistical analysis using tract-based spatial statistics (TBSS). As a whole-brain voxelwise method TBSS provides a sensitive way of investigating specific patterns of white matter damage produced by TBI. In this study, DTI was employed to investigate the structure of white matter in 28 patients (>2 months post-injury), compared to a group of 26 age-matched healthy controls. A subgroup of 14 patients had microbleed evidence of DAI. Twenty patients had injuries classified as moderate/severe TBI (definite), whilst eight patients had mild TBI (probable). Group differences in white matter structure were tested between: (a) patients and controls, (b) patients with and without microbleeds, (c) patients without microbleeds and controls, and (d) patients with mild injuries and controls. Patients showed lower fractional anisotropy, as well as elevated mean and axial diffusivities, in widespread loci compared with controls. This was despite the limited amounts of cortical and white matter damage observed on standard brain imaging. A stratified analysis based on the presence or absence of microbleeds revealed DTI to be more sensitive than gradient-echo imaging to diffuse axonal damage. Patients with mild injuries also had lower FA and elevated mean and axial diffusivities compared with controls. Using TBSS it was possible to characterise the diffuse nature of white matter abnormalities more fully than could have been achieved by alternative methods.

4.1 Introduction

4.1.1 Diffuse axonal injury in TBI. Diffuse axonal injury is the predominant neuropathological mechanism in as many as 40-50% of all patients who are hospitalised following TBI (Meythaler et al., 2001). The extent and severity of strain injury to axons increases with increasing severity of head injury (Lux, 2007), and severe disability or even vegetative state can result from DAI (Adams, Ghaham, & Jennett, 2000). Although DAI occurs in a more widespread pattern than in the zone of focal injuries, some regions seem to be more vulnerable than others. Petechial haemorrhages (i.e. microbleeds) that are surrogate markers of DAI primarily affect the white matter of the corpus callosum, superior frontal regions, gray-white matter junctions of the frontal and temporal lobes, and the brain stem (Lux, 2007; Scheid et al., 2003). In addition, the long axonal pathways connecting the brain stem with cortical regions (i.e. the corticospinal tracts) are particularly vulnerable (Lux, 2007). Diffuse axonal injury is believed by some to be the primary mechanism to cause persistent cognitive impairment following TBI (Lux, 2007; Scheid, 2006; Sugiyama et al., 2007).

4.1.2 Pathophysiology of DAI. Diffuse axonal injury occurs in TBI secondary to the rotational movement when the head is rapidly accelerated and decelerated as often happens in high velocity road traffic accidents. The angular or rotational forces cause shearing and tearing of the delicate axonal fibres. Axons incorporate *microtubules* and *neurofilaments*. Microtubules are intracellular components that are composed of subunits known as tubulin dimmers, assembled into linear arrays. Due to the head-to-tail configuration of its subunits, microtubules are inherently polarised (i.e. have a “plus” and a “minus” end), with the plus ends being dynamically instable, that is, cycling through periods of growth and shrinkage. Due to their dynamic instability, microtubules can be used to probe other features of the intracellular space (Mitchison and Kirschner, 1984; in Dent & Gertner, 2003). However, the dynamic properties of microtubules could also make axons more susceptible to mechanical trauma, as in DAI associated with TBI (Tang-Schomer et al., 2010). Neurofilaments are proteins that are widely recognised to have a role in the radial growth of myelinated axons, and, via this function to contribute to nerve conduction velocity (Hoffman et al., 1987; in Julien, 1999). Together, microtubules and neurofilaments make up the *neuronal cytoskeleton*, and this can break down

in axonal injury (possibly due to an influx of calcium). This, in turn, causes *axoplasmic flow,* the continuous movement of the cytoplasm between the cell body of a neuron and the axon fibre*,* to be interrupted, leading to a subsequent accumulation of organelles (Adams, Graham, Gennarelli, & Maxwell, 1991; Arfanakis et al., 2002; Povlishock, 1992).

In Figure 4-1A the top axon is healthy, but in the axon below, depicting the effects of DAI a short time post-injury, cytoskeletal misalignment and disarray of neurofilaments can be seen. Neurofilamentous disruption has long been identified as one of the key mechanisms in human DAI (Christman, Grady, Walker, Holloway, & Povlishock, 1994). The bottom axon of Figure 4-1B illustrates how organelles accumulate in the injured region due to disrupted axonal transport, and cause local swelling. The interruption of axoplasmic flow between the cell body and the supplying axon can result in degeneration of the unsupplied section, and subsequently lead to disconnection (Arfanakis et al., 2002; Povlishock, 1992). Through the disconnection, DAI has

the potential to impair neural network function (Beretta et al., 2008).

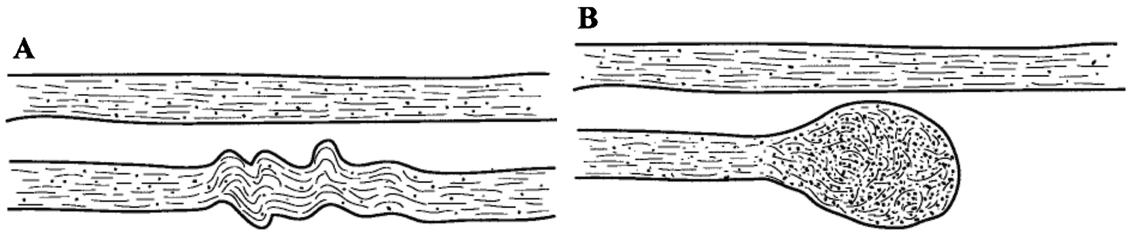

Figure 4-1. Axons before and after cytoskeletal disruption due to mild TBI. The dimensions do not necessarily correspond to reality. Reprinted from AJNR May 2002, 23, Arfanakis, K., Haughton, V. M., Carew, J. D., Rogers, B. P., Dempsey, R. J., & Meyerand, M. E., Diffusion tensor MR imaging in diffuse axonal injury, 794–802, Copyright (2011), with permission from AMERICAN SOCIETY OF NEURORADIOLOGY.

4.1.3 Severity of DAI. Some time following the initial observations by Strich (1956) and Nevin (1967) of shearing injury and diffuse changes in human white matter after head injury, Gennarelli et al. (1982) studied DAI lesions in detail using a primate model of controlled acceleration of the head. By accelerating the primate (monkey) head in one of three directions they found that the amount of DAI that this caused was directly related to the amount of coronal head motion applied. They then went on to identify three grades of DAI severity: *Grade I* characterised by microscopic evidence of diffuse axonal injury without focal lesions, *Grade II* also including a focal lesion in the corpus callosum, and *Grade III* including yet another focal lesion in the midbrain/brain stem. Clinical studies have demonstrated that as deeper brain

structures become involved, prognosis becomes poorer (Beretta et al., 2008).

4.1.4 Diffusion tensor imaging of DAI. Apart from traumatic microbleeds visible as small hypointense signal abnormalities on T2*-weighted MRI, DAI can cause more subtle disruption of structural integrity of white matter tissue, difficult (or even impossible) to detect using standard clinical imaging sequences (Symms et al., 2004). Brain white matter consists of a large number of axons forming compact white matter tracts, and the principal direction of water diffusion in white matter normally corresponds well with the main orientation of these tracts (Beaulieu, 2002). The degree of directionality of water diffusion in brain white matter can be quantified using metrics derived from DTI. This has been shown to be a sensitive way to detect microstructural abnormalities in white matter tissue (Le Bihan, 2003).

In general, diffusion at each imaging voxel can be described as *isotropic* (i.e. occurs equally in all directions) or *anisotropic* (i.e. occurs primarily in one direction). *Fractional anisotropy* (FA), the most frequently studied index of white matter structure, is a scalar measure (0-1.0) based on all three diffusivity eigenvalues that characterise the diffusion tensor. Higher FA indicates a stronger degree of directionality of diffusion. *Mean diffusivity* (MD) is another frequently used scalar measure, and normally shows the opposite relationship with white matter structure to that of FA. These two non-specific but relatively sensitive markers of pathological changes in white matter are complemented by measures of the longitudinal and transverse eigenvalues, representing diffusion parallel (i.e. *axial diffusivity*) and perpendicular (i.e. *radial diffusivity*) to the primary direction.

There is now a relatively extensive literature describing regional changes in DTI metrics associated with TBI. For example, studies have shown DTI to be more sensitive to microstructural axonal injury in TBI than standard structural neuroimaging techniques (Lipton et al., 2008; Nakayama et al., 2006; Niogi, Mukherjee, Ghajar, Johnson, Kolster, Sarkar et al., 2008; Rugg-Gunn et al., 2001). In addition, there is some evidence to suggest that DTI metrics may be sensitive in detecting the effects of injury severity on the extent of white matter damage (Benson et al., 2007).

Nakayama et al. (2006) used DTI to investigate white matter abnormalities in a group of 23 TBI patients with cognitive impairments in the absence of large-scale structural damage observed on standard neuroimaging compared with 23 age- and gender-matched healthy

volunteers. The patients were in the post-acute/chronic stage (on average 14 months post-injury) following a high-velocity, high-impact TBI resulting from a motor vehicle accident. Employing a combined voxel-based and region of interest (ROI) approach they showed that FA was reduced within the corpus callosum and the column of the fornix in the patient group compared with controls. In general, they found that FA was higher in healthy tissue where diffusion is highly anisotropic. By contrast, when axons are injured, FA is reduced and average diffusivity increased, possibly due to restricted axoplasmic flow along and increased flow across the axonal membrane (see Kou et al., 2010).

Benson et al. (2007) employed DTI to investigate global white matter structure in a heterogenous sample of TBI patients. They compared histograms of spatially normalised FA maps from a sample of 20 TBI patients (6 mild) and 14 age-matched healthy controls. They observed a shift to the left (i.e. reduced FA) in histograms of global FA values for the patients as compared with controls. Notably, however, the patient FA histograms were also significantly more variable than the control histograms. Although the patients in the Benson et al. (2007) study were scanned on average 35 months post-injury, some of the patients were scanned within only days of injury, and others during the first two months post-injury. This is not ideal, because the early neuropathological events are likely to be different from those occurring later after TBI, and the inclusion in the sample of these acute patients may have importantly contributed to the global FA abnormality observed by Benson et al. (2007). Although they did not find time since injury to significantly predict their global measure of average FA, this does not exclude the possibility that it does impact on whole-brain abnormalities measured via other DTI metrics or regional white matter abnormalities. When Benson et al. (2007) investigated other DTI metrics, including axial (parallel) and radial (transverse) diffusivities, they found a difference between groups in radial diffusivity only, which was significantly higher for the patients.

Previous work has shown that TBI produces early reductions in axial diffusivity associated with axonal injury and disrupted axonal transport (MacDonald et al., 2007; Song et al., 2002; Song et al., 2003; see Figure 4-1). However, axial diffusivity has also been shown to gradually normalise over time following TBI (e.g. Sidaros et al., 2008; Wang et al., 2009), which could explain the lack of abnormality overall in the Benson et al. (2007) heterogenous patient sample that consisted of acute as well as post-acute/chronic cases. There is also likely to be

considerable regional variability in the indices of white matter structure across different locations, which a whole-brain histogram approach would not be adequately sensitive to. In addition, when Benson et al. (2007) plotted FA values from individual patients against control values, their results may have been critically influenced by inter-individual variability in FA values across white matter loci within the control group. It may be preferable to restrict this kind of analysis to only those white matter tracts that show low inter-individual variability in the healthy brain. Despite these criticisms, the Benson et al. (2007) preliminary study advanced the field of investigating white matter damage in TBI, suggesting that DTI is a sensitive technique to investigate abnormalities in white matter structure even in a small number of cases and across different injury mechanisms, variable patterns of focal brain damage, a spectrum of TBI severity, and variable injury to scan intervals.

4.1.5 Effects of severity of injury on outcome after TBI. Patterns of recovery and outcome after TBI vary greatly across individuals (Chu et al., 2007). One of the factors that has been investigated in terms of the extent and persistence of functional impairments following TBI is the initial severity of injury. Severity of TBI can be classified based on the lowest Glasgow Coma Scale (GCS; Teasdale & Jennett, 1974) score within the first 24 hours post-injury, the duration of post-traumatic amnesia (PTA), or the length of coma (see Chapter 1 for further discussion). Studies investigating the impact of injury severity on outcome have generally shown that duration of PTA and length of coma predict early cognitive outcome (Dikmen, Machamer, Temkin, & McLean, 1990; Lannoo, Colardyns, Jannes, & De Soete, 2001), but not necessarily long-term outcome (see Chu et al., 2007). The GCS, in particular, has been shown to predict level of function initially and at one year post-injury, but to have limited value in predicting cognitive outcome in the long term (Dikmen et al., 1990; Levin et al., 1990). Thus, the determinants of cognitive dysfunction in the more chronic stages of TBI remain less well understood (Chu et al., 2007). Benson et al. (2007), who investigated the relationships between white matter damage indexed by global FA abnormalities and injury severity in their mixed group of TBI patients, found that FA abnormalities across the brain were correlated with severity of injury. Severity as measured by the duration of PTA was found to be more strongly correlated with whole-brain FA changes than were GCS scores. As expected, they found that longer PTA duration was associated with lower average FA, whilst a higher GCS score was associated with

higher global FA.

4.1.6 Whole-brain versus region of interest approach to analysing white matter tract structure. Previous studies have demonstrated using an ROI approach that the structure of brain white matter is altered after TBI in a number of regions (e.g. Arfanakis et al., 2002; Huisman et al., 2004; Inglese et al., 2005). This appears to have behavioural relevance (Kennedy et al., 2009; Kraus et al., 2007; Little et al., 2010; Niogi, Mukherjee, Ghajar, Johnson, Kolster, Lee et al., 2008; Niogi, Mukherjee, Ghajar, Johnson, Kolster, Sarkar et al., 2008). These studies have focused their analysis on a limited number of ROIs, whereas tract-specific abnormalities following TBI in microstructural properties of white matter have not been previously studied using a whole-brain voxelwise method.

Although the ROI approach has succeeded in identifying white matter damage, it is limited to assessment of a tiny amount of the total white matter (see e.g. Niogi, Mukherjee, Ghajar, Johnson, Kolster, Lee et al., 2008). Moreover, due to anatomical factors that vary between individuals such as the presence of differently oriented fibres crossing at specific locations, closeness to non-white matter tissue, as well as factors such as axon packing densities and diameters, white matter integrity can vary considerably along the length of the tract (Pierpaoli, Jezzard, Basser, Barnett, & Chiro, 1996). Calculation of average anisotropy or diffusivity values within a limited number of tracts may thus not be the optimal starting point for an analysis of group differences.

Analysis of white matter structure across participants can also be problematic for ROI approaches that involve the registration to individual brain space of normalised, standard space tracts (e.g. derived from white matter atlases or based on the groupwise average), as well as for voxelwise analyses carried out in standard space, which necessitates prior spatial smoothing of the data to an arbitrarily determined extent (e.g. in VBM, see below). When white matter tracts from multiple participants are registered to a common space, the individual differences lead to a high likelihood for errors, and the subsequent conclusions drawn from the analysis are not necessarily based on spatially invariant anatomical locations (Smith et al., 2006). In TBI, the heterogenous and often complex pattern of damage at variable locations across individuals further complicates these issues, and makes it difficult to decide *a priori* where to 'look' for the white matter disruption. A failure to identify significant damage to certain white matter tracts may

result simply from the investigation focusing on a small selection of tracts, covering only a fraction of the total white matter. This could lead to, for example, altogether missing behaviourally relevant changes in white matter tracts involved in supporting cognitive function (Mesulam, 1998).

Tract-based spatial statistics (TBSS; Smith et al., 2006) is a voxel-based technique for analysing white matter structure across the major tracts and allows the investigation of complex patterns of white matter disruption. Another voxelwise approach that has been previously applied to studying white matter structure is VBM (Ashburner & Friston, 2000), quantifying brain tissue density. Previous VBM studies have reported widespread white matter pathology following TBI (e.g. Bendlin et al., 2008; Xu et al., 2007), as well as a relationship between regional increases in diffusivity after TBI and impaired learning and memory performance (Salmond, Menon, Chatfield, Williams et al., 2006). However, VBM involves some degree of spatial smoothing of individual participants' data to enable a voxelwise analysis to be carried out across participants. This and potential spatial misalignment of anatomical structures between participants could produce erroneous results.

Moreover, due to problems with spatial normalisation and coregistration of regions affected by atrophy and on the edges of tissue classes, such as near CSF, VBM is not robust to partial volume effects. The risk of misregistration between tissue classes is particularly problematic when comparing tract-specific white matter structure of patients to that of healthy controls. If what is classed as white matter in the patient group also partially contains grey matter or CSF, false positive errors are possible because anisotropy is much lower in CSF and grey matter than in the normally highly anisotropic white matter (Benson et al., 2007). For example, although Xu et al. (2007, p. 753) report significant white matter abnormalities in chronic TBI patients compared with healthy controls in "major white matter tracts", it is not possible using their voxel-based method to ascertain whether the voxels in which the differences were observed are fully contained within white matter tracts, even when excluding voxels with extremely low FA, as they did.

The two-stage registration process of TBSS aims to overcome the two main problems of VBM: statistical calculations are first performed at each point within an individual's white matter 'skeleton', which has been registered to a standard space using nonlinear warping, and individual white matter tracts are then aligned across participants to a spatially invariant mean

FA skeleton (see Chapter 2 for further details). Whereas most ROI analyses have calculated the mean within particular tracts of measures such as FA or MD, TBSS allows the analysis of the DTI metrics to explore complex spatial patterns of abnormalities of white matter structure along multiple tracts.

4.1.7 Aims of the present study. In summary, unlike standard clinical neuroimaging of TBI, DTI has been shown by several studies to be a sensitive to DAI (Lipton et al., 2008; Nakayama et al., 2006; Niogi, Mukherjee, Ghajar, Johnson, Kolster, Sarkar et al., 2008; Rugg-Gunn et al., 2001), and has shown promise in detecting the effects of injury severity on its extent (Benson et al., 2007). However, it has not yet been studied whether, when assessed using DTI, abnormalities of white matter structure are also seen in patients with TBI but no traumatic microbleeds visible on standard MRI, compared with patients with microbleeds. Further research is also needed to investigate in more detail the correspondence of the DTI metrics with conventional indices of injury severity. In particular, DAI is the primary neuropathology also in mild TBI (Gennarelli & Graham, 1998; Povlishock & Katz, 2005). Many patients with a mild TBI have unremarkable standard clinical neuroimaging results, but may still experience persisting symptoms characteristic of TBI. Use of DTI as a way of providing objective evidence of axonal injury in such cases is receiving increasing support (Belanger, Vanderploeg, Curtiss, & Warden, 2007). The current study aims to address these issues via the following:

- TBSS is used to investigate differences in white matter structure between a group of post-acute/chronic TBI patients and an age-matched control group.
- A stratified analysis based on the presence or absence of microbleeds, a marker of DAI, is carried out to investigate if patients with microbleeds show more extensive white matter abnormalities than those without microbleeds.
- It is also explored if those patients with no evidence of microbleed evidence of white matter damage on standard MRI show abnormalities in white matter microstructure as compared with healthy controls.
- Finally, it is explored whether patients whose TBI is defined as ‘mild’ using a standard classification system also show evidence of white matter disruption relative to controls.

4.1.8 Hypotheses. TBSS analyses of DTI data will show between-groups differences in FA, MD, axial diffusivity, and radial diffusivity. Specifically, it is hypothesised that:

1) White matter disruption following TBI will be spatially distributed, as indexed by lower FA and higher diffusivities in the patient than the control group in a number of white matter tracts.
2) Increasing time since injury within the TBI group will be associated with more extensive abnormalities of white matter structure.
3) Patients with microbleed evidence of DAI will show more extensive white matter disruption than patients without microbleeds.
4) Patients without microbleeds will also show DTI evidence of white matter damage compared with controls.
5) A subgroup of patients classified as having sustained a mild TBI, compared with controls, will show DTI abnormalities of white matter structure.

4.2 Methods

4.2.1 Design. Patients with definite or probable TBI were compared with healthy controls on neuroimaging indices of white matter structure (FA, MD, axial diffusivity, radial diffusivity). In addition, the effects of traumatic microbleeds (MB) were evaluated within the patient group (MB vs. Non-MB), as well as the effects of clinical indicators of TBI severity (Mild TBI vs. Control). The presence of microbleeds was assessed using gradient-echo T2*-weighted MRI. Injury severity was classified based on the Mayo Traumatic Brain Injury Severity Classification System (Malec et al., 2007; which draws on the duration of loss of consciousness, length of PTA, lowest recorded GCS score in the first 24 hours, and/or CT or MR imaging results (see Chapter 2 for further information). Recruitment procedures have been described in Chapter 2, and the inclusion and exclusion criteria, as outlined in Chapter 2, also apply here.

4.2.2 Participants. Twenty-eight TBI patients in the post-acute/chronic phase (21 males, mean age ± standard deviation: 38.9±12.2 years), and an age-matched group of 26

healthy controls (12 males, 35.4±11.1 years) took part. Table 4-1 shows the main clinical characteristics of each of the 28 patients.

Table 4-1

Clinical Characteristics of the Patients

Patient	Age [a]	Gender	Cause of injury	Lowest GCS	PTA (days)	Contusions	White matter lesions [b]	Superficial siderosis
1	45	M	Assault	NK	<24 h	+	+	−
2	25	M	Assault	14	<24 h	−	−	−
3	18	M	Fall	NK	<24 h	−	−	−
4	23	M	Assault	NK	12	+	−	+
5	54	M	Fall	NK	<24 h	+	+	+
6	37	M	Fall	4	52	+	+	−
7	50	M	RTA	4	180	+	−	+
8	41	M	RTA	6	30	+	+	−
9	34	F	Fall	6	30	+	−	+
10	34	M	RTA	3	42	+	−	+
11	49	M	Assault	NK	<24 h	+	−	+
12	39	F	RTA	6	30	+	+	+
13	47	M	Assault	NK	5	+	−	+
14	47	F	RTA	5	42	+	+	−
15	36	M	Fall	NK	11	+	+	−
16	23	M	Sports	14	<24 h	+	+	−
17	53	M	Fall	15	<24 h	−	−	−
18	66	F	RTA	NK	77	+	−	+
19	29	F	RTA	NK	<24 h	+	+	+
20	33	M	RTA	14	<24 h	+	+	−
21	52	M	Assault	3	<24 h	+	+	+
22	42	F	RTA	12	63	−	−	−
23	34	M	Assault	NK	42	+	+	+
24	53	F	Sports	15	<24 h	−	−	−
25	24	M	Assault	NK	<24 h	+	−	−
26	24	M	Assault	NK	<24 h	+	+	−
27	26	M	Fall	NK	<24 h	−	−	−
28	50	M	Assault	6	5	+	+	−

Notes: [a] Age at assessment, [b] Microbleeds or other white matter injury as observed on gradient-echo T2*-weighted magnetic resonance imaging. TBI = traumatic brain injury. RTA = road traffic accident. GCS = Glasgow Coma Scale. PTA= post-traumatic amnesia. NK = not known/information not available.

All patients were at least two months post-injury (average 25 months). Head injuries were secondary to assaults (36%), road traffic accidents (32%), falls (25%) and sports-related incidents (7%). There were 20 moderate/severe (definite), and eight mild (probable) cases of TBI based on the Mayo Traumatic Brain Injury Severity Classification System (Malec et al., 2007). Apart from one moderate/severe (definite) and five mild (probable) cases of TBI, all patients showed evidence of residual brain injury on MR imaging on the day of the research scans (for MRI results, see section 4.3.2, pp. 140-141). These six patients had all experienced a head trauma, as documented in their medical records, followed by a period of at least

momentary (<30 min) loss of consciousness and/or subsequent PTA (<24 hours), and in the moderate/severe case a lowered GCS score. At the time of the research assessment, all patients were documented to have or subjectively reported to be experiencing some physical and/or cognitive sequelae symptomatic of TBI.

4.2.3 Structural MR imaging data acquisition and analysis.

4.2.3.1 Standard MRI. The MRI sequences were described in Chapter 2. The lesion mapping carried out to determine the presence of focal lesions was described in Chapter 3. The groupwise lesion overlap images are shown below in Figure 4-2.

The Microbleed Anatomical Rating Scale (MARS; Gregoire et al. (2009) was used for classifying microbleeds identified on gradient-echo T2*-weighted MRI according to their anatomical location (infratentorial, deep, or lobar), and to distinguish them from various types of microbleed mimics.

4.2.3.2 Diffusion tensor imaging. For DTI, 72 diffusion-weighted volumes with gradients applied in 16 non-collinear directions were collected in each of the four DTI runs, resulting in a total of 64 directions. The specific parameters used for the data acquisition were described in Chapter 2, as well as the data preprocessing steps. Fractional anisotropy (FA) and mean diffusivity (MD) maps were generated using the Diffusion Toolbox (Behrens et al., 2003) of FSL, as well as images for each of the eigenvalues (λ1, λ2, and λ3) representing the magnitude of diffusion along the three principal axes of the diffusion tensor. Axial (D_{ax}) and radial (D_{rad}) diffusivity images were then derived from these eigenvalues ($D_{ax} = \lambda 1$, $D_{rad} = \lambda 2 + \lambda 3 \div 2$).

4.2.3.3 DTI data analysis. Voxelwise analysis of the FA, MD, and axial and radial diffusivity data was carried out using TBSS and Randomise within FSL. This involved a number of steps: (a) nonlinear alignment of all participants' FA images into common FMRIB58_FA template space; (b) affine-transformation of the aligned images into standard MNI152_1mm space; (c) averaging of the aligned FA images to create a 4D mean FA image; (d) thinning of the mean FA image to create a mean FA 'skeleton' representing the centre of all white matter tracts, and in this way removing partial-volume confounds; and (e) thresholding of the FA

skeleton at FA ≥ 0.2 to suppress areas of extremely low mean FA and exclude those with considerable inter-individual variability.

Similar steps for processing non-FA images were then carried out to obtain the mean, axial and radial diffusivity images. Non-parametric permutation-based statistics were employed with threshold-free cluster enhancement (TFCE) and 5000 permutations (Nichols & Holmes, 2002; Smith & Nichols, 2009). A threshold of $p < .05$ was then applied on the results, corrected for multiple comparisons across the white matter tract skeleton voxels.

Age and gender were included as 'covariates of no interest' (see Appendix C, for more information) in all TBSS analyses, because both may have independent effects on white matter integrity (see e.g. Hsu et al., 2008; Giorgio et al., 2010; Madden, Bennett, & Song, 2009; Westerhausen et al., 2011). The aim of including these in the design matrices was to attenuate their potential confounding effects on the variables of interest.

The reader is referred to Chapter 2 and Appendix C for more detailed descriptions of TBSS and the various DTI data analysis steps.

4.2.4 Assessment of intellectual functioning. All participants also underwent comprehensive neuropsychological assessment (as described in Chapter 2), including assessment of premorbid and current intellectual ability. The Wechsler Test of Adult Reading (WTAR; Wechsler, 2001) was used to provide an estimate of premorbid intellectual ability. Current verbal and nonverbal reasoning ability were measured using the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999) Similarities and Matrix Reasoning subtests. These neuropsychological data are presented here for the sole purpose of demographically characterising the patient and control groups (described in section 4.3.1). The relationships between DTI and neuropsychological measures will be the focus of Chapter 5.

4.3 Results

4.3.1 Participants. Tables 4-2a and 4-2b provide the group averages for the demographic variables and patient clinical variables, and show the results of the group comparisons on these variables.

Table 4-2a

Characteristics of the TBI and Control Groups

	TBI	CON	TBI vs. CON
Group sixe (*N*)	28	26	-
Mean Age (years)	38.9±12.2	35.4±11.1	$t = 1.08^{ns}$
Age range (years)	18-66	19-60	-
Gender (M:F)	21:7	12:14	$\chi^2 = 4.72$ *
Mean time since injury (months)	25.4±21.8	n/a	n/a
Time since injury range (months)	2-69	n/a	
Severity of injury (severe/moderate:mild) [a]	20:8	n/a	
Premorbid IQ/WTAR age-scaled score (*M*÷SD)	108.0±8.1 (*N*=27)	115.0±9.2 (*N*=26)	$t = -2.95$ *
Current verbal IQ/WASI Similarities age-adjusted *T*-score (*M*÷SD)	57.5±5.5 (*N*=28)	52.7±8.9 (*N*=26)	$t = 2.38$ *
Current nonverbal IQ/WASI Matrix Reasoning age-adjusted *T*-score (*M*÷SD) [b]	61.1±5.0 (*N*=27)	55.9±8.4 (*N*=26)	$t = 2.73$ **

Notes: [a] On the Mayo Classification System for Traumatic Brain Injury Severity (Malec et al., 2007). [b] Following Exploratory Data Analysis/boxplots, one extreme outlier score (≥ 3.0 x interquartile range outside the middle half of the sample) was excluded. TBI = Traumatic brain injury. CON = Control. WTAR = Wechsler Test of Adult Reading. WASI = Wechsler Abbreviated Scale of Intelligence. n/a = not applicable. * $p < .05$. ** $p < .01$. ^{ns} not significant at $p < .05$.

Table 4-2b

Characteristics of TBI Subgroups Stratified Based on Microbleed Presence/Absence

	TBI: MB	TBI: Non-MB	MB vs. Non-MB
Group sixe (*N*)	14	14	-
Mean Age (years)	38.9±9.9	38.9±14.5	$t = 0.00^{ns}$
Age range (years)	23-54	18-66	-
Gender (M:F)	11:3	10:4	$\chi^2 = 0.19^{ns}$
Mean time since injury (months)	26.3±23.1	24.5±21.3	$t = 0.21^{ns}$
Time since injury range (months)	2-69	2-68	-
Severity of injury (severe/moderate:mild) [a]	11:3	9:5	$\chi^2 = 0.70^{ns}$
Median (range) number of microbleeds [b]	7 (1-19)	n/a	n/a
Premorbid IQ/WTAR age-scaled score (*M*÷SD)	105.5±8.7 (*N*=13)	110.4±7.1 (*N*=14)	$t = -1.61^{ns}$
Current verbal IQ/WASI Similarities age-adjusted *T*-score (*M*÷SD)	55.1±5.9 (*N*=14)	59.9±3.9 (*N*=14)	$t = -2.52$ *
Current nonverbal IQ/WASI Matrix Reasoning age-adjusted *T*-score (*M*÷SD)	58.9±10.2 (*N*=14)	61.1±4.5 (*N*=14)	$t = -0.75^{ns}$

Notes: [a] On the Mayo Classification System for Traumatic Brain Injury Severity (Malec et al., 2007). [b] Microbleed identification was based on the Microbleed Anatomical Rating Scale (MARS; Gregoire *et al.*, 2009). TBI = Traumatic brain injury. MB = Microbleed evidence of diffuse axonal injury. Non-MB = No microbleed evidence of diffuse axonal injury. WTAR = Wechsler Test of Adult Reading. WASI = Wechsler Abbreviated Scale of Intelligence. n/a = not applicable. * $p < .05$. ^{ns} not significant at $p < .05$.

The TBI and control groups were well matched for age ($t(52) = 1.08$, *ns*), but a goodness of fit test indicated that the proportions of each gender differed in the two groups ($\chi^2(1, N = 54) = 4.72$, $p < .05$). There were more males than females in the TBI group, whilst the control group was more balanced between the genders. The controls had a significantly higher

premorbid IQ (as assessed by the WTAR) than the patient group. Unexpectedly, however, the patients outperformed the controls in terms of current intellectual functioning, as assessed by the WASI. This will be considered further in the Discussion and in Chapter 5, where the relationships between DTI and neuropsychological measures are explored in detail.

When the patient group was divided into two subgroups based on the presence or absence of microbleed evidence of DAI (MB vs. Non-MB), the MB and non-MB groups were well matched for age, gender, severity of injury, time elapsed since the injury, and premorbid intellectual functioning. Whilst these patient groups did not differ in current nonverbal reasoning ability, as measured by WASI Matrix Reasoning, the Non-MB group outperformed the MB group in terms of current verbal reasoning ability, measured via WASI Similarities.

4.3.2 Standard MR imaging. High-resonance T1-weighted imaging was normal for the majority of patients (61%) and gradient-echo T2*-weighted imaging normal for seven (25%). Six patients (21%) had normal T1- and T2*-weighted scans.

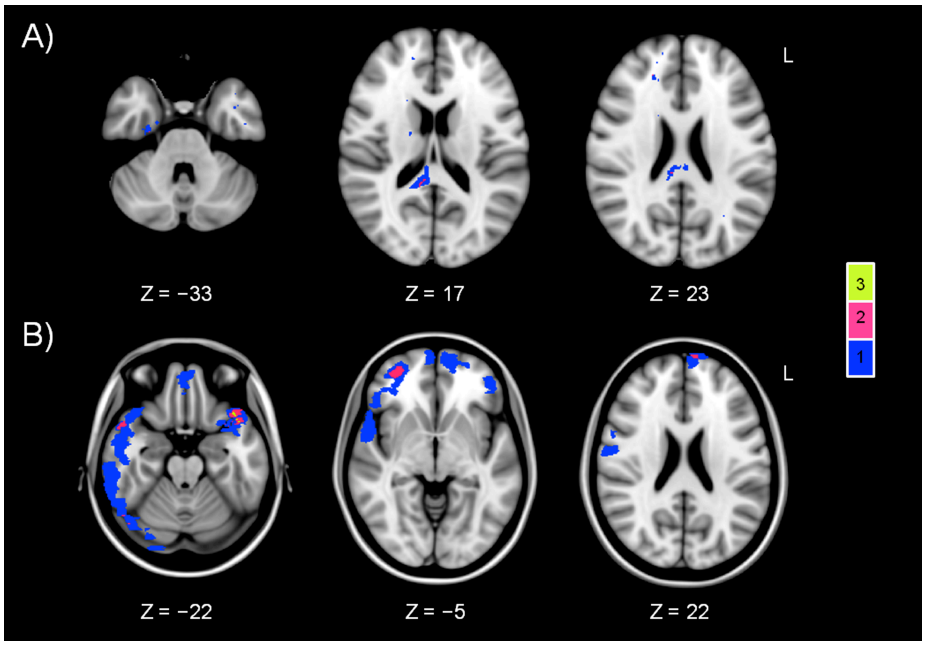

Figure 4-2. Distribution and frequency of focal lesions. (A) white matter lesions visible on gradient-echo imaging and (B) contusions. The colour bar indicates the number of patients who had lesions at each site. Green-yellow indicates where lesions were present in three (11%) of the 28 TBI patients, pink indicates where they were present in two (7%), and blue where a lesion was found in one patient only. Reprinted from Brain February 2011, 134/2, Kinnunen, K.M., Greenwood, R., Powell, J.H., Leech, R., Hawkins, P.C., Bonnelle, V, Patel M.C., Counsell, S.J., Sharp, D.J., White matter damage and cognitive impairment after traumatic brain injury, 449-463, Copyright (2011), with permission from Oxford University Press.

Definite and possible intraparenchymal microbleeds (MB) indicative of DAI were found

in 50% of the patients. The median number of microbleeds in the MB group, identified using the Microbleed Anatomical Rating Scale (MARS; Gregoire et al., 2009), was seven (range 1-19). Microbleeds were mainly found in frontal and temporal white matter bilaterally. There was little overlap in the location of white matter damage observed across patients (see Figure 4-2A). Table 4-3 shows the proportion of the 28 patients with white matter injury indicative of DAI found in each white matter region, observed on T2*-weighted MRI scans.

Cortical lesions were found in 39% of all TBI patients and were also mainly seen in frontal and temporal regions. Again, there was a relatively small amount of lesion overlap across patients with contusions (see Figure 4-2B). Signal abnormality indicative of superficial siderosis was found in nearly half (43%) of the patients' MR images, mainly overlying the frontal cortex bilaterally and the right temporal cortex. Its presence is likely to be secondary to chronic haemosiderin deposition, resulting from subdural or subarachnoid haemorrhage at the time of injury.

Table 4-3

Number/Percentage of Patients with T2 Evidence of Diffuse Axonal Injury*

Evidence of DAI[a] by anatomical location	**Right hemisphere**		**Left hemisphere**		**Totals by WM region**	
	Number	Percentage	Number	Percentage	Number	Percentage
Infratentorial WM/Totals	**1**	**4%**	**2**	**7%**	**3**	**11%**
Brainstem	-	-	1	4%	1	4%
Cerebellum	1	4%	1	4%	2	7%
Deep WM/Totals	**4**	**14%**	**1**	**4%**	**5**	**18%**
Basal Ganglia	-	-	1	4%	1	4%
Thalamus	-	-	-	-	-	-
Internal Capsule	-	-	-	-	-	-
External Capsule	1	4%	-	-	1	4%
Corpus callosum	2	7%	-	-	2	7%
Deep/periventricular	1	4%	-	-	1	4%
Lobar WM/Totals	**10**	**36%**	**12**	**43%**	**14**	**50%**
Frontal	10	36%	8	29%	13	46%
Temporal	6	21%	5	18%	7	25%
Parietal	3	11%	3	11%	6	21%
Occipital	2	7%	1	4%	2	7%
Insula	1	4%	1	4%	2	7%
One or more WM regions	**10**	**36%**	**12**	**43%**	**14**	**50%**

Notes: [a]Traumatic microbleeds or other white matter lesions observed on gradient-echo T2*-weighted MRI. DAI = diffuse axonal injury. WM = white matter.

4.3.3 TBSS analysis of DTI data. Diffusion tensor imaging metrics of fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (D_{ax}), and radial diffusivity (D_{rad}) were investigated voxelwise. Two contrasts per each of the four DTI metrics characterising the diffusion tensor were calculated to test for group differences in white matter tract structure.

4.3.3.1 Hypothesis 1: White matter disruption following TBI will be spatially distributed, as indexed by lower FA and higher diffusivities in the patient than the control group in a number of white matter tracts. Comparison of TBI patients and age-matched controls revealed that the majority of the white matter tracts showed some evidence of disruption in the TBI group, as shown in Figure 4-3 that presents axial slices of the results of (a) FA, (b) MD, (c) axial diffusivity, and (d) radial diffusivity contrasts between the TBI and Control groups (Control > TBI or TBI > Control). All results, as shown, have been thresholded at $p < .05$, corrected for multiple comparisons across voxels.

The between groups differences were most clear for FA and MD, with less extensive but still marked differences seen for axial diffusivity, and much more limited differences seen for radial diffusivity.

Higher FA was found in the control than the TBI group in interhemispheric fibres (genu, body, and splenium of the corpus callosum) and intrahemispheric association fibres of the uncinate fasciculi, inferior and superior longitudinal fasciculi, inferior fronto-occipital fasciculi, and the cingulum bundle. Higher FA for the controls was also found in projection fibres of the corticopontine and corticospinal tracts, as well as in the fornices, the anterior and posterior thalamic projections, the forceps major and minor, the anterior and posterior limbs of the internal capsule, and the anterior corona radiata (Figure 4-3A).

The opposite contrast showed higher MD for the patients in similar locations (Figure 4-3B) as those showing higher FA for the controls, but more extensively in the left superior longitudinal fasciculus and the external capsules bilaterally.

Higher axial diffusivity in the TBI than control group was seen in several tracts including the corpus callosum, bilateral uncinate fasciculi, the right superior and inferior longitudinal fasciculi, bilateral cingulum bundles underlying the posterior cingulate cortex, both corticospinal tracts, the fornices, the anterior thalamic radiations bilaterally, the forceps major and minor, and the anterior and posterior limbs of the internal capsule (Figure 4-3C).

Radial diffusivity was found higher for the patients than controls in the corpus callosum, the right superior longitudinal fasciculus, the right posterior/medial parietal white matter underlying the posterior cingulate/precuneus cortices, the fornices, bilateral anterior thalamic radiations, and the forceps minor, but to a limited extent only (Figure 4-3D).

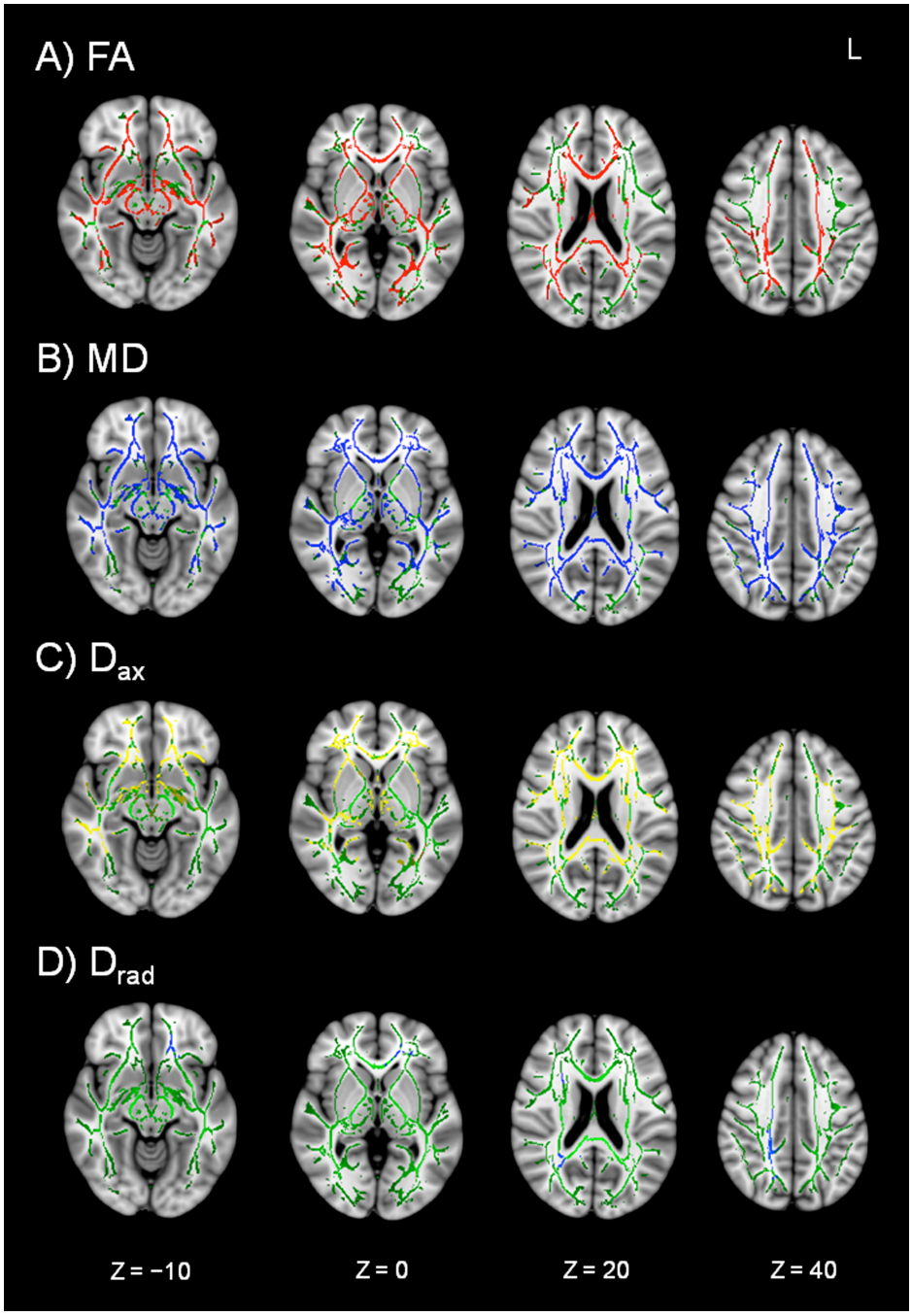

Figure 4-3. Widespread white matter disruption following TBI. (A) FA (red): Control > TBI; (B) MD (dark blue): TBI > Control; (C) D_{ax} (yellow): TBI > Control; and (D) D_{rad} (light blue): TBI > Control. The results are overlaid on a standard MNI152 T1 1mm brain and the mean FA skeleton (in green; with display thresholds set to FA = 0.2-0.8). All results shown here are significant at $p < .05$. Reprinted from Brain February 2011, 134/2, Kinnunen et al., White matter damage and cognitive impairment after traumatic brain injury, 449-463, Copyright (2011), with permission from Oxford University Press.

There were no white matter regions that showed either significantly higher FA for the patients than the controls, or significantly higher mean, axial or radial diffusivities for the controls than the patients (see Table 4-4). Table 4-4 summarises for each group contrast the anatomical locations of the white matter clusters (≥100 voxels) showing the peak test-statistics in the voxelwise analyses as well as their associated *p*-values.

Table 4-4

TBSS Group Differences between the TBI and Control Groups

Group contrast	DTI index	WM region*)	Side	P-value	Peak coordinates (MNI)		
					x	y	z
Control > TBI	FA	CB/CST	L	<0.001	−15	−35	55
		SLF/CST	L	<0.001	−22	−35	53
		CC (splenium)	R	<0.001	26	−54	13
	MD	-		0.98, *ns*			
	D_{ax}	-		0.68, *ns*			
	D_{rad}	-		0.60, *ns*			
TBI > Control	FA	-		0.98, *ns*			
	MD	CST	L	<0.001	−9	−28	66
		CST	R	<0.001	21	−20	62
		Medial frontal	L	<0.001	−4	44	−24
	D_{ax}	CB/ CR	R	<0.001	20	−41	37
		CST/CR	R	<0.001	25	−24	32
		Orbitofrontal	L	<0.001	−15	23	−21
	D_{rad}	IFOF/CR	R	<0.05	26	−41	26
		UF	L	<0.05	−25	30	−7
		ATR/CR	R	<0.05	26	14	19
		Fornix	L	<0.05	−2	−6	11

Notes: Anatomical locations of the peak test statistics for each contrast and their significance values. WM = white matter. FA = fractional anisotropy; MD = mean diffusivity; D_{ax} = axial diffusivity; D_{rad} = radial diffusivity. CB = cingulum bundle; CST = corticospinal tract; SLF = superior longitudinal fasciculus; CC = corpus callosum; CR = corona radiata; IFOF = inferior fronto-occipital fasciculus; UF = uncinate fasciculus; ATR = anterior thalamic radiation. *ns* = not significant. *) Regions with clusters larger than 100 voxels only are listed.

4.3.3.2 Hypothesis 2: Increasing time since injury within the TBI group will be associated with more extensive abnormalities of white matter structure. Distinct effects of TBI on white matter structure early versus late post-injury have been previously reported, especially as relates to axial diffusivity (see MacDonald et al., 2007; Sidaros et al., 2008; Song et al., 2002; Song et al., 2003; Wang et al., 2009). Therefore, it was next examined whether the lower FA and higher mean and axial diffusivity values observed in the TBI group relative to controls were also associated with time since injury.

The influence of time since injury was seen in axial and mean diffusivity, but not in FA. In the regions where axial diffusivity was found elevated in the patients compared with controls, it also positively correlated with increasing time since injury ($R_{partial}$ = 0.45, p < .05), an effect that was present after controlling for patient age (as a way of controlling for age-related effects on white matter structure; for a review see Madden et al., 2009). Similarly, MD showed a

positive relationship with increasing time since injury in the regions where it was found elevated in the patient group relative to controls ($R_{partial}$ = 0.46, p < .05, patient age controlled for).

4.3.3.3 Hypothesis 3: Patients with microbleed evidence of DAI will show more extensive white matter disruption than patients without microbleeds. As expected, the comparison of patients with microbleed evidence of DAI (MB) and those without microbleeds (Non-MB) revealed evidence of more severe disruption in the MB group in large parts of the white matter. Figure 4-4 shows the results of (a) FA, (b) MD, (c) axial diffusivity, and (d) radial diffusivity contrasts between the MB and Non-MB patient groups (Non-MB > MB or MB > Non-MB), all but the axial diffusivity contrast significant at p < .05 (corrected for multiple comparisons).

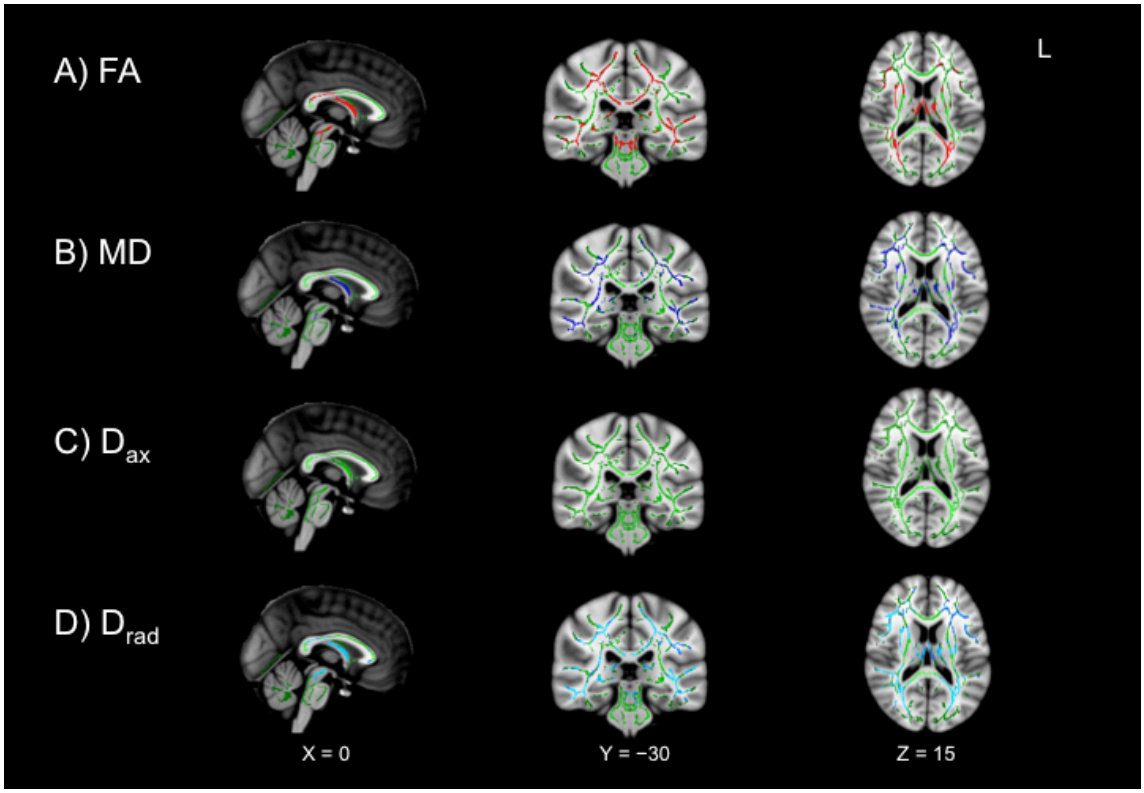

Figure 4-4. Patients with microbleed evidence of DAI (MB) show more extensive white matter damage than patients without microbleeds (Non-MB). (A) FA (red): Non-MB > MB (p < .05); (B) MD (dark blue): MB > Non-MB (p < .05); (C) D_{ax}: neither MB > Non-MB nor Non-MB > MB contrasts were significant at p < .05; and (D) D_{rad} (blue-light blue): MB > Non-MB (p < .05). The results are overlaid on a standard MNI 152 T1 1mm brain and the mean FA skeleton (in green).

Significantly higher FA for the contrast of Non-MB versus MB was observed in the body and splenium of the corpus callosum, as well as bilaterally within the inferior longitudinal fasciculi, the corticopontine/corticospinal tracts, the fornices, the thalamic radiations, the internal

and external capsules, and within white matter structures of the midbrain, such as the decussation of the superior cerebellar peduncles (Figure 4-4A).

There was also higher MD for the MB patients than Non-MB patients in loci largely corresponding to those where the Non-MB patients showed higher FA, including bilateral inferior longitudinal fasciculi, the corticospinal tracts bilaterally, the fornices, bilateral anterior thalamic radiations, the posterior limbs of the internal capsules, and the external capsules. In addition, higher MD for the MB patients was observed in the superior longitudinal fasciculi, bilateral cingulum bundles underlying the posterior cingulate and retrosplenial cortices, and the forceps major and minor, but in the corpus callosum only in the posterior body and the splenium (Figure 4-4B).

The MB patients also showed significantly elevated radial diffusivity in several white matter tracts, compared with the Non-MB patients. These corresponded to the tracts showing either higher FA in the Non-MB group or higher MD in the MB group (or both), apart from the superior longitudinal fasciculi and the forceps minor on the right, these tracts showing no group difference in radial diffusivity despite showing higher MD in the MB than in the Non-MB group (Figure 4-4D).

Table 4-5

TBSS Group Differences between the Microbleed and Non-microbleed TBI Groups

Group contrast	DTI index	WM region*)	Side	P-value	Peak coordinates (MNI)		
					X	y	Z
Non-MB > MB	FA	ILF/IFOF	R	<0.01	39	−46	−8
		CR	L	<0.05	−12	29	−11
	MD	-		0.99, *ns*			
	D_{ax}	-		0.98, *ns*			
	D_{rad}	-		0.99, *ns*			
MB > Non-MB	FA	-		0.96, *ns*			
	MD	IFOF/ILF	R	<0.05	40	−17	−10
		CC (splenium)	L	<0.05	−17	−47	14
		CB	L	<0.05	−26	−19	30
	D_{ax}	-		0.08, *ns*			
	D_{rad}	CST/CR	L	<0.01	−27	−16	30
		ILF/IFOF	R	<0.01	27	−84	−9

Notes: Anatomical locations of the peak test statistics for each contrast and their significance values. MB = Microbleed group; Non-MB = Non-microbleed group. WM = white matter. FA = fractional anisotropy; MD = mean diffusivity; D_{ax} = axial diffusivity; D_{rad} = radial diffusivity. ILF = inferior longitudinal fasciculus; IFOF = inferior fronto-occipital fasciculus; CC = corpus callosum; CB = Cingulum bundle; CST = corticospinal tract; CR = corona radiata. *ns* = not significant. *) Regions with clusters larger than 100 voxels only are listed.

There were no group differences between the MB and Non-MB groups in axial diffusivity (Figure 4-4C), and no white matter tracts that showed either higher FA in the MB group than in

the Non-MB group or higher mean or radial diffusivities in the Non-MB group than in the MB group (see Table 4-5).

4.3.3.4 Hypothesis 4: Patients without microbleeds will also show DTI evidence of white matter damage compared with controls. To assess whether DTI is more sensitive to the presence of white matter damage than gradient-echo imaging, patients without microbleeds were compared with controls. Figure 4-5 shows the results of (a) FA, (b) MD, (c) axial diffusivity, and (d) radial diffusivity contrasts between the Non-MB and Control groups (Control > Non-MB or Non-MB > Control). The results shown, apart from the radial diffuvisity result, are significant at $p < .05$ (corrected for multiple comparisons).

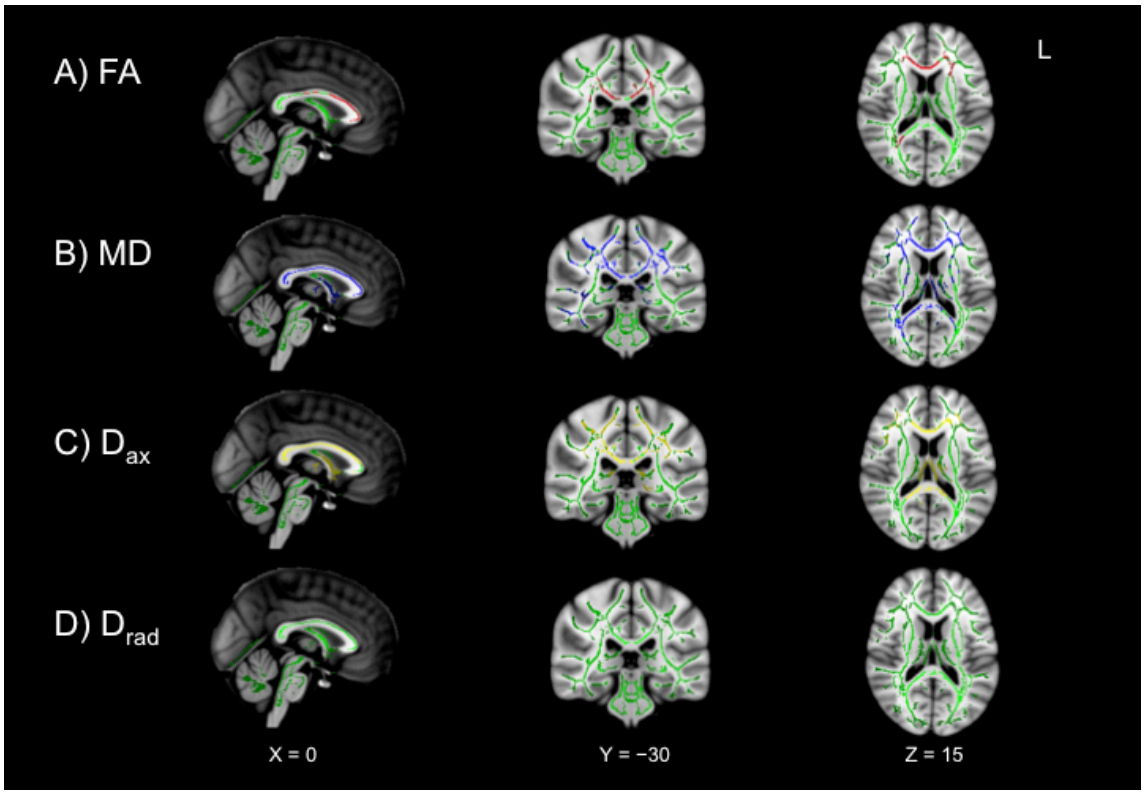

Figure 4-5. Patients without microbleeds show DTI evidence of white matter injury. (A) FA (red): Control > Non-MB; (B) MD (dark blue): Non-MB > Control; (C) D_{ax} (yellow): Non-MB > Control; and (D) D_{rad}: neither Non-MB > Control nor Control > Non-MB contrasts were significant at $p < .05$. All contrasts are overlaid on a standard MNI 152 T1 1mm brain and the mean FA skeleton (in green).

Significantly lower FA, compared with controls, was found in the Non-MB group in the body and genu of the corpus callosum, both corticopontine tracts, and the right forceps major (Figure 4-5A).

Mean diffusivity was significantly elevated in the Non-MB group compared with controls

in several tracts, including the corpus callosum, the cingulum bundle bilaterally, the corticopontine/corticospinal tracts, the fornices, the forceps major and minor, and the anterior and posterior limbs of the internal capsules, particularly on the right (Figure 4-5B).

Axial diffusivity was found higher for the Non-MB group in locations largely corresponding to those in which MD was also higher in this group than for controls, including the corpus callosum (apart from the rostrum), the fornices, and bilateral cingulum bundles, but not in the internal capsules or the forceps major and minor (Figure 4-5C).

There were no significant group differences in radial diffusivity (Figure 4-5D), and no regions where FA was higher, or mean or axial diffusivities lower in the Non-MB than the Control group (see Table 4-6).

Table 4-6

TBSS Group Differences between the Non-microbleed TBI and Control Groups

Group contrast	DTI index	WM region*)	Side	P-value	Peak coordinates (MNI)		
					x	y	z
Control > Non-MB	FA	ATR	R	<0.01	10	18	21
		CC (body)	R	<0.01	8	−23	25
	MD	-		0.98, *ns*			
	D_{ax}	-		0.83, *ns*			
	D_{rad}	-		0.66, *ns*			
Non-MB > Control	FA	-		0.89, *ns*			
	MD	CST	R	<0.001	19	−38	54
		Frontal (SFG)	L	<0.001	−18	5	48
	D_{ax}	CC (splenium)	R	<0.001	16	−41	10
		ATR	L	<0.001	−20	30	18
	D_{rad}	-		0.37, *ns*			

Notes: Anatomical locations of the peak test statistics for each contrast and their significance values. Non-MB = Non-microbleed group. WM = white matter. FA = fractional anisotropy; MD = mean diffusivity; D_{ax} = axial diffusivity; D_{rad} = radial diffusivity. ATR = anterior thalamic radiation; CC = corpus callosum; CST = corticospinal tract; SFG = superior frontal gyrus. *ns* = not significant. *) Regions with clusters larger than 100 voxels only are listed.

4.3.3.5 Hypothesis 5: A subgroup of patients classified as having sustained a mild TBI, compared with controls, will show DTI abnormalities of white matter structure. The relationship between TBI severity as defined using the Mayo classification system (Malec et al., 2007) and microstructural white matter damage was next examined. As shown in Figure 4-6, patients who are clinically classified as having a 'mild' TBI also show abnormalities in the structure of several white matter tracts when compared with healthy controls. The results of (a) FA, (b) MD, (c) axial diffusivity, and (d) radial diffusivity contrasts between the Mild TBI and Control groups are shown (Control > Mild TBI or Mild TBI > Control), thresholded at $p < .05$ (corrected for multiple comparisons).

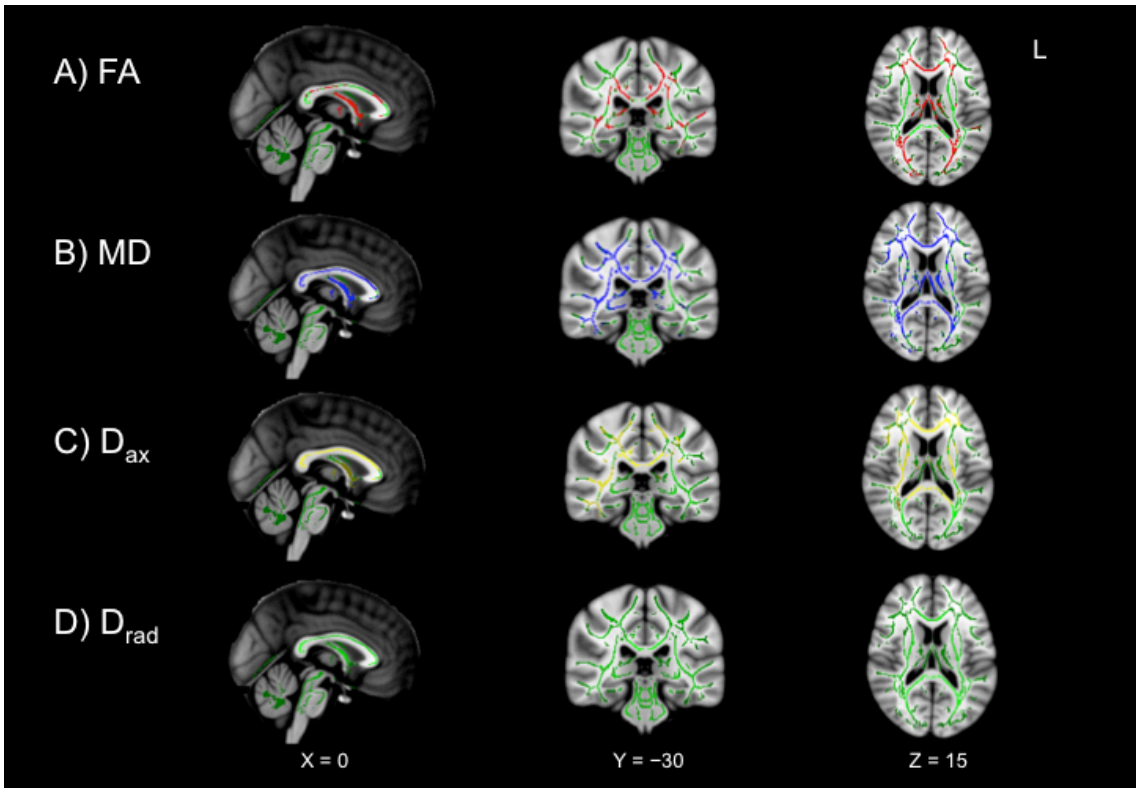

Figure 4-6. Patients with TBI classified as 'mild' also show white matter abnormalities. (A) FA (red): Controls > Mild TBI; (B) MD (dark blue): Mild TBI > Controls; (C) D_{ax} (yellow): Mild TBI > Controls; and (D) D_{rad}: neither Mild TBI > Controls nor Controls > Mild TBI contrasts were significant at $p < .05$. All contrasts are overlaid on a standard MNI 152 T1 1mm brain and the mean FA skeleton (in green).

Although the mild (probable) TBI group consisted of only eight patients, they showed significantly lower FA compared with controls in a wide range of tracts (Figure 4-6A). These included the fornices, the cingulum bundle bilaterally, the corpus callosum, the anterior limb of the right internal capsule, the left external capsule, the inferior fronto-occipital fasciculi, the left superior longitudinal fasciculus, the forceps major and minor bilaterally, bilateral anterior thalamic radiations, and the corticospinal tracts.

The Mild TBI group also showed elevated MD compared with the Control group in similar, but a greater number of tracts, with additional group differences seen in the internal and external capsules bilaterally, and in the superior longitudinal fasciculi (Figure 4-6B).

Axial diffusivity was higher for the Mild TBI group relative to controls in the corpus callosum, the inferior fronto-occipital fasciculi, the posterior cingulum bundles, the left superior longitudinal fasciculus, the posterior limbs of the internal capsule bilaterally, the corticospinal tracts, and both anterior thalamic radiations (Figure 4-6C).

Radial diffusivity measurements did not differ significantly between the Mild TBI and Control groups (Figure 4-6D), and there were no tracts that showed higher FA or lower mean or

axial diffusivities in the Mild TBI group compared with controls (see Table 4-7).

Table 4-7

TBSS Group Differences between the Mild TBI and Control Groups

Group contrast	DTI index	WM region*)	Side	P-value	Peak coordinates (MNI)		
					x	y	z
Control > Mild TBI	FA	ATR	L	<0.01	−15	−16	18
		IFOF	L	<0.05	−11	−79	4
		Forceps minor	R	<0.05	17	56	5
	MD	-		0.65, *ns*			
	D_{ax}	-		0.42, *ns*			
	D_{rad}	-		0.75, *ns*			
Mild TBI > Control	FA	-		0.82, *ns*			
	MD	CC (genu)	R	<0.001	15	24	22
		CST	L	<0.001	−20	−22	54
		CB	L	<0.001	−10	−32	54
	D_{ax}	Forceps minor	L	<0.001	−10	56	23
		ATR	R	<0.01	3	−7	11
	D_{rad}	-		0.13, *ns*			

Notes: Anatomical locations of the peak test statistics for each contrast and their significance values. WM = white matter. FA = fractional anisotropy; MD = mean diffusivity; D_{ax} = axial diffusivity; D_{rad} = radial diffusivity. ATR = anterior thalamic radiation; IFOF = inferior fronto-occipital fasciculus; CC = corpus callosum; CST = corticospinal tract; CB = cingulum bundle. *ns* = not significant. *) Regions with clusters larger than 100 voxels only are listed.

4.3.4 The extent of the white matter abnormalities. Table 4-8 summarises the extent of white matter abnormalities detected using TBSS across the mean FA skeleton, both after TBI and in association with microbleed evidence of DAI. All between-groups differences reported here were significant at $p < .05$ (corrected for multiple comparisons) for the percentage of skeleton voxels shown.

Table 4-8

Percentage/Total Skeletonised White Matter Showing Each Significant Group Difference

Contrast	**FA**	**MD**	**D_{ax}**	**D_{rad}**
Control vs. TBI	46.4% Control > TBI	64% TBI > Control	40.5% TBI > Control	2.8% TBI > Control
Non-MB vs. MB	23.6% Non-MB > MB	31.8% MB > Non-MB	0%	38% MB > Non-MB
Control vs. Non-MB	7.8% Control > Non-MB	32.5% Non-MB > Control	18.8% Non-MB > Control	0%
Control vs. Mild TBI	22.4% Control > Mild TBI	47.2% Mild TBI > Control	26.5% Mild TBI > Control	0%

Notes: TBI = traumatic brain injury patients. MB = patients with microbleed evidence of diffuse axonal injury. Non-MB = patients with no microbleed evidence of diffuse axonal injury. FA = fractional anisotropy. MD = mean diffusivity. D_{ax} = axial diffusivity. D_{rad} = radial diffusivity.

Comparing the overall TBI group with healthy controls resulted in the most extensive

abnormalities in the DTI metrics: The patients showed significantly lower FA and higher mean and axial diffusivities in a large percentage of tracts.

Interestingly, when patients with and without microbleeds were compared, white matter abnormalities were found as indexed by FA, MD and radial diffusivity, but not by axial diffusivity. This is in contrast to the overall TBI group showing extensive abnormalities indexed by axial diffusivity and very limited abnormalities indexed by radial diffusivity, compared with controls.

Compared with controls, patients who did not have microbleed evidence of DAI and patients with TBI clinically classified as mild showed more extensive abnormalities of white matter tract structure measured by mean as well as axial diffusivities than when measured by FA. Tract radial diffusivity did not differentiate either of these patient subgroups from the healthy controls.

Out of the four DTI metrics MD appeared to be the most sensitive to detecting abnormalities of white matter tract structure. It showed the most extensive group differences across contrasts, apart from when patients with and without microbleeds were compared, resulting in slightly more extensive abnormalities indexed by radial diffusivity than by MD.

4.4 Discussion

This work builds on previous studies, which have shown that DTI is a sensitive technique for imaging white matter damage in TBI (e.g. Inglese et al., 2005; Kennedy et al., 2009; Kraus et al., 2007; Niogi, Mukherjee, Ghajar, Johnson, Kolster, Sarkar et al., 2008; Salmond, Menon, Chatfield, Williams et al., 2006; Sidaros et al., 2008). In general these studies have used an ROI approach. This involves the investigation of a limited amount of white matter, within regions that are defined on the basis of *a priori* judgements of regions that are believed to show the effect of interest. Here, for the first time, TBSS (a voxel-based approach), was used to explore in a data-driven manner the effects of TBI on white matter microstructure of the whole brain. Using TBSS it was possible to investigate the impact of TBI on the structure of all major white matter tracts and at the same time control problematic partial volume effects by focusing on the tract centres only. Widespread white matter abnormalities were found, also in patients with no microbleed evidence of DAI on gradient-echo MRI and in patients with TBI clinically

classified as ‘mild’.

4.4.1 Widespread white matter disruption following TBI. These results demonstrate that widespread white matter abnormalities can persist following TBI that are distinct from the limited, and largely non-overlapping, patterns of focal cortical damage.

The DTI abnormalities in the TBI patients were generally seen in the expected directions. Radial diffusivity, however, showed relatively little difference between the TBI patients and age-matched healthy controls. One determinant of radial diffusivity is the degree of axonal myelination (Beaulieu, 2002), and as this increases radial diffusivity should in principle be reduced. Conversely, where there is breakdown of myelin, such as following brain injury, radial diffusivity should in principle increase due to there being less myelin to restrict diffusion perpendicular to the principal direction of axonal fibres. These results suggest that the relationships between different elements of axonal injury and the magnitude and directionality of water diffusion as indexed by the DTI metrics are more convoluted. Further animal studies may be needed to determine in detail the complex relationships between the pathological effects of TBI and different DTI measures.

Some structures, including the fornix, showed white matter abnormalities more consistently than others. The frequent involvement of the fornix as a location showing disruption of white matter structure is not surprising given its arch-like shape and long fibre tracts, which may make it particularly vulnerable to the shearing and tearing forces of TBI (Tate and Bigler, 2000). One previous DTI study examined the effect of TBI on the fornix specifically, and found that fornix FA was significantly reduced in post-acute/chronic TBI patients compared with age- and gender-matched healthy volunteers (Nakayama et al., 2006). However, given its anatomical location close to the ventricles, which makes it prone to CSF contamination, some of the group differences observed in fornix structure may reflect partial volume effects (Metzler-Baddeley, O’Sullivan, Bells, Pasternak, & Jones, 2012). Brains affected by atrophy would be expected to be particularly affected by such partial volume effects due ventricular exapansion. Metzler-Baddeley et al. (2012) recently investigated the effects of CSF-based partial volume artefacts on fornix FA and diffusivity measurements from a TBSS analysis of data from 39 older adults (age range = 53–93 years). In addition to showing that advancing age is associated with FA decreases and diffusivity increases, they demonstrated that in detecting the effects of ageing on

fornix white matter structure, FA is more robust to partial volume effects than the three diffusivity metrics. As a degree of brain atrophy is both associated with advanced age and likely after TBI, some caution in interpreting the group differences observed here in the diffusivity indices may be warranted, particularly those in areas in the immediate vicinity of the ventricles.

4.4.2 Microbleed versus diffusion tensor imaging evidence of axonal injury. Diffusion tensor imaging is extremely sensitive to white matter damage following TBI. The results show that DTI can detect white matter damage not seen using the standard MR techniques. When the analysis of white matter abnormalities was stratified based on the presence or absence of microbleeds, evidence of microstructural white matter disruption was found irrespective of their presence or absence.

The presence of microbleeds on gradient-echo T2* MRI is often used as a marker of DAI, and indicates the presence of more severe white matter injury (Scheid et al., 2003). As expected, patients with microbleeds showed more widespread white matter abnormalities than those who did not have microbleeds, but patients without microbleeds also showed significant abnormalities of white matter microstructure, compared with controls. This highlights the limitation of relying on the presence of microbleeds as a marker of often more subtle white matter damage, and demonstrates that conventional clinical neuroimaging of TBI is insensitive to the full extent of axonal injury.

4.4.3 Patients with a 'mild' TBI show abnormalities of white matter tract microstructure. The analysis was further stratified on the basis of TBI severity, defined based on the Mayo system (Malec et al., 2007) for the comparison of patients clinically classified as 'mild' with healthy controls. Relative to controls, the mild patients showed lower FA and higher mean and axial diffusivities in a number of white matter tracts. These results demonstrate the sensitivity of DTI in identifying significant white matter abnormalities also in patients with clinically mild TBI. Unlike the microbleed analysis, however, the comparison of mild patients and controls did not reveal a group difference in radial diffusivity measurements. This implies that transverse diffusion does not remain abnormally elevated in the post-acute stages of mild TBI. Why the other DTI metrics appear more sensitive to mild TBI than radial diffusivity may relate to different pathophysiological mechanisms being at play in different degrees of injury severity and

these affecting the DTI metrics in distinct ways (see section 4.4.5).

Although the Mayo system includes some aspects of structural brain damage in its criteria, it does not integrate sensitive MR measures of white matter damage. Three out of the eight mild TBI patients had microbleeds on gradient-echo MRI. Therefore, it is likely that the results in this small group reflect the inclusion in this group of patients with macrostructural DAI. However, the results also suggest that clear white matter abnormalities may be present following TBI even in those patients whose standard MRI scans are normal. This is consistent with previous studies that have reported abnormalities of white matter structure in the absence of macroscopic damage on conventional MR imaging (Lipton et al., 2008; Nakayama et al., 2006; Niogi, Mukherjee, Ghajar, Johnson, Kolster, Sarkar et al., 2008; Rugg-Gunn et al., 2001). These results highlight the limitation of existing severity classifications for TBI that fail to include specific measures of white matter damage. Use of DTI as a basis of TBI severity classification, however, remains to be further clinically validated (Saatman et al., 2008).

4.4.4 Changes in DTI metrics over time after TBI. Previous research has indicated that structural abnormalities of white matter may change dynamically over time after TBI. In general, anisotropy has been shown to be reduced and overall diffusivity increased acutely after TBI (e.g. Newcombe et al, 2007). Previous longitudinal work has shown that TBI tends to produce early reductions in axial diffusivity that gradually normalise over time (e.g. Sidaros et al., 2008; Wang et al., 2009). This normalisation of axial diffusivity has been suggested to reflect re-organization within the white matter, due to axonal recovery or even regrowth (Voss et al., 2006; Sidaros et al., 2008).

In the current study, by comparison with controls, TBI patients showed markedly elevated axial diffusivity, which correlated positively with time since injury. Axial diffusivity was also found elevated in patients clinically classified as having a mild TBI. Patients in the current study were scanned longer after their injury than patients in the Sidaros and colleagues' study, and were on average two years post-injury. Apart from showing lower FA patients showed higher mean and axial diffusivities, suggesting that overall diffusivity, mainly driven by axial diffusivity, may continue to rise after the acute phase of TBI. Moreover, far from normalising, axial diffusivity appears to remain at a supranormal level well into the post-acute/chronic stages after TBI. The pathological significance of this variability in axial diffusivity from the acute to the

post-acute/chronic stages of TBI remains unclear, in part due to a lack of relevant animal studies (although see Wang et al., 2009) and, to date, very limited longitudinal work in humans.

When the relationships of the DTI abnormalities in patients (relative to controls) and time post-injury were specifically explored, increasing time since injury correlated not only with elevated axial but also with elevated mean diffusivity; these correlations were present after controlling for possible effects of patient age on the DTI metrics. The observation that FA did not significantly correlate with time since injury is consistent with Sidaros and colleagues' longitudinal findings in that they found abnormally low FA to persist rather than change over time after TBI, whilst changes observed in axial diffusivity were more dynamic over time (Sidaros et al., 2008). The current results further highlight the potential significance of axial diffusivity changes, in particular when investigating evolving tissue damage.

4.4.5 Possible pathophysiological mechanisms. In experimental models of TBI early reductions in FA and axial diffusivity normally emerge in the first few hours after a cortical contusion (MacDonald et al., 2007), and these early changes reflect axonal damage (Budde et al., 2008; Budde et al., 2009; Song et al., 2003). Tissue injury evolves over time with the development of macrophage infiltration, tissue oedema and demyelination, and these pathological changes are reflected in DTI parameters after TBI (MacDonald et al., 2007; Sidaros et al., 2008). In general, low FA has been found to persist over time after TBI, accompanied by an increase in radial diffusivity which leads to high mean diffusivity. Interestingly, here, elevated MD overall was associated with elevated radial diffusivity to a much lesser extent than with elevated axial diffusivity (apart from the patients with microbleeds).

This study was not specifically designed to elucidate the underlying pathophysiological mechanisms, but recent *in vitro* evidence from an axon culture study points toward microtubule damage as the primary mechanism of axonal cytoskeleton breakdown in TBI (Tang-Schomer et al., 2010). Recent developments in high-resolution optical methods have made such sub-cellular level imaging of cytoskeletal dynamics possible (Dent & Gertler, 2003). Previously, a reduction in the number of microtubules of axons has been observed following mechanically-induced trauma (Maxwell & Graham, 1997; Pettus & Povlishock, 1996). However, unlike these studies, suggesting the influx of calcium post-trauma to play a key role, Tang-Schomer et al. (2010) believe that mechanical rupture of the microtubules occurs due to the dynamic axonal

damage in TBI, which subsequently leads to ‘delayed elasticity’, a gradual recovery of axons following trauma to their original orientation, and progressive microtubule disarray. This, in turn, could impair axonal transport, leading to the accumulation of proteins and, ultimately, to degeneration of the unsupplied parts of the axons (Arfanakis et al., 2002; Christman et al., 1994; Povlishock, 1992; Tang-Schomer et al., 2010). To what extent these findings can explain the evolving pathophysiology that determines the DTI abnormalities observed after human TBI remains to be further explored.

4.4.6 Limitations. One limitation associated with all DTI analyses is the presence of partial volume effects. This is potentially problematic when investigating TBI patients in the chronic phase, as these patients frequently show a degree of brain atrophy. This means that the changes in DTI measures such as lower FA may reflect partial volumes, resulting from contamination of the white matter measurements by CSF. The TBSS approach employed here attempts to limit the impact of this problem via the analysis involving ‘skeletonisation’ of the white matter tracts, restricting the subsequent statistical analysis to the centres of these tracts (Smith et al., 2006). This removes the white matter at the junctions with CSF and gray matter that is particularly prone to partial volume effects. Hence, the TBSS approach is arguably more robust to the effects of brain atrophy than approaches such as ROI or VBM analyses.

However, despite TBSS being able to deal with partial volume effects in the case of large white matter tracts, smaller fibre tracts such as the fornix are more problematic. For the projection of individual FA values onto the spatially invariant skeleton structure, in order to ‘fill’ the skeleton and construct that individual’s white matter tract skeleton, the search for the maximum FA value proceeds perpendicularly. For thin tracts such as the fornix, no wider than skeleton structure voxel dimensions, the FA value extracted is not likely to be the true ‘peak’ value, but is instead likely to additionally reflect some partial voluming from the surrounding CSF. In addition, as well as change in the DTI metrics, brain contusions or DAI may cause remote atrophy, which could exacerbate the partial volume effects in brains affected by these lesions. It follows that these effects may have played a role in some of the differences observed in the DTI metrics between the patient and control groups, as well as between the patients with and without microbleeds. By contrast, the DTI abnormalities observed here in patients without any macroscopic lesions relative to healthy controls should be relatively unaffected by atrophy-

related partial volumes, but still dependent on the accuracy of the projection of individual FA values onto the tract skeleton step of TBSS.

A related limitation is the use of scalar measures of FA and MD to provide insight into microstructural properties of white matter (Basser and Pierpaoli, 1996). Although in general FA should become lower and MD higher as the proportion of damaged relative to healthy axons increases within a voxel (Kou et al., 2010), these relationships are not quite as simple. A major limitation of scalar measures such as FA and MD is that they do not directly reflect features of tissue microstructure, but instead are sensitive to a variety of factors that affect its integrity and structural organization. The size and packing density of axons, as well as the permeability of cell walls and membranes, can affect these measures. The distribution of fibre orientations within an imaging voxel also affects the measured average anisotropy and diffusivity. Thus, a change observed in these statistics does not necessarily correspond to any specific changes in tissue microstructure (Alexander et al., 2010).

Investigating multiple DTI metrics between different participant subgroups naturally results in multiple comparisons being carried out. All TBSS analyses were corrected for comparisons carried out across multiple imaging voxels contained within the white matter skeleton. No additional correction was made to account for the separate analyses of the four DTI metrics and those of the different patient subgroups. This is based on the premise that all of these comparisons ask the same overall question, and their results lead to the same conclusion – relative to healthy individuals TBI patients show white matter disruption (indexed by lower FA and higher diffusivities), which is greater in those patients with macrostructural white matter injury. The results of all comparisons, including those producing non-significant results are included in the results section. It can be seen from the tables that list these results that their general pattern is very consistent, pointing to the conclusion stated above.

A limitation stemming from participant recruitment is that the TBI group consisted of predominantly males, whilst the control group had more equal proportions of males and females. As differences between male and female brains may exist that may in turn influence the DTI metrics (see e.g. Hsu et al., 2008), an attempt was made to statistically control for the effects of this possible confound on the relationship between group membership and white matter structure. It is acknowledged, however, that linear statistical approaches may not be able to deal with confounds involved in complex multifactorial relationships, such as those relating to

gender differences in brain structure. Therefore, in retrospect, it would have been preferable to selectively recruit more males than females for the control group in order to achieve better balanced gender ratios for the patient and control groups.

A surprising discrepancy between the TBI and control groups was that the patients significantly outperformed controls in current verbal and nonverbal reasoning ability. Furthermore, they did so whilst having, on average, a significantly lower WTAR-estimated premorbid level of intellectual ability than the control group. Whether this has any implications for the interpretation of the DTI comparisons between the patients and controls depends on the existence and causal direction of a relationship between IQ and white matter structure. Whilst it is of course possible that having a higher IQ leads to a higher structural integrity of white matter connections, potentially contributing to the group differences observed here in the DTI metrics, this is not likely to be of major concern considering the patients' superior current intellectual function. In fact, that the patients have a higher current IQ than the controls potentially makes the current results more striking and the analysis more conservative. That the patients' intellectual function should improve as a consequence of a TBI is peculiar, however, and a more likely explanation is that use of the WTAR has in this study produced artificially low estimates of the patients' pre-injury IQ, especially given their currently high level of intellectual functioning. There are a number of possible reasons that could lead to such unreliability of the WTAR estimates.

A salient explanation is that good performance on this test is dependent upon correct pronunciation of English words, whilst English was not the first language of all participants (see Chapter 2; Green et al., 2008). In addition, some have questioned the use of scores on a word pronunciation tests as an index of the kind of intellectual ability that putatively is resistant to the effects of head injury (Riley & Simmonds, 2003). It has been suggested, for example, that the level of impairment on the WTAR can vary as a function of severity of injury (Mathias et al., 2007). It is also possible that there was some improvement in cognitive function after TBI, which could partly explain why the patient group on average were not found impaired in current reasoning ability (see Green, Melo et al., 2008, for further discussion). The observations by Mathias et al. (2007), suggesting that greater injury severity after TBI may sometimes be associated with worse WTAR performance, highlight that some caution may be necessary when premorbid IQ is estimated in a mixed sample of patients of various levels of injury severity, as

this could potentially lead to underestimation of premorbid intellectual ability in some more severely injured patients.

4.4.7 Conclusions. To summarise, widespread FA, MD, and axial diffusivity differences were found between TBI patients and healthy controls using TBSS. This study also demonstrates the presence of white matter abnormalities in patients with no evidence of microbleed evidence of DAI on gradient-echo MRI and in patients with clinically mild TBI. These results suggest that DTI is extremely sensitive to microstructural white matter damage following TBI and that these abnormalities of white matter tract structure are more widespread across the brain than previously demonstrated by ROI studies that have limited their analysis to a fraction of the total white matter.

Importantly, these results also suggest that DTI can detect white matter abnormalities in cases where tools used in standard clinical care fail to do so despite there being factors that may make the presence of a TBI possible. However, whilst use of DTI for research purposes is on the increase, it remains to be further validated clinically before it can be used to support diagnosis of TBI (Saatman et al., 2008). Given the susceptibility of axonal fibres to damage through TBI, not necessarily visible on conventional MRI as identifiable white matter lesions, DTI could be particularly useful in identifying and understanding mild and very mild TBI, where subtle forms of structural brain damage are possible (see Bigler & Bazarian, 2010).

Many patients with mild TBI who have normal conventional scans experience cognitive problems, some of which are persistent (Lipton et al., 2008; Nakayama et al., 2006; Rugg-Gunn et al., 2001). These impairments can have many important consequences such as limiting the return to productive employment or education (Drake, Gray, Yoder, Pramuka, & Llewellyn, 2000). Early and accurate identification of TBI patients who are likely to have long-lasting problems would thus not only be valuable clinically but also socioeconomically. More generally, improved early detection of axonal damage after TBI could inform subsequent care and rehabilitative interventions. Possible better outcomes that could be achieved by early and well targeted interventions include, for example, reduced length of stay in acute care, improved cognitive function and increased functional independence (Edwards, McNeil, & Greenwood, 2003; Mackay, Bernstein, Chapman, Morgan, & Milazzo, 1992; Wagner et al., 2003).

In conclusion, the results suggest that a flexible approach such as TBSS may be best

suited to analysing the complex patterns of disruption to the structure of the brain's white matter tracts following TBI. Regional white matter damage that DTI (but not necessarily standard MRI) can detect has previously been found to correlate with cognitive dysfunction after TBI (e.g. Kraus et al., 2007). The voxelwise approach used here may, however, be better suited than ROI approaches to investigating the relationships between DTI indices of white matter tract structure and their relevance for high-order cognitive function, likely to be supported by spatially distributed brain networks. This will be the focus of the next chapter that explores the relationships of the DTI metrics studied here with measures of cognitive function.

CHAPTER 5: Relationships between white matter tract structure and cognitive functions

Diffusion tensor imaging and tract-based spatial statistics (TBSS) combined may provide a sensitive way of investigating the complex relationships between axonal injury and cognitive functions that are supported by large-scale neural networks. It has not been previously investigated across the whole brain how damage to specific white matter tracts may affect cognitive performance following TBI. Here, the TBSS approach described in the previous chapter was extended to explore whether the structure of specific white matter connections is associated with performance within particular cognitive domains. These relationships were investigated in the same sample of 28 TBI patients and 26 age-matched controls as in Chapter 4. Specific patterns of white matter abnormality predicted performance on some tasks. The structure of the fornix was correlated with associative learning and memory performance across the patient and control groups, as was the structure of fronto-temporal/parietal connections with immediate logical memory. By contrast, the structure of frontal connections showed relationships with set-shifting ability, which differed in the two groups: patients showed a relationship between stronger directionality of diffusion within these tracts and better performance, whilst controls did not. In addition, patients showed a relationship between greater axial diffusivity in a region that contains descending perceptual-motor fibres and faster responses on a simple choice-reaction task. These results highlight the complexity of relationships between structural properties of white matter and different cognitive functions. Although widespread and sometimes chronic white matter abnormalities are identified after TBI, their impact on high-level cognitive function is likely to depend on damage to key pathways that link nodes in the distributed brain networks supporting the specific cognitive processes involved.

5.1 Introduction

5.1.1 Outline. Whilst Chapter 1 provided a general overview of the anatomical substrates to verbal learning and memory, executive control, and information processing speed, and Chapter 3 explored their relationships with standard MRI indices, the present chapter will focus on exploring how the more subtle axonal injury in TBI, affecting the structure of the brain's white matter connections (as seen in Chapter 4), may relate to specific cognitive functions within these domains.

5.1.2 White matter connections and verbal learning and memory. Episodic memory, critically needed in everyday life, is one the most commonly impaired domains of cognitive function following brain injury. Impairments of verbal learning and memory are often found after TBI (e.g. Draper & Ponsford, 2008; Scheid et al., 2006). Most studies of the anatomical substrates of verbal episodic memory have focused on the role of the medial temporal lobe. Impairments of learning and memory have been consistently described after damage to both the medial temporal (hippocampal) and midline diencephalic regions (e.g. Ariza et al., 2006; Bigler et al., 1996; Scoville & Milner, 1957; Squire, 2004; Wheeler, Stuss, & Tulving, 1997). There is evidence from human lesion studies to support a specific role for the hippocampal formation in recollection (vs. familiarity-based judgements) as well as in associative recall versus free recall of items (Squire, 2004; Dickerson & Eichenbaum, 2010). Human neuroimaging research also implicates the hippocampal formation in the encoding and retrieval of semantic associations (Prince, Daselaar, & Cabeza, 2005), specifically of new associations (Henke, Buck, Weber, & Wieser, 1997; Henke, Weber, Kneifel, Wieser, & Buck, 1999). Hippocampal-dependent consolidation of memories has been suggested to begin immediately upon encountering new information (Zola-Morgan & Squire, 1990), and this region believed to support the maintenance, elaboration, and storage of information over the short term. Considering that the medial temporal lobe often sustains damage in TBI, a hippocampal-dependent difficulty to consolidate learned information may in some patients explain their verbal memory impairments (Vanderploeg, Crowell, & Curtiss, 2001).

Regarding possible interactions of the hippocampal region with other regions, a recent account (Nee & Jonides, 2011) suggests that (i) whilst the hippocampi support the processing of

material actively maintained in short-term memory; (ii) top-down control processes of the PFC are also required for the retrieval of information from the long-term store; and (iii) accessing information that is in the focus of attention further engages the inferior temporal and posterior parietal cortices. Extrapolating from this model, the integrity of hippocampal white matter should be particularly relevant for the initial learning, and interconnections of the prefrontal, inferior temporal and posterior parietal cortices potentially critical for the subsequent retrieval of memories.

Aggleton and Brown (1999) proposed a model for understanding the neural substrates of episodic memory in which central importance is placed on the 'extended hippocampal formation', comprising the hippocampus, the fornix, the mammillary bodies, and the anterior thalamic nuclei. This formation is also referred to as the hippocampal-diencephalic system. White matter fibres carrying neural signals from the hippocampus via the fornix to the diencephalon (the thalamus and the hypothalamus) are viewed in the model as playing a critical role in the efficient encoding and subsequent recall of information. The fornix, a compact bundle of white matter fibres, critically mediates interactions of the hippocampi with other regions (Aggleton, 2008; Aggleton & Brown, 1999; Tsivilis et al., 2008). Fornix damage in humans has been shown to produce impairments of learning and memory (Gaffan & Gaffan, 1991; Kesler et al., 2001; McMackin, Cockburn, Anslow, & Gaffan, 1995; Park, Hahn, Kim, Na, & Huh, 2000), providing further support for the importance of this structure for episodic memory. Moreover, reduced white matter integrity within the hippocampal region has been previously suggested to predict impaired association formation after TBI (Salmond, Menon, Chatfield, Williams et al., 2006). Findings from a recent study by Metzler-Baddeley, Jones, Belaroussi, Aggleton and O'Sullivan (2011) point to a particular role for the structure of the fornix in supporting episodic memory. They investigated, in a sample of 40 healthy older adults (age range: 53-93 years), how age-related changes in the structure of three white matter tracts (the fornix, the hippocampal part of the cingulum bundle, and the uncinate fasciculus) may relate to age-related decline of episodic memory. Out of the three tracts studied, degradation of fornix structure only was specifically associated with age-related decline in episodic memory recall.

Elsewhere, the PFC has been implicated in the application of semantic and executive strategies during verbal encoding and episodic learning (e.g. Miotto et al., 2006). Prefrontal lesions can impair episodic memory, but sometimes memory deficits following frontal damage

may not be apparent due to the presence of executive impairments that are more traditionally linked with frontal injury (Ranganath & Blumenfeld, 2008; Simons & Spiers, 2003). A more common view has been that memory deficits associated with prefrontal damage reflect a failure to recruit executive control processes (e.g. strategic information processing) during storage or retrieval (see e.g. Blumenfeld & Ranganath, 2007, for a review). Earlier neuroimaging studies of the functional correlates of executive control functions supporting memory retrieval in healthy individuals found evidence for the involvement of the ventrolateral PFC (Buckner et al., 1995; Petrides, Alivisatos, & Evans, 1995), while more recent findings have suggested that the effortful application of strategic memory processes may engage the dorsolateral and orbitofrontal cortices (Frey & Petrides, 2002; Miotto et al., 2006). Frey and Petrides (2002) argue that hippocampal-orbitofrontal interactions may be particularly important for successful memory encoding. Such interactions between the medial temporal lobe and subregions of the PFC are supported anatomically by white matter tracts, with the fornix implicated as one of these connections (Croxson et al., 2005).

The contributions of other brain regions to episodic memory have also been extensively studied, including posterior/medial and lateral parietal involvement. Activity in regions including the posterior cingulate, precuneus, and retrosplenial cortices as well as more lateral parietal regions has been frequently observed in functional neuroimaging studies of healthy individuals during retrieval of episodic information (Cabeza et al., 2008; Dickerson & Eichenbaum, 2010). On the other hand, parietal lesions do not severely impair episodic memory (Cabeza et al., 2008). Therefore, an attentional account has been suggested that attributes parietal activity during episodic memory processing to its involvement in attention, an impairment of which may act to weaken the formation of episodic memories (Cabeza, 2008; Cabeza et al., 2008). Given the evidence indicating prefrontal and parietal contributions to episodic memory, it is likely that the long white matter tracts interconnecting the frontal and parietal regions are also involved. Specifically, the superior longitudinal fasciculus structurally connects the posterior parietal and dorsomedial/dorsolateral prefrontal regions that have been implicated in attentional/executive control processes involved in the formation and retrieval of memories.

Previous studies using region of interest (ROI) approaches have found evidence for the involvement of both fronto-temporal and fronto-parietal pathways in episodic memory. Bendlin et al. (2008) studied the relationship between white matter structure and an aggregate index of

memory that tapped verbal learning and visuospatial memory (immediate and delayed) in 35 TBI patients and 36 healthy controls. As expected, they found FA to be significantly lower and mean diffusivity (MD) to be higher for patients than controls in a number of brain regions. However, when the relationships between these DTI indices and memory function were assessed, both groups separately showed a relationship between higher MD (indexing lower structural integrity) in the dorsal posterior cingulate, middle frontal gyrus and fronto-parietal regions and worse memory performance. Neither group showed correlations between FA-indexed integrity of any brain structure and memory function. Although this study demonstrates the potential relevance of degradation of white matter structure to memory dysfunction in both the injured and the healthy brain, it has two major limitations. The first is that a composite measure of memory was used, which averaged performance across diverse tasks that included immediate and delayed recall involving both visual and verbal material. The underlying cognitive processes supporting these functions differ somewhat and, thus, may also have different principal white matter correlates. The second important limitation of the Bendlin et al. (2008) study is their use of VBM (Ashburner & Friston, 2000), which due to data preprocessing can result in considerable partial voluming of white matter structures. This may partly explain the null result for the relationship between FA and memory and complicates the interpretation of their results, as relates to both the group differences reported in FA and MD measures from various brain structures and the behavioural relevance of the structure of some of these regions.

Findings from normally ageing older adults suggest that the structure of parietal as well as temporal white matter connections supports episodic memory (e.g. Ziegler et al., 2010). Bucur et al. (2008), however, suggest that there is a relationship between perceptual speed and episodic retrieval, which the structure of prefrontal white matter connections may mediate. They found evidence for such mediating roles for fractional anisotropy (FA) of the genu of the corpus callosum and pericallosal/frontal white matter in a study comparing 19 older (63-78 years) and 19 younger (20-28 years) healthy individuals on tasks tapping memory retrieval and perceptual speed. Apart from the common problems in all ROI investigations relating to the arbitrary selection of ROIs and partial volume effects in sampling the white matter tracts as well as in sampling a given tract across individuals (as discussed elsewhere in this thesis), a particular problem for this study is that although long-coursing white matter tracts (e.g. the cinglum

bundles) were investigated, a small section of the tract represented each ROI from which FA was measured. This is potentially problematic as FA can vary considerably in different parts of a long white matter tract such as the cingulum bundle, which may have relevance to how its structural integrity is observed to relate to cognitive processes. Thus, apart from a true absence of a mediating role for the structure of five out of the total of seven tracts that Bucur et al. (2008) investigated, their negative findings may also relate to how their ROIs were defined. Moreover, their tract ROIs were all bilateral and FA averaged across the two hemispheres, which masks potential specific contributions of the structure of tracts involved in lateralised brain networks such as the right-lateralized fronto-parietal attentional network, known to support processing speed on some tasks (e.g. Rypma et al., 2006; Perry et al., 2009), possibly including those that involve episodic memory retrieval.

In sum, findings from previous studies suggest a role in verbal episodic memory and associated executive control processes for the structure of white matter tracts connecting the medial temporal, the prefrontal and parietal regions. The fornix has been identified as particularly relevant, but several other pathways, including the cingulum bundles, the uncinate fasciculi, the anterior limbs of the internal capsule, the genu of the corpus callosum, the superior and inferior longitudinal fasciculi, and the inferior fronto-occipital fasciculi have been implicated (see Figure 5-1). Even though some consistent patterns appear to emerge from the literature, amongst other potential limitations (such as those highlighted above), sample sizes have mostly been small, which is why firm conclusions will have to wait until further research has been carried out.

Amongst the diverse strategies contributing to successful encoding and retrieval of information are elaborative processing and semantic clustering of material, cue specification, response selection and monitoring, maintenance of retrieved information, and blocking of distracting information (Simons & Spiers, 2003), any of which could be affected by brain injury. Tests that tap the various elements of episodic memory formation and recall involve, for example, presenting the examinee with a set of target items (e.g. words to be studied), and subsequently asking them to generate the targets, initially (immediate recall) or after a period of delay (delayed recall). Common ways to measure recollection are paired associate learning and free recall. Whilst paired associate learning involves learning to link together two items and produce the second when provided with the first as a cue, in free recall the examinee is required

to retrieve as many items as possible in any order (Baddeley, 2002).

The People Test (Baddeley et al., 1994) is a measure of verbal associative learning and recall that involves learning of four name-face pairs that are retrieved on cue, immediately after presentation. Another widely used test of verbal memory is Logical Memory I (Wechsler, 1997), which involves free recall: the examinee is required to listen to two narrative stories, with their recall then tested immediately following presentation (see Chapter 2, for further description). These two tests are used as measures of episodic memory in the current study.

5.1.3 White matter connections and executive function. As discussed in Chapter 1, executive control processes are central to the control of voluntary and complex actions, and allow performance to be monitored and adjusted. Widely used tests that tap executive functions include the Trail Making Test (Reitan, 1958; Reitan & Wolfson, 1985) and subtests of the Delis-Kaplan Executive Function System (D-KEFS; Delis et al., 2001) such as the Color-Word Interference test and the Verbal Fluency test (see Chapter 2 for more detail). What these tests have in common is that they require high-order cognitive processing such as the ability to inhibit prepotent stimulus-response associations, to maintain a mental set over an extended period of time, and to switch between alternative stimulus-response mappings (Gilbert & Burgess, 2008). Therefore, problems with any of these aspects of executive function can contribute to impaired test performance (Miyake et al., 2000).

Impairments of executive function are common after TBI, and these deficits have traditionally been attributed to frontal lobe damage. However, diffuse axonal shearing often occurs in TBI, and this is likely to be relevant as it may damage white matter pathways between prefrontal and more posterior temporal and parietal regions (Mesulam, 1998; Miller, 2000; Miller & D'Esposito, 2005). As reviewed in Chapter 1, characteristic deficits include those of planning, goal formulation, initiating a response, self-monitoring of behaviour and errors, suppression of prepotent responses, maintaining and shifting a set, and sustained attention (Godefroy, 2003; Stuss & Benson, 1984). Miller and D'Esposito (2005) argue that the executive impairments seen after TBI are consistent with failures of top-down control. Perlstein, Larson, Dotson and Kelly (2006) used a modified Stroop task to investigate the behavioural correlates of cognitive control in a small group of 11 severe TBI patients in the chronic phase post-injury (average of 99 months), compared with 11 healthy controls. The response requirement (i.e. naming the colour

vs. reading the word) was provided in the task by a cue that preceded each target, with the target then presented either after a short or a longer delay. Apart from showing generalised slowing of responses and increased error rates on incongruent trials relative to controls, unlike the controls, patients did not benefit from a long versus a short delay between the cue and the target on incongruent trials, which the authors interpreted as suggestive of post-TBI difficulties with using context information to support the processing of incongruent items. Findings by Seignourel et al. (2005) from a related study of patients with closed-head injury that used the same Stroop task, corroborate the results of Perlstein et al. (2006), which according to the authors suggest that context maintenance problems, which lead to a difficulty to maintain task instructions, may play a role in cognitive control failures that often follow brain injury. In the healthy brain, the neural correlates of response inhibition, another important requirement of Stroop-like tasks, as assessed using functional neuroimaging, typically include the right lateral orbitofrontal cortex, the medial orbitofrontal cortex, cingulate gyrus, superior temporal gyrus, and the inferior parietal lobule, predominantly on the right side (Horn et al., 2003).

Performing cognitively demanding tasks also requires attention, essential for all high-level cognitive functions. Attention is thought to be a distributed mental process that is commonly attributed to two major neural networks, both of which interact with the PFC to support executive control. Fox, Corbetta, Snyder, Vincent and Raichle (2005) postulate that one is a bilateral dorsal system (i.e. the dorsal attentional network) consisting of the intraparietal sulci and the frontal eye fields that is involved in top-down orienting of attention, whilst the second is a right-lateralized ventral system (i.e. the ventral attentional network) that includes the right inferior frontal gyrus and is involved in directing attention towards salient or unexpected stimuli. This model of neural substrates of attention is currently very influential and under investigation by several groups (see e.g. Sharp et al., 2010). The white matter tracts interconnecting the cortical and subcortical nodes of these distributed networks may play an integral role in supporting executive control, with efficient network function depending on the bottom-up and top-down neural interactions (Miller & D'Esposito, 2005). The breakdown of these connections may therefore critically influence network function (Mesulam, 1998).

Disctinct structural connectivity patterns have been identified in the macaque monkey (N = 4) and humans (N = 10) between different subregions of the PFC; both species showing largely similar patterns of PFC-subcortical connectivity (see Croxon et al., 2005). Croxson et al.

(2005) used diffusion-weighted imaging and fibre tracking to investigate the connectivity of the PFC, and distinguished between several pathways: 1) PFC-hippocampal formation via the fornix, 2) PFC-hippocampi/other medial temporal structures via the cingulum bundle, 3) PFC-anterior temporal cortex via the uncinate fasciculus, 4) PFC-posterior temporal cortex via the inferior fronto-occipital fasciculus/extreme capsule, 5) PFC-amygdala, 6) PFC-parietal cortex via the superior longitudinal fasciculus, and 7) PFC-striatum (putamen and caudate nucleus). The cingulum bundles also importantly connect the medial prefrontal with posterior medial/parietal regions (Greicius et al., 2009; van den Heuvel et al., 2008). In addition, the inferior fronto-occipital fasciculi, superior and inferior longitudinal fasciculi, and uncinate fasciculi (see Figure 5-1) may also mediate the functional integration between frontal and more posterior brain regions, critical for a variety of high-order cognitive processes (Fuster, 2004).

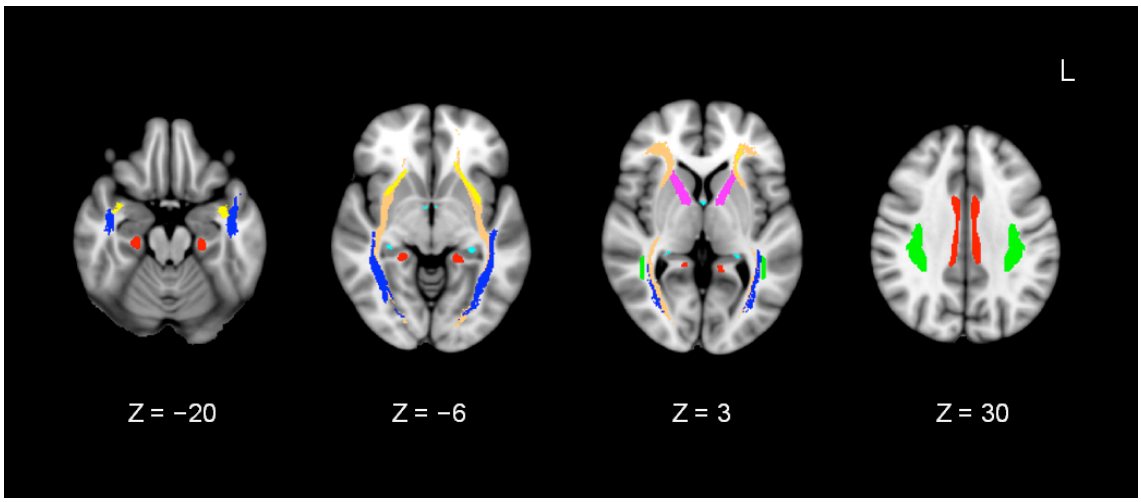

Figure 5-1. White matter tracts putatively involved in verbal learning and memory and executive functions. In red: cingulum bundles. In yellow: uncinate fasciculi. In dark blue: inferior longitudinal fasciculi. In copper: inferior fronto-occipital fasciculi. In light blue: fornix. In green: superior longitudinal fasciculi. In pink: anterior limbs of the internal capsule. The cingulum bundles, superior longitudinal fasciculi and anterior limbs of the internal capsule (all thresholded at 0.5) were derived from the ICBM-DTI-81 White Matter Labels Atlas (Mori et al., 2005). The inferior longitudinal fasciculi, inferior fronto-occipital fasciculi and uncinate fasciculi (all thresholded at 25) were taken from the JHU White Matter Tractography Atlas (Wakana et al., 2007; Hua et al., 2008), and the fornix (thresholded at 60) from the Jülich Histological Atlas (Bürgel et al., 1999; Bürgel et al., 2006). The tracts are overlaid on a standard MNI 152 1mm skull-extracted brain from FSL (displayed as axial slices with MNI Z-coordinates shown). L = Left.

Previous studies have demonstrated a relationship between executive dysfunction and age-related degradation of white matter structure within frontal (Davis et al., 2009; Kennedy & Raz, 2009; Madden et al., 2009; O'Sullivan et al., 2001; Perry et al., 2009; Ziegler et al., 2010) and parietal (Kennedy & Raz, 2009) connections. Kennedy and Raz (2009) studied a sample of 52 healthy adults between the ages of 19 and 81 and found that age-related reduction in FA of

prefrontal white matter predicted worse task-switching performance, both when the task required the participant to switch between stimuli (left vs. right) and to switch between tasks (target to respond to being either <5 vs. >5 or odd vs. even). They also found that lower FA in parietal white matter predicted worse ability to inhibit prepotent responses on a Stroop task. Forstmann et al. (2008) observed a relationship between the structure of the right inferior fronto-occipital fasciculus and better performance by healthy individuals on a visuospatial response inhibition task. Other studies have suggested that the structure of the superior longitudinal fasciculus specifically may mediate performance on simple decision-making tasks (Boorman, O'Shea, Sebastian, Rushworth, & Johansen-Berg, 2007; Gold, Powell, Xuan, & Hardy, 2007).

Hartikainen et al. (2010) explored, in 18 patients following mild/moderate TBI, whether white matter damage, measured using DTI, would be associated with persistent executive impairment. Their outcome measures were reaction times on an 'executive reaction time test' and an aggregate 'executive' measure based on scores on several neuropsychological tests. These scores and DTI indices of the structure of midbrain white matter were found to distinguish patients with persistent cognitive symptoms from patients deemed fully recovered. Although the sample size was small and relationships of their executive measures with only midbrain white matter studied, the findings suggest that disrupted white matter structure within the fronto-striatal network may play a role in executive dysfunction after TBI. Little et al. (2010) studied the relationship between cognitive function and the structure of thalamic white matter projections in 24 TBI patients (12 mild and 12 moderate) and in 12 age- and education-matched healthy controls. Better executive function was reported to correlate with more coherent structure of thalamic fibres in both groups, and in the control group similarly correlate with the structure of the genu of the corpus callosum. A difficulty in interpreting the findings by both Hartikainen et al. (2010) and Little et al. (2010) is that an 'executive domain' score was used that, as well as aggregating across diverse executive abilities such as inhibition and set-shifting, inherently included complex information processing speed as one component. Neither of these studies investigated the possible mediating role of processing speed on the observed relationships between the structure of midbrain/thalamic connections and 'executive function'.

Niogi, Mukherjee, Ghajar, Johnson, Kolster, Lee et al. (2008) investigated the relationship between the white matter structure after TBI and performance on a modified version of a visuospatial two-choice reaction task. On the task (that included both spatially congruent

and incongruent trials), a group of 43 patients with mild TBI showed a positive relationship between the integrity of left anterior corona radiata fibres and conflict processing ability. The authors additionally found no relationship between the structure of left anterior corona radiata and memory performance, or between the structure of the uncinate fasciculus and conflict processing ability, which they interpreted as evidence for a specificity of the observed relationship, a 'correlational dissociation'. Such specificity can hardly be determined by comparing two white matter regions, though, as the structure of any of those regions that Niogi, Mukherjee, Ghajar, Johnson, Kolster, Lee et al. (2008) did not study could have also showed a relationship with conflict processing ability. To answer the question whether *any* other white matter regions are involved requires the use of a whole-brain approach such as TBSS.

5.1.4 White matter connections and information processing speed. As outlined in Chapter 1, information processing speed can be broadly classified as 'simple' or 'complex'. Briefly, simple information processing speed refers to the speed at which respondents react to straightforward stimuli (e.g. present/absent) and is thus determined by basic perceptual, motor and attentional processes. One type of simple processing speed is choice-reaction time on a visuospatial task that involves presentation of target stimuli to which a fast finger-press response is required, according to the spatial location (left/right) of the target stimuli (see Chapter 2 for a description of the task used here). Such choice-reaction tasks are sufficiently simple to reasonably attribute individual differences in response times to the efficiency of basic mental operations. At the same time, these tasks are also sufficiently demanding in the sense of tapping cognitive efficiency instead of being based on purely sensorimotor operations (Rypma et al., 2006). Complex information processing speed, which refers to the speed of responses to stimuli that require more complex judgements to be made, interacts with high-order cognitive processes including executive control (Chiaravalloti, Christodoulou, Demaree, & DeLuca, 2003). From here on these are referred to as 'simple processing speed' and 'complex processing speed'. 'Cognitive processing speed' (e.g. Kochunov et al., 2010; Turken et al., 2008) can then be deduced from the subtraction of simple processing speed from complex. For example, the Trail Making Test (TMT; Reitan, 1958; Reitan & Wolfson, 1985; see Chapter 2) is a widely used measure of executive function, where performance on Trail A (TMT-A) depends on simple processing speed, and performance on the more demanding Trails B (TMT-B) on complex

processing speed, thereby allowing such deduction.

Impairments of processing speed are common following moderate to severe TBI, and are also observed in mild TBI in early stages post-injury (Frencham et al., 2005). In general, when brain-injured individuals are compared with healthy controls, they are substantially slower on a range of processing speed measures, including simple and more complex tasks (Fong, Chan, Ng, & Ng, 2009). Where no particular distinction has been made between types of processing speed, post-TBI deficits have been shown to increase with increasing severity of injury as well as with increasing task difficulty (Tombaugh, Rees, Stormer, Harrison, & Smith, 2007).

Efficient inter-regional communication within the fronto-parietal attentional network is believed to act as the neural basis of processing speed across a broad range of cognitive tasks (Rypma et al., 2006). For example, Perry et al. (2009) who studied white matter tract structure and complex processing speed on the TMT Trails B in normal cognitive aging found that higher anisotropy within the inferior fronto-occipital fasciculi, especially on the right, correlated with faster performance, independently of age. Speed of information processing may thus partly depend on the integrity of this and other white matter tracts connecting the frontal with more posterior regions (see Figure 5-1).

White matter pathways that interconnect the distinct regions of the motor network are involved in the integration of sensory input and motor output functions, and may thus play an important role in perceptual and motor processing speed. The motor network is a well-defined neural system that predominantly consists of the primary motor cortex, supplementary motor area, and the cerebellum (Kasahara et al., 2010). The corpus callosum is the principal connective structure between the brain's two hemispheres and also connects the left with the right primary motor cortex (Wahl et al., 2007). The corpus callosum contains large numbers of myelinated fibres running in parallel (Shimony et al., 1999), a feature of white matter that in general supports its integrity (Beaulieu, 2002). Based on a relationship between white matter integrity and nerve conduction velocity (Jack, Noble, & Tsien, 1983), overall processing speed on a given task could be mediated by the structure of tracts that support the various perceptual, motor and cognitive processes involved. Several DTI studies have shown that the structural coherence of the corpus callosum is reduced after TBI, which could compromise the interhemispheric functional interactions within the motor network. In healthy individuals the

integrity of the corpus callosum has been suggested to predict individual differences in visuomotor processing speed (Schulte, Sullivan, Muller-Oehring, Adalsteinsson, & Pfefferbaum, 2005), as well as the decline in processing speed associated with normal cognitive ageing on a range of simple tasks (e.g. Bucur et al., 2008; Kennedy & Raz, 2009; Kochunov et al., 2010; Sullivan et al., 2001; Sullivan, Rohlfing, & Pfefferbaum, 2010).

Niogi, Mukherjee, Ghajar, Johnson, Kolster, Sarkar et al. (2008) explored the relationship between white matter structure and processing speed on a visuospatial choice-reaction task (indexed by the average reaction time for the congruent and incongruent conditions) in a sample of 34 patients with mild TBI, compared with 26 healthy controls (the same task was used in Niogi, Mukherjee, Ghajar, Johnson, Kolster, Lee et al., 2008 to index conflict processing ability). In patients, as the number of white matter structures showing reduced integrity increased, average choice-reaction times also increased. The predominant regions showing reduced anisotropy as compared with the healthy controls were the anterior corona radiata, the uncinate fasciculi, the genu of the corpus callosum, the inferior longitudinal fasciculi, and the cingulum bundles. Given the involvement of other frontal connections, in addition to the motor fibres of the anterior corona radiata, these results may reflect the executive components of the task used. All in all, these studies are consistent in suggesting a role for the structure of fronto-parietal, motor, and interhemispheric connections in information processing speed that may also underpin the deficits observed following TBI.

The present study will investigate the relationship between white matter structure and simple processing speed only. This is both because of the involvement of executive processes in complex processing speed, which would undesirably complicate exploring the relationship between the structure of specific white matter tracts and processing speed itself, and because post-TBI impairments have been demonstrated across a range of measures, including the simple ones.

5.1.5 Investigating the relationship between white matter tract structure and cognitive function using TBSS. Investigation of white matter abnormalities within a limited number of regions of interest (ROI) is unlikely to clarify complex relationships between axonal injury and cognitive impairment after TBI, given the diffuse pathology. As the connecting white matter pathways of structures involved in cognitive processes sustain damage in TBI, reduced

efficiency of information transfer within functional brain networks may follow. It is also likely that structural abnormalities in white matter tracts have differential effects on cognitive functions depending on which specific tracts are damaged. Verbal learning and memory, executive function and information processing speed are all believed to depend on distributed network function. An ROI approach would thus critically limit the analysis of the structural causes of acquired impairments in these cognitive domains. These issues are compounded by our limited knowledge of how white matter tract structure relates to cognitive function in the normal brain, making it important to assess white matter abnormalities after TBI with as comprehensive spatial coverage as possible. However, a downside of a whole-brain approach is that statistical power to detect significant relationships can be weak, particularly if sample sizes are small and a large number of variables studied, but this naturally depends on the size of the effect studied and the variance in the measures of interest. This study is the first to use TBSS, a voxelwise whole-brain approach, to investigate how the structure of specific white matter tracts may relate to particular cognitive impairments after TBI.

Voxelwise analysis of white matter properties typically relies on scalar measures derived, for example, from a tensor model fit to diffusion-weighted MRI data (Jbabdi et al., 2010). Several voxel-based methods can be used, of which VBM has previously been used in TBI (Salmond, Menon, Chatfield, Williams et al., 2006). The problems associated with VBM-type analysis have been discussed in previous chapters, and Chapter 4 found tract-based spatial statistics (TBSS) to be sensitive to white matter abnormalities after TBI. As detailed in Chapter 2, TBSS uses nonlinear registration of each individual's data that is optimised for the registration of diffusion data, and these data are subsequently projected onto an alignment invariant 'skeleton' representing the group's average white matter tract structure. This has some clear advantages over the VBM method. Briefly, TBSS reduces alignment and registration errors between the individual and standard brain spaces and across participants. This reduces partial white matter volume effects, making the results more interpretable (Jones, Symms, Cercignani, & Howard, 2005; Smith et al., 2006). Finally, as opposed to restricting the analysis to a limited number of small ROIs, TBSS allows complex patterns of white matter disruption to be identified across the brain's white matter tracts, and relationships of tract-specific abnormalities with specific cognitive functions to be studied.

5.1.6 Aims of the present study. Here, TBSS is used for the first time to investigate relationships between anatomically distributed white matter damage after TBI and impairments of verbal learning and memory, executive function, and information processing speed. Specifically, this study explores whether structural properties of particular white matter tracts (FA, MD, axial diffusivity and radial diffusivity) are correlated with cognitive functions within the three domains commonly impaired following TBI.

5.1.7 Hypotheses. It is hypothesised that greater white matter disruption following TBI will be associated with greater cognitive impairment, and that particular patterns of white matter disruption will be associated with distinct types of impairment. The following hypotheses are tested:

1) Structural abnormalities of hippocampal and prefrontal connections (more than of other tracts) will be correlated with worse associative learning and memory performance.
2) Abnormalities of medial temporal lobe white matter tracts and tracts interconnecting the frontal and more posterior temporal and parietal cortices (more than of other tracts) will be associated with worse logical memory performance.
3) Abnormalities of frontal white matter tracts and tracts connecting the frontal to posterior medial/parietal regions (more than of other tracts) will be associated worse executive function.
4) Abnormalities of fronto-parietal, motor, and interhemispheric pathways (more than the integrity of other tracts) will be associated with slower information processing speed.

5.2 Methods and Materials

5.2.1 Design. A cross-sectional correlational study was carried out to investigate the relationships between DTI indices of white matter structure and measures of cognitive function in TBI patients and healthy controls.

5.2.2 Participants. The 28 post-acute/chronic TBI patients and 26 age-matched healthy controls who participated in the present study were the same as those in the study reported in

Chapter 4. The reader is therefore referred to the previous chapter for clinical details on each patient (p. 136) and summary demographic information about each group (p. 139). Chapter 2 described the procedures for participant recruitment, as well as the general and group-specific inclusion and exclusion criteria. All participants gave a written informed consent according to the Declaration of Helsinki (World Medical Association, 2008). The present study was approved by the Hammersmith, Queen Charlotte's and Chelsea Research Ethics Committee and the Departmental Ethics Committee of Goldsmiths Psychology Department.

5.2.3 Neuropsychological assessment. All participants completed a battery of neuropsychological tests sensitive to cognitive impairments associated typically with TBI. This has been fully described in Chapter 2. The specific tests and measures used in the current study are listed below.

5.2.3.1 Measures of general intellectual ability:

- Premorbid IQ: Wechsler Test of Adult Reading (WTAR; Wechsler, 2001), age-scaled score
- Current verbal and nonverbal reasoning ability: Similarities and Matrix Reasoning (WASI; Wechsler, 1999), age-adjusted *T*-scores

5.2.3.2 Measures of theoretical interest:

- Verbal (associative) learning and memory: People Test (Doors and People battery; Baddeley et al., 1994), immediate recall index
- Verbal learning and memory (logical/structured material): Logical Memory I (WMS-III; Wechsler, 1997), first recall total score
- Set-shifting ability: Trail Making Test (TMT; Reitan, 1958; Reitan & Wolfson, 1985), completion time for TMT-B minus completion time for TMT-A = 'alternating switch-cost index'
- Cognitive flexibility/susceptibility to interference: Color-Word Interference test (D-KEFS; Delis et al., 2001), inhibition/switching completion time minus a baseline of

the average completion time for the color naming and word reading conditions = 'Color-Word interference index'

- Word generation fluency: D-KEFS Verbal Fluency/letter fluency, total correct score for letters F, A and S
- Information processing speed: A computerised choice-reaction task (CRT; see Chapter 2 for details), median reaction time on correct trials

Subcomponents of the TMT and D-KEFS Color-Word Interference test tapped different aspects of processing speed (visual search: completion time for TMT-A; complex information: completion time for TMT-B; and speed of naming and reading: completion times for Color-Word Interference baseline conditions, color naming and word reading), but these were not measures of interest in the present analyses.

5.2.4 Structural imaging. The conventional T1- and T2*-weighted MRI sequences were as described in Chapter 2 and the DTI sequence as described in Chapter 4 (and in more detail in Chapter 2).

5.2.5 DTI data analysis. Voxelwise analysis of the FA, MD, and axial and radial diffusivity data was carried out using TBSS in FSL (Smith et al., 2004; Smith et al., 2006) as described in detail in Chapter 2. Mean FA and 'skeletonised' FA, MD, axial diffusivity and radial diffusivity images were derived. Permutation-based non-parametric significance testing was performed using randomise with threshold-free cluster enhancement (TFCE) and 5000 permutations, and a threshold of $p < .05$ (corrected for multiple comparisons) was applied (Nichols & Holmes, 2002; Smith & Nichols, 2009). Possible effects of age and gender were attenuated in the TBSS analyses by including them as covariates of no interest, because these have been shown previously to correlate with the diffusion parameters used here (e.g. Giorgio et al., 2010; Madden et al., 2009; Westerhausen et al., 2011).

5.2.6 Analyses of relationships between white matter structure and cognitive function. The relationships between four indices of white matter structure (FA, MD, axial diffusivity and radial diffusivity) and six measures of cognitive function (as set out in section

5.2.3.2) were explored.

The effects of group and each cognitive variable were modelled using general linear modelling (GLM) in FSL (as described in more detail in Appendix C). This allowed TBSS analysis of relationships between the structure (indexed by the DTI metrics) of all of the brain's major white matter tracts and specific cognitive functions to be conducted.

Voxel-based correlations between each of the four DTI metrics and the six cognitive variables were tested within a combined sample of patients and controls and within each group separately. Group interactions were also tested to explore whether the two groups showed distinct relationships between white matter structure and cognitive function. Based on exploratory data analysis, one patient and one control were excluded from the set-shifting ability (alternating-switch cost index) and cognitive flexibility/susceptibility to interference (Color-Word interference index) analyses due to extreme scores (score ≥ 3 interquartile ranges outside scores for the middle half of the sample). Permutation-based significance testing was then carried out as described above (section 5.2.5).

Where these whole-brain analyses produced significant results, the relevant FA or diffusivity values within the voxels showing the significant effect were extracted for each participant from the skeletonised white matter image and plotted against their cognitive scores to clarify the group effects.

5.3 Results

5.3.1 Participants. The same sample of TBI patients and age-matched healthy controls participated in the current study as described in the previous chapter. Table 4-2a in Chapter 4 (p. 139) provided the summary demographic characteristics of the patient and control groups. Clinical characteristics within the patient group, including MRI evidence of residual brain injury, were also described in the previous chapter.

5.3.2 Cognitive function. Although the cognitive variables used in the analyses of relationships with white matter structure are confined to those set out in section 5.2.3.2, the table below additionally shows the scores of the two groups on related indices that are

subcomponents of the measures of interest, and that tap different aspects of processing speed.

Table 5-1

Scores on Cognitive Measures of Theoretical Interest and Their Subcomponents

COGNITIVE DOMAIN	COGNITIVE VARIABLE	TBI Mean±SD[a)]	CONTROL Mean±SD[a)]	TBI vs. CONTROL[b)]
Measures of theoretical interest				
Verbal memory: Associative learning and memory	People Test immediate recall total	24.8±4.9 (*N*=28)	29.9±4.0 (*N*=24)	*t* = −4.03 ***
Verbal memory: Logical memory	LM I first recall total (stories A + B immediate)	28.9±5.7(*N*=27)	27.2±8.5(*N*=26)	*t* = 0.94 [ns]
Executive function: Set-shifting ability	TMT Trails B minus A (s) = Alternating-switch cost	34.2±26.5 (*N*=26)	22.2±9.9 (*N*=24)	*t* = 2.10 *
Executive function: Cognitive flexibility/susceptibility to interference	Color-Word inhibition/switching minus baseline of (CN + WR) ÷ 2 (s) = Interference	38.0±15.4 (*N*=27)	27.9±10.4 (*N*=25)	*t* = 2.74 **
Executive function: Word generation fluency	Verbal Fluency/letter fluency F + A + S total correct	43.1±9.8 (*N*=28)	49.6±10.0 (*N*=24)	*t* = − 2.40 *
Processing speed: Choice reaction time	Choice-reaction task median RT (ms)/correct trials	449±75 (*N*=27)	393±52 (*N*=26)	*t* = 3.00 **
Subcomponents of main measures				
Processing speed: Visual search	TMT Trail A (s)	28.3±9.5 (*N*=27)	19.8±4.3 (*N*=25)	*t* = 4.36 ***
Processing speed: Complex	TMT Trails B (s)	70.2±40.1 (*N*=28)	40.5±10.5 (*N*=23)	*t* = 3.74 ***
Processing speed: Complex	Color-Word inhibition/switching (s)	67.2±18.8 (*N*=27)	54.1±10.6 (*N*=25)	*t* = 3.13 **
Processing speed: Naming/Reading	Color-Word color naming (CN) (s)	34.2±8.6 (*N*=27)	28.2±5.6 (*N*=26)	*t* = 3.30 **
	Color-Word word reading (WR) (s)	23.6±4.0 (*N*=27)	22.5±4.5 (*N*=25)	*t* = 0.90 [ns]

Notes: [a] Following Exploratory Data Analysis (EDA) using boxplots, outlier scores ≥ 1.5 x interquartile range outside the middle half of the sample were excluded variable-wise. [b] All significant group differences indicate that controls outperformed patients. LM = Logical Memory. TMT = Trail Making Test. Color-Word (inhibition/switching, color naming, and word reading) and Verbal Fluency/letter fluency are from the D-KEFS. * indicates $p < .05$. ** indicates $p < .01$. *** indicates $p < .001$. [ns] not significant at $p < .05$.

Given the group differences (as reported in Chapter 4) in premorbid IQ (controls outperformed patients) and current reasoning ability (patients outperformed controls), individual differences in IQ were controlled for in the comparisons of all other cognitive variables. Due to reasons including the unexpected discrepancy between group averages for estimated premorbid versus current IQ and potential problems with the WTAR-estimates of premobid IQ in the present research (as discussed in Chapter 4), a decision was made to use an index of current intellectual ability for this purpose instead of WTAR scores. Thus, a composite measure

of current IQ was derived: standardised scores for WASI Similarities and Matrix Reasoning were summed and the result divided by two. Each cognitive variable was then regressed against this aggregate index of IQ, and the standardised residuals of these analyses subjected to comparison via independent samples *t*-tests, current IQ thus regressed out. Where Levene's Test for Equality of Variances was significant, adjusted *t*-values are reported in the table. As the purpose of this enquiry was exploratory, no multiple comparisons correction of the *p*-value was applied.

Possible effects of current IQ thus controlled for, the patients showed a pattern of specific cognitive impairments characteristic of TBI (Draper & Ponsford, 2008; Frencham et al., 2005; Levin, 1995; Levin & Kraus, 1994; Ponsford & Kinsella, 1992; Scheid et al., 2006). Specifically, they showed (i) impaired associative memory on the People Test; (ii) impaired executive functioning (shown by inefficiencies on the TMT, the Color-Word Interference test inhibition/switching condition, and the Verbal Fluency/letter fluency condition); and (iii) impaired information processing speed, indexed by slower responses on the CRT, as well as on all additional measures of processing speed apart from the word reading baseline condition of the Color-Word Interference test.

5.3.3 The relationship between white matter structure and cognitive function. As mentioned in section 5.2.5**,** possible effects of age and gender were statistically controlled for in all the following TBSS analyses (see Appendix C for details). The DTI and cognitive indices used in these analyses were as set out in section 5.2.6. Although TBSS tests for voxelwise relationships between the structure of all the tracts of the white matter 'skeleton', the structure of certain white matter tracts was predicted in the present study to correlate with specific cognitive functions more than the structure of other white matter tracts. In particular, the following four hypotheses were tested:

5.3.3.1 Hypothesis 1: Structural abnormalities of hippocampal and prefrontal connections (more than of other tracts) will be correlated with worse associative learning and memory performance. As shown by Figure 5-2A, evidence was found in the TBSS analyses of the combined sample of patients and controls that more coherent fornix structure (indexed by higher FA) correlated with better associative learning and memory performance

(higher scores for People Test immediate recall). Thus, as expected, individuals with less anisotropic white matter within the fornix showed worse associative memory performance. With the stringent permutation test employed, corrected for multiple comparisons across all voxels of the white matter skeleton, this relationship was of borderline significance ($p < .06$ in the right fornix/peak voxel: MNI x = 7, y = −5, z = 9 and $p < .07$ in the left fornix/peak voxel: MNI x = −2, y = −16, z = 17).

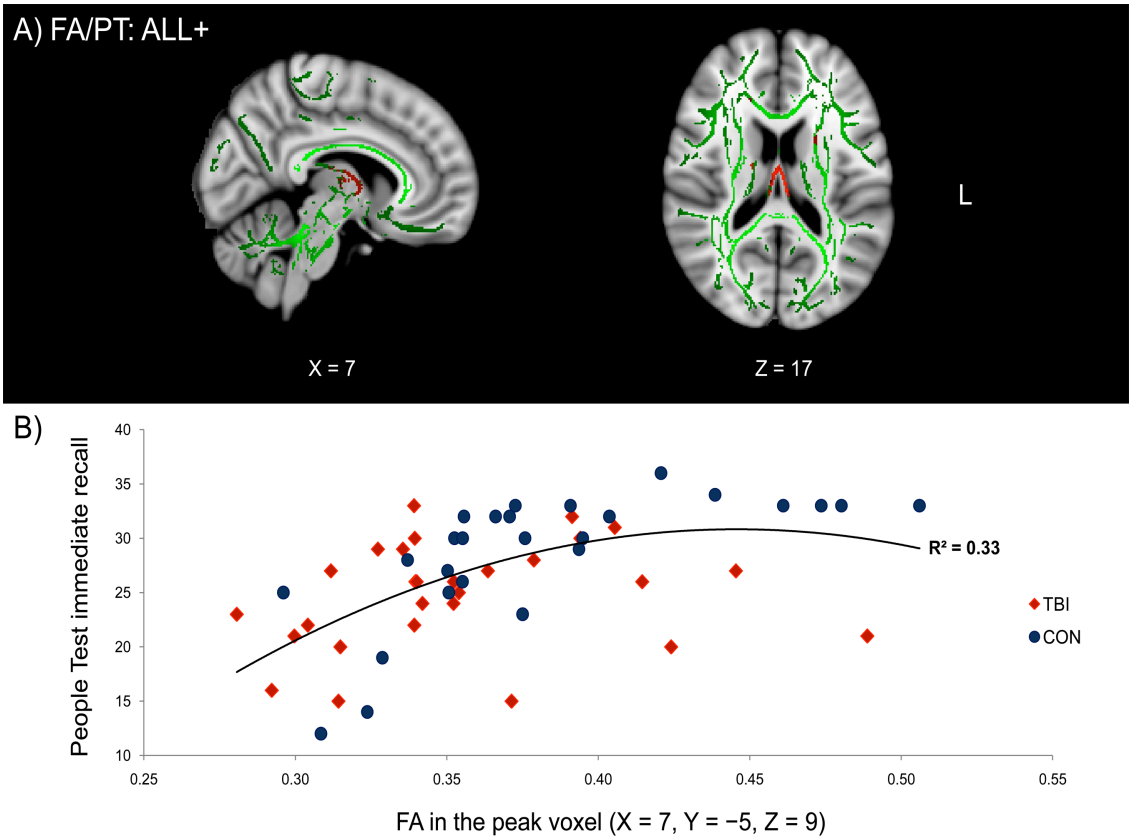

Figure 5-2. Correlation between associative learning and memory and FA. A) Areas where FA is positively correlated with immediate recall on the PT are indicated in red. The result is overlaid on a standard MNI 152 T1 1mm brain and the mean FA skeleton (in green). For display purposes $p < .1$. B) Graph showing individual data points for the correlation between PT total score and FA in the peak voxel of the whole-brain result (MNI x = 7, y = −5, z = 9). The second order polynomial regression fit is shown. MNI coordinates are shown. PT = People Test. L = Left. Reprinted from Brain February 2011, 134/2, Kinnunen et al., White matter damage and cognitive impairment after traumatic brain injury, 449-463, Copyright (2011), with permission from Oxford University Press.

As seen in Figure 5-2B, when individual participant's FA values were extracted from the voxel within the right fornix that showed the strongest effect, these values were significantly correlated with the participants' scores on the People Test in the combined sample of patients

and controls (R^2 = .25, p < .001). However, whilst TBSS tests for linear relationships between white matter structure and the cognitive variable, this relationship may not always be best modelled linearly, as the data in Figure 5-2B suggest. Thus, a second-order polynomial regression slope was fitted instead, which models the data more accurately (R^2 = .33, p < .0001). The relationship between FA within the voxel showing the peak effect and People Test immediate recall remained significant ($R_{partial}$ = .48, p < .001) after possible effects of current IQ were controlled for (using the WASI index as detailed above). This relationship was also specific to white matter of the hippocampal formation, with no regions outside it showing significant (or near-significant) correlations between FA and People Test scores.

Associative learning and memory performance did not significantly correlate with any of the three diffusivity measures of white matter structure analysed using TBSS (MD, axial diffusivity, or radial diffusivity) across the two groups, as shown in Table 5-2 Thus, one out of the four voxelwise analyses within the combined sample of patients and controls produced positive results.

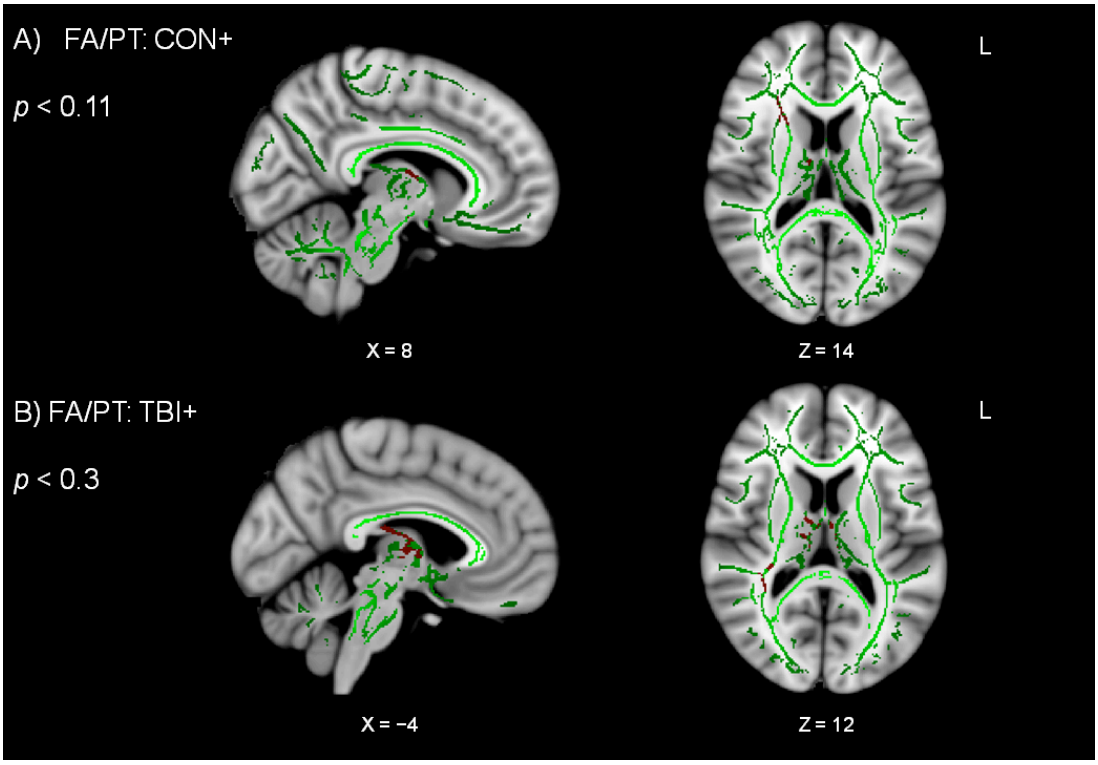

Figure 5-3. Group-specific correlations between associative learning and memory and FA. A) A positive correlation between FA and PT total score within the control group (CON+); for display purposes thresholded at p < .11. B) The same correlation within the patient group (TBI+); for display purposes thresholded at p < .3. Areas where FA is positively correlated with immediate recall on the PT are indicated in red. The result is overlaid on a standard MNI 152 T1 1mm brain and the mean FA skeleton (in green). MNI coordinates. PT = People Test. L = Left.

When the voxelwise relationships of each DTI metric with People Test immediate recall were explored within the two groups separately, both groups showed non-significant trends for a positive relationship between anterior thalamic radiation/fornix FA and associative learning and memory, whereby higher FA was associated with better performance. A stronger trend was seen in the control than in the patient group. This is illustrated by Figure 5-3 above, showing these group-specific results thresholded at a sub-threshold level required to display the peak effects.

No other DTI metrics showed significant (or near-significant) relationships with People Test scores within either the patient or the control group analysed separately (see Table 5-2).

5.3.3.2 Hypothesis 2: Abnormalities of medial temporal lobe white matter tracts and tracts interconnecting the frontal and more posterior temporal and parietal cortices (more than of other tracts) will be associated with worse logical memory performance. As shown in Figure 5-4A, the patient and control groups combined showed significant voxelwise relationships (p < .05, whole-brain corrected) between better logical memory performance (higher LM I immediate recall scores) and higher tract FA. Higher FA within the right superior longitudinal fasciculus/inferior fronto-occipital fasciculus correlated with better LM I performance.

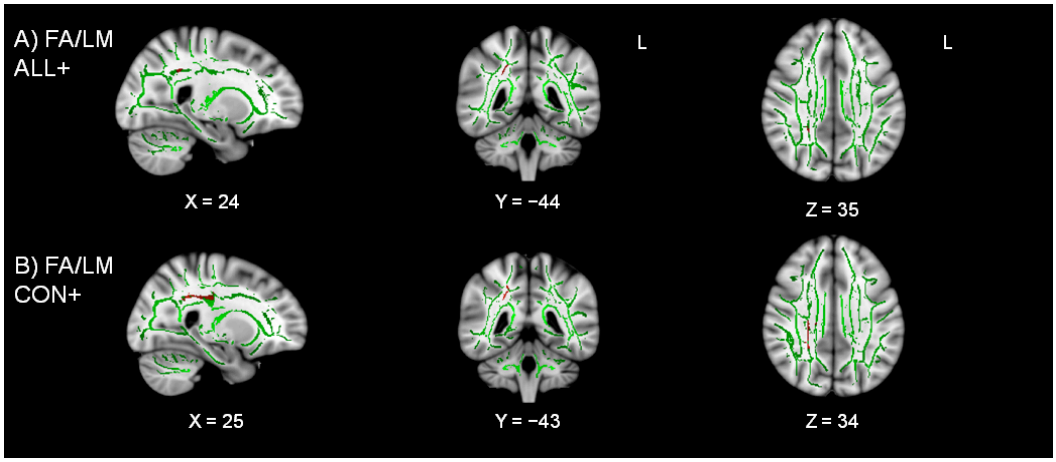

Figure 5-4. Group-specific correlations between FA and Logical Memory I performance. A) A positive correlation between FA and immediate recall on LM I across the patient and control groups (ALL+). B) The same correlation within the control group only (CON+). Areas where FA is positively correlated with LM I performance are indicated in red, showing the white matter tract locations where significant correlations (p < .05, corrected for multiple comparisons) were observed. The results are overlaid on a standard MNI 152 T1 1mm brain and the mean FA skeleton (in green). MNI coordinates. LM = Logical Memory. L = Left.

When voxelwise relationships between tract FA and LM I performance were analysed using TBSS within each group separately, the control group showed a significant correlation ($p < .05$, whole-brain corrected) in a location similar to the across-both-groups result (Figure 5-4B). Although indicating a similar set of voxels, the relationship between FA and LM I performance within the patient group alone was not statistically significant. As shown in Table 5-2, LM I did not significantly correlate with any of the other indices of white matter structure (MD, axial or radial diffusivity), either in the combined sample of patients and controls or within the groups separately.

5.3.3.3 Hypothesis 3: Abnormalities of frontal white matter tracts and tracts connecting the frontal to posterior medial/parietal regions (more than of other tracts) will be associated with worse executive function. Of the three indices of executive function whose voxelwise relationships with white matter structure were assessed, two (cognitive flexibility assessed by D-KEFS Color-Word Interference and word generation fluency assessed using D-KEFS letter fluency) showed no associations with the structure of any white matter tracts (indexed by FA, MD, axial diffusivity or radial diffusivity).

For the remaining index of executive function, set-shifting ability (TMT alternating switch-cost index), significant relationships were observed in the voxelwise analyses with tract MD and radial diffusivity, but not with tract FA or axial diffusivity. No relationships were observed between tract MD or radial diffusivity and set-shifting in the combined sample of patients and controls. Instead, the relationships between both of these DTI metrics and the TMT alternating switch-cost index differed between the two groups.

As expected, the patient group showed a correlation between lower white matter integrity (indexed by higher MD) and worse set-shifting performance; this relationship was observed for white matter tracts in the left superior frontal region. A subsequent analysis of MD values extracted from only those voxels that showed the significant relationship, confirmed that higher MD was associated with higher alternating switch-cost, indicating worse performance ($R_{partial} = .75$, $p < .001$, controlling for current IQ). The control group, when analysed separately, did not show any significant relationships between tract MD and set-shifting ability. Despite the relationships between tract MD and set-shifting thus differing in the patient and control groups, no white matter tracts showed significant group interaction effects for MD and set-shifting, but

near-significant trends (p = 0.06) were seen in the left superior and medial frontal WM, left superior longitudinal fasciculus (temporal part), and left superior parietal WM (see Table 5-2).

The voxelwise relationships between tract radial diffusivity and set-shifting ability were also analysed within the patient and control groups separately. No significant relationships were observed between radial diffusivity and set-shifting performance within the patient group only. Within the control group, an unexpected negative relationship was observed: higher radial diffusivity was significantly associated with better set-shifting performance (p < .01, whole-brain corrected). This relationship was observed within parts of the right cingulum bundle, right superior longitudinal fasciculus, and the body of the corpus callosum. Figure 5-5 shows the spatial maps for these correlations for each group separately.

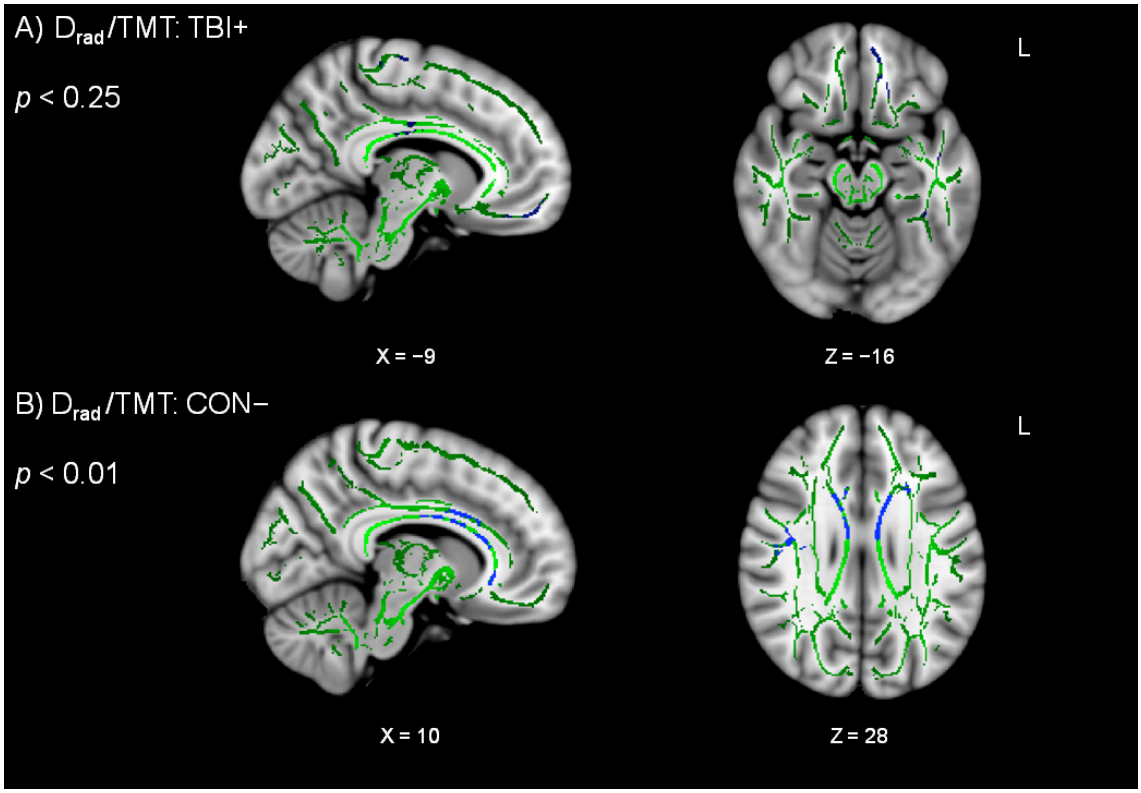

Figure 5-5. Group-specific correlations between radial diffusivity (D_{rad}) and set-shifting performance (TMT alternating-switch cost). A) A correlation between higher D_{rad} and higher alternating-switch cost (worse TMT performance) in the patient group (TBI+); for display purposes thresholded at p < .25. B) A correlation between higher D_{rad} and lower alternating-switch cost (better TMT performance) in the control group (CON−). Areas where D_{rad} is correlated with TMT performance are indicated in blue-lightblue. The results are overlaid on a standard MNI 152 T1 1mm brain and the mean FA skeleton (in green). MNI coordinates. TMT = Trail Making Test. L = Left.

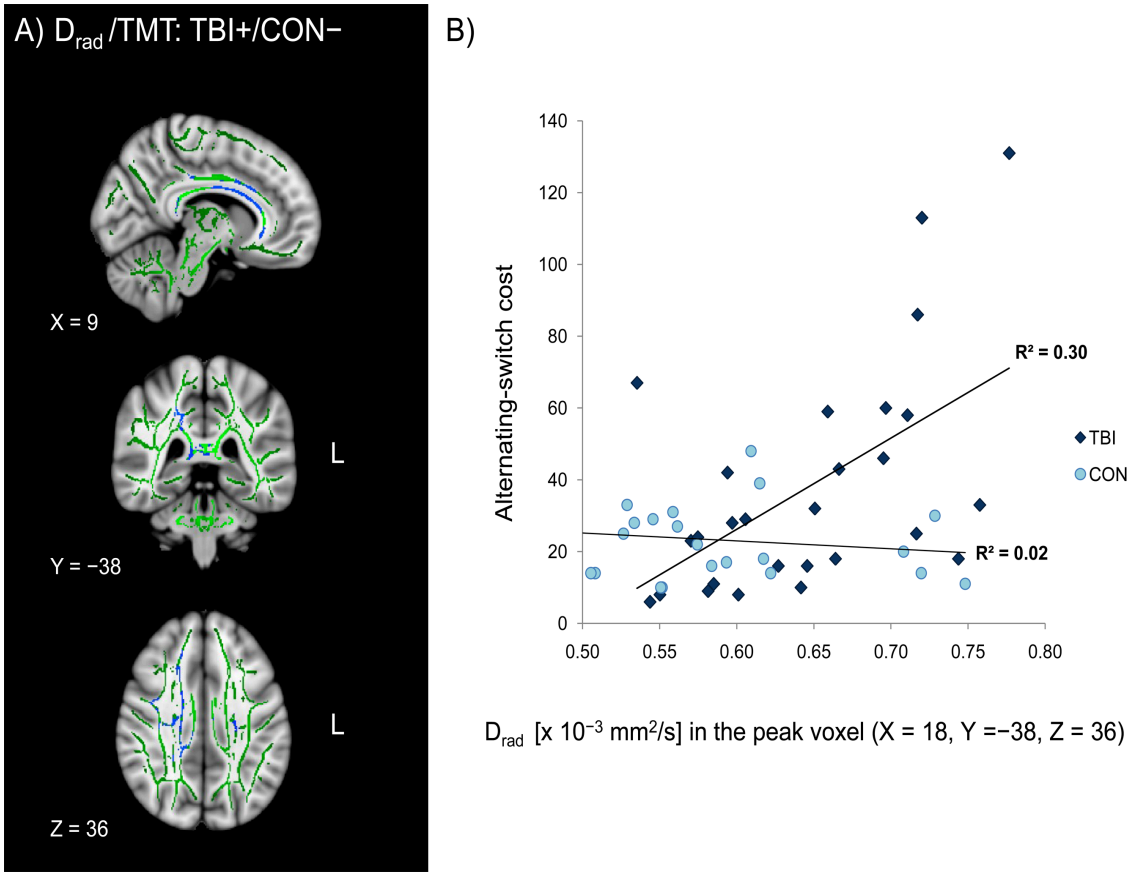

Figure 5-6. Group interaction: the relationship between radial diffusivity (D_{rad}) and set-shifting performance (TMT alternating-switch cost). A) Areas showing significant effects in the whole-brain analysis are shown in blue-lightblue. The results are thresholded at $p < .01$ (corrected for multiple comparisons) and overlaid on a standard MNI 152 T1 1mm brain and the mean FA skeleton (in green). B) Graph illustrating linear regression slopes for each group and individual data points for TMT alternating-switch cost against D_{rad} in the peak voxel (MNI x =18, y = −38, z = 36) of the interaction effect. The D_{rad} values are expressed as $mm^2/s \times 10^{-3}$ for convenience of display. MNI coordinates. TMT = Trail Making Test. L = Left. Reprinted from Brain February 2011, 134/2, Kinnunen et al., White matter damage and cognitive impairment after traumatic brain injury, 449-463, Copyright (2011), with permission from Oxford University Press.

When the relationships between tract radial diffusivity and TMT set-shifting ability shown by the two groups were directly contrasted, a highly significant group interaction (i.e. distinct relationships for patients and controls) was observed; as seen in Figure 5-6A above this was seen in white matter tracts connecting the frontal with more posterior brain regions ($p < .01$, whole-brain corrected). In particular, the tracts showing the group interaction included the right cingulum bundle and the superior longitudinal fasciculus, as well as the body and genu of the corpus callosum and the right corticospinal tract. The set of voxels showing this group interaction effect only partially overlapped with those showing the relationship observed within the control group alone, with the peak effect intensity for this large cluster found in the right posterior parietal/ medial white matter, between the superior longitudinal fasciculus and the cingulum bundle ((MNI x = 18, y = −38, z = 36).

When the radial diffusivity values were extracted for each participant from this peak voxel, the patient group showed a significant correlation between radial diffusivity at that voxel and higher scores on the TMT alternating-switch cost index (R^2 = .30, p < .01). These measues were not significantly correlated within the control group only (see Figure 5-6B). A similar result emerged when all voxels showing the significant group interaction effect were examined in the same way: a significant relationship was found in the patient group between average radial diffusivity values across these voxels and alternating-switch cost (R^2 = .17, p < .05), but no such relationship was found in the control group. These relationships observed in the patient group between radial diffusivity and alternating-switch cost remained significant after controlling for current IQ: $R_{partial}$ = .53, p < .01 (within the peak voxel) and $R_{partial}$ = .43, p < .05 (across all significant voxels).

5.3.3.4 Hypothesis 4: Abnormalities of fronto-parietal, motor, and interhemispheric pathways (more than of other tracts) will be associated with slower information processing speed. Information processing speed, as indexed by median RT for correct responses on the choice-reaction task (CRT), was not found significantly associated with the structural integrity of any white matter tracts in the TBSS analyses of FA, MD, axial diffusivity and radial diffusivity data.

Table 5-2

Correlations between the DTI Indices and Cognitive Measures

Cognitive measure	DTI index	Contrast	WM region*)	Side	P-value	Peak coordinates (MNI)		
						x	y	z
People Test/ immediate recall total	FA	CON+	ATR/Fornix	R	0.08	10	2	−1
		TBI+	SLF/CST	R	0.18	21	−19	46
		ALL+	Fornix	R	0.06	7	−5	9
		CON−	-		0.77			
		TBI−	-		0.85			
		ALL−	-		0.87			
	MD	CON+	-		0.77			
		TBI+	-		0.58			
		ALL+	-		0.58			
		CON−	-		0.53			
		TBI−	-		0.68			
		ALL−	-		0.34			
	D_{ax}	CON+	-		0.68			
		TBI+	-		0.61			
		ALL+	-		0.86			
		CON−	-		0.83			
		TBI−	-		0.39			
		ALL−	-		0.60			
	D_{rad}	CON+	-		0.99			
		TBI+	-		0.66			
		ALL+	-		0.88			
		CON−	CST	L	0.06	−7	−29	−37
		TBI−	-		0.34			
		ALL−	CST	L	0.08	−5	−31	−38

Cognitive measure	DTI index	Contrast	WM region*)	Side	P-value	Peak coordinates (MNI)		
						x	y	z
Logical Memory I/ 1st recall total	FA	CON+	SLF	R	<0.05	25	−43	34
		TBI+	-		0.73			
		ALL+	SLF/IFOF	R	<0.05	25	−44	35
		CON−	-		0.77			
		TBI−	-		0.64			
		ALL−	-		0.93			
	MD	CON+	-		0.86			
		TBI+	-		0.55			
		ALL+	-		0.69			
		CON−	-		0.56			
		TBI−	-		0.59			
		ALL−	-		0.45			
	D_{ax}	CON+	-		0.50			
		TBI+	-		0.53			
		ALL+	-		0.48			
		CON−	-		0.77			
		TBI−	-		0.47			
		ALL−	-		0.76			
	D_{rad}	CON+	-		0.79			
		TBI+	-		0.55			
		ALL+	-		0.83			
		CON−	-		0.26			
		TBI−	-		0.28			
		ALL−	-		0.16			
TMT/ Alternating-switch cost index	FA	CON+	Frontal/IFOF	R	0.09	28	22	22
		TBI+	-		0.78			
		CON−	-		0.90			
		TBI−	SLF	R	0.08	37	−31	32
		CON+/TBI−	Frontal/IFOF	R	0.06	28	22	22
	MD	CON+	-		0.97			
		TBI+	SCT/superior frontal	L	0.05	−14	−23	65
		CON−	CC (genu)	L	0.18	−1	28	1
		TBI−	-		0.43			
		CON−/TBI+	Frontal/parietal	L	0.05	−29	−15	47
	D_{ax}	CON+	-		0.93			
		TBI+	CC (genu)	R	0.12	3	31	5
		CON−	-		0.61			
		TBI−	-		0.51			
	D_{rad}	CON+	-		0.98			
		TBI+	-		0.21			
		CON−	CC (genu)	R	<0.01	10	27	−7
		TBI−	-		0.34			
		CON−/TBI+	Posterior/medial parietal	R	<0.01	18	−38	36

Notes: The cognitive measures showing a significant correlation with at least one of the DTI indices and the anatomical locations of the largest effect clusters. CON = Controls. SLF = superior longitudinal fasciculus; ILF = inferior longitudinal fasciculus; IFOF = inferior fronto-occipital fasciculus; CST = corticospinal tract; Cing = Cingulum bundle; *) For the significant results, the anatomical location of the peak test statistic for clusters larger than 100 voxels only is shown. For the results showing trends towards significance, only the location associated with the lowest *p*-value is shown.

5.4 Discussion

Relationships were found between white matter abnormalities, as assessed using DTI, and cognitive function in two domains commonly affected by TBI: verbal learning and memory and executive function. The present study was the first to have used tract-based spatial statistics (TBSS), a whole-brain approach, to explore these relationships after TBI. The flexible voxelwise data analyses conducted here allowed the effect of TBI to be investigated across the

brain's major white matter tracts using a number of complementary DTI measures, and at the same time provided the opportunity to study the effects on the specific cognitive functions of damage to particular white matter tracts.

5.4.1 White matter structure underlying associative learning and memory. Whilst the results of Study 2 (Chapter 4) showed that widespread white matter abnormalities persist following TBI, the present results suggest that the pattern of damage to specific white matter tracts has particular behavioural relevance. Variability observed in associative memory function in the patients cannot be explained by the limited, and largely non-overlapping, pattern of focal cortical damage found in these patients. By contrast, across both the patient and control groups, the structure of the fornix correlated with associative learning and memory performance. Previous work has suggested that the fornix, an important connecting pathway of the hippocampus, may have a key role in supporting episodic memory function (Aggleton, 2008; Tsivilis et al., 2008). Clearly identifiable fornix damage in humans, resulting from surgical transection, atrophy or infarction, has been previously shown to produce verbal memory deficits (Gaffan & Gaffan, 1991; Kesler et al., 2001; McMackin et al., 1995; Park et al., 2000). In the monkey, the fornix has been shown to be critical for the rapid learning of new spatial and nonspatial associations (Brasted, Bussey, Murray, & Wise, 2002; Brasted, Bussey, Murray, & Wise, 2003; Kwok & Buckley, 2010). It has been suggested that a difficulty to consolidate into long-term memory newly learned information may underlie the verbal memory deficits that are often observed following TBI (Vanderploeg et al., 2001), and that hippocampal activity supports such initial consolidation (Zola-Morgan & Squire, 1990). After brain injury, the extent of hippocampal damage has been shown to correlate with the severity of memory impairment (Tate & Bigler, 2000) and degradation of the structure of the hippocampal region has been found to correlate with deficits of paired associate learning (Salmond, Menon, Chatfield, Williams et al., 2006).

Observations by previous ROI studies are extended here by demonstrating that the structure of the fornix in particular is correlated with the efficiency of associative learning and memory, in the absence of correlations with the structure of other white matter tracts. Moreover, this relationship was not specific to the injured brain, but was also found in healthy controls. Given that a cross-sectional study design was used, the possibility that there may have been

premorbid differences in fornix structure between the two groups cannot be completely excluded, but this seems like a highly unlikely explanation for the results; particularly, when one considers that the patients, compared with controls, showed a specific pattern of cognitive impairment typical for TBI in association with better current reasoning ability. Instead, the results suggest that FA within the fornix is positively correlated with associative memory performance in the healthy brain as well as after TBI. It appears that TBI may modulate a normal relationship between fornix structure and associative memory function by disrupting the structure of the fornix and thereby shifting patients along an existing continuum into a less efficient structure-function relationship than what exists in the healthy brain. Importantly, although the fornix is a thin structure that can be prone to partial volume effects from the surrounding CSF, especially in brains affected by atrophy, TBSS has recently been shown to reliably measure changes in fornix FA associated with ageing (Metzler-Baddeley et al., 2012).

Mechanical factors may be important in explaining the prevalence of this type of memory impairment after TBI, as the shearing and tearing forces at the time of injury are likely to damage the fornix due to its delicate arch-like shape and long fibre tracts (Tate & Bigler, 2000). The three-state model of memory (Nee & Jonides, 2011) and its suggestion that the hippocampus is primarily involved in short-term association formation and retrieval provides a potential explanation as to why fornix damage may produce a particular cognitive deficit. Interestingly though, during the People Test immediate recall some patients qualitatively showed intra-list intrusion by mixing up the names of the four people, associating the wrong person with the wrong profession, as well as extra-list intrusion by, for example, producing the name of a doctor they had recently encountered in response to the doctor item. Such intrusions could suggest inefficient use of executive control strategies during retrieval, particularly of response selection and blocking of distracting information. The execution of these strategies has been attributed to the more lateral aspects of the PFC (see e.g. Fletcher & Henson, 2001) not directly connected by the fornix. Here, white matter structure of lateral PFC connections was not, however, found to correlate with People Test performance, unlike fornix structure. The current results thus appear more consistent with an explanation that attributes potential problems after TBI with associative interference to impaired hippocampal-dependent processes, such as short-term consolidation (Vanderploeg et al., 2001; Zola-Morgan & Squire, 1990). However, such memory deficits are also linked with impaired retrieval accuracy (Kramer et al.,

2005; Prince et al., 2005).

5.4.2 White matter abnormalities and logical memory performance. The patient and control groups combined showed significant voxelwise relationships between better logical memory performance (higher Logical Memory I (LM I) immediate recall scores) and higher tract FA in the right superior longitudinal fasciculus/inferior fronto-occipital fasciculus. In the current research, the superior longitudinal fasciculi were also amongst the tracts in which white matter abnormalities, including lower FA, were most consistently observed in the patients relative to healthy controls. It should be studied in larger samples in the future whether the relationship observed here between FA and logical memory performance can again be identified.

The involvement of the superior longitudinal fasciculus, a long-coursing white matter tract that connects the frontal with more posterior parietal brain regions in the correlation between white matter structure and logical memory performance may be associated with the role of the prefrontal regions in episodic memory, most likely due to their role in implementing executive control processes during encoding and retrieval (Ranganath & Blumenfeld, 2008). Evidence for functional specialisation within the PFC suggests that the lateral aspects in particular support the goal-directed executive processes involved in memory encoding and retrieval (Fletcher & Henson, 2001). Unlike the relationship observed for associative learning and memory, the structure of the fornix, based on these analyses, did not appear critical for logical memory. However, this is not entirely surprising given that the hippocampal formation is believed to be particularly involved in association formation (Henke et al., 1997; Henke et al., 1999). On the other hand, the hippocampal formation is implicated in both initial encoding and conscious recollection of information, which is inconsistent with the absence of a role for hippocampal white matter in the logical memory result.

Recall on complex tasks such as the LM involves the mental manipulation of contextual information and depends on a number of cognitive processes that are supported by anatomically distributed brain regions. It is suggested, given the apparent specificity of tracts whose structure showed relationships with logical memory performance and other cognitive functions as outlined above, that an ROI approach is unlikely to capture the complexity of the patterns in which white matter structure may support high-order cognitive functions, including verbal learning and memory. Although a whole-brain approach that investigates the structural

properties of thousands of voxels and applies a stringent correction for multiple comparisons across these voxels presents its own challenges for detecting possible real relationships with cognitive variables, it can still be argued to be better suited to investigating how white matter abnormalities may affect cognitive functions supported by distributed neural networks. If the analysis had been restricted to the analysis of a few pre-selected ROIs, only a fraction of potentially relevant white matter tracts would have been investigated, and interesting relationships may have been missed altogether.

5.4.3 White matter structure associated with executive function. In contrast to the similar relationships observed for both patients and controls between white matter structure and verbal memory performance, the groups showed distinct relationships between white matter structure and set-shifting ability, one of the three executive functions investigated. As expected, after TBI increasing mean and radial diffusivities (indexing lower integrity) were associated with executive impairment of set-shifting ability and these effects were seen in white matter tracts connecting the frontal regions. However, no such relationships were observed for controls (further discussed in section 5.4.4).

These results reveal that white matter tracts show variable structure-function relationships in the healthy and damaged brain, suggesting that highly complex relationships exist between white matter microstructure and high-order cognitive functions. Therefore, it appears that determining these relationships in the healthy brain and then using this information to guide an ROI analysis in a TBI group (e.g. Niogi, Mukherjee, Ghajar, Johnson, Kolster, Lee et al., 2008) may not be optimal. Although Niogi, Mukherjee, Ghajar, Johnson, Kolster, Lee et al. (2008) showed that regional DTI abnormalities after TBI can relate to a specific cognitive deficit in the absence of a relationship with a deficit in another domain, this approach does not exclude the possibility that the structure of white matter tracts that were not investigated would have also shown a relationship with performance on either of their two cognitive measures, in addition to the ‘specific’ ROI. Their demonstration of a ‘correlational dissociation’ does, however, suggest that the structure of certain critical tracts may be more relevant than that of others in terms of specific cognitive functions, which the current results lend support to.

The flexible voxelwise approach employed here appears particularly suitable for the investigation of complex relationships between white matter microstructure and cognitive

function. First, a standard ROI approach requires *a priori* knowledge of the likely locations of effects of interest, which is both difficult and restrictive, particularly at this relatively early stage of research. Second, if the ROIs to be investigated after brain injury are first defined in healthy individuals, it is assumed by default that similar relationships exist in the healthy and injured brain. This is problematic as the current understanding of these relationships is limited and the subtle white matter damage after TBI often diffuse, limiting the usefulness of an ROI approach. The TBSS approach used in the present study does not require the placement of specific ROIs and allows the relationship between the DTI and cognitive variables to be modelled within the framework of a general linear model, which can be adapted to look at various DTI metrics across all major white matter tracts. As discussed in Chapter 4, TBSS is also more robust to the effects of brain atrophy on white matter tracts than some widely used alternatives as it restricts the analysis to tract centres only and thus reduces the impact of atrophy around the edges avoiding such partial volume effects.

Increases in MD and radial diffusivity, potential indices of white matter injury, have been previously reported following TBI (Kraus et al., 2007; Sidaros et al., 2008; Kennedy et al., 2009). The involvement of tracts connecting the frontal with posterior medial and parietal regions suggests that axonal damage after TBI can disrupt the integrity of what has been dubbed the brain's 'structural core'. This core is densely connected with the temporal and frontal cortices via white matter tracts (Hagmann et al., 2008). The most prominent 'node' of this structural core is the precuneus/posterior cingulate cortex that also corresponds to the posterior component of the human 'default mode network' (DMN), a functional network found engaged in a variety of neuroimaging paradigms across a wide range of cognitive states (Honey et al., 2009; Chapter 1). Here, reduced structural integrity (indexed by high radial diffusivity) of white matter tracts interconnecting these posterior with frontal regions was found to be associated with worse set-shifting ability after TBI. This is consistent with the proposal that executive dysfunction following brain injury is partly the result of disconnection between frontal and more posterior brain regions involved in executive processes (Miller & D'Esposito, 2005). Converging evidence comes from studies of normally ageing older adults that have shown age-related decline in the structural coherence of frontal connections to predict executive dysfunction (Davis et al., 2009; Kennedy & Raz, 2009; O'Sullivan et al., 2001; Perry et al., 2009).

A common cognitive impairment linking TBI patients and healthy older individuals is

slowed information processing on a range of tasks, including the 'executive'. A related consideration comes from functional neuroimaging research into cognitive efficiency (Rypma et al., 2006). In particular, Rypma et al. suggest that patterns of neural interaction within the fronto-parietal attentional network may critically mediate individual differences in cognitive efficiency. Specifically, individual variability in processing speed across a range of tasks may depend on prefrontal activity through the PFC exerting more control over the more posterior brain regions in slow-performing than in fast-performing individuals. Therefore, the structural integrity of connections between the frontal and more posterior parietal regions could be more important after brain injury for achieving optimal cognitive efficiency than it is in the healthy brain. This could partly explain the distinct relationships observed in the TBI and control groups between radial diffusivity in the specific tracts and set-shifting performance. The predominantly right-lateralized pattern of these results could also be related to the known involvement of the right-lateralized fronto-parietal network in attentionally demanding tasks such as those requiring set-shifting (Fox et al., 2005).

5.4.4 Unexpected findings and limitations. Whilst the involvement of white matter within the hippocampal formation, specifically in the fornix, was observed here in association with verbal associative memory, the structure of prefrontal white matter was not found to predict associative memory performance. This is surprising in that cognitive control processes modulated by the prefrontal areas are believed to support episodic memory encoding and retrieval (Nyberg, 2008; Ranganath & Blumenfeld, 2008; Tulving, 2002). This may mean that hippocampal-dependent consolidation better distinguished between individuals with more versus less efficient structure-function relationships. It may be for this reason that the voxelwise analysis revealed a correlation between fornix structure and associative memory, whilst the structure of frontal connections was not as consistently involved and thus did not show significant effects in the whole-brain analysis. Another consideration is that both human and animal research has shown that the strategy implemented during learning is also important in terms of which memory system is engaged (Squire, 2004). It is possible that the strategies used are not always optimal in terms of task performance, and therefore the task does not engage the system that supports the use the optimal strategies. Performance on the test of associative memory employed here is likely to have involved several memory processes, and it is possible

that the structure of only the fornix was relevant to an extent that its role was reflected in retrieval success. It is of course also possible that the structure of white matter tracts connecting prefrontal regions (other than the involvement of the fornix in connecting the hippocampus with the PFC) is irrelevant for performance on the People Test and that the relationship was not observed because it simply does not exist.

Another unexpected result was that despite the impairments observed in the TBI patients across all but one measure of processing speed, no relationship was observed in the whole-brain analysis between white matter disruption and processing speed as indexed by median reaction times on the choice-reaction task (CRT). Involvement of white matter organisation of callosal fibres as well as descending motor tracts was expected based on previous studies in healthy individuals (e.g. Bucur et al., 2008; Schulte et al., 2005; Sullivan et al., 2001), but this was not found for either controls or patients in the TBSS analysis. The relatively small sample size may have contributed to the lack of some of the expected relationships, including this one, by rendering the statistical power insufficient after the stringent whole-brain correction for voxelwise comparisons. A related possibility is that inter-individual variability in the localisation of function may prevent the TBSS approach from identifying adequate consistency in the patterns of white matter organisation underlying cognitive function to survive the whole-brain correction. It is also possible, although unlikely given the role of intact anatomic connections in efficient network function, that the cognitive operations required for performing the CRT did not critically depend on white matter organisation as expected, meaning that the null result could reflect a true lack of structure-function relationship.

In general, white matter abnormalities in the patients were seen in the expected directions. The healthy controls, however, showed a relationship between higher radial diffusivity in certain white matter tracts and better set-shifting ability as indexed by alternating-switch cost on the TMT. This was unexpected, because one determinant of radial diffusivity is the degree of axonal myelination (Beaulieu, 2002), and as this increases one might expect reduced radial diffusivity and faster nerve conduction times (Jack et al., 1983), which should lead to more efficient executive function. As mentioned above, one factor that may have affected the present results is the possibility of inadequate statistical power, and more research, preferably first in a large sample of healthy individuals, is needed to further investigate these unexpected relationships.

A related consideration is that multiple comparisons were carried out between the DTI metrics and cognitive measures studied here - an issue, which increases the risk of producing false positive results. Although often not adequately dealt with in neuroimaging studies, leading to upwardly biased effect sizes being reported, accounting for multiple comparisons (other than across multiple imaging voxels) would ensure that the results produced by studies are reliable and interpretable. Conventional approaches to multiple comparisons correction such as Bonferroni correction for familywise error rate may however be unsuitable. In any case these approaches control for the probability of false positives only, which may again lead to reduced statistical power and more false negative results.

Here, multiple DTI measures were studied relative to each cognitive variable which inflated the likelihood of finding a chance relationship with at least one of these measures - compared to if, for example, FA alone had been studied. It was important, however, for this first investigation after TBI of relationships between cognitive function and white matter structure across the whole TBSS-derived skeleton to study all four DTI measures. This allowed for a more comprehensive investigation of how abnormalities of the different elements of the diffusion tensor may relate to cognitive dysfunction following TBI. Previous studies had typically investigated some of the measures only, or measured them from a limited number of ROIs/tracts. Nevertheless, given that a number of contrasts were carried out between each DTI metric and cognitive variable, it remains a possibility that some of the findings were due to random chance. As outlined in the above discussion of the current understanding of how white matter structure, indexed by the DTI measures, may relate to the cognitive functions of interest here, it is however also possible that the identified relationships are real. Firm conclusions will have to wait until future studies have replicated the current findings.

All in all, relationships between DTI indices of white matter structure and cognitive function appear to be complex. Considering this apparent complexity and difficulty in interpreting some of the white matter structure-cognitive function relationships identified here, it is not surprising that similar unexpected relationships have been reported previously (e.g. Tuch et al., 2005; Wu et al., 2010). Recently, Wu et al. (2010) investigated the structure of the left cingulum bundle and word list learning and recall in a group of 12 adolescents with mild TBI and 11 healthy controls. The patients all had unremarkable CT imaging, but showed significantly lower FA and higher apparent diffusion coefficient (i.e. the overall magnitude of diffusion) in the

cingulum bundle relative to controls. Surprisingly, Wu et al., (2010) found that in the patient group anisotropy of the cingulum bundle was negatively correlated with delayed verbal recall and apparent diffusion positively correlated with immediate recall. These results are another demonstration that the functional significance of the various DTI metrics in terms of complex cognitive functions such as memory is not yet well understood.

As discussed in Chapter 4, several features of the tissue microstructure can influence the values observed for the DTI measures of anisotropy and diffusivity. For example, the resolution of DTI images normally achieved is considerably lower than the actual scale of the fibres under examination would require. One voxel can contain several distinct fibre populations, some of which may be crossing, branching, or touching (Tuch et al., 2003). The crossing-fibre issue is more problematic in some regions than in others, and can sometimes make the interpretation of changes in DTI metrics difficult. Until imaging techniques capable of producing higher resolution data and analysis methods able to model complex fibre architecture can be applied to investigate the human brain in a way that is feasible for most research protocols, it cannot be determined to what extent the approach employed here was affected by these issues. Advanced methods such as q-ball imaging (Tuch et al., 2003; Tuch, 2004) can resolve multiple fibre orientations within a single voxel, and could potentially help to untangle the fibre crossing issue in specific regions, but these are not currently as widely used as DTI. More research is also required to better understand the morphological, cellular and molecular mechanisms that underlie the complex white matter architecture. As more sophisticated techniques are developed, it may become possible to study how exactly the changes in the different DTI metrics after TBI reflect alterations in the physiological features of fibre pathways and what their functional relevance is.

5.4.5 Conclusions. Specific patterns of white matter structure were found to correlate with individual differences in verbal learning and memory (associative learning and memory and memory for logical/structured material) and executive function (set-shifting ability). More coherent fornix structure, indexed by higher FA, was associated with better associative memory performance in both the TBI and control groups. The structure of other tracts, particularly higher FA, was associated with better immediate recall of logically structured material, also in both patients and controls. By contrast, distinct patterns were observed in the two groups for the

relationship between the structure of frontal connections and set-shifting ability: patients' performance benefited from lower radial diffusivity, but controls' performance did not.

Apart from illustratating the complexity of relationships between white matter microstructure and cognitive functions, these results highlight the importance of flexibly analysing patterns of disruption across the whole brain using several complementary DTI measures. The type of neuropsychological deficit that a patient experiences is likely to depend on damage to key pathways that link nodes in the distributed networks supporting high-level cognitive functions such as verbal memory and executive functions. It is not necessarily the extent but rather the particular pattern of white matter abnormalities in specific connections within a brain network that is critical in determining the presence, type and degree of cognitive impairment after TBI. The development of diffusion tensor imaging has made it possible to test this assumption *in vivo*, and the findings of the current investigation may help to explain why traditional lesion-focused studies have generally shown weak correlations with cognitive impairment following TBI. The main reason for this may be that such studies have not been able to capture the spatially distributed pattern of subtle white matter disruption that can damage the structural connections of large-scale brain networks, and thus impair their function after TBI.

To conclude, widespread abnormalities of white matter structure (indexed by FA, MD and axial diffusivity) were observed for the TBI patients in the previous study (Chapter 4). Variability across participants in the DTI metrics in particular anatomical locations correlated with individual differences in associative learning and memory, logical memory, and one of the three indices of executive function. White matter structure of the fornix predicted associative memory performance across both groups, whereas a more distinct pattern was observed for the relationship between the structure of frontal connections and executive function in the two groups. This approach reveals the complexity of the relationships between indices of white matter structure and cognition, and shows the importance of flexibly analysing patterns of disruption across the whole brain.

CHAPTER 6: General discussion

This chapter first summarises the main findings from the three studies forming the current research programme. It then briefly discusses the principal implications of these findings with reference to the central themes of the research. Before presenting the final conclusions the chapter postulates some alternative approaches to and future directions for investigations of the relationships between the neural and the cognitive sequelae of traumatic brain injury.

6.1 The Research and Its Main Findings: A Summary

The understanding of the neural underpinnings of impairments of cognitive function that often follow traumatic brain injury (TBI), including those of learning and memory and executive functions, remains limited. Since the development of diffusion tensor imaging (DTI; Basser et al., 1994), and its application to the investigation of white matter abnormalities after TBI, evidence has started to accumulate suggesting that white matter damage, detected by DTI, may play an important role in the post-injury cognitive impairments. High-order cognitive functions depend on the efficient recruitment and coherent functioning of large-scale brain networks, the nodes of which are anatomically connected by the brain's white matter tracts. These tracts can sustain considerable damage following TBI via the mechanical impact, as the movement of the head and the brain cause the delicate axonal fibres to shear and tear, leading to the more subtle problems with axonal function, as discussed earlier. Due to the widespread nature of diffuse axonal injury (DAI) after TBI, a whole-brain voxelwise method such as tract-based spatial statistics (TBSS; Smith et al., 2006) is well suited to studying its extent and exploring the relationships between local white matter structure and cognitive functions in as comprehensive a manner as possible.

To address these themes empirically, the present research programme explored, using various *in vivo* neuroimaging markers, structural brain abnormalities after TBI and their relationship to neuropsychological function. Neuroimaging methods including high resolution T1-weighted and gradient echo T2*-weighted MRI and DTI were used to assess brain macro- and microstructure and various neuropsychological measures were used to assess cognitive function, both after brain injury and in healthy individuals. In particular, the purpose of this work has been to advance our understanding of white matter damage after TBI and to explore how this may be related to sometimes persistent cognitive impairments. By doing so, the current research has also aimed to define appropriate questions for future investigations.

Thus, the sensitivity of DTI to abnormalities of white matter structure not identified by standard MRI was explored; and TBSS was used to analyse the anatomical distribution and degree of these abnormalities across the brain's white matter tracts. The thesis then explored how structural abnormalities in particular white matter tracts relate to specific cognitive deficits. Three empirical studies investigated the following issues:

- Study 1 (Chapter 3) used standard MRI in TBI patients to characterise whole-brain atrophy and the type, anatomical location and degree of 'focal' brain damage, and explored relationships with neuropsychological function.

- Study 2 (Chapter 4) used TBSS to compare TBI patients and healthy controls on DTI indices of white matter tract structure (fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity and radial diffusivity). The study also compared these DTI metrics between patient subgroups stratified by clinical indices of TBI severity.

- Study 3 (Chapter 5) explored, again using TBSS, relationships between the same four DTI metrics of white matter tract structure and indices of neuropsychological function in the domains of verbal learning and memory, executive function and information processing speed.

The research programme was framed around the following five principal questions:

1) After TBI, are verbal learning and memory, executive function and information processing speed associated with the degree of whole-brain atrophy?
2) Does performance in these cognitive domains correlate with 'focal' lesions in specific anatomical locations or with volume/number of lesions?
3) Do patients with TBI show abnormalities of white matter tract structure when compared with age-matched healthy controls?
4) Are the presence of brain microbleeds and/or clinical severity of TBI associated with abnormalities of white matter tract structure?
5) Are specific cognitive deficits after TBI associated with breakdown of the structural integrity of specific white matter tracts?

The following paragraphs summarise the main findings from each study.

6.1.1 Study 1 (Chapter 3). This study found that neither MRI measurements of whole-brain tissue volume (grey matter, white matter, total brain tissue) nor the specific anatomical location of focal lesions, or their volume/number, were good predictors of cognitive function after

TBI. Relative to age-matched healthy controls, the patient group showed significantly lower residual grey matter, white matter and total brain tissue volumes, indicating a significant level of tissue atrophy. In addition, despite having generally quite good cognitive function, the patients showed deficits in information processing speed. However, only weak correlations were identified in the patient group between MRI indices of structural brain abnormalities and neuropsychological measures. These findings suggest that other neuropathological factors, not detected by standard MRI, may impact cognitive function after TBI. A likely candidate is the widespread white matter disruption potentially caused by DAI (as discussed in section 6.2.1).

6.1.2 Study 2 (Chapter 4). This study demonstrated that structural abnormalities of white matter (as indexed by FA, MD, axial diffusivity and radial diffusivity values) can be detected in TBI patients using DTI. Tract-based spatial statistics (TBSS) revealed these to be much more widespread than previous ROI analyses have shown. In addition to characterising the extent of white matter disruption, TBSS also allowed quantification of tract-based structural abnormalities at each imaging voxel, providing key information about their degree. Moreover, the analyses revealed that axonal injury after TBI is not constrained to brain regions that are affected by traumatic microbleeds, which were found to be poor indicators of the extent and degree of white matter damage. Abnormalities detected by DTI were also found in a number of white matter tracts in patients who did not have standard MRI evidence of microbleeds. In particular, in these patients, increases in axial diffusivity rather than radial diffusivity appeared to contribute to changes in FA and MD (the two scalar measures often used respectively to index white matter integrity and loss of integrity). Finally, TBSS revealed significant white matter abnormalities in patients with no standard MRI evidence of brain injury, and in patients clinically classified as having ‘mild’ TBI. The main implications of these findings are discussed in section 6.2.2.

6.1.3 Study 3 (Chapter 5). The final empirical study explored the relationships between the DTI indices of white matter structure and cognitive function in the healthy and injured brain. The data suggested that reduced white matter integrity after TBI in specific structural connections is associated with impairments of verbal learning and memory and executive function.

Specifically, associative learning and memory, indexed by the People Test, correlated with the structure of the fornix, an important connecting pathway of the hippocampal formation. This result extends previous findings that have implicated the fornix in episodic memory by demonstrating - for the first time in humans with TBI - that white matter disruption of the fornix specifically is associated with worse verbal associative memory performance (Kinnunen et al., 2011). Healthy controls showed a similar relationship between higher fornix FA and better associative memory performance. In addition, in the combined sample of patients and controls higher FA within the right superior longitudinal fasciculus was associated with better immediate recall assessed via the Logical Memory test. Finally, in the patient group, as expected, higher radial diffusivity (possibly indexing reduced white matter integrity) was associated with worse set-shifting ability on the Trail Making Test (indexed by the increment in completion time for the more complex Trails B relative to Trail A); by contrast this was not found for the controls.

Hence, the two groups showed similar relationships between FA and associative memory, and between FA and logical memory, but distinct relationships between radial diffusivity and set-shifting ability. This underlines that the relationships between DTI indices of white matter structure and high-order cognitive functions are complex. Section 6.2.3 further discusses the implications of these findings.

6.2 Discussion

Three principal themes arising from the main findings of the current research are briefly discussed in the following sections.

6.2.1 'Focal' lesions versus diffuse brain injury and cognitive sequelae of TBI. The diffuse nature of the structural brain abnormalities typically found after TBI has been one of the overarching themes of this thesis. Standard clinical neuroimaging of TBI can identify focal 'lesions', but damage to the brain tissue is likely to extend beyond the boundaries of such seemingly isolated abnormalities (Bigler, 2001a). The present findings illustrate this in two principal ways. Firstly, in Study 1, T1- and T2*-weighted MRI identified focal lesions (cortical contusions, white matter lesions or both) in under 50 per cent of the patients; and in those

patients with these lesions there was little overlap between different patients in the anatomical location of lesions. By contrast, in Study 2 DTI detected very widespread, more subtle white matter damage. At the group level this was seen across virtually all of the brain's major white matter tracts. Secondly, Study 2 found DTI-detected white matter damage both in patients with normal conventional MRI scans and in patients with no white matter lesions visible on gradient echo T2*-weighted MRI, which provides a good contrast for the detection of traumatic microbleeds (i.e. petechial haemorrhages) that are visible as hypointense signal abnormalities. The presence of microbleeds is linked with, but as shown here is not a perfect correlate of, DAI.

Consistent with previous reports of rather weak correlations between brain abnormalities identified using standard structural MRI and neuropsychological function, Study 1 found little evidence that the presence of either focal lesions or brain atrophy was associated with specific deficits of verbal learning and memory, executive function, or information processing speed. It is suggested here that this may be because a key determinant of cognitive outcome after TBI is white matter damage that disrupts the brain's structural connections. Bigler (2001b) notes that there may be a lack of correspondence after TBI between macrostructural (i.e. MRI-defined) brain damage and even focal neurological deficits such as speech or language difficulties because these brain abnormalities are likely to be superimposed on more diffuse neuropathology. Study 2 showed that DTI is sensitive to this more subtle axonal damage, that it can be very extensive after TBI, and that it may be present in patients whose TBI is classified as 'mild' clinically, as well as in those with more severe injuries. The diffuse pattern of injury is believed to result from shearing and tearing forces at the time of injury, which disrupt the cytoskeletal components of axons. The resultant abnormalities of white matter microstructure may ultimately lead to axonal degeneration (Büki & Povlishock, 2006), an important mechanism in structural disconnection (Li et al., 2010).

6.2.2 Future use of DTI in TBI? The second central theme of this thesis has been to explore the potential of DTI in the assessment of the neural sequelae of TBI, especially those that are more subtle than can be detected by standard MRI. The results indicate that DTI can indeed detect imaging abnormalities in the white matter, and that such damage is more extensive than previously shown by ROI studies which have assessed only a limited number of white matter locations.

Diffusion tensor imaging may therefore hold promise as a potential tool for determining the presence, degree, and anatomical distribution of white matter injury in patients with mild head injuries (see Sharp & Ham, 2011, for a recent review). Some such patients show no objective evidence of TBI in standard neurological and neuroimaging assessments, but may still feel that problems with everyday cognitive functions have emerged since their head injury. The future integration of advanced structural and functional neuroimaging techniques into assessment of the neural sequelae of TBI may offer new insights into the prediction of functional outcome. This will require large-scale investigations to further validate DTI as a tool with potential to provide clinically and functionally relevant information, and to help predict which patients are at risk of long-term cognitive impairments (Niogi & Mukherjee, 2010).

Abnormalities of axial diffusivity in particular showed interesting patterns in the current sample of relatively high-functioning patients who were at least two months post-TBI. Whilst FA was abnormally low and MD abnormally high, axial diffusivity varied across different patient subgroups. Abnormalities of axial diffusivity in patients relative to healthy controls also positively correlated with increasing time since injury. In experimental models of TBI *reductions* in FA and axial diffusivity have been observed acutely after cortical impact (MacDonald et al., 2007), and these early changes have been shown to reflect axonal damage (Budde et al., 2008; Budde et al., 2009; Song et al., 2003). *Increases* in axial diffusivity after TBI have been suggested to reflect possible axonal recovery (Sidaros et al., 2008; Voss et al., 2006). These potentially adaptive changes in axial diffusivity over time after TBI remain to be further investigated. Longitudinal studies are needed to elucidate the evolution of microstructural white matter changes over time after TBI and to clarify the functional and symptomatic relevance of abnormalities such as those observed here (between groups) and in other studies (between groups and over time). Section 6.2.5 briefly considers how future studies could investigate these issues.

The finding that DTI can detect white matter damage over and above that evident on standard MRI suggests that its use would increase the accuracy with which the structural impact of TBI is currently evaluated. According to the well-known DAI severity grading system developed by Gennarelli et al. (1982), Grade I DAI is indicated by only microscopic evidence of axonal injury; Grade II DAI by the presence of corpus callosal lesions and microscopic evidence; and Grade III DAI by the additional presence of midbrain lesions. The additional

evidence of axonal injury provided by DTI could influence TBI severity classification, and specifically improve the detection of subtle DAI.

However, there are a number of barriers to the routine use of DTI in clinical assessment of TBI. Preprocessing of diffusion-weighted data involves a number of steps before quantitative information such as FA or MD can be extracted and further research is still needed to evaluate the functional significance of abnormalities of the various DTI metrics. The cost-efficiency of the use of DTI in clinical settings also needs to be ascertained. In particular this would involve establising what additional benefits DTI, compared with standard methods, can yield in terms of determining the presence of brain damage, informing acute and long-term management of brain injury, as well as in in predicting functional outcome.

6.2.3 Relationships between DTI findings and cognitive function after TBI. It has been proposed here that the widespread abnormalities of white matter structure observed after TBI may have relevance to the common cognitive deficits. A voxelwise analysis using TBSS revealed that the structure of particular white matter tracts is associated with specific cognitive functions. However, not all the expected relationships were observed, and some of the relationships that were observed were unexpected. Associative learning and memory performance showed similar relationships with fornix FA for patients and controls, and FA within the superior longitudinal fasciculus correlated with memory for logically structured verbal material (short stories). By contrast, partly surprising relationships were observed between radial diffusivity and set-shifting ability. Thus, whilst patients with higher radial diffusivity in frontal white matter tracts showed more impairment, this was not the case for healthy individuals.

These results highlight that more work is needed to establish how each DTI metric relates to the structural integrity of white matter (in the healthy and injured brain), and how this in turn may relate to cognitive function and dysfunction.

6.2.4 Methodological considerations. The three empirical chapters discussed the potential limitations of the methods employed by the research as relates to each study and considered their implications for the interpretation of the results in those studies. This section briefly summarises the possible methodological limitations of the research programme.

Although the sample size was relatively large for this type of work, a larger sample would have provided added statistical power to detect more subtle effects and potentially contributed to the generalisability of the findings. In particular, sample size in some analyses comparing subgroups of patients was small, which may have contributed to the lack of some positive findings, although it is also entirely possible that the effects simply were not there. However, it is noted that sample sizes in the main analyses of the current research are comparable to or larger than those in previous studies employing similar methods, which have detected effects. A related consideration here is participant sampling. The patients in the current research were cognitively relatively high-functioning, with selective impairments in the domains of verbal learning and memory, executive function and information processing speed. The subtlety of their residual impairments may have limited the power of the studies to detect some differences from the controls, or relationships between the neural and cognitive variables within the patient group(s).

Demographic matching of the patients and healthy controls was also imperfect, with more men than women in the patient sample. Whilst this reflects the higher incidence of TBI in men, in retrospect it would have been desirable to selectively recruit more male controls to achieve better matching. This possible confounder may have influenced the results, despite the efforts to control for its effects statistically. Ideally this would have been dealt with within in the study design, as it is possible that even after statistical control for the effects of gender, differences between male and female brains may have affected the results of the non-gender-matched comparisons. Men are reported to have approximately 12% larger total TIV (Buckner et al., 2004) and on average 10% larger brain volumes than women (Dekaban, 1978). Other differences may of course exist, for example in how strongly directional the diffusion of water molecules (i.e. the degree of anisotropy) across different white matter regions is (see e.g. Hsu et al., 2008).

Furthermore, linear statistical approaches may not be able to adequately deal with confounds linked with multifactorial issues or variables that may interact in complex ways (e.g. gender, aging and brain volume). For example, a nonlinear relationship has been suggested to exist between age and brain volumetric decline (e.g. Scahill et al. 2003). Whilst good age-matching between groups was achieved for the majority of group comparsions, in the brain volume analyses the patients were on average older than the controls. Due to the possible

nonliearity of the relationship between age and brain volume, it is possible that, even after linear statistical control of this confound, the results were slightly biased.

A surprising discrepancy was also observed in the patient group between premorbid and current intellectual function. This may be partly explained by the potential unreliability of the premorbid IQ estimates in the current sample, as discussed in Chapter 4, but may nevertheless limit the generalisability of the present data. Importantly, however, despite the good overall intellectual functioning of the patients (and in some cases superior to that of controls), a pattern of specific cognitive deficits characteristic of TBI was observed. Thus, the patients showed impaired associative memory, impaired executive functioning, and impaired information processing speed relative to the healthy controls. Slowed processing speed was the most pronounced cognitive deficit in this sample of patients. This pattern of impairments is consistent with previous studies, some showing long-term impairments after TBI within these domains of cognitive function (e.g. Draper & Ponsford, 2008 Frencham et al., 2005; Levin, 1995; Levin & Kraus, 1994; Ponsford & Kinsella, 1992; Scheid et al., 2006).

The scope of any research project is naturally limited by its choice of particular measures and techniques. The neuropsychological measures included in the test battery here consisted of some widely used and well-validated measures of verbal learning and memory, executive functions and processing speed, as well as an experimental measure of choice-reaction times. These or similar measures have been used in numerous previous studies to detect cognitive impairment following brain injury, and were thus appropriate for the purposes of the present research. The power to detect differences between groups is of course importantly determined by the sensitivity and specificity of the measures used, apart from sample size, the size of the investigated effect, and the likelihood of error. This makes test selection a critical part of the study design. Whilst this aspect of the current research was constrained by the practical considerations and research questions of the larger programme (as explained in Chapter 2, section 2.6.1, pp. 63-65), other measures could have been included if a more experimental approach had been taken to the study of cognitive impairment following TBI. For example, these may have included measures that are likely to better tap cognitive processes relevant for everyday function than some of the more traditional neuropsychological measures (see e.g. Burgess et al., 2006).

The particular choice of neuroimaging methods in any study also limits the kind of

questions that can be answered, and determines the analyses that can be carried out. Here, the lesion method used in Study 1 only had the potential to test whether specific regions are necessary for performing a particular cognitive task. Although DTI (used in Studies 2 and 3) is particularly sensitive to white matter damage, believed since early connectionist accounts to be a major determinant of the cognitive impairments, it is not without its limitations. Being sensitive to characteristics of the displacement of water molecules (including the principal direction of diffusion and its anisotropy) depending on hindrances to diffusion, DTI can be used for probing the local structure of white matter tracts (Le Bihan, 2003). It does not, however, directly quantify tract integrity or structural connectivity. Moreover, the spatial resolution of DTI is limited in terms of probing white matter microstructure, and regions that have a complex fibre architecture involving' kissing' or crossing fibres present a particular challenge.

Another consideration is that higher-order cognitive processes are underpinned not only by intact structural connectivity but also by efficient network function. Although the anatomical distribution and degree of structural brain damage is likely to be associated with cognitive outcome, functional brain imaging is required to investigate brain network function. Thus, both structural and functional brain imaging methods are needed to better address the questions of which brain structures are critical for efficient cognitive function in the healthy brain, how these structures are damaged as a consequence of head injury and why/how certain cognitive impairments develop and are more frequent than others.

Functional imaging techniques include task-related functional MRI (fMRI), which images patterns of cerebral blood flow during the performance of a cognitive task, and resting state fMRI, which images these patterns in the absence of external stimuli. These techniques that measure brain activation rather than its structure can be used to infer which brain regions appear to be involved in given mental processes (Rorden & Karnath, 2004). Resting state fMRI in particular, although currently in need of further validation, could prove useful in the assessment of abnormalities of brain network function after TBI, because patients do not need to perform a task; it is thus feasible to conduct with most patients.

Our group (Sharp et al., 2011) recently used fMRI to investigate which brain regions were activated during performance of a simple choice-reaction task (CRT) and to capture the patterns of interactions between regions within a network ("network functional connectivity"). Resting state fMRI data were also collected, along with DTI data, to explore whether axonal

injury and its effects on structural connectivity are reflected in the functional connectivity patterns of critical brain regions. The relationships of these two types of connectivity to behaviour were also explored. Twenty patients with longstanding TBI (at least six months post-injury) were compared with age-matched controls. Neuropsychological assessment revealed typical impairments of information processing speed in the patient group relative to controls on a variety of measures, including the CRT. Although similar brain regions were activated during task performance in both groups, patients showed greater deactivation of the default mode network (DMN; see Chapter 1, section 1.7) during rest: a multivariate analysis of the resting state data revealed that DMN functional connectivity increased in the patient group. Patients with the highest functional connectivity were the least impaired cognitively. Patterns of functional connectivity at rest also predicted brain activation during subsequent task performance. Moreover, compared with controls, patients showed evidence of widespread white matter damage, and those with more disruption to the structure of the posterior corpus callosum, located adjacent to the brain's 'structural core' (precuneus/posterior cingulated cortex = the posterior part of the DMN) showed lower functional connectivity within the DMN. This was the first study of the relationships between structural connectivity, functional connectivity, task activation patterns, and behaviour in the same group of patients after TBI; it demonstrated the feasibility of combining complementary methods to explore the neural and functional sequelae of TBI and to offer new insights into how TBI may disrupt both types of brain connectivity, and alter cognitive function.

It is also worth noting here that the present research was not designed or powered to investigate numerous other interrelationships that potentially exist between brain structure and cognitive function. The choice of a specific and necessarily limited set of neuropsychological indices, like the choice of the particular neuroimaging techniques, of course also sets certain boundaries. A more comprehensive investigation using more indices of brain structure, functional connectivity, and neuropsychological function would require a considerably larger sample of patients, with more heterogeneity in patterns and degree of cognitive impairment. Future research using measures that tap the various levels of the brain-behaviour puzzle are thus needed to further increase our understanding of the relationships between pathophysiology of TBI, network disruption, and cognitive outcome.

A number of other factors may of course also contribute to the development and

maintenance of cognitive impairment following TBI. Whilst the current research has focused on how abnormalities of brain structure – and those of white matter microstructure in particular – relate to cognitive impairment after TBI, it recognises that this is far from the whole story. For instance, there has been recent interest in the "dopamine hypothesis" of cognitive dysfunction. Specifically, it has been suggested that abnormalities in the expression of dopamine, a central nervous system neurotransmitter and neuromodulator, may play an important role in persistent cognitive and behavioural dysfunction following TBI (Bales, Wagner, Kline, & Dixon, 2009). In addition, preliminary findings from a PET study suggest that cholinergic function in several brain regions is abnormal one year following TBI and that this may be associated with cognitive impairments (Östberg et al., 2011). Electrophysiological markers of cognitive impairment after TBI also show promise as a method of investigation (Dockree & Robertson, 2011).

6.2.5 Future directions. During the past few years TBI research into injury mechanisms, neuropathological processes, types and degree of brain trauma, and clinical outcome has intensified, leading to an increased awareness of many issues, including the need to clarify the diagnostic criteria for mild TBI and its neural and functional consequences (Menon et al., 2010). It is now increasingly recognised that even mild TBI can result in subtle cognitive, behavioural or emotional problems and that a lack of evidence of brain damage from standard clinical neuroimaging is by no means conclusive. As already noted, the present findings confirm previous reports that white matter disruption can be identified by DTI where MRI does not detect damage (Lipton et al., 2008; Nakayama et al., 2006; Niogi et al., 2008; Rugg-Gunn et al., 2001). A few possible directions for further work in this field are next proposed, keeping in mind the ultimate goal of achieving better targeted care and rehabilitation for individual patients.

Outcomes after TBI show great variation from individual to individual, and assessing outcome requires a multifaceted approach. The widely used Glasgow Outcome Scale (GOS; Jennett et al., 1981) classifies outcome in terms of one of five discrete levels: 1) dead, 2) vegetative state, 3) severely disabled, 4) moderately disabled, or 5) good recovery. To more sensitively quantify improvements in function over time a variety of measures are needed to tap diverse cognitive, behavioural, emotional and social problems (Nortje & Menon, 2004). Currently, although there is some understanding of how factors including age, clinical severity of injury, and CT/MRI abnormalities relate to outcome, the prediction of long-term outcome after

TBI remains very imprecise (Lingsma, Roozenbeek, Steyerberg, Murray, & Maas, 2010). One possible avenue for future longitudinal research would be to utilise DTI in assessing white matter damage in individual patients and to investigate whether particular patterns of damage predict specific cognitive impairments. If such white matter 'signatures' could be identified, this could illuminate a wide range of issues. In particular, DTI would be of clinical utility in identifying at an early stage whether a patient is likely to make a good or poor recovery.

In recent years, computational biomechanical models have increasingly provided new insights into the injury mechanisms of TBI, to complement more traditional human histologic and neuroimaging studies and experimental animal or *in vitro* models of axonal injury (e.g. Tang-Schomer et al., 2010). Such models simulate the biomechanics of the impact loading in TBI and can, for example, be designed to simulate the axonal effects of rapid rotational acceleration/deceleration movement of the brain. In general, these models use several measured mechanical parameters to infer the tolerance levels for the occurrence of, and neural responses to, head injury (Colgan, Gilchrist, & Curran, 2010). However, a limitation of most computational analyses as applied to inferring the responses of the human brain is that these models are often based on a *linear* model, which is unlikely to accurately reflect the responses of heterogeneous brain structures (Bandak, 1996; Meythaler et al., 2001). Nevertheless, such work has advanced understanding of mechanics of head injury, and a link has recently been suggested to exist between tissue strain injury and the microstructural properties of white matter tracts, which Colgan et al. (2010) studied via a 'viscoelastic' model of TBI to predict the response of brain tissue to rotational strain. They developed this model by first reviewing previous literature to derive parameters which were then used to describe the viscous and elastic properties of neural tissue within a *nonlinear* model. This model was best able to predict the response of neural tissue to injury where diffusion was modelled as highly anisotropic, suggesting a possible mediating role for microstructural properties of different brain regions in neural sequelae of TBI.

Future nonlinear models could help to further advance the understanding of the complex relationships between TBI, DAI, changes in the microstructural properties of white matter, and the extent and degree of brain injury; they could also begin to explain unexpected relationships with indices of cognitive function, especially those that depend on the coordinated function of a number of distinct but interconnected brain structures. Even with the availability of

more advanced neuroimaging techniques such as DTI, studying the complex relationships between the response of neural tissue to TBI and its cognitive sequelae will undoubtedly require a multifaceted approach.

6.3 Conclusion

In conclusion, this thesis has used a combination of neuroimaging and neuropsychological methods to show that widespread and sometimes chronic white matter abnormalities are present after TBI and that these are associated with impairments of high-order cognitive functions. Standard structural MRI, which detects primarily focal lesions, cannot identify this DTI-detected more subtle damage that impacts the integrity of the brain's structural connections. The findings therefore highlight the importance of flexibly analysing patterns of disruption to brain structure across the whole brain using various complementary indices of its macro- and microstructure.

Overall, the findings suggest that cognitive impairments are not simply related to the presence or degree of structural brain abnormalities per se, but that they may be largely determined by the extent to which TBI disrupts the normal structural connectivity patterns of large-scale brain networks whose function supports complex cognitive processes.

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A

Characteristics of individual patients

Table A1

Summary Clinical Details for All 40 Patients Participating in the Research Programme

Patient	Age[a]	Gender	Cause of injury	Lowest GCS	PTA (days)	Contusions	White matter lesions[b]	Superficial siderosis
1	45	M	Assault	NK	<24 h	+	+	−
2	25	M	Assault	14	<24 h	−	−	−
3	18	M	Fall	NK	<24 h	−	−	−
4	23	M	Assault	NK	12	+	−	+
5	54	M	Fall	NK	<24 h	+	+	+
6	37	M	Fall	4	52	+	+	−
7	56	M	Other	3	NK	-	-	+
8	50	M	RTA	4	180	+	−	+
9	41	M	RTA	6	30	+	+	−
10	34	F	Fall	6	30	+	−	+
11	34	M	RTA	3	42	+	−	+
12	49	M	Assault	NK	<24 h	+	−	+
13	39	F	RTA	6	30	+	+	+
14	47	M	Assault	NK	5	+	−	+
15	47	F	RTA	5	42	+	+	−
16	36	M	Fall	NK	11	+	+	−
17	35	M	RTA	4	NK	+	-	+
18	23	M	Sports	14	<24 h	+	+	−
19	53	M	Fall	15	<24 h	−	−	−
20	66	F	RTA	NK	77	+	−	+
21	29	F	RTA	NK	<24 h	+	+	+
22	33	M	RTA	14	<24 h	+	+	−
23	52	M	Assault	3	<24 h	+	+	+
24	42	F	RTA	12	63	−	−	−
25	34	M	Assault	NK	42	+	+	+
26	53	F	Sports	15	<24 h	−	−	−
27	24	M	Assault	NK	<24 h	+	−	−
28	24	M	Assault	NK	<24 h	+	+	−
29	26	M	Fall	NK	<24 h	−	−	−
30	50	M	Assault	6	5	+	+	−
31	45	M	Assault	4	4	−	−	−
32	33	M	Assault	14	NK	+	−	−
33	49	M	NK	14	NK	+	−	+
34	47	F	Fall	15	NK	+	−	−
35	44	M	Sports	9	NK	−	+	+
36	21	F	RTA	NK	NK	−	−	+
37	25	M	Assault	14	NK	+	-	+
38	47	M	RTA	15	<24 h	−	−	−
39	34	F	RTA	15	NK	+	-	-
40	50	M	Fall	15	<24 h	−	−	−

Notes: [a] Age at the time of research assessment, [b] Presence of microbleeds (identified using the Microbleed Anatomical Rating Scale; Gregoire et al., 2009) or larger white matter lesions observed on gradient-echo T2*-weighted MRI. RTA = road traffic accident. GCS = Glasgow Coma Scale. PTA = post-traumatic amnesia. NK = not known/information not available.

B

Additional/self-report measures

This Appendix lists the self-report measures that were included in the battery of psychological measures as part of a wider research programme, but not included in any of the analyses reported in this thesis.

These measures included:

- The Hospital Anxiety and Depression Scale (HADS; Zigmond and Snaith, 1983)
- A modified, 18-item version of the Profile of Mood States (POMS; McNair, Lorr, & Droppleman, 1971, 1981)
- The Emotional Processing Scale (EPS; Baker, Thomas, Thomas, & Owens, 2007; Baker et al., 2010)
- The Frontal Systems Behavior Scale (FrSBe; Grace & Malloy, 2002)
- The Brain Injury Community Rehabilitation Outcome-39 (BICRO-39; Powell et al., 1998)
- The Barratt Impulsiveness Scale, Version 11 (BIS-11; Patton, Stanford & Barratt, 1995)

C

DTI preprocessing and tract-based spatial statistics (TBSS): Extended methods

Pre-processing of diffusion tensor imaging data:

DTIStudio (Jiang, van Zijl, Kim, Pearlson, & Mori, 2006) was used for computing the diffusion tensor by applying a least-squares fitting solution to solving an over-determined linear equation system (Jiang et al., 2005). This required the 'raw data' (i.e. the Philips Achieva PAR and REC files), and the scan sequence-specific gradient files, the bvals and bvecs determining the magnitude of diffusion weighting and the gradient directions. The parameters used for the DTIStudio setup were: image width = 128, image height = 128, image slice = 73, slice orientation = axial slice sequencing, inferior-superior, voxel size: FOV width = 224, FOV height = 224, slice thickness = 2, pixel size width = 1.75, pixel size height = 1.75, *b*-value = 1000, slices to be processed: start at 0, end at 72, partial slices, image sequence = gradient by gradient.

First, the image with no diffusion weighting (b = 0) was analysed and saved. Then, starting from the first DTI run and applying the vectors corresponding to the 16-directions encoded during that DTI run, the first set of 16 diffusion-weighted images was analysed and saved as unregistered DTI data (.hdr/.img pairs). The same was then done with the next three DTI runs, each containing 16 diffusion-weighted images. All images were checked for quality and the presence of artefacts or other visible problems. Together, the resulting four sets of unregistered data contained the data collected by applying gradients in 64 noncollinear directions. In order to concatenate the four unregistered .hrd images in time and create a single nifti (nii.gz) image, fslmerge from FSL was used.

Next, eddy current correction from the FDT was applied on the image that contained the newly concatenated data. This was done in order to correct for stretches and shears in the diffusion-weighted images caused by eddy currents of the gradient coils. Affine registration of the diffusion-weighted images to the reference (b = 0) image was also performed using FLIRT to correct for head in the scanner. The resulting nifti (nii.gz) image was an eddy current- and head motion-corrected registered image containing the 64-direction DTI data. Brain extraction was then performed on the b = 0 images as described in Chapter 2.

Finally, dtifit from the FSL's Diffusion Toolbox (FDT; Behrens et al., 2003) was used to

perform the three-dimensional fitting of the tensor model on the data. This resulted in a series of 3D images describing various properties of the diffusion tensor, including dti_L1 (the first eigenvalue (parallel or 'axial diffusivity'), mean diffusivity (MD), and fractional anisotropy (FA). To create an image characterising diffusivity perpendicular to the main axis of the diffusion tensor (i.e. 'radial diffusivity'), FSL's fslmaths was used to add together the dti_L2 and dti_L3 and then divide the resulting image by two to generate a dti_radial image (see Chapter 2 for a full list of these outputs).

The TBSS analyses consisted of:

1) Preparing the data:

   The individual FA (and MD, axial diffusivity and radial diffusivity) maps derived from the pre-processing stages of DTI data analysis were processed and moved into a newly generated folder called 'origdata'.

2) Registering the individual-space FA data to standard space:

   The FMRIB's Nonlinear Registration Tool (FNIRT; Andersson 2007a, 2007b), was used to perform non-linear registration of the individual-diffusion space images to standard FMRIB58_FA diffusion space, based on averaging 58 FA volumes collected from healthy individuals (see http://www.fmrib.ox.ac.uk/fsl/data/FMRIB58_FA.html, for further information)

3) Post-registration processing:

   As well as the nonlinear transform of each participant's FA image to standard diffusion-space, affine transform of the original FA images to standard 1mm brain space (Montréal Neurological Institute (MNI) template MNI152_1mm) was performed. The standard-space versions of each participant's FA image were then merged into a single 4D image file (all_FA), moved into a newly created 'stats' folder. Next, a mean_FA image was created from the mean of all individual FA images, and then thinned to create a mean_FA_skeleton image.

4) Pre-stats:

   A threshold of FA ≥ 0.2 was applied on the mean FA skeleton to create a skeleton mask image that exclude fibres of extremely low FA and, thus, high uncertainty due to partial volume effects and high inter-individual variability.

5) Projecting the pre-aligned FA data onto the ‘mean FA skeleton’:

A ‘distance map’ was first created from the skeleton mask and this map then used to guide the projection of each individual’s FA onto the skeleton (see Smith et al., 2006, for more detail). The 4D all_FA image, containing all participant’s aligned FA data, was then used for projecting each individual’s FA data (taken from the centres of white matter tracts) onto the spatially invariant mean_FA_skeleton by searching each participant’s aligned FA data (perpendicularly to the local tract direction) for the maximum FA values. This step created another 4D image (all_FA_skeletonised) that now contained all participants’ registered FA skeletons as individual ‘time points’.

6) Checking the results:

FSLView was used to load, one at a time, each participant’s FMRIB58_FA template-registered FA image, overlaid on the FMRIB58_FA image to check for the quality of the FNIRT registrations. The 4D all_FA and all_FA_skeletonised images were also viewed in FSLView and the accuracy of the alignment of each individual’s registered FA skeleton with the mean_FA_skeleton inspected.

7) Projecting the MD, axial diffusivity and radial diffusivity data onto the FA skeleton:

The non-linear transformation matrices, the FA skeleton and vectors applied for the skeleton-projection of individual FA data, derived from the previous steps, were used to align the other DTI metric data onto the white matter skeleton. This step, applied one metric at a time, produced the 4D all_MD_skeletonised, all_axial_skeletonised and all_radial skeletonised images that contained projected and skeletonised diffusion data from all participants. These skeletonised images, like the all_FA_skeletonised, could then be fed into voxelwise nonparametric permutation-based analyses.

8) Voxelwise statistics:

First, a GLM design matrix was set up to specify the contrasts to test hypotheses of the format ‘there will be a regionally specific group difference in white matter structure between TBI patients and healthy controls anywhere on the FA skeleton’ or ‘there will be a regionally specific correlation between white matter structure and cognitive function anywhere on the FA skeleton’. Examples of the designs and contrasts are provided below. Statistical analysis was performed using Randomise Version 2.1.

First, to specify a contrast to test whether there were any white matter regions where healthy controls (CON) had, for example, higher FA than TBI patients (TBI), the CON column of the matrix was assigned a +1 and the TBI column a −1. The opposite contrast, whereby, for example, the CON group were expected to have a lower MD than the TBI group, was then simply specified by assigning the CON column a −1 and the TBI column a +1. Potential confounding variables (here referred to as 'covariates of no interest'), such as age and gender, were mean centered (i.e. the mean deducted from each participant's score/value) before entering these into the design, data from each group modelled as separate EVs. When specifying the contrasts these columns were assigned zeros so that the effects of the potential confounds on the variables of interest would be attenuated in the analysis and not explored in their own right.

Second, to test for statistical association, for example whether there were any white matter regions that showed a significant correlation between FA and the neuropsychological variable of interest, each group membership, the neuropsychological variable, and possible covariates of no interest were all modelled as separate EVs, one column per group. The neuropsychological variable was first mean centered, and then, to set up the correlations to test, the EV for this variable was assigned a +1 or a −1, depending on the direction of its expected relationship with the DTI metric. Again, covariates of no interest were also mean centered and assigned a zero in the design matrix so that their effect on the relationship of interest would be attenuated.

Saving the designs thus generated the design.mat and design.con files that were then fed into the statistical analysis using Randomise, together with the skeletonised 4D image of the DTI metric being tested (e.g. all_FA_skeletonised) and the mean FA skeleton mask.

D

Publications

The following is a list of publications by the Traumatic Brain Injury Team to which the author has contributed and that were published during the course of the doctorate.

Journal papers

Bonnelle, V., Ham, T. E., Leech, R., Kinnunen, K. M., Mehta, M. A., Greenwood, R. J., & Sharp, D. J. (2012). Salience network integrity predicts default mode network function after traumatic brain injury. *Proceedings of the National Academy of Sciences of the United States of America, 109*(12), 4690-4695. doi:10.1073/pnas.1113455109

Bonnelle, V., Leech, R., Kinnunen, K. M., Ham, T., Beckmann, C. F., De Boissezon, X., . . . Sharp, D. J. (2011). Default mode network connectivity predicts sustained attention deficits after traumatic brain injury. *Journal of Neuroscience, 31*(38), 13442-13451. doi:10.1523/JNEUROSCI.1163-11.2011

Ham, T. E., de Boissezon, X., Leff, A., Beckmann, C., Hughes, E., Kinnunen, K. M. ., . . . Sharp, D. J. (2012). Distinct frontal networks are involved in adapting to internally and externally signaled errors. *Cerebral Cortex*. doi:10.1093/cercor/bhs056

Kinnunen, K. M., Greenwood, R., Powell J. H., Leech, R., Hawkins, P. C., Bonnelle, V., . . . Sharp, D. J. (2011). White matter damage and cognitive impairment after traumatic brain injury. *Brain, 134*(2), 449-463. doi:10.1093/brain/awq347

Ramlackhansingh, A. F., Brooks, D. J., Greenwood, R. J., Bose, S. K., Turkheimer, F. E., Kinnunen, K. M., . . . Sharp, D. J. (2011). Inflammation after trauma: Microglial activation after traumatic brain injury. *Annals of Neurology, 70*(3), 374-383. doi:10.1002/ana.22455

Sharp, D. J., Beckmann, C. F., Greenwood, R., Kinnunen, K. M., Bonnelle, V, De Boissezon, X., . . . Leech, R. (2011). Default mode network functional and structural connectivity after traumatic brain injury. *Brain, 134*(8), 2233-2247. doi:10.1093/brain/awr175

Published conference abstracts

Baxter, D., Leech, R., Midwinter, M., Kinnunen, K. M., Ham T., Bonnelle, V., . . . Sharp, D. J. (2011). White matter damage secondary to military blast and civilian traumatic brain injury: A comparative study. *Journal of Neurotrauma, 28*(5), A89-A90. doi:10.1089/neu.2011.9946

Bonnelle, V., Leech, R., Kinnunen, K. M., Ham, T., Beckmann, C. F., De Boissezon, X., . . . Sharp, D. J. (2011). Default mode network connectivity predicts sustained attention deficits following traumatic brain injury. *Journal of Neurotrauma, 28*(5), A73-A74. doi:10.1089/neu.2011.9946

Ham, T., Bonnelle, V., Barber, T., Kinnunen, K., De Boissezon, X., Leech, R., . . . Sharp, D. (2012). The neural basis of performance neglect after traumatic brain injury. *Brain Injury, 26*(4-5), 727. doi:10.3109/02699052.2012.660091

Ham, T., Bonnelle, V., Barber, T., Leech, R., Kinnunen, K. M., Beckmann, C. F., . . . Sharp, D. J. (2011). The neural basis of impaired self-awareness after traumatic brain injury. *Journal of Neurotrauma, 28*(5), A7-A8. doi:10.1089/neu.2011.9946

Pandit, A., Expert, P., Lambiotte, R., Leech, R., Turkheimer, F., Kinnunen, K., . . . Sharp, D. (2012). Traumatic brain injury impairs small world connectivity. *Brain Injury, 26*(4-5), 513. doi:10.3109/02699052.2012.660091

Sharp, D. J., Ham, T. E. (2011). Investigating white matter injury after mild traumatic brain injury. *Current Opinion in Neurology, 24*(6), 558-563. doi:10.1097/WCO.0b013e32834cd523

Sharp D., Powell, J., Leech, R., Bonnelle, V., Beckmann, C. F., De Boissezon, X., . . . Kinnunen, K. (2010). Altered structural and functional network connectivity predicts cognitive function after traumatic brain injury. *Journal of Neurology, Neurosurgery & Psychiatry, 81*, e23. doi:10.1136/jnnp.2010.226340.25